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Molecular Dynamics Simulation of Membrane Proteins

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In the present review we summarize a few results from modeling and simulation of proteins embedded in membranes of biological cells. In particular, we report on the distribution of internal water forming the hydrogen-bonded network in the proton pump bacteriorhodopsin, and on the permeation and selectivity of ion channels.

1 Introduction

The response of intracellular processes of biological cells to extracellular stimuli can only be achieved by correct signal transductions across the plasma membrane of the cell. Since the membrane is impermeable for almost all molecules, the signal transduction is mediated by membrane proteins which are integral parts of a lipid bilayer membrane.

In general, there are two types of transmembrane signaling : the intracellular response of extracellular ligand-induced structural changes of membrane proteins, and the transmembrane transport of ions, protons or other small molecules. The transport of ions, e.g., can take place either down the electrochemical gradient of a specific ion (passive transport), or by "uphill" transport that calls for an energy input in order to overcome the energy barrier (active transport). Respective protein structures are accordingly classified into ion channels[1,2] and ion pumps, respectively.

The advantage of computer simulation over experimental techniques has been widely seen in the possibility to explore the dynamical aspects of the protein structure, and hence its functional properties, which are in most cases difficult to adressed experimentally. However, in order to perform reliable computational studies, a high atomic resolution of the protein structure is a necessary prerequisite. Although the molecular structure determination of major membrane proteins is still a generally non-solved problem, a few structures became available during the last decade. Since the computational facilities have gained more power, the theoretical modelling and simulation approach has shifted from previous simple models to realistic one including fully hydrated lipid membranes, containing up to 10^5 atoms. This has, for example, been seen after significant recent progress in the field of structure determination of potassium ion channels[3] and the proton pump bacteriorhodopsin (bR) (e.g. refs.4,5). Simulations of these two membrane proteins are addressed in the present review.

At present, the combination of atomistic resolution structures with highly sophisticated computational methods is considered to be a leading way in oder to improve our

understanding of fundamental properties of signal transduction processes across cell membranes.

2 Proton Pump

The protein bacteriorhodopsin (bR) resides in the membrane of the archaebacterium *Halobacterium salinarum* and uses photonic energy for transmembrane proton pumping. Proton transport is a critical process in physical chemistry and biology. The protein contains a certain molecule (retinal chromophore) which absorbs light around 568 nm. Photoexcitation triggers a certain structural change (isomerization) of the retinal, which leads to a cascade of conformational transitions of the whole protein bR. These conformational changes lead to a rearrangement of the internal hydrogen-bonded network built by various charged residues and internal water molecules. This rearrangement induces a vectorial transfer of a proton along the hyrogen-bonded network, leading to the release of a proton at the extracellular side and an uptake from the cytoplasmic side. Our current knowledge of the structure and the photocycle of bR has been reviewed in detail by several authors, e.g. ref.[6]. However, certain dynamical features of bR cannot be captured by crystallographic techniques, and therefore molecular dynamics simulations had been used to elucidate, among others, conformational fluctuations[7–9] and bR-water mobility[10,11]. Very recently[12] new details of the amount and the distribution of internal water molecules, and of the related hydrogen-bonded networks in bR that constitute proton pathways, has been reported. The results provide new information on hydrogen-bonded networks in bR fluctuating on the ps to ns time scale, which has not been seen in crystallographic studies. The procedure of the molecular dynamics (MD) simulations followed essentially the protocol as used in other simulation of membrane proteins[13].

