Published in final edited form as:

Curr Opin Struct Biol. 2009 April ; 19(2): 128–137. doi:10.1016/j.sbi.2009.02.011.

Molecular dynamics simulations of membrane channels and transporters

Fatemeh Khalili-Araghi^{1,4}, James Gumbart^{1,4}, Po-Chao Wen^{3,4}, Marcos Sotomayor⁵, Emad Tajkhorshid^{2,3,4}, and Klaus Schulten^{1,3,4,*}

¹ Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801

² Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801

³ Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

⁴ Beckman Institute for Advanced Studies, University of Illinois at Urbana-Champaign, IL 61801

⁵ Howard Hughes Medical Institute and Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Abstract

Membrane transport constitutes one of the most fundamental processes in all living cells with proteins as major players. Proteins as channels provide highly selective diffusive pathways gated by environmental factors, and as transporters furnish directed, energetically uphill transport consuming energy. X-ray crystallography of channels and transporters furnishes a rapidly growing number of atomic resolution structures, permitting molecular dynamics (MD) simulations to reveal the physical mechanisms underlying channel and transporter function. Ever increasing computational power today permits simulations stretching up to 1 μ sec, i.e., to physiologically relevant time scales. Membrane protein simulations presently focus on ion channels, on aquaporins, on protein-conducting channels, as well as on various transporters. In this review we summarize recent developments in this rapidly evolving field.

Introduction

Molecular dynamics (MD) simulations have become a key research tool in membrane biology, a tool that complements physiological experiments and structural analysis. While until recently membrane biology suffered from lack of structural information, one finds often today that both physiological and structural data are available, but that the mechanisms underlying the selectivity and gating of membrane channels or the coupling of energy sources to transport nevertheless remain unknown. For elucidating these mechanisms no method seems to be more suitable than MD simulation, the reason being simply that the functions of channels and transporters involve dynamic processes that cannot be captured by any experimental method today. Clearly, MD simulations suffer from shortcomings, a short (today microsecond) time scale and inaccuracies in the force field describing physical interactions. However, simulations have evolved dramatically in regard to size scale (they can describe today individual membrane

* To whom correspondence should be addressed; Email: E-mail: kschulte@ks.uiuc.edu; Phone: 217 244-1604; Fax: 217-244-6078.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

proteins in their native environment), ability to image mean electrostatic potentials, and proven value of their contributions (many recent discoveries in the field are due to simulations). Indeed, the vital role of molecular modeling in membrane biology is reflected in numerous studies and discoveries reported during the past few years in the fields of membrane channels and transporters. Another review described recently advances in coarse-grained simulations of membrane proteins as well as protein-membrane interactions (1); here we focus on all-atom MD simulations of membrane channels and transporters reported in the past two years.

Membrane Channels

Membrane channels control the influx and efflux of materials across cellular membranes through high selectivity combined with high conductivity and through gating that is sensitive to essential environmental factors. Channels furnish optimal selectivity, conductivity, and sensitivity, but do not use energy to advance motion across a membrane, i.e., motion is energetically “down-hill”. Since channels usually, but by no means always, engage only in small motions during conduction events, that often happen over time scales shorter than microseconds, these events are rather readily studied by MD methods. Over the last two years significant simulation studies have been reported on the seven channels depicted in Fig. 1. The channels differ widely in architecture and function, ranging from highly selective ion channels (e.g. Kv1.2) to less selective channels (e.g. nAChR and ASIC1) and to channels (Sec Y) that conduct entire proteins, yet in all cases molecular modeling furnished great insights into the various channel mechanisms.

Aquaporins

Aquaporins, providing or “acting as” membrane channels for water, small linear alcohols, and gases proved to be ideal objects of investigation for molecular modeling. On the one hand, the channels are mechanically rather inert and their function, e.g., water translocation, is fast enough for simulation; on the other hand, the conduction mechanisms, in regard to observed selectivities and gating, pose challenges that can best be met by a combination of simulation and experiment, but not by experiment alone. Reported simulations date back seven years ago, i.e., to the time when the first well resolved structures were reported. Today simulations of aquaporins and related channels are commonplace and indeed important discoveries have been published again during the past two years.

Simulations had shown that water transport through aquaporins involves concerted motion of several (~ ten) water molecules aligned often in a single file, the concertedness regarding orientation and translocation. This behavior was illustrated qualitatively or introduced schematically. A recent publication (2) furnished an elegant, concise description of concerted transport. Extending a general statistical mechanical framework (3) for water conduction in channels, the authors expressed the osmotic permeability of water through a diffusion tensor that relates water motion in all channel segments. The treatment reminds one of the correlation matrix analysis method for protein motion. Interestingly, channels that sport the same water permeability are found to differ in regard to the new correlation analysis. Investigated were several aquaporins along with synthetic carbon nanotubes that also conduct water single file.

The wide selectivity of aquaporin made it a favored subject of free energy calculations, which characterize the pathway of solute through channels, utilizing steered MD (4), combinations of steered MD and quantum chemistry calculations (5), implicit ligand sampling (6), the somewhat dated umbrella sampling (7), and the more recent adaptive biasing force sampling (8). The latter method showed that glycerol may display different behavior when pulled through the channel as in (4) than when left to move freely. While the steered MD in (4) confirmed crystallographically established equilibrium points, adaptive biasing force calculations found that reorientation and isomerization of glycerol arises over the same time scale as translocation

itself. Clearly, long-time simulations will be needed to settle the dispute involving the same authors on both sides of the issue. Still a hotly debated topic in the aquaporin field is the nature of the high selectivity barrier against proton transport. Combining classical steered MD simulations to sample intermediate geometries with advanced quantum chemistry calculations to describe the Grotthuss mechanism of proton charge delocalization, the authors in (5) elucidated the mechanism of proton blockage in aquaporins. It has been suggested also that aquaporins are involved in gas (O_2 , CO_2 , and NO) conduction, investigated in (6,7). The authors in (6) suggest that gas conduction involves the central pore of aquaporin, which strangely has not been attributed yet a clearly confirmed function other than that it is a natural geometric feature of the aquaporin tetramer.

