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Review

Proton transfers in the bacteriorhodopsin photocycle

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Abstract

The steps in the mechanism of proton transport in bacteriorhodopsin include examples for most kinds of proton transfer reactions that might occur in a transmembrane pump: proton transfer via a bridging water molecule, coupled protonation/deprotonation of two buried groups separated by a considerable distance, long-range proton migration over a hydrogen-bonded aqueous chain, and capture as well as release of protons at the membrane–water interface. The conceptual and technical advantages of this system have allowed close examination of many of these model reactions, some at an atomic level.
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1. Introduction

In the past three decades, bacteriorhodopsin has become a model for the simplest and most essential features necessary in an active proton transporter. Proton transport in this small (25 kDa) integral membrane protein is based on the stepwise reactions that accompany the thermal reisomerization of the photoisomerized 13-cis,15-anti retinal to the initial all-trans configuration (the "photocycle"). A huge effort, from many laboratories, on the spectroscopic, mutational, and crystallographic aspects of these steps has given a description, to a large extent at atomic resolution, of the transport mechanism. The sheer volume of information to be covered, and the many lively controversies that have arisen (for example [1]), make it difficult to give a comprehensive account of this field in a single, brief review article. Instead, the intent here is to call attention to what has been learned by the author about the individual proton transfer steps in bacteriorhodopsin that might be of interest to the larger field of proton pumps. Other novel aspects of bacteriorhodopsin, such as analysis of the complex photocycle kinetics, nature of the protonation switch, cascades of conformational changes and their causes at the active site, and the flow of energy through the system, have been discussed at length in numerous review articles elsewhere [2–21].

The photocycle (Fig. 1) is described by a sequence of states, all well characterized by spectroscopic and most also by crystallographic methods: $\mathrm{BR}\text{-}h\nu \rightarrow \mathrm{K} \leftrightarrow \mathrm{L} \leftrightarrow \mathrm{M}_1 \leftrightarrow \mathrm{M}_2 \leftrightarrow \mathrm{M}_2' \leftrightarrow \mathrm{N} \leftrightarrow \mathrm{N}' \leftrightarrow \mathrm{O} \rightarrow \mathrm{BR}$. The interconversions of these states reflect the physical steps necessary in moving a proton from one membrane surface to the other in this system, and the retinal and protein conformations they represent have been described in much detail, and the reader should consult the review articles cited. Description of the protein and the retinal in these states is essential to understand the transport mechanism, but here they will be discussed only in the context of proton transfer reactions.

The structure of bacteriorhodopsin is available from cryo-electron microscopy, and in the past few years also from high-resolution (to 1.4 Å) X-ray diffraction. This hydrophobic protein contains seven transmembrane helices (A though G), which span the membrane at small angles to the perpendicular and surround the centrally located retinal linked to helix G by a protonated Schiff base with lys-216 [22–25]. The crucial steps in the transport take place at the active center, which comprises wat402 that receives a hydrogen-bond from the Schiff base and donates hydrogen-bonds to the anionic asp-85 and asp-212 [26]. Deprotonation of the Schiff base of the photoisomerized retinal to protonate asp-85 moves a proton to the extracellular side, where it

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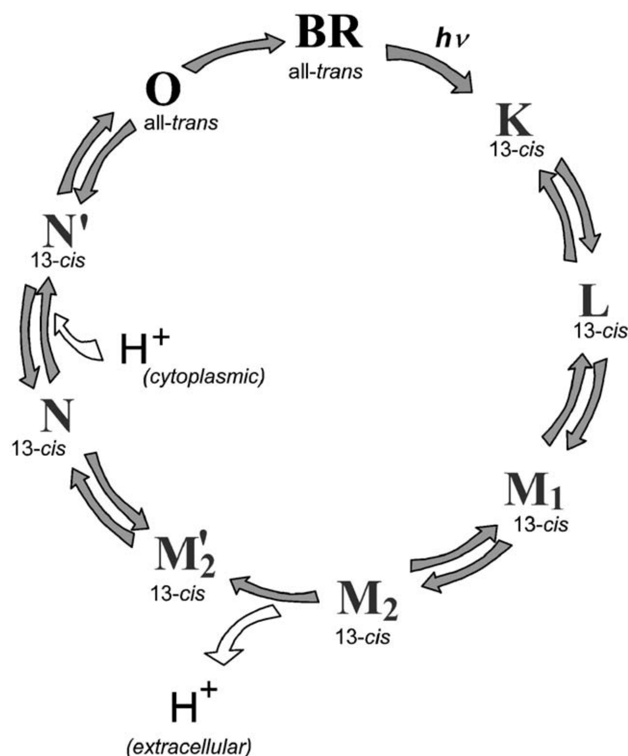

Fig. 1. Schematic representation of the bacteriorhodopsin photocycle. The isomeric state of the retinal in each state is as indicated. The release and uptake of protons are discussed in the text.

causes the release of another proton to the surface. Replacement of the proton of the Schiff base from the cytoplasmic side and the proton released on the extracellular side complete the cycle. Release and uptake of the proton are accomplished differently in the two halves of the protein. To the extracellular direction of the Schiff base there is an extensive hydrogen-bonded network of polar side-chains and bound water [23,24]. It extends to the aqueous interface where the proton is released, and constitutes a recognizable pathway for proton transfers in the BR state already. In contrast, the region on the cytoplasmic side of the Schiff base contains few polar groups and is largely devoid of bound water [23,24]. This is the hydrophobic barrier in the resting pump [27]. During the photocycle, transient entry of water into this part of the protein creates a pathway for proton transfers between the surface, where the proton is taken up, and the Schiff base.

