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Tools for channels: moving towards molecular calculations of gating and permeation in ion channel biophysics

Thomas B. Woolf [a,*], Daniel M. Zuckerman [b], Nandou Lu [a], Hyunbum Jang [a]

[a] *Department of Physiology, Johns Hopkins University, School of Medicine, Biophysics 206, 725 N. Wolfe Street, Baltimore, MD 21205, USA*
[b] *Department of Environmental and Occupational Health and Center for Computational Biology and Bioinformatics, Biomedical Science Tower W1041, University of Pittsburgh, Pittsburgh, PA 15261, USA*

Abstract

Recent X-ray structures of voltage gated potassium channels provide an exciting opportunity to connect molecular structures with measured biological function. Two of the most important connections for these channels are: first, to the molecular basis behind selectivity and the associated free energy profile underlying ionic current flow and, second, to a true molecular understanding of the large-scale conformational transitions that underlie voltage dependent gating. But, existing computational tools need to be further developed to reach these goals. In this contribution to the symposia on sampling methods we outline our dynamic importance sampling method for sampling large-scale conformational transitions as well as our studies with non-equilibrium work events and equilibrium overlap sampling (OS) methods for sampling events related to the calculation of relative free energies.

Keywords: Molecular dynamics; Ion channels; Importance sampling; Kinetics; Permeation; Relative free energy; Overlap sampling; Non-equilibrium free energy

1. Introduction

More than 50 years ago voltage clamp experiments were developed to understand the electrical activity of nervous tissue [1]. The approach led to the Hodkgin–Huxley equations coupled differential equations that described the non-linear, time, and voltage dependent conductance properties of the biomembrane. In particular, the conductances were postulated to have separate pathways for sodium and potassium cations [1,2]. With the molecular biology revolution, the primary sequences for these channels were determined and initial attempts made to relate the electrical measurements to channel structure [2]. In particular, a large volume of work was performed addressed at site-directed mutations and comparison of primary sequence data from many different organisms. But, despite the information available from experiments and the primary sequence, it is clear that a full description of the complexities of gating requires a three-dimensional view of the protein structure *and* a description of the molecular motions in the membrane medium that defines the protein [3,4]. Only with the recent X-ray structures of potassium channels does it become possible to start addressing the possible details of how these gating events occur on a molecular level [5–10]. Yet the small number of available structural snapshots (from experiments) do not begin to describe the *dynamical* complexity of the permeation process. Large issues remain for the simulation community. How do we define a gating transition in the absence of a clear reaction path? How can we sample on the permeation pathway to compute a meaningful relative free energy?

In the formulation developed by Hodgkin and Huxley [1], potassium current flow is described electrically as a time and voltage dependent conductance (inverse of resistance) that is driven by the reversal potential for potassium. In other words, the current flow is an Ohms law ($V = IR$) flow where the maximum is set by a physical property containing the flow ($\bar{g}_k$) and the flow modulated by a time and voltage dependent "gate" that is described through the behavior of four "n" particles:

$$I_k = n^4 \bar{g}_k (E - E_k)$$

In this electrical and differential equation approach to explain the voltage clamp data, the "n" particle moves between two positions with voltage dependent properties set by the rate constants (α and β).

$$\text{“}1 - n\text{”} \underset{\beta_n}{\overset{\alpha_n}{\leftrightarrow}} \text{“}n\text{”}$$

* Corresponding author. Tel.: +1-410-614-2643; fax: +1-410-614-4436.
E-mail address: woolf@groucho.med.jhmi.edu (T.B. Woolf).

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Solving the first order differential equation for the behavior of *n*, essentially involves curve fitting to the data set from the voltage clamp records, and this led to the ability to recreate on a computer the current flow of sodium (with a similar model involving two particles: *m* and the *h*) and potassium that can be measured as action potentials. A whole generation of models for neural activity and neural circuitry then followed this pioneering work on the electrical circuit simulation of sodium and potassium current flows [2].