The aquaporin family impresses through a wide variety of channel types in many life forms. Particularly fascinating among the human aquaporins is AQP0, the protein most ubiquitous in the lens of our eyes. Here the numerous proteins need to satisfy actually two functions, namely, to provide sites at which lens cells dock together and to conduct water between cells, and possibly also glycerol as lens cells are mainly devoid of mitochondria and rely on the glycolytic pathway metabolically. Interestingly, AQP0 conducts water rather poorly, a property on which experimentalists agree with two recent simulation studies (7,9). In (9) the authors studied single membrane tetrameric AQP0 and double membrane octameric AQP0, establishing that linking of two membranes through the docking of two juxtaposed tetramers does not significantly affect water permeation. The amazing water files crossing two membranes in an aquaporin octamer are shown in Fig. 2.

The aquaporin field is rather mature and has moved beyond the stage where a structure of yet another aquaporin creates much of a stir. The excitement of the field will derive from a systematic approach that combines structural, physiological, and mechanistic (i.e., simulation) approaches. It is safe to predict, therefore, that modeling will play a still larger role than it has done so far in the aquaporin field.

Potassium Channels

Potassium channels are membrane proteins that are highly selective for the conduction of K^+ ions across the cell membrane. These channels are responsible for maintaining the membrane resting potential and open in response to a variety of stimuli, such as changes in the transmembrane potential, intracellular pH, or binding of ligands. The selectivity filter of the channel constricts the conduction pore, and provides four binding sites for K^+ ions. The ions in the binding sites are coordinated mainly by carbonyl oxygens from the protein backbone. MD simulations show that changing the number or dipole moment of the coordinating ligands directly affects the selectivity of the binding sites (10). Fowler et al. (11) have tested the predictions of various hypotheses for channel selectivity and their compatibility with the results of MD simulations of KcsA, a highly selective potassium channel, and NaK, a non-selective cation channel that conducts both Na^+ and K^+ ions. Their results suggest that loss of selectivity in NaK is primarily due to an anomalous flexibility of the filter and a reduced number of coordinating ligands around Na^+ ions in this channel. Interestingly, the authors observed that in the presence of K^+ , the selectivity filter of NaK adopts a conformation that is similar to the conformation of the filter in KcsA and other K^+ -selective channels. The results were independent of the choice of the forcefield, and were robust in multiple simulations of the channel, indicating that the conformation of the filter at physiological temperatures is probably different from the crystallographic structure.

Conformational changes in the selectivity filter have been attributed to inactivation of the K^+ channels. Cordero-Morales et al. (12) have used a combination of experiment and simulation to characterize the inactivated state of the KcsA potassium channel. The simulations showed that the conductive conformation of the filter is intrinsically unstable, and identified a low free

energy state corresponding to an inactivated conformation of the filter in which isomerization of the peptide bonds disrupts one of the binding sites for K^+ ions. The authors have suggested that when the channel gate is open, the selectivity filter fluctuates between these alternative conformations and functions as a secondary (fast) gate for the conduction of the K^+ ions. Alternative (non-conductive) conformations of the filter are also identified in the simulations of KirBac1.1 channel (13).

The transmembrane domains of voltage-gated potassium channels include highly charged segments within their voltage-sensing domains (VSD) that are positioned inside the membrane. These charged segments contain multiple positively charged residues, known as gating charges, that are positioned uniformly at every fourth position on one of the transmembrane helices. The voltage-sensor domains move in response to changes in the electrostatic potential and, thus, couple the transmembrane voltage to the conformational change of the conduction gate. Simulations of the voltage-sensor domain of potassium channels, either as individual domains or in the context of the full channel, show that these charged residues (mainly arginines) are fully solvated inside the membrane. These charges are stabilized either by interaction with the lipid headgroups or by salt bridge interactions within the protein (14–17). Coarse-grained simulations of the voltage-sensing domain also showed significant deformation of the lipid bilayer around the protein (18). The transmembrane potential sensed by the charged residues in the voltage-sensor domain has been calculated based on continuum electrostatic approximations (16) and all-atom simulations (17). The calculations show that the membrane electric field is focused within the voltage-sensor domains, resulting in a sharp voltage-gradient across the membrane.

Mechanosensitive Channels

Mechanosensitive (MS) channels, involved in hearing, touch sensation, and cell-volume regulation, control the passage of ions and solutes through the cellular membrane in response to mechanical forces conveyed by other proteins or the membrane itself. The short time scale of MS channel gating ($<100\mu s$), the large structural rearrangement of the proteins involved, and the availability of crystallographic models for two bacterial MS channels, MscL and MscS, make this family of channels particularly suitable for all-atom MD simulations.