2. Short-distance exchange of protons: protonation of asp-85

Although the reaction mechanism of what might be considered the simplest proton transfer, between the Schiff base and asp-85, cannot be resolved into smaller steps and is therefore not accessible to direct measurement, there is much indirect evidence to suggest why and how it occurs. The proton affinities of the Schiff base and asp-85 are very high [28] and very low [29], respectively, in the BR (resting) state. From a thermodynamic cycle, the free energy to be provided for this proton transfer was calculated to be 30–33 kJ/mol [30], or about 2/3 of the excess energy in the K state [31]. The equilibrium that keeps the proton away from its acceptor, asp-85, is ensured by the large number of hydrogen-bonds [32,33] that pervade the extracellular region. How is this equilibrium upset by the free energy accumulated upon photoisomerization of the retinal from all-trans to 13-cis,15-anti?

Crystal structures of the trapped photocycle intermediates K and L [34,35] suggest that energy is conserved in a distorted retinal bond (at C_{12}—C_{13}=C_{14}) and in twists of two double bonds (C_{13}=C_{14} and C_{15}=NZ). The relaxed 13-cis,15-anti retinal would have a bent contour, and the N—H bond turned approx. 180°, from facing wat402 and the two anionic carboxyl groups toward the hydrophobic cytoplasmic side, but this configuration is not realized in the K state because the binding site does not yield to accommodate the change of shape and increased charge separation. The relaxed state can be reached only by appropriate distortions in the matrix around the retinal, and importantly for the proton transport, loss of the polar nature of the positively charged protonated Schiff base and its Coulombic interaction with the extracellular network through wat402 [35,36]. Transfer of its proton to asp-85 in the L↔M_1 reaction (τ=10 μs) accomplishes both.

The crystallographic structure provides a ready explanation why asp-85, and not the nearly symmetrically located asp-212, is the proton acceptor. The anionic states of both side-chains are stabilized by their hydrogen-bonds. The carboxylate group of asp-212 retains its low pK_a as it is hydrogen-bonded to tyr-57 and tyr-185, bulky residues that do not move during the photocycle. The low pK_a of asp-85, on the other hand, depends on its hydrogen-bond to wat401 and the side-chain of thr-89, and both of these are mobile and move, in fact, as the M states are reached.

In a series of X-ray diffraction structures for trapped intermediates [34,35,37,38], the N—H bond (and after deprotonation of the Schiff base N:) continues to point to the extracellular direction throughout the K↔L↔M_1 sequence. In the K state, it loses its hydrogen-bond to wat402, but regains it in L as the twist in the retinal spreads along the polyene chain. In M_1, the hydrogen-bond is lost again because the Schiff base is deprotonated and wat402 has moved away, by 1 Å, toward asp-85 (now protonated) and asp-212. In M_2, the deprotonated Schiff base nitrogen turns to the cytoplasmic direction, and wat402 is no longer observed in the structure. These observations suggest that the driving force for the Schiff base deprotonation is the relaxation of the retinal, and that the proton transfer to asp-85 occurs via wat402.

In diffraction structures from other laboratories, however, the Schiff base N—H bond is turned to the cytoplasmic side in the K state [39], or in the L state [40] already. In the first of these reports, wat402 was not seen in the K state at all, and this would require direct proton transfer from Schiff base to asp-85 [39,41]. According to the second report [40], wat402 migrates from its initial location to the cytoplasmic side of the Schiff base in the K to L transition. The latter

would be consistent with FTIR spectra of the L state [42–44] where an O–H stretch band from water appears that is affected both by mutations in the cytoplasmic region (and therefore argued to be on the cytoplasmic side of the Schiff base) and the Schiff base. The path of the proton from Schiff base to asp-85 is somewhat problematical in the mechanisms implied by these conflicting observations [1]. However, calculations of the reaction barriers [45] suggested that movement of the proton from the Schiff base, facing the cytoplasmic direction, to asp-85, on the extracellular side, is made feasible both by passing through asp-212 in one mechanism and by transient protonation of the side-chain OH of thr-89 in another.

A radically different mechanism for the $L \rightarrow M_1$ reaction has been proposed in the hydroxyl ion hypothesis [38,46]. According to this novel idea, inspired by the analogy between bacteriorhodopsin and halorhodopsin, the light-driven chloride pump [15,47,48], wat402 dissociates to protonate asp-85 while the OH^- ion moves to the cytoplasmic side of the Schiff base. The latter loses its proton to form water in this postulated concerted reaction. The OH^- ion hypothesis accounts also for the fact that the D85T mutant of bacteriorhodopsin, which binds a halide ion near the Schiff base and transports the anion like halorhodopsin [49]. Although attractive, there are numerous problems with this idea. Dissociation of water to yield H^+ and OH^- is difficult to accomplish, and the question is whether polarization of wat402 by the surrounding dipoles decreases its proton affinity sufficiently. Further, none of the three different sets of X-ray diffraction structures support it: in one, wat402 remains on the extracellular side after deprotonation of the Schiff base [35], in another, wat402 has moved to the cytoplasmic side even before protonation of asp-85 [40], and in the third, wat402 is absent well before the proton transfer takes place [39,41]. The analogy with halorhodopsin is coming under fire also. In a recent model based on FTIR spectra [50], the Schiff base in halorhodopsin was found to be hydrogen-bonded to water, and not Cl^- during translocation of the anion as would be expected from the OH^- hypothesis for bacteriorhodopsin.

