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TRANSPORT PROPERTIES OF SYNTHETIC NANOPORES

BY

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DISSERTATION

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Abstract

In recent years, advances in nanotechnology have allowed researchers to manufacture pores in synthetic membranes with subnanometer precision, so-called synthetic nanopores. Immersed in aqueous solution, these nanopores can be deployed to study the translocation of charged molecules and ions, a process of main importance in biology. However, researchers run into problems to build and control such small devices. Surface properties, thermal fluctuations and the discreteness of matter dominate at the nanoscale, producing phenomena not observed in the microscale. Indeed, little is known about the molecular level dynamics inside nanopores or the mechanism by which molecules and ions interact with nanopore walls. Molecular dynamics simulations can provide detailed atomistic images involving nanopores and physiological solutions. The work presented in this thesis uses molecular dynamics simulations to study synthetic nanopores made with two different materials: silica and polyethylene terephthalate. For simulated silica nanopores, the lack of suitable force fields to represent the interactions of the silica with other molecules in solution is a mayor problem. To address this problem, a novel silica force field was developed, which accurately reproduces the hydrophobicity of realistic amorphous silica surfaces. Such force field was employed to study the ionic conduction through silica nanopores. The simulations revealed that the atomic topography of the silica nanopore plays a major role in ionic conduction, producing phenomena such as ionic rectification. For polymeric polyethylene terephthalate nanopores, this thesis presents a protocol to assemble atomic models of polymeric bulk materials, which are then used to sculpt polymer nanopore models that reproduce the key features of experimental devices, namely a conical geometry and a negative surface charge density. The polymeric nanopore models were used to study on a novel phenomenon reported in nanopores, so called nanoprecipitation oscillations. The simulations unveiled the atomic detail mechanism of the nanoprecipitation phenomenon.
Acknowledgments

One of the things I learned in graduate school, is to strive to be brief and precise, concise and clear. I will try to apply such principle here, in the acknowledgments section. First of all, I would like to thank my advisor Klaus Schulten, Director of the Theoretical and Computational Biophysics Group (TCBG) at the University of Illinois at Urbana-Champaign (UIUC). His great support, seek for perfection, and passion for science have been truly inspirational to me. Specially, I thank him for teaching me how to write scientific papers, skill that improves with each paper. Also, I would like to thank Oleksii (Alek) Aksimentiev, a non-official co-advisor during my first years, who guided me through my first projects. A special thanks to Cindy Dodds, the Administrative Coordinator for the Center for Biophysics and Computational Biology (CBCB) at UIUC, her friendship and support have been constant since my first day in UIUC. I would also like to thank the other two members of my thesis committee, Gregory Timp and Narayan Aluru.

I have to thank all members of the TCBG without exception, for providing a stimulating scientific environment. I own an infinite list of thanks to Amy Shih, my officemate for more than five years: for proofreading my papers, for answering my random questions, for thousands of calls just to find my cell phone. I own another infinite list of thanks to James Conrad von Gumbart III, last heir of the Macomb-Habsburg royalty. I particularly thank Eric Lee, our vivacious discussions improved my spoken english and forced me to change the soft tone of my voice. I must mention Elizabeth Villa, Ying Yin, Anton Arkhipov, Robert Brunner, Markus Dittrich, David Hardy, Barry Isralewitz, Eduard Schreiner, Tim Skirvin, Marcos Sotomayor, John Stone and Leonardo Trabuco, who directly or indirectly contributed to this thesis. I thank David Brandon, Joyce Lucas and Jo Miller, who organize the TCBG behind the scenes. Also, I would also like to acknowledge a former TCBG member, Thorsten Ritz, who together with Zuzanna Siwy, introduced me to the polymeric nanopore system. I have to extend thanks to the new TCBG members: Xueqing Zou and Chaitanya Sathe, who reminded me the joy of teaching.
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<th>Description</th>
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<tr>
<td>CHARMM</td>
<td>Chemistry at Harvard macromolecular mechanics software and force-field.</td>
</tr>
<tr>
<td>C₉</td>
<td>Characteristic ratio for polyethylene terephthalate 9-mer.</td>
</tr>
<tr>
<td>DA</td>
<td>Dangling atom.</td>
</tr>
<tr>
<td>DO</td>
<td>Dangling oxygen.</td>
</tr>
<tr>
<td>DSi</td>
<td>Dangling silicon.</td>
</tr>
<tr>
<td>g(r)</td>
<td>Radial distribution function.</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamics.</td>
</tr>
<tr>
<td>MSD</td>
<td>Mean square deviation.</td>
</tr>
<tr>
<td>NAMD</td>
<td>Software for nanoscale molecular dynamics.</td>
</tr>
<tr>
<td>NVT</td>
<td>Canonical ensemble. Constant particle number, volume, and temperature.</td>
</tr>
<tr>
<td>NpT</td>
<td>Isothermal-isobaric ensemble. Constant particle number, pressure, and temperature.</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene terephthalate.</td>
</tr>
<tr>
<td>PME</td>
<td>Particle-mesh Ewald summation.</td>
</tr>
<tr>
<td>SPC</td>
<td>Single point charge water model.</td>
</tr>
<tr>
<td>TIP3P</td>
<td>Transferable intermolecular potential 3-point water model.</td>
</tr>
<tr>
<td>VMD</td>
<td>Software for visualization of molecular dynamics.</td>
</tr>
<tr>
<td>WCA</td>
<td>Water contact angle.</td>
</tr>
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</table>
God is in the details

- Ludwig Mies van der Rohe

the Devil is in the details

- Hyman G. Rickover
Chapter 1

Introduction

During the last decade, nanotechnology has gained popularity in the scientific community, mainly due to the development of promising technological tools, such as carbon nanotubes [1], nanowires [2], and nanopore sensors [3]. At the same time, nanotechnology has fostered the combination of techniques from different fields, such as material science and molecular biology, allowing us to craft and study devices with a precision below 100 nm. Nevertheless, building and controlling such small systems impose many problems. As pointed out by Feynman in 1959 [4], new difficulties can arise as we go down in size. All physical properties do not scale down proportionally. Surface effects, thermal fluctuations and the discrete nature of matter start to dominate at nanometer length scales, producing phenomena that can not be observed on the microscale. Currently available experimental techniques are not sensitive enough to elucidate the atomic-level dynamics of all these factors, providing many opportunities for theoretical and computational research to complement experiments [5, 6].
Synthetic nanopores are among the most popular nanodevices under research, mainly because of their putative use as a low-cost and ultrafast genome sequencing technique [7, 8]. Synthetic nanopores are small pores with nanometer scale radii, made of synthetic materials, such as silica [9], silicon nitride [10] or polymeric plastics [11]. Because of their apertures of just few nanometers, nanopores usually translocate a single charged biopolymer at a time; for example, a strand of nucleic acid. As the molecules thread their way through a nanopore, they produce electrical signals characteristic of their structure. Such signals have been used to differentiate single-stranded from double-stranded DNA [12, 13], to detect hairpin formation in RNA [14], to recognize methylation in DNA [15], and to study DNA-protein complexes [16, 17]. Recently, researchers have started to add chemical modifications to the pore surface, opening the possibility for more complex applications, such as nanofluidic diodes that control ion flow [18, 19], protein-coated channels to study cellular transport [20], and DNA-coated nanopores that switch the diameter depending on pH conditions [21]. Even though the number of nanopore applications is increasing, our understanding of the physical processes inside the nanopore is still in its early stages. In this regard, an atomic level description of the interactions with the pore surface is essential to improve technical uses of nanopores. Those interactions are enhanced by the high surface-to-volume ratio inside nanopores, affecting the selectivity of the pore as well as the dynamics of the translocating molecules.

Molecular dynamics (MD) simulations can provide a detailed atomistic description of systems with lengths up to hundreds of nanometers and time scales up to several microseconds, becoming valuable tools for studying nanopores. A brief description of the MD methodology is included in Appendix A. However, MD simulations of nanopore systems are not straightforward. Historically, methodologies for MD simulations of nanopore materials -such as silica glasses and polymeric plastics- and biomolecules -such a DNA, proteins and even water- have been developed independently from each other. As a result, they use incompatible models and the consolidation of both areas demands a great effort.

This thesis presents MD studies of synthetic nanopores manufactured in two different materials: silica (SiO$_2$) and polymeric polyester-terephthalate (PET). The outline of the remainder of this thesis is as follows: Chapter 2 presents the research performed to integrate silica with biomolecular models. A key point in simulating these systems is determining the interactions between the silica surface and water. This Chapter reports the parameterization of a force field for amorphous silica surfaces using an approach proposed by Werder et al. [22], making the hydrophobicity of the surface the main target of parameterization (see Fig. 1.1). In Chapter 3, the resulting silica force field
is deployed to study the ionic conduction through silica nanopores, focusing on the ionic current rectification phenomena. Chapter 4 presents a study on nanopores made from plastic polymers, namely polyethylene terephthalate (PET). MD simulations of PET have been previously used to study PET materials [23–25]. Nevertheless, most of the work has been focused on studying the bulk properties and there is still a lot to be learned about the interactions of the PET surface with water and ions. This Chapter presents a procedure to build an accurate atomic description of the polymer nanopore surface in solution, which was not available before. Furthermore, KCl conduction through PET pores and the effect of Ca\(^{+2}\) ions is presented. Finally, Chapter 5 presents a study of a recent phenomenon reported in PET nanopores, so-called nanoprecipitation oscillations. Overall, the four following Chapters highlight the relevance of MD simulations as imaging modalities to study and improve the capabilities of nanodevices.

Figure 1.1: Water droplet. The figure shows an atomic-scale model of a water droplet resting on top of a silica surface. The hydrophobicity of the silica surface determines the shape of the droplet.
Chapter 2

A water-silica model for simulating nanodevices


2.1 Introduction

Amorphous silica (SiO$_2$) is an inorganic material commonly used in semiconductor circuits to isolate different conducting regions. Due to its mechanical properties, high dielectric strength, and selectivity for chemical modification, amorphous silica has become a key material in microelectronics [26] and chromatography [27]. In recent years, the convergence of molecular biology and nanotechnology has opened up opportunities for many applications that involve macromolecules and silica, such as nanoelectronics [28], self-assembly of nanostructures [29], microfluidics [30], and DNA microarray technology [31]. An atomic level understanding of the interactions between biomolecules and silica is now central for further development of bionanotechnology applications. A combination of spectroscopic and molecular biology tools allows one to infer such interactions in principle; however, no experimental technique is yet sensitive enough to resolve atomic dynamics at the amorphous interface. Molecular dynamics (MD) simulations can provide detailed interfacial properties involving silica and physiological solutions. Such simulations have been used previously to study systems composed of biomolecules and inorganic crystals [32] and they can be tailored to study non-crystalline inorganic systems.

Until recently, atomic-scale simulations of inorganic and biomolecular materials, such as silica and DNA, have mostly evolved independently and the integration of both inorganic and biomolecular models is still a great challenge. Key in modeling these hybrid systems are the interactions between the solid surface, biopolymers, and water [22, 33]. These interactions are defined through the potential energy function of the system, the force field, that returns, in simulations, the interacting...
forces between atoms as a function of their atomic coordinates.

Since interactions with biomolecules in all present biotechnical applications of silica occur in an aqueous environment, an empirical force field for silica should reproduce its surface wetting properties. However, existing force fields for silica [34–37] were parameterized to describe primarily its bulk physical properties correctly, and then refined to reproduce the geometry of the silica surface [38], but the force fields were not validated to describe interactions with biopolymers in aqueous solution. Furthermore, the functional form of most available silica force fields is not compatible with the water models used in biomolecular simulations, i.e., TIP3P [39] or SPC [40].

Because of the lack of a suitable force field, silica potentials have been used beyond their validated conditions to study the interactions of water [41–43], small organic molecules [44], and ions [45] with silica nanopores. Although those studies have captured different experimental features of the confined nanopore environment, combining different silica force fields with the same water model can produce completely different outcomes as illustrated rather dramatically in Fig. 2.1.

In this Chapter, a novel silica force field compatible with TIP3P [39] and CHARMM [46] force field is presented. The force field was parameterized using an approach proposed by Werder et al. [22] This approach selects force field parameters such that macroscopic properties, namely the hydrophobicity of the surface, are reproduced well. For this purpose, the contact angle of a water droplet on a silica surface, referred to as water contact angle (WCA), is used to tune the intermolecular silica-water interactions. This Chapter describes the protocols to build atomic-scale models of amorphous silica surfaces, as well as the procedure to parameterize a force field using the WCA.
2.2 Building silica structures

The schematic procedure for building silica structures is shown in Fig. 2.2. First, a model of bulk amorphous silica was built. Then, pieces of the bulk material were sliced to create slabs of amorphous silica. Subsequently, the sliced systems were annealed.

The program Cerius$^2$ v. 4.9 [47] from Accelrys was used to obtain amorphous silica structures. The GLASS$2.01$ and GLASSFF$1.01$ force fields provided in Cerius$^2$ were employed. The GLASS$2.01$ force field includes two contributions, a two-body and a three-body nonbonded potential:

\[
U_{\text{total}} = \sum_{i<j} U_2(i,j) + \sum_{i<j<k} U_3(i,j,k) , \tag{2.1}
\]

\[
U_2(i,j) = A_{ij} \exp \left[ -\frac{r_{ij}}{\rho} \right] + \frac{Z_i Z_j e^2}{r_{ij}} \text{erfc} \left( \frac{r_{ij}}{\beta_{ij}} \right) , \tag{2.2}
\]

\[
U_3(i,j,k) = h(r_{ij}, r_{ik}, \phi_{ijk}) + h(r_{jk}, r_{ji}, \phi_{kji}) + h(r_{ki}, r_{kj}, \phi_{ikj}) . \tag{2.3}
\]

The two-body potential $U_2(i,j)$ is composed of a repulsive van der Waals (vdW) term and an electrostatic Coulombic term screened with a complementary error function (erfc) that reduces the

![Figure 2.2: Building amorphous silica structures. (a) A crystoballite unit cell is replicated, filling a cube of 57 Å side. (b) To obtain amorphous bulk, the crystalline silica is annealed, increasing the temperature to 8000 K and then lowering it back to 300 K. The cube of bulk material was sliced and annealed to create silica surfaces. (c) A 20 Å-thick slab of amorphous silica (black rectangle) is annealed further to produce amorphous silica surfaces. Atoms located 4 Å from the bottom of the slab were fixed (shown in green). By varying the annealing conditions (Table 2.1), different concentrations of dangling atoms at the surface (shown in blue) were obtained.](image)
Annealing Cycle

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<tr>
<th></th>
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<th>Initial Time (ps)</th>
<th>Cooling Rate (K/ps)</th>
<th>Total Time (ps)</th>
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<td>5000</td>
<td>20</td>
<td>100/5</td>
<td>255</td>
</tr>
<tr>
<td>SURF.3b1</td>
<td>5000</td>
<td>20</td>
<td>100/5</td>
<td>255</td>
</tr>
<tr>
<td>SURF.3b2</td>
<td>1000</td>
<td>10</td>
<td>700/10</td>
<td>20</td>
</tr>
<tr>
<td>SURF.3b3</td>
<td>300</td>
<td>50</td>
<td>–</td>
<td>50</td>
</tr>
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<td>SURF.3b5</td>
<td>300</td>
<td>10</td>
<td>–</td>
<td>10</td>
</tr>
<tr>
<td>SURF.Raw</td>
<td>–</td>
<td>–</td>
<td>–</td>
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Results

<table>
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<tr>
<th></th>
<th>[DO] (nm⁻²)</th>
<th>[DSi] (nm⁻²)</th>
<th>[DA] (nm⁻²)</th>
<th>Accessible Surf. Area (nm⁻²)</th>
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<tr>
<td>SURF.2b</td>
<td>0.031</td>
<td>0.062</td>
<td>0.092</td>
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<tr>
<td>SURF.3b1</td>
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<td>0.462</td>
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<td>42.731</td>
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<tr>
<td>SURF.3b2</td>
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<td>1.108</td>
<td>2.093</td>
<td>42.384</td>
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<tr>
<td>SURF.3b3</td>
<td>1.016</td>
<td>1.200</td>
<td>2.161</td>
<td>42.984</td>
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<tr>
<td>SURF.3b4</td>
<td>1.108</td>
<td>1.631</td>
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<td>SURF.3b5</td>
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<td>1.662</td>
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<td>SURF.Raw</td>
<td>3.232</td>
<td>2.585</td>
<td>5.817</td>
<td>50.411</td>
</tr>
</tbody>
</table>

Table 2.1: Annealing simulations for silica surfaces. Simulations were performed assuming an NVT ensemble. The final annealing temperature was 300 K in all cases. Concentrations of dangling atoms [DA], dangling oxygens [DO], and dangling silicons [DSi] were calculated using a constant area of 32.5 nm². The solvent accessible surface area was calculated using the SASA command provided in VMD [48] with expanded atom radius of 2 Å. To obtain SURF.2b, the annealing was performed using GLASSFF_1.01 force field; all other simulations were performed using GLASS_2.01 force field. For slabs SURF.3b2 and SURF.3b3, atoms 4 Å from the bottom were fixed; for slabs SURF.3b4 and SURF.3b5, atoms 15 Å from the bottom were fixed. SURF_Raw was neither minimized nor annealed, yielding the surfaces with the highest concentrations of DAs.

interaction between the ionic charges $Z_i$ and $Z_j$. $e$ is the elementary charge, $r_{ij}$ is the distance between atoms $i$ and $j$, and $A_{ij}$, $\beta_{ij}$, and $\rho$ are adjustable parameters. The three-body potential $U_3(i,j,k)$ introduces a directional contribution to induce a tetrahedron arrangement of the silica glass. Here, $h(r_{ij}, r_{ik}, \phi_{jik})$ is defined through

$$h(r_{ij}, r_{ik}, \phi_{jik}) = \begin{cases} \lambda_i \exp \left[ \frac{\gamma_i}{r_{ij} - r_i} + \frac{\gamma_i}{r_{ik} - r_i} \right] (\cos \phi_{jik} - \cos \phi_{jik}^c)^2, & r_{ij} < r_i^c \text{ and } r_{ik} > r_i^c \\ 0, & r_{ij} \geq r_i^c \text{ or } r_{ik} \leq r_i^c \end{cases}$$

(2.4)

This term produces an energy bias towards the angle $\phi_{jik}^c$; the angle is determined by the atoms $j$ and $k$, with a vertex at $i$, only when the atoms $j$ and $k$ are within the radial distance $r_i^c$. $\lambda_i$ and $\gamma_i$ are adjustable parameters. The force field GLASSFF_1.01 is an earlier version of GLASS_2.01 and includes only the two-body potential (Eq. 2.2).

The simulations to obtain silica structures were performed using an integration time step of 1 fs and assuming periodic boundary conditions as well as an NVT ensemble (unless stated otherwise). Temperature and pressure were controlled using a Nosé-Hoover thermostat. To permit full electrostatic calculations via PME summation, the systems were made periodic in all directions. To maintain electroneutrality of the systems, a few atoms were removed from the surfaces, enforcing a ratio of two oxygen atoms per one silicon atom.

To create bulk amorphous silica, a low-crystoballite unit cell was replicated $11 \times 11 \times 8$ times,
filling a box of 57 Å side, yielding a system of 11,616 atoms. This step was followed by the annealing cycle 2-VIII proposed by Huff et al. [49] that employed the silica force field GLASS_2.01. The final amorphous system had a periodicity of 57.3 Å. The connectivity was calculated for each atom using a cut-off radius of 2 Å. 99.51% of the oxygens were bi-coordinated and 97.22% silicons were four-coordinated, which is in good agreement with Huff et al.’s results.

In order to furnish different silica surfaces, a slab of 20 Å height comprising 3,981 atoms was cut from the 3D periodic bulk silica and annealed using either GLASS_2.01 or GLASSFF_1.01 force fields and different heating and cooling rates. For all annealing cycles, atoms 4 Å from the bottom in the Z-direction were fixed [50] and the periodicity of the original bulk cell was kept constant, which created a 30 Å slab of vacuum above the silica surface.