The crystallographic model of MscL from *Mycobacterium tuberculosis* revealed a pentameric channel in a closed conformation, with each subunit featuring two transmembrane helices. Multiple models derived from experimental and all-atom simulation data suggested that the MscL channel opens through rotation and tilting of its transmembrane helices moving in an iris-like fashion. Recently, MscL has been used as a proof-of-concept for finite-element calculations (19) and coarse-grained simulations (20). These two novel approaches yielded results that are consistent with previous experimental and simulation results, while partially bridging the gap between the time- and length- scales accessible to all-atom MD simulations and to experiments. At the same time, the finite element framework and the coarse-grained models provide a natural connection to all-atom MD simulations when needed. However, perhaps the most novel finding related to MscL gating comes from elegant but generic continuum mechanics models (21) describing how interactions between neighboring MscL proteins may affect their open probability, tension sensitivity, and clustering. The challenge now is to test these predictions using a combination of all-atom and continuum/CG models to simulate multiple MscL channels in liposomes.

In the case of MscS, all-atom MD simulations have played an important role in determining the functional conformation depicted by its first resolved crystallographic model. This model depicted *E. coli* MscS as a heptamer with three transmembrane helices per subunit, a large cytoplasmic domain, and a transmembrane pore that was suggested to be wide enough to allow a 1 nS conductance. All-atom MD simulations with explicit transmembrane potentials (22)

suggested, however, that significant widening of the MscS transmembrane pore was required to have a fully-conducting channel. The MD simulations also revealed how ions permeate multiple side-portals of MscS's large cytoplasmic domain, hinting that it might serve as a molecular sieve (a suggestion that remains to be tested). In light of these results, multiple models for a truly open state were obtained using purely computational techniques (22–25) or by incorporating experimental data as constraints during MD simulations (26,27). Although the models differ in some details, most of them featured wider pores with straightened, and closely packed transmembrane helices. A recent crystal structure of an *E. coli* MscS mutant (28) in a putative open state confirms some of the motions seen in simulations, but features a transmembrane pore with lateral apertures that is distinct from what has been proposed through modeling. Further characterization of this structural model through simulations might clarify its functional conformation. The seemingly complex gating mechanism of MscS seems to be now the next challenge for mixed fine/coarse-grained MD simulations.

Other Channels

A rather unique channel, the translocon is one that, in conjunction with a partner, e.g., the ribosome, translocates nascent proteins across the membrane when destined for secretion or threads proteins into the membrane in the case of membrane proteins. The complexity and slowness of the translocon's function (~0.1–1 second per amino acid translocated) is a great challenge for modelers and after a first “wave” of publications only two simulation studies were reported during 2007/2008. One study investigated how the translocon opens its lateral gate that permits nascent proteins to leave the channel for the membrane (29). Through steered MD, gate opening was enforced and a clamp-like underlying motion of the channel was identified. Interestingly, the open gate does not permit lipids to stream into the channel; rather, lipids remain outside for extended periods of time (microseconds, achieved through coarse-grained MD). A second study (30) investigated how the translocon manages to keep its channel sealed against water and ion flow, yet opens to entering proteins: by simulating native and mutant translocons, a constriction ring in the center of the channel was found to close the channel when needed, while a plug, thought to be the gate, has actually mainly a stabilizing function for the constriction ring.

The cation-selective nicotinic acetylcholine receptor (nAChR) channels are crucial for the propagation of signals between nerve cells and their targets at the peripheral synapses. MD simulations of an EM structure of nAChR (obtained at 4 Å resolution) revealed that the transmembrane domain of the pentameric channel contains internal binding sites for cholesterol, and occupation of these binding sites stabilizes the experimentally observed structure (31). The potential of mean force (PMF) for the conduction of ions through the channel pore in two homologous models of nAChR showed that the main barrier for cation conduction in nAChR is hydrophobic in nature (32). The selectivity of the channel is also attributed to the interaction of ions with charged residues near the entrance and exit of the pore that favor or disfavor the passage of ions solely based on their charge (32).

Acid-sensing ion channels (ASICs) are cation channels whose gating is controlled by extracellular pH. Equilibrium MD simulations of ASIC1 at different ionic solutions and concentrations examining multiple titration states of various acidic residues have been used to identify potential proton and cation binding sites and to study cation/H⁺-induced protein conformational changes (33).

Membrane Transporters and Carriers

In contrast to membrane channels which provide a passive permeation pathway for their substrates, transport in membrane transporters is mediated by close interaction and engagement of the protein and the substrate. This is necessary due to the active (energy-dependent) nature

of the transport process during which the energy provided by various sources, e.g., ATP hydrolysis or ionic gradient across the membrane, is used to actively “pump” the substrate across the membrane, often against its electrochemical gradient. Shown in Fig. 3, membrane transporters are structurally much more diverse than membrane channels, as they need to harvest various sources of energy in the cell and efficiently couple them to substrate transport. They are also far slower than channels, since several stepwise protein conformational changes of various magnitude are usually involved in their mechanism. Along with the recent availability of structures for several different membrane transporters, MD simulations have been employed to investigate dynamical properties and details of the mechanism of function. Although the time scale of the entire transport cycle proves to be usually beyond the reach of transporter MD simulations, such simulations have proven successful in describing individual steps and transitions involved in such cycles.

ABC Transporters

ATP-binding cassette (ABC) transporters use ATP to drive active transport of substrates across the membrane. ATP binding and hydrolysis in the nucleotide binding domains (NBDs) drive conformational changes of the transmembrane domains (TMDs), thus switching substrate accessibility between the cytoplasmic and extracellular sides of the membrane. Elucidating the conformational changes induced by ATP binding and hydrolysis in the NBDs and the coupling of NBDs and TMDs constitute two major themes in simulation studies of ABC transporters.