1.1. Recent X-ray structures

Over the last several years the MacKinnon group at Rockefeller has solved a set of K-channel structures that provide a wonderfully exciting view on the molecular world of ion channels (Figs. 1 and 2) [5–10]. When the X-ray work was first started, the mindset of many in the community was that X-ray structures of ion channels were decades away from the reality. The most recent work used antibodies to stabilize mobile elements of the protein structure, and provides insight into the possible mechanism of gating (Fig. 2) [7,8]. The first structure provides insight into permeation and the molecular substrate for selectivity of potassium (Fig. 1) [9]. These structures, as a set, provide a whole host of next-generation questions on both a computational and an experimental level. In this contribution we will mainly focus on the possible sets of calculations that can be considered from the existence of the structures. In particular, the challenge for the simulation community is: can we predict the conformational changes that underlie the gating transition? In particular, can we compute meaningful permeation paths and conformational changes that predict and explain measured current flow under a variety of voltage and amino acid mutational conditions? Meeting this set of challenges requires consideration of sampling for kinetics, pathways, and free energies. In addition, it requires consideration of the environmental conditions that the channels operate under the heterogenous and electrically complex membrane bilayers. If we improve sampling without understanding the underlying solvation environment, then the improved sampling will not lead to meaningful and insightful answers. Thus, the nature of the challenge is both sampling related (the main topic of this contribution and this special volume) and environment related (getting the setting right).

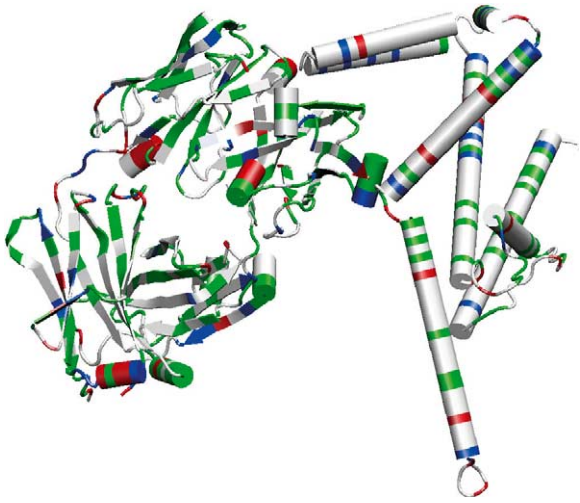

Fig. 2. The voltage gating paddles of the K-channel were stabilized by an antibody in the X-ray structure [7,8]. The helix-turn-helix motif (top center) is believed to move in response to voltage changes and in turn gate the channel pore from open to closed. Note that the full structure is a tetramer of these units. Simulations of gating will need to consider the nature of the voltage change, the motions of the paddle in the membrane bilayer and the coupling between paddle motion and pore access for cations.

1.2. Connecting static protein structure with function

In general, the X-ray structure tells us the average conformation of a state without telling us how the protein and

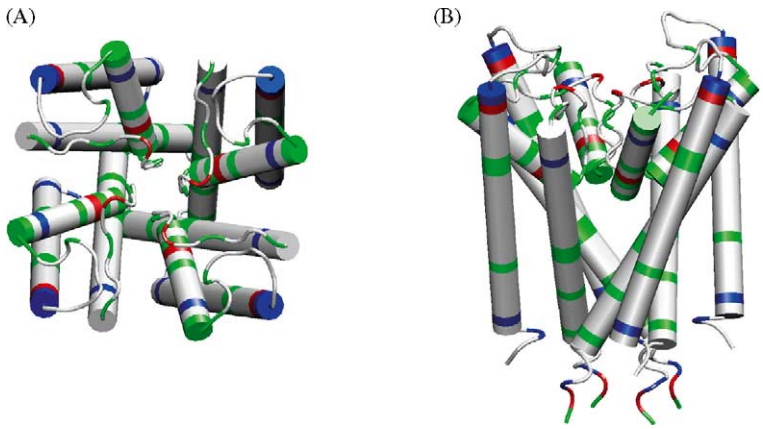

Fig. 1. KcsA K-channel structure [9]. Part A is a view down the pore axis, while part B is from the side. Note that the structure is drawn in cartoon form with the color scheme indicating the amino acid residue type (VMD). The selectivity filter is seen most clearly in the top half of Part B and is stabilized by aromatics. Permeation calculations need to consider motion through the pore as defined by the channel as well as the electrostatics of the salt solutions and the response of the membrane bilayer.