After annealing, the silica atoms in the resulting structures were classified according to their connectivity. Two atoms were considered covalently bonded if they had a separation distance of 2 Å or less. Oxygens with two or more bonds and silicons with four or more bonds were classified as non-dangling atom (non-DA) type. Oxygens with less than two bonds and silicons with less than four bonds were classified as dangling oxygen (DO) type and dangling silicon (DSi) type, respectively, or just as dangling atom (DA) type. These classifications were used extensively in the analysis of material properties. Table 2.1 defines the seven surfaces produced and lists the respective concentration of DAs, DOs, and DSis. These surfaces will be denoted as annealed surfaces.

Higher temperature and longer annealing using the GLASS_2.01 force field reduces the number of DAs, but not to the extent sufficient to obtain a silica surface with no DAs. In order to obtain a silica surface with very few DAs, i.e. a minimum DA concentration, the force field GLASSFF_1.01 was used. Bakaev et al. [51, 52] were able to obtain hydrophobic silica surfaces with few DAs using non-linear annealing schemes. In their original study, Bakaev employed an ionic pair potential (Eq. 2.2) with an additional dispersive term [34]. Due to the directionality added by the three-body potential in GLASS_2.01, DAs at the surface can easily be trapped into local minima corresponding to the tetrahedron configuration. The advantage of GLASSFF_1.01 is that it takes only two-body interactions into account and allows enough distortion of the tetrahedron arrangement to obtain a surface of silica without DA.

Once the annealed surfaces were obtained, they were replicated 2 × 2 times in the X- and Y-directions and used as templates to build silica slabs with four extra surface modifications, denoted as surfaces of type I, II, III, and IV. All silica surfaces are illustrated in Fig. 2.3. Figure 2.3a shows the annealed surface, with DOs and DSis present at the surface. Figure 2.3b shows the surface of
Figure 2.3: Five models for silica surfaces. All surfaces are composed of non-dangling atoms along with five different modifications: (a) Annealed surface with dangling silicon and oxygen atoms (opaque yellow and red atoms, respectively). (b) Surface of type I with only dangling oxygens (opaque red atom). (c) Surface of type II with silanols. (d) Surface of type III with vicinal silanols. (e) Surface type IV with deprotonated vicinal silanols, a hydrogen bond between each pair of silanols being enforced (green dashed line, see also Fig. 2.4).

For annealed surfaces and surfaces of type I and II, the number of DAs and, consequently, the number of surface modifications, is determined by the annealing cycles. In order to have control of the silanol concentration, silanols were also generated by randomly breaking siloxane bonds (−SiO−) starting with a surface with very few DAs (cf. surface Surf_2b in Table 2.1). Figure 2.3d shows the surface of type III; in this case each broken −SiO− bond that produces a pair of DAs was converted to silanol groups by adding a hydrogen to the DO and a hydroxyl group to the DSi. Figure 2.3e shows the surface of type IV, created in the same way as the surface of type III, but with one silanol of each pair of vicinal groups being deprotonated; a harmonic bond between hydrogen and oxygen was enforced to mimic a hydrogen bond (green dashed line, see below).

The annealed surfaces provided DA concentration in a range between 0.092 and 2.832 nm$^{-2}$ and were employed to simulate water-silica systems. The annealed surfaces modified into types I-IV were also employed this way. Water discs were placed on top of the silica surfaces; then, the resulting systems were employed to parameterize the intermolecular interactions using a CHARMM compatible silica force field.

### 2.3 Functional form of water-silica force field

To parameterize a force field that can account for silica-water interactions, a potential energy function compatible with the CHARMM force field was used. Previously existing parameters for silicon and oxygen atoms were used as starting points, but refined to reproduce the interactions of silica
with water. The functional form used is:

\[ U_{\text{total}} = U_{\text{bond}} + U_{\text{angle}} + U_{\text{vdW}} + U_{\text{Coulomb}} \]  

(2.5)

The first two terms on the right hand side of Eq. 2.5 are harmonic potentials used to describe bond stretching and bending:

\[ U_{\text{bond}} = \sum_{\text{bonds } \alpha} k_{\alpha}^{\text{bond}} (r_{\alpha} - r_{0\alpha})^2 \]  

(2.6)

\[ U_{\text{angle}} = \sum_{\text{angles } \beta} k_{\beta}^{\text{angle}} (\theta_{\beta} - \theta_{0\beta})^2 \]  

(2.7)

Here the sums run over all bonds and bond angles; the parameters \( k_{\alpha}^{\text{bond}}, k_{\beta}^{\text{angle}}, r_{0\alpha}, \) and \( \theta_{0\beta} \) describe the equilibrium values of the degrees of freedom. The bonded interactions of silica were taken from Hill et al. [35], but adjusted to fit Eq. 2.6 and Eq. 2.7.

The last two terms in Eq. 2.5 describe the vdW and electrostatic non-bonded interactions that are the main focus of this work:

\[ U_{\text{vdW}} = \sum_i \sum_{j>i} \varepsilon_{ij} \left( \frac{r_{ij}^{\text{min}}}{r_{ij}} \right)^{12} - 2 \left( \frac{r_{ij}^{\text{min}}}{r_{ij}} \right)^6 \]  

(2.8)

\[ U_{\text{Coulomb}} = \sum_i \sum_{j>i} \frac{q_i q_j}{4 \pi \epsilon_0 r_{ij}} \]  

(2.9)

The vdW interactions are represented through a Lennard-Jones 6-12 potential, with two adjustable parameters: \( r_{ij}^{\text{min}} \), the distance at which the energy between atoms \( i \) and \( j \) is minimal, and \( \varepsilon_{ij} \), the well depth. In the case of the oxygen atom, vdW parameters were taken from the CHARMM force field, assigning 3.5 Å to \( r_{ij}^{\text{min}} \) and 0.15 kcal/mol to \( \varepsilon_{i} \). For silicon, \( r_{ij}^{\text{min}} \) was set to 4.3 Å, corresponding to the silicon atomic radius [53, 54]. The value of \( \varepsilon_{Si} \) was estimated from the wettability of silica. The electrostatic interactions were calculated using Eq. 2.9, \( q_i \) and \( q_j \) being the partial atomic charges of atoms \( i \) and \( j \), and \( \epsilon_0 \) the vacuum permittivity. The charges corresponding to silicon (\( q_{Si} \)) and oxygen (\( q_O \)) were tuned to reproduce the silica wettability. TIP3P water model was used, since the CHARMM force field works best with this choice of model and its functional form conforms to Eq. 2.5.
2.4 Silica-water systems

The molecular dynamics program NAMD 2.5 [55] was used to simulate silica-water systems. Cylindrical volumes of water were generated from a pre-equilibrated water box. The silica structures were maintained rigid by fixing or restraining atomic coordinates to their original positions (see below), while water molecules were allowed to move freely. All simulations involving silica-water systems were carried out using a CHARMM compatible form of the potential energy function (Eq. 2.5). The details of the MD protocols are summarized in Appendix A.

To simulate the WCA of silica, a water disc with a radius and height of 30 Å composed of 1981 molecules, was placed on top of each surface. All systems were minimized for 1000 steps of 1 fs using the conjugate gradient method and then equilibrated for 2 ns in the \( NVT \) ensemble. The elementary cell extension of the systems was 11.5 nm\( \times \)11.5 nm\( \times \)15.8 nm. To keep the amorphous slab rigid, the silica atoms were either fixed or restrained to their original position by applying a harmonic force with a force constant of 1 kcal/mol/Å\(^2\). Table 2.2 and 2.3 summarize the parameters used for the various silica slabs and specify which atoms were either fixed or restrained.

In order to evaluate the electrostatic effect of silanols on wetting, the respective charges were divided according to the scheme (see Fig. 2.4):

\[
q_{\text{total}} = q_{\text{vicinal}} + q_{\text{neigh,O}},
\]

where \(q_{\text{total}}\) is the total charge of each pair of silanol groups and its neighboring oxygens, \(q_{\text{neigh,O}}\) is the half of the charge corresponding to six oxygens, and \(q_{\text{vicinal}}\) is the charge of each pair of vicinal silanols. The initial charges for the silanol hydroxyl group were taken from the serine hydroxyl

<table>
<thead>
<tr>
<th>Surface</th>
<th>Fixed</th>
<th>Parameters for surface modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>annealed all atoms</td>
<td>( q_{\text{Si}}: 0.1, 0.3, 0.5, 0.7, 0.9, 1.1 \ e )</td>
<td>( \varepsilon_{\text{Si}}: 0.06, 0.1, 0.2, 0.3, 0.4 \text{ kcal/mol} )</td>
</tr>
<tr>
<td>type I all atoms except ( DO )</td>
<td>( q_{\text{DO}}: -0.0, -0.1, -0.3, -0.5, -0.7, -0.9, -1.1 \ e )</td>
<td>( \varepsilon_{\text{DO}}: 0.3 \text{ kcal/mol} )</td>
</tr>
</tbody>
</table>

Table 2.2: WCA simulations of silica slabs without explicit silanol groups. The table presents a summary of the conditions and parameters used. For annealed surfaces, bulk and surface atoms have the same parameters. For surfaces of type I, parameters for bulk atoms were fixed to the values \( q_{\text{Si}}: 0.9 \ e, \varepsilon_{\text{Si}}: 0.3 \text{ kcal/mol}, r_{\text{Si}}^{\text{min}}: 4.3 \ \text{Å}, q_{\text{O}}: -0.45 \ e, \varepsilon_{\text{O}}: 0.15 \text{ kcal/mol}, r_{\text{O}}^{\text{min}}: 3.5 \ \text{Å}, \) while the charge of \( DO, q_{\text{DO}} \), was changed as shown in the table.
Figure 2.4: Charge distribution for silanol groups on surfaces of type III and IV. (a) In the surface of type III, \( q_{\text{vicinal}} \) gets contributions from six atoms. (b) In the surface of type IV, \( q_{\text{vicinal}} \) is composed of five atoms, a hydrogen-bond is enforced between vicinal silanols (dashed line) with a spring constant of 5.5 kcal/mol/Å² and an equilibrium distance of 3.5 Å. In both cases, (a) and (b), \( q_{\text{neigh}} \) is the charge of the half of six oxygens and the total charge \( q_{\text{total}} \) was modified from 0 to -1.0 \( e \) according to Eq. 2.10. The resulting charge was evenly distributed over the silanol atoms in \( q_{\text{vicinal}} \).

group of the CHARMM force field. Subsequently, \( q_{\text{total}} \) was changed from 0 to -1 \( e \) in decrements of 0.25 \( e \) according to Eq. 2.10 and the resulting charge was evenly distributed over the silanol atoms included in \( q_{\text{vicinal}} \).

To perform MD simulations with full electrostatics under periodic boundary conditions, the total charge of the system has to be zero. However, when adding modifications to annealed surfaces, different proportions of atoms are added, resulting in a total charge not equal to zero. Electronu- trality was recovered by scaling down the charge for all silica atoms below the modifications [56]. For all charges modified, the adjustment was less than 0.5% of the initial charge value.

In the case of surfaces of type III and IV, oxygen atoms directly connected to silanols were allowed to move while the rest of the silica was restrained. Such relaxation was required for the following reasons: The silanols were generated by breaking bonds and adding hydroxyl groups; however, the latter gave rise to strong repulsive forces that relaxed only when the first layer of atoms connected to the silanol groups were free to rearrange.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Fixed</th>
<th>Harmonic Restraints</th>
<th>Parameters for surface modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>type II</td>
<td>all atoms except hydroxyl groups</td>
<td>–</td>
<td>OH from CHARMM, OH from TIP3P</td>
</tr>
<tr>
<td>type III</td>
<td>all atoms except SiOH groups and oxygens bonded to SiOH</td>
<td>OH from CHARMM, but ( q_{\text{total}} ) varied according to Eq. 2.10 from 0 to -1 ( e ) in decrements of 0.25 ( e )</td>
<td></td>
</tr>
<tr>
<td>type IV</td>
<td>all atoms except SiOH groups and oxygen bonded to SiOH</td>
<td>OH from CHARMM, but ( q_{\text{total}} ) varied according to Eq. 2.10 from 0 to -1 ( e ) in decrements of 0.25 ( e )</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3: WCA simulations of silica slabs with explicit silanol groups. The table presents a summary of the conditions and parameters used. Parameters for bulk atoms were fixed to the values \( q_{\text{Si}}: 0.9 \ e \), \( \varepsilon_{\text{Si}}: 0.3 \) kcal/mol, \( r_{\text{Si-O}}^{\text{min}}: 4.3 \) Å, \( q_{\text{O}}: -0.45 \ e \), \( \varepsilon_{\text{O}}: 0.15 \) kcal/mol, \( r_{\text{O}}^{\text{O}}^{\text{O}}: 3.5 \) Å. Bond bending terms for OH were taken from either CHARMM or TIP3P. In the case of OH from CHARMM, the hydroxyl group was taken from the serine group.
2.5 The water contact angle

The calculation of the water contact angle [57] is schematically presented in Fig. 2.5. The density profile was computed using horizontal layers of 3 Å height. Each layer was displaced by 0.5 Å in the Z-direction, starting from the base and ending at the top of the droplet. Within each layer, the radial density was calculated using circular bins of 3 Å thickness, that were concentric around the center of mass of the droplet; for each bin, the inner boundary was displaced by 0.5 Å along the horizontal axis until 20 bins with zero density were reached. To find the droplet boundary, each layer was fitted to the function

$$\rho(x) = a \cdot \left[ 1 - \tanh \left( \frac{x - x_0}{b} \right) \right], \quad \text{(2.11)}$$

which represents the decrease of the radial density. Here $\rho(x)$ is the radial density, $a$ and $b$ are proportional to the bulk liquid density and the thickness of the interface, respectively, and $x_0$ is the layer boundary. The layer boundary $x_0$ resulting from matching Eq. 2.11 to the radial density was fitted to a circular segment, the boundary within 5 Å from the bottom being excluded.

Figure 2.5: Calculation of the water contact angle. The contour plot shows the radial density profile of a water droplet (gray tones). First, the surface of the water droplet (crosses) is calculated from the density profile using Eq. 2.11. Then, the resulting surface is fitted to a circular segment (dashed line). The water contact angle ($\theta$) is the angle between the tangent of the fitted circle and the silica surface. The water below 5 Å from the silica surface (dotted line) is excluded from the calculations.
Figure 2.6: Variation of the WCA during MD equilibration. The figure shows the value of the WCA ($\theta$) using an annealed surface (cf. Fig. 2.3a). The error bars reflect the deviation of the droplet’s contact line from a circle. (a) Initial configuration with a water disc. The snapshots taken after (b) 0.1, (c) 0.5, and (d) 2.0 ns of equilibration are shown. The WCA values during the last 0.2 ns (gray background) were averaged and used as an equilibrium value.

Figure 2.6 shows the time dependence during a 2 ns MD simulation of the WCA for a droplet of 1981 water molecules resting on an annealed silica surface. A similar time dependence was seen for all annealed surfaces, showing an initial decrease within the first 1 ns, followed by an equilibrium state over the next 1 ns. The WCA was averaged over the last 0.2 ns for each MD simulation. A few water molecules eventually evaporated physically from the droplet; water molecules 7 Å away from the droplet were not taken into account.

2.6 Influence of droplet size on water contact angle

In using the WCA as a selection criterion of force field parameters, the question arises if simulated WCAs may depend on the size of droplets considered such that results may be biased. The effect of the nanometer size of the water droplet on the WCA was evaluated using a procedure employed by Werder et al. [22] which is briefly described below. For a macroscopic droplet, the contact angle at equilibrium with an inert solid surface is determined by Young’s equation, i.e., by:
\[ \gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta_{\infty}, \]  

(2.12)

where \( \theta_{\infty} \) is the macroscopic WCA and \( \gamma_{LV}, \gamma_{SV}, \gamma_{SL} \) are the surface tensions at the liquid-vapor, solid-vapor, solid-liquid interfaces, respectively. To describe a microscopic droplet, a free energy correction to Eq. 2.12 needs to be taken into account. Macroscopic and microscopic contact angles can be correlated to each other using a modification of Young’s equation [58]

\[ \begin{align*}
\gamma_{SV} &= \gamma_{SL} + \gamma_{LV} \cos \theta + \frac{\tau}{r_B}, \\
\cos \theta &= \cos \theta_{\infty} - \frac{1}{\gamma_{LV} r_B} \frac{\tau}{r_B}.
\end{align*} \]  

(2.13, 2.14)

where \( \theta \) is the microscopic WCA, \( \tau \) is the line tension, \( \gamma_{LV} \) is the surface tension at the liquid-vapor interface, and \( r_B \) is the radius of the base of the droplet.

Using Eq. 2.14, the variation of the WCA with droplet size can be determined by plotting \( \cos \theta \) as a function of \( 1/r_B \). The plot has a slope proportional to \( \tau \), the sign of \( \tau \) indicating if the WCA either increases or decreases with decreasing droplet size. Experimentally, the sign of \( \tau \) is difficult to determine; its magnitude has been estimated to lie between \( 1 \times 10^{-12} \) and \( 1 \times 10^{-10} \) J m\(^{-1}\) [59], which is at the limit of experimental resolution. MD simulations using argon droplets on ideal smooth surfaces have shown that \( \tau \) can be positive, negative, or even close to zero, depending on the magnitude of the intermolecular interactions between liquid and solid.[60]

Surface SURF.2b was employed to calculate the slope \( \tau/\gamma_{LV} \) in Eq. 2.14 (cf. Table 2.1), which has the lowest solvent accessible area and a minimum effect of surface roughness. In addition to the system of 1981 water molecules, two further systems were built: a smaller one with a water disc of height and radius of 22 Å (1039 water molecules) and a larger one with a water disc of height and radius of 35 Å (4059 water molecules). In the latter system, the annealed surface was replicated 4×4 times in the X- and Y-directions; the systems were simulated for 2 ns and 5 ns, respectively, under the conditions described in Table 2.2. The slope \( \tau/\gamma_{LV} \) showed no clear correlation with droplet size. Then, the WCA was assumed to be independent of the droplet size, and the silica force was parameterized using a droplet of 1981 water molecules.
2.7 Dangling atoms enhance electrostatic silica-water interactions

After the annealed surfaces were obtained and the size of the water-silica system specified, the silica surfaces were used to investigate how the force field parameters affect the wettability of different silica surfaces. Figure 2.7 shows the dependence of the WCA on the silica intermolecular parameters for three silica surfaces: two annealed surfaces with DA concentrations of 0.092 and 2.093 nm$^{-2}$ and a surface corresponding to a “raw cut” of bulk amorphous silica without any minimization or annealing applied, with a DA concentration of 5.817 nm$^{-2}$. The parameter for the silicon well-depth ($\varepsilon_{Si}$) was varied from 0.06 to 0.4 kcal/mol while the oxygen and silicon charges were changed simultaneously, keeping their ratio constant, namely at a value of -1:2.

The WCA determined for the surface with the lowest DA concentration (Fig. 2.7a) is seen to depend only on the magnitude of the vdW interaction and is insensitive to the partial charges of silicon and oxygen atoms. For a non-zero DA concentration, the partial charges influence the WCA. Figure 2.7b shows a surface with a DA concentration of 2.093 nm$^{-2}$; in this case, the WCA indeed is seen to depend to the same extent on vdW and electrostatic interactions, an increment in the magnitude of either of these interactions reducing the WCA. Figure 2.7c shows a surface with the highest DA concentration, corresponding to a “raw cut” of bulk amorphous silica; in this case the effect of the electrostatic interactions dominate.