The dimeric structures of the NBDs of maltose transporter (MalK) and an archaeal ABC transporter (MJ0796) have been extensively used in simulation studies. Earlier MD simulations of MalK performed on the three crystal forms of MalK verified the nucleotide dependence of opening and closing of the NBDs (34). Simulations on the order of 20 ns performed on different nucleotide-bound forms of MJ0796 identified the rotation of the helical subdomain as the primary response to ATP replacement by ADP (35), while longer simulations (30–50 ns) were employed to investigate the mechanism of dimer separation (36). Using even longer simulations (~70 ns) of MalK, and through simulating the immediate effect of ATP hydrolysis (conversion to ADP-P_i), it was proposed that the hydrolysis reaction itself is the initial trigger for dimer opening (37). It was also shown that despite the presence of two nucleotide-binding sites, only one ATP hydrolysis reaction is needed for dimer opening (37). MD simulations of NBD1 of CFTR, an ABC chloride channel, showed that the mutation $\Delta F508$ confers greater flexibility and larger surface area to the protein, which likely results in premature proteolysis of the mutant protein (38).

Reported MD simulations of complete ABC transporters to date are essentially limited to BtuCD, which is involved in transport of vitamin B₁₂. Rather short simulations (10–15 ns) of BtuCD after docking of the substrate binding protein, BtuF, and/or ATP-Mg²⁺, showed that ATP-driven NBD dimerization is BtuF dependent in the complete transporter, likely in an asymmetric manner (39). Other simulations focused on the coupling between BtuC (TMD) and BtuD (NBD) using a perturbed anisotropic network model and essential dynamics sampling, capturing large scale conformational changes of the TMDs (40). From these simulations, it was suggested that the closure of NBDs is coupled to the cytoplasmic opening of the TMDs and *vice versa* (the “MalK-based” transport model), in contrast to the BtuCD-based model in which the NBD closure is coupled to the periplasmic opening.

Interestingly, MD simulations performed on the withdrawn crystal structure of MsbA resulted in a significant distortion of the protein in the membrane (41), suggesting that MD can be used for validating experimentally suggested structures of membrane proteins. Along with the growing number of crystal structures of ABC transporters in different functional states, large scale simulations of entire ABC transporters on longer time scales should be able to provide

deeper and more detailed insight into the conformational coupling between the NBDs and the TMDs, the structural transitions during the transport cycle, and substrate translocation pathways.

Secondary Transporters

In contrast to ABC transporters which utilize ATP, secondary transporters couple the transport of a substrate to co-transport of ions (e.g., Na^+ , H^+) down their concentration gradient. All secondary transporters are believed to function via an alternating access mechanism in which the cytoplasmic-open (C_i) state is distinct from the periplasmic-open (C_o) state. Two further mechanistic subdivisions have been put forward: the “rocker-switch”, in which a large motion between the two halves (domains) of the protein is required to switch between the C_o and the C_i states, and the “gated-pore”, in which smaller, localized conformational changes (gates) control access. Currently, no transporter family has been imaged in both its C_i and C_o states, although a recent structure of a galactose transporter, vSGLT, in its C_i state revealed similarities to the structure of the unrelated LeuT, captured in its C_o state (42).

One family of secondary transporters, the major facilitator superfamily (MFS), is among the largest, but only three unique atomic-resolution structures have been resolved (LacY, GlpT, and EmrD). The lactose permease LacY has been most extensively studied via simulations, exploring the lactose binding site and transport pathway (43–46). Manipulation of a key residue, Glu269, either by protonation (43) or loss of interactions with the substrate (45), led to closure of the cytoplasmic half-channel in multiple simulations. Although MFS members have been proposed to function via the rocker-switch mechanism, steered MD simulations probing translocation of lactose across the protein hinted at the possible involvement of smaller scale motions in LacY during transport (44). A combination of simulations and experiments has also been used to characterize the substrate binding site in the G3P (glycerol-3-phosphate) transporter GlpT (47). In both GlpT and LacY, salt-bridge dynamics have been proposed to control the transition between the C_i and C_o states (43,47).

Neurotransmitter sodium symporters (NSS), in contrast to MFS members, have been proposed to function via the gated-pore mechanism (48). The bacterial leucine transporter LeuT is the only structurally known NSS, although it has also been used as a template for the homologous dopamine transporter (DAT) (48,49). LeuT was captured in an “occluded” state in which the substrate is bound on the extracellular side, but closed off from the bulk water (50). Although distinct from the NSS family, the glutamate transporter Glt has also been crystallized in an occluded state (51,52).

Simulations have been used to study the binding of the substrate (leucine, dopamine, or glutamate) and Na^+ ions, and in what order they bind (48–54). For example, steered MD simulations of LeuT revealed that the conversion from the C_o state to the occluded state likely involves only minor conformational changes; additionally, an initial binding site for leucine in the C_o state of LeuT was identified, in agreement with previous results for DAT (49,50, 53). Experiments based on the simulations confirmed the existence of the secondary binding site, showing that both sites can be occupied simultaneously and that binding of leucine to the secondary site triggers release of leucine from the primary site (53). Simulations combined with experiments also implicated a conserved salt bridge in DAT in controlling the C_o to C_i conversion, in line with the proposed role for salt bridges in MFS members (43,47,48).

