Response to "Comment on 'Free energy simulations of single and double ion occupancy in gramicidin A' " [J. Chem. Phys. 128, 227101 (2008)]

Turgut Baştuğ[1,a)] and Serdar Kuyucak[2,b)]
[1]*Faculty of Arts and Sciences, TOBB University of Economics and Technology, Ankara 06560, Turkey*
[2]*School of Physics, University of Sydney, New South Wales 2006, Australia*

(Received 19 March 2008; accepted 28 April 2008; published online 13 June 2008)

[DOI: 10.1063/1.2931571]

In the preceeding Comment, Roux *et al.*[1] criticized our use of one-dimensional (1D PMF) in calculation of the binding constants and argue that this renders our results on the gramicidin A channel unreliable.[2–7] While citing all our work on application of 1D PMF to ion channels, they have neglected to mention that there are many other papers on the subject, which may inadvertently give the impression that we are the only group who has used 1D PMF in ion channels. To give proper credit, we note that 1D PMF was first used in the gramicidin A channel by Roux and Karplus,[8,9] and since then, it has been used in ion channels by many others, see, for example, Refs. 10–16. What sets our work apart from the others is that we have presented detailed comparisons of the calculated properties of the gramicidin A channel with experiments and drew attention to substantial discrepancies. These are likely to arise from the neglect of the polarization interaction in the CHARMM and other rigid force fields. We suggested, therefore, that polarizable force fields should be seriously considered for molecular dynamics simulations of channel proteins. Ironically, in all other applications of 1D PMF to ion channels, no attempt was made to justify it, whereas we had actually justified the use of 1D PMF approximation in calculation of the binding constants both in Ref. 1 for which the Comment was written, and in an earlier paper.[4] These results are clearly relevant to the discussion at hand but are not mentioned in the Comment. Therefore, it will be worthwhile to go over them in some detail first and then return to the arguments made in the Comment.

Without loss of generality, we can assume that the PMF, $W(\mathbf{r})$, vanishes in the bulk, and write for the binding constant

$$K = \int_{\text{site}} e^{-W(\mathbf{r})/kT} d\mathbf{r}. \tag{1}$$

That is, the binding constant is given by a 3D integral of $\exp[-W(\mathbf{r})/kT]$ over a region covering the binding site. Computation of $W(\mathbf{r})$ in a 3D grid would be prohibitively expensive, and therefore, one has to look for approximate evaluations of the integral. An obvious approximation is suggested by the exponential dependence of the integral on $W(\mathbf{r})$, which ensures that the major contribution to the integral comes from the binding site where $W(\mathbf{r})$ has a minimum. For the gramicidin A channel, this was indicated in Fig. 1 and the accompanying text in Ref. 2, which showed that 95% of the integral value was attained by $z=13$ Å. This roughly corresponds to the border of the binding site. In the binding pocket, the ion is restrained by the peptide atoms to a radial distance of about $R=2$ Å from the channel axis. Beyond that, $W(\mathbf{r})$ rises sharply due to the repulsive Lennard–Jones interactions of the ion with the peptide atoms, rendering contributions beyond $R>2$ Å negligible. Thus, it is intuitively clear that a good approximation to the 3D integral can be obtained from the 1D integral of the PMF along the central axis of the channel

$$K = \pi R^2 \int_{z_1}^{z_2} e^{-W(z)/kT} dz, \tag{2}$$

where the integration limits z_1 and z_2 are taken in the bulk region on either side of the channel where PMF vanishes. The only potential problem with this approximation is that the 1D PMF must be calculated correctly. It is crucial that the well depth with respect to the bulk is calculated correctly, otherwise, there could be large errors arising from the exponential factor.

We had first addressed this issue in Ref. 4, where the ion conduction properties of the gramicidin A channel were determined from the PMF calculations. To ensure the accuracy of the 1D PMF, we compared the barrier height at the channel center with respect to the bulk, with the free energy of translocating the ion from the bulk to the channel center, obtained from free energy perturbation and thermodynamic integration calculations. The fact that there was good agreement between the two free energy difference calculations and the barrier height obtained from the PMF proved that there were no problems associated with the 1D PMF calculations with respect to the bulk reference value. It is worthwhile to stress that the latter two methods are considered to be the most accurate and reliable among the free energy calculation methods. In fact, the accuracy of the PMF calculations is questioned by some (e.g., Ref. 12), whether they are 1D or 3D. Thus, comparison of the PMF with the more accurate free energy methods goes beyond the 3D to 1D reduction issue, and provides a much stronger justification for the calculated PMF.

In the more recent paper studying ion binding in gramicidin A,[2] we provided a similar justification for the 1D PMF. As pointed out above, the main issue here was the well depth with respect to the bulk. Therefore, the well depth determined from the 1D PMF, was compared to the free energy of translocating the ion from the bulk to the binding site, obtained from the thermodynamic integration calculations. The

results were (see Fig. 3 and the text below it in Ref. 2): −4.5 and −4.6 kT, respectively, for the PMF and thermodynamic integration. Again, this proved that there were no problems with the 1D PMF calculation, and the well depth—crucial for the correct estimation of the binding constant—was calculated correctly. Thus, the assertion in the Comment that the unbiased restraint introduces an unknown error into the calculation of the binding constant is not true. It can be and has been checked by independent free energy calculations and inaccuracies introduced by the 1D approximation is found to be negligible.