Distribution of Water Molecules
Using molecular dynamics simulations the number of internal water molecules in bR have been estimated and demonstrate that bR is divided in an extracellular and a cytoplasmic part separated by a water-impermeable structural boundary. This is demonstrated in Fig.1. There, the average volume occupied by a single bR protein is represented by the gray three dimensional surface. Superimposed on the gray surface are depicted by blue and yellow triangulated nets, the surfaces of the volumes accessible to diffusive and trapped water molecules, respectively. The red balls indicate the locations of water molecules as found by crystallographic studies[4]. From the location of the potential proton acceptor Asp96 in the cytoplasmic (upper) part of bR (denoted in Fig.1 by the upper white molecule), it can be seen that water molecules in the ground state of bR cannot provide hydrogen-bonds to be used during proton transfer to Asp96. The two separate distributions of diffusive water molecules (blue nets) indicate that a migration of water between the extracellular and cytoplasmic part does not take place. The corresponding gap reflects a kind of structural “watershed” in the protein. The existence of this impenetrable structural interface between the cytoplasmic and the extracellular part of bR was already concluded from crystallographic data and is a necessary feature of the protein in its ground state, prohibiting a spontaneous transport of protons across the membrane. The distribution, however, of internal water molecules (diffusive and trapped), as detected by crystallographic data and as found during our simulations, have been compared recently[12] and found to differ con-

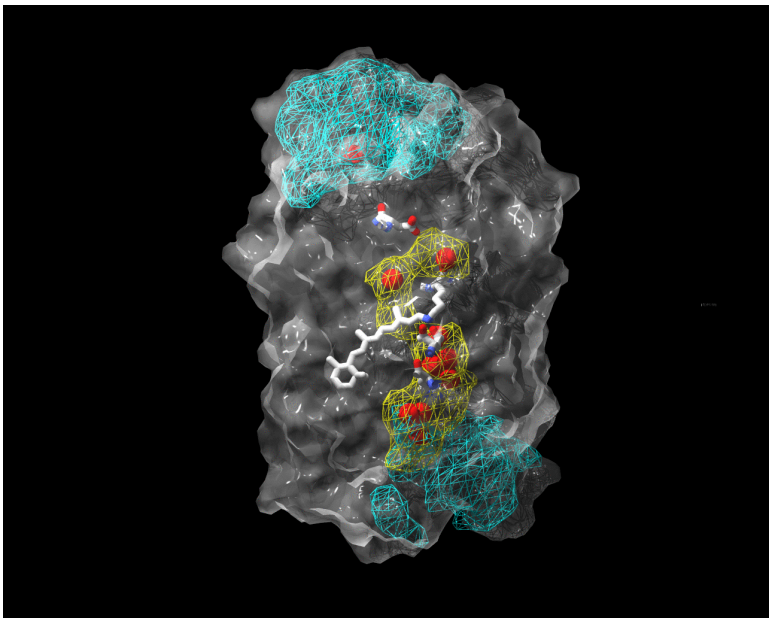

Figure 1. Accessible volumes for internal water molecules of a trimeric bR. The surfaces of the volumes for trapped and diffusive water molecules are represented by yellow and blue triangulated nets, respectively. The white molecules represent Asp96, retinal, and Asp85, from top to bottom. The red balls represent the locations of trapped water molecules as identified by crystallographic studies.

siderably from a quantitive point of view. The crystallographic data of Sass et al.[4] contain in total 77 water molecules, out of which only 18 are inside the protein, whereas the simulation data provide a much higer amount, on the average 36. The explanation for the large discrepancy of the number of water molecules, as found during simulation and as compared to the crystallographically found, is the high exchange rate of water molecules between internal and external locations. Therefore, it is of interest to characterize some time-dependent properties of internal water molecules in terms of typical residence times and correlation times. The residence time τ_{res} is the time which one of the diffusive water molecules spends inside the protein. Then, $N(\tau_{res})$ is the number of water molecules with residence time τ_{res} found in a given ensemble of diffusive water. The average over many ensembles, taken from simulation data, exhibits a distribution of $N(\tau_{res})$ which is well described by a power law within a certain time range, for trimeric and monomeric bR (Fig.2). Trimeric and monomeric bR refer to systems consisting of a single bR and an aggregate of three bR, respectively. From this distribution $N(\tau_{res})$ the average residence time of a water molecule had been calculated to $\langle\tau_{res}\rangle < 52$ ps. Again, this result explains why it is very difficult to detect the accurate amount of water molecules inside bR by crystallographic methods. On the other hand it demonstrate the advantage of computer simulations.

