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Molecular dynamics simulations of proteins in lipid bilayers

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Abstract

With recent advances in X-ray crystallography of membrane proteins promising many new high-resolution structures, MD simulations become increasingly valuable for understanding membrane protein function, as they can unleash dynamic behavior concealed in the static structures. Dramatic increase in computational power in synergy with more efficient computational methodologies allows one today to carry out molecular dynamics simulations of any structurally known membrane protein in its native environment, covering the time scale of up to 0.1 μ sec. At the frontier of membrane protein simulations are ion channels, aquaporins, passive and active transporters, and bioenergetic proteins. In this review we summarize recent developments in this rapidly evolving field.

Keywords

membrane proteins; channels; molecular dynamics

Introduction

The successful solution of membrane protein structures permits molecular dynamics (MD) investigations elucidating the physical mechanisms behind manifold processes associated with cellular membranes. The membrane environment through electrostatic and steric interactions as well as through the membranes' internal pressure influence the functions of membrane proteins and, therefore, the environment needs to be properly taken into account in MD studies. The resulting calculations incorporating proteins, lipid bilayer, water, and ions need to cover between 50,000 atoms for the smallest proteins and up to 300,000 atoms for the largest one yet studied. The large simulation volume poses a major computational challenge, yet computational biologists have succeeded recently in carrying out the required calculations and have been rewarded with discoveries and insights into our understanding of membrane processes. Here we review selectively the reported investigations, focusing largely only on MD simulations that describe integral membrane proteins in realistic lipid bilayers. Figure 1 compares some of the membrane proteins covered in the simulations reviewed, two single-channel proteins (bacteriorhodopsin and KcsA), two multi-channel proteins (aquaporin and OmpF) as well as a large multimeric channel, MscS. Presently, the structure-function relationship of membrane proteins is not well understood and there are great opportunities for new fundamental insights. It is likely that MD simulations will play a great role in realizing this potential. Indeed, the examples presented below reveal already significant successes.

Ion Channels

Ion channels present a unique and difficult challenge for molecular dynamics. While appearing simpler than channels transporting small solutes, they are in some ways more complicated because of the very precise electrostatic interactions required between ion, protein, and solvent. This highlights the need for exact force field parameters in order to produce accurate results. As in many MD simulations, the difference in the timescale available to simulations as compared to that needed to calculate experimentally-measurable properties can also present a problem. However, great advancement has been made in the last few years in circumventing or overcoming these issues.

The potassium or K^+ channel has been at the forefront of ion channel simulations, with the goal to understand the energetics of ion transport as well as channel selectivity and gating mechanisms. The effects of different singly-ionized alkali metals (Na^+ , K^+ , Rb^+ , and Cs^+) on the pore structure have been studied, demonstrating flexibility of the pore [1]. In fact, this flexibility has been proposed to play a role in the selectivity, perhaps the most impressive property of the channel; the potassium channel can conduct K^+ at a rate thousands of times greater than that of the smaller (by .38 Å) sodium (Na^+) ion [2]. It has been suggested that this selectivity arises from the coordination of ions into select energy minima by the carbonyl groups of backbone residues lining the pore; it was found that fluctuations of the pore do not destroy selectivity but rather keep it by allowing the channel to relax to its optimal conformation [2]. K^+ channels can also alternate between conducting and non-conducting states, controlled by various external influences. The structure of the voltage-gated K^+ channel, KvAP, contains segments referred to as “paddles” which are known to regulate the channel’s permeability [3]. These paddles were rotated in a 10 ns steered MD simulation demonstrating a potential coupling mechanism between chloride ions and conserved arginine residues not in the pore itself [3]. Another more general gating mechanism has been observed through simulations totaling 50 ns of a K^+ channel which revealed a correlation between ion concentration and the pore’s flexibility [4]. The role of the selectivity filter in gating was recently explored demonstrating that the orientation of two peptide linkages affects ion permeability [5]. Targeted MD studies have led to the proposal that the conformational change in the filter between the closed and open states proceeds in a “ziplike” fashion [6].

