



Molecular dynamics simulations of gramicidin A in a lipid bilayer: From structure–function relations to force fields

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Abstract

Molecular dynamics simulations of membrane proteins have become a popular tool for studying their dynamic features, which are not easily accessible by experiments. Whether the force fields developed for globular proteins are adequate for this purpose is an important question that is often glossed over. Here we determine the permeation properties of potassium ions in the gramicidin A channel in a lipid bilayer from free energy simulations, and compare the results to experimental data. In particular, we check the dependence of the free energy barriers ions face at the channel center on the membrane size. The results indicate that there is a serious problem with the current rigid force fields independent of the membrane size, and new, possibly polarizable, force fields need to be developed to resolve this problem.

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1. Introduction

Due to lack of molecular structure, membrane proteins have rarely been considered in molecular dynamics (MD) simulations until recently. This also meant that the MD force fields have been optimized by fitting their parameters to the properties of globular proteins. The recent determination of the crystal structure of the KcsA potassium channel (Doyle et al., 1998), followed by many others, have generated a great deal of interest in MD simulations of membrane proteins (for a recent review see Ash et al., 2004). Most of these MD simulations are carried out using one of the available force fields such as AMBER (Pearlman et al., 1995), CHARMM (MacKerell et al., 1998) or GROMACS (Berendsen et

al., 1995), which are rigid, that is, they do not include the polarization interaction explicitly. Because the polarization characteristics of water and lipid are widely different, it is not clear from the outset that the force fields developed for globular proteins will work for membrane proteins as well. Clearly suitability of these force fields for simulation of membrane proteins need to be carefully checked before investing heavily on such simulations. Because the structure–function relations are much better quantified for ion channels, they offer an ideal testing ground for this purpose.

Here we perform such a test using the antibacterial peptide gramicidin A (gA), whose dimer in a lipid bilayer forms an ion channel. Structure of gA has been known for a long time—the initial β -helical dimer model proposed by Urry (1971) was later confirmed and refined using solution (Arseniev et al., 1986) and solid state NMR (Ketchum et al., 1993). At present there are two high-resolution structures of gA that are commonly used in

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MD simulations, namely, PDB:1MAG (Ketchum et al., 1997) and PDB:1JNO (Townesley et al., 2001). Functional properties of the gA channel also have a long history and have been very well determined from numerous physiological experiments (for reviews see Andersen and Koeppe, 1992; Busath, 1993). For our purposes, the most important permeation property of the gA channel is that it conducts monovalent cations at near diffusion rates. The conductance of the gA channel for potassium ions is about 10 pS at physiological conditions, which corresponds to a current of ~ 1 pA and a transit time of 160 ns per ion. Considering that many conductance events have to be observed, simulations of the order of several microseconds are required to obtain a single conductance value, and even more to construct an I – V curve.

The current capabilities of MD simulations are in the nanosecond time domain, hence a direct calculation of the channel conductance is not feasible at present. Nevertheless we can still relate the channel structure to its function by calculating the potential of mean force of ions from MD simulations first and then using this potential in Brownian dynamics simulations. In fact, a potential profile that reproduces the conductance properties of K^+ ions in the gA channel has already been determined by inverting the available data using the Brownian dynamics simulations (Edwards et al., 2002). This potential profile has two binding sites at the either pore entry with a depth of 8 kT and a central barrier of height 5 kT (relative to the binding site). While these values are not unique, extensive parameter studies in Edwards et al. (2002) indicate that the channel ceases to conduct when the barrier height is larger than the well depth. That is, the free energy for translocating a K^+ ion from bulk water to the channel center – the so-called stabilization energy – must be negative. In the following we will use this criterion in testing the MD force fields.

