The Structures of Organelles and Reticula: Localised Bioenergetics and Metabolism

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Introduction

In this article I shall draw attention to the requirement to re-examine bioenergetics of cells in the light of recent observations concerning cellular structures which localise the flow of ions such as the proton and calcium[1, 2] as well as that of ATP.

The flow of protons is concerned with the maintenance of different pH values and potentials in different compartments, for example the cytoplasm, vacuoles and vesicles, and their coupling to the energisation of the uptake (or rejection) of both organic and inorganic materials, and to the capture of energy from light in chloroplasts and from oxidation of carbohydrates in mitochondria.[3] The two organelles are found as compartments of eukaryote cells but must supply energy to all other compartments, usually indirectly through ATP production from proton gradients.[3] Thus the flow of protons is central to bioenergetics. The idea that such ion gradients as that of protons (and of sodium, potassium, chloride, calcium ions and many organic molecules) could be created from hydrolysis of ATP has been well demonstrated for more than fifty years and the reverse effect of driving ATP formation from an organelle proton gradient was proposed more than forty years ago.[3, 4, 5] The remaining problem is the local control of flow in and between these ion and ATP networks. We start from ATP formation.

There are two ways in which to analyse the relationships between the formation of ATP and an ion gradient in an organelle. The simplest described by Williams and Mitchell[4,5] is to assume an equilibrium, which is not the same as a steady state, is closely approached. This gives Equations (1) and (2) first published by Mitchell[4] from which the total phosphate *thermodynamic potential energy*, ΔG can be determined.

$$RT \ln \frac{[ATP]}{|ADP||P_i|} = RT \ln X \qquad (1)$$

At equilibrium ΔG can be equated to the proton gradient energy, the protomotive force, generated across or in mitochondrial or thylakoid membranes [Eq. (2)], where μ is the *electrical potential* across a membrane space due to electrogenic movement of charge (electrons or H^+) and ΔpH is the concentration difference across the same space.

$$2.303\,RT \log X = \mu + 2.303\,RT\,(\Delta pH) \qquad (2)$$

It was supposed by Mitchell that the ATP synthase which connected the two worked in equilibrated balance throughout *a whole organelle*. (It is now known[6, 7] that there is a mechanical machine connecting the protomotive force and ATP but we shall ignore the fact that no machine can work at equilibrium, that is at 100% energy efficiency). The alternative approach is that μ and ΔpH do not equilibrate in the complete organelle space, but two-dimensional flow of protons in and along organelle membranes gives ATP locally (Figure 1).[3] The structure, size and shape of the organelle is critical in this distinction (see below). Note that in principle any ion or molecule gradient can replace that of H^+, for example Na^+. (Note that flows can be in a steady state not close to a thermodynamic equilibrium).

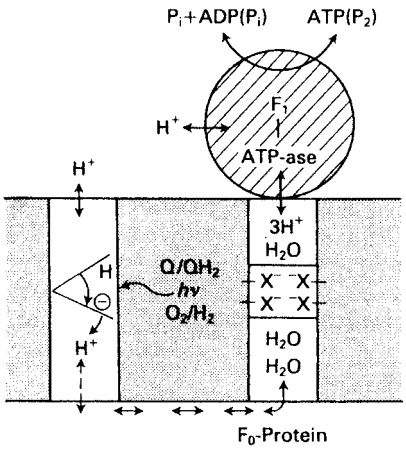

Figure 1. *The proposal of proton flow in particles, the sources and sinks of protons, in membranes. Note electron flow is of shorter distance. Further proton flow is along membrane surfaces and not in bulk aqueous phases.*

The mode of production of a pH gradient across other membranes for example the cytoplasmic or vacuole membranes, or indeed of a gradient of ions such as those of Na^+, K^+, Cl^- or Ca^{2+} or even of molecules, is quite different and uses ATP in P-type ATP-ases.[8] These ATP-ases operate far from equilibrium and are effectively irreversible and are controlled by feedback from the ion gradients themselves. The ion currents which create ATP and those which use it flow continuously throughout cells but to various degrees locally and are interactive in networks. For example the release of a *local* current (flow) of calcium from an initial receptor in a plasma membrane *across the cytoplasm* is usually to a local channel opening in a reticulum (ER) membrane where it generates an amplified calcium current which flows more strongly in the cytoplasm. This is the well-known calcium pulse. It is often the case that the membranes of the reticulum and of the cytoplasm are in close juxtaposition so as to reduce losses which would arise if the ion were to diffuse in useless space. ATP is used to restore the calcium in the

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reticulum and to remove it from the cytoplasm by Ca^{2+}-activated ATP-ase (P-type) judiciously placed close to the release sites.[9, 10] Some of the Ca^{2+} is also removed to mitochondria (see below).