Chloride (Cl^- , specifically ClC in these studies) channels are an anion counterpart to K^+ channels. However, they do not display the same remarkable specificity as K^+ channels (likely due to a physiological lack of other anions in significant concentrations) [7]. A mechanism of ion permeation similar to that of K^+ channels was proposed where ions move between key energy minima within the channel, confirmed by the calculations of the potential of mean force [7]. Through this, it was also elucidated that permeation most likely occurs by a “king-of-the-hill” mechanism where an ion only moves from the central energy minimum in the presence of another ion [7]. The gating mechanism of the channel has been explored in MD simulations showing that the protonation of a highly conserved residue (Glu148) near the entrance of the channel is crucial to Cl^- conduction [8].

Gramicidin A (gA) channels are among the simplest and as such, they represent a paradigm for testing ion-channel modeling. The potential of mean force both radially and axially was calculated for K^+ ions inside gA with 100 ns of MD, allowing extension to longer timescales using less-detailed methods [9]. From this, it was confirmed that a single-file of water molecules plays a crucial role in ion conduction, both in lowering the energy barrier as well as in influencing the rate of permeation due to the orientation of the water molecules [9]. However, more recent calculations of channel energetics suggest that the flexibility of the protein can have a great effect on the potential of mean force inside the channel [10].

In the case of an acetylcholine receptor (a ligand-gated ion channel), partial gating was seen to occur through the arrangement of side-chains in 35 ns of simulation [11]. MD has also been used to investigate changes in conformations of this channel leading to the proposal of an asymmetric gating mechanism [12].

Other Selective Channels

In addition to ions, living organisms have evolved channels that provide selective pathways for passive permeation of other substrates. It has been suggested that cells have selective channels for permeation of certain nutrient molecules, such as glycerol, gas molecules, and even water. The best known family of such channels are membrane water channels known as aquaporins (AQPs). The solved structures of several AQPs at high resolution are indicative of a conserved protein architecture in the whole family. All members of the family form homotetramers in the membrane in which four functionally independent pores provide highly selective, yet efficient pathways for water permeation across the low dielectric barrier of lipid bilayers. Some AQPs have the additional capability of conducting small neutral molecules, such as glycerol, and are, therefore, called aquaglyceroporins. The most prominent member of the latter family is the *E. coli* glycerol uptake facilitator GlpF.

The availability of high resolution structures of AQPs and their uncomplicated biological function, i.e., acting as passive pores for permeation of small substrates, has made them an ideal application for molecular dynamics simulations. In fact, no other family of membrane proteins has been studied as extensively as AQPs. Computational studies have contributed significantly to our current understanding of the mechanism of substrate permeation and selectivity in these channels.

The first simulations performed on AQPs investigated the mechanism and dynamics of glycerol [13] and water permeation [14,15]. By pulling glycerol through the GlpF channel, Jensen et al. [13] calculated the associated free energy profile, providing a quantitative picture of the glycerol permeation event.

Water permeation through AQP1 and GlpF was investigated using 10 ns MD simulations of tetrameric models of the proteins in membrane [14]. Given the natural time scale of water permeation, the simulations could capture several full permeation events of water, which were used to calculate kinetic properties of the event without the need to apply external forces.

Similar simulations on both wild-type and mutant forms of tetrameric GlpF in membrane succeeded in simulating diffusive water permeation through these channels, but also paid more attention to the mechanism of proton blocking in these channels, and proposed a unique configuration of water molecules as a novel mechanism of proton exclusion [15]. Further computational studies using different methodologies elaborated on the details of the proposed mechanism [16,17,18,19]. The claim in [20] that proton exclusion is independent of electrostatic interactions, and hence, water orientation, is based on the comparison of a full electrostatics and a zero electrostatics simulation; the lack of realism of the latter simulation makes the claim questionable.

The resolution of the available X-ray structures turned out to be very important in calculating the right permeation rate for water in these channels. Earlier simulations of AQP1 in a membrane using a lower resolution structure (3.8 Å) of the channel had found the conformation of critical amino acids lining the pore to be unstable and, thus, failed to correctly describe the permeation speed [21]. Law et al. [22] conducted a computational experiment in which they compared the stability of various experimental and homology models of AQP1 using 37 ns of MD simulation. In agreement with other computational studies [14,21] the authors concluded that the low resolution structure of AQP1 did not provide an accurate enough model for full

atomic simulations. Interestingly, the homology model of AQP1, which was constructed using the X-ray structure of GlpF, was found to be able to provide a better description of water inside the channel, thus indicating that a similar approach may be taken to model and simulate AQPs for which crystallographic structures have not yet been solved.

