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Through the Channel and around the Channel: Validating and Comparing Microscopic Approaches for Evaluation of Free Energy Profiles for Ion Penetration through Ion Channels

Mitsunori Kato and Arie Warshel*

Department of Chemistry, University of Southern California, 3620 McClintock Ave., Los Angeles, California 90089-1062

Abstract

Microscopic calculations of free energy profiles for ion transport through biological ion channels presents a very serious challenge to modern simulation approaches. The main problem is due to the major convergence problems associated with the heterogeneous landscape of the electrostatic environment in ion channels and with the need to evaluate the profile associated with the transfer of the ion from bulk water to the channel environment. This problem is compounded by the lack of reliable and relevant benchmarks that can discriminate between alternative approaches. The present study is aimed at reducing the above problems by defining benchmarks that are directly relevant to ion channels and can also give converging results. This is done by constructing a series of models of a truncated gramicidin channel with different numbers of water molecules and by comparing the profiles for going around the channel and through the channel. These discriminating models are then used to validate and compare the adiabatic charging free energy perturbation (FEP) approach combined with an umbrella sampling approach (Warshel *J. Phys. Chem.*, 86, 2218, 1982) and the potential of mean force (PMF) approach used frequently in studies of ion channels. It is found that both approaches work quite well until one moves to the fully solvated channel case. In this limit the PMF approach starts to give different results for the overall work of going through the channel and around the channel while the FEP approach gives physically consistent results. The present benchmark also indicates that the weighted histogram analysis method (WHAM) approach does not offer a significant advantage over earlier approaches at least as much as studies of ion channels are concerned. Finally, it is concluded that the FEP approach may be more useful in evaluating the overall barrier for moving ions from water to ion channels and that in some cases it might be beneficial to use the FEP approach for selective points along the channel and then to connect these points by PMF calculations.

I. Introduction

Movements of ions through specific transmembrane channels underlie many important biological functions ranging from oxidative phosphorylation to electric signaling in neural and muscular systems¹. Although a large amount of electrophysiological data provides a qualitative picture of the action of such channels (*e.g.* 1⁻⁴), a detailed molecular picture of the control of ion permeation is still a partially unresolved problem.

The recent elucidation of the crystal structure of a bacterial K⁺ channel (KcsA from *Streptomyces lividans*)⁵ and other experimental studies (*e.g.* 6,7) has offered the exciting possibility of structure-based studies of biologically relevant channels. Here the ultimate

*Corresponding author E-mail: warshel@usc.edu

challenge involves a detailed correlation of the structure of ion channels with the corresponding ion current. This includes understanding the control of ion currents by external factors such as voltage, pH, concentration gradient and drugs. It also includes the ability to predict and analyze the effects of various mutations.

Although macroscopic (*e.g.* 8-11) and semimacroscopic approaches 12-14 can provide a reasonable description of the energetics of ion permeation in ion channels, it is clear that microscopic simulations are essential in order to obtain a detailed quantitative description of such systems. An early attempt to perform microscopic calculations was unable to provide a reasonable energy profile 15. However, the introduction of a consistent electrostatic treatment and a free energy perturbation (FEP) treatment led to the first consistent microscopic study of the energetics of an ion channel 16 and overcame the challenge of obtaining reasonable barriers. Subsequent PMF studies 17 also seemed promising despite the fact that they overestimated significantly the observed barriers. Nevertheless, fundamental problems remained with regards to the convergence of the calculations as well as with regards to long-range treatments and the need for use of a polarizable force fields (see discussion in 12,13,18).

The above mentioned long-range issue could be reduced drastically by using spherical polarization constraints 19, boundary conditions 19,20 and the local reaction field method 21, and similarly the use of polarizable force fields (introduced in 22) is in fact simpler than what is commonly assumed particularly when one uses the noniterative method 23.

