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Topical Review

Hydrogen Bonded Chain Mechanisms for Proton Conduction and Proton Pumping

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Introduction

This review focuses on the question of how protons are transported across membranes during bioenergetic processes. It has been found that proton transport through membranes is a central feature of several bioenergetic systems, such as bacteriorhodopsin, which pumps protons to higher free energies [115], and the ATP synthase, which consumes energetic protons [90]. In addition, it may be noted that proton transport has been implicated in transhydrogenase [28, 91], cytochrome oxidase, and the bc_1 redox loop in the mitochondrial respiratory chains [29, 90, 119]. ATP-driven proton pumps may be utilized in a variety of biological systems: evidence for them has been found in kidney [69] and liver [97] lysosomal vesicles, maize apical meristem plasmalemma [13], chromaffin granules [2], turtle bladder [1], *Escherichia coli* and *Streptococcus lactis* [68], and *Neurospora crassa* [36]. In addition, H^+/K^+ antiport has been suggested to occur in alkaline tolerant strains of *Bacillus firmus* [38], in gastric parietal cells [102], and H^+/β-galactoside symport in *E. coli* [89].

While the question of proton transport mechanisms was stimulated by the delocalized chemiosmotic theory [72], it should be emphasized that the question is even more relevant for localized theories [120]. Indeed, for localized theories one requires proton transport for long distances along the membrane [49, 50, 86], not just proton transport across the membrane. It is also possible to imagine that even more extensive networks of proton pathways exist in the cell, perhaps utilizing the cytosol microstructure [5, 6]. Finally, proton transport may be a part of other bioenergetic mechanisms such as muscle action [75] or flagellar motion [34, 52].

It will not be our purpose to review the theories of Mitchell [72] and Williams [120]. These theories, which are very important, are phenomenological. They attempt to describe where various ions go and which enzymes and substrates are utilized, but they do not attempt to describe molecular mechanisms. One danger of purely phenomenological theories is that they may require processes for which no physical mechanisms exist. Our purpose will be to review theories of mechanisms for proton transport which can then be utilized within the framework of any phenomenological theories.

Various kinds of active transmembrane proton transport can be envisaged (Fig. 1). One would involve a carrier molecule that would attach a proton and carry it across the membrane (Fig. 1 *A*). Another would involve a transmembrane protein

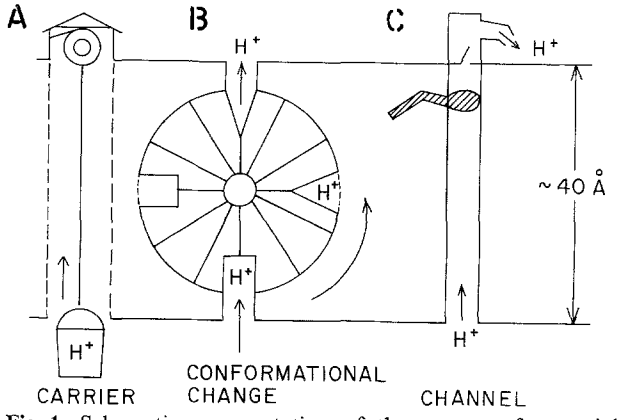

Fig. 1. Schematic representation of three types of vectorial proton transporters suggested by practical devices. (*A*): Conventional water well suggests a carrier mechanism. (*B*): Irrigation water wheel suggests a large conformational change. (*C*): Standard pump suggests a thin channel with an active site at the handle

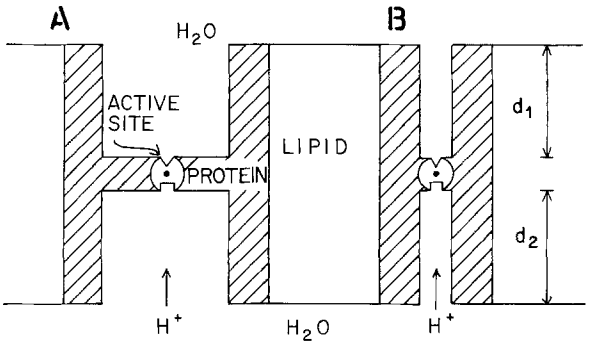

Fig. 2. (*A*): Wide water pore proton channel leading to the localized active site. Such a channel is not ion selective and subjects the active site to dielectric breakdown. (*B*): H_2O molecules in a narrow channel are unlikely to behave as bulk water

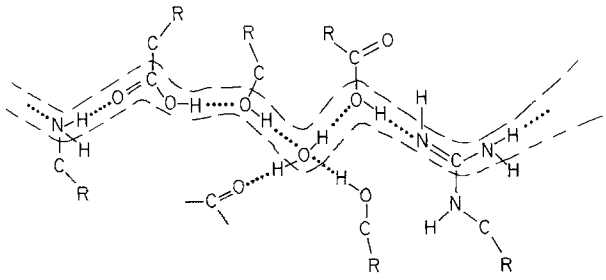

Fig. 3. Hydrogen bonded chain formed from amino acid side chains from membrane proteins and some bound water molecules. The symbol *R* refers to the remainder of the amino acid side chains leading to the protein backbone. The proton channel containing the HBC is outlined with dashed lines

that would attach a proton, then undergo large conformational changes that would take the proton to the other side of the membrane and finally release it (Fig. 1 *B*). A third kind of transport, the one that will be the focus of this review, involves a proton channel through a membrane protein (Fig. 1 *C*). Channels are appealing because they reduce the requirements on the active chemical site. In particular, the active chemical site can have dimensions much smaller than 40 Å, the bilayer thickness, and any conformational changes may be small compared to those in the second kind of transport, shown in Fig. 1 *B*. The first kind of transport (Fig. 1 *A*) defers the problem of vectoriality for active transport to the carrier.

In this review we will concentrate primarily on the proton channel in Fig. 1 *C* and only secondarily upon the active site. Although the active site is at least as important as the channel, we believe that channels are also interesting and nontrivial. In particular, the channels are unlikely to be as wide as the simple water channel shown in Fig. 2 *A*, because the electric field across the active site would then exceed the dielectric breakdown field of most materials ($\sim 10^6$ V/cm) [75]. (Incidentally, the correlation between maximum transmembrane electrical potentials, ~250 mV [29], and dielectric breakdown fields in biological lipid bilayers, $\sim 5 \times 10^5$ V/cm [81], is noteworthy.) One way to avoid dielectric breakdown is to make the water channel narrower (Fig. 2 *B*). In this case most of the water molecules become hydrogen bonded to more or less fixed hydrophilic groups of the protein that form the interface of the pore [22, 31], so that describing the channel as being filled with simple aqueous water is not appropriate. An even smaller proton channel need not involve any water at all, as we now review.

Hydrogen Bonded Chain Structure

In the 60's Onsager suggested that the side chains of proteins could form networks of hydrogen bonds that would provide a hydrophilic environment for transport of ions through biological lipid membranes [82, 83]. A particularly simple network, consisting of a single chain of hydrogen bonds is depicted in Fig. 3. This structure will be called HBC for *Hydrogen Bonded Chain*. Amino acid side chains that are expected to be able to form a HBC are Ser, Thr, Tyr, Glu, Asp, Gln, Asn, Lys, Arg and His, and a HBC could involve bound water molecules as well. Since each hydrogen bond is ~2.5–3.5 Å long and not all bonds would be parallel to the membrane normal, the number of residues required for a HBC to traverse a 40-Å membrane would be about 20. Onsager was primarily interested in HBCs for passive Na^+ and K^+ transport in nerve axons. He knew quite well that of all the ions, HBCs transport H^+ best, but that was before chemiosmosis was the accepted paradigm in bioenergetics! It rapidly became clear in the late 60's (L. Onsager and J.F. Nagle, *unpublished*) that HBCs cannot transport the requisite current of Na^+ or K^+ ions, although Onsager believed that HBCs may provide the gating current for the nerve action potential, as discussed by one of Onsager's coworkers [43]. The connection of Onsager's HBCs to bioenergetics was made by Morowitz [76], who coined the name "proton wires" in the expectation that HBCs could perform the same function for protonic circuits at the membrane micro scale that metallic wires perform for conventional electronic macro circuits. In this review, the name proton wire will refer to any structural entity which can perform biological proton transport and the name HBC will refer to