The Na^+/H^+ antiporter NhaA is distinct from other secondary transporters, particularly in its pH-dependence (55,56). Based on extended simulations (up to 100 ns each) and supporting experiments, two aspartate residues, Asp164 and Asp163, have been proposed to be the Na^+ -binding site and to control its alternating access, respectively (56). How the channel is controlled by pH is less clear, although two, possibly coexisting mechanisms have been put

forth. Simulations of NhaA at pH 4 and pH 8 revealed a pH-dependent kink in helix X (55); alternatively, protonation of a third aspartate (Asp133) was found to align two helical dipoles, preventing access of Na⁺ to its binding site (56). Helical dipoles likely play a role in other transporters as well; the alignment of two helical dipoles in GlT was shown to contribute to the formation of a Na⁺ binding site, illustrated in Fig. 4 (52), rather than abolishing one as in NhaA.

Other Transporters

In addition to the major classes of transporters discussed above, the structures of members of different families of transporters have also been solved experimentally, prompting simulation studies to investigate their dynamics and mechanisms.

BtuB is an example of an outer membrane transporter that relies on the inner membrane for its function via an unknown mechanism. A set of SMD simulations (57) put to the test the viability of a mechanical coupling as the mechanism of energy transduction, and showed that partial unfolding due to such coupling can provide a permeation pathway for the substrate in BtuB.

Recent studies on the mitochondrial ADP/ATP carrier (AAC) independently discovered that AAC possesses an “electrostatic funnel”, responsible for bringing the substrate (here, ADP) to its binding site, although some disagreement on the precise site remains (58,59). It was shown that this strong electrostatic funneling of the substrate likely plays a similar role in all mitochondrial carriers (58). Simulations have also implicated three proline residues in a concerted hinge motion, which may be required for conversion of AAC from the cytoplasm-open to the matrix-open state (58,60).

Conclusion

Membrane protein simulations have contributed considerably to our understanding of the phenomena and processes involved in transport of materials across biological membranes. Molecular simulation provides temporal and spatial resolutions currently unattainable by experimental methods, thus offering a powerful complementary approach toward unraveling molecular mechanisms of membrane proteins.

The quality and accuracy of membrane protein simulations can be improved further in various respects. The dynamics of a simulated membrane protein can be largely affected by the treatment of the surrounding lipid bilayer. Inaccuracies associated with, e.g., the lack of polarizability and imperfect transferability of force field parameters, can result in different geometrical and dynamical properties of the simulated lipid bilayers. For example, commonly used force fields for biomolecular simulations underestimate the area per lipid for membranes and introduce too much order in the lipid tail region, problems that became particularly apparent after extended simulations became feasible. Such inaccuracies may affect the dynamics of the embedded protein, and even hamper relevant large scale protein motions, e.g., during the transport cycle of membrane transporters or gating of channels. Fortunately, polarizable force fields are being developed and tested for membranes. A first successful test on the dipole potential of a DPPC monolayer has recently been published (61).

The composition of the lipid bilayers is another aspect that might be of importance in this regard. Almost all biological membranes are composed of various types of lipids, even exhibiting asymmetry with respect to the lipid composition of their two leaflets. Due to the short duration and finite size of the membrane used in simulations, however, most studies have used a homogeneous (single lipid) composition of the bilayer. Given that the majority of the studied membrane proteins retain their main function after reconstitution in artificial bilayers, the problem of lipid composition is less of a concern. In some cases, however, it is known that

particular lipids are of critical importance for the function; for instance, binding and activation of coagulation factors and signaling proteins are highly dependent on anionic lipids, e.g., PIP2. In such cases, these effects need to be taken into account by including a faithful representation of the natural environment of the protein in the simulation.

Ion conduction and selectivity constitute other examples of key attributes in membrane channels that might be affected by force field approximations. Although several simulation studies employing traditional force fields have revealed key principles of selectivity and permeation in membrane channels, often in close agreement with experiments, electronic polarization effects, e.g., those induced by a permeating ion in the channel protein, are expected to be significant, and, therefore, a complete picture of the phenomena involved in such processes can only emerge after these effects are included in the simulation. For example, MscS simulations exhibit a discrepancy with experiment in regard to channel ion selectivity which might be resolved by including the effect of polarization.

The best years of molecular modeling in membrane biology are yet to come as the methodology becomes more closely tied to physiology and structural analysis. Leading experimental laboratories have started their own modeling efforts or have begun close collaborations with modelers. Modeling is seen to enter into the research process already at the inception of new projects. Advances in computational technology (e.g., petascale computing and graphics-processor based computing) and force fields promise that the simulation time scale will be extended towards the millisecond (10 μ sec has been reached already (62)) and the accuracy of energetics greatly improved.

Acknowledgements

This work was supported by the National Institutes of Health (P41-RR05969 and R01-GM067887). MS is a Howard Hughes Medical Institute Fellow of the Helen Hay Whitney Foundation at the laboratories of D. P. Corey and R. Gaudet.