Hydrogen-Bonded Network

During the photocycle, protons are vectorially transported from the cytoplasmic side to the extracellular environment. During this process the proton is captured by the Schiff

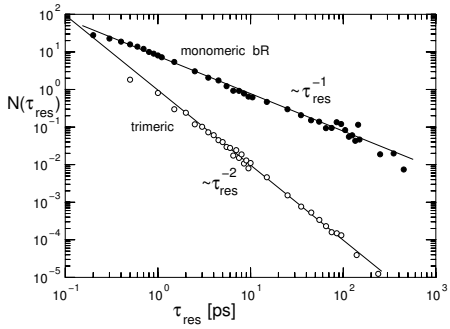

Figure 2. Average number $N(\tau_{res})$ of water molecules as function of their residence time τ_{res}. The full lines indicate the power laws for the trimeric and monomeric bacteriorhodopsin.

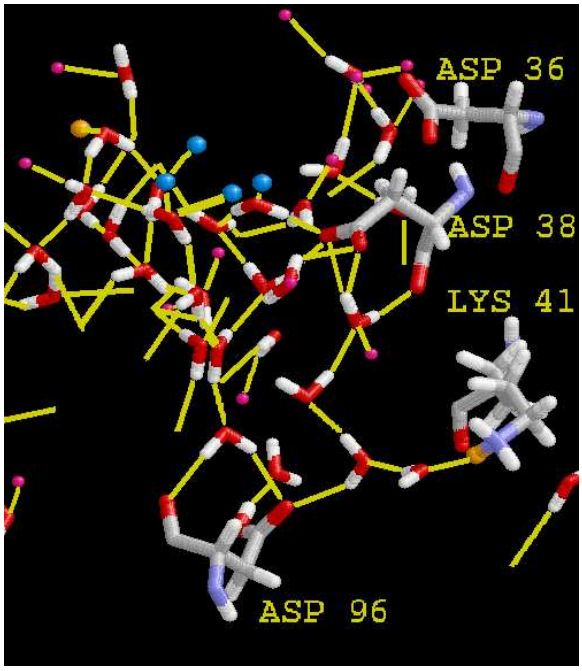

Figure 3. Snapshot of a hydrogen-bonded network of a monomeric bR, connecting the protein surface and Asp96. The yellow lines denote hydrogen bonds. The blue balls denote external water molecules to which Asp96 is connected via hydrogen bonds. Pink balls denote water molecules to which Asp96 is not connected. Orange balls denote other residues not denoted in the figure.

base of the retinal. This implies that hydrogen-bonded pathways must exist between the cytoplasmic surface of the protein and the Schiff base via the side chain Asp96 as shown by infrared spectroscopy. With regard to the previous section, concerned with the water population in bR, one can address the question about the contribution of water molecules

to generate hydrogen-bonded pathways between the bR's aqueous environment and the core of bR. It is useful to considere the constructs of hydrogen-bonded chains using the "Grotthuss relay mechanism" for proton transport. A large amount of literature exist on this subject, e.g., refs. 14, 15. One path consists of an alternating sequence of hydrogen bonds between water molecules, $H\cdots O$, separated by O–H bonds of water molecules. In this case, the protons are assumed to hop in a rate-limiting process along such a path which results in a reorientation of the participating water molecules. Many refinements of this model have been proposed[16]. In spite of the simplicity of the Grotthuss model, its application, in particular to biological systems, has lead to many valuable insights into proton transport governed by the concerted actions of spontaneously forming hydrogen-bonded networks and the structural fluctuations of the embedding proteins[17,18]. In Fig. 3 we have depicted a typical snapshot of a hydrogen-bonded network connecting the protein surface and Asp96 in the case of a single (monomeric) bR embedded in the membrane. A more quantitative description of the network has been provided[12] by the average number and lengths of Grotthuss paths connecting the protein surface to various charged residues of bR.