the surrounding system adopts with voltage changes (like the application of a voltage clamp and the current flow that follows the open gating transition). To learn more about the molecular details of how the X-ray structure relates to conformational change, either molecular dynamics (MD) or Monte Carlo (MC) sampling is usually performed. In the MC framework, only energies are needed and with a move set obeying detailed balance defined, conformations are updated by comparison of energy changes and the Metropolis acceptance criteria. Molecular dynamics depends on both energies and forces from the empirical energy surface and uses a discrete update based on a small time step. Conformations are thus sampled in a temporal domain and kinetics, in principle, could be determined by a sufficiently long MD trajectory calculation (and also by MC under certain assumptions). In practice, for MD, the free energy barriers between states are frequently sufficiently large that the multiple time-scale problem is encountered, where sampling within a state is very long relative to sampling of regions outside of the states. For large protein systems, e.g. K-channels where the time-scale for gating transitions is on the order of milli-seconds, it is not realistic to expect that a straight forward MD calculation will be able to sample on the kinetic transition. Monte Carlo methods, though less restricted, in principle, to a particular region of phase space, nonetheless have frequent problems with finding good transitions from one multiple minimum state to other multiple minimum states and need to be further supplemented with other calculations for determination of kinetics and pathways. Further, complicating the molecular analysis of the situation, for the K-channel and many other protein systems, it is commonly recognized that a one-dimensional reaction coordinates for the kinetic transition can't be readily defined. In fact, limiting sampling to a single pre-defined reaction coordinate may often create a systematic error, since it may reflect a poorly chosen slice of a multi-dimensional surface while many different paths contribute to the observed kinetic rate.

1.3. Membrane environment of potassium channels

The simulation of an ion channel requires a decision about the solvent setting for the protein. Is the simulation in a vacuum setting? Numerous simulation studies have shown that at least some solvent interactions are required for the phase space sampling of the MC or MD trajectories to be meaningful on comparison to experiment [11]. A trade-off is frequently required between a large all-atom and explicit solvent simulation with many lipid and water molecules and a less expensive calculation with implicit solvent. Fig. 3 shows a recent simulation of rhodopsin in an explicit lipid bilayer [12]. In this calculation, illustrating the insights that can come from all-atom simulations, a coupling between local events near the retinal and large-scale changes of the helices was observed. This type of coupling is believed to be important for gating transitions in

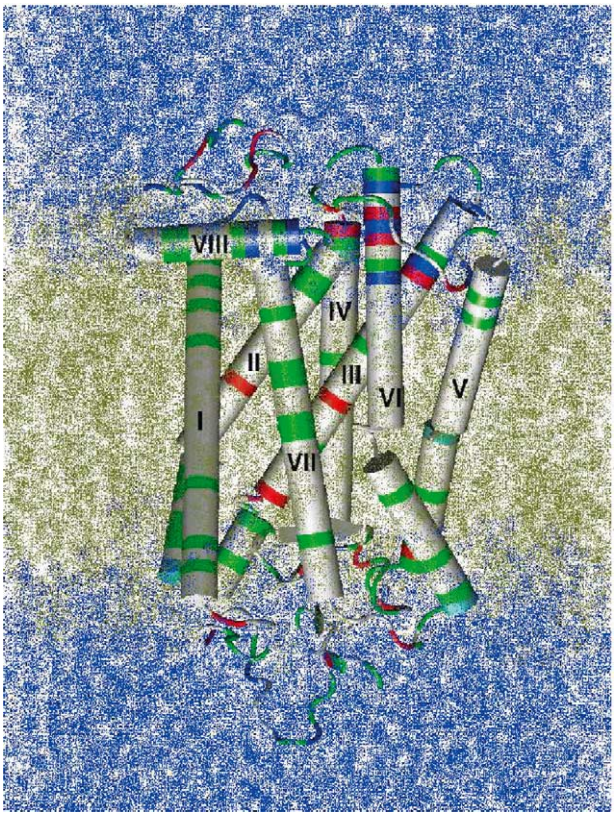

Fig. 3. A recent molecular dynamics simulation of rhodopsin in an explicit solvent environment [12]. Water (7441 TIP3) is shown in blue and lipid (99 DOPC, two palmitate) is shown in tan. The protein is illustrated in cartoon form with the helices labeled and demonstrates the relative position of the protein in the bilayer. The cytoplasmic side is to the top, with helix VIII an amphipathic helix. Basic residues are colored blue, acidic residues red, polar residues green, and non-polar residues are white. The total system has 41,623 atoms in all.

K-channels and other membrane systems. In each case the details of the solvent, and especially the electrostatic response may be key to understanding, correctly, the details of the large-scale motions and the connections from a detailed structure to a molecular model of function. Getting the long-range electrostatics right may be possible in implicit solvent models, but there are many trade-offs in getting the electrostatics correctly (taking more computer time) along with appropriate sampling (also taking computer time). We developed a lattice dipole model of the membrane setting that attempts to capture the electrostatic heterogeneity of the membrane setting while enabling faster sampling [66] (Fig. 4). Other groups have applied the generalized born approach with accessible surface area arguments to this same type of calculation [75] or used an effective energy function that models the electrostatics and near-field effects [74]. Probably, some combination of near-field atomic accuracy with long-range electrostatic response will be required to understand large-scale conformational motions [66,76].