3. Coupling of protonation states: proton release to the extracellular surface

Asp-85 remains protonated until the last step of the photocycle, but a proton is released to the extracellular surface in what is described [38,51] as the M_2 to M_2' step (Fig. 1, τ = ca. 100 μs). In a linked equilibrium with the $M_1 \leftrightarrow M_2$ reaction, this reaction appears as the second component in the multiphasic rise of the M state. Coupling between the protonation states of asp-85 and another group was demonstrated by the complex titration behavior of the aspartic acid in the dark [52]. The nature of this coupling is that between pH 5 and 9 either group may be protonated but not both, and thus forced protonation of asp-85 results in dissociation of the other group that is proposed to be the one to release the proton during the photocycle. In the model that reproduces the titration, the pK_a of this group is 9–10 when asp-85 is anionic, but becomes 5–5.5 when asp-85 is protonated [52,53]. Although in these titrations the retinal is all-trans, the calculated pK_as agree well with those measured for the then unidentified proton release group in the BR state and during the photocycle. There is good agreement also between the influence of numerous single-site mutations, such as arg-82, tyr-57, glu-194 and glu-204, on a) the coupled titration behavior and b) the proton release.

The origin of the released proton had long been controversial, however. The various proposed proton release sites, from FTIR spectra, include glu-204 [54], arg-82 [55], and an aqueous extracellular network that contains a delocalized proton [56,57]. Recently, conclusive evidence was obtained in favor of the last of these [58], as follows. Decrease of the amplitude of an IR continuum correlates well with the temporal behavior of proton release in the wild type and in numerous mutants. It is absent when proton release is blocked, at pH <6 or in mutants. A novel method was developed for determining the protonation states of the relevant acidic residues by difference FTIR spectroscopy without relying on their dissociation. It appears from these results that the excess proton in the release site fluctuates rapidly between two hydrogen-bonded water molecules, or a “Zundel water” [59], stabilized by the symmetrical arrangement of the anionic side-chains of glu-194 and glu-204 that face one another. The delocalized proton binding site is abolished not only by replacement of either glutamates with glutamine, but also by replacement of either glutamate with an aspartate which alters the symmetry. In the latter kind of mutants, the proton release occurs but the proton is a localized one. For example, in the E194D mutant it will originate from glu-204, and FTIR spectra show first protonation of asp-194 as asp-85 is protonated and then its deprotonation, coincident with a proton detected at the surface with a pH indicator dye [60].

The diffraction structures of M states [37,38,61] indicate that the mechanism of coupling between this site and asp-85 is the shuttling of the side-chain of arg-82. The guanidinium group is connected to the Schiff base region by its electrostatic interaction with the anionic asp-85 and a hydrogen-bond to wat401 via wat406. Upon collapse of this network because of protonation of asp-85, the positively charged arg-82 moves away from the protonated asp-85 and toward the glu-194/glu-204 pair in the crystal structures of all reported M states [37,38,61] after M_1 [62]. As expected, replacement of arg-82 with glutamine or alanine abolishes proton release during the lifetime of M [63]. The proton released to the surface upon approach of the positive charge of arg-82 may pass by ser-93, but the details of this are not clear.

4. Creation of a new hydrogen-bonded chain of water: reprotonation of the retinal Schiff base

The proton donor to the Schiff base in the $M_2' \rightarrow N$ reaction is asp-96, located at a distance of 10 Å from the

Schiff base and about 6 Å from the cytoplasmic surface. There are two obstacles to overcome in this long-range proton transfer: the high (>11) initial pK_a of the donor [64] and the lack of a proton-conducting pathway. Both are accomplished by entry of water into this region. The formation of a cluster of bound water molecules at asp-96, and its growth, begin once the Schiff base is deprotonated [35,62,65]. Its cause appears to be cavities of increasing size, the result of repacking of side-chains between helices F and G as the isomerized retinal and the attached lys-216 side-chain assume their changed shape [38]. In M_2, the hydrogen-bond between the protonated carboxyl and the thr-46 side-chain is replaced by a water molecule that bridges these groups, but is connected also to two other water molecules in a partial chain that extends toward the Schiff base [38]. Presumably, at this stage the pK_a of asp-96 is lowered to the observed value of about 7.5 [66–68], and the pK_a of the Schiff base is about 8 [69]. However, proton equilibration between donor and acceptor can take place only after a fourth water completes the single-file chain to the retinal [65].

The chain of four water molecules (wat503–wat502–wat506–wat505) is observed in the crystal structure of the trapped N′ state [65], where the Schiff base is reprotonated, as is asp-96, but the retinal is still 13-cis. The chain thus includes wat502, present in the BR state already, that remains hydrogen-bonded to the displaced peptide carbonyl of lys-216. It is moved to a location suited to form hydrogen-bonds with wat503 and wat506. Other than this connection to lys-216, however, the only hydrogen-bonds are those that connect water molecules to each other along the chain and to the proton donor ands acceptor at either end. This kind of an arrangement appears to be well suited for conducting protons [70].