Unfortunately, the convergence problem presented an extremely serious and frequently overlooked problem. Despite the emergence of promising strategies 24-27, it is not clear that microscopic simulations of ion transport in ion channels give converging results (see discussion in 12,13,18, see also 28). What seems to be missing is a simple benchmark that can tell us which approach actually works and what is required for a reasonable convergence. Here it is not sufficient to consider recent studies of different free energy calculations 29,30, since these studies used test cases that are not directly relevant to the electrostatic free energy of ion channels. More specifically, instructive test cases such as studying the energetics of rotation around the bond of alanine dipeptide 31,32, mutating methane to methanol 33, or ethane to ethanol 29, are not so useful in telling us if a given model will be able to properly sample the complex energy landscape associated with the penetration of ions through ion channels. One may try to establish statistical estimates of convergence (*e.g.* 29), but in our opinion and experience this is not the main issue and in general it is extremely hard to establish the convergence limit in complex biophysical systems without persistent consideration of the effect of different factors (*e.g.* ionized residues), boundary conditions and initial configurations. In general, in studies of biophysical problems the key issue is the convergence of the specific method in the specific system. This issue must, of course, be tested in the relevant system. Unfortunately, to the best of our knowledge no cooperative study has been reported as much as ion channels are concerned.

In looking for an effective benchmark we cannot start with a full-blown solvated channel since in this case it is not clear what is the “correct” answer for the given force field. Here we need to search for a closely related benchmark but restrict the search to sufficiently simple systems that will allow a convergence of different models. Obtaining the same free energy profile by different simulation approaches will guarantee that we obtain the correct results for the given model system and will allow us to compare the effectiveness and validity of different treatments. This work focuses on the above idea and generates several benchmarks, with increasing levels of complexity, for studies of ion transfer through ion channels.

Section II will outline our benchmark systems, Section III will describe our simulation methods and section IV will compare the performance of the different approaches in evaluation the free

energy profile for the different model system. The conclusions that emerge from our study will be summarized in section V.

II. Defining benchmarks

As stated in the introduction the aim of this work is to introduce simple yet discriminative benchmarks for studies of ion channels. In doing so we generated four models with differing levels of complexity (see Fig. 1). The first model (Model A) has been constructed by taking the structural model of gramicidin A (gA) and its environment, removing the side chains, the membrane and all the solvent molecules, and including one Na⁺ ion. Position constraints of 10 kcal/mol·Å² are introduced on the main chain Cα atoms to create a stable benchmark, because the membrane is removed and the model channel can not keep the shape by itself. This generated the simple polar/non-polar narrow channel depicted in Fig. 1A.

The second model (Model B) and the third model (Model C) were generated from model A by adding one (Model B) or four (Model C) water molecules before and after the ion (Fig. 1B,C). This introduced an additional complexity and as will be seen below a significantly more complex landscape.

Finally we generated a more realistic model (model D), where we shortened the channel in model A to a half by taking one of two symmetric units, and then immersed it in an SCAAS water model¹⁹ with a radius of 14 Å. Making the channel shorter allowed us to solvate it completely in the water sphere and to reduce problems associate with calculations of a PMF in a partially solvated channel.

III. Simulation methods

In this work we will compare the performance of two methods that are described schematically in Fig. 2. The first method involves an adiabatic charging by free energy perturbation (FEP) approach^{16,34} coupled with an umbrella sampling approach³⁵. This approach changes the charge of the system from 0 to Q₀ in an adiabatic charging (AC) process and evaluates the corresponding free energy by using a mapping potential of the form

$$V_m(\lambda_m) = (1 - \lambda_m) V_a + \lambda_m V_b + E_{cons}(z) \quad (1)$$

where V_a and V_b are the potential of the solute – solvent interaction for Q=0 and Q=Q₀ respectively. The additional potential E_{cons}(z) keeps the system at a given region of the channel axis, z, and is given by E_{cons}(z) = K(z-z₀)². The free energy associated with the charging process is then evaluated by (e.g.^{36,37})

$$\begin{aligned} \Delta\Delta G(\lambda_m \rightarrow \lambda_{m+1}) &= -\frac{1}{\beta} \ln [\langle \exp \{-(V_{m+1} - V_m)\beta\} \rangle_m] \\ \Delta G(V_a \rightarrow V_b) &= \Delta G(\lambda_0 \rightarrow \lambda_n) = \sum_{m=0}^{n-1} \Delta\Delta G(\lambda_m \rightarrow \lambda_{m+1}) \end{aligned} \quad (2)$$

where λ_m changes in 51 steps between zero and one.