Most simulations studying permeation properties of AQPs have modeled the channel in its tetrameric form, which seems to be the functional form of the channel. In order to reduce the computational cost of the simulations, which would allow one to run longer simulations, some researchers studied the protein in its monomeric form. A 10 ns simulation of monomeric GlpF in an octyl glucoside micelle environment found a larger degree of fluctuation for the channel [23]. Longer simulations of monomeric GlpF in a lipid bilayer (unpublished results) found an unstable water file in the channel. However, for short simulations, or in combination with constraints applied to remote regions of the protein, the monomeric form provides a very efficient model for studying various aspects of permeability and selectivity in AQPs. The selectivity of GlpF for linear sugar molecules was investigated in such models using interactive molecular dynamics simulations [24]. The study applied interactive forces to guide different stereoisomers of a 5-carbon sugar, ribitol and arabitol, through the pore and found that a combination of size restriction and the number and strengths of multiple hydrogen bonds can explain the selectivity of GlpF for ribitol. In another study, the free energy profile associated with water permeation through AQPs was studied in a monomeric model [25].

Water permeation through most AQPs is a very fast phenomenon and can be observed on nanosecond time-scales in MD simulations under equilibrium conditions. While such simulations allow one to calculate equilibrium properties of water channels, most experimental results for water permeation through AQPs have been obtained under osmotic pressure conditions, i.e., nonequilibrium conditions. In order to simulate the channel under similar conditions, a novel computational method was developed [26,27]. The method takes advantage of applying small forces to bulk water molecules along the membrane normal to generate a hydrostatic pressure gradient across the membrane, thus changing the chemical potential of water on the two sides of the membrane. Under such conditions, net flow of water across the membrane is observed. Using this method the pressure difference between the two sides of the membrane can be readily changed. Applying different pressure gradients, the osmotic permeability of GlpF [26] and AQP1 [27] has been calculated and found to be in good agreement with experimentally measured values.

Along with the discovery of novel functions for AQPs, and the availability of more structures for the members of the family, one has begun to understand the physical mechanisms of specific functions of these channels. A recent comparative study, for instance, investigated the energetics associated with the permeation of glycerol through two structurally highly homologous, but functionally different AQPs from *E. coli* [28]. A membrane-embedded model of tetrameric AqpZ, which is a pure water channel, was used to examine the energetics associated with artificially induced permeation of glycerol. Comparison of the results with similar calculations for GlpF [13] clearly shows that there are much larger barriers against the permeation of glycerol in AqpZ, which is expected, as it is a pure water channel. Examination of the substrate at the positions of barriers show that the energy barriers against glycerol permeation are mainly steric in nature, due to a much narrower pore of AqpZ along the whole channel. The study suggests that the size of the pore is the primary mechanism determining whether or not an AQP can function as a glycerol channel [28].

Non-Selective Channels and Outer Membrane Proteins

Non-selective membrane channels facilitate passive permeation of ions and other small solutes through lipid bilayers, selecting for permeation only those solutes that fit geometrically into

the channel's pore. Although referred to here as non-selective, most of the channels in this class exhibit minor to moderate selectivity to either cations or anions. Three types of non-selective channels are discussed below: mechano-sensitive (MS) channels, pore-forming toxins, and outer membrane porins. Other β -barrel outer membrane proteins are discussed at the end of this section.

MS channels transform mechanical signals into electrical current by changing their conductance in response to mechanical stress. According to their maximum conductance, three types of MS channels have been identified: MscL, MscS and MscT, corresponding to MS channels of Large, Small and Tiny conductance. The structure of the first two channels has been determined to atomic resolution. In search of the molecular mechanism of mechano-transduction, MD simulations determined the pressure profile across the lipid bilayer, with and without a lateral tension applied [29]. Lipids were found to shift the lateral pressure to the head groups; stretching or shrinking of the lipid bilayer increases dramatically the pressure in the same region. Derived from the pressure profile, lateral forces were applied to Eco-MscL (MscL from *E. coli*), driving the channel into a fully expanded state within about 10 ns [30]. The expanded state was found to agree well with a previously proposed model of MscL gating, in which the iris like expansion of the pore is accompanied by tilting of the transmembrane helices. Steered molecular dynamics simulations [31] provided a detailed account of the initial stages of the Tb-MscL opening, that involved tilting of the helices followed by the enlargement of the pore, allowing water to penetrate through. The lipid composition was found to affect the MscL structure [32], supporting the hypothesis about the hydrophobic matching between MscL and the lipid membrane.