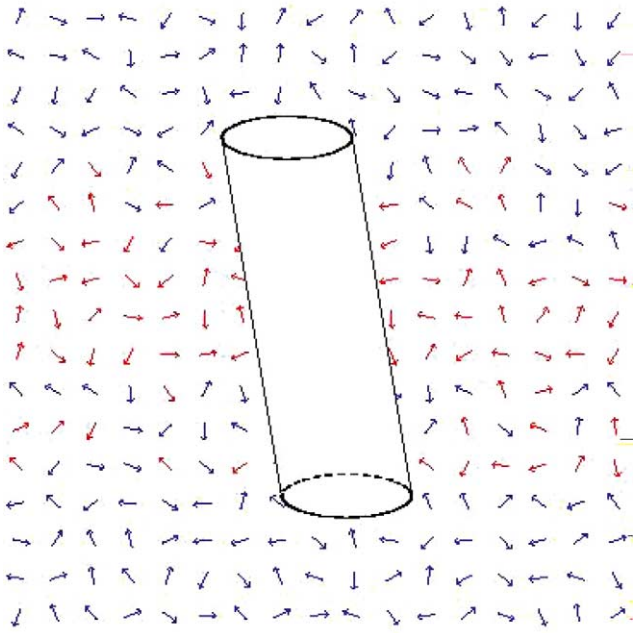

Fig. 4. An implicit solvent model of the membrane bilayer [66]. A mesoscopic electrostatic response is simulated by a lattice of Langevin dipoles. The properties of the dipoles change with position relative to the normal to the bilayer plane. Those furthest from the bilayer center represent water, while those in the center are more alkane like in their electrostatic response. This type of model captures the complexity of the membrane interface: a region that can vary from 10 to 15 Angstroms in thickness and that contains the headgroup and glycerol regions of the lipid bilayer.

2. Methods aimed at gating

2.1. Reaction path computations

The late 1980s saw the first strategy, developed by Pratt, for side-stepping the problem of short MD time-scales [13]. The key insight was to distinguish the waiting time between "transition" (e.g. permeation) events from the relatively short duration of the event itself, and attempt to focus computer time on the latter. Pratt devised an approach to generate an ensemble of transition path trajectories solely from knowledge of the starting and ending states, but with no knowledge of the intermediate states or with a defined reaction coordinate. In the immediate years after the paper, a few authors suggested algorithms, but no particular candidates were defined [14–19]. Certainly, the reigning wisdom was that a special path-finding algorithm should first define a reaction coordinate [20–22] and then other special purpose algorithms should be run to define a relative free energy, finally a reactive flux calculation for the transition path along the reaction coordinate would be used to determine the kinetics [23]. While this prescription works well for simple systems, it is not clear whether it will work for a complex bio-system where multiple pathways may well contribute to the final measured rate. Thus, for potassium channels, this type of approach to gating calculations would suggest first performing a reaction path calculation along an adiabatic (zero effective temperature path), the parameterization of the conformational changes in the adiabatic path would then be used for a relative free energy calculation. Following the determination of the transition state along the reaction path, the kinetics would then be estimated (and compared to experiment) with a third calculation for the rate of barrier crossing.

In the late 1990s, several groups independently defined approaches for the computation of intermediate states that connected determined starting and ending points [24–27]. All the current approaches take their conceptual starting point from Pratt's idea to focus computer time on transition events [28–43].

2.2. Transition path sampling

Pratt's approach was most directly embodied in the Chandler group's "transition path sampling" protocol, in which an initial path of arbitrary quality is used to initiate metropolis sampling of a properly distributed *ensemble* of transition paths [24]. The Chandler group's contributions include determining a general algorithm for rapidly sampling in the space of paths, as well as developing a separate calculation for computing the transition rate from the ensemble of paths [44–51]. Thus far, the transition path sampling approach has been applied to systems with variable sizes where the largest is an explicitly-solvated alanine dipeptide.

2.3. Dynamic importance sampling, an alternative approach

Other approaches use methodology independent of Pratt's Metropolis scheme [24,26,52]. A primary motivation for non-Metropolis methods is to avoid the possibility of "trapping" in a restricted part of path space—a risk inherent in any Metropolis procedure.