In the next step, we obtain the free energy probability at the Q₀ state at different points along the channel axis by the approach using^{16,38}

$$\Delta g_b(z)_{AC} = -\frac{1}{\beta} \ln [\exp \{-\Delta G(V_a \rightarrow V_b)\beta\} \langle \delta(z' - z) \exp \{E_{cons}(z)\beta\} \rangle_b] \quad (3)$$

The second approach is a potential of mean field (PMF) approach, where the free energy of moving the ion from one position to another is evaluated by the cycle of Fig. 3. This is done by using the umbrella sampling in the implementation used in our studies of electron transfer

reactions^{35,38} and in the evaluation of the free energies of the EVB approach (e.g.³⁹). In this approach, we use a mapping potential of the form

$$\varepsilon_m = (1 - \lambda_m) \varepsilon_1 + \lambda_m \varepsilon_2$$

$$\varepsilon_1(z) = E_g(z) + E_{1, \text{cnstr}}(z) = E_g(z) + K(z - z_0^{(1)})^2$$

where

$$\varepsilon_2(z) = E_g(z) + E_{2, \text{cnstr}}(z) = E_g(z) + K(z - z_0^{(2)})^2 \quad (4)$$

where $z_0^{(1)}$ and $z_0^{(2)}$ are two neighboring points on the z axis and $E_g(z)$ is the total potential of the systems (i.e. the solvated Na^+) without the constraints. We thus use our FEP/US formula³⁵

$$\begin{aligned} \Delta g(z)_{\text{PMF}} &= -\frac{1}{\beta} \ln \left[\exp \{ -\Delta G(\lambda_m) \beta \} \langle \delta(z' - z) \exp \{ - (E_g(z) - \varepsilon_m(z)) \beta \} \rangle_{\varepsilon_m} \right] \\ &= -\frac{1}{\beta} \ln \left[\exp \{ -\Delta G(\lambda_m) \beta \} \langle \delta(z' - z) \exp \{ E_{m, \text{cons}}(z) \beta \} \rangle_{\varepsilon_m} \right] \end{aligned} \quad (5)$$

where $E_g(z) - \varepsilon_m = E_{m, \text{cons}}(z)$

In using the PMF approach, one encounter frequently enormous hysteresis, which will be also illustrated in this work. This effect can be reduced by different approaches (e.g. 40). In this work we found that the hysteresis can be drastically reduced by evaluating the $\Delta G(\lambda_m)$ and the $\langle \delta(z' - z) \exp \{ E_{m, \text{cons}}(z) \beta \} \rangle_{\varepsilon_m}$ terms of Eq. 5 as the average of the forward and backward runs, using

$$\begin{aligned} \Delta \Delta G_{\text{ave}}(\lambda_m \rightarrow \lambda_{m+1}) &= \frac{1}{4} \left(\{ \Delta \Delta G(\lambda_m \rightarrow \lambda_{m+1}) - \Delta \Delta G(\lambda_{m+1} \rightarrow \lambda_m) \}_{\text{forward}} \right. \\ &\quad \left. + \{ \Delta \Delta G(\lambda_m \rightarrow \lambda_{m+1}) - \Delta \Delta G(\lambda_{m+1} \rightarrow \lambda_m) \}_{\text{backward}} \right) \\ \langle \delta(z' - z) \exp \{ E_{m, \text{cons}}(z) \beta \} \rangle_{\varepsilon_m, \text{ave}} &= \frac{1}{2} \left(\langle \delta(z' - z) \exp \{ E_{m, \text{cons}}(z) \beta \} \rangle_{\varepsilon_m, \text{forward}} \right. \\ &\quad \left. + \langle \delta(z' - z) \exp \{ E_{m, \text{cons}}(z) \beta \} \rangle_{\varepsilon_m, \text{backward}} \right) \end{aligned} \quad (6)$$

Of course the AC procedure also involve well-known hysteresis which is minimized here by evaluating the average of the forward and backward mapping.