We mention that two previous such tests have been carried out for the CHARMM force field (Allen et al., 2003a, 2004). In Allen et al. (2003a), the 1MAG structure of Ketchum et al. (1997) was employed and the well depth and barrier height were found as 6 and 22 kT, respectively. While in Allen et al. (2004), the 1JNO structure of Townesley et al. (2001) was employed and the corresponding well and barrier energies were 3 and 20 kT. In both cases the barrier-well difference (or the stabilization energy) is around 16–17 kT, which is too high to account for the experimental data. For example, a simple estimate of conductance from the Boltzmann factor indicates errors of the order of 10^7 . Allen et al. (2004) also estimated the effect of several simulation artifacts from continuum electrostatics that help to reduce the central barrier. They found that inclusion of finite size

effects and polarization of lipid molecules reduced the barrier height from 20 to 14 kT. The well depth, however, remained at about 3 kT, which is somewhat shallow to explain the binding of K^+ ions to gA. Most importantly though, the stabilization energy of 11 kT is still too high and points to 4–5 orders of magnitude error in conductance. A much smaller discrepancy in conductance (only a factor of 30) was estimated in Allen et al. (2004) using the 1D Nernst–Planck equation. As pointed out earlier (Levitt, 1999; Kuyucak et al., 2001), the 3D Brownian dynamics simulations provide a more reliable estimate of the channel conductance compared to the 1D Nernst–Planck equation. Thus despite some improvements, there are still substantial errors in the calculated gA properties, and they need to be understood to ensure the reliability of MD simulations of membrane proteins.

In this paper we explore the effect of the membrane size on the simulation results. Using a large number of lipid molecules severely limits the length of MD simulations. Thus the tendency is to use as small number of lipids as possible, especially when simulations lasting many nanoseconds are desired. For example, in Allen et al. (2003a), 48 lipids per layer was employed, which limited the simulation time to 100 ps per window in the potential of mean force (PMF) calculations. In contrast, Allen et al. (2004) were able to increase the simulation time to 1 ns per window by using only 10 lipids per layer. Because the two simulations differ in simulation time and the gA structure employed as well as the membrane size, it is impossible to assess the impact of the latter on the results. Here we address this issue by using four gA systems that are identical in all respects except in the number of lipid molecules per layer, which varied from 10 to 16, 32 and 64. For each system the free energy difference for translocating a K^+ ion from bulk water to the channel center is calculated. The system with 16 lipids appears to be the minimal system for obtaining stable results. We have used this system in long MD simulations (up to 1 ns per window) to study the convergence properties of the PMF of a K^+ ion. For testing purposes, we have also used rigid gA structures in the free energy calculations. Comparison of the free energies obtained using fixed and flexible structures provides interesting information on the role of peptide flexibility in ion permeation.

2. Methods

2.1. Model system and MD simulations

The simulation systems are constructed using the VMD suit of software developed by the Schulten group (Humphrey et al., 1996). Initially a bilayer of DMPC molecules with 128 lipids per layer is constructed and

the 1JNO structure of gA (Townsend et al., 2001) is embedded in the middle of this system. The lipids at the periphery of this system are gradually removed – while preserving a rectangular shape – to obtain the four systems with 64, 32, 16 and 10 lipids per layer. During the initial phase, the gA atoms are constrained to their initial positions. Each system is hydrated and placed in an orthorhombic periodic box. Following an energy minimization of 10,000 steps, they are equilibrated with surface-tension coupling until the surface area converged to the experimental lipid density of 60 \AA^2 per lipid (Nagle and Tristram-Nagle, 2000). At this stage, a number of water molecules are replaced with K^+ and Cl^- ions so that the solution has the physiological concentration of about 150 mM. In the remaining simulations, the periodic box is fixed in the x and y directions and a pressure coupling of 1 atm is applied in the z -direction. The simulation parameters thus obtained are listed in Table 1 for each system. Each system is further equilibrated for 1 ns (the z size is obtained from its average during this equilibration period). These systems with the original 1JNO gA structure are employed in free energy calculations with a rigid gA structure. The restraints applied to the gA atoms are then gradually relaxed in MD simulations lasting 3 ns. These systems with a flexible gA structure are employed in free energy calculations as well as a PMF calculation for the 16-lipid system.