Another misrepresentation of our results in the Comment is the statement that we obtained the PMF only with a restraint and did not unbiase it. In fact, we repeated the PMF calculations with and without radial restraints and found no discernible difference between the two calculations (e.g., see line 8 on the third page of Ref. 2). The fact that the restrained and unrestrained PMFs yield the same results within the accuracy of calculations ($\approx$1 kT) provides further evidence that the error caused by the weak restraint is negligible, and this is not an issue in the present context of the 1D PMF calculations in ion channels.

Returning to the calculation of the 1D PMF using a restraining cylinder,[17] while there is no problem with the formalism given for the 3D to 1D reduction of the PMF, we believe that the way it is applied to the gramicidin A channel is problematic. We have previously studied dependence of the PMF on radial restraints and found negligible change among the PMFs, including the case with no restraints. The reason is that the simulation time used in the PMF calculations (about 1 ns) is too short for the restraints to have the effect discussed in the Comment. Thus, when a large restraining radius (e.g., $R=8$ Å) is employed, the ion simply does not have sufficient time to probe such a large area properly. Unless one is willing to do much longer simulations (because of the quadratic dependence on R), it is important to choose a restraining radius that is commensurate with that of the channel, which makes the major contribution to the binding constant. Otherwise, R can be chosen as large as one wishes, the PMF will change little in 1 ns simulations, and the binding constant will grow as R^2. That will obviously help to reduce the discrepancy with the experimental binding constant found in our work,[2] but the meaning of such a result is questionable.

As demonstrated in our earlier papers[2,4] and discussed above, there is no problem with the 1D approximation of the PMF as it is employed in the binding constant calculations. Thus, our results showing that there are substantial discrepancies in the calculated properties of the gramicidin A channel remain intact.[2–7] Finally, in the Comment, a blanket statement is made as if the issue raised affects all our results. It is worthwhile to stress that the major discrepancy—namely, the 20 kT central barrier relative to the binding site that leads to the suppression of conductance over a million fold compared to the experimental value—is not affected at all by the 1D PMF approximation because the corresponding PMF is calculated entirely in the channel (a similar value for the barrier is also found by the authors of the Comment[17]). The 1D PMF approximation concerns only the binding constant calculations, for which the discrepancies found are relatively minor, i.e., calculations deviate from the experimental values by factors of 10–100 rather than 10^6. These discrepancies are presumably due to the neglect of the polarization interaction in the current force fields, and therefore, construction of polarizable force fields is urgently needed to address these issues.

This work was supported by grants from the Australian Research Council.

[a]Electronic mail: tbastug@etu.edu.tr.
[b]Electronic mail: serdar@physics.usyd.edu.au.

[1] B. Roux, O. S. Andersen, and T. W. Allen, J. Chem. Phys. **128**, 227101 (2008).
[2] T. Bastug and S. Kuyucak, J. Chem. Phys. **126**, 105103 (2007).
[3] S. M. Patra, T. Bastug, and S. Kuyucak, J. Phys. Chem. B **111**, 11303 (2007).
[4] T. Bastug and S. Kuyucak, Biophys. J. **90**, 3941 (2006).
[5] T. Bastug, S. M. Patra, and S. Kuyucak, Chem. Phys. Lipids **141**, 197 (2006).
[6] T. Bastug and S. Kuyucak, Eur. Biophys. J. **34**, 377 (2005).
[7] T. W. Allen, T. Bastug, S. Kuyucak, and S. H. Chung, Biophys. J. **84**, 2159 (2003).
[8] B. Roux and M. Karplus, J. Phys. Chem. **95**, 4856 (1991).
[9] B. Roux and M. Karplus, J. Am. Chem. Soc. **115**, 3250 (1993).
[10] T. B. Woolf and B. Roux, Biophys. J. **72**, 1930 (1997).
[11] W. Im and B. Roux, J. Mol. Biol. **322**, 851 (2002).
[12] M. Kato and A. Warshel, J. Phys. Chem. B **109**, 19516 (2005).
[13] O. Beckstein and M. S. P. Sansom, Phys. Biol. **3**, 147 (2006).
[14] M. S. Lee and M. A. Olson, Biophys. J. **90**, 864 (2006).
[15] L. Charlier, C. Nespoulous, S. Fiorucci, S. Antonzack, and J. Golebiowski, Phys. Chem. Chem. Phys. **9**, 5761 (2007).
[16] B. Corry, J. Phys. Chem. B **112**, 1427 (2008).
[17] T. W. Allen, O. S. Andersen, and B. Roux, Proc. Natl. Acad. Sci. U.S.A. **101**, 117 (2004).