The results described are consistent with previous MD studies [51, 61] which showed that DAs at the surface, like DOs and DSis, are very hydrophilic. The low affinity of bi-coordinated oxygens (non-DAs) towards water has also been confirmed by experiments [62]. Fully coordinated atoms are partially buried at the surface; hence, their electrostatic contribution is effectively screened by their neighbors. Conversely, DAs are more exposed and the charges of $qO$ and $qSi$ produce oriented dipoles that act as adsorption sites for water.
Figure 2.7: Concentration of dangling atoms affects the wetting properties of silica. The first row shows a top view of three silica slabs: two annealed surfaces with dangling atom (DA) concentration of 0.09 nm$^{-2}$ (a) and 2.09 nm$^{-2}$ (b), and a “raw cut” surface with 5.82 nm$^{-2}$ (c). Dangling atoms are colored in blue and non-dangling atoms are colored in red. The three contour plots in the second row illustrate the dependence of the WCA on silica intermolecular parameters. The value of the WCA is color-coded according to the colorbar at the right. The abscissa represents the variation of the vdW parameters for silica. The parameter for the silicon well-depth ($\varepsilon_{Si}$) varies from 0.06 to 0.4 kcal/mol. The ordinate represents the partial charge of the silicon atoms. The oxygen and silicon charges are changed simultaneously, keeping their ratio at -1:2. The charge of the oxygen atoms ($q_O$) varies from -0.05 to -0.55 e, while the charge of the silicon atoms ($q_{Si}$) varies from 0.1 to 1.1 e.
2.8 Parameterization of intermolecular interactions

The main goal of this study was to optimize force field parameters for the interactions of silica and water. The first step in judging modeled silica-water interactions is to establish the experimental observable that will serve as the criterion for optimality, namely the observed WCA. In the case of amorphous silica, the observed WCA can assume a range of values, depending on the concentration of silanol groups at the silica surface. Lamb et al. [63] have established a heuristic relationship between WCA and the area fraction, $a_{SiOH}$, of silanols in polished silica plates:

$$\cos \theta = 0.257 \ a_{SiOH} + 0.743$$  \hspace{1cm} (2.15)

Here $a_{SiOH}$ is the number of silanols per nm$^2$ divided by 4.6 nm$^{-2}$ (the latter being the maximum silanol surface density) [64]. From Eq. 2.15, a completely dehydroxylated silica surface without any silanols ($a_{SiOH}=0$) would exhibit a WCA of 42°, whereas a fully hydroxylated surface ($a_{SiOH}=1$) is completely hydrophilic and has a WCA of zero. In the following, Eq. 2.15 will be used as a reference for experimentally observed WCAs for silica surfaces with different silanol concentrations. Comparison of the results of Eq. 2.15 with the results from simulations will permit us to select optimal force field parameters.

Annealed surfaces were used as starting points to model the wetting properties of silica. In case of these surfaces, two parameters are unknown and, hence, can be varied: $\varepsilon_{Si}$ and one of the partial atomic charges, either $q_{Si}$ or $q_{O}$. First, the surface with very few DAs was used, where $a_{SiOH}=0$ (surface SURF 2b in Table 2.1). Figure 2.7a shows such a surface (that is similar to a completely dehydroxylated silica surface) and the dependence of $\varepsilon_{Si}$ and $q_{Si}$ with WCA. As can be seen, the WCA is independent of $q_{Si}$ (or $q_{O}$) and depends only on $\varepsilon_{Si}$; therefore, the optimal value for one of the two parameters was found, namely $\varepsilon_{Si}$. In fact, an $\varepsilon_{Si}$ value of 0.3 kcal/mol reproduces the WCA of about 42° suggested by Eq. 2.15.

The next step is to parameterize the partial atomic charges $q_{Si}$ and $q_{O}$. Figures 2.7b and 2.7c show that, as the DA concentration increases, the effect of $q_{Si}$ and $q_{O}$ on wetting also increases. Therefore, a straightforward strategy consisted in choosing a range of surfaces with different DA concentrations, and use them to find parameters for the partial atomic charges that match Eq. 2.15. This procedure for determining partial atomic charge deviates from the standard ab initio methods. Ab initio procedures estimate the point charges by fitting the electrostatic potential of small model fragments. However, amorphous materials are fairly heterogeneous. Clearly, one will have to define
Figure 2.8: Dependence of the WCA on the concentration of surface defects. The contour plot shows the value of the WCA (color coded), the lower X-axis shows the concentration of surface defects, either DA (a) or DO (b). The Y-axis shows the variation of the electrostatic parameters. The black crosses indicate the experimental WCAs for different silanol concentrations based on Eq. 2.15. (a) For annealed surfaces, the wettability of silica can be reproduced using a single set of charges, 1.0 e for silicon and -0.5 e for oxygen (white line). (b) For surfaces of type I, two values of $q_{DO}$ can reproduce the experimental wetting curve: -0.044 and -0.423 e.

The WCA approach provides a straightforward method for calculating single set of charges that reproduce the surface hydrophobicity.

Figure 2.8a depicts the dependence of the WCA on the DA concentration and silica charges. It is assumed that DAs control the adsorption properties of silanols. Even though those surfaces did not have explicit silanols, exposed DOs and DSIs mimic the behavior of silanols in the sense that they act as adsorption centers of diverse intensity [65] with different affinities towards water [51, 61], representing realistic amorphous silica where silanols also have different adsorption properties due to the heterogeneity of the silica surface. Figure 2.8a shows that an increment in either the partial charges or the DA concentration decreases the WCA. One can recognize from Fig. 2.8a that point charges $q_{Si} = 1.0$ e and $q_{O} = -0.5$ e (white line) reproduce closely the experimentally observed WCAs as described by Eq. 2.15.

For surfaces of type I (cf. Fig. 2.3b), the WCA has to be found by varying only $q_{DO}$, the charge of DOs, that was assumed to be different from the charge of oxygen in the bulk. Figure 2.8b present the relevant results. The parameters assumed for the bulk atoms are given in Table 2.2. The WCA shows a clear dependence on the DO concentration. However, the effect of $q_{DO}$ is not straightforward. As $q_{DO}$ becomes more negative, the WCA shows an initial increase; at a $q_{DO}$ value
Figure 2.9: Silanol groups enhance surface hydrophilicity. Surface silanols (pictured as opaque yellow, red and white points) produce complete wetting, independent of the charge parameters. Snapshots correspond to (a) 0, (b) 0.12, (c) 0.64, and (d) 2.0 ns of a MD simulation of a water disc (blue) resting on top of the silica surface with a silanol concentration of 1.0 nm$^{-2}$. Panels (b) and (c) show that initial spreading of the water molecules over the surface is being guided by the locations of surface silanols. Panel (d) shows complete wetting of the silica surface.

around -0.3 |e| there is a change in behavior and the WCA starts to decrease continuously. One can recognize from Fig. 2.8b that the experimental wettability described by Eq. 2.15 can be reproduced for two values of $q_{DO}$, namely -0.044 and -0.423 e.

The initial increase in the hydrophobicity of surfaces of type I with decreasing $q_{DO}$ is due to a change in direction of the oriented dipole of the exposed SiO$_4$ tetrahedra containing DOs. For a perfect SiO$_4$ tetrahedron with vanishing dipole moment, each oxygen atom contributes a charge of $-q_{Si}/4$. In the case of surfaces of type I, the charge of $q_{Si}$ was set to 0.9 e. Assuming a tetrahedron with perfect symmetry, a value of $q_{DO} = -0.225$ e cancels out the dipole moment. Any value of $q_{DO}$ different from -0.225 e produces a non-vanishing dipole moment; for $q_{DO} < -0.225$ e the direction of the dipole is to the exterior of the surface, whereas for $q_{DO} > -0.225$ e to the interior. In Fig. 2.8b, the change in the wetting behavior occurs around $q_{DO} = -0.3$ e. The difference to the estimated value of -0.225 e should be due to distortions of the tetrahedron as well as due to the amorphous topography. The wetting properties of a surface are strongly dependent on the dipole moment at the solid surface [66, 67]. Furthermore, Bakaev et al. [51] have shown that dipoles originating from surface defects, such as DOs, produce strong hydrophobic sites. Thus, it is likely that the switch in the wetting behavior observed around $q_{DO} = -0.3$ e is due to a change in the dipole moment.

Up to this point, the WCA was found to be reproducible for non-hydroxylated surfaces: the annealed surfaces and surfaces of type I. The next logical step was to consider silica surfaces of type II, III and IV (cf. Fig. 2.3c-e), which have hydroxyl groups and offer an opportunity to parameterize the silanol groups. For all parameter combinations tested (cf. Table 2.3), the silanol group acted as a strong hydrophilic center and produced a WCA value of 0°. Complete wetting ($\theta = 0$) was
Table 2.4: Parameters that reproduce silica wetting for annealed and type I surfaces.

<table>
<thead>
<tr>
<th>Surface</th>
<th>$\varepsilon_{Si}$ (kcal/mol)</th>
<th>$\varepsilon_{O}$ (kcal/mol)</th>
<th>$r_{Si}^{min}$ (Å)</th>
<th>$r_{O}^{min}$ (Å)</th>
<th>$q_{Si}$ (e)</th>
<th>$q_{O}$ (e)</th>
<th>$q_{DO}$ (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>annealed</td>
<td>0.3</td>
<td>0.15</td>
<td>4.3</td>
<td>3.5</td>
<td>1.0</td>
<td>-0.50</td>
<td>-</td>
</tr>
<tr>
<td>type I (1\textsuperscript{st} solution)</td>
<td>0.3</td>
<td>0.15</td>
<td>4.3</td>
<td>3.5</td>
<td>0.9</td>
<td>-0.45</td>
<td>-0.044</td>
</tr>
<tr>
<td>type I (2\textsuperscript{nd} solution)</td>
<td>0.3</td>
<td>0.15</td>
<td>4.3</td>
<td>3.5</td>
<td>0.9</td>
<td>-0.45</td>
<td>-0.423</td>
</tr>
</tbody>
</table>

Judging by agreement of the measured WCAs with Eq. 2.15, the best surface models are given in Table 2.4. The wetting properties of silica can be reproduced using annealed surfaces and surfaces of type I. In the latter case, two sets of parameters reproduce wetting, likely due to a change on the surface dipole when changing the charge $q_{DO}$. Parameterization of silanol groups requires a different approach which should take into account the different types of silanols and their protonation states.

2.9 Conclusions

In this Chapter, a novel force field for amorphous silica surfaces was presented. The silica force field reproduces experimental wetting properties of silica surfaces with different concentration of silanols based on the observable WCA. The route used for parameterization is different from conventional schemes. The main results are summarized in Table 2.4. It was found that atomic level details of the silica surface, such as dangling atoms (DAs), need to be taken into account when one seeks to describe accurately silica-water interactions. Our simulations have shown that annealed silica surfaces with DAs capture the heterogeneity of realistic surfaces and are good representations of the silica structures. Therefore, that the surface reconstruction through annealing cycles is a necessary step for simulating amorphous silica-water systems. For building hydrophobic silicas, annealings with two-body potentials are recommended over the three-body ones, as they permit larger distortions of the surface geometry. The proposed atomic-level silica model is appropriate for MD simulations of confined environments such as nanopores, where the silica topography is an important factor.
Chapter 3

Ionic current rectification through silica nanopores


3.1 Introduction

Solid-state nanopores are small pores with nanometer scale radii, made of synthetic materials, such as silica [9], silicon nitride [10] or carbon polymers [11]. Immersed in aqueous solution, nanopores can be used as electrical tweezers to study the translocation of charged species, such as ions or nucleic acids [12, 14, 56] (see Fig. 3.1). To date, most applications of nanopores are based on measuring the changes in the ionic current. Therefore, understanding the ion dynamics through nanopores is desirable. Many interesting results that challenge our understanding have been reported, such as current dependence on DNA translocation and salt concentration [68], conductance-noise variations attributed to nanobubbles [69], surface charge inversion [70, 71], and nanoprecipitation [72].

Under a given voltage, a nanopore can produces striking different ionic currents, depending on the polarity of the applied potential, enhancing the ionic current for a given voltage but reducing it for the same voltage magnitude with opposite polarity. This phenomenon, known as ionic current rectification, has been proposed as the basis for a nanofluidic diode [18, 19, 73], a starting point for developing nanofluidic electronic devices [74]. There has been extensive research on the rectification phenomenon specifically for polymer nanopores [75, 76] in which case the asymmetric behavior has been attributed to various factors, such as the surface charge, the ionic double layer confined in the small nanopore space and the different electrostatic distribution due to structural/chemical asymmetry at the two openings. Several models to explain rectification have been proposed [77, 78]. These models were mostly based on continuous macroscopic approaches, but were refined to
Figure 3.1: Atomic model of a silica nanopore. The figure shows a silica nanopore of 1 nm radius immersed in a KCl solution. The nanopore is sliced in the middle. The gray surface covers the bulk silica region, permitting a view of the pore interior. Blue and red beads represent K$^+$ and Cl$^-$ ions, respectively. Water molecules are not shown. The system is divided into three regions: cis compartment (a), nanopore region (b) and trans compartment (c).

include the peculiarities of the surface system, the latter represented as a continuous surface charge. However, the applicability of continuum descriptions at the scale of a few nanometers is a subject of debate [79–81], since continuous approaches do not take into account the discreteness of the solvent nor the influence of the surface topography. Molecular dynamics (MD) simulations can provide a detailed atomistic description of the interactions between the solvated ions and the solid surface, offering a valuable tool for studying the rectification phenomenon.

This Chapter presents a systematic MD study of the ionic-current rectification phenomenon in nanopores of 1 nm radius. All nanopores have different surface topographies and were build using a silica model that was designed to mimic the heterogeneity of realistic surfaces [82] (cf. Chapter 2). The silica model reproduces the wetting properties of amorphous silica based on the concentration of non-fully coordinated silica atoms, so-called dangling atoms. The definition of a dangling atom is the following: A silicon and an oxygen atom are considered covalently bonded if they have a separation distance of 2 Å or less; oxygens with less than two bonds and silicons with less than four bonds are classified as dangling atoms. In this Chapter, all-atom MD simulations are used to relate the ionic-current rectification phenomenon to the atomic events inside the nanopore, namely surface-ion interactions, binding of ions to the solid surface and the electrostatic potential.
3.2 Building silica nanopores

To produce amorphous silica pores we employed the MD programs Cerius$^2$ v. 4.9 [47]. Four silica nanopores of 1 nm radius (Fig. 3.2a-d) were considered in this work and classified into three different categories according to the arrangement of surface atoms: crystalline pores, raw cut amorphous pores, and annealed amorphous pores. The protocols to fabricate atomic-scale models of amorphous silica are summarized below.

To create crystalline pores, a silica cube was produced by replicating a cristoballite unit cell, filling a cube with length of 57 Å along each side. Then, the periodicity in the Z-axis was removed, exposing the (001) cristoballite face upwards. One nanopore, called pore A, was built by removing atoms from a pore through the cube, producing a cylindrical pore of 1 nm radius parallel to the Z-axis (10368 atoms, Fig. 3.2a). To create amorphous silica pores, the crystalline silica cube was randomized using an annealing procedure [82] in Cerius$^2$. The resulting amorphous bulk had a periodicity of 57 Å×57 Å×58 Å. A raw cut pore of 1 nm radius, called pore B (10488 atoms, Fig. 3.2b), was obtained by removing the periodicity along the Z axis of the amorphous cube and

![Figure 3.2: Four silica nanopores with 1 nm radius and different surfaces. Figures show silica systems sliced along the Z-axis. Gray surfaces cover the bulk regions, colored beads show the pore surface. Red and yellow beads represent bicoordinated oxygens and four-coordinated silicons, respectively. Blue represents dangling atoms, either dangling oxygens or dangling silicons. Pore A was cut from a crystal structure (a). Pores B was cut from amorphous bulk silica (b). Pores C and D were obtained after applying annealing cycles (c,d).](image-url)
deleting atoms from the center. To preserve electroneutrality, we enforced a ratio of two oxygen atoms per one silicon atom by removing a few extra atoms from the surfaces. Annealed pores were created by applying simulated annealing procedures to pore B [82] using two silica force fields: GLASSFF.1.01 and GLASSFF.2.01. Both force fields have been described in the previous Chapter 2, section 2.2. The annealing cycle started with a minimization, followed by MD equilibration at 5000 K for 10 ps. After that, the temperature was gradually reduced to 300 K, with a cooling step of 2 ps per -100 K. Two annealed pores were produced, such pore C (Fig. 3.2c) and pore D (Fig. 3.2d), annealed with GLASSFF.1.01 and GLASSFF.2.01 respectively.

3.3 Measuring ionic current from MD simulations

To simulate systems composed of silica, water and ions we used the program NAMD 2.6 [55]. Silica parameters were taken from the silica force field presented in Chapter 2, which reproduces the hydrophobicity of amorphous silica [82]. Parameters for ions were taken from the CHARMM force field [46], the TIP3P [39] model being used for water. The details of the MD protocols are summarized in Appendix A.

To study the ionic conduction, the silica nanopores were solvated and ionized. To solvate silica nanopores, rectangular water boxes were generated using the Solvate plugin of VMD [48] and placed above and below the silica nanopores. The dimensions of the water boxes match the X and Y periodic lattice vectors of the silica systems and have 60 Å length along the Z-axis. To induce water permeation, the systems were minimized for 400 steps using the conjugate gradient method and then equilibrated for 1 ns of molecular dynamics (MD) simulation in the NpT ensemble. Subsequently, the VMD plug-in Autoionize was used to place ions randomly, corresponding to 1M KCl concentration. The systems were then minimized (400 steps) and equilibrated for 1 ns. The last frames of the equilibration simulations were used to study ionic conduction. The final systems composed of silica, water and ions are periodic, with unit cell vectors of about 57 Å×57 Å×150 Å. All silica atoms were restrained to their original positions using a harmonic force with a spring constant of 5 kcal/mol/Å², while water molecules and ions were allowed to move freely. To induce ionic current through the nanopore, a uniform electrostatic field \( E_z \) was applied to all atoms in the Z-direction [56, 83]. The voltage bias \( \varepsilon \) across the simulated system is

\[
\varepsilon = -L_z \cdot E_z,
\]  

(3.1)
where \( L_z \) is the length of the periodic system along the \( Z \)-axis. To obtain an ionic current-voltage (I-V) curve, ten voltage biases were applied: \( \pm 0.12 \) V, \( \pm 0.24 \) V, \( \pm 0.5 \) V, \( \pm 1 \) V and \( \pm 2 \) V. The voltage signs are defined relative to the cis solvent compartment (see Fig. 3.1). A positive voltage bias induces cations to move from the trans solvent compartment to the cis solvent compartment.

The ionic currents were computed using [56]

\[
I(t) = \frac{1}{\Delta t L_z} \sum_{i=1}^{N} q_i (z_i(t + \Delta t) - z_i(t))
\] (3.2)

where \( q_i \) is the charge of atom \( i \) and \( z_i \) is its \( Z \)-coordinate, \( L_z \) is the length of the system along the \( Z \)-axis and \( \Delta t \) is 1 ps. The sum runs over all ions in the system. Non-annealed pores (\( A \), \( B \), and \( E \)) require from 2 to 5 ns to reach a steady-state current, depending on voltage. Therefore, the average current was calculated from the last 4 ns of each 10 ns trajectory.

Even though MD simulations provide a detailed atomic description, there are inherent discrepancies between the MD model and the real physical system, such as membrane thickness, surface charge and time scale of measurements [56]. The silica surfaces used are approximate models that reproduce wettability and surface topography quite accurately, but do not reproduce other physical features equally well. For instance, the silica model employs the same set of partial charges for dangling and bulk atoms; a more accurate representation would include different partial charges based on the connectivity and local environment of the dangling atoms [84]. The polarizability of silica has not been taken into account either [85]. Furthermore, the varying properties of the surfaces are due to the presence of defects on the surface, such as dangling atoms, and not to explicit deprotonated silanols. It is noteworthy to mention that a variety of defects have been extensively reported in the literature [86], such as defects produced when the silica surface is damaged by mechanical stress [87] or by ion and electron beams [88, 89]. One indeed expects that such defects, in contact with water, would produce deprotonated silanols. Unfortunately, silica models that reproduce all such surface defects in detail and can be used with the TIP3P water model are not yet available. Recent work focused on fully hydroxylated silica models [45, 90], which are inadequate at neutral pH conditions. A most recent publication has used a model of deprotonated silanol [71]. Nevertheless, the integration of silica models and MD models employed in biomolecular simulations is at an early stage of development and there is still much need for improvement.
3.4 Ionic current through silica nanopores

Silicon-based nanopores can produce linear ohmic responses [10, 56, 91]; however, ion-current rectification is not unusual. The presence of rectification has been reported in Si$_3$N$_4$ nanopores coated with SiO$_2$ [92] and glass nanopipettes [93]. In the respective studies, the rectification can be abolished either by lowering the pH to neutralize the surface charge or by coating the surface with Al$_2$O$_3$, showing the dependence of rectification on the physical characteristics of the nanopore surface.