References

1. Lindahl E, Sansom MSP. Membrane proteins: molecular dynamics simulations. *Curr Opin Struct Biol* 2008;18:425–431. [PubMed: 18406600]
2. Hashido M, Kidera A, Ikeguchi M. Water transport in aquaporins: osmotic permeability matrix analysis of molecular dynamics simulations. *Biophys J* 2007;93:373–385. [PubMed: 17449664]
Osmotic permeability of water in aquaporins is described by a matrix that can be decomposed into contributions from each local region of the channel
3. Zhu F, Tajkhorshid E, Schulten K. Collective diffusion model for water permeation through microscopic channels. *Phys Rev Lett* 2004;93:224501. [PubMed: 15601094]
4. Jensen MØ, Park S, Tajkhorshid E, Schulten K. Energetics of glycerol conduction through aquaglyceroporin GlpF. *Proc Natl Acad Sci USA* 2002;99:6731–6736. [PubMed: 11997475]
5. Chen H, Ilan B, Wu Y, Zhu F, Schulten K, Voth GA. Charge delocalization in proton channels. I. The aquaporin channels and proton blockage. *Biophys J* 2007;92:46–60. [PubMed: 17056733]
Mechanism of proton blockage in aquaporins is investigated by a combination of steered MD simulations and quantum chemistry calculations
6. Wang Y, Cohen J, Boron WF, Schulten K, Tajkhorshid E. Exploring gas permeability of cellular membranes and membrane channels with molecular dynamics. *J Struct Biol* 2007;157:534–544. [PubMed: 17306562]
7. Hub J, de Groot B. Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proc Natl Acad Sci USA* 2008;105:1198–203. [PubMed: 18202181]
8. Henin J, Tajkhorshid E, Schulten K, Chipot C. Diffusion of glycerol through *Escherichia coli* aquaglyceroporin GlpF. *Biophys J* 2008;94:832–839. [PubMed: 17921212]

9. Jensen M, Dror R, Xu H, Borhani D, Arkin I, Eastwood M, Shaw D. Dynamic control of slow water transport by aquaporin 0: implications for hydration and junction stability in the eye lens. *Proc Natl Acad Sci USA* 2008;105:14430–5. [PubMed: 18787121]
10. Noskov S, Roux B. Importance of hydration and dynamic on the selectivity of the KcsA and NaK channels. *J Gen Physiol* 2007;129:135–143. [PubMed: 17227917]
11. Fowler PW, Tai K, Sansom MS. The selectivity of K⁺ ion channels: Testing the hypotheses. *Biophys J* 2008;95:5062–5072. [PubMed: 18790851]
- 12••. Cordero-Morales JF, Jogini V, Lewis A, Vasquez V, Cortes DM, Roux B, Perozo E. Molecular driving forces determining potassium channel slow inactivation. *Nat Struct Mol Biol* 2007;14:1062–1069. [PubMed: 17922012]A combination of experiment and simulation is used to identify the inactivated state of the potassium channel, KcsA
- 13•. Domene C, Klein ML, Branduardi D, Gervasio FK, Parrinello M. Conformational changes and gating at the selectivity filter of potassium channels. *J Am Chem Soc* 2008;130:9474–9480. [PubMed: 18588293]The free energy of ion conduction through the potassium channel is calculated along a pathway, in which the conformation of the selectivity filter changes according to the lowest free energy path obtained in metadynamics simulations
14. Treptow W, Tarek M. Environment of the gating charge in the Kv1.2 Shaker potassium channel. *Biophys J* 2006;90:L64–L66. [PubMed: 16533847]
15. Sands ZA, Sansom MS. How does a voltage sensor interact with a lipid bilayer? Simulations of a potassium channel domain. *Structure* 2007;15:235–244. [PubMed: 17292841]
16. Jogini V, Roux B. Dynamics of the Kv1.2 voltage-gated K⁺ channel in a membrane environment. *Biophys J* 2007;93:3070–3082. [PubMed: 17704179]
- 17•. Nishizawa M, Nishizawa K. Molecular dynamics simulation of Kv channel voltage sensor helix in a lipid membrane with applied voltage. *Biophys J* 2008;95:1729–1744. [PubMed: 18487312] Extensive simulations of the voltage-sensor domain revealed the initial steps of potassium channel gating. The environment of the gating charge is characterized from electrostatic calculations
18. Bond PJ, Sansom M. Bilayer deformation by the Kv channel voltage sensor domain revealed by self-assembly simulations. *Proc Natl Acad Sci USA* 2007;104:2631–2636. [PubMed: 17301243]
- 19•. Tang Y, Cao G, Chen X, Yoo J, Yethiraj A, Cui Q. A finite element framework for studying the mechanical response of macromolecules: Application to the gating of the mechanosensitive channel MscL. *Biophys J* 2006;91:1248–1263. [PubMed: 16731564]A finite element methodology is used to model the gating transition of *M. tuberculosis* and *E. coli* MscL under equi-biaxial tension and axisymmetric bending. Modeling predicts full opening of MscL under equi-biaxial tension with structural transitions that are consistent with previous experimental and theoretical models. This technique provides a continuum description with a natural connection to specific all-atom models
20. Yefimov S, van der Giessen E, Onck PR, Marrink SJ. Mechanosensitive membrane channels in action. *Biophys J* 2008;94:2994–3002. [PubMed: 18192351]
- 21•. Ursell T, Huang KC, Peterson E, Phillips R. Cooperative gating and spatial organization of membrane proteins through elastic interactions. *PLoS Computational Biology* 2007;3:803–812.A generic continuum mechanics model is used to study how interactions between neighboring channels may affect MscL gating. The model predicts cooperative gating and an effect in average separation between two proteins due to their conformational changes
22. Sotomayor M, Vasquez V, Perozo E, Schulten K. Ion conduction through MscS as determined by electrophysiology and simulation. *Biophys J* 2007;92:886–902. [PubMed: 17114233]
23. Akitake B, Anishkin A, Sukharev S. Straightening and sequential buckling of the pore-lining helices define the gating cycle of MscS. *Nat Struct Mol Biol* 2007;14:1141–1149. [PubMed: 18037888]
24. Anishkin A, Akitake B, Sukharev S. Characterization of the resting MscS: Modeling and analysis of the closed bacterial mechanosensitive channel of small conductance. *Biophys J* 2008;94:1252–1266. [PubMed: 17981908]
- 25•. Anishkin A, Kamaraju K, Sukharev S. Mechanosensitive channel MscS in the open state: Modeling of the transition, explicit simulations, and experimental measurements of conductance. *J Gen Physiol* 2008;132:67–83. [PubMed: 18591417]Models of MscS in open and closed conformations are presented (23, 24), characterized by MD simulations, and partially validated through patch-clamp experiments and mutagenesis