Early impressions of organelle structures

We return now to the production of ATP in the chloroplast and the mitochondrion. As stated the immediate need is to consider the structures of the two organelles. At the time when equilibrium chemiosmotic synthesis of ATP was developed[3–5] the mitochondria were considered to be small elliptical objects, even smaller than bacterial cells (Figure 2). Note

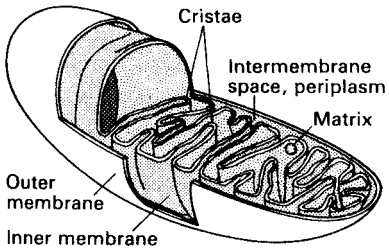

Figure 2. *The early impression of mitochondrial structure. Note the small size, approximately 1 μm in all dimensions, and the lack of connection from cristae to the periplasm.[11]*

the apparent lack of access of the interior of the cristae to the periplasm. Evidence in favour of the model came from isolated mitochondrial and thylakoid particles, which are minute *spherical* vesicles of the inner membranes,[11] from isolated mitochondria,[11] which in vitro are also small, or from spherical swollen isolated inner membrane preparations.[12] *Steady state kinetics close to equilibrated systems* using these preparations with external pH controls apparently gave convincing support to in vivo delocalised equilibrium.

The modern view of organelle structure

It is now apparent that the early descriptions of the structure of mitochondria were incorrect inferences resulting from the methods of their study. In these studies single cross sections of cells were used for electron microscopy studies which naturally gave the impression of elliptical small organelles as well as leading to images such as those of small spherical calcizomes many, if not all of which, are now known to be part of long weaving tubes of reticula. Once serial sectioning with tilted-stage electron microscopy was developed mitochondria and chloroplasts in vivo were shown to be long weaving reticula structures (Figure 3,[13] and Figure 4[14, 15]), which had indeed been suggested in outline earlier but the idea had been ignored.[16] The cristae membranes are even longer weaving internal units of a variety of structures and they have, very restricted, connection with the periplasmic space (Figure 3).[13]

A further feature of the structures of the mitochondrial cristae is that there are several distinct cristae not necessarily directly connected to one another in a single mitochondrion (Figure 3). The only