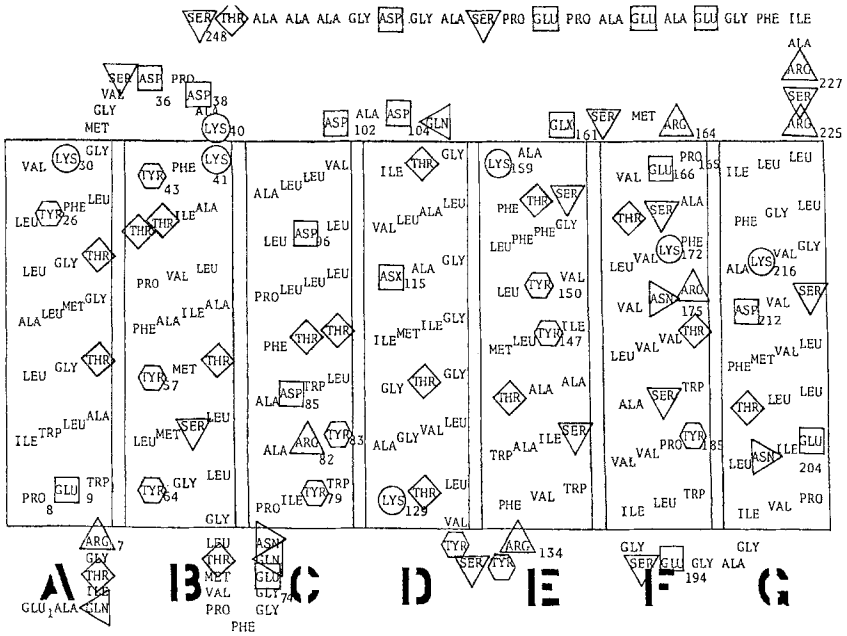

Fig. 4. Model of the secondary structure of bR [116]. Groups capable of forming HBCs are enclosed in symbols. The primary sequence is that of Khorana et al. [53]. Compared to other models of the secondary structure [26, 45, 88] this model reflects some small changes due to additional information gained from chemical modification studies [116, 117], but the overall organization is similar

the specific structural suggestion of Onsager as described above.

The first question concerning the hypothesis of HBC structures is: Can they exist? Is it possible for a protein to satisfy the rules of stereochemistry, including favorable rotational angles about the bonds of the amino acid side chains as well as the peptide backbone, and also to have good hydrogen bonding angles and distances between the side chains? The answer is yes, since one molecular model that satisfies these constraints has been built based on a β-sheet structure [76]. This model involves a HBC between serine side chains; all other side chains are hydrophobic and in contact with lipid. It is more complicated to build models of HBCs based on α-helices. Burres and Dunker [11] have reported molecular models of HBCs with good hydrogen bonding distances and angles between two pairs of nearly parallel α-helices, although they note that systematic consideration was not given to eclipsed side chain conformations which could cost as much or more energy than is gained by the hydrogen bonding between side chains. However, allowing more than two helices adds many more possibilities for longer HBCs.

The second question is: Should HBCs exist? Is such a structure energetically favored? Why should hydrogen bonding groups exist in the interior of a protein? To answer this question in its entirety is to answer the difficult question of protein structure which we do not propose to attempt. But in the case of the single protein in the purple membrane of *Halobacteria halobium*, bacteriorhodopsin, we know that the protein *does* place many side chains capable of forming H-bonds on the inside of the lipid membrane [88] as shown in Fig. 4. For such a protein, sometimes called an "inside-out" protein [27], many workers [19, 63, 118] have stated that the protein will aggregate so as to place hydrophobic residues in contact with lipid and to sequester hydrophilic groups in the protein interior as well as at the aqueous interface. Under such circumstances hydrogen bonding between the side chains in the interior is favored energetically and HBCs become likely. The energy of formation of a HBC of about 20 hydrogen bonds would provide about 20×6 kcal/mole protein. Such considerable energies could even compete with other better known and generally more appreciated structural forces [67]. Indeed, the energy of formation of H-bonds between side chains is just as favorable as the formation of backbone hydrogen bonds in α-helices or β-sheets [76]. While this is not the major consideration for water-soluble proteins for which most of the hydrogen bonding residues are in contact with water, for membrane proteins it should become a much more important consideration.

The third question is: Do HBCs exist in bioenergetic membrane proteins? The answer is, unfortunately, not known since high resolution structures of integral membrane bound proteins from X-ray or neutron diffraction studies do not exist. A likely candidate for a protein containing HBCs is the light-activated proton pump, bacteriorhodopsin (bR). bR is especially promising since it has been shown to involve a channel [95] in a highly ordered two-dimensional crystalline mem-

brane [8] and is probably less subject to large scale dynamic fluctuations [54, 59, 96, 98] than water soluble proteins [92, 94]. In fact, the largest motion during photocycling activity [100] appears to be the *trans-cis* isomerization [10] of the retinal chromophore. Therefore, permanent proton channels of the HBC kind, which should be the easiest kind of channels to find by structural studies, are more likely to exist in bR than in those water-soluble proteins whose structures have been determined. It has been suggested by Merz and Zundel [70] that a HBC stretching part way through the purple membrane could be formed from six tyrosines that are on three different helices, and we have verified the stereochemical possibility of their suggestion. However, two of the tyrosines are on a helix which is remote from the other two, according to the best models of Engelman et al. [26] and Katre et al. [46]. Burres and Dunker [11] mention that they have found several likely proton wires in bR and will report on them in the future. Our own efforts at building HBCs in a model of the bR structure are still in progress. It is clear that there are many degrees of freedom that need systematic exploration.

Dunker and Jones [19] proposed a HBC in murein lipoprotein based on a knobs-in-holes approach. While this approach is useful to screen for favorable candidates, a model for murein lipoprotein that successfully places the knobs into the holes does not yield a completely stereochemically successful HBC model (Mille, *personal communication*; [11]). As is described by Dunker [18], the knobs-in-holes approach led Dunker and Marvin [20] to conclude that HBCs are important structural features of membrane proteins and that they may play a role in proton and other transport.

Unlike murein lipoprotein for which no evidence supports proton translocation, the F_0 portion of the ATP synthase is well established as a transmembrane proton transporter. F_0 has been resolved as a complex containing a few types of protein subunits [41], and evidence supporting the protonophoric activity of one of these subunits, the DCCD-binding proteolipid, has been obtained in reconstituted systems using mitochondrial [16] or chloroplast [78] proteolipid. Based upon their amino acid sequence data for the DCCD binding subunit (mol wt 8,000) of *N. crassa, S. cerevisiae* and *E. coli*, Sebald and Wachter [111] have proposed that this proteolipid subunit consists of two largely hydrophobic sequences each of which traverses the membrane. A polar sequence separating the two hydrophobic sequences and another polar sequence on the N-terminal end are suggested to be in contact with the aqueous phase. Sebald and Wachter [111] comment that there are too few hydrogen bonding groups in the hydrophobic segments to form a HBC. However, given the proposal of a hexameric complex for this subunit [110], the total number of hydrogen bonding side chains is $6 \times 6 = 36$. A HBC composed of 36 hydrophilic groups from six proteolipid subunits is not unreasonable, provided that placement of the proteolipid subunits within the membrane compensates for a hydrophobic region. In addition, conformational changes during proton transport as shown in chloroplast F_0 [25] may allow H-bonding to bridge the hydrophobic region, or one or two bound water molecules may bridge this region. In native *E. coli* membranes, the long hydrophobic region may be compensated for by a second type of subunit, mol wt 19,000, since Loo and Bragg have shown the requirement for two types of subunits to translocate protons [66]. There is yet another possibility. Dunker *(personal communication)* points out, "that the proteolipid is soluble in organic solvents, implying that no part of the molecule in that native structure need be in contact with water, and furthermore that the *polar* connecting link in the model of Sebald and Wachter [111] is at least as hydrophobic as the transmembrane helices in bacteriorhodopsin. These features lead to a different model for the F_0 proteolipid, a model in which the central *polar* sequence provides a transmembrane helix that is flanked by two neighboring transmembrane helices (e.g., in this model, the protein crosses the membrane three times, not twice). Aggregation of the relatively polar segments in a fashion analogous to bacteriorhodopsin would enable the proteolipid to form an HBC across the hydrophobic region of the bilayer [18]."