In order to compare the two approaches in test cases that introduce water molecules inside the ion channels it is sometimes important to use the cycle of Fig. 3.

All simulations were performed with the MOLARIS program package. The SCAAS boundary conditions were used for model D. The specific parameter set used is summarized in Table 1. The AC process includes typically 51 steps at 300°K and with 1fs time steps. The specific simulation times will be given in the captions of the figures that describe the different results.

IV. Results and Discussion

In the first step we examined the first benchmark (Model A) composed of the ion channel and no solvent. The results obtained by the two approaches are summarized in Fig. 4. Apparently both methods work without any problem in this case. We used the cycle A of Fig. 3 for model A.

$$\Delta G_{\text{PMF}}(\text{Na}^+(z \rightarrow z + \delta z)) \approx \Delta G_{\text{AC}}(\text{Na}^{(0)}(z + \delta z) \rightarrow \text{Na}^+(z + \delta z)) - \Delta G_{\text{AC}}(\text{Na}^{(0)}(z) \rightarrow \text{Na}^+(z)) \quad (7)$$

In the second step, we used as a benchmark models B and C (the channel, the ion, and either two or eight water molecules). In these cases we had to use the cycle of Fig. 3B,C and the relationship:

$$\Delta G_{\text{PMF}}(\text{Na}^+(z \rightarrow z+\delta z)) - \Delta G_{\text{PMF}}(\text{Na}^{(0)}(z \rightarrow z+\delta z)) = \Delta G_{\text{AC}}(\text{Na}^{(0)}(z+\delta z) \rightarrow \text{Na}^+(z+\delta z)) - \Delta G_{\text{AC}}(\text{Na}^{(0)}(z) \rightarrow \text{Na}^+(z)) \quad (8)$$

where $\Delta G_{\text{PMF}}(\text{Na}^{(0)}(z \rightarrow z+\delta z))$ is not negligible in these cases.

The results obtained for model B and C are summarized in Figs. 5 and 6. Again both methods appear to work without major problems.

Next we moved to the much more challenging benchmark of model D (the channel the ion and a sphere of water molecules). The corresponding results are summarized in Fig. 7. Here we started to obtain different results from the two approaches, which meant that we could not determine which one was the correct approach. To resolve this problem we considered the cycle described in Fig. 8 and obtained the results depicted in the same figure. The most important point that we try to convey in Fig. 8 is that using the two paths between 1 and 2 should allow one to evaluate the hysteresis by PMF. When a Na^+ ion goes through the channel and comes back around the channel to the original point as shown in Fig. 8, the free energy at the identical point should be the same if there is no hysteresis. Considering the little hysteresis for the PMF that goes through path 2 (moving through the solvent) of Fig. 8. We can assess the hysteresis of the PMF that drags the ion through path 1 (through the channel) by requiring it to give the same results as those obtained in path 2. This provides a practical way of assessing the relationship between the heterogeneity of the environment and the resulting hysteresis. It is also useful to note here that we report quite long simulation times (up to 1.12 μs using 100 CPUs of Pentium III 1.0GHz processors for about a week). These long simulation times were possible in part because of the use of the SCAAS boundary condition.