3 Ion Channels

The membrane lipid bilayer is highly impermeable to ions, because the energy barrier for transferring a hydrated ion to the low dielectric environment inside the membrane is prohibitively large. This enables the cell to maintain the intracellular ionic concentrations very much different to the extracellular ones. In general, potassium has about 30-fold higher intracellular concentration than extracellular, while sodium, chlorine and calcium are more concentrated outside. Nevertheless, every cell has to have a pathway for ionic flow through the membrane in order to maintain proper physiological functioning. This is provided by ion channels[1,2]. As the direction of the ion flow through the channel is down the electrochemical gradient, the process is often refered to as a passive transport. Since the net flow of electric charges gives rise to a rapid change in transmembrane potential, ion channels play a key role in generating and propagating action potentials in the nervous system. Moreover, it is well established that ion channels play an important role in the pathophysiology of various diseases and thereby present the primary targets for pharmacological drug design.

In order to control transmembrane ionic flow, ion channels have to be i) highly selective towards specific ion type, and ii) have to have well defined gating control mechanism by, e.g., ligands or mechanical stress. Since the rate of ion transport ranges from 10^6 to 10^9 ions/s, a simple calculation indicates that this rapid transport is almost at the diffusion rate. This fact indicates that an open channel represents an almost barrierless pathway for selected ion flow. This phenomenon is not well understood and a complete physical theory is still not yet available.

At present, no single computational technique can describe all the functional properties of an ion channel. The choice of the level for the theoretical approach and the use of the computational technique to describe processes of selectivity, permeation and gating of an ion channel, depend essentially on the timescale of the process itself. While the timescale of the permeation process for typical channels is in the order of few hundreds of ns, the gating takes time in the order of ms. At the present state of computational speed neither

of the processes can be completed using MD techniques. On the other hand, Brownian dynamics (BD) simulations have provided usefull data on the total permeation process of ions through channels[19], but their reliability concerning details is limited due to the coarse-graining of the channel structure and applications of mean-field approximations of water and lipids. Nevertheless, based on highly resolved structures of the bacterial potassium channel KcsA[3,20–23], MD simulations[24–30] have provided valuable insights into dynamical details of the ion channel.

Structure

In order to support the reliability of MD simulations of a full all-atom model of an ion channel (including channel, water and lipid bilayer), it is important to test the stability of the simulated structure with respect to the crystallographically determined one. It has been shown that the simulated KcsA channel structure[26,28,29] is still very similar to the original structure. The r.m.s. deviation of the C_α atoms between the crystal structure and the simulated structure is about 3.7 Å [29], which is comparable to the crystal structure of 3.2 Å resolution[3]. Since the potassium ions, which are localized in the selectivity filter, represent an integral part of the crystal structure, it is of interest to compare their possible locations as predicted by X-ray analysis[3,21] to simulation findings. Based on the most recently published structure[21], it is conjectured that the K^+ ions may occupy seven different locations : 4 inside the selectivity filter, one in the cavity and 2 ions weakly bound to the extracellular mouth. MD simulations, however,[26,28,29] indicate that only one or at most two ions may occupy the filter simultaneously. Some MD results report on only one ion in the filter, where second ion exits the filter either to the intracellular side[26] or to the extracellular side[29]. The MD simulations predict an average number of water molecules within the cavity of about 21[29], which is enough to solvate the cavity ion, but which is in contrast to the conjecture of 50 water molecules based on solving the finite difference Poisson equation[31]. However, the latter result has to be viewed in the light of the recently reported shortcomings of the Poisson-Boltzmann theory applied to inhomogeneous systems[32].