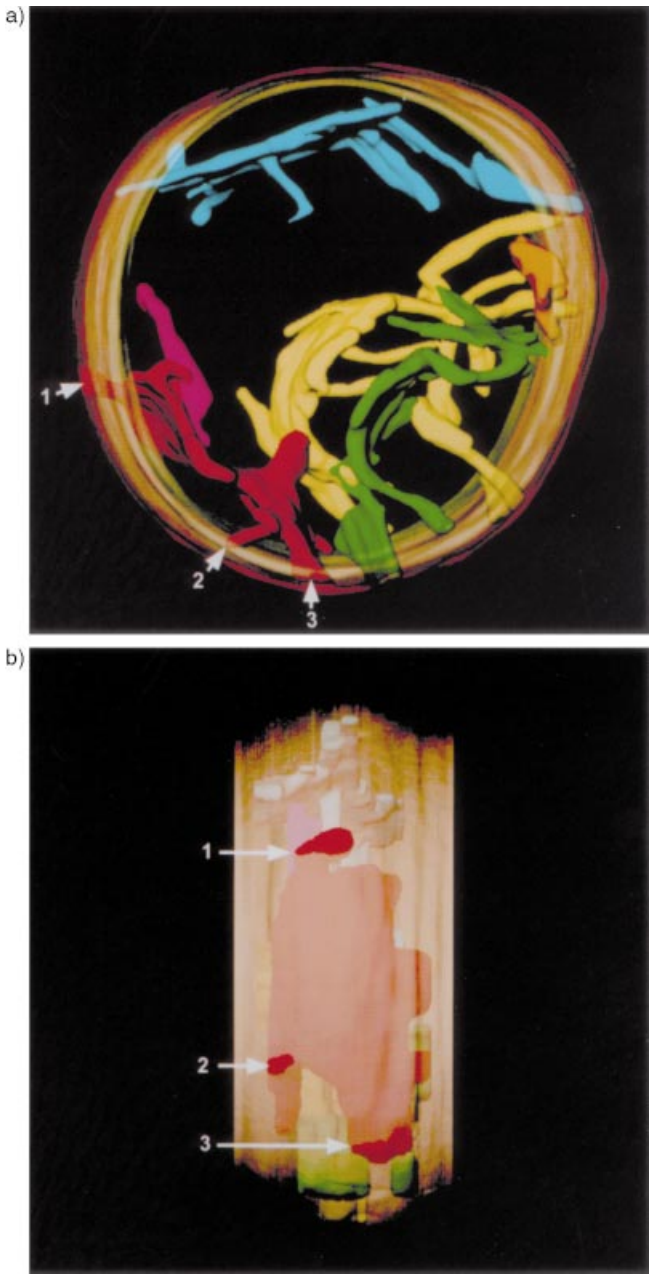

Figure 3. *a) A cross-section of a cylindrical mitochondrion with depth of focus. The outer membrane is the concentric circles, while the six (different) cristae are shown as weaving membrane structures. Note small open contacts to the periplasmic space. The diameter of the cylinder is about 1.0 μm. The cristae have a total width (two membranes plus intramembrane space) of less than 300 Å. b) A longitudinal view of part of the same mitochondrion cylinder following one of the cristae, marked by arrows in both a and b. Effectively cristae are isolated from one another. Note that the structures are dynamic. These figures were provided by Dr. C. A. Manuella (see acknowledgement).*

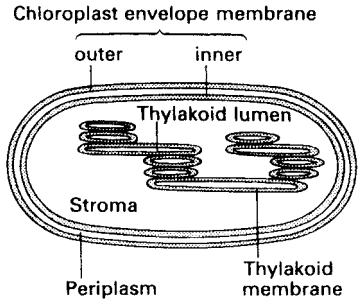

Figure 4. *A view of the chloroplast inner membrane, the thylakoid, once it was seen to be extremely extensive.*

connection between them appears to be through the periplasmic space which suggests that to a large degree they are independent units.[13] It is extremely unlikely that such structure could give either a quick or a totally delocalised response in the whole organelle. Let us consider the significance of the revised structures of the organelles while firstly reminding ourselves of other structures inside cells.

Linking organelle and other membrane structures

I shall refer again now to calcium and other ion gradients energised by ATP. They are found across both the outer cell membranes and the inner membranes (in a reverse sense) of reticula, vesicles and vacuoles. The pumped gradients associated with *resting states* of cells may be of fixed value over large regions of the cell or vesicle membranes and can be given a *thermodynamic potential*. (This is not an equilibrium across a membrane). As stated we know that cells also have localised switched-on activities when ion flows enter cell cytoplasm. We need to consider next the relative disposition of the membranes of the plasma, reticula and of mitochondria (or chloroplasts) from which ATP must come to be applied to the transport systems so as to restore the ion gradients. Here and there the long structures of the mitochondria (see Figure 3b of reference [13]) have been shown recently to approach locally both the outer and the reticula (or vesicle) membranes.[17] Thus the organelle and other membrane, for example the ER, are locally adjacent and therefore inhomogeneous. Consider the effect of a *localised* input of Ca^{2+} to the cytoplasm of the cell and then an organelle at one such point of juxtaposition of the two membranes. The response of the *nearby* part of the mitochondrial membrane is now known to take in some of this localised pulse of calcium aiding relaxation. We need to see whether the calcium in the *local* part of the cytoplasm of the mitochondrion, that is in a part of a cristae, which is known to activate dehydrogenases and in turn generate substrates for oxidative phosphorylation will produce local proton potentials and concentration gradients. The gradients could then produce ATP for export to the cytoplasm locally. We can appreciate the possibilities by examining the diffusion of charge in cell membranes and as can be seen by studying diffusion in aqueous solutions.

Pathways of electrons and protons in membranes

The proton gradient in mitochondrion arises from an initial flow of electrons from oxidation of substrates. The electron pathway is between molecules in cell membranes. These transfers are then extremely localised in circuits even down to the Q-cycle and apply generally to all organelle bioenergetics. Electronic potentials are then created locally in membrane particles. The electron flow is by tunneling at rates which are modulated by the distance between these electron-transfer centres.[18, 19] Electron transfer over 100 Å is fast, that is $< 10^{-3}$ s, so that electron transfer is usually not rate limiting for energy capture. Now in the membranes of mitochondria and chloroplasts, both of which use electron flow initially, it was maintained by Mitchell,[4] and subsequently it is stated in many textbooks, that electrons flow across the whole of organelle membranes to generate proton gradients only in the aqueous media of the cristae, that is the black space in Figure 3. This would leave the problem of any proton flow, apart now from considerations of equilibria, to that in bulk aqueous solutions only. Williams considered this mechanism but preferred one in which that the electron flowed a limited distance in the membrane and that connected subsequent proton flow could be in or on the membrane surface (Figure 1).[3–5] This was later called a proton wire (see reference [20] and references therein). It is now known that proton wiring does exist in the membranes of mitochondria and chloroplasts so that proton currents are measurable events in organelle membrane structures and in the ATP synthase (Figure 5). The flow of protons in the membrane (even their existence in such media was repeatedly described as impossible in the period 1961 – 1980[21]) is in part through bound