Figure 3.3 presents the I-V curves for pores A, B, C and D. In general, the presented MD simulations provide a good qualitative description of the ionic-current phenomena: for Si$_3$N$_4$ pores of 1 nm radii, the experimental ionic conductance has been estimated to be 1.09 nS [10] (or 1.09 nA at 1 V) assuming a linear response, which compares well with the simulated ionic-current. Figure 3.3 shows that the overall shape of the I-V curve depends strongly on the type of pore employed. Pores A and B (Fig. 3.3a) shows an asymmetric I-V curve with a clear hindrance in the ion conduction under positive and negative voltages, respectively. At low voltages (±0.125 and ±0.25 V) the magnitudes of the current do not show any significant rectification. However, at higher voltages (±0.5, ±1 and ±2V), rectification appears and increases with the magnitude of the applied field. For pores C and D (Fig. 3.3b), both with annealed surfaces, the I-V curves are symmetric and no rectification is observed.

An estimate of the degree of the ionic-rectification is given by the rectification factor $f_{rec}$, defined as the absolute value of the ratio between the electric current measured for a given voltage with

![Figure 3.3: I-V curve. Figures present the I-V curves for 1 nm-radius pores immersed in 1 M KCl. Figure (a) shows the I-V curves for pores A (blue) and B (red), both pores producing rectification. Figure (b) shows the I-V curves for pores C (red) and D (blue), both pores producing a symmetric curve without rectification. Error bars show the ± standard error, defined as $\sigma/\sqrt{n}$, where $\sigma$ in the standard deviation and $n$ is the number of MD frames used for calculating the average, namely 4000.](image)

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negative and positive signs. The closer $f_{rec}$ to 1, the lower the rectification. Table 3.1 shows the rectification factor at ±1 V for six nanopores. The values were computed using

$$f_{rec} = \frac{|I(-1 V)|}{|I(+1 V)|}.$$  \hspace{1cm} (3.3)

Pores A and B have considerable rectification, while pores C and D have lower rectification (Table 3.1, second column).

Table 3.1 also shows the number of ions inside each nanopore under +1V and -1V biases, averaged over the last 4 ns of each 10 ns trajectory. Nanopores that produce rectification (A and B) accommodate different number of ions for each voltage polarity, the total number of ions being proportional to the ionic current. For crystalline pore A, the number of ions is higher at -1V than at +1V. Pore A also allows passage of a higher number of K$^+$ ions compared to Cl$^-$ ions. Under +1V bias, the number of Cl$^-$ ions is negligible. Conversely, for raw cut pore B, the number of ions is higher at +1V than at -1V, the number of K$^+$ and Cl$^-$ ions inside the pore being similar. Annealed pores C and D show the same trend: there is no significant difference between the number of ions at +1 and -1 V, and the number of K$^+$ is close to the number of Cl$^-$ ions.

### 3.5 Ionic distribution inside silica nanopores

Previous MD studies with crystalline Si$_3$N$_4$ nanopores [12, 13, 56] have shown that mainly K$^+$ ions adhere to the nanopore walls. It was hypothesized that the preference for K$^+$ over Cl$^-$ adsorption is due to a Si$_3$N$_4$ fixed negative charge in the pore as well as due to the smaller size of K$^+$ to access voids in the corrugated nanopore wall [13]. Since the proportion of nitrogens is higher than silicons, the Si$_3$N$_4$ model exposes more negatively charged atoms, which translates into a negatively charged surface. The MD simulations with SiO$_2$ pores show that the ion adsorption is due to surface topography and dangling atoms at the surface. Simulations involving crystalline pore A and raw
cut B exhibited ion binding to the silica walls, similar to the ion binding observed in former studies of Si$_3$N$_4$ pores [12, 13, 56]. However, simulations with annealed pores C and D did not show any ion binding. The latter pores have a very low or negligible concentration of dangling atoms, revealing the roles of dangling atoms in ion adsorption.

Figure 3.4 shows the ionic density averaged over the last 4 ns for two voltage biases (+1 V and -1 V). The radial ion density changes depending on pore surface and voltage polarity. The same pattern is observed for all positive and negative voltage biases; therefore, only ±1 V bias are presented. In the case of the crystalline and raw cut pores, a dramatic change is observed for different polarities. The crystalline pore A (Fig. 3.4a) shows a significant increment in the radial ionic concentration for -1 V bias. The opposite is observed in the raw cut pore B (Fig. 3.4b), the ionic concentration being lower for -1 V bias than for +1 V bias. In contrast, annealed pores C and D show the same radial density profiles for +1 V and -1 V (Fig. 3.4c and 3.4d).

During the course of the simulations, ions adsorbed temporally to the silica surface. To study the
ion-adsorption process, the residence time of ions in contact with the surface was computed. The residence time was calculated by counting the time each ion spent in contact with the silica surface over the entire 10 ns trajectory. For these calculations, the entire silica surface was considered, not only the exposed surface at the nanopore region, but also at the cis and trans compartments. An ion was considered in contact with the surface if the distance between the ion and the silica surface was less than 5 Å. If an ion left that region and then returned, it was counted as a different ion. Figure 3.5 shows the ionic residence times at +1 V bias. A similar pattern was obtained for a -1 V bias; therefore only the +1 V bias are presented. The adsorption of ions is intermittent, depending on arrangement of dangling atoms at the surface and random collisions with other ions. The majority of ions have short residence times, ranging from 10 to 100 picoseconds (see insets in Fig. 3.5). These ions can not be considered adsorbed by the surface, since such short residence times can be produced by random collisions. For an ion to be adsorbed, it has to be retained for

Figure 3.5: Residence times for +1 V bias. The plots show the residence times for pores A (a), pore B (b), pore C (c) and pore D (d) under +1 V bias. The residence time for K⁺ ions is shown in blue and that of Cl⁻ ions in red. The ordinate axis shows the number of ions, the abscissa axis the residence time from 100 to 10000 ps. The insets show numbers of ions with residence times from 10 to 100 ps.
Figure 3.6: Ionic current near nanopore wall. Figures show the percentage of the cumulative ionic current produced near the nanopore wall for pores A (blue), B (red), C (green) and D (black) under +1 V (a) and -1 V (b) biases. Plots were obtained by dividing the nanopore region in cylindrical rings. The outer surface of the ring is the nanopore wall while the inner surface is displaced from the wall towards the nanopore center (abscissa axis). The ionic current was computed for each cylindrical ring using Eq. 5.1 and divided by $I_{\text{total}}$, the ionic current through the entire nanopore volume. Plots present the average values over the last 4 ns.

several hundred picoseconds. For pores A and B (Fig. 3.5a and 3.5b, respectively), ions that move close to the silica surface are eventually adsorbed and then released. In these pores, ions can reach residence times of up to hundreds of picoseconds and even of several nanoseconds. Pore C and D do not attach ions to the surface, therefore their residence times being therefore shorter.

By comparing Fig. 3.4 and Fig. 3.5, it becomes clear that the surfaces of non-annealed pores (A and B) exhibit significant affinities for ions. The question arises, how much ions near the surface contribute to the total current. The concentration of ions near the wall is higher, therefore, the ionic current should be higher in that region. However, since the residence time is longer, the mobility is lower, which implies a lower current near the wall. Figure 3.6 shows the percentage of ionic current near the nanopore wall. If the ionic current is produced within 5 Å from the silica surface, its is considered in the “wall region”, otherwise it is considered in the “center region”. For non-annealed pores A and B, the current near the wall is about 25% of the total current. For annealed pores C and D, the current near the wall ranges from 10% to 17%. Even though pores A and B have higher ionic current near the wall, the majority of the current (about 75%) is still produced in the center of the pore.
3.6 Ion-silica interactions

To study the effect of the silica surface on the ion dynamics without the influence of colliding ions, four single ion simulations were performed. A single ion, either K\(^+\) or Cl\(^-\), was placed in the cis compartment, 40 Å above the top surface of two nanopores, one with a high number of dangling atoms (pore B) and the other with a negligible number of dangling atoms (pore C). To induce translocation from the cis to the trans compartment, the systems were simulated under a voltage of +1 V for a Cl\(^-\) ion and -1 V for a K\(^+\) ion. To avoid the ion diffusing away from the nanopore opening, the ion movement was limited to a cylinder of 15 Å radius and 140 Å length concentric with the pore axis, using a phantom surface [13, 94] that imposes boundary limits on the ion, while allowing water molecules to diffuse freely. When the ion reaches the phantom surface, it experiences an elastic collision that pushes it back towards the interior of the cylinder. To maintain electroneutrality, a counterion was located 40 Å below the bottom pore surface and 10 Å away from the phantom surface; the counterion was restrained to its original position using a harmonic force with a spring constant of 5 kcal/mol/Å\(^2\). After the simulations were completed, the intermolecular energies were calculated on two different selections: ion with all silica and ion with all water (Fig. 3.7). This calculation allowed us to disentangle the influence of ion-water and ion-silica interactions while not accounting for ion-ion interactions.

The experimental values for the hydration free energies for K\(^+\) and Cl\(^-\) ions have been estimated around -80 and -85 kcal/mol [95, 96]. The values obtained with NAMD energy exceed experimental values for several reasons: (i) NAMD energy only calculates the potential energy component (U), which is computed between two selections of atoms, ignoring the rest of the system; (ii) then exist inaccuracies in the silica force field as well as in the water and ion models; (iii) details in the simulation protocol (cut off distance, periodic conditions) also affect calculated energy values. Even though the computed energies are affected to a great extent by the stated shortcomings, they provide nevertheless a valuable qualitative description of the energetic variations actually experienced by ions.

The ion movement across the nanopore and the associated ion-water and ion-silica energies are presented in Fig. 3.7. Figure 3.7b shows translocation of a K\(^+\) ion through pore B. Initially, the K\(^+\) ion moved randomly for 5.7 ns in the cis compartment. In that region, the interaction energy of ion-water was dominant (Fig. 3.7a), while the interaction energy of ion-silica was zero (region i). From 5.7 ns to 7 ns, the ion reached and touched the upper surface without yet entering into the pore, producing a negative increase of the ion-silica energy (region ii). When touching the surface, a
Figure 3.7: Voltage-driven translocation of a single K\(^{+}\) ion through silica nanopores. The plots on the left hand side show the potential energy (a,c) and location (b,d) of a single K\(^{+}\) ion voltage-driven through raw cut pore B (a,b) and annealed pore C (c,d). Magenta lines show the ion location along the Z-axis (scale on the left ordinate axis), green lines show the radial distance between the ion and the nanopore wall (scale on the right ordinate axis) as a function of time (abscissa axis). The horizontal black dashed lines are reference lines to indicate the boundaries of the silica pores. For a complete ion translocation from the cis to the trans compartment, the magenta line has to cross both black lines. The blue and red lines show the associated ion-water and ion-silica potential energies, respectively. The snapshots on the right hand side show the K\(^{+}\) ion at a specific moment during translocation. K\(^{+}\) is pictured in blue. Silica atoms are colored as follows: non-dangling silicon in yellow, non-dangling oxygen in red, dangling silicon in green and dangling oxygens in purple. Water molecules are shown in licorice representation (oxygen red, hydrogen white). Atomic events in regions (i-vi) are described in the text.

partial removal of the ion’s solvation shell produced a decrease in the absolute value of the ion-water energy and increased in the ion-silica energy. After that initial collision, the K\(^{+}\) moved back into the cis solvent compartment (region iii), the negative increment in the ion-water energy indicates that the water shell is restored. Then, the ion moved again near the nanopore opening at 9.5 ns. The sharp spikes observed in the energy profiles (region iv) were due an initial ion binding to the surface. The ion was released from the surface at 10 ns and quickly moved through the nanopore (region v) until it was absorbed again by the surface at 10.8 ns, 10 Å away from the pore exit. The snapshot on the right hand side shows the K\(^{+}\) ion in direct contact with two dangling oxygens (pictured in purple) and its water shell partially removed. Subsequently, the ion bounced between solvent and
surface (region vi), as evident through the oscillations in the ion-water and ion-silica energies.

Figure 3.7d shows the translocation of a \( \text{K}^{+} \) ion though pore \( C \). The single \( \text{K}^{+} \) ion reaches the nanopore opening at 3.3 ns, moves through the nanopore, and is finally released into the trans compartment at 4.9 ns. The snapshot on the right hand side shows the \( \text{K}^{+} \) ion in the middle of the pore. During translocation, the \( \text{K}^{+} \) ion keep its solvation shell. The ion-water potential energies remained at the same level during the entire simulation, while the ion-silica potential energies were negligible throughout the translocation. Similar scenarios were observed for conduction of a single \( \text{Cl}^{-} \) ion through pores \( B \) and \( C \).

The presented results provide a complete picture of ion translocation and surface binding processes. To capture an ion, the surface has to provide a location that can compensate the energetic cost of removing the ion’s water shell [97]. Due to their accessibility, dangling atoms have higher affinities for ions; however, they need to arise in a group of two or more to effectively bind an ion. Since the gaps on the raw cut and crystalline surfaces were previously filled with silicon and oxygen atoms, \( i.e. \), are wide enough to permit access to ions, the patches of dangling atoms are geometrically optimized for retaining ions. When a uniform electric field is applied, ions move through the pore. If an ion moves near a patch of dangling atoms, it is adsorbed. The adsorption is intermittent and affects the ionic density profile inside the pore. On the other hand, surfaces of annealed pores are not able to dehydrate ions. After annealing, the silica surface rearranges and the patches of dangling atoms are removed. The interaction energy between an ion and an annealed surface can not compensate for the energetic penalty of removing the hydration shell; therefore, ions are not adsorbed.

### 3.7 Electrostatic potential of the system

The ionic current is affected not only by the external applied potential, but also by the electrostatic potential generated by the location of all charged species in the system, such as silica, ions and water. To investigate how these potentials alter the ionic current, the mean electrostatic potential at voltage \( \varepsilon \), \( V_{\text{mean}}^{\varepsilon} \), was computed. \( V_{\text{mean}}^{\varepsilon} \) is defined through

\[
V_{\text{mean}}^{\varepsilon}(\mathbf{r}) = \langle V_{\text{intr}}^{\varepsilon}(\mathbf{r}) + V_{\text{appl}}^{\varepsilon}(\mathbf{r}) \rangle_{4ns}.
\]

where \( V_{\text{intr}}(\mathbf{r}) \) refers to the intrinsic electrostatic potential determined by the location of all charged atoms and has been computed with the VMD plugin \( \text{PMEpot} \). The details of the procedure employed in \( \text{PMEpot} \) are described elsewhere [98]. \( V_{\text{appl}}^{\varepsilon}(\mathbf{r}) \) refers to the external, \( i.e. \), applied, potential defined
through

\[ V^\varepsilon_{\text{appl}}(\mathbf{r}) = -\varepsilon \cdot [Z - Z_{\text{min}}], \] (3.5)

where \( \varepsilon \) is the voltage bias (Eq. 3.1) and \( Z_{\text{min}} \) is the beginning of the periodic cell along the \( Z \)-axis. Note that \( V^\varepsilon_{\text{appl}}(\mathbf{r}) \) depends only on the \( Z \)-coordinate. Equation 3.4 states that \( V^\varepsilon_{\text{mean}}(\mathbf{r}) \) is an average (denoted by \( \langle \cdots \rangle_{4ns} \)) over the last 4 ns of each MD simulation.

\( V^\varepsilon_{\text{mean}}(\mathbf{r}) \) describes the mean electrostatic potential for every point \( \mathbf{r} \) in the simulation space. However, of main interest is the variation of the average potential along the pore axis, i.e., along the \( Z \)-axis. The associated potential, denoted by \( V_{\text{fit}}^{\varepsilon}(Z) \), has been calculated by determining first the average potential along the \( Z \)-axis

\[ V^\varepsilon_{\text{avg}}(Z) = \langle V^\varepsilon_{\text{mean}}(\mathbf{r}) \rangle_{\text{cylinder along } Z-\text{axis}}, \] (3.6)

where \( \langle \cdots \rangle_{\text{cylinder along } Z-\text{axis}} \) denotes the average of \( V^\varepsilon_{\text{mean}}(\mathbf{r}) \) over cylinders concentric with the pore axis, each cylinder of 1 \( \AA \) height and 3 \( \AA \) radius centered at \( Z \). The radius of 3 \( \AA \) was chosen to avoid distortions of the potential due to the silica wall. \( V_{\text{fit}}^{\varepsilon}(Z) \) was assumed to adopt the analytical function

\[ V_{\text{fit}}^{\varepsilon}(Z) = \frac{\varepsilon}{\pi} \cdot \arctan(-b \cdot [Z - Z_0]), \] (3.7)

where \( b \) and \( Z_0 \) are fitting parameters determined through matching \( V_{\text{fit}}^{\varepsilon}(Z) \) to \( V^\varepsilon_{\text{avg}}(Z) \). \( V_{\text{fit}}^{\varepsilon}(Z) \) is a smooth differentiable function that represents the key variation of the electrostatic potential along the continuous \( Z \)-axis at \( \varepsilon \) bias [13].

In order to study the electrostatic contribution of all charged species in the system discounting the influence of \( V_{\text{appl}} \), the potential \( V_{\text{system}}(Z) \) was computed. \( V_{\text{system}}(Z) \) was calculated through

\[ V_{\text{system}}(Z) = \frac{1}{2} \cdot \left[ V_{\text{fit}}^{+1\varepsilon}(Z) + V_{\text{fit}}^{-1\varepsilon}(Z) \right], \] (3.8)

where \( V_{\text{fit}}^{+1\varepsilon}(Z) \) and \( V_{\text{fit}}^{-1\varepsilon}(Z) \) correspond to \( V_{\text{fit}}^{\varepsilon}(Z) \) at +1 V and -1 V biases, respectively. \( V_{\text{system}}(Z) \) cancels out the effect of the applied potential and reflects only the intrinsic electrostatic contribution of the system without the influence of the applied bias.

As shown in Fig. 3.8, \( V_{\text{system}}(Z) \) exhibits an asymmetric \( Z \)-dependence for non-annealed pores \( A \) and \( B \) (Fig. 3.8a,b). It had been proposed that electrostatic potentials of such asymmetric "saw-tooth" shape produce rectification [76], where the height of the electrostatic barrier is shifted
Figure 3.8: Electrostatic potential of the system. Plots show the electrostatic potential of the system ($V_{\text{system}}$) for pores A (a), B (b), C (c) and D (d) along the Z-axis. $V_{\text{system}}$ was computed using Eq. 3.8. Trans, nanopore, and cis regions are located in the intervals $[0-50]$ Å, $[50-100]$ Å and $[100-150]$ Å, respectively. 

depending on the polarity of the applied voltage. The different shifts increase the ion conduction in one direction and decrease it in the opposite direction, producing rectification. For annealed pores C and D, $V_{\text{system}}(Z)$ exhibits low values and a symmetric Z-dependence (Fig. 3.8c,d).

3.8 Effect of dangling atom distribution on ionic rectification

In the previous section, it was shown that ionic rectification is related to an asymmetric electrostatic potential. In the following, the origin of this asymmetry is explained, focusing on the atomic details of the nanopore surface. Specifically, the following questions are discussed: how an asymmetric electrostatic potential arises in a system with a seeming front-back symmetry and why the rectification observed in pores A and B enhances ionic current in opposite directions.

The direction of the ionic rectification in pore A can be explained through the dangling atom distribution and the resulting adsorbed ions. Pore A was obtained from a crystoballite silica cube:
on the cis side of the solvent compartment, crystalline silica exposes the (001) face covered with negatively-charged dangling oxygens, while on the trans side of the solvent compartment, crystalline silica exposes its (00\(\bar{1}\)) face covered with positively-charged dangling silicones. In the case of pore A, ions attach to the surface in response to the ordered structure of the dangling atoms, with K\(^+\) and Cl\(^-\) ions adsorbed near the (001) and (00\(\bar{1}\)) silica faces, respectively. The increment in the number of K\(^+\) ions near the cis pore opening and Cl\(^-\) ions near the trans pore opening enhances ionic current at negative biases, where K\(^+\) ions translocate from cis to trans and Cl\(^-\) ions from trans to cis.