Permeation and Selectivity

The goal of modelling the permeation process is to understand the physical processes underlying the permeation of ions through the channel, with the ability to reproduce present and predict future experimental observations. The time scale of permeation is too long to enable reproducing the experimental data on the process using MD calculations, so the modelling which attempts to reproduce experimental data usually employs more coarse-grained methods like continuum electrostatics and/or BD simulations. Nevertheless, among the computational methodologies used on describing the permeation process, only molecular dynamics has the advantage of providing the data on the molecular kinetic details at the atomic resolution. So far, none of the simulation studies with explicit lipid environment was able to complete the permeation process for the KcsA channel.

Several theoretical studies have addressed the multi-ion transport mechanism of permeation where several ions are located at the same time in cavity and selectivity filter. Unfortunately, standard MD simulations, usually restricted to phenomena of a few nanosecond time scale, are not suitable to reproduce ion conduction taking place at tenths or hundreds of nanoseconds. However, applying an “alchemical” method[29] where water molecules and ions can exchange their places, the whole permeation process of a single ion can be mon-

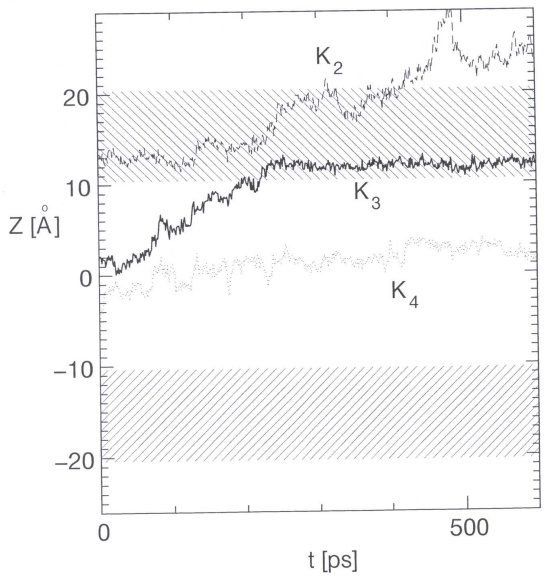

Figure 4. Z-component of trajectories of three potassium ions as function of time. The ions K4 and K3 are initially located in the cavity within $-5 < z < 7$, the ion K2 in the slectivity filter at $7 < z < 20$. The shaded regions represent the head group regions of the POPC bilayer.

itored. The alchemical method can be considered as a variant of the "particle insertion" method, well known in computational physics[33]. Fig.4 shows a typical example of the z-component of trajectories of three potassium ions as a function of time. The ions K4 and K3 are initially located in the cavity, which is in the range $-5 < z < 7$, and the ion K2 in the selectivity filter at $7 < z < 20$. From the various positions of the ions one can conclude that the permeation process can be described by a "supplanting" process where the electrostatic repulsion between K3 and K4 leads to an expulsion of K3 into the selectivity filter, where it supplants the ion K2, which itself dissociates from the filter and moves into the extracellular mouth of the channel.

The interaction energy at various positions along the axis of the channel (energy profile), give some indication for favorable positions of the ion. Energetic considerations for the KcsA channel have been reported previously[31,24,34] using free energy perturbation calculation. The complete energy profile including the contribution of a fully hydrated lipid bilayer membrane along the whole channel axis can also be calculated from MD simulations[29] using the non-equilibrium approach of steered molecular dynamics (SMD).