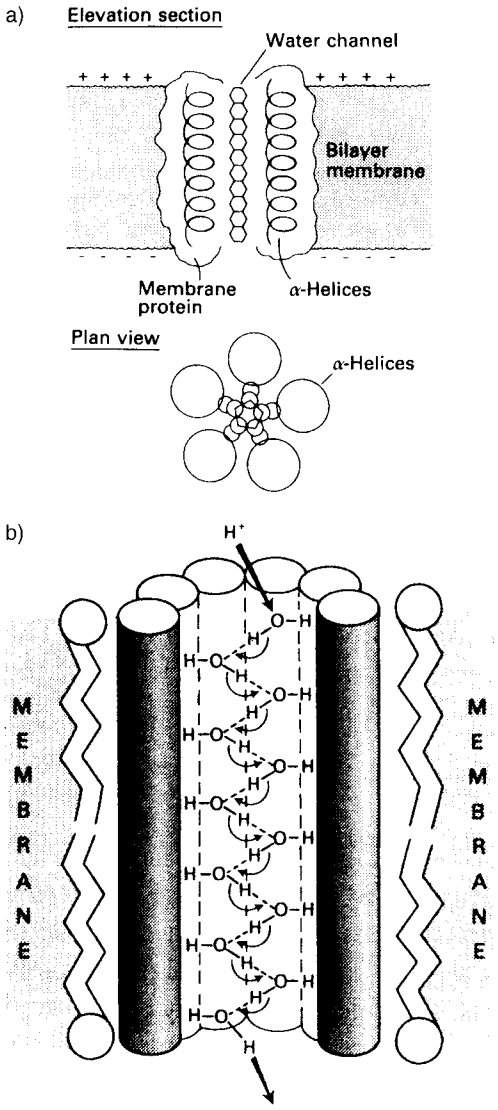

Figure 5. *a) The nature of proton flow proposed by Williams[3, 5] can be through water channels or through protein sidechains. b) A detailed proposal for a proton channel using water molecules and subsequently called a proton wire.*

H_2O channels as shown in Figure 5. The question arises as to the connection between the proton wires of the ATP synthase and the proton wires of the photo and oxidative systems of the membranes of the organelles. It is this section of the current flow which will produce ATP by mitochondria and chloroplasts ensuring that it is fast and could be local. The pathway rates are limited by ion diffusion which we must consider next.

Diffusion pathways in aqueous compartments

We wish to know how far diffusion of ions limits communication and hence cooperative activity in the cellular compartments. The diffusion constant D in water at 25 °C of the proton and a simple cation such as Ca^{2+} are 9.3×10^{-9} and 2.0×10^{-9} $m^2 s^{-1}$, respectively.[22–24] The diffusion distance in time t is given by $X = \sqrt{2Dt}$ m. Now the turnover time of the cytochrome chain and the ATP synthase in all organelles is greater than 10^3 s^{-1} so that we can put $t < 10^{-3}$ s when $X < 5.0 \times 10^{-6}$ and 3.0×10^{-6} $m s^{-1}$ for H^+ and K^+ or Ca^{2+}, respectively, that is the greatest linear distance an ion could diffuse freely in water during turnover is much less than 10^5 Å s^{-1}. In itself this is less than the length of the aqueous volumes now seen in the connected cristae and thylakoids or within the ER (Table 1).[13] This diffusion distance ignores volume dilution of the concentration of proton and calcium ions and trapping of the ions by proteins in the aqueous phases by membranes and by the anionic lipids present.[25] Experiment shows that in fact proton diffusion is largely restricted to the membrane surfaces and hence between sources and ATP synthases.[26–29] The ATP synthase which is highly concentrated also contains many negative charges (Figure 1). Given the limited possibility of free diffusion, it is no longer possible to conceive that the uptake of protons by the ATP synthases will be at any greater distance than say 10^4 Å from their origin. The diffusion of Ca^{2+} is likely to be less before it bonds to dehydrogenases. The same problems arise with descriptions of Ca^{2+} ion diffusion in the ER. Here there are densely packed units of calciquestrin throughout the ER which are sometimes seen even as ordered molecular arrays. Calciquestrin, the structure of which is known, can bind up to 40 Ca^{2+} ions and release them in a localised patch.[30] Finally diffusion of ATP is slower than that of Ca^{2+} and acts locally at kinases, for example creatine kinase, associated with adjacent membrane structures.