Function of HBCs: Proton Wires

Given the uncertainty of the existence of HBCs, it may seem to some to be idle to discuss their putative properties. However, the history of science is filled with examples of structures and entities which could not be experimentally verified until long after they were introduced to rationalize experience. Such theoretical constructs have provided guidelines for future development. Accordingly, a moderate theoretical effort has been expended to quantitate the proton transporting properties of HBCs. Of course, if such properties appear to be unfavorable for bioenergetic processes, then this line of theory should be abandoned. While there are many unanswered ques-

tions and much research to be done, there are strong grounds for optimism as we now review.

Most of the papers that mention HBCs or proton channels assume that such channels will transport fast enough and efficiently enough for bioenergetic purposes. Only a few papers [55, 74, 75, 76] have seriously addressed the crucial question of kinetic competence. Also, in most papers the detailed two-process mechanism of proton transport has been ignored. However, this mechanism has important consequences when interpreting measurements of charge movement. For the sake of simplicity we will call this two-process mechanism the *hop/turn* process of proton transport.

The first question concerning the functional possibility of HBCs is: Can HBCs conduct protons? The concept of HBCs acting as proton wires is based on conductivity and dielectric studies in hydrogen bonded crystals (abbr. HBX). The most common and most thoroughly studied HBX is ice, which consists of extended three-dimensional networks of hydrogen bonds. In addition, there are a number of HBXs which have hydrogen bonded networks of lower dimensionality. Of particular interest are lithium hydrazinium sulfate (LiHzS) [108] and imidazole [47]; both have one-dimensional chains of hydrogen bonds, all of which run in the same direction. Both crystals conduct protons, as is evidenced by the bubbling off of H_2 at the cathode adjoining the crystal. (Incidentally, the loss of H_2 at the cathode implies that a proper anode must inject hydrogen; these experiments have been complicated by difficulty in finding suitable anodes.) Both LiHzS and imidazole have substantially higher ($\times 10^3$) conductivities in the direction along the HBC than in the perpendicular directions. Schmidt et al. [108] note that LiHzS is as good a proton conductor as ice itself.

The second question is: How do HBCs conduct protons? The naive notion that an excess proton simply hops along from one hydrophilic group to the next as shown in Fig. 5*A* does not incorporate hydrogen bonding and does not provide a way to solvate the excess charge. Such solvation is required to lower its electrostatic energy for entry of charge into the membrane protein (*vide infra*). Most importantly, the breaking of the OH covalent bond and the streaking of a naked proton as suggested by Freund [33] would involve a very large activation energy which seems unlikely in biological systems. The standard theory of proton conduction in HBXs reviewed by Runnels [101] and Onsager [85] consists of the alternating two-process hop/turn mechanism shown in Fig. 5*B* and

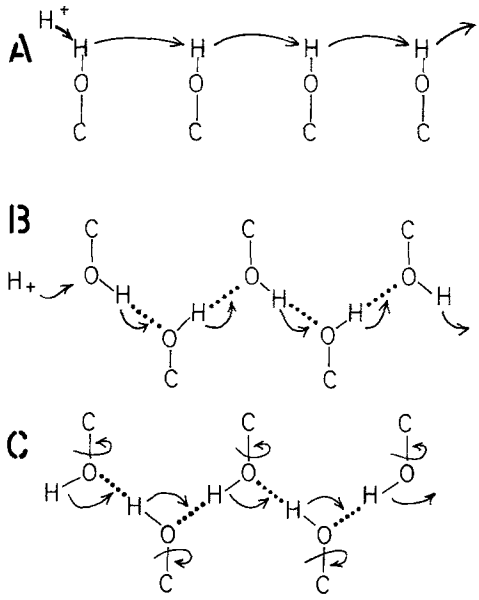

Fig. 5. Mechanisms of proton conduction. (*A*): Unlikely mechanism in proteins. (*B* and *C*): Conventional alternating hop/turn mechanism. In *B*, an ionic defect (proton) first *hops* from group to group as shown. In *C*, the bonding defect *turns* to assume the configuration in *B*, thereby translocating one net proton

C. One process involves the passage of an ionic defect (an excess proton *hops*) as shown in Fig. 5*B*. Ionic defects may be of two types, positive (excess proton on a group) or negative (deficient proton on a group). The HBC is then blocked to passage of another ionic defect until a second process occurs. This second process is the passage of a different kind of defect, called a bonding defect (a group adjacent to the bonding defect *turns*), shown in Fig. 5*C*. Bonding (Bjerrum) defects also may be of two types, L (no protons on a bond) or D (two protons on a bond). The hop/turn mechanism may begin with either the hop process or the turn process, but thereafter they must strictly alternate. Most papers of a general nature show models [20, 48, 60, 113] that indicate the ionic defect hopping, but not the bonding defect turning. It should be stressed that transport of the bonding defect is absolutely necessary for repeated proton transport along the same chain. Especially serious in the regard are Kayalar's [48] and Krimm and Dwivedi's [60] proposals to use backbone hydrogen bonds on α-helices to transport protons, because turning of the bonding defect is difficult to imagine chemically and must involve prohibitively large activation energies; Onsager [84] specifically discounted the possibility of such proton transport.

The two defect processes (hopping and turning) have been shown to be separated in time [85, 101],

and recent spectroscopic studies [99] have been able to follow the time course of the defects in ice. The ionic defect carries only a fraction of the full protonic charge e [42, 85, 106]. This fraction depends very much upon the kind of HBC or HBX one has; in ice $e_I = 0.62\,e$ and in KH_2PO_4, e_I is much smaller, $\sim 0.2e$ [82]. The remainder of the charge, $e_B = e - e_I$, is carried by the bonding defect. One of the advantages of partitioning the full protonic charge between the two different kinds of defects is that it lowers the electrostatic energy barrier for moving protons through a membrane of low dielectric constant [76, 82]. Nagle and Morowitz [76] considered an ionic diameter of 3 Å and a dielectric constant of 10, which, when compared to lipid bilayers with a dielectric constant of 2–3, assumes the presence of polarizable protein groups such as the backbone H-bonds and polarizable side chains. Nevertheless, the electrostatic energy barrier for bringing a full protonic charge into the membrane protein is still 16 kT; this would make such entry too infrequent for bioenergetic mechanisms. However, the partitioning of the full charge into two half charges by the HBC reduces this entry energy to only 4 kT; this allows sufficiently frequent passage, while still making it unlikely that two defects are on the HBC at the same time. This lowering of the electrostatic energy for defect entry may be described as a solvation of the defect by the HBC, thus providing a local dielectric constant higher than the average value for the membrane protein.

The partitioning of charge in the two-process hop/turn mechanism alters the interpretation of measurements of charge movements such as those of Keszthelyi and Ormos [51] because a movement of a full positive charge $1\,e$ over a distance of 10 Å would give the same electrical result as the movement of a positive ionic defect of charge $0.67\,e$ over a distance of 15 Å, or as the movement of an L bonding defect of negative charge $0.33\,e$ over a distance of 30 Å in the opposite direction. Furthermore, in proton pumping one should generally expect four separate charge movements when only one proton is pumped across a biological cell membrane (two separate hop/turn processes) [74]. One charge movement involves an ionic defect (charge e_{I1}) along the chain segment (length d_1 in Fig. 2*B*) which connects the active site to the cytoplasm and another involves a bonding defect (charge e_{B1}) along the same chain segment. Movement of the ionic defect (charge e_{I2}) and bonding defect (charge e_{B2}) along the chain segment (length d_2) which connects the active site to the extracellular space yields two more charge movements. Notice also that e_{I1} and e_{I2} are not necessarily equal if the two HBC segments have a different composition and bond lengths.