26. Vasquez V, Sotomayor M, Cortes DM, Roux B, Schulten K, Perozo E. Three dimensional architecture of membrane-embedded MscS in the closed conformation. *J Mol Biol* 2008;378:55–70. [PubMed: 18343404]
- 27•. Vasquez V, Sotomayor M, Cordero-Morales J, Schulten K, Perozo E. A structural mechanism for MscS gating in lipid bilayers. *Science* 2008;321:1210–1214. [PubMed: 18755978] Electron paramagnetic resonance spectroscopy data is used to drive MD simulations and obtain an experimentally determined open model of MscS. Standard MD simulations are then used to thoroughly characterize open and closed (22, 26) conformations of MscS
28. Wang W, Black SS, Edwards MD, Miller S, Morrison EL, Bartlett W, Dong C, Naismith JH, Booth IR. The structure of an open form of an *E. coli* mechanosensitive channel at 3.45 Å resolution. *Science* 2008;321:1179–1183. [PubMed: 18755969]
29. Gumbart J, Schulten K. Structural determinants of lateral gate opening in the protein translocon. *Biochemistry* 2007;46:11147–11157. [PubMed: 17760424]
30. Gumbart J, Schulten K. The roles of pore ring and plug in the SecY protein-conducting channel. *J Gen Physiol* 2008;132:709–719. [PubMed: 19001142]
- 31•. Brannigan G, Henin J, Law R, Eckenhoof R, Klein ML. Embedded cholesterol in the nicotinic acetylcholine receptor. *Proc Natl Acad Sci USA* 2008;105:14418–14423. [PubMed: 18768796] Simulations showed that binding of cholesterol is essential for maintaining the stability of the EM structure of nAChR. Such instability was not identified in previous (short time-scale) simulations of the same structure
32. Ivanov I, Cheng X, Sine SM, McCammon JA. Barriers to ion translocation in cationic and anionic receptors from the cys-loop family. *J Am Chem Soc* 2007;129:8217–8224. [PubMed: 17552523]
33. Shaikh SA, Tajkhorshid E. Potential cation and H⁺ binding sites in acid sensing ion channel-1. *Biophys J* 2008;95:5153–5164. [PubMed: 18790845]
34. Oloo EO, Fung EY, Tieleman DP. The dynamics of the MgATP-driven closure of MalK, the energy-transducing subunit of the maltose ABC transporter. *J Biol Chem* 2006;281:28397–28407. [PubMed: 16877382]
35. Jones PM, George AM. Nucleotide-dependent allostery within the ABC transporter ATP-binding cassette. *J Biol Chem* 2007;282:22793–22803. [PubMed: 17485460]
36. Jones PM, George AM. Opening of the ADP-bound active site in the ABC transporter ATPase dimer: Evidence for a constant contact, alternating sites model for the catalytic cycle. *Proteins: Struct, Func, Bioinf.* 2008In press
37. Wen PC, Tajkhorshid E. Dimer opening of the nucleotide binding domains of ABC transporters after ATP hydrolysis. *Biophys J* 2008;95:5100–5110. [PubMed: 18790847]
38. Wieczorek G, Zielenkiewicz P. ΔF508 mutation increases conformational flexibility of CFTR protein. *J Cyst Fibros* 2008;7:295–300. [PubMed: 18234567]
39. Ivetac A, Campbell JD, Sansom MS. Dynamics and function in a bacterial ABC transporter: simulation studies of the BtuCDF system and its components. *Biochemistry* 2007;46:2767–2778. [PubMed: 17302441]
- 40•. Sonne J, Kandt C, Peters GH, Hansen FY, Jansen MO, Tieleman DP. Simulation of the coupling between nucleotide binding and transmembrane domains in the ATP binding cassette transporter BtuCD. *Biophys J* 2007;92:2727–2734. [PubMed: 17208973] This paper describes two simulation methods (perturbed anisotropic network model and essential dynamics sampling) to facilitate large conformational changes of a transporter in designed directions and to interpret its mode-of motion. Using knowledge of its structural component as a guide, the motion of the complete protein assembly can be successfully annotated with both methods
41. Ivetac A, Sansom MS. Molecular dynamics simulations and membrane protein structure quality. *Eur Biophys J* 2008;37:403–409. [PubMed: 17960373]
42. Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, Abramson J. The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na⁺/sugar symport. *Science* 2008;321:810–814. [PubMed: 18599740]
43. Yin Y, Jensen MØ, Tajkhorshid E, Schulten K. Sugar binding and protein conformational changes in lactose permease. *Biophys J* 2006;91:3972–3985. [PubMed: 16963502]