The general approach of non-Metropolis importance sampling can be seen by considering the average of a quantity g over a phase space x. With unbiased sampling the average is simply the relative probability of visiting each location $Q(x)$ and the value at that location $g(x)$. A biased sampling can be used, with correction, to increase the efficiency of sampling. In that setting, a new distribution $D(x)$ is created and samples collected under $D(x)$ are then corrected relative to $Q(x)$ to give the original unbiased distribution.

$$\langle g \rangle = \int g(x)Q(x)\mathrm{d}x = \int \frac{g(x)Q(x)}{D(x)} D(x)\mathrm{d}x$$

While importance sampling has a long history in the Monte Carlo community, and variants of it have been used for free energy calculations with umbrella sampling in molecular dynamics, there has not been an easy way to generate pure dynamic trajectories that efficiently start at one state and end at another. To improve this situation, we developed a method

that we call dynamic importance sampling (DIMS) [26,52]. In our formulation of the problem there is no need to define a reaction coordinate in advance. This is a major aid for large systems, like proteins, where it is difficult to clearly define a single reaction coordinate. In fact, the method will create a set of properly weighted trajectories with their associated probabilities of occurrence, through application of weighting functions that are corrected at each time-step. The resulting set of information can be used for determination of temperature refined pathways, kinetics, and relative free energies between the defined starting and ending states.

The DIMS method can be illustrated in the context of Brownian dynamics simulations. In unbiased Brownian dynamics simulations (simulations where the protein is represented in full detail, while the solvent degrees of freedom are represented approximately by a random force and friction coefficient from a temperature coupled bath), the first order differential equation that describes the time evolution of each atom of the system is provided by:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{f}{m\gamma} + R(t)$$

where the m is the mass of atom, f the force on the atom, γ the friction coefficient, and $R(t)$ is a sampled random force from the bath degrees of freedom. The time evolution of this type of equation will show a random walk due to the bath coupled with the deterministic walk of the protein itself at the thermal temperature chosen for the simulation. With sufficient computer time (and a good implicit solvent model) the protein will visit all the important states of the system. That is, this type of simulation would show permeation and gating, on a sufficiently long time-scale. (Such time-scales, in *unbiased* Brownian simulation, are however still well out of reach.)

For computations, the equation is written in a discrete form:

$$x_{j+1} = x_j + \left(\frac{f_j}{m\gamma}\right)\Delta t + \Delta x_{\mathrm{R}}$$

In general, for each time step, the transition probability to the next x-position can be determined. In an equilibrium system, with no bias, the transition density will be a Gaussian, centered on the current x-position and with a mean and deviation determined by the systematic (protein) force and the random (solvent) force. This directly means that a full distribution of possible moves can be assigned from a current position to the next position. In other words, the trajectory can be viewed as a realization of a particular walk through the probability space of possible moves, or as a sequence of unbiased probable walks that start at one time point and end at another.

$$T_{\Delta t}(x_{j+1}|x_j) = \frac{1}{\sqrt{2\pi\sigma^2}}\exp\left\{\frac{-[(x_{j+1}-x_j)-(f_j/m\gamma)\Delta t]^2}{2\sigma^2}\right\}$$

In a slightly more formal manner the full probability of the trajectory can be viewed as the product of independent steps along the path (all of the same step size Δt). The unbiased probability of the trajectory is then determined:

$$\widetilde{Q}(\zeta) = \prod_{j=0}^{n-1} T_{\Delta \mathrm{t}}(x_{j+1}|x_j)$$

Using ideas that are rooted in importance sampling, we realized that the dynamic trajectory itself can be biased at each time step, by using a separate distribution that is more likely to lead to the desired end-point. *In other words, if a biased step is taken and corrected for at each time step, then an un-biased estimate of the probability of reaching a particular end-point after a set number of steps (n) can be defined*:

$$P_{\mathrm{B}}(t_n|0;x_0) = \int \widetilde{Q}(\zeta)h_{\mathrm{B}}(x_n)\mathrm{d}\zeta = \int \frac{\widetilde{Q}(\zeta)}{D(\zeta)}D(\zeta)h_{\mathrm{B}}(x_n)\mathrm{d}\zeta$$

Where h_{B} is an indicator function to show that the end-state has been reached. This expression hides the difficult choice of determining an optimal distribution (D) that can be used to bias the walk from the starting point to the end-point. We have studied several different possible forms for this distribution. The simplest is to consider the situation for a model system in one or two-dimensions. In that situation, the biasing distribution is relatively simply defined by starting with a weak bias generally towards the end-point with a broad Gaussian shape and then as the simulation time advances, a more narrow (stronger bias) Gaussian is used to further bias the distribution of trajectories to end at the desired end-point near to the desired end-time. Our experience with this approach, and other similar formulations, led us to realize that we had to understand more about the nature of the transition event [53,54], in order to create biasing distributions that differed as little as possible from the unbiased probability distributions. If the biasing distribution became too different from the unbiased probability of the same move in a transition event, then the penalty for the biased walk is large and the resulting trajectory a poor sample of the true (unbiased) transition events [53].