This mechanism for proton transport involves HBCs that have the property that each amino acid side chain involved is simultaneously a donor d of a hydrogen on one of its two bonds on the HBC, and an acceptor a of a hydrogen on the other bond. Such HBCs will be called *da*-HBCs. As an example, the use of neutral carboxyl and amino groups in Fig. 3 constitutes a *da*-HBC. Since the pK's of amino acid side chains inside a lipid membrane will be shifted to favor the neutral form, the restriction to *da*-HBCs for bioenergetic systems is conceivable. For instance, the electrostatic energy of burying a charged group of diameter 5 Å in a membrane of dielectric constant 5, corresponds to shifting the pK by 10 units in favor of the neutral form. Chains of uniformly charged side groups (e.g., all carboxyls or all lysines) have been proposed [58] but the additional repulsive electrostatic energy would shift the pK of such groups even further in favor of the neutral form and hence the charged form would not exist within membranes. HBCs with alternating charged carboxyl and amino groups have also been suggested [P. Mowery, *personal communication*]. Their conduction mechanism would be quite different and more complicated than that of the *da*-HBC and our investigations (M. Mille and J.F. Nagle, *unpublished*] have indicated that they would not be conducive to fast, efficient proton transport.

The third question is: How fast do HBCs conduct? Are they kinetically competent for bioenergetics? The bR photocycle, which consists of many intermediates [77, 115] lasts between 10 and 100 msec, and it has been estimated that proton conduction through the mitochondrial ATPase has a half-life between 10 and 700 msec [73], so proton conduction processes should probably not exceed the msec time scale. The first estimate of proton transit times for biological HBCs was made by Nagle and Morowitz [76]. These calculations used the mobilities for ionic ($\mu_I = 10^{-3}$ cm^2/V sec) and bonding ($\mu_B = 10^{-4}$ cm^2/V sec) defects from ice and the simple relation for the transit time, $\tau = \mathrm{d}/\mathrm{v} = \mathrm{d}^2/\mathrm{V}\mu$, where the thickness of the membrane $\mathrm{d} = 50$ Å and the defect drift velocity $v = \mu(\mathrm{V}/\mathrm{d})$. The effective driving field was V/d and the effective potential V was assumed to be only 1 mV so that the remaining more than 99% of the potential could be used for biochemical energy transduction. This gave the basic conduction time across a 50-Å membrane for ionic defect hopping, $\tau_I = 0.25$ μsec, and for bonding defect turning, $\tau_B = 2.5$ μsec. Such

rapid conduction times across biological membranes imply that HBCs are kinetically competent proton wires.

Discussion of Kinetics of HBCs

As noted by Nagle and Morowitz [76], the preceding, very simple estimate of transit times should be elaborated upon and discussed, and this has been done [55, 75]. In this section several of these points are reviewed.

1. Use Of Defect Mobilities From Ice

a. The measurement of mobilities of defects in ice is a demanding task with many pitfalls and the best values have varied over the years. Eigen et al. [23] obtained 75×10^{-3} cm^2/V sec for $\mu_{H^+} + \mu_{OH^-}$, but such a large value was apparently due to surface conduction. The mobility of negative ionic defects μ_{OH^-} would appear to be about 10 times smaller than μ_{H^+} [14]. A much lower value of 0.27×10^{-3} cm^2/V sec [12] has been given for μ_{H^+} than was inferred by Eigen et al. [23], but this probably reflects a good deal of trapping of ionic defects. This latter problem is avoided by the most recent measurement of Kunst and Warman [61] who give $\mu_{H^+} = 6 \times 10^{-3}$ cm^2/V sec. There has been less fluctuation in the value of the mobility of the L bonding defect which was given by Granicher [37] as 2×10^{-4} cm^2/V sec and more recently as 5×10^{-4} cm^2/V sec [12]. The values of the mobilities for ice used in [76] appear to be conservatively slightly lower than the best current values.

b. The next concern is that HBCs are ordered one-dimensional structures whereas ice is a disordered three-dimensional crystal. Defects in ice can "go astray" in a myriad number of directions. Consequently, even with a dissipation-free tunnelling Hamiltonian, the ionic defect has a finite mobility in ice [14]. The same tunnelling Hamiltonian would give infinite mobility for a one-dimensional HBC. There are, of course, various dissipative forces in both ice and HBCs [32]. However, if all other factors were equal, mobilities would be expected to be greater in HBCs than in ice, owing to the disorder in ice. It would be interesting to know the mobilities of defects in HBCs in HBXs such as LiHzS. However, while the conductivities σ have been measured [108], the concentrations c of defects are not known so the mobilities μ cannot be found from $\sigma = ce\mu$. Direct measurements for the mobilities of the ionic defects, as were carried out for ice [21, 61], have not been performed yet for one-dimensional HBXs.

c. Activation energies for bonding defects in HBCs may be different from those in ice. In ice the bonding defect proceeds by turning an HO_1H molecule around a stationary $O_2 \cdots H - O_1$ axis. This involves an activation energy which has been thought to involve breaking a hydrogen bond, or which might proceed via a bifurcated intermediate hydrogen bonded state [79]. The activation energies for protein side chains would involve a similar activation energy term, but may also involve additional contributions. For example, rotation around the C–O axis of hydroxyl side chains could involve energies of eclipsed hydrogens of ~ 1 kcal [30]. On the other hand, the ground state might already contain an unfavorable rotation angle compensated by formation of the hydrogen bonds in the HBC, thereby decreasing the rotation energy. In contrast to the L defect, a D bonding defect would be delocalized on a HBC and might have a coherence length longer than the HBC: this would facilitate more rapid transport, which could be especially important for the questions to be discussed under 2 below. Interestingly, a current paradigm in theoretical physics is the soliton, or solitary wave, which propagates with little dissipation of energy [7]. Some of the defect structures in HBCs may fit into this paradigm [J.A. Krumhansl, *personal communication*] and the D bonding defect seems to be the most promising in this regard.

d. Obtaining fundamental rate constants for defect movements requires interpretation of experiments. The basic rate constant for hopping of the positive ionic defect, $k_I = 10^{11}$/sec, is obtained from the analysis of Chen et al. [14], as explained by Nagle et al. [75]. A more exact value is $k_I = 2.36 \times 10^{11}$/sec if $\mu_{H^+} = 10^{-3}$ cm^2/V sec, and more recently, $k_I = 1.4 \times 10^{12}$/sec when a new value of μ_{H^+} is used [61]. It is important to observe that this calculation of k_I does not depend upon the use of the dissipation-free tunnelling Hamiltonian, which is the main object of the study of Chen et al. [14]. This calculation is consistent with thermally activated hopping, which is the mechanism assumed by Nagle et al. [75] and Knapp et al. [55], and mainly depends on the experimental value of the mobility. The basic rate constant, $k_B \sim 10^{10}$/sec, for turning of the bonding defect corresponds to the smaller measured mobility [12, 37]. Knapp et al. [55] used essentially the same k_I but they used a much smaller value for k_B, $\sim 7 \times 10^6$/sec, which they obtained from a lengthy derivation which is presented as applying to ice. However, this calculated value of k_B is incorrect for ice since equal preexponential factors (entropy term) were assumed [55] in the

activation energy formulae for the rates. This emphasizes the difficulty of calculating basic rates for elementary hopping and turning steps and the necessity to rely on experiment. (Once the basic rates are given, the further calculation of rates for processes consisting of many elementary steps is relatively straightforward conceptually, though mathematically challenging [55, 75].) While the basic rates assumed by Knapp et al. [55] are inappropriate for ice-like channels, their calculations for processes consisting of many elementary steps are of interest in illustrating possible extreme effects of the extra energies impeding bonding defects mentioned above in c. Even with their much smaller rate for the bonding defect, they obtain fluxes of several net protons per millisecond.

e. There are ways to avoid the problem suggested in c and d, that transport of the bonding defect may be severely rate limiting. As noted by Nagle and Morowitz [76], in a less rigid HBC, μ_I will decrease and μ_B will increase. The same effects are achieved by increasing the hydrogen bond length in the HBC. Recent *ab initio* quantum mechanical calculations by Scheiner [103–105] show quantitatively how the energy barrier to ionic defect propagation increases with bond length. Also, the hydrogen bond strength decreases for long hydrogen bonds, so thermally activated transfer of bonding L defects will be made easier. Another way to avoid this problem emerges in the following discussion.