MD simulations are carried out using the NAMD code, version 2.5 (Kale et al., 1998) with the PARAM27 version of the CHARMM force field (MacKerell et al., 1998), which provides a complete set of parameters for all the atoms in the system. An NpT ensemble is used with periodic boundary conditions. Pressure is kept at 1 atm using the Langevin piston method with a damping coefficient of 5 ps^{-1} (Feller et al., 1995). Similarly, temperature is maintained at 298 K through Langevin damping with a coefficient of 5 ps^{-1} . Electrostatic interactions are computed using the particle–mesh Ewald algorithm. The list of non-bonded interactions is truncated at 13.5 \AA , and a switching cut off distance of 10 \AA is used for the Lennard–Jones interactions. A time-step of 2 fs is employed for all simulations. Trajectory data is written at 1 ps intervals during both equilibration and production runs.

Table 1
System parameters used in the MD simulations

Lipid #	Surface area (\AA^2)	Size (x, y, z) (\AA)	Water #	Ion #
10	840	29, 29, 72	1156	2
16	1210	38, 32, 72	1532	4
32	2280	52, 44, 72	3050	8
64	4090	64, 64, 70	5210	16

2.2. Free energy difference calculations

While the PMF calculations provide a detailed free energy profile for an ion permeating along the reaction coordinate, they are very time consuming and require an inordinate amount of computing resources. If one is mainly interested in the height of the central barrier and the well depth at the binding site for a given ion, these quantities can be computed with much less effort from free energy difference calculations. Naturally one needs to find the binding site first, but that can be achieved through a local simulation, i.e., a full PMF is not necessary. Because they require less time, the free energy difference calculations can be carried out for longer periods and hence offer a better handle for checking the convergence of the results.

Here we calculate the free energy difference for translocating a K^+ ion from bulk water to the center of the channel. We use the thermodynamic integration method for this purpose (Beveridge and DiCapua, 1989). The free energy difference is obtained from

$$\Delta G = \int_0^1 \left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_{\lambda} d\lambda, \quad (1)$$

where $H(\lambda) = (1 - \lambda)H_0 + \lambda H_1$, with H_0 and H_1 representing the Hamiltonians of the initial and final states, respectively (e.g., if the initial state is an ion in the channel and a water molecule in bulk, in the final state these two are interchanged). The integral in Eq. (1) is performed using a Gaussian quadrature (Press et al., 1989). We have experimented with various numbers of quadrature points (e.g., 3, 5, 7 and 12 points), and found that 7-point quadrature provides sufficient accuracy for our purposes. This value is used in all subsequent calculations. Each system is equilibrated for at least 200 ps (longer in some cases) before production runs. In all cases, the integrals are evaluated from 700 ps of production runs.

When a K^+ ion is in the channel center, the dipoles of water molecules in the pore point away from the ion, whereas when there are only water molecules in the channel they all point in the same direction. Thus an alchemical transformation of a K^+ ion in the channel center to a water molecule disrupts the orientation of half of the water molecules. The resulting fluctuations in the free energy calculations can be reduced by performing the transformation via an intermediate state with no charge, which we choose as a water molecule with the partial charges set to 0 (denoted as W_0). Thus we perform two calculations, $\Delta G(\text{K}^+ \rightarrow \text{W}_0)$ and $\Delta G(\text{W}_0 \rightarrow \text{W})$, whose sum gives the desired free energy change for the $\text{K}^+ \rightarrow \text{W}$ transformation. Note that a similar transformation, $\text{W} \rightarrow \text{W}_0 \rightarrow \text{K}^+$ is carried out in bulk simulta-

neously to find the ionic free energy difference between the channel and bulk water.

2.3. Potential of mean force

The PMF of a K^+ ion along the gA channel axis is calculated using umbrella sampling (Torrie and Valleau, 1977) together with the weighted histogram analysis method (Kumar et al., 1992; Souaille and Roux, 2001). As the method was explained in Allen et al. (2003a) in some detail, we give a brief account here stressing only the differences in the present work. Using an umbrella potential, we sample an ion's position at equal intervals along the channel axis during MD simulations of the system. The biased ion distributions obtained from the production runs are then unbiased and combined using the weighted histogram analysis method. In all cases, the ion coordinates are measured with respect to the center of mass of gA.