2.4. Successful DIMS algorithms

By considering, in some detail, the nature of successful transitions, we realized that a large gain in efficiency could be realized by constructing the biasing to have a similar form to that found in unbiased crossing events [53,54]. Thus, one algorithm, that was shown to work well in simple model systems, consists of two steps: (i) waiting until unbiased fluctuations bring a trajectory to an "edge" of the initial state, and (ii) then applying an optimal bias, based on Onsager–Machlup theory, to force the full transition. The optimality of the forcing bias ensures the net penalty for the bias is small, resulting in a large gain in efficiency.

While the same general approach could be applied for protein systems, it is more difficult to define a general form for the biasing distribution. We therefore developed a more general algorithm, the '*soft-ratcheting*' approach [55]. This

algorithm generates independent trajectories, avoiding the potential trapping problems of Metropolis path sampling, but it does so using a hybrid of Brownian and Metropolis-like *intrinsic* dynamics to generate trajectories. (The intrinsic, step-by-step dynamics are to be distinguished from the path sampling algorithm.) In particular, unbiased Brownian dynamics steps are generated in the usual way, but these are accepted or rejected according to a Metropolis-like criterion. The acceptance criterion, defined by the user, accepts all steps proceeding towards the desired final state, and also accepts a fraction of other events. The resulting "ratcheting" is soft because the system need not proceed monotonically toward the target state. Furthermore, the Metropolis probabilities may be used to estimate a weight (score) for the transition trajectory; full details are given in Zuckerman and Woolf [55]. Conventional targeting schemes do not associate scores with the trajectories generated.

We show some results from this type of 'soft-racheting' in the CHARMM program for the alanine dipeptide (Fig. 5). A strength of the approach is that multiple pathways may be determined without trapping. Note, in particular, that the trajectories can start off in any direction from the initial state and thus they sample a large region of conformation space–space that would be hard to sample without the biasing distribution. Additionally, scores are associated with all paths, which permits the determination of more probable pathways and weighted averages.

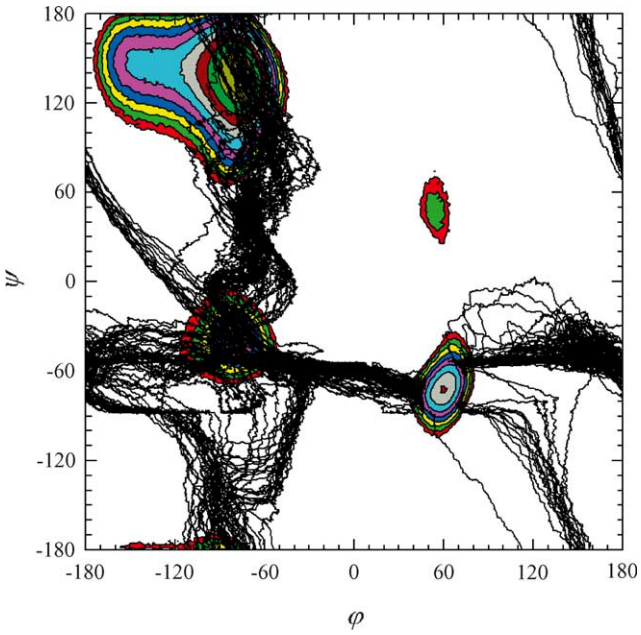

Fig. 5. Dynamic importance sampling used to sample possible transitions from the αR state to the c7axial state of the alanine dipeptide [26,52,55]. These are the states in the lower left (start) and the lower right (end) of the figure. The alanine dipeptide has proven to be an excellent model system for testing ideas for protein simulation methods before application to larger systems. In this case, note that trajectories are sampled in many directions from the starting (αR) state. The trajectories terminate on entrance to the final (c7ax) state. Relative weights associated with each trajectory show the likelihood of each.

2.5. Gating summary

Thus, the calculation of gating events requires the development of special sampling methods that will focus the computational resources on the relatively brief transition events and not on the sampling of events within the stable states themselves.