The X-ray structure of the MscS channel revealed a ~ 7 Å transmembrane pore. MD simulations addressed stability of the X-ray structure and investigated the permeability of the pore for water and ions. When the conformation of the channel is restrained to the X-ray structure coordinates, water cannot form a stable continuous passage through the transmembrane pore, creating a large energy barrier for the ions [33]. When the channel is not restrained to the X-ray structure, it undergoes a conformational change to a closed state [34]. Applying a lateral tension to the lipid bilayer was found to reverse the channel closure. Both studies suggest that the X-ray structure captured the MscS channel in a closed state.

α -hemolysin of *Staphylococcus aureus* is a 232.4-kDa toxin that self-assembles from seven soluble monomers into a water-filled transmembrane channel, shown in Fig. 2a. The structure of α -hemolysin was determined in 1996, but only recently was the first all-atom MD simulation of the channel in a lipid environment reported [35]. This study provided a milestone in the development of the computational technology, as for the first time the current/voltage dependence of a membrane channel was computed directly, starting from the X-ray structure. Figure 2 illustrates procedures involved in a calculation of a current/voltage dependence. The reported values of the simulated currents, of the osmotic permeability for water, and of the electroosmotic effects are in excellent agreement with experiment. The study also pioneered the computation of the average electrostatic potential from MD trajectories, providing the first images of the electrostatic potential distribution in a membrane channel, like the one shown in Fig. 2b.

Outer membrane proteins (OMP) are found at the outer membrane of many procaryotic organisms and in certain organelles of eucaryotic cells. Most of them have β -barrel architecture. The most common function of β -barrel OMPs is to provide a pathway for intake of nutrients or disposal of waste, although these similar (by architecture) proteins can also function as enzymes, active transporters, or pathogenic recognition agents. Over twenty structures of OMPs are known. The most studied one is OmpF, the cation-selective matrix porin forming a trimer of 16-stranded β -barrel pores. MD simulations investigated distribution and passage of

K⁺ and Cl⁻ ions [36], permeation of small dipolar molecules (alanine and methylglucose) [37], and of antibiotics (ampicillin) [38].

OmpA is one of the most abundant outer membrane protein in *E. coli*. Its membrane spanning domain has a relatively simple structure of an eight-stranded anti-parallel β -barrel. The X-ray structure did not reveal a continuous water-filled pore, adding controversy as several experiment groups had recorded ionic currents with reconstituted OmpA. MD simulations were performed to find out whether or not OmpA can form a pore in a lipid bilayer [39] and in a micelle environment [40]. Although the structural fluctuations observed in the simulations might lead to a putative open form of the channel, it is yet to be established if such a form exists.

OmpLA is a 12-stranded β -barrel outer membrane enzyme that catalyzes the splitting of a phospholipid by the addition of water. The enzyme activation requires calcium-induced dimerisation as well as a perturbation of the bilayer. As the X-ray structures of the enzyme in active and inactive form appear very similar, MD simulations probed dynamics of the enzyme in both forms [41] revealing a more stable conformation of the binding site in the active (dimeric) form. Another outer membrane enzyme is endoprotease OmpT that cleaves two consecutive basic amino acids. Its 10-beta stranded membrane part, located below the binding site, was found to contain water in the X-ray structure. MD simulations [42] investigated dynamics of water in the β -barrel, indicating that, in the state captured by the X-ray structure, water can exchange between the intracellular face of the membrane but not with the extracellular mouth. The simulations supported the current catalytic model of OmpT.

FhuA is a 22-beta stranded siderophore receptor that upon binding of the substrate facilitated the energy-dependent transport of the substrate across the outer membrane. The X-ray structure of the transporter with and without a bound substrate (ferrichrome) provided some insights into the initial stage of the ferrichrome uptake but did not reveal how the substrate is transported inside the cell. MD addressed [43] the conformational stability of the protein, revealing the lack of a continuous water passage through which the ferrichrome could permeate without significantly disrupting the structure.