It is useful to note that the results reported in Fig. 7 already reflect the averaging (Eq. 6) of the large hysteresis of the PMF calculations. This fact is illustrated in Fig. 9 which provides the hysteresis without averaging the forward and backward PMF. It is also useful to note that the PMF hysteresis before averaging is about 6kcal/mol as compare to 3kcal/mol in the AC treatment that did not involve averaging

Armed with the information from the above validation studies we explored the possibility that the problems with our PMF calculations are related to the way we handle the statistics obtained from the simulations. In particular, we addressed the assumption that the so called “weighted histogram analysis method” (WHAM) approach^{24,32,40} offers a major improvement in convergence of PMF and related calculations. This approach has formulated a relatively rigorous way for connecting the free energy segments obtained from sequential simulations with different coupling parameters. It has been argued however, that it is not clear that the WHAM approach provides an improved convergence relative to the use of the mapping free energy as the tool of overlapping the simulation results obtained with different windows^{35, 41}. Fig. 10 compares the PMF obtained with the WHAM protocol to that obtained with our FEP/US procedure (Eq. 5). As appears from the figure, both approaches give basically identical results.

V. Concluding Remarks

There seems to be a perception that the error range in biological simulations can be estimated by statistical analysis of the given simulation and that the use of sophisticated formulations such as the Jarzynski's formula²⁷ or the WHAM approach²⁴ guarantees the reliability of the simulated results. There is also an apparent tendency to use standard benchmarks such as the alanine dipeptide in validating free energy calculations (*e.g.*^{31,32}) and then to assume that the convergence obtained in the test case is relevant to studies of very different systems.

Unfortunately, in our experience for a long time (*e.g.* ^{42,21,43}), the convergence and reliability of microscopic and macroscopic free energy calculations cannot be validated by a specific formulation or by running a simulation with a single set of initial conditions. It appears that the convergence of such calculations is system dependent and that the only way to establish their reliability is to use directly relevant benchmarks, to compare different approaches, to start from different initial conditions and if possible to use experimental benchmarks.

The present work illustrates our points of view, while focusing on the assessment of the validity and accuracy of different methods for evaluation of the free energy profile for ion penetration through ion channels. This was done by constructing benchmarks that are simple enough to allow for actual convergence, yet sufficiently related to the problem of ion penetration through ion channels.

Because there is no rigorous way to define a general analytical limit for the convergence of free energy studies of ion channels we define this limit by requiring different methods to give the same results. At the point where this becomes difficult to accomplish we introduce the requirement of getting the same results for moving the ion through the channel and around the channel (see also below).

Our study demonstrated that the FEP/US based AC and PMF approaches converge at times of 100ns and 1 μ s respectively for very simple solvated ion channels. However, the PMF approach suffers from inability of detecting whether the model is actually correct. That is, with the PMF we cannot detect any problem with long-range treatments since it evaluates only the relative energy of the ion in different sites without having any clue about the absolute energy. Unfortunately, it is the absolute energy that can give an indication about incorrect electrostatic treatments.

Another interesting finding of the present work is the demonstration that the WHAM approach ²⁴ does not seem to give improved results relative to the earlier FEP/US approach ³⁵ of Eq. 5. This finding is important in view of the impression that the WHAM presents a major advance that allows for a superior connection of the umbrella sampling segments and thus its implementation may help to overcome the problems of some early PMF calculations of the free energy profile in ion channels.

Since the present work compares different approaches for evaluation of the free energy profile in ion channels it is useful to comment here about the perceived merit of using the Jarzynski's formula and related approaches ^{27,44}. Here we note that while this formulation might be useful for infinitely long runs or for a very large ensemble it must suffer all the same convergence problems experienced by the PMF approach (see also discussion in ³⁰). In view of the problem identified here with regards to the PMF approach we believe that the use of Jarzynski's formula will lead to exactly the same problems. Thus it may be useful if proponents of this approach will attempt to evaluate the PMF in our benchmark or related problems and compare it to the performance of other approaches.

One may argue that the PMF calculations can be improved by considering in addition to the *z* coordinate the generalized solvent coordinate that reflects both the water and channel polarization (see ⁴⁵ for a description of this coordinate). However, the present paper deals with the standard approach in PMF calculations of ion channel and with the problematic perception that this is a reliable approach.