2. *Movement of Defects over Energy Barriers*

As emphasized by Nagle and Morowitz [76] the first estimate of transit times assumed that each step of a defect in the desired direction on a HBC involves a small decrease in energy of the defect. This was true for the molecular motor model and the active chain proton pump model [76]. For proton pump models generally, at least after the pump has been working for a while and a potential has been established, additional pumped protons must move *against* a transmembrane electric field, not down it. In the active chain model this opposing electric field is overcome by the energy difference between the two states of an asymmetric HBC (energy difference between OH···OH···OH and HO···HO···HO). An asymmetric HBC energy can always overcome the electric field energy for one of the two kinds of defects. (For example, this can speed up the transport of that defect which is rate limiting [76].) The transport was made to run downhill step-by-step for both kinds of defects in the active chain proton pump model by the use of active conformational changes that altered the asymmetry of the HBC. However, in other proton pump models such as the kind shown schematically in Fig. 1*C*, active conformational changes in the chain are not assumed. This confronts one with the problem of estimating transit times for step-by-step diffusion of at least one defect over a potential barrier (due to the opposing electric field) and into solution. The overall process involves a decrease in *free* energy even though specific intermediate steps involve an increase in energy. This led Nagle et al. [75] to a considerably different and more complicated kinetic calculation. The main result was that for energy barriers of 450 mV (10 kcal) the transit times are still less than 1 msec, assuming basic hopping and turning times of $\sim 10^{11}$/sec.

3. *Heterogeneity of the HBC*

Even the calculated estimate of transit times not against a gradient [75] makes the assumption that all residues along the HBC are essentially identical. If the HBC is heterogeneous as shown in Fig. 3, then uniform stepping of a defect along the HBC involves both substantial increases and decreases in energy rather than a smooth increase or decrease. Calculations for these cases have not yet been done.

4. *Multiple Defects Occurring Simultaneously*

Since transfer of each defect from solution to the HBC costs at least 3 kcal at normal pH [75], the probability of multiple defects is usually small. In some previous calculations [75, 76] it is assumed that there is only one defect on the HBC at a time, although it is noted [75] that multiple inclusion of defects will only serve to increase transport rates. In contrast, Knapp et al. [55] formally pursue the multiple defect possibility and in their explicit calculations they include one multiple defect, namely a bonding L defect paired to a negative ionic defect. While this multiple defect makes very little difference at normal pH and small net proton motive force (pmf), they show that the saturation values of proton flux at large pmf and high pH are dominated by the paired defect and a double saturation plateau results. As also noted in [55] there are domains of pH and potential within which the net transport rates are smoothly varying because the dominating defects are constant (e.g., negative ionic defect and D bonding defect). In this case, the rate of proton flux changes relatively abruptly when entering another pH and potential domain, because at least one of the dominant defects changes and assumes a different

mobility (e.g., positive ionic defect replaces negative ionic defect when pH is lowered). Observation of these proton flux changes could provide clues to the dominant types of defects on the HBC.

5. Statistical Fluctuations

One characteristic of these theories [55, 75, 76] that is worth emphasizing is the statistical nature of defect transport. It does *not* consist of steady uniform passage of a defect from group i to group $i+1$ to group $i+2$ along the HBC. Rather, it is a fluctuating, dynamic process that involves much *to-and-fro* motion. Technically, it is a thermally activated random walk in various potentials. For example, with an electric potential $\Delta\psi \sim 400$ mV *driving* the transport, a defect hops or turns 300 times *on average* before reaching the end of a HBC 20 residues long. In contrast, to climb a barrier of 400 mV on a similar chain requires 10^8 hops or turns on average. Even with such a large number of hops and turns these processes are still kinetically competent for biological processes (*see* Fig. 7 in [75]).

Coupling of HBCs to Active Sites

Assuming from the preceding discussion that HBCs can perform the function of kinetically competent proton wires in membrane bioenergetics, one can turn to the exciting problem of constructing complete theoretical models for bioenergetic functions which utilize HBCs or proton wires [3, 9, 15, 24, 40, 62, 64, 71, 74, 75, 76, 109, 113]. This involves the coupling or connection of HBCs to the active site of proteins. It is becoming apparent that there is a wide variety of possibilities which do not exclude each other.

ATP Synthase Models

In the simplest form of coupling the HBC acts as a passive proton wire to conduct protons efficiently and quickly to a suitable site for chemical action. Morowitz [73] has used this form of coupling in a model of ATP synthase. His model requires a proton wire through the F_0 subunit and a reaction chamber associated with the F_1 subunit which is separated from the cytoplasm, for bacterial ATP synthase, by a membrane permeable only to divalent anions. The function of the proton wire is to equilibrate the electrochemical potential of protons in the reaction chamber with that of the extracellular space. This gives the reaction chamber a pH of 3–4 which Morowitz shows will favor the forward reaction, $ADP + P \rightarrow ATP$. The free energy driving the reaction is supplied by the linked transport of two protons from pH 3–4 in the reaction chamber to pH 7–8 in the cytoplasm. In addition, Morowitz [73] advances two arguments why a simple water pore would not work in his model. The first of these, namely that the flow of protons along a water pore must be highly dissipative, is hard to reconcile with the fact that the mobility of protons in water, 3×10^{-3} cm^2/V sec, [17], is about the same as in ice and therefore as in the HBC proton wire. However, the second argument, namely that a water pore is also permeable to salt ions, suffices; in a wide pore one would short circuit the electrical potential and have the dielectric breakdown problem, and in a narrow pore the presence of salt ions in the channel would greatly reduce the mobility of protons.

In contrast to the simple passive proton wire, Nagle and Morowitz [76] have proposed a coupling in which the HBC is an integral part of the active site. This model is called the active chain model. A defect of one type is driven along an asymmetric HBC; this stores the transmembrane electrochemical potential for protons (*ca.* 200–400 mV or 4–9 kcal) on the HBC. This energy then drives a conformational change in the protein. Transport of a defect of the second type drives the reverse conformational change, giving rise to a molecular motor or engine of a back and forth type. Gating control of the entry of defects onto the HBC is accomplished via the protein conformational change, which lengthens or shortens the hydrogen bonds at the ends of the HBC; the energy barrier to proton hopping as a function of bond length has since been computed by Scheiner [103] using *ab initio* methods. An active chain model is more feasible when groups forming the HBC are entirely protein side chain groups rather than bound waters because then the coupling of the high energy asymmetric HBC that drives the conformational change in the protein is through strong covalent bonds rather than through weaker hydrogen bonds. This active chain could be used as a molecular motor to do mechanical work, to cleave a substrate or to force ADP and P together to form ATP. However, no detailed molecular models for the coupling of the driven protein conformational change to specific bioenergetic functions have yet been devised.

The analysis of the active chain model [75] raises an interesting question in bioenergetics. It is usually assumed that bioenergetic functions should proceed at the same rate for a fixed total electrochemical potential $\Delta\mu$ regardless of the specific ratio $R = e\Delta\Psi/kt\Delta\text{pH}$ of electrical/chemical po-

tential, from the corresponding well-known result for aqueous solution. Indeed, for any system the rates are the same in the limit of small driving potentials and the active chain model obeys this theorem. But for transmembrane driving potentials large compared to $RT = 0.6$ kcal/mole, one is no longer operating in the linear transport domain. In this nonlinear domain the rates are greater for the active chain model if the driving potential is electrical than if it is chemical. Similar results obtain for an entirely different set of purely kinetic models [39]. Therefore, one should not assume that there is a blunder in an experiment if the measured rates are different for different values of R, as concluded in one report on bR [93], nor is such a result incompatible with delocalized chemiosmosis (since the active chain model *is* compatible with both delocalized and localized chemiosmosis). On the other hand, if the measured rates are the same for different values of R, this only means that the rate limiting steps are not the proton transport and it is not a refutation of the active chain model. In view of the result of the last section, that the kinetics of proton transport would appear to be faster than bioenergetic cycling times, independence of the rates on the ratio R of electrical/chemical potential is probably the most likely result, but it is by no means an inevitable one.

Proton Pump Models

The active chain model [76] can be reversed to operate as a proton pump. In this case cyclic back and forth conformational changes of the protein drive the protonic defects along the HBC. The direction of the pump is determined by the coupled states of the protein, the asymmetric HBC and the gating bonds. While experiments on bR indicate that conformational changes in the protein are not large enough to be consistent with the scheme shown in Fig. 1 *B*, they do indicate that a model in which all the conformational changes is localized on the retinal chromophore is also not correct [4, 57, 87]. The active chain model is a compromise between the schemes in Fig. 1 *B* and *C* in that only small conformational changes in parts of the protein are necessary to activate the HBC.