The reason for the direction of the rectification in pore B is not straightforward. In pore B, the structure is amorphous and the number of dangling atoms and the respective ion adsorption patterns do not have the simple organization as seen for pore A. Another set of simulations was performed to investigate why the amorphous raw cut surface of pore B produces an ion current rectification opposite to the one observed in pore A. Annealed pore C was used as a template to produce two nanopores that expose an amorphous raw cut surface only in the nanopore region. The first pore, called C1, was built by removing atoms located within a 1.3 nm distance from the Z-axis of pore C. The second pore, called C2, was built by removing 7 dangling silicones and 14 dangling oxygens from the amorphous surface of pore C1; the deletion of the dangling oxygens creates new dangling silicones, and vice versa. Consequently, both pores C1 and C2 expose amorphous raw cut surfaces at the nanopore region and have 1.3 nm-radius pores, but differ only in the distribution of the dangling atoms. Figure 3.9 shows the nanopores and the number of dangling atoms along the Z-axis.

Pores C1 (b) and C2 were solvated, ionized with 1 M KCl and simulated under ±1 V biases. The simulations were performed for 30 ns to provide a wider sample for the computed average currents. Table 3.2 presents the ionic currents and the rectification factor, \(f_{rec}\). The rectification effect for pore C1 is similar to that for pore A, enhancing the ionic current in the case of a negative voltage. In the case of pore C2, the rectification was inverted relative to pore C1, producing a rectification effect similar to pore B. The different rectification factors \(f_{rec}\) confirm that the distribution of dangling atoms determines the direction of the ionic rectification for raw cut amorphous pores. Pore B was obtained by cutting the surface of an amorphous cube and the particular location of dangling atoms was determined by chance. A different cut would expose a different surface and would have a different effect on the ionic current and rectification.

Several models for rectification have been proposed \([77–79]\) based on interactions between ions translocating a negatively charged pore, the pore being narrow enough to allow ion-surface interactions (for a detailed review, see \([76]\) and references within). In all cases, the ion current rectification
Figure 3.9: Atomic models of nanopores $C_1$ and $C_2$. Figures at left show two nanopores, namely pores $C_1$ (a) and $C_2$ (c). Both pores present a raw cut surface in contact with the nanopore region and annealed surface in contact with the cis and trans compartments. Dangling silicons are shown in yellow, dangling oxygens in red, non-dangling oxygens and non-dangling silicons in gray. Plots at right show the number of dangling silicon (green lines) and dangling oxygen (purple lines) atoms along the $Z$-axis for pores $C_1$ (b) and $C_2$ (d).

arises when the symmetry of the electrostatic potential is broken. The scenario presented here is alike those described in [77–79], but instead of breaking the electrostatic symmetry through conical geometry or surface charges, the simulated nanopores exhibit an asymmetric distribution of dangling atoms with different affinities for cations and anions. Furthermore, MD simulations provide new insights into the rectification phenomenon. In nanopores with rectification, desolvation and temporal adsorption of ions is needed to redistribute the voltage drop inside the pore and produce rectification. Moreover, rectification was associated with different total ionic concentration at opposite voltage biases (Table 3.1). Ion selectivity can be observed (pore $A$) but was not necessary for
### Table 3.2: Rectification factor for pores C1 and C2.

<table>
<thead>
<tr>
<th>Nanopore</th>
<th>$I(+1V)$ (nA)</th>
<th>$I(-1V)$ (nA)</th>
<th>$f_{rec}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>2.2</td>
<td>-3.0</td>
<td>1.4</td>
</tr>
<tr>
<td>C2</td>
<td>3.3</td>
<td>-2.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Nanopores are listed in the first column. Ionic currents under +1V and -1V are shown in the second and third columns, respectively. The rectification factor ($f_{rec}$) is shown in the fourth column. The average ionic currents were calculated over the last 20 ns of each 30 ns MD simulation.

rectification (pore B). Finally, the atomic detail provided by MD simulations allowed us to perform thorough analyses, such as inspecting the radial ionic concentration inside the pore, residence times for ions, amount of current near the wall and electrostatic contributions of every component of the system.

## 3.9 Conclusions

Through MD simulations, a systematic study of ion current rectification in silica nanopores has been performed. For this purpose, six silica nanopore models were built, four of 1 nm radius and two of 1.3 nm radius, and simulated them immersed in KCl solution for a variety of voltage biases. The MD simulations showed that ion current rectification is due to an asymmetric distribution of ion-binding spots on the silica surface. An atomic-level description of the rectification phenomenon is presented. Nanopores that produce ion current rectification expose ion-binding spots, namely dangling atoms. These spots have the ability to dehydrate ions and temporally bind them to the surface, affecting the ion distribution inside the pore. An asymmetric distribution of dangling atoms generates then an asymmetric electrostatic potential and, when the polarity is inverted, it produces different electrostatic profiles and consequently current rectification. Conversely, nanopores that lack dangling atoms are unable to dehydrate and retain ions, exhibit symmetric electrostatic potentials and produce symmetric ohmic responses. Our results validate the use of MD simulations as valuable tools for studying nano-scale systems, where the atomic details of the solvent and the solid surface play a major role.
Chapter 4

Molecular control of ionic conduction in polymeric nanopores


4.1 Introduction

Polyethylene terephthalate (PET) is a carbon-based linear polymer widely used in synthetic fibers, beverage bottles, and other plastic containers. Due to its unique properties, such as high melting point, high mechanical strength, and non-reactivity to many chemicals [99], PET is an excellent material for nanotechnology applications. In particular, PET membranes have been used for building nanopores. An atomic model of a polymer nanopore immersed in electrolyte solution is shown in Fig. 4.1. PET nanopores have emerged as a nano-scale model system of interest, because of the experimental observation of multiple effects allowing for the control of ion transport properties, i.e., ion current rectification [76, 100], selectivity [79, 101–103], as well as the realization of ionic diodes [18, 19] and ionic transistors [104]. Furthermore, PET nanopores have also been used as models to test fundamental theories about ion dynamics in nanoscale confinements [105, 106].

Recently, it has been shown that local charge inversion can be a powerful tool to control ionic transport properties in PET nanopores [107]. In a PET nanopore with negative walls, charge inversion results in switching the selectivity properties of the nanopore. While a negatively charged nanopore in contact with *e.g.* KCl, would be cation selective, the same nanopore in contact with multivalent cations, such as Ca$^{2+}$, will become selective for anions. Such a selectivity switch induced through charge inversion has also been observed in protein pore OmpF [108] as well as in PET nanopores [107]. Therefore, understanding the interactions of multivalent and monovalent ions with the pore structure is of main importance for gaining a better understanding of induced charge
inversion.

Studying ionic transport on the nanoscale is crucial not only for controlling the flow of ions across the nanopore [3, 18, 19, 105, 106, 109], but also for understanding how biological channels function. In this regard, theoretical and computational modeling have been of critical importance, since experimental techniques are not yet able to resolve the dynamics of the few ions contained in the nanopore volume. Modeling based on the Poisson-Nernst-Planck (PNP) equations, Monte Carlo (MC) as well as molecular dynamics (MD) simulations have accompanied experimental efforts. The continuum approach of PNP successfully described the effect of ion current rectification observed in asymmetric pores [79, 110, 111] as well as ionic selectivity, i.e., a pore preference for transporting one type of ion, either cations or anions [101, 102]. However, continuum modeling cannot provide a molecular understanding of how ions compete for space and surface charges in the restricted volume of a nanopore. Monte Carlo simulations, which take into account the finite size of ions, offer more detail on the behavior of ions in nanopores. Applied to calcium-selective channels these simulations successfully described channel selectivity properties [112]. MD approaches reveal most details about ionic trajectories and their interactions with other ions, water and nanopore surface in equilibrium as well as non-equilibrium conditions. Increases in computational power have extended the duration of MD simulations, approaching today a time scale that is accessible in ion current measurements. Indeed, MD simulations successfully described transport properties of proteinaceous nanopores such as α-hemolysin [94, 98, 113] and solid-state silicon-based nanopores [13, 14, 91, 114]. The MD methodology had not been extended initially to polymer nanopores due to the lack of an accurate model for the chemical features of the pore surface, a key factor that determines the transport properties.

This Chapter presents a procedure to build all-atom models of PET nanopores that accurately replicates experimental devices. The simulated PET bulk model is compared with previously reported PET models and the expected values from experimental PET materials. After validation of the PET bulk, a model of PET nanopore is built and used to study the dynamics of K\(^+\), Cl\(^-\) and Ca\(^{2+}\) ions through the pore at different pH conditions. All MD simulations were performed using the program NAMD [55]. The details of the MD protocols are summarized in Appendix A. The following questions are discussed: How does the PET nanopore surface affect the ratio of the current carried by K\(^+\), Cl\(^-\) and Ca\(^{2+}\) ions? How does Ca\(^{2+}\) interact with the charged groups of the pore walls?
Figure 4.1: Atomic model of a polymer nanopore. The figure shows a polyethylene terephthalate (PET) nanopore immersed in electrolyte solution. The nanopore bulk material is shown in gray color. PET residues with deprotonated carboxyl groups are colored in purple. K\(^{+}\), Cl\(^{-}\), and Ca\(^{2+}\) ions are pictured as blue, red and green beads, respectively. Water molecules are shown in white. A single PET polymer chain is highlighted, colored in cyan (carbon atoms), red (oxygen atoms), and white (hydrogen atoms).
4.2 Building PET bulk

A PET monomer can exist as a \textit{trans} or a \textit{cis} isomer, depicted in Fig. 4.2a and 4.2b, respectively. Monomers connect to form polymers and terminate in carboxyl groups that are protonated at pH 4 and deprotonated at pH 7 (\textit{cf.} Fig. 4.2c and 4.2d). Several MD models of PET polymers had already been developed [23, 115] and successfully used to study the atomic origin of PET mechanical properties [24, 25, 116]. However, these models had been designed to describe properties of the bulk material and used force fields incompatible with explicit water models. To develop a PET model suitable for simulation of ion currents in a nanopore system, a PET moiety was built with the widely used CHARMM force field [46]. This force field was employed extensively to study a variety of biomolecular channels [83, 98, 117, 118], the limitations of the model in representing interactions with water and ions being well-known. The force field parameters for PET were obtained in analogy to previously parameterized model compounds.

In previous studies [24, 119], PET bulk models were built using a stepwise procedure [120], where monomers are added to a growing chain confined within a periodic cell. A disadvantage of such a method is that the accessible space is reduced as the polymer grows, which can lead to residues crossing through the center of aromatic rings. To avoid this problem, a periodic PET bulk was built using a collapsing/annealing MD procedure, presented schematically in Fig. 4.3.

First, ten PET polymers were generated. The number of subunits for each polymer was set to nine, corresponding to the most abundant polymer length observed in amorphous PET materials [24]. The initial conformation for each 9-mer was linear, the isomeric form (\textit{cis} or \textit{trans}) for each residue

![Figure 4.2: PET chemical structure.](image)

\textbf{Figure 4.2:} PET chemical structure. Shown are the chemical structure of a PET monomer in (a) \textit{trans} and (b) \textit{cis} isomeric forms. Also shown are the terminal PET monomers with (c) protonated and (d) deprotonated carboxyl groups, respectively.
Figure 4.3: Building PET bulk. The figure illustrates the collapsing/annealing procedure used to build amorphous PET. The first stage of the procedure involved collapsing a grid of PET 9-mers. (a) Model of a PET 9-mer. (b) Initial conformation of the PET grid. Ten different 9-mers were randomly located on a grid of $7 \times 7 \times 5$ points. Each 9-mer is presented in a different color. Subsequently, the grid was collapsed into a rectangular box. The snapshots at (c), (d) and (e) present the collapsing simulation after 0.4, 0.7 and 2 ns, respectively. (f) Enlarged view of the collapsed PET structure. The second stage of the procedure involved annealing the PET structure, in order to convert it into a periodic, amorphous box of homogeneous density. (g) Collapsed PET structure, colored in red, placed in a periodic box. The periodic images are colored in blue. The first step of the annealing cycle consisted of equilibration at 1000 K. Snapshots (h) and (i) show the simulation after 1 and 5 ns, respectively. The system was then cooled until reaching room temperature. The last frame of the annealing cycle is shown in (j); the PET polymers are seen to be tightly braided across the periodic cells.

was assigned randomly, and carboxyl groups at both ends were protonated. To randomize the structure, each 9-mer was minimized for 200 steps and equilibrated for 1 ns at 1000 K. A single PET 9-mer obtained by this method is shown in Fig. 4.3a. Subsequently, one of the ten PET 9-mers was randomly selected, reoriented in space, and assigned to a point on a $7 \times 7 \times 5$ grid with 75 Å spacing between grid points. After that, the grid was collapsed into a 90 Å×90 Å×60 Å rectangular box by applying forces of 5 pN towards the center of the box to each aromatic carbon atom located outside of the box. This simulation was performed at 1000 K to avoid folding of the 9-mers when they were moving toward the rectangular region. The collapsing grid at 0, 0.4, 0.7 and 2 ns is shown in Fig. 4.3b, 4.3c, 4.3d and 4.3e, respectively. The collapsed structure was placed within a periodic cell of dimension 120 Å×120 Å×90 Å, i.e., sufficiently large to avoid contacts among periodic images, as shown in Fig. 4.3g. To obtain homogeneous density, the PET structure was annealed in the $NpT$
ensemble. First, the PET cell was simulated for 5 ns at 1000 K. At this temperature, the PET structure is a fluid and fills the entire cell volume. Snapshots of that simulation after 1 and 5 ns are shown in Fig. 4.3h and 4.3i, respectively. After that, the temperature was gradually reduced to 400 K, with a cooling step of 0.2 ns per -100 K. The annealing cycle finished with 5 ns of equilibration at 300 K. The resulting amorphous PET is shown in Fig. 4.3j, the periodic cell having a dimension of 107 Å×84 Å×64 Å.

To compare the resulting bulk material with other PET models and experimental PET material, the volume density, the so-called characteristic ratio for a 9-mer, $C_9$, and the glycol dihedral angle

![Figure 4.4: Analysis of the annealing cycle for periodic PET bulk. Panel (a) shows the variation of temperature during the annealing cycle and the respective variation in density (b), characteristic ratio (c) and percentage of glycol dihedral in trans conformation (d). Vertical black guidelines divide each panel into three regions: the first region corresponds to 5 ns of equilibration at 1000 K, the second region to 1.2 ns when cooling from 900 K to 400 K, and the third region to 5 ns of equilibration at 300 K. In panel (b), the density values shown were computed for two cubic regions of equal volume (see text), an inner cube (x marks) and an outer cube (solid line). The inset in panel (b) shows a closer view of the density variation during cooling.](image-url)
between two joint PET residues were computed. These three measurements are commonly used to characterize PET bulk properties.

The density of the PET bulk model during the annealing cycle is shown in Fig. 4.4b. At the initiation of the annealing cycle, densities in the center (x marks) and periphery (solid line) of the bulk cube differed significantly, but both converged to a constant value, demonstrating homogeneity of the bulk material. The equilibrium density of 1.20 g cm\(^{-3}\) is in good agreement with previously reported PET models [23, 24] with densities of 1.28 g cm\(^{-3}\) and 1.13 g cm\(^{-3}\), but still slightly lower than the experimental value [25] of 1.34 g cm\(^{-3}\).

The characteristic ratio is defined as the ratio between the mean-square end-to-end length \(R\) of a polymer and the length \(l_i\) of each rigid bond within the polymer [23]. The characteristic ratio, \(C_9\), was calculated according to

\[
C_9 = \frac{\langle R^2 \rangle}{\sum_i l_i^2}.
\] (4.1)

At 1000 K, the PET bulk behaves as a fluid and \(C_9\) fluctuates, reaching values up to 5. As the system becomes more dense, \(C_9\) decreases, obtaining a final value of 3.96, which is close to the experimental value of 4 and in good agreement with previously reported simulations [23].

Panel 4.4d shows the percentage of glycol dihedral in the \textit{trans} conformation, the \textit{trans} angle being defined as 180° ± 20°. At the end of the annealing cycle, the PET bulk has 39.4% of \textit{trans} content, such value differs from previous atomistic models and experimental values that report 25% \textit{trans} [24]. The percentage of \textit{trans} content is strongly influenced by the values of the O-C-C-O dihedral parameters. Cail \textit{et al.} [116] have reported that for dihedrals with barrier heights of 1.40, 1.00 and 0.24 kcal mol\(^{-1}\), the \textit{trans} content at 541 K is 19%, 24% and 39%, respectively. The dihedral constant used was taken from the propane-1,2-diyl diacetate compound model present in CHARMM, which has a barrier height of 0.2 kcal mol\(^{-1}\). In order to obtain 25% \textit{trans} content, a further refinement of this dihedral parameter would be needed.

Finally, the accuracy of the bulk model was evaluated using the radial distribution function \(g(r)\). Figure 4.5 shows \(g(r)\) for all carbon-carbon pairs. The overall shape of the curve compares well with previous studies [23]. The peaks observed between 1 and 4 Å describe the carbon-carbon distances defined by the connectivity within each polymer [120]. The inset in Fig. 4.5 shows the \(g(r)\) computed for carbon-carbon pairs, where each carbon belongs to a different polymer chain. The shoulder around 5 Å is related to the van der Waals contact between two carbons that are not covalently bonded.
Figure 4.5: Radial distribution function of carbon-carbon pairs in PET bulk. The figure shows $g(r)$ for C-C pairs separated by a distance $r$. $g(r)$ was computed over the last 2 ns of a 5 ns MD equilibration at 300 K. The inset shows the $g(r)$ for C-C pairs, each carbon belonging to different PET 9-mers.

4.3 Building PET nanopore

Experimentally, single nanopores in PET are prepared by the track-etching technique [76]. Briefly, PET films are irradiated with single swift heavy ions, which were accelerated to a total kinetic energy of 2.2 GeV. This irradiation process causes the formation of a single damage track through the films. It is expected that the impact of the heavy ion modifies the biaxially-oriented PET into an isotropic amorphous structure [99]. Conically shaped nanopores are obtained by asymmetric etching of the irradiated foils in 9 M NaOH while the other side of the membrane is in contact with an acidic stopping medium [121]. The heavy ion irradiation and chemical etching cause formation of 1 carboxyl group per nm$^2$.

An atomic-model of a PET nanopore should be built from amorphous bulk material, rather than from crystalline material. A nanopore was molded into the PET material by defining a conical pore geometry [76], according to

$$x^2 + y^2 = [(z + \frac{h}{2}) \tan \theta + R_{\text{min}}]^2, \quad -\frac{h}{2} \leq z \leq \frac{h}{2} \quad (4.2)$$

Here, $\theta$ is the angle between nanopore wall and pore axis, $h$ is the height of the pore and $R_{\text{min}}$ is the minimum radius of the cone. Through deletion of residues, a pore was created with $R_{\text{min}}=16$ Å, $h=105$ Å, $\theta=3^\circ$. The height of the pore was achieved through replication of the bulk structure unit.
Figure 4.6: Nanopore radius. Panel (a) shows a snapshot of a solvated PET nanopore. The PET structure is pictured as gray beads and water as a white surface. Panel (b) shows the radius of the PET nanopore along the Z-axis at two different stages of creation and after solvation. Circles denote the radius after the initial cut and patching of terminal residues. Squares denote the radius after rearrangement of the surface in vacuum. The solid line denotes the radius after solvation. The radius values were computed using the program HOLE [122]. Solid line and square symbols represent the average values over the last 2 ns of MD simulation at each stage.

cell, followed by deletion of residues above $z = h/2$ and below $z = -h/2$. Protonated carboxyl groups were added to all polymer terminals.

Deletion of residues cut some of the 9-mers into smaller segments. To prevent that small segments would be released from the nanopore surface, polymers with a length of three subunits or less were removed. To relax the surface while preserving the optimal geometry, a force of 1 pN guided non-terminal residues entering the pore volume back into the PET bulk during 2000 steps of minimization and 1 ns of simulation. After that, the system was equilibrated in a constant volume simulation without guiding forces for 4 ns. Figure 4.6b shows the radius of the pore along the Z-axis after the initial cut (circles) and after surface rearrangement (squares). As it can be observed, the conical geometry is preserved during the entire procedure. The narrowest pore region has a radius of 1 nm, resembling the smallest nanopores that can be manufactured by track-etching [11].