3. Methods aimed at permeation

3.1. Permeation and selectivity

Ion channels, for example the potassium channel, have the ability to discriminate between different cation types and furthermore to mediate a high flux rate of that type through the channel. How the protein is able to lower the free energy barrier for a charged ion over a membrane bilayer and to simultaneously discriminate charge type is an ongoing research question for ion channel structure function connections. The computation that is needed, unlike that behind gating, has a more natural reaction coordinate [56,57]. The cation (or anion) can be described as following an essentially normal to the bilayer path and the potential of mean force (integrated work) for the traversal of the ion through the protein medium can thus, in principle, be calculated. But, given a relatively simple sounding prescription, it does not follow that the computation is efficient. There are several approaches that might be followed. First, a "slow-growth" type of calculation might be used where the ion is restricted to small changes along the axis normal to the bilayer plane and the small changes are then integrated and averaged over trajectories to get a final estimate. The problem, as is known historically, with "slow-growth" as applied directly is that the Hamiltonian can lag the system, and systematic errors result [77]. A second method is to use umbrella sampling or a similar biasing potential to collect data within windows along the direction normal to the bilayer plane. Using the weighted histogram analysis method (WHAM) the bias introduced by the set of potentials can then be removed and the underlying free energy profile computed [58]. While this is probably the method of choice in many groups, we would like to suggest that at least two other methods should be considered for this type of calculation.

3.2. Method 1: overlap sampling for permeation

Understanding overlap sampling (OS) starts with a brief review of free energy perturbation (FEP) calculations. In a free energy perturbation [67] computing the free energy difference (ΔA) between two states (designated as 0 and 1), is performed where the free energy change is estimated using ensemble average of a small perturbation (u)

$$\exp(-\beta\Delta A) = \langle \exp(-\beta u) \rangle_0$$

A large change between the initial and final states can be divided into a series of smaller ones (i.e., multiple stages), and the FEP formula can be applied to compute relative free energies for each stage. For example, this might be a series of changes along the reaction coordinate for permeation of potassium through the potassium channel. Note that for a given perturbation pair, the FEP can be conducted in two opposite directions which are usually referred to as forward (e.g. from 0 to 1) and reverse (from 1 to 0). It is known that the forward and reverse calculations do not produce identical estimate of free energy, due to different finite sampling errors [68,69]. However, the free energy simulation can be designed to greatly reduce this type of sampling error; one effective design is to combine the sampling of forward and reverse calculations in an overlap sampling method.

Finding the optimal approach for the free energy difference between two nearby states was first investigated by Bennett [59]. The statistical error in the estimate of ΔA can be minimized by combining sampling data from the two simulations, one on state 0 and perturbing to state 1, the other on state 1 and perturbing to state 0. In this picture of the problem, it differs from WHAM in that it considers only the free energy difference between two points along a reaction coordinate. Recently, we considered the reliability (due both to statistical and to systematic errors) of free energy calculations (Fig. 6). We realized that to reduce the systematic error of free energy calculation, one has to ensure appropriate sampling for both the reference state (e.g. 0) and target state (1) [69]. The overlap sampling method [70] was devised based on this principle. The OS method starts with a pair of systems (again designated as 0 and 1) sharing common phase space regions (in other words, phase spaces of 0 and 1 systems partially overlap), and it constructs an intermediate (M) between 0 and 1, it then performs two separate perturbations to compute the free energy difference: one from 0 to M, the other from 1 to M. A key point is that the phase space of M is accessible either from system 0 or system 1, thus good sampling in both FEP calculations can be ensured. The general working formula for the OS method is

$$\exp(-\beta\Delta A) = \frac{\langle w(u)\exp(-\beta u/2)\rangle_0}{\langle w(u)\exp(+\beta u/2)\rangle_1}$$

In this formula $w(u)$ is a weighting function associated with the perturbation (u) from state 0 to state 1 and vice versa. This formula is correct for any arbitrary choice of $w(u)$. Note that the working formula involves no quantities of the intermediate M, thus neither an explicit definition nor a simulation of the intermediate M is needed. From this point of view, OS can be seen as a technique to combine sampling on states 0 and 1 for a better free energy estimate. This feature also allows the freedom to adjust $w(u)$ for an optimal free energy calculation without changing the simulations performed on states 0 and 1. One particular choice of $w(u)$

$$w(u) = \frac{1}{\cos h[\beta(u - C)/2]}$$

creates a OS working equation with a form mathematically identical to that of Bennett's original formulation. It can be shown that the free energy estimate produced using this method is not only the most precise, but also the most accurate [70,71]. If needed, multiple overlap sampling calculations could be employed in computing the overall free energy difference between the initial and the final states. This makes it similar to a WHAM calculation.

In general, we believe that an intelligent set of sampled distributions placed along a reaction coordinate, combined with overlap sampling may be more efficient for sampling of events than a series of perturbations with an arbitrary and equally spaced series of changes along a reaction coordinate. It may be the case that WHAM and umbrella sampling can be improved in efficiency, by consideration of the optimal spacing between window locations (perturbation locations), rather than by checking after the calculations (or assuming) that the information from all umbrella windows has covered the phase space well. In other words, the overlap sampling method provides an approach to ensure sufficient sampling between non-uniform spaced locations along a reaction coordinate. This ensures strong sampling of events all along the reaction coordinate and provides an efficient and less error-prone estimate of the relative free energy differences.