We employ umbrella potentials with a force constant of $17 \text{ kT}/\text{\AA}^2$ at 0.5 \AA intervals. To avoid potential equilibration problems associated with dragging of an ion in the channel (Allen et al., 2003a), here we have replaced individual water molecules in the pore with a K^+ ion. This way we obtain 10 configurations with the ion placed at regular intervals along the channel axis. The K^+ ion in each configuration then needs to be pushed by only $\sim 1 \text{ \AA}$ to either side in order to generate the full set of windows required in the PMF calculations. Outside the channel, where equilibration is not a problem, the ion is pushed along the central axis. A total of 81 windows covering the range $[-20, 20] \text{ \AA}$ are employed for the K^+ PMF. For each window, the system is equilibrated for 200 ps and the trajectory data for ion positions is collected for 1 ns. Thus the PMF of an ion is determined from the ion distributions between $z = \pm 20 \text{ \AA}$. Neither the equilibrated gA structure nor the PMF obtained from this structure are symmetric around the gA center of mass at $z = 0$. Fluctuations of the center of mass of gA (up to an Angstrom) appear to be the major reason for this asymmetry, which persists even after a simulation of 1 ns. As discussed in more detail below, one way to deal with this problem is to symmetrize the ion densities, i.e., use $\rho_{\text{sym}}(z) = [\rho(z) + \rho(-z)]/2$ in the calculation of the PMF. We will also adapt this procedure here and provide some justification for its use.

3. Results and discussion

3.1. Barrier energies from free energy differences

We have calculated the free energy difference for translocating a K^+ ion from bulk to the channel cen-

ter using both a rigid gA structure and a flexible one. The reason for the former is that a rigid gA system offers a more controlled environment for assessing the effect of the membrane size on the peptide function. Comparison of the rigid vs. flexible results also gives valuable information on the influence of protein flexibility on its function, which is an important consideration in establishing the validity of coarse-grained models of ion channels and other proteins (Baštuž et al., 2006). Of the two legs of the transformation, $K^+ \rightarrow W_0 \rightarrow W$, we find the free energy difference for the second leg is negligibly small (much smaller than the $\sim 1 \text{ kT}$ statistical error) regardless of the system size. Therefore in the following, we consider only the free energy difference for the first leg, i.e., $\Delta G(K^+ \rightarrow W_0)$.

The free energy difference results are summarized in Table 1. The fixed gA results for ΔG_{av} are all consistent with each other if we allow the statistical errors of about 1 kT. That is, they are all within 1 kT of the average value of 56.5 kT. More importantly, there is no systematic dependence on the lipid numbers. Thus the fixed gA results suggest that the number of lipids in the simulation system does not play a crucial role in the peptide function. The flexible gA results suggest a similar trend but due to increased fluctuations, they are not as clear cut as the fixed ones. To make these differences clearer, we show the running averages of the free energy differences for the forward (ΔG_+) and backward ($-\Delta G_-$) directions in Fig. 1. We see that unlike the other three cases, the relatively large hysteresis effect for the system with 10 lipids persists throughout the 700 ps simulation. This suggests that using only 10 lipids per layer may not be sufficient to provide an adequate membrane environment for the peptide when it moves freely. The hysteresis

Table 2

Free energy differences for translocating a K^+ ion from bulk to the channel center (ΔG_+), negative of the reverse transfer ($-\Delta G_-$), and their average (ΔG_{av})

Lipid #	ΔG_+	$-\Delta G_-$	ΔG_{av}
Fixed gA			
10	52.9 ± 0.7	58.0 ± 1.1	55.4 ± 0.9
16	58.7 ± 1.4	55.2 ± 1.2	57.0 ± 1.3
32	54.6 ± 0.9	60.4 ± 0.8	57.5 ± 0.9
64	56.9 ± 1.0	54.3 ± 1.1	55.6 ± 1.1
Flexible gA			
10	11.1 ± 1.0	15.9 ± 0.9	13.5 ± 1.0
16	11.4 ± 0.9	13.3 ± 0.9	12.4 ± 0.9
32	16.0 ± 1.2	17.1 ± 1.1	16.6 ± 1.2
64	13.5 ± 0.9	13.9 ± 1.0	13.7 ± 1.0

The top part shows the free energy barriers obtained from a fixed gA structure and the bottom part shows the same for a flexible gA structure. All free energies are given in units of kT.