Transduction of gradients and electric potentials

The picture of cellular bioenergetic systems is therefore one of localised stimulated interactive currents and networks of ions and ATP much as in a computer where diffusion rates are controlled by resistors and capacitors. Thus it is to current flow controlled by wires, resistors and capacitors to which we must turn to understand bioenergetics.[10] Relaxation times for calcium binding have been analysed quantitatively[10] but those for protons are less well established. We need theoretical treatments of reaction diffusion systems involving capacitors and resistors in very difficult media and structures. (I thank the referee for this comment).

The transduction of energy from a pH gradient or a potential occurs in the ATP synthase. The machinery of this device is now well known and involves a coupled F_0 proton channel in a membrane and an enzyme (F_1) for the condensation reaction. Initially it was considered by Mitchell[4] that the electric field potential ψ could drive the reaction without protons entering the machinery. This still causes confusion. The potential is transduced as is a pH gradient by proton flow *in the* membrane,[3][5] which creates mechanical action. The electric potential ψ in a spherical volume is delocalised but in a structure such as that of mitochondrial vesicles will be localised. Driving reactions by ψ (or ΔpH), locally confined, requires less charge transfer across a membrane than the creation of an equivalent energy source due to an equilibrium gradient and is possibly the general method of transduction. (Note ψ drives local ion flow in membranes to transfer energy to ATP).[5]

The essential features of all these flows of H^+, Ca^{2+} or ATP is that they connect in localised micro-circuits within organelle, vesicle and plasma membranes.

Conclusion

Clearly this localised treatment of flow of ions and ATP is not in agreement with delocalised thermodynamic equilibrium treatments of energy production in whole mitochondria or chloroplasts.[31] However none of this description detracts from the use of Equation 2 if local reaction sites are treated as close to local equilibrium in micro-volumes no matter what the mode of transfer of protons between places of creation and use of protons (see Figure 3 and references in reference [5]). Note however that to activate large volumes of a compartment is in many circumstances just wasteful of energy.

The great disadvantage of using equilibrium theories for the understanding of biological compartmentalisation is that they lose sight of a fundamental requirement of cellular activities. A cell is an integrated system which can only be understood by seeing that energy is constantly applied and used *locally* in connected networks. The treatment is then one of diffusion in systems not of necessity close to equilibrium, whether in resting or pulsed states. Looking at the now known structures of cell compartments, such as the membranes of the plasma, reticula, and particularly of mitochondria and chloroplasts, and their here-and-there juxtaposition with one another allows us insight into the reasons for their newly revealed tube- or plate-like con-

Table 1. *The essential structural features of diffusion restrictions on localised ionic activities.*

System	Vesicle diameter	Vesicle length
mitochondria[a]	~100Å	$> 10^5$Å
chloroplasts[a]	~100Å	$> 10^5$Å
endoplasmic and sarcoplasmic reticula[b]	~100Å	$> 10^6$Å
nerves[b]	10^4Å	metres

[a] These systems are disc-like or tube-like. [b] These systems are tube-like.

struction.[13, 17] They can transfer material and energy differentially by specific micro-routes in cells much as the electron diffuses in computer circuits.[32, 33]