The PET nanopore was solvated in a rectangular water box using the Solvate plugin of VMD [48].
To allow the solvent molecules fill all small voids at the PET surface, the system was simulated for 5 ns at 300 K in the \( NpT \) ensemble, with the PET structure restrained using harmonic forces with a spring constant of 5 kcal/mol/\( \text{Å}^2 \). Subsequently, the restraints were removed, and the system simulated further for 7 ns at 300 K in the \( NpT \) ensemble. The radius of the pore along the \( Z \)-axis after solvation is shown in Fig. 4.6b (solid line). A snapshot of the solvated nanopore is presented in Fig. 4.6a.

### 4.4 Ionic current simulations

The solvated nanopore was used to study ionic conduction at pH 4 and pH 7. Simulations of ionic currents closely follow protocols used in earlier MD studies [55, 83, 98, 114, 117, 118]. To mimic pH 7 conditions, terminal PET residues in contact with water were deprotonated, resulting in a surface charge of \(-1.03 \, e \, \text{nm}^{-2}\), which agrees well with experimental values. The system was neutralized by adding counter ions, either \( K^+ \) or \( Ca^{2+} \), using the program *cionize* [123] that located the ions according to the electrostatic potential of the PET structure. To mimic pH 4 conditions, all carboxyl groups remained protonated. Subsequently, \( K^+ \) and \( Cl^- \) ions were randomly placed in solvent compartments, corresponding to 1 M KCl concentration. After adding ions, the systems were equilibrated at 300 K for 1 ns in the \( NpT \) ensemble, followed by 1 ns equilibration in the \( NVT \) ensemble. The final systems have periodic lattice vectors of \( 107 \, \text{Å} \times 84 \, \text{Å} \times 265 \, \text{Å} \).

To simulate ionic currents, a uniform electrostatic field \( E_Z \) was applied to all atoms of the system along the \( Z \)-axis. The voltage difference \( \varepsilon \) across the simulated cell is

\[
\varepsilon = -L_Z \cdot E_Z, \tag{4.3}
\]

where \( L_z \) is the dimension of the system in the \( Z \)-direction. Table 4.1 presents a summary of the ion conduction simulations performed. The voltage signs are defined relative to the lower solvent compartment which is in contact with the narrowest pore opening. A +1 V bias induces cations to move from the lower to the upper solvent compartment. To prevent the PET membranes from drifting, the benzene carbons located in a belt region of 40 Å height and 30 Å away from the pore axis were restrained by harmonic forces with a spring constant value of 1 kcal/mol/\( \text{Å}^2 \).

As mentioned in the introduction, MD simulations were employed to obtain information about how the surface charge of the pore walls affects the ion current passing through the pore, and about how the presence of divalent cations such as \( Ca^{2+} \) changes the ionic selectivity of the pore.

Ten simulations were performed, varying pH values between pH 4, where the carboxyl groups are
Table 4.1: Ionic current simulations of PET nanopores. The table shows the conditions for the various simulations. The number of K\(^+\) ions was 994 for all simulations. The number of Ca\(^{2+}\) ions in simulations 5-8 is sufficient to saturate half of the carboxyl groups, and sufficient to saturate all carboxyl groups in simulations 9 and 10. Cl\(^-\) were added in order to obtain charge neutrality in each of the simulations. MD simulations were performed under constant volume conditions. For pH 4, the simulations were performed for 10 ns (simulations 1, 2, 5 and 6); for pH 7, the simulations were performed for 20 ns (simulations 3-4 and 7-10). Averaged ionic currents and their standard error were calculated using Eq. 5.1 for the last 5 ns for each simulation.

<table>
<thead>
<tr>
<th>Sim</th>
<th>pH</th>
<th>ε (V)</th>
<th>Ca(^{2+}) (# ions)</th>
<th>Cl(^-) (# ions)</th>
<th>(I_{K^+}) (nA)</th>
<th>(I_{Cl^-}) (nA)</th>
<th>(I_{Ca^{2+}}) (nA)</th>
<th>(I_{Total}) (nA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>+1</td>
<td>0</td>
<td>994</td>
<td>2.02 ± 0.19</td>
<td>1.79 ± 0.18</td>
<td>0</td>
<td>3.81</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>-1</td>
<td>0</td>
<td>994</td>
<td>-1.60 ± 0.18</td>
<td>-1.85 ± 0.18</td>
<td>0</td>
<td>-3.45</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>+1</td>
<td>0</td>
<td>703</td>
<td>3.09 ± 0.17</td>
<td>0.83 ± 0.15</td>
<td>0</td>
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</tr>
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<td>-0.80 ± 0.15</td>
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<td>145</td>
<td>1285</td>
<td>1.33 ± 0.18</td>
<td>2.24 ± 0.21</td>
<td>0.50 ± 0.08</td>
<td>4.07</td>
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<tr>
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<td>1285</td>
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<td>-2.27 ± 0.21</td>
<td>-0.42 ± 0.08</td>
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<td>995</td>
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<td>1.44 ± 0.18</td>
<td>0.06 ± 0.07</td>
<td>3.12</td>
</tr>
<tr>
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<td>995</td>
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<td>-1.56 ± 0.18</td>
<td>-0.05 ± 0.07</td>
<td>-3.31</td>
</tr>
<tr>
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<td>1286</td>
<td>1.24 ± 0.18</td>
<td>1.98 ± 0.21</td>
<td>0.12 ± 0.10</td>
<td>3.34</td>
</tr>
<tr>
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<td>-1.71 ± 0.21</td>
<td>-0.22 ± 0.10</td>
<td>-2.97</td>
</tr>
</tbody>
</table>

The MD simulations suggest a strong dependence of ion selectivity properties of PET nanopores on the nanopore surface charge. At pH 4, when the nanopore surface is not charged, the anionic and cationic currents are of about the same magnitude (simulations 1 and 2), as shown in Fig. 4.7a and Table 4.1. As expected, there is no ion current selectivity in a neutral nanopore. At pH 7 however, where the carboxyl groups are negatively charged, the ion current carried by K\(^+\) is about three times larger than the current carried by Cl\(^-\) (simulations 3 and 4), as shown in Fig. 4.7b and Table 4.1. Ion selectivity is typically expressed as the ratio of the current carried by cations and the total current carried by cations and anions. The ion selectivity from simulations is 0.79, which agrees well with the values measured experimentally at elevated KCl concentrations [107, 124]. Due to the comparatively small volume accessible in all atom simulations, the ion concentration of 1 M...
KCl is chosen larger than the concentrations typically used in experimental measurements. However, experimental values of ion currents and ion selectivity have been determined at a very wide range of KCl concentrations including 1 M KCl [107, 124]. One should note that due to the required electroneutrality of the system, the number of Cl\(^-\) ions changes from 995 in simulations 1 and 2 (pH 4) to 703 in simulations 3 and 4 (pH 7). This change can account for some of the reduction in anionic current. However, the reduction in anionic current by a factor of two is larger than would be expected solely due to the changed concentration.

The ion currents in the presence of both KCl and CaCl\(_2\) were also studied. In a control condition at pH 4 (simulation 5 and 6), with an uncharged surface, addition of CaCl\(_2\) had little effect on the total current and the ion selectivity when compared with a pure KCl solution (simulation 1 and 2). The cationic current was simply split between K\(^+\) and Ca\(^{2+}\) ions with a ratio roughly corresponding to their relative concentrations. The increase of the anionic current compared to the results of simulations 1 and 2 is due to the increase of the Cl\(^-\) concentration (Fig. 4.8c).

At pH 7, with negatively charged surfaces due to the deprotonated carboxyl groups in the system, the addition of CaCl\(_2\) resulted in a more complex and interesting behavior. In simulations 7 and 8, 145 Ca\(^{2+}\) ions were added to neutralize the surface charge. In simulations 9 and 10, 291 Ca\(^{2+}\) ions were added, which could, in principle, saturate every carboxyl group, thereby possibly resulting in overcharging of the system walls. In all of the simulations 7-10, Ca\(^{2+}\) competed with K\(^+\) ions for locations close to the negative carboxyl groups (see below) [125]. During the simulations, most Ca\(^{2+}\) ions remained practically immobile, in contact with carboxyl groups. For the smaller number of 145 Ca\(^{2+}\) ions, indeed only a very small fraction of Ca\(^{2+}\) ions was mobile and their contribution to the total ion current was negligible, as shown in Fig. 4.7c and Table 4.1. With Ca\(^{2+}\) ions linked to carboxyl groups, the system is effectively neutralized (simulations 7 and 8). Differences between cationic and anionic currents are within the standard error of the simulations, indicating that there is no selectivity of ion current in this case.

Addition of 291 Ca\(^{2+}\) ions (simulations 9 and 10) resulted in a further change of the ionic conduction behavior. The currents carried by Cl\(^-\) are now larger than the combined K\(^+\) and Ca\(^{2+}\) currents (Table 4.1 and Fig. 4.7d). This suggests that the nanopore in the latter system has changed selectivity, possibly due to charge inversion in the nanopores. To analyze the reason underlying the changed ionic currents, the ionic concentration profile in the pore as well as the electrostatic potential along the Z axis of the nanopore were calculated.
Figure 4.7: Ionic currents through PET nanopores. The figure shows the ionic currents computed using Eq. 5.1, each point representing an average over 0.5 ns. The ionic currents were calculated for each ionic species, K\(^+\) (blue), Cl\(^-\) (red) and Ca\(^{2+}\) (green). Two different voltages are used: +1 V (positive currents) and -1 V (negative currents): (a) shows simulations 1 and 2, (b) shows simulations 3 and 4, (c) shows simulation 5 and 6, (d) shows simulations 7 and 8, (e) shows simulations 9 and 10. Simulation conditions are listed in Table 4.1.
4.5 Ionic distribution inside PET nanopores

The pH dependence of the ion selectivity properties of the PET pore can also be seen by analyzing the concentration profiles for different ion species. Figure 4.8 shows the ionic density as a function of distance from the center of the nanopore. As expected, at pH 4 (Fig. 4.8a), the concentrations of K$^+$ and Cl$^-$ ions are practically identical, while at pH 7 (Fig. 4.8b) there is a huge increase of the K$^+$ concentration, which agrees well with the observed increase of the cationic current (cf. Table 4.1).

Furthermore, an analysis of the ion densities in the nanopore shows that Ca$^{2+}$ outcompeted K$^+$ in the region close to the pore walls, the K$^+$ concentration being significantly less than in the situation without Ca$^{2+}$ (compare Fig. 4.8b with 4.8d). However, the Ca$^{2+}$ concentration at the pore walls increased only slightly when the overall concentration was changed from 145 to 291 ions (compare Fig. 4.8d with 4.8e). This indicates that the fraction of the carboxyl groups with an adsorbed Ca$^{2+}$ did not change much for the higher Ca$^{2+}$ concentrations, i.e., the pore walls remained roughly electroneutral. Figure 4.8e shows an increase of the total Ca$^{2+}$ concentration in the pore, accompanied by a corresponding increase in the amount of chloride ions. The distribution of the Cl$^-$ ions, however, does not show any maximum in the vicinity of the position with the increased Ca$^{2+}$ concentration. Such maximum of Cl$^-$ concentration near the pore wall is typically indicative of charge inversion [126]. Since one does not see such a maximum here, one can rule out overcharging of the nanopore walls as the cause for increased anionic currents (cf. Table 4.1).

It is expected that the adsorbed Ca$^{2+}$ should cause changes in the electrostatic potentials. These changes, together with the increased concentration of Cl$^-$ ions, can explain the increased anionic current. The presented MD results are in agreement with experimental data [125, 127] and previous modeling [128], which showed that the charge inversion and ion selectivity switch do not occur if a system contains a high concentration of monovalent cations. This effect, called salting-out, is explained by the competition of K$^+$ and Ca$^{2+}$ ions for the negative charges at the walls. The K$^+$ ions concentration at the wall remains high enough so that surface overcharging does not occur.
Figure 4.8: Ionic concentrations as a function of radial distance. Concentrations are averaged over an 8 nm [-40; 40 Å] compartment along the Z-axis. The figures show the ion concentration inside the pores for each MD simulation at +1 V bias: (a) simulation 1, (b) simulation 3, (c) simulation 5, (d) simulation 7, (e) simulation 9. K\(^+\), Cl\(^-\) and Ca\(^{2+}\) concentrations are depicted in blue, red and green, respectively. Simulation conditions are listed in Table 4.1.
4.6 Electrostatic potential of the system

Finally, to study how the adsorbed ions affect the electrostatic properties of the nanopore channel, the electrostatic potential along the pore Z-axis, $V_{\text{system}}(Z)$, was computed. The procedure to calculate $V_{\text{system}}(Z)$ has been described in detail in Chapter 3, section 3.7. Briefly, the averaged three-dimensional electrostatic potential was computed for the entire system using the PMEpot plugin [98] available in VMD [48] for the last 5 ns of each trajectory. Then, the three-dimensional electrostatic potentials was used to compute the electrostatic potential across the pore Z-axis at +1 V and -1 V biases, denoted as $V^{+1V}(Z)$ and $V^{-1V}(Z)$, respectively. Then, $V_{\text{system}}(Z)$ was calculated using:

$$V_{\text{system}}(Z) = \frac{V^{+1V}(Z) + V^{-1V}(Z)}{2}. \tag{4.5}$$

Adding $V^{+1V}(Z)$ and $V^{-1V}(Z)$ cancels out the influence of the external applied voltage biases +1 V and -1 V, leaving only the electrostatic contributions from all charged species in the system. Therefore, $V_{\text{system}}$ describes the electrostatic potential of the system itself acting on a line coincident with the pore Z-axis.

Figure 4.9 shows $V_{\text{system}}$ for all systems. There is a significant change in $V_{\text{system}}$ at different pH conditions. At pH 4, the PET surface is not charged, there is no ion adsorption, and the resulting $V_{\text{system}}$ is small and positive (Fig. 4.9a and 4.9c). However, at pH 7, the PET surface becomes negatively charged and in the presence of 1 M KCl, $V_{\text{system}}$ becomes negative (Fig. 4.9b), which implies cation selectivity of the pore. After adding Ca$^{2+}$, the negatively charged surface binds the Ca$^{2+}$ ions, $V_{\text{system}}$ becomes positive again as shown in Fig. 4.9d and 4.9e. The reversal of the sign of $V_{\text{system}}$ after Ca$^{2+}$ binding suggests that charge inversion has occurred; however, the selectivity for Cl$^-$ ions, a characteristic feature of charge inversion, was not observed. The absence of Cl$^-$ selectivity in the simulations can be due to the high ionic concentration assumed; only at a sufficiently low ionic concentration would the effect of charge inversion and Cl$^-$ selectivity arise.
Figure 4.9: Electrostatic potential of the system. The figures show the electrostatic potential of the system ($V_{\text{system}}$) for the systems at: (a) pH 4 and 1 M KCl, (b) pH 7 and 1 M KCl, (c) pH 4 and 1 M KCl and 145 Ca$^{2+}$ ions, (d) pH 7 and 1 M KCl and 145 Ca$^{2+}$ ions, (e) pH 7 and 1 M KCl and 291 Ca$^{2+}$ ions. $V_{\text{system}}$ defined in Eq. 4.5. Lower solvent compartment, nanopore region, and upper solvent compartment are located in the intervals [-150; 50 Å], [-50; 50 Å], and [50; 150 Å] along the Z axis, respectively.
4.7 Conclusions

This Chapter presented the first all-atom MD simulations of PET polymer nanopores. The study presents several methodological and scientific accomplishments and opens an avenue for future research. First, a collapsing-annealing procedure was developed to produce polymeric PET bulk structures. This procedure overcomes the difficulties identified for the growing-chain methodology [120] and can be easily used to build other polymeric bulk structures. Secondly, a model of PET nanopore was built, which accurately reproduces geometry and surface charge density of the experimental systems. Third, the MD model of PET is based on the CHARMM force field, which permits extension for future simulations, for instance, to study functionalized PET nanopores [19, 21]. The all-atom MD simulations give insight into transport properties of these pores, such as the ion dynamics inside PET nanopores at different pH conditions, which correspond to different surface charge properties of the pore walls. The results reproduced the cation selectivity observed at neutral pH conditions, at which the PET surface is negatively charged. Also, a very high affinity of Ca$^{2+}$ ions to the surface deprotonated carboxyl groups was observed, which is important for understanding the effect of charge inversion. The simulations showed that mobility of Ca$^{2+}$ ions in the pore is very low and that Ca$^{2+}$ ions close to the carboxyl groups are practically immobile. Since the simulations were performed in the presence of a high concentration of KCl, charge inversion could not be observed.
Chapter 5

Nanoprecipitation oscillations in polymeric nanopores

Reproduced in part from Eduardo R. Cruz-Chu and Klaus Schulten. The role of protonable surface residues on nanoprecipitation oscillations. Submitted.

5.1 Introduction

Polymeric nanopores are manufactured drilling through synthetic membranes made of plastic materials [76, 121], such as polyethylene terephthalate (PET). Figure 5.1a depicts a model of a PET polymer nanopore in electrolytic solution. In a recent study [72], Powell and colleagues described a new phenomenon occurring in polymeric PET nanopores, namely the formation of transient precipitates inside the pore lumen, so-called nanoprecipitation. This phenomenon occurs when a voltage bias is applied across a polymeric nanopore membrane immersed in phosphate buffer solution. If the solution contains monovalent cations, such as K\(^+\), the ions flow through the pore establishing a constant ionic current. However, if the solution contains sub-millimolar concentrations of divalent cations, such as Ca\(^{2+}\), Co\(^{2+}\) or Mg\(^{2+}\), the ionic current is not constant but oscillates, reflecting a sequence of low and high conductance states. Figure 5.1b shows a schematic representation of the ionic current oscillations. Powell \textit{et al.} demonstrated that the oscillations are due to the formation of small precipitates containing divalent cations and phosphate ions. The low conductance state is caused by a precipitate clogged in the nanopore, obstructing the ion flow, while the high conductance state is caused by the dissolution of the precipitate, resetting the ion flow.

The discovery of nanoprecipitation inside nanopores provides us with a clear example of the interplay between pore surface and translocating molecules. The chemical structure of the nanopore walls plays a key role in the formation of the nanoprecipitates. As a result of the fabrication process, the pore surface is covered by PET terminal residues, which contain negatively charged carboxyl groups [76, 121] (see Fig. 5.1). Using theoretical modeling based on Poisson-Nernst-Planck (PNP) equations, Powell \textit{et al.} proposed that the combined effect of the negative nanopore surface and the applied voltage bias produces a high accumulation of ions inside the nanopore, leading to the
formation of a nanoprecipitate blocking the pore. Even though the PNP modeling captures the physical insight of the phenomenon, it lacks an atomic description of the nanopore and assumes a smooth pore surface with continuous negative charge. Therefore, a molecular understanding of the nanoprecipitation mechanism is still needed.

Molecular dynamics (MD) simulations can be used to elucidate the atomic origin of nanoprecipitation. MD simulations provide an all-atom description of the nanopore surface, ions and solvent molecules, on time scales that can be extended up to microseconds. Indeed, MD simulations have been extensively employed in nanopore research [12, 13, 15, 56, 91, 114, 129], providing accurate dynamic images used to complement experiments. This Chapter presents a systematic study of nanoprecipitation, the MD simulations were performed using an all-atom PET nanopore model immersed in electrolytic solution containing divalent cations and phosphate ions. The choice of divalent cation was Ca\(^{2+}\), used also in the experimental studies [130, 131] leading to the discovery of nanoprecipitation in polymeric nanopores. The PET force field parameters [132] are based on the CHARMM force field [46], and have been described in Chapter 4, section 4.2. K\(^{+}\), Cl\(^{-}\), and Ca\(^{2+}\) parameters were taken from the CHARMM force field. H\(_2\)PO\(_4\)\(^{-}\) parameters were taken from Yang et al. [133]; HPO\(_4^{2-}\) parameters were obtained by homology using model compounds in the CHARMM force field. The details of the MD protocols are summarized in Appendix A.