Movement of a proton over such a chain of water molecules is in the ns time-domain, yet reprotonation of the Schiff base (the $M_2' \rightarrow N$ reaction) has a time-constant of a few ms. There are three possible, not all mutually exclusive, alternative reasons for this. First, the slow time-constant might reflect the conformational shift in the interior of the protein that creates the conditions for the hydrogen-bonded proton-conducting chain. Second, the hydrogen-bonded aqueous chain may flicker on and off as the result of conformational fluctuations of the protein, and it is populated very infrequently. Third, the existence of a hydrogen-bonded chain might not be sufficient for proton transfer, because the latter depends on overcoming the Coulombic barrier of separating the proton from the carboxylate of asp-96. The crystallographic results [65], together with earlier studies of the thermodynamics of the Schiff base reprotonation [71] suggest that while the conformational shift that allows formation of the proton-conducting pathway is gradual, with distinct stages, the main barrier is the deprotonation of asp-96. Replacing asp-96 with an asparagine makes the proton transfer very slow and pH dependent [71–74], suggesting that the proton now originates at the membrane surface. In this case there is no separation of charges, and the enthalpy of activation is so strongly decreased that it accounts for the 6 orders of magnitude difference between the expected and observed time-constants [71]. The greatly increased entropic barrier may reflect conformational requirements for capturing a proton without the negative charge of the anionic asp-96 in the D96N mutant, or difficulties of forming a hydrogen-bonded chain without nucleation by this residue.

5. Proton capture at the membrane surface: reprotonation of asp-96

In the wild-type photocycle, reprotonation of asp-96 from the cytoplasmic surface is virtually coincident with the thermal reisomerization of the retinal to all-trans, yielding the O state. For this reason, a second N state with protonated asp-96, i.e., N′, usually cannot be detected. The suspicion that this is caused by coupling between the isomeric state of the retinal and the protonation state of asp-96 is supported by several observations. The lifetime of the N state (i.e., the reisomerization reaction) is influenced by mutations not only near the retinal but also near asp-96 [68]. Somewhat more direct evidence is from the properties of the D85N/F42C mutant [75], which resembles N because the negative charge of asp-85 is removed as in N (where it is protonated) and the pK_a of asp-96 is lowered by the second, nearby mutation so it can be deprotonated by raising the pH to 8 and it is then anionic as in N. In this protein designed to test the hypothesis of coupling, the isomeric state of the retinal depends on the pH. When the pH is shifted to 8 where asp-96 is deprotonated, the retinal changes, within tens of ms and in the dark, to 13-cis,15-anti, an isomeric state assumed otherwise only upon illumination. In this artificial system it is the pH that regulates the isomerization of the retinal though the protonation state of asp-96, but in the photocycle it should be the reverse: the coupling will ensure that asp-96 reprotonates when the retinal returns to its all-trans configuration.

The means of this coupling, over the 10 Å distance that separates the Schiff base and asp-96, must be the four water molecules that form the hydrogen-bonded chain. The hydrogen-bond of the proximal water molecule, wat505, with the Schiff base requires that the direction of the N–H bond be toward the cytoplasmic side, and the chain will collapse when it turns back to face the extracellular side. If water molecules leave the vicinity of asp-96, this would increase its proton affinity and be responsible for its reprotonation. Importantly, it is inherent in this mechanism that it does not allow the reprotonation of asp-96 to be by the Schiff base, i.e., by reversal of the rise of the N state. The pK_a of asp-96 at this stage has been estimated as ca. 7.5 from the pH dependence of the decay of the N state [66] or the rise of the O state [67]. The same value is obtained more directly when the photocycle of the V49A mutant, which is perturbed in retinal reisomerization and therefore in the coupling, is examined [68]. In this mutant,

the lifetime of the N state is increased from ca. 10 ms to several seconds, and below pH 7 (but not above), protonation of asp-96, as followed with the appearance of its C═O stretch band, precedes reisomerization by several orders of magnitude of time. With increasing pH along a titration curve with a pK_a of 7, an increasing fraction of asp-96 shows slower protonation that is delayed until the end of the cycle. It is not clear, however, at what stage the very high (>11) initial pK_a of asp-96 is regained.

The pathway of the proton from the cytoplasmic surface to asp-96 is also unclear. Low-resolution electron diffraction maps suggested an open channel that leads to asp-96 [76], but if there is such a pore it cannot be open during reprotonation of the Schiff base because the Schiff base has a pK_a of about 8 [62], yet it fully reprotonates independent of pH, up to at least pH 10. Further, mutagenesis of the residues that line the putative pore that decrease the volume of the side-chains results in blocking proton uptake rather than accelerating it [68]. It seems likely that there are specific structural changes in the hydrophobic lid that separates asp-96 from the surface, and a second chain of water might accumulate transiently in this region during the N→N′ reaction.

Analysis of the time-course of the events after flash-initiated pulses of protons from pyranine had suggested that the numerous acidic residues on the cytoplasmic surface (asp-36, asp-38, asp-102, asp-104, and glu-166) interact as buffering groups [77–79]. During the photocycle, they would capture the proton collectively as an "antenna," and facilitate funneling the proton to asp-96. However, replacing these residues with their non-protonatable equivalents, singly and in groups, produced little change or changes in the opposite direction from what is expected, in the lifetime of the N state [79,80]. It appears that the rate of the protonation of asp-96 is determined by factors other than the efficient capture of protons.

6. Use of an existing network of water: reprotonation of the extracellular proton release group

In the last step of the photocycle, asp-85 reprotonates the vacant extracellular proton release group. This is a strongly downhill reaction, the principal one identified in the photocycle after the K state [81,82]. The driving force for this reaction must be the reestablishment of the low pK_a (about 2.5) of asp-85 and the high pK_a (about 9) of the proton release group as the initial geometry of the extracellular region recovers. There being no crystal structure for the O state, all ideas on how it occurs are indirect, however.