44. Jensen MØ, Yin Y, Tajkhorshid E, Schulten K. Sugar transport across lactose permease probed by steered molecular dynamics. *Biophys J* 2007;93:92–102. [PubMed: 17434947]
45. Holyoake J, Sansom MSP. Conformational change in an MFS protein: MD simulations of LacY. *Structure* 2007;15:873–884. [PubMed: 17637346]
46. Klauda JB, Brooks BR. Sugar binding in lactose permease: Anomeric state of a disaccharide influences binding structure. *J Mol Biol* 2007;367:1523–1534. [PubMed: 17320103]
47. Law CJ, Almqvist J, Bernstein A, Goetz RM, Huang Y, Soudant C, Laaksonen A, Hovmöller S, Wang DN. Salt-bridge dynamics control substrate-induced conformational change in the membrane transporter GlpT. *J Mol Biol* 2008;378:828–839. [PubMed: 18395745]
48. Kniazeff J, Shi L, Loland CJ, Javitch JA, Weinstein H, Gether U. An intracellular interaction network regulates conformational transitions in the dopamine transporter. *J Biol Chem* 2008;283:17691–17701. [PubMed: 18426798]
49. Huang X, Zhan CG. How dopamine transporter interacts with dopamine: insights from molecular modeling and simulation. *Biophys J* 2007;93:3627–3639. [PubMed: 17704152]
50. Celik L, Schiott B, Tajkhorshid E. Substrate binding and formation of an occluded state in the leucine transporter. *Biophys J* 2008;94:1600–1612. [PubMed: 18024499]
51. Shrivastava IH, Jiang J, Amara SG, Bahar I. Time-resolved mechanism of extracellular gate opening and substrate binding in a glutamate transporter. *J Biol Chem* 2008;283:28680–28690. [PubMed: 18678877]
52. Huang Z, Tajkhorshid E. Dynamics of the extracellular gate and ion-substrate coupling in the glutamate transporter. *Biophys J* 2008;95:2292–2300. [PubMed: 18515371]
53. Shi L, Quick M, Zhao Y, Weinstein H, Javitch JA. The mechanism of a neurotransmitter:sodium symporter – inward release of Na⁺ and substrate is triggered by substrate in a second binding site. *Mol Cell* 2008;30:667–677. [PubMed: 18570870] Simulations combined with experiments reveal the existence of a secondary substrate binding site in the leucine transporter LeuT. Binding of leucine to the secondary binding site triggers release from the primary one, while antidepressants, known to bind to the secondary site, are suggested to prevent release
54. Noskov SY, Roux B. Control of ion selectivity in LeuT: Two Na⁺ binding sites with two different mechanisms. *J Mol Biol* 2008;377:804–818. [PubMed: 18280500]
55. Olkhova E, Padan E, Michel H. The influence of protonation states on the dynamics of the NhaA antiporter from *Escherichia coli*. *Biophys J* 2007;92:3784–3791. [PubMed: 17350999]
56. Arkin IT, Xu H, Jensen MØ, Arbely E, Bennett ER, Bowers KJ, Chow E, Dror RO, Eastwood MP, Flitman-Tene R, Gregersen BA, Klepeis JL, Kolossváry I, Shan Y, Shaw DE. Mechanism of Na⁺/H⁺ antiporting. *Science* 2007;317:799–802. [PubMed: 17690293] Long timescale simulations verified by mutagenesis experiments are used to characterize the entire transport cycle and pH dependence of the Na⁺/H⁺ antiporter NhaA
57. Gumbart J, Wiener MC, Tajkhorshid E. Mechanics of force propagation in TonB-dependent outer membrane transport. *Biophys J* 2007;93:496–504. [PubMed: 17449669]
58. Wang Y, Tajkhorshid E. Electrostatic funneling of substrate in mitochondrial inner membrane carriers. *Proc Natl Acad Sci USA* 2008;105:9598–9603. [PubMed: 18621725] The first report in which spontaneous ligand binding to a protein was captured in 0.1 microsecond simulations and the unknown binding pocket along with initial protein conformational changes triggered by the substrate were identified. The study characterizes electrostatic forces as a common mechanism of substrate recruitment in all mitochondrial carriers
59. Dehez F, Pebay-Peyroula E, Chipot C. Binding of ADP in the mitochondrial ADP/ATP carrier is driven by an electrostatic funnel. *J Am Chem Soc* 2008;130:12725–12733. [PubMed: 18729359]
60. Johnston JM, Khalid S, Sansom MSP. Conformational dynamics of the mitochondrial ADP/ATP carrier: a simulation study. *Mol Membr Biol* 2008;25:506–517. [PubMed: 18988066]
61. Harder E, MacKerell AD, Roux B. Many-body polarization effects and the membrane dipole potential. *J Am Chem Soc*. 2009;10.1021/ja806825g In press
62. Freddolino PL, Liu F, Gruebele M, Schulten K. Ten-microsecond MD simulation of a fast-folding WW domain. *Biophys J* 2008;94:L75–L77. [PubMed: 18339748]

Figure 1. Membrane channels studied recently. Proteins are shown in cartoon representation, with the lipid bilayer in the background. The protein subunits are colored differently, and the dominant permeating molecules are indicated in the case of each channel.

Figure 2. Simulations of AQP0-mediated membrane junction (9). Cross-section of an AQP0 octamer embedded in a double lipid bilayer. Two pairs of interlocked AQP0 monomers that connect the two cells are shown. Permeating water is red and white. Other waters are shown as a white surface. Figure courtesy of Morten Jensen.

Figure 3.

Membrane transporters studied recently. Shown in the same format as in Fig. 1, each transporter is colored according to domain with substrates and direction of transport indicated. These transporters are found in a variety of cellular membranes including the cytoplasmic membrane (e.g., MalefGK), the bacterial outer membrane (BtuB), and the mitochondrial inner membrane (AAC).

Figure 4.

Initial steps in the transport cycle of the glutamate transporter GlT (lower left). Binding of the substrate to the apo state (upper left) induces a focusing of two helical dipoles (middle right) which then permits binding of a Na^+ ion (lower right) (52). Figure courtesy of Zhijian Huang.