A simple and elegant model of the proton pump for bR was proposed by Stoeckenius [112, 113, 115] and by Schulten and Tavan [109]. It utilizes two simple passive proton wires with a gap in between. This model places two requirements on the active site, which is assumed to be the retinal located in the gap: (1) The active site must undergo a conformational change which takes it from one proton wire (leading to one side of the membrane) to the other proton wire (leading to the other side); thus, the retinal acts as a shuttle. (2) The active site undergoes an internal pK change so that it preferentially releases a proton to one wire and picks up a proton from the other wire. This model does not require any activity or conformational change in the protein.

Related models of proton pumps that impose only requirement (1) *or* requirement (2) on the active site are also possible. These related models involve the HBC and the protein in a more central and less passive way. The active injector model [74] of proton pumps requires only (2) that the active site X undergo a cyclic pK change so that X ejects (or takes up) a proton at one stage of the cycle and then X takes up (or ejects) a proton at a later stage. Vectoriality is obtained from an asymmetric HBC. No gating bonds are required. This is the simplest model of proton pumps. It has been proposed [65, 74] that a conformational change of the protein that brings a charge close to a group X on the HBC could change its pK. Recently Dunker [18] has proposed that the active site X might be a "proton hole" formed at specialized proline sites. During the cycle, the proton affinity of the proton hole becomes modulated primarily by changes in proton affinity of the proline nitrogen, which becomes more or less basic depending on changes in the imide electron resonance energy resulting from conformational distortions at the proline imide locus. Very recently, L.C. Allen *(personal communication)* has proposed that X may be a carboxyl group hydrogen bonded to the retinal Schiff base. Figure 6 shows a new integral injector model. The injector X is taken to be tyrosine since it is thought that tyrosine deprotonates during the photocycle [44] and since chemical modification studies indicate tyrosines to be essential for proton translocation in bR [35, 56, 107]. Tyrosine deprotonates in this model due to an electrostatic interaction [65, 74] with nearby arginine 82, which becomes positively charged when the Schiff base deprotonates. Although the ejection of the Schiff base proton onto arginine 82 initiates pumping on the HBC, it is not the Schiff base proton that is pumped, an idea noted in [74] and also incorporated into Allen's model.

Another related model has been called the switch proton pump model [74], although the name *shuttle model* might be more appropriate. This proton pump model requires only (1) above, that the active site shuttle across a gap between two proton wires. This model illustrates two important points. The first is that the breaking of

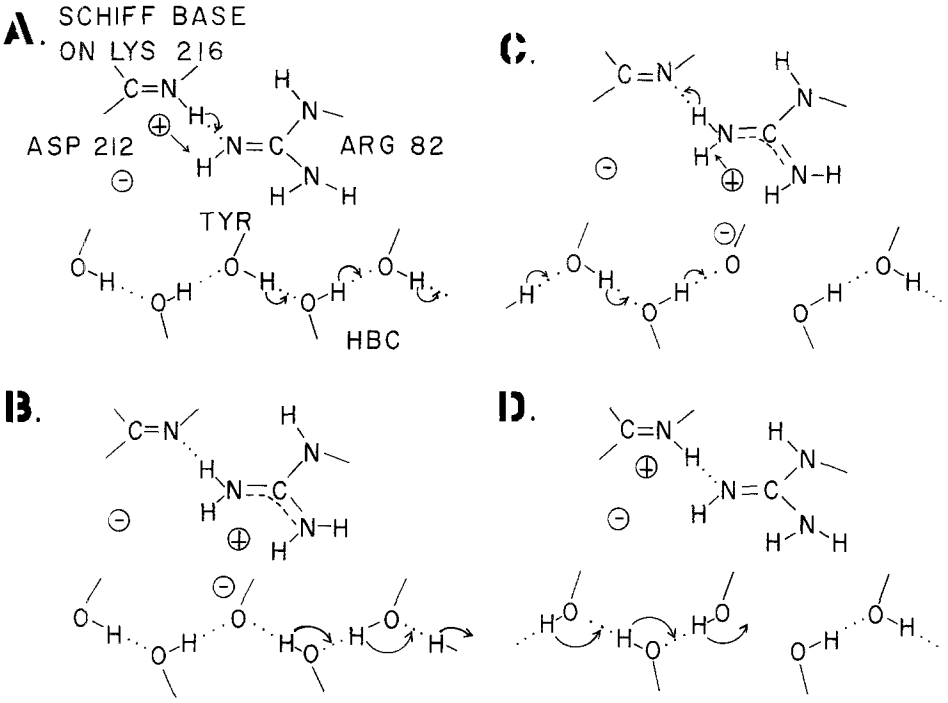

Fig. 6. An integral injector model [74] of a proton pump in bR. In this model, the protonation of arginine by transfer of the Schiff base proton causes a nearby tyrosine on the HBC to deprotonate. (*A*): Light-driven ejection of the Schiff base proton onto ARG 82 initiates ionic defect transport on the right half of the HBC. (*B*): The asymmetric HBC drives the bonding defect along the right half of the HBC. (*C*): Reprotonation of the Schiff base occurs as TYR picks up the nearest proton from the HBC, thereby creating a negative defect which migrates along the left half of the HBC. (*D*): Relaxation of the left half of the HBC via transport of a bonding defect returns the model to its original configuration in *A*

a hydrogen bond can be a form of energy storage. The second is that the making and breaking of a hydrogen bond can lead to kinetic trapping which yields vectorial transport. One very important principle that must be satisfied in any model of active transport is that vectorial transport cannot occur utilizing thermal fluctuations only; otherwise the second law of thermodynamics is violated. The reason that the kinetic trapping mechanism with thermal fluctuations does not transport vectorially is that the probability of purely thermal shuttling of X depends upon where the proton is. This makes the probability of forward transport equal to that of backward transport. In contrast, for active pumping the time when the active site shuttles is determined by the activity of the protein, not the proton position. Along with kinetic trapping and energy storage in hydrogen bonds, this can provide vectorial transport.

A related important principle is that vectoriality is a statistical concept which is not guaranteed in every single cycle of any real system or of any acceptable model. Indeed, such strict vectoriality is guaranteed *not* to occur unless an infinite amount of free energy is consumed. This has some important implications for measured stoichiometries. In the simple models of proton pumps the "ideal" stoichiometry is one H^+/cycle/wire. Such an ideal stoichiometry will never be achieved because some fraction of the cycles must necessarily end in misfiring or even backfiring. In the simple active injector model the only way to prevent absolutely the diffusion of defects off the wrong end of the HBC is to change the pK of X from infinitely positive to infinitely negative. Furthermore, as the "load" on the pump increases, that is, as the opposing transmembrane electrochemical potential builds up, the stoichiometry monotonically decreases. Therefore, excessive emphasis upon measured stoichiometries in bioenergetics may be misleading as noted also by Williams [121].

Concluding Perspectives

This review has focused upon proton transport through membranes and proteins as an essential part of bioenergetic mechanisms. Research in this area has concentrated upon permanent structural transport channels which could function as protonwires. The hydrogen bonded chain (HBC) has emerged as a candidate because it requires a minimum of space, its hydrogen bonding is ener-

getically favorable, and it appears to be kinetically competent. HBCs involving only hydrogen bonds between side chains of the protein are especially appealing because they are reasonably definite structures which would allow intimate coupling to the protein and consequently control by the protein over proton transport. Nevertheless, one should not lose sight of other less well-defined possibilities. In particular, it seems likely that at least some bound waters may be involved in HBCs and the proportion of bound waters could be fairly large. Proton channels could also involve networks, not just single chains, of hydrogen bonds. Furthermore, the proton channel may not consist of a permanent structural wire, rather, it could be a dynamic, flexible channel. One extreme possibility is completely transient HBCs of water molecules; these appear to be necessary to explain the anomalously high H^+ permeability of lipid bilayers [80]. Nevertheless, control of the directionality of such channels for vectorial processes in bioenergetics is difficult to envisage. Another less radical possibility is that the proton wire has several gaps. During a bioenergetic process each gap could be bridged by a conformational change [114] or alternatively, by thermal fluctuations. Despite these alternatives, the theoretical study of permanent HBCs does establish a most important result, namely, a plausible mechanism for kinetically competent vectorial proton transport through proteins and membranes. This supports phenomenological theories and paradigms of bioenergetics that involve proton transport through nonaqueous cellular components and membranes. Whether nature is obliging regarding the actual use of HBCs remains to be seen.