Mutations in the extracellular region affected the rate of recovery of the BR state in the same manner as they affected the rate of deprotonation of asp-85 when the pH was raised, in pH jump experiments in the dark, from 2 to 6 [83]. This suggested that deprotonation of asp-85 does not require structural rearrangements unique to the photocycle, although one could argue that at pH 2 where asp-85 is uncharged, as in some asp-85 mutants, the structure might resemble that of the O state [84]. The appearance of a C═O stretch band attributable to the partial protonation of asp-212 in the O state suggested transient proton equilibration between asp-85 and asp-212 [85]. Thus, asp-212 may be in the pathway of the proton before it reaches its destination. Residues whose replacements slow deprotonation of asp-85 include arg-82, glu-194, and glu-204, indicating that all of these are involved in both the deprotonation and the reprotonation of the proton release site.

It should be mentioned that at pH below 6, i.e. below the pK_a of the proton release group during the photocycle, proton is not released at the normal time. Instead of being concurrent with the rise of M (in the M_2 to M_2' reaction), proton release to the extracellular membrane surface is delayed until the last photocycle step [51, 86], and assumed to be directly from asp-85. This is true also in the mutants that block proton release. Interestingly, transport function is not affected: either proton release on one side or proton uptake on the other may occur first in the sequence.

References

[1] J.K. Lanyi, What is the real crystallographic structure of the L photointermediate of bacteriorhodopsin? Biochim. Biophys. Acta 1658 (2004) 14–22.

[2] J.K. Lanyi, G. Varo, The photocycles of bacteriorhodopsin, Isr. J. Chem. 35 (1995) 365–385.

[3] J.K. Lanyi, Understanding structure and function in the light-driven proton pump bacteriorhodopsin, J. Struct. Biol. 124 (1998) 164–178.

[4] J.K. Lanyi, Bacteriorhodopsin, Int. Rev. Cytol. 187 (1999) 161–202.

[5] J.K. Lanyi, Progress toward an explicit mechanistic model for the light-driven pump, bacteriorhodopsin, FEBS Lett. 464 (1999) 103–107.

[6] J.K. Lanyi, Molecular mechanism of ion transport in bacteriorhodopsin: insights from crystallographic, spectroscopic, kinetic and mutational studies, J. Phys. Chem., B 104 (2000) 11441–11448.

[7] J.K. Lanyi, A. Pohorille, Proton pumps: mechanism of action and applications, Trends Biotechnol. 19 (2001) 140–144.

[8] J.K. Lanyi, X-ray crystallography of bacteriorhodopsin and its photointermediates: insights into the mechanism of proton transport, Biochemistry (Mosc.) 66 (2001) 1192–1196.

[9] J.K. Lanyi, H. Luecke, Bacteriorhodopsin, Curr. Opin. Struct. Biol. 11 (2001) 415–419.

[10] J.K. Lanyi, Molecular mechanism of ion transport in bacteriorhodopsin: insights from crystallographic, spectroscopic, kinetic, and mutational studies, J. Phys. Chem., B 104 (2003) 11441–11448.

[11] J.K. Lanyi, B. Schobert, Local–global conformational coupling in a heptahelical membrane protein: transport mechanism from crystal structures of the nine States in the bacteriorhodopsin photocycle, Biochemistry 43 (2004) 3–8.

[12] J.K. Lanyi, X-ray diffraction of bacteriorhodopsin photocycle intermediates, Mol. Membr. Biol. 21 (2004) 143–150.

[13] J.K. Lanyi, A structural view of proton transport in bacteriorhodopsin, in: M. Wikström (Ed.), Biophysical and structural aspects of bioenergetics, Royal Soc. Chem. U.K., in press.

[14] U. Haupts, J. Tittor, E. Bamberg, D. Oesterhelt, General concept for ion translocation by halobacterial retinal proteins: the isomerization/switch/transfer (IST) model, Biochemistry 36 (1997) 2–7.

[15] D. Oesterhelt, The structure and mechanism of the family of retinal proteins from halophilic archaea, Curr. Opin. Struct. Biol. 8 (1998) 489–500.

