

Directional Proton Transfer in Membrane Proteins Achieved through Protonated Protein-Bound Water Molecules: A Proton Diode**

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Dedicated to Professor Manfred Eigen

The key function of energy-transducing membrane proteins is the creation of a proton gradient by directional proton transfer. The role of protein-bound water molecules herein is not fully understood, as X-ray diffraction analysis has resolved the positions of oxygen, but not of hydrogen atoms in such protein–water complexes. Here we show, now time-resolved at atomic resolution, how a membrane protein achieves directional proton transfer via protein-bound water molecules in contrast to random proton transfer in liquid water. A combination of X-ray structure analysis, time-resolved FTIR spectroscopy, and molecular dynamics (MD) simulations elucidates how directionality is achieved. Using the proton-pump bacteriorhodopsin as the paradigm, we show how controlled conformational changes of few amino

acid residues rearrange preordered water molecules and induce directional proton transfer. This mechanism is analogous to an electronic diode: a “proton diode”.

According to the chemiosmotic theory, the creation of a proton gradient in photosynthesis^[1] and oxidative phosphorylation^[2–4] by means of directional proton transfer is the key step for energy transduction in living cells. ATPases use this proton gradient to produce ATP, the fuel for life. In contrast to this directional mechanism in proteins, proton transfer in liquid water is random.^[5,6] Bacteriorhodopsin (bR), a protein that belongs to the microbial rhodopsin family,^[7,8] achieves this directional proton transfer by a light-driven proton-pumping mechanism. Like other microbial rhodopsins, bR exhibits a structural motif of seven transmembrane α -helices and a retinal chromophore covalently bound to a lysine through a protonated Schiff base. The light-induced retinal isomerization from all-*trans* in the ground state (BR) to the 13-*cis* conformer drives bR through a photocycle with intermediates named J, K, L, M, N, and O in order of their appearance.^[8]

During the L to M transition, the protonated Schiff base (C=NH⁺), the central proton-binding site, deprotonates and protonates its counterion Asp85^[9] (step 1 in Figure 1a). Protonation of Asp85 breaks its salt bridge to Arg82, which then moves towards Glu194/Glu204 (step 2). The orientation of Arg82 depends on the protonation state of Asp85.^[10] The arginine movement destabilizes a protonated water cluster between Arg82, Glu194, and Glu204 (step 3 in Figure 1a), and a proton is released to the bulk.^[11,12]

However, the detailed nature of the proton-release group is still under debate. QM/MM simulations of the proton-release group propose a shared proton between Glu194 and Glu204,^[13] a Zundel cation with two water molecules (H₃O₂⁺),^[14] or an asymmetric Eigen cation of four water molecules (H₅O₄⁺).^[15] From time-resolved FTIR experiments with site-directed mutations around the protonated water cluster and H/D-exchange experiments we have concluded that the proton-release group forms a protonated water cluster, most likely an asymmetric Eigen ion as shown in Figure 1a in purple.^[12] Glu194 and Glu204 are clearly deprotonated in the bR ground state.^[11] This experimental result was recently confirmed by Lórenz-Fonfría et al.^[16] Nevertheless, the exact nature of the protonated water cluster and the release mechanism has still to be determined.

Here, we used X-ray structure analysis to determine the positions of the water oxygen atoms and FTIR difference spectroscopy to determine the dynamics of the corresponding water hydrogen atoms. The proton release to the bulk in the L

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