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References

1. Al-Awqati, Q., Dixon, T.E. 1982. *In:* Current Topics in Membranes and Transport. C.L. Slayman, editor. Vol. 16, pp. 163–174. Academic Press, New York
2. Apps, D.K., Schatz, G. 1979. *Eur. J. Biochem.* **100:**411–420
3. Baltscheffsky, H. 1978. *In:* Energy Conservation in Biological Membranes. G. Schaefer and M. Klingenberg, editors. pp. 3–18. Springer-Verlag, Berlin-Heidelberg
4. Beece, D., Bowne, S.F., Czégé, J., Eisenstein, L., Frauenfelder, H., Good, D., Marden, M.C., Marque, J., Ormos, P., Reinisch, L., Yue, K.T. 1980. *Photochem. Photobiol.* **33:**517–522
5. Berry, M.N. 1980. *FEBS Lett.* **117:**K106–K120
6. Berry, M.N. 1981. *FEBS Lett.* **134:**133–138
7. Bishop, A.R., Krumhansl, J.A., Trullinger, S.E. 1980. *Physica D* **1:**1–44
8. Blaurock, A.E., Stoeckenius, W. 1971. *Nature New Biol.* **233:**152–154
9. Bogomolni, R.A. 1980. *In:* Bioelectrochemistry. H. Keyzer and F. Gutmann, editors. pp. 83–95. Plenum, New York
10. Braiman, M., Mathies, R. 1980. *Biochemistry* **19:**5421–5428
11. Burres, N., Dunker, A.K. 1980. *J. Theor. Biol.* **87:**723–736
12. Camplin, G.C., Glen, J.W., Paren, J.G. 1978. *J. Glaciol.* **21:**123–141
13. Castro, P.R.C. 1979. Mechanism of auxin action. *An. Esc. Super. Agric. "Luiz de Queiroz". Univ. Sao Paulo* **36:**621–634
14. Chen, M.-S., Onsager, L., Bonner, J., Nagle, J.F. 1974. *Chem. Phys.* **60:**405–419
15. Christensen, H.N. 1979. *Adv. Enzymol.* **49:**41–101
16. Criddle, R.S., Packer, L., Shieh, P. 1977. *Proc. Natl. Acad. Sci. USA* **74:**4306–4310
17. Daniels, F., Alberty, R.A. 1975. Physical Chemistry. (4th Ed.) p. 372. John Wiley and Sons, New York
18. Dunker, A.K. 1982. *J. Theor. Biol.* **97:**95–127
19. Dunker, A.K., Jones, T.C. 1978. *Membrane Biochem.* **2:**1–16
20. Dunker, A.K., Marvin, D.A. 1978. *J. Theor. Biol.* **72:**9–16
21. Eckener, U., Helmreich, D., Engelhardt, H. 1973. *In:* Physics and Chemistry of Ice. E. Whalley, S.J. Jones, and L.W. Gold, editors. pp. 242–245. Royal Society, Ottawa
22. Eigen, M., De Maeyer, L. 1958. *Proc. R. Soc. London A* **247:**505–533
23. Eigen, M., De Maeyer, L., Spatz, H.-Ch. 1964. *Ber. Bunsenges. Phys. Chem.* **68:**18–29
24. Eisenbach, M., Caplan, S.R. 1979. *In:* Current Topics in Membranes and Transport. F. Bronner and A. Kleinzeller, editors. Vol. 12, pp. 166–248. Academic Press, New York
25. Ellenson, J.L., Pheasant, D.J., Levine, R.P. 1978. *Biochim. Biophys. Acta* **504:**123–135
26. Engelman, D.M., Henderson, R., McLachlan, A.D., Wallace, B.A. 1980. *Proc. Natl. Acad. Sci. USA* **77:**2023–2027
27. Engelman, D.M., Zaccai, G. 1980. *Proc. Natl. Acad. Sci. USA* **77:**5894–5898
28. Ernster, L., Schatz, G. 1981. *J. Cell Biol.* **91:**227*S*–255*S*
29. Fillingame, R.H. 1980. *Annu. Rev. Biochem.* **49:**1079–1113
30. Fink, W.H., Allen, L.C. 1967. *J. Chem. Phys.* **46:**2261–2275
31. Finney, J.L. 1977. *Phil. Trans. R. Soc. London B.* **278:**3–32
32. Fischer, S.F., Hofacker, G.L., Sabin, J.R. 1969. *Phys. Kondens. Mater.* **8:**268–278
33. Freund, F. 1981. *Trends Biochem. Sci.* **6:**142–145
34. Glagolev, A.N., Skulachev, V.P. 1978. *Nature (London)* **272:**280–282
35. Gogel, G., Lewis, A. 1981. *Biochem. Biophys. Res. Commun.* **103:**175–181
36. Gradmann, D., Hansen, U.-P., Slayman, C.L. 1982. *In:* Current Topics in Membranes and Transport. C.L. Slayman, editor. Vol. 16, pp. 257–276. Academic Press, New York
37. Granicher, H. 1963. *Phys. kondens. Materie* **1:**1–12
38. Guffanti, A.A., Blanco, R., Berenson, R.A., Krulwich, T.A. 1980. *J. Gen. Microbiol.* **119:**79–86
39. Hansen, U.-P., Gradmann, D., Sanders, D., Slayman, C.L. 1981. *J. Membrane Biol.* **63:**165–190
40. Hobbs, A.S., Albers, R.W. 1980. *Annu. Rev. Biophys. Bioeng.* **9:**259–291