[16] M. Kataoka, H. Kamikubo, Structures of photointermediates and their

implications for the proton pump mechanism, Biochim. Biophys. Acta 1460 (2000) 166–176.
[17] H. Kandori, Role of internal water molecules in bacteriorhodopsin, Biochim. Biophys. Acta 1460 (2000) 177–191.
[18] L.S. Brown, Proton transport mechanism of bacteriorhodopsin as revealed by site- specific mutagenesis and protein sequence variability, Biochemistry (Mosc.) 66 (2001) 1249–1255.
[19] J. Herzfeld, J.C. Lansing, Magnetic resonance studies of the bacteriorhodopsin pump cycle, Annu. Rev. Biophys. Biomol. Struct. 31 (2002) 73–95.
[20] R. Neutze, E. Pebay-Peyroula, K. Edman, A. Royant, J. Navarro, E.M. Landau, Bacteriorhodopsin: a high-resolution structural view of vectorial proton transport, Biochim. Biophys. Acta 1565 (2002) 144–167.
[21] J.P. Cartailler, H. Luecke, X-ray crystallographic analysis of lipid–protein interactions in the bacteriorhodopsin purple membrane, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 285–310.
[22] N. Grigorieff, T.A. Ceska, K.H. Downing, J.M. Baldwin, R. Henderson, Electron-crystallographic refinement of the structure of bacteriorhodopsin, J. Mol. Biol. 259 (1996) 393–421.
[23] H. Belrhali, P. Nollert, A. Royant, C. Menzel, J.P. Rosenbusch, E.M. Landau, E. Pebay-Peyroula, Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 A resolution, Struct. Fold. Des 7 (1999) 909–917.
[24] H. Luecke, B. Schobert, H.T. Richter, J.P. Cartailler, J.K. Lanyi, Structure of bacteriorhodopsin at 1.55 A resolution, J. Mol. Biol. 291 (1999) 899–911.
[25] L. Essen, R. Siegert, W.D. Lehmann, D. Oesterhelt, Lipid patches in membrane protein oligomers: crystal structure of the bacteriorhodopsin–lipid complex, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 11673–11678.
[26] H. Luecke, H.T. Richter, J.K. Lanyi, Proton transfer pathways in bacteriorhodopsin at 2. 3 angstrom resolution, Science 280 (1998) 1934–1937.
[27] M. Wikstrom, Proton translocation by bacteriorhodopsin and heme-copper oxidases, Curr. Opin. Struct. Biol. 8 (1998) 480–488.
[28] S. Druckmann, M. Ottolenghi, A. Pande, J. Pande, R.H. Callender, Acid–base equilibrium of the Schiff base in bacteriorhodopsin, Biochemistry 21 (1982) 4953–4959.
[29] C.H. Chang, J.G. Chen, R. Govindjee, T. Ebrey, Cation binding by bacteriorhodopsin, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 396–400.
[30] L.S. Brown, L. Bonet, R. Needleman, J.K. Lanyi, Estimated acid dissociation constants of the Schiff base, Asp-85, and Arg-82 during the bacteriorhodopsin photocycle, Biophys. J. 65 (1993) 124–130.
[31] R.R. Birge, T.M. Cooper, A.F. Lawrence, M.B. Masthay, C.F. Zhang, R. Zidovetzki, Revised assignment of energy storage in the primary photochemical event in bacteriorhodopsin, J. Am. Chem. Soc. 113 (1991) 4327–4328.
[32] Y. Gat, M. Sheves, A mechanism for controlling the pK_a of the retinal protonated Schiff base in retinal proteins. A study with model compounds, J. Am. Chem. Soc. 115 (1993) 3772–3773.
[33] L.S. Brown, Y. Gat, M. Sheves, Y. Yamazaki, A. Maeda, R. Needleman, J.K. Lanyi, The retinal Schiff base–counterion complex of bacteriorhodopsin: changed geometry during the photocycle is a cause of proton transfer to aspartate 85, Biochemistry 33 (1994) 12001–12011.
[34] B. Schobert, J. Cupp-Vickery, V. Hornak, S. Smith, J. Lanyi, Crystallographic structure of the K intermediate of bacteriorhodopsin: conservation of free energy after photoisomerization of the retinal, J. Mol. Biol. 321 (2002) 715–726.
[35] J.K. Lanyi, B. Schobert, Mechanism of proton transport in bacteriorhodopsin from crystallographic structures of the K, L, M-1, M-2, and M-2′ intermediates of the photocycle, J. Mol. Biol. 328 (2003) 439–450.
[36] J. Herzfeld, B. Tounge, NMR probes of vectoriality in the proton-motive photocycle of bacteriorhodopsin: evidence for an ‘electrostatic steering’ mechanism, Biochim. Biophys. Acta 1460 (2000) 95–105.
[37] H. Luecke, B. Schobert, H.T. Richter, J.P. Cartailler, J.K. Lanyi, Structural changes in bacteriorhodopsin during ion transport at 2 angstrom resolution, Science 286 (1999) 255–261.