CNG Channels[30]

Cyclic nucleotide-gated channels play a central role in both visual and olfactory sensory transduction. They exist in vertebrate rod and cone photorecepters and olfactory neurons. In the visual system, Na^+ and Ca^{2+} flow into the rod outer segment through CNG channels in darkness. Light absorption by the visual pigment trigger a chain of enzymatic reactions and decrease the concentration of a cyclic nucleotide, the cyclic guanosine monophosphate

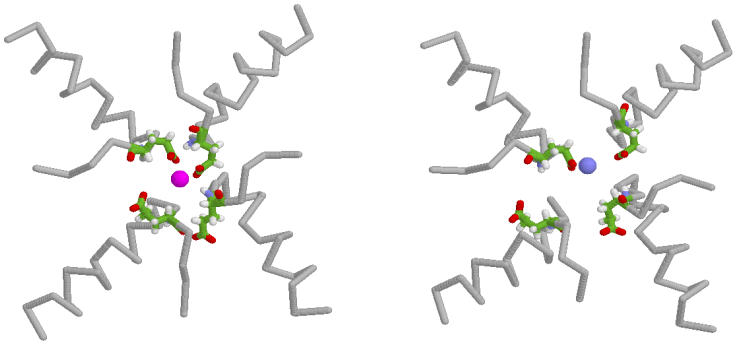

Figure 5. Ions in the selectivity filter viewed from the extracellular side: Ca^{2+} (left), Na^{+} (right). The side chain of the four glutamates (green) are also shown.

(cGMP), and so closes the channels. The closing of the CNG channels causes a hyperpolarization of the cell and triggers neural excitation[35, 2, 36].

The amino acid sequences of CNG channels from several species have been determined by cDNA cloning. The sequence homology analysis shows that the pore part of CNG channels present structural similarity to *Shaker*-like potassium channels[37]. Contrary to the high selectivity of potassium channels, however, CNG channels are non-selective between alkali monovalent cations, and are permeant to both monovalent and divalent cations[38–40]. The permeability sequence of alkali monovalent cations is different from the ion mobilities in water. And when both monovalent and divalent cations are presented, the current flow of monovalent cations is blocked. It indicates that ions interact with a binding site in the channel pore during the permeation process, and divalent cations have higher affinity to the binding site than monovalent cations.

Experiments show that the permeation properties of a potassium channel can be altered by mutating certain residues. The mutant channel displays only little selectivity between monovalent cations, i.e. it turns to be a CNG-like channel[41]. Based on this fact, we take the KcsA channel (PDB file 1BL8) as a template and construct a homology model of CNG channels by mutating a few residues in the selectivity filter. Molecular dynamics simulations of the model CNG channels with different cations are conducted. Experiments show that the four glutamates in the extracellular vestibule are responsible for the binding effect of the channels. Our simulation shows flexibility of the glutamate side-chains. The orientation of the side-chains are adjusted according to the ions presented. Fig. 5 shows the interaction of the four glutamates with Ca^{2+} and Na^{+}. The Ca^{2+} is stably bind to two glutamates of neighboring subunits during the simulation, while the Na^{+} moves between different glutamate side-chains and fluctuation of glutamate side-chains are also higher.

Gating

Ion channels regulate the selective transfer of ions across the membrane in response to different types of stimuli, as e.g. changes of pH, transmembrane potential, mechanical stress or ligand binding. A channel can generally assume two stabile conformations, the

open and the closed one, accompanied by conformational changes in the protein ("gating"). The structural and dynamical details of the gating mechanism are the least known properties of ion channels, mostly because of the fact that an opened state of the channel is a transient one, thus not easily fixed to be isolated by cristallization. Structural details of a gating mechanism for the KcsA channel were suggested by Perozo and coworkers[42–44] base on experiments. Results from these experiments indicate that the channel undergoes a "twisted" motion where each of the four inner helices o the KcsA channel tilts away from the symmetry axis of the channel. However, the origin of the pH-mediated driving force is still unclear. It has been conjectured[30] that the four long cytoplasmic chains of the channel may play the crucial role, because some of the charged residues may change their protonation state during pH variation and hence provide the necessary variation in their inter-chain Coulomb interaction. Unfortunately the gating mechanism cannot be studied by standard MD simulation because the time scale of gating is in the order of at least microseconds. Therefore, the simulation of the channel gating is still one of the "grand challenges" in biophysics.

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