41. Houštěk, J., Kopecký, J., Svoboda, P., Drahota, Z. 1982. *J. Bioenerg. Biomembr.* **14:**1–13
42. Hubmann, M. 1979. *Z. Physik. B* **32:**127–139
43. Hui, C.S. 1977. *Biosystems* **8:**207–212
44. Kalisky, O., Ottolenghi, M., Honig, B., Korenstein, R. 1981. *Biochemistry* **20:**649–655
45. Katre, N.V., Stroud, R.M. 1981. *FEBS Lett.* **136:**170–174
46. Katre, N.V., Wolber, P.K., Stoeckenius, W., Stroud, R.M. 1981. *Proc. Natl. Acad. Sci. USA* **78:**4068–4072
47. Kawada, A., McGhie, A.R., Labes, M.M. 1970. *J. Chem. Phys.* **52:**3121–3125
48. Kayalar, C. 1979. *J. Membrane Biol.* **45:**37–42
49. Kell, D.B. 1979. *Biochim. Biophys. Acta* **549**:55–99
50. Kell, D.B., Clarke, D.J., Morris, J.G. 1981. *FEMS Microbiol. Lett.* **11:**1–11
51. Keszthelyi, L., Ormos, P. 1980. *FEBS Lett.* **109:**189–193
52. Khan, S., MacNab, R.M. 1980. *J. Mol. Biol.* **138:**599–614
53. Khorana, H.G., Gerber, G.E., Herlihy, W.C., Gray, C.P., Anderegg, R.J., Nihei, K., Biemann, K. 1979. *Proc. Natl. Acad. Sci. USA* **76:**5046–5050
54. Kinsey, R.A., Kintanar, A., Tsai, M.-D., Smith, R.L., Janes, N., Oldfield, E. 1981. *J. Biol. Chem.* **256:**4146–4149
55. Knapp, E.-W., Schulten, K., Schulten, Z. 1980. *Chem. Phys.* **46:**215–229
56. Konishi, T., Packer, L. 1978. *FEBS Lett.* **92:**1–4
57. Konishi, T., Tristram, S., Packer, L. 1979. *Photochem. Photobiol.* **29:**353–358
58. Kouyama, T., Kumura, Y., Kinosita, K., Ikegami, A. 1981. *FEBS Lett.* **124:**100–104
59. Kozlov, I.A., Skulachev, V.P. 1977. *Biochim. Biophys. Acta* **463:**29–89
60. Krimm, S., Dwivedi, A.M. 1982. *Science* **216:**407–408
61. Kunst, M., Warman, J.M. 1980. *Nature (London)* **288:**465–467
62. Lauger, P. 1979. *Biochim. Biophys. Acta* **552:**143–161
63. Lenard, J., Singer, S.J. 1966. *Proc. Natl. Acad. Sci. USA* **56:**1828–1835
64. Lewis, A. 1978*a*. *In:* Frontiers of Biological Energetics. P.L. Dutton, J.S. Leigh and A. Scarpa, editors. Vol. I, pp. 297–306. Academic Press, New York
65. Lewis, A. 1978*b*. *Proc. Natl. Acad. Sci. USA* **75:**549–553
66. Loo, T.W., Bragg, P.D. 1981. *Biochem. Biophys. Res. Commun.* **103:**52–59
67. Lumry, R., Biltonen, R.L. 1969. *In:* Structure and Stability of Biological Macromolecules. S. Timasheff and G. Fasman, editors. pp. 67–122. Marcel Dekker, New York
68. Maloney, P.C. 1982. *In:* Current Topics in Membranes and Transport. C.L. Slayman, editor. Vol. 16, pp. 175–193. Academic Press, New York
69. Mego, J.L. 1975. *Biochem. Biophys. Res. Commun.* **67:**571–575
70. Merz, H., Zundel, G. 1981. *Biochem. Biophys. Res. Commun.* **101:**540–546
71. Metzler, D.E. 1979. *Adv. Enzymol.* **50:**1–40
72. Mitchell, P. 1961. *Nature (London)* **191:**144–148
73. Morowitz, H.J. 1978. *Am. J. Physiol.* **235:**99–114
74. Nagle, J.F., Mille, M. 1981. *J. Chem. Phys.* **74:**1367–1372
75. Nagle, J.F., Mille, M., Morowitz, H.J. 1980. *J. Chem. Phys.* **72:**3959–3971
76. Nagle, J.F., Morowitz, H.J. 1978. *Proc. Natl. Acad. Sci. USA* **75:**298–302
77. Nagle, J.F., Parodi, L.A., Lozier, R.H. 1982. *Biophys. J.* **38:**161–174
78. Nelson, N., Eytan, E., Notsani, B.-E., Sigrist, H., Sigrist-Nelson, K., Gitler, C. 1977. *Proc. Natl. Acad. Sci. USA* **74:**2375–2378
79. Newton, M.D. 1983. The use of *ab initio* calculations for small water clusters in elucidating structural, energetic and kinetic features of ice. *J. Phys. Chem. (in press)*
80. Nichols, J.W., Deamer, D.W. 1980. *Proc. Natl. Acad. Sci. USA* **77:**2038–2042
81. Ohki, S. 1972. *Ann. N.Y. Acad. Sci.* **195:**457–477
82. Onsager, L. 1967. *In:* The Neurosciences. F.O. Schmitt, editor. pp. 75–79. Rockefeller University Press, New York
83. Onsager, L. 1969. *Science* **166:**1359–1364
84. Onsager, L. 1970. *In:* Physical Principles of Biological Membranes. F. Snell, J. Wolken, G. Iverson, and J. Lam, editors. pp. 137–141. Gordon & Breach, New York
85. Onsager, L. 1973. *In:* Physics and Chemistry of Ice. E. Whalley, S.J. Jones, and L.W. Gold, editors. pp. 7–12. Royal Society, Ottawa
86. Ort, D.R., Dilley, R.A., Good, N.E. 1976. *Biochim. Biophys. Acta* **449:**108–124
87. Ort, D.R., Parson, W.W. 1979. *Biophys. J.* **25:**355–365
88. Ovchinnikov, Y.A., Abdulaev, N.G., Feigina, M.Y., Kiselev, A.V., Lobanov, N.A. 1979. *FEBS Lett.* **100:**219–224
89. Page, M.G.P., West, I.C. 1981. *Biochem. J.* **196:**721–731
90. Papa, S. 1982. *J. Bioenerg. Biomembr.* **14:**69–86
91. Pennington, R.M., Fisher, R.R. 1981. *J. Biol. Chem.* **256:**8963–8969
92. Perutz, M.F. 1970. *Nature (London)* **228:**726–734
93. Quintanilha, A.T. 1980. *FEBS Lett.* **117:**8–12
94. Quiocho, F.A., Lipscomb, W.N. 1971. *Adv. Prot. Chem.* **25:**1–78
95. Racker, E., Hinkle, P.C. 1974. *J. Membrane Biol.* **17:**181–188
96. Razi Naqvi, K., Gonzalez-Rodriguez, J., Cherry, R.J., Chapman, D. 1973. *Nature New Biol.* **245:**249–251
97. Reeves, J.P., Reames, T. 1981. *J. Biol. Chem.* **256:**6047–6053
98. Rice, D., Herzfeld, J., Griffin, R.G. 1981. *Biophys. J.* **33:**103*a*
99. Ritzhaupt, G., Devlin, J.P. 1980. *J. Chem. Phys.* **72:**6807–6808
100. Rothschild, K.J., Zagaeski, M., Cantore, W.A. 1981. *Biochem. Biophys. Res. Commun.* **103:**483–489
101. Runnels, L.K. 1966. *Sci. Am.* **215:**118–126
102. Sachs, G., Wallmark, B., Saccomoni, G., Rabon, E., Stewart, H.B., DiBona, D.R., Benglindh, T. 1982. *In:* Current Topics in Membranes and Transport. C.L. Slayman, editor. Vol. 16, pp. 136–159. Academic Press, New York
103. Scheiner, S. 1981. *J. Am. Chem. Soc.* **103:**315–320
104. Scheiner, S. 1982. *J. Phys. Chem.* **86:**376–382
105. Scheiner, S., Harding, L.B. 1981. *J. Am. Chem. Soc.* **103:**2169–2173
106. Scheiner, S., Nagle, J.F. 1983. *Ab initio* molecular orbital estimates of charge partitioning between Bjerrum and ionic defects in ice. *J. Phys. Chem. (in press)*
107. Scherrer, P., Packer, L., Seltzer, S. 1981. *Arch. Biochem. Biophys.* **212:**589–601
108. Schmidt, V.H., Drumheller, J.E., Howell, F.L. 1971. *Phys. Rev.* **4:**4582–4597
109. Schulten, K., Tavan, P. 1978. *Nature (London)* **272:**85–86
110. Sebald, W., Graf, T., Lukins, H.B. 1979. *Eur. J. Biochem.* **93:**587–599
111. Sebald, W., Wachter, E. 1979. *In:* Energy Conservation in Biological Membranes (Colloqium Mosbach Ser) G. Schafer and M. Klingenberg, editors. Vol. 29, pp. 228–236. Springer-Verlag, New York
112. Stoeckenius, W. 1976. *In:* The structural basis of membrane function. Y. Hatefi and L. Djavadi-Ohaniance, editors. pp. 39–44. Academic Press, New York
113. Stoeckenius, W. 1979. *In:* Membrane Transduction Mech-

anisms. R.A. Cone and J.E. Dowling, editors. pp. 39–47. Raven Press, New York
114. Stoeckenius, W., Bogomolni, R.A. 1982. *Annu. Rev. Biochem.* **52:**587–615
115. Stoeckenius, W., Lozier, R.H., Bogomolni, R.A. 1979. *Biochim. Biophys. Acta* **505:**215–278
116. Tristram, S.A. 1981. The Role of the Positively Charged Amino Acids in Bacteriorhodopsin, Ph.D. Dissertation. p. 230. University of California, Berkeley
117. Tristram-Nagle, S., Packer, L. 1981. *Biochem. Int.* **3:**621–628
118. Wallach, D.F.H., Zahler, P.H. 1966. *Proc. Natl. Acad. Sci. USA* **56:**1552–1559
119. Wikstrom, M., Krab, K., Saraste, M. 1981. *Annu. Rev. Biochem.* **50:**623–655
120. Williams, R.J.P. 1961. *J. Theor. Biol.* **1:**1–17
121. Williams, R.J.P. 1978. *Biochim. Biophys. Acta* **505:**1–44

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