Membrane Proteins for Bioenergetics and Vision

Cellular energy is largely stored and used in the form of a proton gradient across cellular membranes by membrane proteins. The most prominent protein of this type is F₁F₀-ATP synthase that converts the membrane potential into chemical energy stored in ATP. The ATP synthases link a mechano-chemical motor, the F₁ sector, to an electro-mechanical motor, the F₀ sector. F₁ couples the reaction ADP + phosphate $\leftrightarrow$ ATP to a mechanical torque acting on one of its rotating components, the stalk; F₀ converts a proton gradient into torque acting on a rotating complex of ten or more c-subunits. F₀, the rotor ring, is located inside the membrane while F₁ is located outside, the connection between F₀ and F₁ furnished by the F₁ stalk. The F₁F₀-ATP synthases contain furthermore an a-subunit, closely associated with F₀, that jointly with the rotor ring controls the trans-membrane proton current; it also contains b-subunits acting as a stator preventing overall rotation of F₁. The structure of the membrane embedded rotor ring of proton-driven ATP-synthases is not yet available, though structures of the closely related rotor ring of F-type Na⁺-ATPase as well as from the more distantly related V-type Na⁺-ATPase have recently been solved [44,45]. However, Aksimentiev et al. [46] constructed a model of the rotor ring - a subunit complex of F₁F₀-ATP synthase from available structural data and embedded it in a lipid bilayer. The simulation's key results reported have not lost their relevance through the availability of the more recent related crystallographic structures. Aksimentiev et al. demonstrated that application of torque to the F₀ rotor composed of ten c-subunits induces rotation without loss of structural stability. They also demonstrated that a

suggested (based on extensive cross-linking experiments) rotation of the c₂ (outer) transmembrane helices of the c-subunits is entirely feasible physically. Indeed, the authors could relate simulations to a mathematical model for torque generation in the rotor ring + a-subunit system through deprotonation-protonation of Asp61 of the c-subunits, c₂ helix rotation, and rotor rotation, all processes regulated through Arg210 of the a-subunit. Puzzling at present is that the angular orientation of the c₂-helix of the rotor ring suggested by cross-link data and modeling is at variance with the orientation observed in [44] and may turn out to be wrong, but a lasting result of Aksimentiev et al. is the demonstrated success in deriving a model of ATP synthase function from atomic level dynamics rather than from *ad hoc* assumptions.

Harvesting sun light and utilizing it for the generation of a membrane potential and provision of high-potential electrons are prototypical bioenergetic processes located at cellular membranes. Light harvesting proteins contain assemblies of chlorophylls and carotenoids that absorb sun light and transfer it efficiently to the photosynthetic reaction center. One such protein, light harvesting complex LH-II of *Rhodospirillum rubrum* was simulated in a lipid bilayer [47]. The goal of the simulation was to describe in a rigorous physical model light absorption in a dynamically disordered ensemble of chlorophylls. At the reaction center, the light energy drives a series of electron transfer reactions that reduce a quinone group to quinol, leaving the reaction center oxidized. The quinol, released from the reaction center, diffuses to a bc₁ complex where it is converted back to quinone while generating a proton gradient and releasing its electrons to cytochrome c₂. The reduced cytochrome c₂ scoots to the reaction center and reduces it, readying it for another round of electron transfers. Authenrieth et al. [48] simulated the docking of cytochrome c₂ to the photosynthetic reaction center of *Rhodobacter sphaeroides* embedded in a lipid bilayer. The simulation established the interactions guiding the overall docking/undocking processes and revealed a key role of ordered interfacial water molecules in the electron transfer between the two proteins.

The archaeal membrane protein bacteriorhodopsin (bR), acting as a proton pump, converts light excitation directly into a proton gradient. With high resolution crystallographic structures available for the protein in many of its functional states, bR offers great opportunities for molecular dynamics simulations. Complicating factors are, however, that photo-reactions, hydrogen-bonded networks, and proton transfer reactions require actual quantum chemical calculations that are extremely challenging; furthermore, the light-driven proton pump cycle stretches over milliseconds that cannot be covered in simulations. Hence, despite the relatively small size of the protein and its apparently simple function, bR poses extreme modeling demands.