In total, this Chapter presents 23 MD simulations, covering altogether 1.24 \(\mu\)s, which have been organized into four sections. The first section focuses on the formation of calcium phosphate precipitates in solution, without PET nanopore. The second section focuses on the calcium phosphate precipitation in nanopores. In the third section, two proposed mechanisms for removal of the precipitates from the nanopore are evaluated, namely dissolution and voltage-driven translocation of the precipitate. Finally, the fourth section explores a new mechanism for removal of the precipitate, namely the translocation of the precipitate after reprotonation of the PET surface.
Figure 5.1: (a) Atomic model of polyethylene terephthalate nanopore. The snapshot shows a polyethylene terephthalate (PET) nanopore in electrolyte solution, the nanopore sliced along the pore axis. PET material is shown as a gray surface; negatively charged PET residues are shown in purple; K\(^+\), Cl\(^-\), Ca\(^{2+}\) and HPO\(_4^{2-}\) ions are colored in blue, orange, green and red, respectively; water molecules are not shown. (b) Schematic representation of nanoprecipitation oscillations. When a PET nanopore is immersed in electrolyte solution containing Ca\(^{2+}\) and HPO\(_4^{2-}\) ions, an external electric field produces an oscillating ionic current through the pore, characterized by a sequence of low and high conductance states [72].
5.2 Formation of calcium phosphate precipitates in solution

The first objective was to determine whether calcium phosphate precipitation can be observed in MD simulations. In aqueous solution, ionic salts containing weak acids or weak bases precipitate if the product of the ionic activities is higher than the solubility product (K\text{sp}). The K\text{sp} value for CaHPO\textsubscript{4} at ambient condition is in the range of 1 × 10\textsuperscript{-7} mol\textsuperscript{2} L\textsuperscript{-2} [134], which indicates that CaHPO\textsubscript{4} is a sparingly soluble salt and precipitates when its concentration exceeds 0.3 mM. To observe precipitation, five systems were built, containing only water, Ca\textsuperscript{2+} and HPO\textsubscript{4}\textsuperscript{2−} ions, with CaHPO\textsubscript{4} concentrations higher than 0.3 mM. A system with an ionic concentration close to 0.3 mM is not practical for MD studies, as it would contain just a few ions in a very large water box. The range chosen was 0.2 to 2.2 M. Since CaHPO\textsubscript{4} acts as a buffer and the phosphate ions can change their protonation states, systems containing Ca(H\textsubscript{2}PO\textsubscript{4})\textsubscript{2} were also included. The systems were minimized for 1000 steps and then equilibrated for 1 ns in the N\textit{p}T ensemble. The last frames of the MD equilibrations were used as starting structures for further simulations, which were performed under N\textit{VT} conditions. The MD simulations performed are summarized in Table 5.1.

The formation of precipitates was observed in all MD simulations listed in Table 5.1. The transition of the ions from bulk solution into a solid phase can be detected by calculating the mean-square-displacement (MSD) and the radial distribution function (g(r)). The MSD was computed using:

\[
MSD(t) = \frac{1}{N} \sum_{i=1}^{N} [r_i(t) - r_i(0)]^2
\]

where MSD(t) is the MSD at time t, N is the number of phosphorus (P) atoms, and r\textsubscript{i}(t) and r\textsubscript{i}(0) are the positions of P\textsubscript{i} at times t and 0, respectively. The g(r) profiles (Fig. 5.3) were computed

<table>
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<th>Sim</th>
<th># Ca\textsuperscript{2+}</th>
<th># HPO\textsubscript{4}\textsuperscript{2−}</th>
<th># H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−}</th>
<th>M\textsubscript{HPO\textsubscript{4}\textsuperscript{2−}}</th>
<th>M\textsubscript{H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−}}</th>
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<tr>
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<td>2.2</td>
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</tr>
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<td>0</td>
<td>2.0</td>
<td>50</td>
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</table>

Table 5.1: Calcium phosphate precipitation in solvent box. The table lists the CaHPO\textsubscript{4} (1-4) and Ca(H\textsubscript{2}PO\textsubscript{4})\textsubscript{2} (5-6) concentrations used. Each simulation is labeled with a number (first column), followed by the number of ions employed. The fourth and fifth columns present the molar concentration for HPO\textsubscript{4}\textsuperscript{2−} and H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−} ions, respectively.
Figure 5.2: Mean-square displacement for calcium phosphate precipitation in solution. The figure shows the MSD for all phosphorus atoms for HPO$_4^{2-}$ (a) and H$_2$PO$_4^-$ (b) MD simulations listed in Table 5.1.

using the VMD command measure gofr.

Figure 5.2 shows the MSD computed for all phosphorus (P) atoms. A linear increment in the MSD corresponds to random diffusion of solvated ions, while a constant MSD value reveals the existence of a solid phase. For both HPO$_4^{2-}$ and H$_2$PO$_4^-$ ions, concentrations higher than 0.4 M (Sim 2-4, 6-8) show an initial linear increment in the MSD, which reflects the movement of the ions from their random distribution into a solid aggregate. In the last 20-25 ns, the MSD values exhibit constant horizontal slopes due to the formation of a stable precipitate. The subsequent small rises in MSD correspond to the diffusion of both the solid precipitates and of some ions still free in solution. For concentrations lower than 0.4 M (Sim 1 and 5), the MSD values show the formation of precipitates only at the end of the 50 ns MD simulations.

Figure 5.3 shows the variation of $g(r)$ for 1 M phosphate solutions, each color line representing a 10 ns average. Similar $g(r)$ profiles were seen for the other concentrations, only 1 M concentrations being presented. The $g(r)$ profiles not only confirm the formation of a solid phase, but also provide information about the local structure of the precipitate. Figures 5.3a and 5.3b show $g(r)$ for P-P and P-Ca atom pairs, respectively, for a 1.1 M HPO$_4^{2-}$ concentration. As the simulation progresses in time, a P-P peak appears near 4.6 Å (Fig. 5.3a), which is the average distance between two P-P centers capturing a common Ca$^{2+}$ ion. This is followed by a sequence of P-P peaks periodically spaced, such strong correlation being characteristic of a solid crystalline structure. The $g(r)$ for P-Ca pairs (Fig. 5.3b) shows two sharp peaks at 3.0 and 3.4 Å. The first peak occurs when Ca$^{2+}$ is in contact with two oxygens from the same HPO$_4^{2-}$, while the second peak occurs when Ca$^{2+}$ is in contact with only one oxygen of HPO$_4^{2-}$. Moreover, the value of $g_{P-Ca}(r)$ around 4 Å is zero, Ca$^{2+}$
ions being excluded from that region because it is already filled with a P atom.

For 1.0 M H$_2$PO$_4^-$ concentration, $g(r)$ for P-P and P-Ca atom pairs are presented in Figures 5.3c and 5.3d, respectively. A comparison between the $g_{P-P}(r)$ profiles for HPO$_4^{2-}$ (Fig. 5.3a) and H$_2$PO$_4^-$ (Fig. 5.3c) reveals that the change in phosphate protonation state modifies the molecular arrangement of the precipitate. The overall P-P distribution in Fig. 5.3c corresponds to a less structured amorphous solid. The $g_{P-Ca}(r)$ profile (Fig. 5.3d) shows a single sharp peak at 3.5 Å, which corresponds to the P-Ca distance when Ca$^{2+}$ is in contact with only one oxygen of H$_2$PO$_4^-$. There are no contacts between Ca$^{2+}$ and two oxygens from the same H$_2$PO$_4^-$. Both MSD and $g(r)$ display clear evidence of the development of a solid phase, but do not provide information about the number, size and charge of the precipitates. To address this point, a procedure to identify individual precipitates was developed. Such procedure is based on a iterative nearest-neighbor searches and is described below. First, a P atom, denoted as P$_i$, is added to a list, the so-called cluster list. Second, a distance search is performed around P$_i$ and new atoms are added to the cluster list. Taking P$_i$ as a center, a search was performed for P and Ca$^{2+}$ within distances $d_{P-P}$ (6 Å) and $d_{P-Ca}$ (4 Å), respectively. The cutoff distances $d_{P-P}$ and $d_{P-Ca}$ were obtained from the $g(r)$ profiles (Fig. 5.3) and locate the first shell of atoms around P$_i$. Third, distance searches are performed over all new elements in the cluster list. For new P atoms, the search was performed for P and Ca$^{2+}$ within distances $d_{P-P}$ and $d_{P-Ca}$, respectively. For new Ca$^{2+}$, the search was performed for P atoms within distances $d_{P-Ca}$. Fourth, repeated elements are removed from the cluster list. The third and fourth steps are iteratively repeated for all new elements, until the cluster size become constant. The final cluster list contains the components of

![Figure 5.3](https://example.com/figure5.3.png)

Figure 5.3: Radial distribution function for calcium phosphate precipitation in solution. The figures present $g(r)$ for calcium phosphate solutions containing 1.1 M HPO$_4^{2-}$ (a,b) and 1.0 M H$_2$PO$_4^-$ (c,d). Each line presents $g(r)$ averaged over a 10 ns period: 0-10 ns (black), 10-20 ns (gray), 20-30 ns (green), 30-40 (blue) and 40-50 ns (red). The $g(r)$ values were calculated for P-P atom pairs (a,c) and P-Ca atom pairs (b,d).
an individual precipitate, its mass and charge are calculated taking into account the hydrogens of the phosphate ions. The entire procedure is repeated for the remaining P atoms. Due to random collisions, transient aggregates containing only 2 or 3 P atoms can be formed. To avoid counting such insignificant aggregates, clusters containing less than 4 P atoms are discarded.

Figure 5.4 provides a schematic view of each individual precipitate, its size and charge, for the entire range of concentrations. Each precipitate is represented as a circle, the radius being proportional to the cube root of the precipitate’s mass. The charge sign is color coded and the total charge is indicated in each circle. Such snowman-like representations provide a generic picture of the precipitation process. Similar plots will be used in the next section to present the development of precipitates inside nanopores. Figure 5.4, allow us to identify the two stages of precipitation, namely, the initial nucleation of small precipitates and the following fusion and growth. For instance, for solutions with 0.2 M H$_2$PO$_4^-$ (5.4a) and 0.2 M H$_2$PO$_4^-$ (5.4e), nucleation started after about 15-25 ns, but there were not enough ions to observe a significant growth. For 0.5 M H$_2$PO$_4^-$ (5.4b) and 0.4 M H$_2$PO$_4^-$ (5.4f) concentrations, small precipitates appeared within the first 5-10 ns and started to aggregate at 20-30 ns. In the case of 1.1 M H$_2$PO$_4^-$ (5.4c) and 1.0 M H$_2$PO$_4^-$ (5.4g) concentrations, small precipitates appeared immediately and fusion started at about 10 ns. Finally, for 2.2 M H$_2$PO$_4^-$ (5.4d) and 2.0 M H$_2$PO$_4^-$ (5.4h) concentrations, the high ionic concentrations produced a large number of small precipitates from the very beginning, and fusion started after 5 ns.
Figure 5.4: Variation of individual precipitates as a function of time. The figure shows schematic representations of individual precipitates for calcium phosphate solutions with different HPO$_4^{2-}$ (a-d) and H$_2$PO$_4^-$ (e-h) concentrations. Each precipitate is represented as a circle, the radius being proportional to the cube root of the precipitate’s mass. For each given time, the precipitates are sorted based on their weight with the heavier ones at the bottom. The legend shows four reference circles, that represent masses of 1, 5, 10 and 20 kDa. Precipitates heavier than 1 kDa have black contour lines around the circle. The precipitate’s charge is color coded: blue for positive, red for negative and green for neutral. The total charge is displayed in each circle.
5.3 Formation of calcium phosphate precipitates in nanopores

The ionic current oscillations observed in PET nanopores [72] have been attributed to the negative charge on the PET walls that, together with the applied voltage bias, increase the Ca$^{2+}$ concentration inside the nanopore, leading to the formation of transient CaHPO$_4$ precipitates. The precipitation of CaHPO$_4$ should be clearly observable in MD simulations for two reasons: First, in a recent study [132], it was shown that Ca$^{2+}$ adheres to negatively charged PET carboxyl groups present at the nanopore surface, resulting in a high local concentration of Ca$^{2+}$ ions inside the pore. Second, in the previous section 5.2, it was demonstrated that MD simulations can accurately describe the formation of CaHPO$_4$ precipitates. Therefore, it is expected that a MD model composed of phosphate ions, a PET nanopore, and Ca$^{2+}$ adsorbed at the PET surface would display the atomic dynamics of the precipitation process.

To test this hypothesis, six simulations were performed, using a nanopore with 1 nm minimum radius, 10 nm length, and surface charge density of -1 $e$ nm$^{-2}$. The nanopore was solvated and a layer of Ca$^{2+}$ ions was located next to the deprotonated carboxyl group. Six systems were created by adding phosphate ions, using two phosphate protonation states and different spatial distributions. K$^+$ and Cl$^-$ ions were added into the solvent compartments to ensure electroneutrality. The final systems, containing nanopore, water and ions, have elementary cells of dimension 10.7 nm$\times$8.4 nm$\times$26.5 nm, and comprise about 226000 atoms. Systems composed of PET nanopore, water and ions (Sim 9-23) were minimized for 2000 steps, and then equilibrated in the $NpT$ ensemble for 0.2 ns with the PET structure constrained, and for 1 ns without any constraints; after that, the systems were equilibrated in the $NVT$ ensemble for 1 ns. The last frames of the MD equilibrations

<table>
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<tr>
<th>Sim</th>
<th># K$^+$</th>
<th># Cl$^-$</th>
<th># Ca$^{2+}$</th>
<th># HPO$_4^-$</th>
<th># H$_2$PO$_4^-$</th>
<th>Initial phosphate distribution</th>
<th>Phantom surface</th>
<th>Time (ns)</th>
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<tr>
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<td>0</td>
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<td>yes</td>
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Table 5.2: Calcium phosphate precipitation in PET nanopores. The table lists the number of ions and conditions applied to phosphate ions. For simulations 9-13, 145 Ca$^{2+}$ ions were located near the deprotonated carboxyl groups. For simulation 14, instead of Ca$^{2+}$ ions, 290 K$^+$ ions were located near the negatively charged PET surface. The phosphate ions were initially arranged using three different conformations: in the solvent compartments (Sim 9), inside the nanopore region (Sim 10) and inside a cylindrical volume located at the top opening (Sim 11-14). For simulations 11-14, the movement of phosphate ions were restrained using phantom surfaces (see text). MD simulations were performed under +1 V bias.
were used as starting structures for further simulations, which were performed under NVT conditions. Voltage biases were applied along the Z-direction. A positive voltage induces cations to move from the lower solvent compartment to the upper one. To prevent the PET nanopore from moving, the benzene carbons located in a toroidal volume of 4 nm height and 3 nm away from the pore axis were restrained using harmonic forces with a spring constant of 1 kcal mol$^{-1}$ Å$^{-2}$. A summary of the MD simulations performed is presented in Table 5.2. The simulations are organized into two groups: In a first group (Sim 9 and 10), phosphate ions were arranged into two different starting conformations, either in the solvent compartments or inside the nanopore, and were allowed to freely diffuse. In a second group (Sim 11-14), phosphate ions were located at the top pore opening and their displacements were biased towards the nanopore interior.

The purpose of the first group of MD simulations was to produce spontaneous CaHPO$_4$ precipitation without any restraints in the system. In simulation 9, 50 HPO$_4^{2-}$ ions were randomly located in the upper and lower solvent compartments. As it was reported before [132], Ca$^{2+}$ ions remained adsorbed at the PET surface and did not diffuse into the solvent compartments. During the simulation, some HPO$_4^{2-}$ ions moved close to the surface and into the nanopore, binding to the adsorbed Ca$^{2+}$ ions, but the ions did not aggregate to create a precipitate large enough to block the pore opening. Figure 5.1a shows a snapshot of the last frame of the simulation.

In simulation 10, 50 HPO$_4^{2-}$ ions were located inside the nanopore volume, providing a high phosphate concentration near the layer of adsorbed Ca$^{2+}$ ions. In this case, CaHPO$_4$ precipitation was observed, the results being presented in Fig. 5.5a. Within the first 25 ns, three small precipitates appeared bound to negatively charged PET residues. At the same time, some phosphates attached to Ca$^{2+}$ ions, releasing them from the surface. The small growth was due to the union of these released fragments with the precipitates at the surface. An interesting result is that nucleation took place at the surface of the pore. The deprotonated carboxyl groups of PET acted as nucleation centers, providing accessible Ca$^{2+}$ ions for precipitation and retaining the precipitates during their growth. Figure 5.5b shows a snapshot of the last frame of simulation 10; three precipitates are seen, all attached to PET carboxyl groups through Ca$^{2+}$ ions. Another interesting result is that some precipitates contained K$^+$ ions. The final compositions of the three precipitates are 4 HPO$_4^{2-}$/9 Ca$^{2+}$ (0.6 kDa), 7 HPO$_4^{2-}$/9 Ca$^{2+}$/1 K$^+$ (1.1 kDa), and 13 HPO$_4^{2-}$/15 Ca$^{2+}$/1 K$^+$ (1.9 kDa).

The objective of the second group of MD simulations was to create a flow of phosphate ions through the nanopore. For this purpose, the phosphates were initially placed in front of the top pore opening, their starting positions randomly assigned within a cylindrical volume of 2.2 nm
Figure 5.5: Precipitation in PET nanopores. Panel (a) shows a schematic representation of CaHPO$_4$ precipitation observed in simulation 10. Each precipitate is represented as a circle; the radius is proportional to the cube root of the mass, the charge is color coded and labeled in each circle. Panel (b) shows a snapshot of the final frame of the simulation. The nanopore is shown sliced in the middle, the negatively charged PET residues are shown in purple. HPO$_4^{2−}$, Ca$^{2+}$ and K$^+$ ions are colored in red, green and blue, respectively. The three precipitates are highlighted with dashed blue lines. Water molecules and ions that are not part of precipitates are not shown.

radius and 3 nm height concentric with the pore axis. The displacement of the phosphate ions was restrained by the top surface and the lateral walls of the cylinder using phantom surfaces [94, 114]: any phosphate reaching those boundaries experienced an elastic collision toward the interior of the cylinder, as shown schematically in Fig. 5.6a. In this way, the phosphates were forced to diffuse towards the interior of the nanopore. The restraints were removed for all phosphate ions that completely translocated the pore and crossed the periodic cell into the upper solvent compartment.

Three simulations were performed using two different protonation states: one with 50 HPO$_4^{2−}$ ions (Sim 11), one with 50 H$_2$PO$_4^−$ ions (Sim 12), and one with a mixture of 24 HPO$_4^{2−}$ and 26 H$_2$PO$_4^−$ ions (Sim 13). Also, a control simulation was performed, containing 50 HPO$_4^{2−}$ ions but without Ca$^{2+}$ ions (Sim 14). The results are summarized in Fig. 5.6b-e. Precipitation was observed for all three simulations containing calcium and phosphate ions (Sim 11-13). For simulation 11 (Fig. 5.6b), nucleation and growth occurred within the first 30 ns, resulting in the formation of a single massive precipitate of 4.5 kDa, which remained stable for the rest of the simulation. The red curve in Fig. 5.7 shows the $Z$-position of the precipitate from 50 to 150 ns. The precipitate remained attached to the PET surface at the same location, near the pore opening. Its final composition is 34 HPO$_4^{2−}$/25 Ca$^{2+}$/13 K$^+$ (4.8 kDa), covering about 37% of the area at the top pore opening. It can be assumed that if the system had provided more than 50 HPO$_4^{2−}$ ions, the precipitate would
Figure 5.6: Precipitation in PET nanopores using different phosphate protonation states. Panel (a) shows a schematic view of the system. Fifty phosphate ions were randomly distributed within a cylindrical volume located at the top pore opening (red lines). If a phosphate ion crosses the top section or the lateral wall of the cylinder, it experiences an elastic collisions normal to the cylinder surface (black arrows). Panels b, c, d and e show the precipitation process for simulations 11, 12, 13, and 14, respectively.