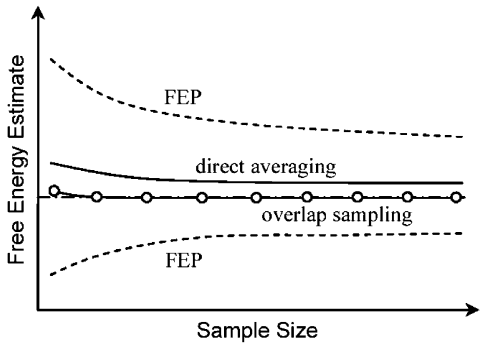

Fig. 6. Overlap sampling, in general, gives faster convergence for a given sample size than does traditional free energy perturbation calculations or direct averaging (i.e. a simple combination of forward and reverse FEP) [60]. The figure summarizes test results from systems of alchemical change for anions and adenosine. We expect that this generalizes to larger protein systems.

3.3. Method 2: non-equilibrium work connections to relative free energy

Jarzynski showed that non-equilibrium work events (requiring energy W) along a reaction coordinate can be combined to give a relative, equilibrium, free energy difference [61,62]:

$$\exp(-\Delta A/kT) = \langle\exp(-W/kT)\rangle$$

In particular, the derivation showed that any system, coupled canonically to a heat bath evolving with MD or MC could

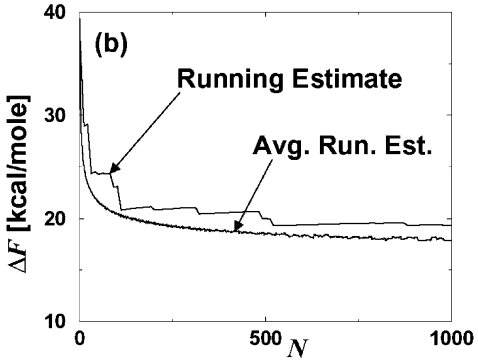

Fig. 7. Due to the sensitivity to rare events, the non-equilibrium work average will reflect large changes when a single running average is considered. In contrast, the block averaged running estimate can be used to create a smooth curve along with error estimates [64,65]. In this case the relative free energy difference between the fatty acids palmitate and stearate were computed. These two fatty acids are common components of the lipids found in biomembranes [78].

generate a distribution of work events that will comment on the free energy difference. In practice, the width of the distribution of work events may become too broad for an accurate estimate of the relative free energy difference and thus a large amount of sampling is required to obtain a good work distribution [63–65,72,73]

We realized that one possible solution to the problem of the work distribution was to estimate the converged distribution by extrapolation from the collected set of work events [64]. In this approach the non-equilibrium work estimate is seen as a a non-linear (exponential) average that will be dominated by the rare events of low work. To enable a smooth curve and extrapolation to be rationally defined, we considered the behavior of a block average [72,73]:

$$\Delta A_n = \frac{1}{m}\sum_{j=1}^{m} - kT \ln\left[\frac{1}{n}\sum_{k=(j-1)n+1}^{jn} \exp(-W^{(k)}/kT)\right]$$

where $W^{(k)}$ is the kth work value. In this formula sets of n data points are considered to form m estimates of the free energy from a total of $N = m \times n$ total work events (see Fig. 7). By graphically plotting the behavior of this curve as a function of $1/n$ (or $(1/n)^a$ with $a \cong 1/4$), a smooth curve that can be extrapolated to an estimate of the free energy can be defined [64].

3.4. Permeation summary

New relative free energy methods can be developed to improve sampling along a defined reaction coordinate. These methods will be important for the application of computational resources to large biomolecular events where both efficiency and accuracy are important. An example of this type of calculation is the permeation free energy along the potassium channel axis.

3.5. Conclusions

The wonderful insights generated by the experimentally determined X-ray structures of K-channels offer both promises and potential pitfalls for those investigators interested in simulating experimental properties derived from the structures. The challenges are essentially related to the large amount of sampling that will be needed to confidently ascertain a particular molecular mechanism for permeation or for gating. At the same time, the molecular insights that can be generated from simulation promise to suggest whole new classes of experiments that are truly based on molecular analysis of important transitions and important amino acids. It is hoped that continuing improvements in computer hardware, software algorithms for sampling and experimental design of questions that can be addressed via simulation will improve our ability to understand the molecular basis of ion channel function.

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