Jang et al. [49] embedded bR in a lipid bilayer in its dark-adapted and its M state. Carrying out equilibrium simulations, they monitored the proteins flexibility, helix tilts, interaction energies and water distribution deriving suggestions for a part of the physical mechanism underlying bR's proton pump cycle. Likewise, Grudinin et al. [50] carried out molecular dynamics simulations of trimeric bR in its so-called ground and its M state, both placed in a lipid bilayer. However, their ground state calculation deviated significantly in regard to water exchange between protein interior and bulk from an earlier simulation by Baudry et al. that painstakingly had simulated the trimer in the native lipid environment of the so-called purple membrane [51]. Grudinin et al. reported differences in water distribution and dynamics, in particular, also a difference between modeled proton conductivities inside bR in its ground and M state. The subject of water dynamics and the proton conduction pathway was investigated also by Kandt et al. in two publications on trimeric bR and N-state bR in a POPC bilayer [52,53]. The authors deduced from their simulations a suggestion for the entire proton conduction pathway in bR, leaving still unsolved the question of how the photo-reaction of retinal in bR is coupled to the vectorial proton transport. This key question, addressed by Onufriev et al. through a static calculation of pK_a values for M and N state bR embedded in a membrane [54], needs further

investigation. The findings in [50,52,53] suggest that the question might be answered eventually through a combination of modeling and observation, but only if the role of the membrane environment is taken into account. A very novel opportunity for the study of bR has been suggested in the experimental-computational study of Shih et al. [55] that placed bR into a discoidal bilayer assembled from truncated lipoprotein. The study employed dipalmitoylphosphatidylcholine forming around bR a disk of 40 Å radius surrounded in a belt-like fashion by lipoprotein. This study demonstrates the convergence of experiment and theory in membrane protein simulations today.

Bacteriorhodopsin is closely related to the visual receptor protein rhodopsin (Rh). After the structure of Rh was solved, the protein became quickly the target of *in situ* molecular dynamics simulations [56,57] that addressed the activation of Rh after its photoreaction. Recent *in situ* molecular dynamics simulations of Rh have taken a step back and focused on Rh in its dark-adapted state. Crozier et al. [58] embedded Rh in a bilayer of DOPC, while Huber et al. [59] embedded Rh into POPC, both author groups carrying out extensive simulations. The authors reported the ensuing flexibility of Rh, the packing of Rh's retinal chromophore in its binding pocket, interaction of retinal with the protein matrix, large scale motions, hydrogen-bond networks, as well as protein contact surfaces and interactions with lipid and water. The main challenge for future investigations remains, namely, to understand how the 11-cis to all-trans photoisomerization of retinal activates the protein and to elucidate the protein conformational changes that lead to the binding of transducin. Simulation has an opportunity to get ahead of crystallography; however, it faces the challenge that the time scale of the transformation is very long (μs to ms). The respective studies should definitely follow in the footsteps of [56, 57,58,59] and account for the effect of a lipid bilayer.

Conclusion

As expressed in the introduction, *in situ* MD simulations of membrane proteins have lived up to the opportunities that offer themselves today when structure analysis permits for the first time detailed glimpses into a molecular world that had been hidden before. Modeling can add tremendous value to newly resolved structures. An example is the mechanosensitive channel MscS: crystallography revealed an open channel, but MD simulation makes it more likely that the structure seen is actually only partially open, as it closes spontaneously when lifted from the crystal context to a lipid bilayer environment [33,34]. The maturity of MD simulations of membrane proteins can be seen in the case of α-hemolysin, one of the experimentally most investigated membrane channels. MD simulations are today capable of sufficient sampling and reproduce accurately observed electrical properties [35]. The accuracy is so impressive that one may want to consider MD simulations as an imaging tool that provides a view into a spatial nanoscale not covered by known imaging methods. Lastly, MD simulations can address physical principles underlying key membrane processes, for example the puzzle connected with channel high selectivity: how can soft and strongly fluctuating systems like proteins be highly selective? The surprising answers given by MD investigations of both aquaporin and KcsA provide the foundation of a new science of biomolecular devices that will deepen our understanding of living systems and will guide our development of biotechnological nanodevices.

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Figure 1.

Some membrane proteins that have been recently studied by molecular dynamics, presented against a lipid bilayer background. From left to right: bacteriorhodopsin, the K^+ channel KcsA, the aquaporin GlpF, the porin OmpF, and the mechanosensitive channel MscS.

Figure 2.

Computing the current/voltage dependence of a membrane channel with all-atom molecular dynamics. **(a)** A microscopic model of a membrane channel is constructed. **(b)** In an MD simulation, a transmembrane potential is generated by applying an external electric field. **(c)** The current is computed by tracing local displacements of the ions. Repeating the simulation at different applied fields yields the current/voltage dependence.