Keep growing until complete blockage of the pore; hence, stopping the ion flow and producing the low conductance state. For simulation 12 (Fig. 5.6c), the change in phosphate protonation state slowed down the aggregation, avoiding the formation of a single large precipitate. As $\text{H}_2\text{PO}_4^-$ ions moved through the nanopore, some of them were absorbed by $\text{Ca}^{2+}$ ions at the surface, resulting in few small precipitates. The blue curve in Fig. 5.7 shows the $Z$-position of the heaviest precipitate. Such precipitate was initially formed near the pore opening but loosely bound to the surface. Therefore, it was released and moved through the pore surface, until it was adsorbed below the middle of the pore. At the end of simulation 12, three precipitates were attached to the surface, whose compositions are $5 \text{H}_2\text{PO}_4^-/3 \text{Ca}^{2+}/1 \text{K}^+$ (0.6 kDa), $7 \text{H}_2\text{PO}_4^-/4 \text{Ca}^{2+}$ (0.8 kDa), and $14 \text{H}_2\text{PO}_4^-/9 \text{Ca}^{2+}$ (1.7 kDa). For simulation 13 (Fig. 5.6d), the presence of $\text{HPO}_2^-$ ions accelerated the aggregation process. At 30 ns, two separate precipitates were seen, their final compositions after 100 ns were $5 \text{H}_2\text{PO}_4^-/3 \text{HPO}_2^-/7 \text{Ca}^{2+}/1 \text{K}^+$ (1.1 kDa) and $8 \text{H}_2\text{PO}_4^-/14 \text{HPO}_2^-/17 \text{Ca}^{2+}/3 \text{K}^+$ (2.9 kDa). The green curve in Fig. 5.7 shows that the heaviest precipitate remained attached to the pore wall. Finally, simulation 14 (Fig. 5.6e), did not produce precipitation, which is in good agreement with experimental results [72, 131]. As precipitation does not occur between $\text{K}^+$ and $\text{HPO}_2^-$ ions; the small circles observed in Fig. 5.6e correspond to $\text{HPO}_2^-$ and $\text{K}^+$ ions that were in close contact due to random collisions, but did not aggregate, and eventually dispersed as they diffused towards the
lower solvent compartment.

5.4 Dissolution and translocation

As presented above, the low conductance state is associated with the formation of a solid precipitate in the nanopore. Naturally, a high conductance state indicates that the flow of ions has resumed (see Fig. 5.1b); thus, high conductance has to be associated with the opening of the pore due to removal of the precipitate. In their original work, Powell et al. [72] considered that pore opening can not be due to the voltage-driven translocation of the entire precipitate and the authors suggested as the most likely scenario the dissolution of the precipitate inside the nanopore. This hypothesis was supported by PNP modeling of $\text{Mg(OH)}_2$ precipitation. The PNP model assumed that both cations and anions are mobile; therefore, the ionic concentrations inside the pore are proportional to the magnitude of the applied voltage. When the pore is blocked, the electric field decreases near the precipitate, causing a drop in the ionic concentrations. Due to the low ionic concentrations, the product of the ionic activities becomes lower than the $K_{sp}$ value and the precipitate dissolves.

In this section, both the dissolution and the voltage-driven translocation hypotheses were tested. A summary of the MD simulations performed is presented in Table 5.3. For these MD simulations, the starting structure was the last frame of simulation 11, which contains the largest precipitate (Fig. 5.8a). The dissolution hypothesis was evaluated in simulation 15. In order to mimic the proposed reduction of the electric field, no voltage bias was applied to the system. In this case, no
<table>
<thead>
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</tr>
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<td>50 HPO$_4^{2-}$, 145 Ca$^{2+}$</td>
<td>+1</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 5.3: Dissolution and translocation hypotheses. The table presents the conditions applied for testing dissolution (Sim 15) and translocation (Sim 16-18) hypotheses. The type of MD simulation is listed in the second column: EQ, cv-SMD and g-SMD denote equilibration, constant velocity SMD and grid-SMD, respectively. The third column contains the number of ions under the influence of SMD forces. For simulations 16 and 17, the steering atoms are part of the precipitate. For simulation 18, all HPO$_4^{2-}$ and Ca$^{2+}$ ions in the system experience g-SMD forces.

dissolution was observed, the precipitate remained attached to the surface at the same position and its size barely changed (Fig. 5.8b). It can be argued, though, that a 25-ns MD simulation is not long enough to observe dissolution. However, it should not be expected to observe dissolution even for a longer simulation. There is a substantial difference between PNP and MD models. In PNP modeling, Ca$^{2+}$ ions are able to move under the influence of the external electric field, whereas in MD simulations, most Ca$^{2+}$ ions remain bound to PET carboxyl groups, resulting in a high number of Ca$^{2+}$ ions available for precipitation even at zero voltage. The possibility of precipitate dissolution should be revised, since the PNP model considered that divalent cations are mobile, which does not seem to be the case in reality.

The voltage-driven translocation hypothesis, which was initially discarded by Powell et al., was also tested. For this purpose, three steered molecular dynamics (SMD) simulations (Sim 16-18) were performed. Different SMD conditions were employed for each simulation (see Table 5.3). In a first attempt (Sim 16), the precipitate was pulled through a single phosphate ion attached to it. The precipitate did not translocate and remained attached to the surface, whereas the phosphate pulled was released from the precipitate (Fig. 5.8c). In a second attempt (Sim 17) all phosphate ions forming the precipitate were pulled. Due to the strong attachment to the PET carboxyl groups, the precipitate tore off three PET polymer chains, damaging the pore (Fig. 5.8d). For both simulations 16 and 17, constant velocity SMD [135] was applied, using a spring constant of 3 Kcal/mol/Å$^2$. The pulling velocities for simulations 16 and 17 were 0.010 Å/ps and 0.025 Å/ps, respectively.

In a third attempt, all phosphate and Ca$^{2+}$ ions (Sim 17) were pulled using grid-SMD (g-SMD) [113]. In g-SMD, the steering forces are calculated from a potential defined on a grid; hence, it can be used to scale up the electrostatic force. The grid employed was the average electrostatic potential of a previously reported 20-ns MD simulation [132] of a PET nanopore immersed in 1 M KCl and under +1 V bias (cf. Chapter 4, Table 4.1-Sim 3). The steering forces derived from the grid
Figure 5.8: Dissolution and translocation. Snapshots show the starting structure (a) and the last frames for simulations 15 (b), 16 (c), 17 (d) and 18 (e). Deprotonated PET residues are pictured as purple lines, HPO$_4^{2-}$, Ca$^{2+}$, and K$^+$ ions are colored in red, green, and blue respectively. The location of the precipitate is highlighted with a black circle. For simulation 16 (c), the position of the pulling HPO$_4^{2-}$ ion is highlighted with a black arrow. For simulations 17 (d) and 18 (e), the precipitate tore three PET chains from the pore, colored in cyan.

were scaled up three times, only the Z-components of the forces were applied. As the precipitate translocated, it collected Ca$^{2+}$ ions, resulting in a decrease in its negative charge. Accordingly, the steering forces acting on the precipitate also decreased, and the precipitate moved only up to the middle of the pore. Similar to simulation 17, three PET chains were torn off from the pore (Fig. 5.8e).

The results of simulations 16-18 led us to discard the voltage-driven translocation. On the one hand, the strong interactions with the PET surface can readily stop the precipitate from moving. On the other hand, the charge of the precipitate is not fixed and it can be decreased or neutralized during translocation, reducing the effect of the applied voltage.

5.5 Surface reprotonation

An alternative scenario for pore opening was considered, which overcomes the strong binding between nanopore and precipitate. In order to release the precipitate from the nanopore surface, deprotonated PET carboxyl groups were reprotonated. The change in protonation state was taken into consideration for two reasons: First, PET carboxyl groups are covalently joined to benzene rings, resembling the structure of benzoic acid, a weak acid that is easily protonated under mildly acidic conditions. Second, phosphate ions coexist in different protonation states and can donate protons to PET carboxyl groups. Based only on the pK$_a$ values of 4.2 for benzoic acid and 7.2 for HPO$_4^{2-}$, this event should not have been considered. However, pK$_a$ values describe the average protonation state of millions of ions in solution; the mechanism in the nanopore, with few phosphate
ions in very close proximity to the PET carboxyl groups, may not be well characterized through the bulk pK\textsubscript{a} values. A previous quantum/classical molecular mechanics study \cite{136} proposed that during the crystallization of calcium phosphates, HPO\textsuperscript{2-} ions act as proton donors. The mechanism described for HPO\textsuperscript{2-} deprotonation involves the aggregation with two Ca\textsuperscript{2+} ions to assemble a [Ca\textsuperscript{2+}···HPO\textsuperscript{2-}···Ca\textsuperscript{2+}]\textsuperscript{2+} ion complex. The positive net charge of the ion complex increases the acidity of the HPO\textsuperscript{2-} ion, releasing a proton. A similar mechanism can be formulated for reprotonation of PET carboxyl groups. The precipitate contains HPO\textsuperscript{2-} ions in direct contact with Ca\textsuperscript{2+} ions. Protons can be released from the precipitate and diffuse towards the PET carboxyl groups located in the near vicinity. It has already been reported that protonated PET residues do not adsorb Ca\textsuperscript{2+} ions \cite{132}; therefore, the precipitate should detach from the nanopore surface and would eventually abandon the pore.

To test the stated hypothesis, five MD simulations were performed. Two approaches were used: in the first one, called total reprotonation, all PET carboxyl groups in the nanopore were reprotonated; in the second one, called gradual reprotonation, only the PET carboxyl group in contact with the precipitate were reprotonated. A summary of the MD simulations is presented in Table 5.4.

<table>
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<tr>
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<td>282</td>
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<td>25</td>
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</table>

Table 5.4: Reprotonation hypothesis. Listed are the starting structures and the number of reprotonated and deprotonated carboxyl groups for total reprotonation (19-20) and gradual reprotonation (21-23) MD simulations. For each simulation, the initial coordinates were taken from the last frame of the simulations listed in the second column (see Table 5.2 for Sim 11).

For the total reprotonation approach, two MD simulations were performed, using +1 V (Sim 19) and -1 V (Sim 20) biases. In both MD simulations, the precipitate exited the pore. Figure 5.9a shows the location of the precipitate for +1 V (blue line) and -1 V (red line) biases. As can be seen, the direction of the translocation is determined by the voltage sign. This is because after reprotonation, there is an excess of Ca\textsuperscript{2+} ions inside the nanopore. These Ca\textsuperscript{2+} ions attach themselves to the precipitate, changing its charge from a low negative value to a high positive one. For a positive charge, the direction of the electrostatic force is upwards for +1 V bias and downwards for -1 V bias. Figure 5.9b shows the charge/mass ratio of the precipitate for simulations 19 (blue) and 20 (red). For comparison, the charge/mass ratio for K\textsuperscript{+} and H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-} are +25.6 and -10.3 e kDa\textsuperscript{-1}, respectively.
Figure 5.9: Total reprotonation. Plots depict the $Z$-position of the precipitate’s center of mass (a) and its charge/mass ratio (b) for simulations 19 (blue) and 20 (red). The snapshots on the left side show: starting conformation for simulations 19 and 20 (i), and final frames for simulations 19 (ii) and 20 (iii). Reprotonated carboxyl groups are pictured as cyan lines. The black horizontal lines in panels (a), (i), (ii) and (iii) are guidelines to locate the top and bottom pore openings.

The gradual reprotonation approach consists of three consecutive MD simulations (Sim 21-23). In this case, a partial translocation was observed. Figure 5.10a shows the position of the precipitate for simulations 21 (blue), 22 (red), and 23 (green). In simulation 21, the precipitate was released from the surface, but did not leave the pore. Figure 5.10i shows the starting conformation for simulation 21, the six PET residues shown in cyan licorice representation have their carboxyl groups reprotonated. Three of those six carboxyl groups were bound to the precipitate, as it was revealed by SMD simulations (see Fig.s 5.8d-e). The other three carboxyl groups did not bind directly, but were located within 1 nm from the precipitate. At the end of simulation 21, the precipitate attached to another deprotonated carboxyl group. Simulation 22 started from the last frame of simulation 21, but the carboxyl group binding the precipitate was reprotonated. The precipitate detached from the surface and moved upwards into the upper solvent compartment (Fig. 5.10a - red curve), but it was caught by two carboxyl groups before complete exit. In simulation 23, the two attaching carboxyl groups were reprotonated. Once more, the precipitate was released from the surface, but did not moved further and remained in about the same position. Figure 5.10ii shows the final frame for simulation 23, the nine protonated carboxyl groups being highlighted. Figure 5.10b shows the precipitate’s charge/mass ratio for simulations 21, 22, and 23. The values registered are negative and small, i.e., the effect of the voltage sign on the direction of translocation is minimal. The
Figure 5.10: Gradual reprotonation. On the left side, plots depict the position of the precipitate’s center of mass (a) and its charge/mass ratio (b) for simulations 21 (blue), 22 (cyan), and 23 (yellow). On the right side, snapshots show the first frame of simulation 21 (i) and the last frame of simulation 23 (ii). Deprotonated PET residues are pictured as purple lines. Reprotonated PET residues are shown in cyan licorice representation. The black horizontal lines in panels (a), (i) and (ii) indicate the location of the top pore opening.

Overall upwards displacement was caused by collisions with K⁺ ions, that pushed the precipitate while exiting the pore through the top pore opening.

The reprotonation simulations described above provide evidence that protonation of carboxyl groups is a likely scenario for the removal of the precipitate. Once the precipitate is out of the pore, the ionic current should be resumed, restating the high conductance state.
5.6 Conclusions

This Chapter described a study of calcium phosphate precipitation in PET nanopores using all-atom MD simulations. In total, 1.24 µs of MD simulations were performed: 0.4 µs to study precipitation in bulk solution and 0.84 µs to study precipitation in PET nanopores. Overall, the results revealed a dynamic interplay between nanopore surface and translocating ions. Key players are the PET carboxyl groups exposed at the nanopore surface. When deprotonated, carboxyl groups retain Ca$^{2+}$ ions and act as nucleation centers for precipitation. The precipitate grows around carboxyl groups, blocking the pore entrance and decreasing the flow of ions through the pore. Previously, it was assumed that the precipitate would dissolve inside the nanopore. An alternative mechanism is proposed, where the entire precipitate exits the pore after being released from the nanopore surface. The MD simulations showed that the precipitate remains strongly attached to carboxyl groups. However, by reprotonation of the PET carboxyl groups exposed in the nanopore, the precipitate can detach from the PET surface and diffuse out of the pore, leaving the pore open and restoring ion flow.
Appendix A

Methodology for molecular dynamics simulations

The molecular dynamics (MD) simulations method consist of solving the classical equations of motion governing the microscopic time evolution of a many-body system. That is, for a system consisting of \( N \) particles, MD simulations are used to compute the positions \( \vec{r}_1(t), \ldots, \vec{r}_N(t) \) as a function of time \( t \). Then, the position coordinates can be used either to visualize the evolution time of the system or to calculate properties that depend on the relative location of the particles. The fundamentals of the MD methodology are described in detail in numerous books [137–139], only a brief presentation of the underlying concepts is included here.

In MD simulations, the particles of a system move according to Newtonian equations:

\[
m_i \ddot{\vec{r}}_i = -\frac{\partial}{\partial \vec{r}_i} U(\vec{r}_1, \vec{r}_2, \ldots, \vec{r}_N) \quad i = 1, \ldots, N. \tag{A.1}
\]

where \( m_i \) is the mass of particle \( i \), \( \vec{r}_i \) its position, and \( U \) is the potential energy, which depends on the position of all particles. Therefore, to perform MD simulations, the two obligatory requirements are the initial coordinates of the particles and the potential energy function.

For simulations of complex systems, such as nanopores or biomolecules, the particles are the atoms and their initial positions are determined by the structure of the system. The potential energy is represented through the force field, which determines the interaction of all atoms in the system. The force field is described by a function \( U(\vec{R}) \) in terms of the atomic positions \( \vec{R} = (\vec{r}_1, \vec{r}_2, \ldots, \vec{r}_N) \). The contributions to the potential energy \( U(\vec{R}) \) are classified in bonded interactions, that describe interactions between atoms connected by covalent bonds, and non-bonded interactions, that describe interactions between atoms that are not covalently linked or atoms separated by more than three covalent bonds.
In most simulations presented in this thesis, the functional form of the force field complies with the CHARMM model [46], which is:

\[
U(\mathbf{R}) = \sum_{bonds} k_{\alpha}^{bond}(r_{\alpha} - r_{0\alpha})^2 + \sum_{angles} k_{\beta}^{angle}(\theta_{\beta} - \theta_{0\beta})^2 + \sum_{dihedrals} k_{\gamma}^{dihed}(1 + \cos(n_{\gamma}\psi_{\gamma} + \delta_{\gamma})) + \sum_{impropers} k_{\delta}^{impr}(\phi_{\delta} - \phi_{0\delta})^2
\]

\[
+ \sum_{i > j} \varepsilon_{ij} \left[ \left( \frac{r_{ij}^{min}}{r_{ij}} \right)^{12} - 2 \left( \frac{r_{ij}^{min}}{r_{ij}} \right)^{6} \right] + \sum_{i > j} \frac{q_i q_j}{4 \pi \varepsilon_0 r_{ij}}. \tag{A.2}
\]

Bonded interactions include \( U_{bond} \), \( U_{angle} \), \( U_{dihedral} \) and \( U_{improper} \), which describes the bond vibrations, the bending motions, the torsional motions and the planar orientation of one atom relative to three others, respectively. The parameters describe the equilibrium values of the degrees of freedom for the bond \((k_{\alpha}^{bond}, r_{0\alpha})\), angle \((k_{\beta}^{angle}, \theta_{0\beta})\), dihedral \((k_{\gamma}^{dihed}, n_{\gamma}, \delta_{\gamma})\) and improper \((k_{\delta}^{impr}, \phi_{0\delta})\).

The non-bonded terms include \( U_{vdW} \), the pair-wise van der Waals energy and \( U_{Coulomb} \), the pair-wise Coulomb energy between charged atoms. The van der Waals interaction between atoms \( i \) and \( j \) is modeled using the Lenard-Jones 12-6 potential with \( \varepsilon_{ij} \) being the minima of the functions contributing to \( U_{vdW} \), located at \( r_{ij}^{min} \). The electrostatic contribution \( U_{Coulomb} \) accounts for interaction between atomic partial charges \( q_i \) and \( q_j \), \( r_{ij} \) is the separation between them, i.e., \( r_{ij} = |\mathbf{r}_i - \mathbf{r}_j| \) and \( \varepsilon \) is the relative dielectric constant of the medium.

In current MD simulations, the system size usually ranges from hundred thousands to millions of atoms. For such large and complex systems, it is not possible to obtain analytical solutions for the equations of motions. Therefore, Eq. A.1 and A.2 are solve numerically using computational approaches. Multiple methods have been developed to solve these equations; the main objective of those methods is to perform a quick calculation without losing accuracy [137–139].
Most MD simulations presented in this thesis have been performed using NAMD [55], unless otherwise stated. For all simulations, an integration time step of 1 fs was used. NAMD uses a modified version of the Verlet algorithm (velocity-Verlet) to obtain the position and velocity of the next step \((\vec{r}_{n+1}, \vec{v}_{n+1})\) from the current one \((\vec{r}_n, \vec{v}_n)\), using only one force evaluation per timestep [55].

The calculation of the forces acting on every particle requires evaluating the potential energy function (Eq. A.2). To increase computational efficiency, a multiple time stepping algorithm [140, 141] was employed to compute interactions involving covalent bonds every time step, short-range non-bonded interactions every two time steps, and long-range electrostatic forces every four time steps. Such multiple time step protocol has been recommended for \(NVT\) and \(NpT\) ensemble simulations [55].

For van der Waals and electrostatic interactions, a cut-off of 12 Å with a switching function starting at 10 Å was assumed. For periodic boundary conditions, full electrostatic forces were computed using the particle-mesh-Ewald method [142] with a grid density of \(1/\text{Å}^3\) or finer. A Langevin thermostat was used to maintain a control the temperature in \(NVT\) ensemble simulations. Simulations in \(NpT\) ensemble were performed using a hybrid Nosé-Hoover Langevin piston [143] to keep the pressure constant at 1 atm. Coordinates of all atoms were saved every picosecond for later analysis.
Appendix B

Publications

Journal Articles


Book Chapters
References