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[1] J. W. Stucki, E. Sigel in *Integration of Mitochondrial Function* (Eds.: J. L. Lemasters, C. R. Hackenbrock, R. C. Thurman, H. V. Westerhoff), Plenum, New York, **1988**, pp. 169 – 175.
[2] R. J. P. Williams, *Biochim. Biophys. Acta* **1975**, *416*, 238 – 286.
[3] R. J. P. Williams, *J. Theor. Biol.* **1961**, *1*, 1 – 13.
[4] P. Mitchell, *Nature* **1961**, *191*, 144 – 148.
[5] For a history of the analysis and discussion of proton-driven ATP formation see: R. J. P. Williams, *Biosci. Rep.* **1993**, *13*, 191 – 212.
[6] W. Junge, H. Lill, S. Engelbrecht, *Trends Biochem. Sci.* **1997**, *22*, 420 – 423.
[7] J. P. Abrahams, A. G. W. Leslie, R. Lutter, J. E. Walker, *Nature* **1994**, *370*, 621 – 628.
[8] *Na, K-ATPase: Structure and Kinetics* (Eds.: J. C. Skou, J. G. Norby), Academic Press, New York, **2000**.
[9] M. D. Bootman, P. Lipp in *Fluorescent and Luminescent Probes*, (Ed.: W. T. Mason), Academic Press, New York, **2000**.
[10] R. J. P. Williams, *Cell Calcium* **1998**, *24,* 233 – 237.
[11] D. G. Nicholls, S. J. Ferguson, *Bioenergetics*, 2nd ed., Academic Press, London, **1992**.
[12] B. Chazotte, C. R. Hackenbrook in *Integration of Mitochondrial Function*, (Eds.: J. L. Lemasters, C. R. Hackenbrock, R. C. Thurman, H. V. Westerhoff), Plenum, New York, **1988**, pp. 53 – 62.
[13] T. G. Frey, C. A. Manuella, *Trends Biochem. Sci.* **2000**, *25*, 310 – 324.
[14] T. Ogata, Y. Yamasaki, *Cell Tissue Res.* **1985**, *241*, 251 – 256.
[15] L. E. Bakeeva, Yu. S. Chentsov, V. P. Skulachev, *Biochim. Biophys. Acta* **1978**, *501*, 349 – 360and references therein.
[16] V. P. Skulachev, *Trends Biochem. Sci.* **2001**, *26*, 23 – 29.
[17] a) R. Rizzuto, P. Pintou, W. Carrington, F. S. Fay, K. E. Fogarty, L. M. Lifschitz, R. A. Tuft, T. Possan, *Science* **1998**, *280*, 1763 – 1765; and see: b) T. Miyazaki, H. Ohata, M. Yamamoto, K. Momose, *Biochem. Biophys. Res. Commun.* **2001**, *281*, 172 – 179.
[18] R. J. P. Williams, *J. Solid State Chem.* **1999**, *145*, 488 – 495and references therein.
[19] C. C. Moser, J. M. Keske, K. Warnicke, R. Farid, P. L. Dutton, *Nature* **1992**, *355*, 796 – 801.
[20] H. J. Morowitz, *Am. J. Physiol.* **1978**, *235(3)*, 99 – 114.
[21] P. Mitchell, *FEBS Lett.* **1974**, *43*, 189 – 194.
[22] D. T. Edmond, *Eur. Biophys. J.* **1994**, *23*, 133 – 138.
[23] M. Gutman, E. Nachliel, S. Kiryati, *Biophys. J.* **1992**, *63*, 281 – 290.
[24] J. F. Nagle, R. A. Dilley, *J. Bioenerg. Biomembr.* **1986**, *18*, 55 – 68.
[25] Diffusion is analysed quantitatively by some theoretical treatments see references [6], [23], and V. Sachs, Y. Marantz, A. Aagard, S. Checover, E. Nachliel, M. Gutman, *Biochim. Biophys. Acta* **1998**, *1365*, 232 – 240—but they are not realistically related to in vivo structural and compositional restraints.
[26] U. Alexiev, R. Mollaaghabab, P. Scherrer, H. G. Klorana, N. P. Heyn, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 372 – 376 and references therein.
[27] Special issue on proton transfer (Ed.: P. Brzezinski): *Biochim. Biophys. Acta*, **2000**, *1458*, 1 – 219.
[28] L. S. Brown, R. Needleman, J. K. Lanyi, *Biochem.* **2000**, *39,* 938 – 945.
[29] J. Heberle, J. Riesle, G. Thiedemann, D. Oesterhelt, N. A. Dencher, *Nature* **1994**, *370*, 379 – 382.
[30] S. Wang, W. R. Trumble, H. Liao, C. R. Wesson, A. K. Dunker, C. Kang, *Nature Struct. Biol.* **1998**, *5*, 470 – 478.
[31] G. Haim, P. Dimroth, *EMBOJ.* **1999**, *18*, 4118 – 4127.
[32] R. J. P. Williams in *The Enzymes of Biological Membranes Vol. 47,* (Ed.: A. N. Martonosi), Plenum, New York, **1985**, pp. 71 – 110.
[33] R. J. P. Williams, J. J. R. Fraústo da Silva, *Bringing Chemistry to Life*, Oxford University Press, Oxford **1999**.

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