Despite the experience gained in the present study it will be important to extend the above comparative studies to treatments of actual biological ion channels where the convergence problems are far more serious. Here again it will be essential to compare different approaches to examine the validity of the given long range treatment. It is important to point out in this

respect that even FEP approaches are not yet garneted to give completely reliable results in free energy calculations of ion channels. For example, as a part of our careful FEP studies of the KcsA channel (e.g. ref. ¹³) we found that the energetics of moving a K⁺ ion from water to the fourth loading site ([0100] to [0101]) in the notation of ⁴⁶ is about -20kcal/mol (similar results were found in ⁴⁶⁻⁴⁸). However, neutralizing Arg 89 and Asp 80, which are ionized in the regular simulations (see 13), increases [[0100] to [0101]] energy by about 11kcal/mol. Assuming that Glu 71 is ionized instead of Asp 80 (which might be the case when the channel is fully loaded ¹³) and then neutralizing the protein ionized groups increases the [[0100] to [0101]] energy by ~20kcal/mol. This large effect of the protein ionized groups seems to contradict experimental estimates of mutations in other systems that produced much smaller observed effects than the corresponding FEP results ⁴⁹, and might reflect an incomplete compensation by reorganization effects. Interestingly, adding an ion in the central cavity seems to return the calculated binding energy of the K⁺ ion to a reasonable value. At any rate, while the possible overestimate in the FEP calculations can be reduced by improving the simulations, the situation may be much more problematic in PMF calculations (e.g. ⁵⁰), since such approaches do not provide an estimate of the absolute charging energy and may not explore in a satisfactory way the energy of moving the ion from the bulk solvent to the selectivity filter. Thus such approaches may give the impression of low barrier both for the case when the protein ionized groups are ionized and when these groups are neutral, while according to the microscopic FEP simulations these profiles should be different and draw attention to possible problems.

Since we anticipate some difficulties with fully microscopic treatments of ion channels, it would be useful to retain and to refine semimacroscopic approaches and in particular the PDLD/S-LRA approach ^{13,51} that accounts for the protein flexibility. This approach does not assume a rigid protein (in a clear contrast to the implications given in ⁵²) and provides a consistent estimate of the protein reorganization effect by using the equivalent of an end point FEP approach. This allows one to assign a “dielectric constant” that only represents those factors that are not captured by the microscopic simulations due to convergence and sampling problems (see discussion in ⁵³). Forcing the PMF and the PDLD/S-LRA approach to reproduce simultaneously discriminating experimental results may provide another way of validating free energy calculations in ion channels.

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Figure 1.

Simplified channel models. (A) All side chains of the gramicidin A system, the membrane and the water molecules are removed, and position constraints are introduced on Ca atoms in the main chain. (B) and (C), one or four water molecules are added, respectively before and after the Na^+ ion. (D) Half of model A is taken and solvated in a SCAAS water sphere with a $14\text{\AA}$ radius.

Figure 2.

A schematic description of the two methods compared in the present study. (A) An adiabatic charging by FEP coupled with an umbrella sampling method, where the ion charge is changed from 0 to Q^0 and the corresponding free energy is sampled by Eq 3. (B) A PMF approach coupled with an umbrella sampling method. The ion position is moved by a harmonic potential and the free energy of moving the ion is evaluated by Eq 5.

total cycle for the model A,B,C, and D:

