Reply to ''Complementarities and Convergence of Results in Bacteriorhodopsin Trimer Simulations''

In the above-mentioned letter, Baudry et al. compare their earlier molecular dynamics (MD) simulations (Baudry et al., 2001) of a bacteriorhodopsin trimer in a fully hydrated lipid membrane with our more recent MD work (Kandt et al., 2004) on a similar system, and comment on the similarities of the ''key results'' of both studies. Whereas both studies, despite technical implementation differences, focus on similar systems, the questions addressed in, and thus the emphasis of Kandt et al., differ substantially from Baudry et al. Klaus Schulten's group has made significant contributions to the field of bacteriorhodopsin, and Kandt et al. therefore cites their work, including Baudry et al., prominently in its introduction and discussion. However, since Kandt et al.'s ''key results'', in contrast to the view expressed in the above letter, deal with the nature of the proton release group in bacteriorhodopsin—the subject of intense debate in the field—Baudry et al. was not the prime focus of our discussion. We begin with a restatement of the key issue addressed in Kandt et al.

In Rammelsberg et al. (1998), we showed that the proton release group consists of a protonated water cluster stabilized by Glu-204 and Glu-194, but that neither Glu-204 nor Glu-194 deprotonates in the wild-type; they can deprotonate in mutants. pK calculations of an $H_5O_2^+$ complex stabilized by Glu-204 and Glu-194 (Spassov et al., 2001) lend theoretical support to this proposal. Kandt et al.'s MD calculations find the largest internal water density close to Glu-204 and Glu-194 (see Fig. 6 of Kandt et al., 2004). In contrast to Baudry et al.'s simulations, Kandt et al. suggest that Glu-204 and Glu-194 form a barrier against intruding water that persists even at longer simulation times, and that this region of water density is separated by Arg-82 from a water density region close to the Schiff base. Our Figs. 7 and 8 show the H-bond contacts of amino acid residues surrounding and stabilizing the proposed protonated water clusters as suggested by our MD simulations. This allows us to predict which specific mutations will influence the continuum absorbance change in the infrared spectroscopy, which indicates a deprotonation of the protonated water cluster at the proton release site. The predictions in Kandt et al. are now in good agreement with recent Fourier transform infrared spectroscopy experiments on the continuum absorbance change and with the measured proton release kinetics (L. Garczarek and K. Gerwert, unpublished). Again, the proton release mechanism via a protonated water cluster, the central focus of Kandt et al., is neither mentioned in Baudry et al. (2001) nor in their letter, and thus Baudry et al. (2001) was not the focus of Kandt et al.'s discussion. In our view, proton transfer via protonated water clusters may be a critical issue for several proton transporting proteins and, as such, it is Kandt et al.'s ''key result''.

As Baudry et al.'s letter notes, both publications confirm the already well-established role of the protonated Schiff base, Asp-85 and Asp-96, despite some technical differences in the simulations. Aside from this detail, it is worth pointing out an important difference between the two publications. Kandt et al. focus on the dynamics of internal water molecules, as stated in their title. The much longer simulation time, 5 ns over 1 ns, of the latter publication is crucial to equilibrate the system under NPT conditions. This allows us to observe water diffusion that occurs beyond the structural changes, often entailing water influx and outflux, at the outset of the MD calculation. After observing such diffusion, we introduce ''water densities'' to quantify the dynamic behavior of the internal water molecules of bacteriorhodopsin, and therefore extend the view provided by x-ray structural models of water at fixed positions in water densities delocalized within cavities. Fig. 5 of Kandt et al. (2004) details six of such internal water densities in bacteriorhodopsin. Such results are beyond the reach of Baudry et al.'s 1 ns simulations.

In summary, whereas the two MD simulations of bacteriorhodopsin trimers in hydrated lipid membranes both similarly confirm certain important technical checks, they have very different goals. The Kandt et al. article looks at the long time mobility of internal water molecules not accessible to the earlier short time simulations and then hones in on the proton-release group, subjects not addressed by Baudry et al.

REFERENCES

Baudry, J., E. Tajkhorshid, F. Molnar, J. Phillips, and K. Schulten. 2001. Molecular dynamics study of bacteriorhodopsin and the purple membrane. *J. Phys. Chem. B.* 105:905–918.

Kandt, C., J. Schlitter, and K. Gerwert. 2004. Dynamics of water molecules in the bacteriorhodopsin trimer in explicit lipid/water environment. *Biophys. J.* 86:705–717.

Rammelsberg, R., G. Huhn, M. Lubben, and K. Gerwert. 1998. Bacteriorhodopsins intramolecular proton-release pathway consists of a hydrogen-bonded network. *Biochemistry.* 37:5001–5009.

Spassov, V. Z., H. Luecke, K. Gerwert, and D. Bashford. 2001. pK(a) calculations suggest storage of an excess proton in a hydrogen-bonded water network in bacteriorhodopsin. *J. Mol. Biol.* 312:203–219.

Christian Kandt, Juergen Schlitter, and Klaus Gerwert

Lehrstuhl für Biophysik
Ruhr Universitaet Bochum
D44870 Bochum
Germany

Submitted May 5, 2004, and accepted for publication May 13, 2004.

Address reprint requests to Klaus Gerwert, Ruhr Universitaet Bochum, Lehrstuhl für Biophysik, Universitatsstr. 150, Gebaude ND/04-Nord, D44870 Bochum, Germany. Tel.: 49-234-700-4461; E-mail: gerwert@bph.ruhr-uni-bochum.de.

0006-3495/04/08/1396/01 $2.00

doi: 10.1529/biophysj.104.045526