[38] H. Luecke, B. Schobert, H.T. Richter, J.-P. Cartailler, A. Rosengarth, R. Needleman, J.K. Lanyi, Coupling photoisomerization of the retinal in bacteriorhodopsin to directional transport, J. Mol. Biol. 300 (2000) 1237–1255.
[39] K. Edman, P. Nollert, A. Royant, H. Belrhali, E. Pebay-Peyroula, J. Hajdu, R. Neutze, E.M. Landau, High-resolution X-ray structure of an early intermediate in the bacteriorhodopsin photocycle, Nature 401 (1999) 822–826.
[40] T. Kouyama, T. Nishikawa, T. Tokuhisa, H. Okumura, Crystal structure of the L intermediate of bacteriorhodopsin: evidence for vertical translocation of a water molecule during the proton pumping cycle, J. Mol. Biol. 335 (2004) 531–546.
[41] A. Royant, K. Edman, T. Ursby, E. Pebay-Peyroula, E.M. Landau, R. Neutze, Helix deformation is coupled to vectorial proton transport in the photocycle of bacteriorhodopsin, Nature 406 (2000) 645–648.
[42] A. Maeda, S.P. Balashov, J. Lugtenburg, M.A. Verhoeven, J. Herzfeld, M. Belenky, R.B. Gennis, F.L. Tomson, T.G. Ebrey, Interaction of internal water molecules with the Schiff base in the L intermediate of the bacteriorhodopsin photocycle, Biochemistry 41 (2002) 3803–3809.
[43] A. Maeda, F.L. Tomson, R.B. Gennis, S.P. Balashov, T.G. Ebrey, Water molecule rearrangements around leu93 and trp182 in the formation of the L intermediate in bacteriorhodopsin's photocycle, Biochemistry 42 (2003) 2535–2541.
[44] A. Maeda, J. Herzfeld, M. Belenky, R. Needleman, R.B. Gennis, S.P. Balashov, T.G. Ebrey, Water-mediated hydrogen-bonded network on the cytoplasmic side of the Schiff base of the L photointermediate of bacteriorhodopsin, Biochemistry 42 (2003) 14122–14129.
[45] A.N. Bondar, S. Fischer, J.C. Smith, M. Elstner, S. Suhai, Key role of electrostatic interactions in bacteriorhodopsin proton transfer, J. Am. Chem. Soc. 126 (2004) 14668–14677.
[46] H. Luecke, Atomic resolution structures of bacteriorhodopsin photocycle intermediates: the role of discrete water molecules in the function of this light-driven ion pump, Biochim. Biophys. Acta 1460 (2000) 133–156.
[47] G. Varo, Analogies between halorhodopsin and bacteriorhodopsin, Biochim. Biophys. Acta 1460 (2000) 220–229.
[48] D. Oesterhelt, Structure and function in halorhodopsin, Isr. J. Chem. 35 (1995) 475–494.
[49] J. Sasaki, L.S. Brown, Y.S. Chon, H. Kandori, A. Maeda, R. Needleman, J.K. Lanyi, Conversion of bacteriorhodopsin into a chloride ion pump, Science 269 (1995) 73–75.
[50] M. Shibata, N. Muneda, T. Sasaki, K. Shimono, N. Kamo, M. Demura, H. Kandori, Hydrogen-bonding alterations of the protonated Schiif base and a water molecule in the chloride pump of *Natronobacter pharaonis*, Biochemistry 44 (2005) 12279–12286.
[51] L. Zimanyi, G. Varo, M. Chang, B. Ni, R. Needleman, J.K. Lanyi, Pathways of proton release in the bacteriorhodopsin photocycle, Biochemistry 31 (1992) 8535–8543.
[52] S.P. Balashov, E.S. Imasheva, R. Govindjee, T.G. Ebrey, Titration of aspartate-85 in bacteriorhodopsin: what it says about chromophore isomerization and proton release, Biophys. J. 70 (1996) 473–481.
[53] H.T. Richter, L.S. Brown, R. Needleman, J.K. Lanyi, A linkage of the pK_a's of asp-85 and glu-204 forms part of the reprotonation switch of bacteriorhodopsin, Biochemistry 35 (1996) 4054–4062.
[54] L.S. Brown, J. Sasaki, H. Kandori, A. Maeda, R. Needleman, J.K. Lanyi, Glutamic acid 204 is the terminal proton release group at the extracellular surface of bacteriorhodopsin, J. Biol. Chem. 270 (1995) 27122–27126.
[55] Y. Xiao, M.S. Hutson, M. Belenky, J. Herzfeld, M.S. Braiman, Role of arginine-82 in fast proton release during the bacteriorhodopsin photocycle: a time-resolved FT-IR study of purple membranes containing ^{15}N-labeled arginine, Biochemistry 43 (2004) 12809–12818.
[56] R. Rammelsberg, G. Huhn, M. Lubben, K. Gerwert, Bacteriorhodopsin's intramolecular proton-release pathway consists of a hydrogen-bonded network, Biochemistry 37 (1998) 5001–5009.
[57] V.Z. Spassov, H. Luecke, K. Gerwert, D. Bashford, pK(a) calculations suggest storage of an excess proton in a hydrogen-bonded water network in bacteriorhodopsin, J. Mol. Biol. 312 (2001) 203–219.
[58] F. Garczarek, L.S. Brown, J.K. Lanyi, K. Gerwert, Proton binding within a