$$\begin{aligned}
 \text{(A)} \quad & \Delta G_{\text{PMF}}(\text{Na}^+(z \rightarrow z + \delta z)) \\
 &= \Delta G_{\text{AC}}(\text{Na}^{(0)}(z + \delta z) \rightarrow \text{Na}^+(z + \delta z)) - \Delta G_{\text{AC}}(\text{Na}^{(0)}(z) \rightarrow \text{Na}^+(z)) \\
 \text{(B,C)} \quad & \Delta G_{\text{PMF}}(\text{Na}^+(z \rightarrow z + \delta z)) - \Delta G_{\text{PMF}}(\text{Na}^{(0)}(z \rightarrow z + \delta z)) \\
 &= \Delta G_{\text{AC}}(\text{Na}^{(0)}(z + \delta z) \rightarrow \text{Na}^+(z + \delta z)) - \Delta G_{\text{AC}}(\text{Na}^{(0)}(z) \rightarrow \text{Na}^+(z)) \\
 \text{(D)} \quad & \Delta G_{\text{PMF}}(\text{Na}^+(z \rightarrow z + \delta z)) \\
 &= \Delta G_{\text{AC}}(\text{Na}^{(0)}(z + \delta z) \rightarrow \text{Na}^+(z + \delta z)) - \Delta G_{\text{AC}}(\text{Na}^{(0)}(z) \rightarrow \text{Na}^+(z)) \\
 &+ \Delta G_{\text{PMF}}(\text{Na}^{(0)}(z \rightarrow z + \delta z))
 \end{aligned}$$

Figure 3.

The thermodynamic cycles used to relate the PMF and adiabatic charging (AC) results. $\Delta G_{\text{PMF}}(\text{Na}^{(0)}(z \rightarrow z + \delta z))$ is neglected in the model without solvent molecules (model A). This term is used for all other models (model B, C, and D).

Figure 4. Comparing the PMF (blue) and AC (red) results for model A. The simulation times were 10ns and 2.5ns for the PMF and AC treatments, respectively. The region studied along the z-axis is marked on the molecular figure.

Figure 5. Comparing the PMF (blue) and AC (red) results for model B. The simulation times were 60ns and 20ns for the PMF and AC treatments, respectively.

Figure 6. Comparing the PMF (blue) and AC (red) results for model C. The simulation times were 60ns and 20ns for the PMF and AC treatments, respectively.

Figure 7.

Comparing the PMF (thin, long, color lines) and AC (red) results for model D. The figure shows the AC results of 130ns and PMF results for simulations of 140ns, 280ns, 420ns, 560ns, 700ns, 840ns, 980ns, 1.12μs. As seen from the figure the PMF results are slowly converging to the AC results.

Figure 8.

(A) Showing the cycles where the Na⁺ goes through (upper line) and around (lower line) the channel. (B) The PMF for transferring the Na⁺ ion through the channel (blue) and the PMF for the Na⁺ ion going around the channel (green). The charging energy corrected by the PMF of Na⁽⁰⁾ is shown in red. The PMF results are made from 560ns to 1.12μs of the simulation shown in Fig. 7, considering the first half of the simulation as a relaxation run.

Figure 9. Showing the hysteresis in the PMF calculations that does not involve the use of Eq. 6. The forward (magenta: from negative to positive direction in z-coordinate) and backward (gray: from positive to negative direction) runs are illustrated separately for 70ns simulation run each.

Figure 10.

Comparing the calculated by Eq. 5 (red) and by the WHAM approach (blue) for 140ns simulation runs. As seen from the figure the difference between these two methods is not significant. Thus the hysteresis in Fig 7 is not due to the specific mapping method used but the slow convergence of PMF.

Table 1

The parameters used in the present simulations ^(a)

partial atomic charges						
Gly	N	H	C α	H α	C'	O
For	-0.400	0.400	-0.194	0.097	0.550	-0.550
Na ⁺	C	H	O			
	0.550	0.000	-0.550			
Wat	Na					
	1.000					
	Ow	Hw				
	-0.820	0.410				
vdW parameters						
Gly	N	H	C α	H α	C'	O
A	1425.000	0.420	1956.000	4.000	1956.000	793.000
B	42.000	0.575	32.000	0.000	32.000	25.000
For	C	H	O			
A	1956.000	4.000	793.000			
B	32.000	0.000	25.000			
Na ⁺	Na					
A	94.500					
B	3.890					
Wat	Ow	Hw				
A	861.440	0.420				
B	26.040	0.575				

^(a)The intramolecular parameters are given in ref. 23. Hw and Ow designate respectively the hydrogen and oxygen of the water molecule. H α are the hydrogens attached to the C α , and C' is the carbonyl carbon of the Gly main chain.