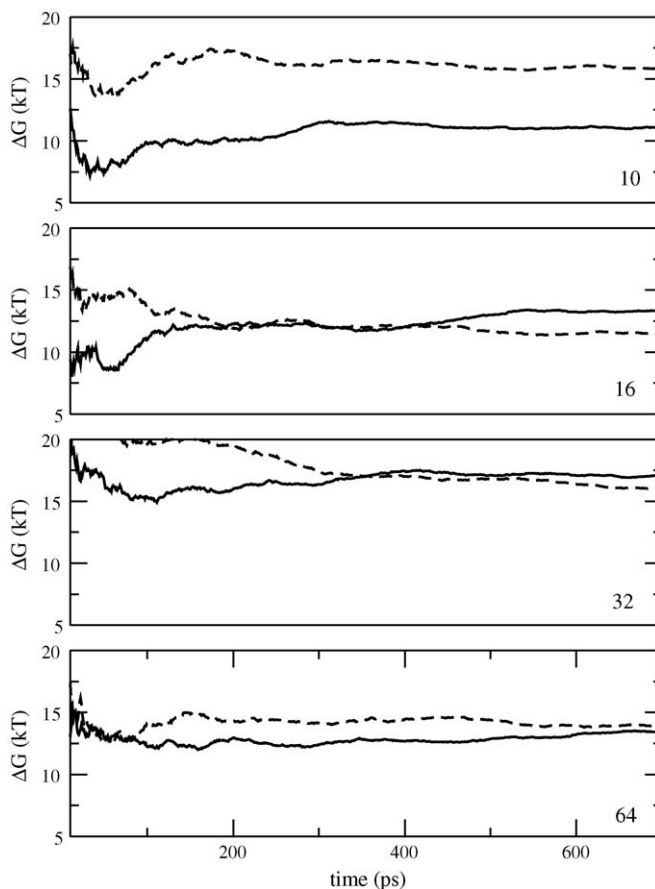


Fig. 1. Running averages of the free energy differences ΔG_+ (solid lines) and $-\Delta G_-$ (dashed lines) for transferring a K^+ ion from bulk to the channel center and the reverse process, respectively. The curve labeled K shows the $K^+ \rightarrow W_0$ leg of the transformation. The $W_0 \rightarrow W$ legs are negligibly small in all cases, and therefore are not shown. The total ΔG is obtained from the average of the two curves.

effect in all other cases are within the statistical fluctuations. Thus using a minimal number of 16 lipids appears to provide an adequate membrane environment for free energy simulations of the gA system. For this system we have also calculated the binding energy of a K^+ ion which yielded 6.8 kT. Adding this to the stabilization energy in Table 2 results in a barrier height of 19.2 kT, which is consistent with the earlier PMF results quoted in the Section 1.

A second observation for the flexible gA results is that the value of the barrier in the 32 lipid case is about 3 kT larger than the others. This is somewhat more than one would expect from statistical fluctuations and suggests that a systematic difference between the structures is likely to be responsible for this increase in energy. While we have not been able to find an obvious difference in structures that would explain this larger barrier so far, the effect is small enough to be caused by a relatively small structural difference. Thus a more detailed comparison of the four systems is needed to understand

the origin of the larger energy barrier in the 32-lipid system.

We next compare the stabilization energies obtained with the fixed and flexible structures, which are about 56 and 13 kT, respectively. Thus allowing flexibility of the peptide reduces the free energy barrier by more than a factor of 4. A similar study has recently been carried out using the 1MAG structure, which resulted in free energy barriers of 34 and 13 kT for the fixed and flexible structures, respectively (Bastug et al., 2006). Both figures point to a very significant contribution from peptide flexibility to the stabilization energy of the ion and should prompt a serious study about the role of protein flexibility on the function of ion channels. While the biological ion channels are wider and hence less likely to exhibit such large effects due to flexibility, even smaller effects could play an important role in ion permeation.