membrane protein by a protonated water cluster, Proc. Natl. Acad. Sci. U. S A. 102 (2005) 3633–3638.
[59] B. Brzezinski, H. Urjasz, G. Zundel, A model molecule of the hydrogen-bonded chain in the active site of bacteriorhodopsin, Biochem. Biophys. Res. Commun. 219 (1996) 273–276.
[60] A.K. Dioumaev, H.T. Richter, L.S. Brown, M. Tanio, S. Tuzi, H. Saito, Y. Kimura, R. Needleman, J.K. Lanyi, Existence of a proton transfer chain in bacteriorhodopsin: participation of Glu-194 in the release of protons to the extracellular surface, Biochemistry 37 (1998) 2496–2506.
[61] H.J. Sass, G. Buldt, R. Gessenich, D. Hehn, D. Neff, R. Schlesinger, J. Berendzen, P. Ormos, Structural alterations for proton translocation in the M state of wild-type bacteriorhodopsin, Nature 406 (2000) 649–653.
[62] J.K. Lanyi, B. Schobert, Crystallographic structure of the retinal and the protein after deprotonation of the Schiff base: the switch in the bacteriorhodopsin photocycle, J. Mol. Biol. 321 (2002) 727–737.
[63] R. Govindjee, S. Misra, S.P. Balashov, T.G. Ebrey, R.K. Crouch, D.R. Menick, Arginine-82 regulates the pK_a of the group responsible for the light-driven proton release in bacteriorhodopsin, Biophys. J. 71 (1996) 1011–1023.
[64] S. Szaraz, D. Oesterhelt, P. Ormos, pH-induced structural changes in bacteriorhodopsin studied by Fourier Transform Infrared Spectroscopy, Biophys. J. 67 (1994) 1706–1712.
[65] B. Schobert, L.S. Brown, J.K. Lanyi, Crystallographic structures of the M and N intermediates of bacteriorhodopsin: assembly of a hydrogen-bonded chain of water molecules between Asp96 and the retinal Schiff base, J. Mol. Biol. 330 (2003) 553–570.
[66] C. Zscherp, R. Schlesinger, J. Tittor, D. Oesterhelt, J. Heberle, In situ determination of transient pK_a changes of internal amino acids of bacteriorhodopsin by using time-resolved attenuated total reflection Fourier-transform infrared spectroscopy, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 5498–5503.
[67] Q. Li, S. Bressler, D. Ovrutsky, M. Ottolenghi, N. Friedman, M. Sheves, On the protein residues that control the yield and kinetics of O(630) in the photocycle of bacteriorhodopsin, Biophys. J. 78 (2000) 354–362.
[68] A.K. Dioumaev, L.S. Brown, R. Needleman, J.K. Lanyi, Coupling of the reisomerization of the retinal, proton uptake, and reprotonation of asp-96 in the n photointermediate of bacteriorhodopsin, Biochemistry 40 (2001) 11308–11317.
[69] L.S. Brown, J.K. Lanyi, Determination of the transiently lowered pK_a of the retinal Schiff base during the photocycle of bacteriorhodopsin, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 1731–1734.
[70] C. Dellago, M.M. Naor, G. Hummer, Proton transport through water-filled carbon nanotubes, Phys. Rev. Lett. 90 (2003) 105902.
[71] Y. Cao, G. Varo, M. Chang, B. Ni, R. Needleman, J.K. Lanyi, Water is required for proton transfer from aspartate-96 to the bacteriorhodopsin Schiff base, Biochemistry 30 (1991) 10972–10979.
[72] H. Otto, T. Marti, M. Holz, T. Mogi, M. Lindau, H.G. Khorana, M.P. Heyn, Aspartic acid-96 is the internal proton donor in the reprotonation of the Schiff base of bacteriorhodopsin, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 9228–9232.
[73] K. Gerwert, B. Hess, J. Soppa, D. Oesterhelt, Role of aspartate-96 in proton translocation by bacteriorhodopsin, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 4943–4947.
[74] A. Miller, D. Oesterhelt, Kinetic optimization of bacteriorhodopsin by aspartic acid 96 as an internal proton donor, Biochim. Biophys. Acta 1020 (1990) 57–64.
[75] A.K. Dioumaev, L.S. Brown, R. Needleman, J.K. Lanyi, Partitioning of free energy gain between the photoisomerized retinal and the protein in bacteriorhodopsin, Biochemistry 37 (1998) 9889–9893.
[76] S. Subramaniam, R. Henderson, Molecular mechanism of vectorial proton translocation by bacteriorhodopsin, Nature 406 (2000) 653–657.
[77] S. Checover, E. Nachliel, N.A. Dencher, M. Gutman, Mechanism of proton entry into the cytoplasmic section of the proton-conducting channel of bacteriorhodopsin, Biochemistry 36 (1997) 13919–13928.
[78] V. Sacks, Y. Marantz, A. Aagaard, S. Checover, E. Nachliel, M. Gutman, The dynamic feature of the proton collecting antenna of a protein surface, Biochim. Biophys. Acta 1365 (1998) 232–240.
[79] S. Checover, Y. Marantz, E. Nachliel, M. Gutman, M. Pfeiffer, J. Tittor, D. Oesterhelt, N.A. Dencher, Dynamics of the proton transfer reaction on the cytoplasmic surface of bacteriorhodopsin, Biochemistry 40 (2001) 4281–4292.
[80] L.S. Brown, R. Needleman, J.K. Lanyi, Functional roles of aspartic acid residues at the cytoplasmic surface of bacteriorhodopsin, Biochemistry 38 (1999) 6855–6861.
[81] K. Ludmann, C. Gergely, G. Varo, Kinetic and thermodynamic study of the bacteriorhodopsin photocycle over a wide pH range, Biophys. J. 75 (1998) 3110–3119.
[82] G. Varo, J.K. Lanyi, Thermodynamics and energy coupling in the bacteriorhodopsin photocycle, Biochemistry 30 (1991) 5016–5022.
[83] H.T. Richter, R. Needleman, H. Kandori, A. Maeda, J.K. Lanyi, Relationship of retinal configuration and internal proton transfer at the end of the bacteriorhodopsin photocycle, Biochemistry 35 (1996) 15461–15466.
[84] S. Rouhani, J.P. Cartailler, M.T. Facciotti, P. Walian, R. Needleman, J.K. Lanyi, R.M. Glaeser, H. Luecke, Crystal structure of the D85S mutant of bacteriorhodopsin: model of an O-like photocycle intermediate, J. Mol. Biol. 313 (2001) 615–628.
[85] A.K. Dioumaev, L.S. Brown, R. Needleman, J.K. Lanyi, Fourier transform infrared spectra of a late intermediate of the bacteriorhodopsin photocycle suggest transient protonation of Asp-212, Biochemistry 38 (1999) 10070–10078.
[86] S.P. Balashov, M. Lu, E.S. Imasheva, R. Govindjee, T.G. Ebrey, B. Othersen III, Y. Chen, R.K. Crouch, D.R. Menick, The proton release group of bacteriorhodopsin controls the rate of the final step of its photocycle at low pH, Biochemistry 38 (1999) 2026–2039.