Another interesting observation is the large difference between the stabilization energies obtained using the fixed 1MAG and 1JNO structures. Recently, it has

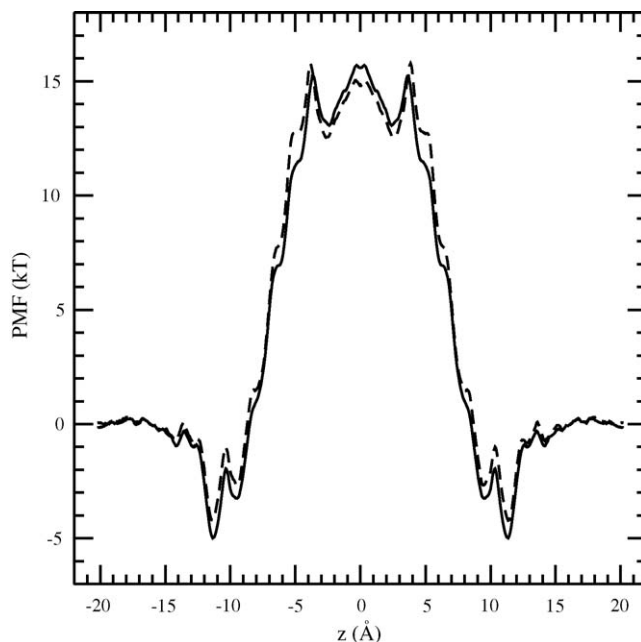


Fig. 2. PMF profiles of a K^+ ion along the central axis of the gramicidin A channel. In the calculation of the PMF, the ion density is symmetrized around $z = 0$. The dashed line shows the PMF obtained from 0.5 ns of MD simulation for each window, and the solid line shows the corresponding PMF when each window is simulated for 1 ns.

been argued on the basis of MD simulations that the 1JNO structure is closer to the equilibrium structure (Allen et al., 2003b). The smaller free energy barrier for the 1MAG structure indicates that, as far as the energetics of ion permeation is concerned, it is closer to the equilibrated gA structure than the 1JNO structure. Most importantly, however, despite employing widely differing initial structures, both MD simulations give the same stabilization energy of 13 kT. This reassures that MD simulations can produce reliable free energy estimates independent of the initial conditions.

3.2. Potential of mean force of a K^+ ion

Here we use the minimal 16-lipid system to calculate the PMF of a K^+ ion in long MD simulations so that its convergence properties can be studied. We find that the calculated PMF is not symmetric around $z = 0$ and exhibits a few kT shift in energy between the left and right sides. Following the evolution of this asymmetry in time indicates that a convergence is not obtained even with a 1 ns simulation of each window, which corresponds to a total MD simulation of the system for 81 ns. As the cause of this asymmetry is likely to be fluctuations of the gA structure around the center of mass, symmetrizing the ion densities around $z = 0$ is a reasonable way to deal with this problem in shorter simulations. In Fig. 2, we show the PMF of a K^+ ion along the central axis of the channel

obtained using the symmetrized ion densities. Comparison of the PMF obtained with 0.5 ns MD simulation per window (dashed line) with that of 1 ns per window (solid line) clearly shows that the PMF results have converged to within statistical accuracy of 1 kT. Thus symmetrizing the densities provides a practical solution to the asymmetry problem encountered in the PMF calculations caused by fluctuations in the system.

From Fig. 2 we see that the well depth and barrier height are about 5 and 20 kT, respectively. These are comparable to the previous results quoted in Section 1 and also to the free energy calculations presented in the previous section. We note that the stabilization and binding energies are calculated with respect to the bulk and therefore they are more easily influenced by fluctuations of the gA system, whereas the barrier energy is calculated with respect to the binding site of gA, hence it is more stable against such fluctuations. Thus while there are sizable variations in the former two, the latter always turns out to be about 20 kT.

4. Conclusions

For the reasons given in Section 1, the gA channel provides one of the best systems for testing the applicability of the MD force fields to membrane proteins. The present free energy and PMF calculations for a K^+ ions in the gA channel demonstrate that membrane size does not have

much influence on its permeation properties. Thus our results confirm those obtained from previous studies for K^+ ions, namely, the rigid force fields can account for the binding of a K^+ ion but have difficulties in explaining the energetics of permeation. The inability of the nonpolarizable force fields to describe conductance of monovalent cations have been noted earlier and despite some recent improvements, this problem has not been resolved satisfactorily. The present work extends those by showing that these negative results remain robust against changes in the membrane size. Thus the solution of this problem is more likely to be found in force fields, in particular, the polarization interaction that is missing from the current force fields (Dorman and Jordan, 2004). We hope that these results will further stimulate construction of polarizable force fields for MD simulations of membrane proteins.

A second interesting result that came out of this work is the significant role played by peptide flexibility in lowering the free energy barrier of an ion at the channel center. A critical question here is the relevance of the results found for gA to other ion channels. Most biological ion channels contain substantially more water molecules, which provide a much better hydration environment for ions compared to the single-file water column in gA. Thus one expects a better screening of the ion–peptide interactions in biological ion channels, which will suppress the changes in the peptide density and fluctuations due to the motion of an ion. Of course, one needs to perform a similar analysis for these channels to show that such expectations are indeed realized, and that the successes of continuum electrostatics in accounting for their permeation properties in several channel models (e.g., Chung et al., 1999; Corry et al., 2001) was not just due to a fortuitous cancellation of errors.

Note added in proof

Further equilibration of the system with 32 lipids resolved the problem with the somewhat larger barrier found in this case (Table 2). The new values for both the forward and reverse free energy differences is 13.5 kT which is consistent with the others.

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References

- Allen, T.W., Bastug, T., Kuyucak, S., Chung, S.H., 2003a. Gramicidin A as a test ground for molecular dynamics force fields. *Biophys. J.* 84, 2159–2168.
- Allen, T.W., Andersen, O.S., Roux, B., 2003b. Structure of gramicidin A in a lipid bilayer environment determined using molecular dynamics simulations and solid-state NMR data. *J. Am. Chem. Soc.* 125, 9868–9877.
- Allen, T.W., Andersen, O.S., Roux, B., 2004. Energetics of ion conduction through the gramicidin channel. *Proc. Natl. Acad. Sci. USA* 101, 117–122.
- Andersen, O.S., Koeppe, R.E., 1992. Molecular determinants of channel function. *Physiol. Rev.* 72, 89–158.
- Arseniev, A.S., Lomize, A.L., Barsukov, I.L., Bystrov, V.F., 1986. Gramicidin A transmembrane ion channel. Three dimensional structure reconstruction based on NMR spectroscopy and energy refinement. *Biol. Membr.* 3, 1077–1104.
- Ash, W.L., Zlomislic, M.R., Oloo, E.O., Tieleman, D.P., 2004. Computer simulations of membrane proteins. *Biochim. Biophys. Acta* 1666, 158–189.
- Bastug, T., Gray-Weale, A., Patra, S.W., Kuyucak, S., 2006. Role of protein flexibility in ion permeation: a case study in gramicidin A. *Biophys. J.* 90, 2285–2296.
- Berendsen, H.J.C., van der Spoel, D., van Drunen, R., 1995. GRO-MACS: a message-passing parallel molecular dynamics implementation. *Comput. Phys. Commun.* 91, 43–56.
- Beveridge, D.L., DiCapua, F.M., 1989. Free energy via molecular simulation: applications to chemical and biomolecular systems. *Ann. Rev. Biophys. Chem.* 18, 431–492.
- Busath, D.D., 1993. The use of physical methods in determining gramicidin structure and function. *Ann. Rev. Physiol.* 55, 473–501.
- Chung, S.H., Allen, T.W., Hoyles, M., Kuyucak, S., 1999. Permeation of ions across the potassium channel: Brownian dynamics studies. *Biophys. J.* 77, 2517–2533.
- Corry, B., Allen, T.W., Kuyucak, S., Chung, S.H., 2001. Mechanisms of permeation and selectivity in calcium channels. *Biophys. J.* 80, 195–214.
- Dorman, V.L., Jordan, P.C., 2004. Ionic permeation free energy in gramicidin: a semimicroscopic perspective. *Biophys. J.* 86, 3529–3541.
- Doyle, D.A., Cabral, J.M., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Chait, B.T., MacKinnon, R., 1998. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* 280, 69–77.
- Edwards, S., Corry, B., Kuyucak, S., Chung, S.H., 2002. Continuum electrostatics fails to describe ion permeation in the gramicidin channel. *Biophys. J.* 83, 1348–1360.
- Feller, S., Zhang, Y., Pastor, R., Brooks, B., 1995. Constant pressure molecular dynamics: the Langevin piston method. *J. Chem. Phys.* 103, 4613–4621.
- Humphrey, W., Dalke, A., Schulten, K., 1996. VMD—Visual Molecular Dynamics. *J. Mol. Graphics* 14, 33–38.
- Kale, L., Skeel, R., Bhandarkar, M., Brunner, R., Gursoy, A., Krawetz, N., Phillips, J., Shinozaki, A., Varadarajan, K., Schulten, K., 1998. NAMD2: greater scalability for parallel molecular dynamics. *J. Comput. Phys.* 151, 283–312.
- Ketchem, R.R., Hu, W., Cross, T.A., 1993. High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR. *Science* 261, 1457–1460.
- Ketchem, R.R., Roux, B., Cross, T.A., 1997. High-resolution polypeptide structure in a lamellar phase lipid environment from solid

- state NMR derived orientational constraints. *Structure* 5, 1655–1669.
- Kumar, S., Bouzida, D., Swensen, R.H., Kollman, P.A., Rosenberg, J.M., 1992. The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. *J. Comput. Chem.* 13, 1011–1021.
- Kuyucak, S., Andersen, O.S., Chung, S.H., 2001. Models of permeation in ion channels. *Rep. Prog. Phys.* 64, 1427–1472.
- Levitt, D.G., 1999. Modeling of ion channels. *J. Gen. Physiol.* 113, 789–794.
- MacKerell Jr., A.D., Bashford, D., Bellott, M., Dunbrack Jr., R.L., Evanseck, J.D., Field, M.J., Fisher, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F.T.K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D.T., Prodhom, B., Reiher III, W.E., Roux, B., Schlenkrich, M., Smith, J.C., Stote, R., Straub, J., Watanabe, M., Wiorkiewicz-Kuczera, J., Yin, D., Karplus, M., 1998. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B* 102, 3586–3616.
- Nagle, J.F., Tristram-Nagle, S., 2000. Structure of lipid bilayers. *Biochim. Biophys. Acta* 1469, 159–195.
- Pearlman, D.A., Case, D.A., Caldwell, J.W., Ross, W.S., Cheatham, T.E., DeBolt, S., Ferguson, D., Seibel, G., Kollman, P., 1995. AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics, and free energy calculations to simulate the structural and energetic properties of molecules. *Comput. Phys. Commun.* 91, 1–41.
- Press, W.H., Flannery, B.P., Teukolsky, S.A., Vetterling, W.T., 1989. *Numerical Recipes*. Cambridge University Press, Cambridge.
- Souaille, M., Roux, B., 2001. Extension to the weighted histogram analysis method: combining umbrella sampling with free energy calculations. *Comput. Phys. Commun.* 135, 40–57.
- Torrie, G.M., Valleau, J.P., 1977. Nonphysical sampling distributions in Monte Carlo free-energy estimation: umbrella sampling. *J. Comput. Phys.* 23, 187–199.
- Townsley, L.E., Tucker, W.A., Sham, S., Hinton, J.F., 2001. Structures of gramicidin A, B and C incorporated into sodium dodecyl sulfate micelles. *Biochemistry* 40, 11676–11686.
- Urry, D.W., 1971. The gramicidin A transmembrane channel: a proposed π_{LD} helix. *Proc. Natl. Acad. Sci. USA* 68, 672–676.