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HISTORICAL CORNER

The two last overviews by Colin Allen Wraight (1945–2014) on energy conversion in photosynthetic bacteria

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Abstract Colin Allen Wraight (1945–2014) was a well-known biophysicist and biochemist of our times—formerly Professor of Biochemistry, Biophysics and Plant Biology, and Head of the Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA. (See a detailed Tribute to him by Govindjee et al., Photosynth Res, 2015.) During the latter part of his life, Colin had (1) given an excellent lecture in 2008 on the overall topic of the molecular mechanisms in biological energy conversion, focusing on how an ubiquinone is reduced to ubiquinol at the so-called "two electron gate", and (2) presented a review poster on the design features of long distance proton transport in biological systems, with focus on photosynthetic bacteria (a pdf file of the original is available from one of us, Govindjee). We present here for historical purpose, a complete transcript of his 2008 lecture and his 2013 poster, which have been annotated and expanded by the authors of this paper. The major theme is: electron and proton transfer in biological systems, with emphasis on bacterial reaction centers. The figures, some of which were prepared by us, are presented in sequence for both the lecture and the poster. A common bibliography is provided at the end of the paper, which is divided into two parts: (I) The Lecture; and (II) The Poster.

Keywords Bioenergetics · Purple bacteria · Reaction center · Ubiquinones · Light induced electron and proton transfer · Two Electron Gate

We begin this presentation with a photograph of Colin Wraight in his office at the University of Illinois at Urbana-Champaign (UIUC; Fig. 1).

I. The lecture

Prologue

We provide here a transcript of a lecture by Colin A. Wraight; this lecture was given on January 16, 2008, at the University of Puerto Rico, School of Medicine; it was the First Biochemistry Symposium there, where Biochemistry chairs spoke about their research. We discovered that this lecture is available free on the internet. The original lecture was first transcribed from the video (see[1]) and edited at

Govindjee initiated the idea of making one of the last lectures and a single-authored poster of Colin Wraight (1945–2014) available to all for the history of research in photosynthesis. This paper is published with the permission of Mary, Colin Wraight's wife. The current manuscript was read and edited by Antony (Tony) Crofts, who pointed out that since it is a lecture and a poster, it couldn't be considered a review of the field, but a historical piece. This paper was approved for publication by Rienk van Grondelle, who serves on the Editorial Board of Photosynthesis Research; he added: It is beautiful, thanks a lot to both of you! Please publish it.

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[1] https://www.youtube.com/watch?v=2IB5e6FxH0M.

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Fig. 1 A photograph of Colin A. Wraight in his office at the University of Illinois at Urbana-Champaign, taken in the 1980s. This photo was provided by Mary Wraight

places; this version differs slightly from the original, although we have retained the spoken version, without making it into a written version. The figures (and the legends) used in the text that follow were prepared by the authors. The video is available at: *https://www.youtube.com/watch?v=2IB5e6FxH0M.

The original video was recorded and edited by the Center for Information Architecture in Research, which was funded by a grant G12RR03051, currently G12 MD007600, from the National Center for Research Resources, National Institutes of Health http://rcmi.rcm.upr.edu.

The Lecture (*Bioenergetics*: *molecular mechanisms in biological energy conversion*) by Colin A. Wraight follows; some of the slides shown in the video are reproduced here with few exceptions; further, the text has been arbitrarily divided into paragraphs, and key references added by the authors of this presentation. A caveat is: it is likely that we may have made errors in understanding the lecture at places; thus, we encourage the readers to write to us in case they have a different opinion. We have attempted to annotate and slightly modify the spoken text to help the readers understand the lecture without having to listen to the video.

Transcript of the 2008 Lecture by Colin Wraight on "Bioenergetics—molecular mechanisms in biological energy conversion"

Colin Wraight began his lecture by saying

My background and perspective is from biophysics and physical biochemistry. Although I am on occasions involved in teaching medical students and I know from that experience that bioenergetics is one of those topics that one meets once and forgets about very quickly or at least never re-encounters. So I'm going to give maybe a fairly extensive introduction with the aim of telling you what I do rather than in very great details and especially why I do it. Bioenergetics, as you will, in general, have met it in the context of mitochondrial activities.

Bioenergetics can be usefully defined as looking at the thermodynamics, the energetics, as well as the mechanisms of how organisms obtain energy from the environment,—it can come in a variety of ways,—and be converted into the energetic 'elements' of metabolism,—the predominant one that one thinks of is ATP,—but also a lot of transport processes and a lot of other so-called high energy chemicals that we encounter.

So in mitochondria the energy comes in the form of food and the process there is to convert the inherent free energy which is bound up in specific kinds of electrons involved that are accessible through oxidation–reduction reactions and to convert that into these other diverse forms. The underlying or sort of grand vision on how this is done is due to this man Peter Mitchell who devised at the time a really radical way of thinking about how organisms do this and he devised something which we now call the chemiosmotic theory of biological energy conversion (Mitchell 1961, 1966, 1979; see Peter Michell in Fig. 2). The idea here is that electrons stripped off from food molecules are fed into a sequence of oxidation–reduction reactions called an electron transport chain which is bound

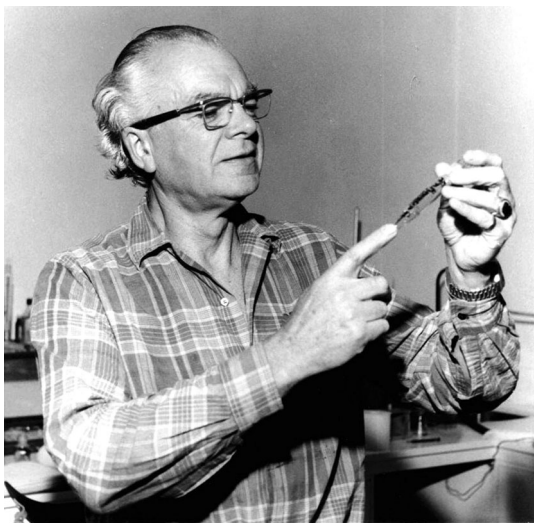

Fig. 2 A photograph of Peter Mitchell, who received Nobel Prize in Chemistry, in 1978, for the chemiosmotic theory of biological energy conversion and transduction. According to this theory, an electron transport chain converts redox free energy into electro-osmotic free energy of a proton (H^+ ion) gradient across the mitochondrial membrane—or bacterial cell membrane. The proton motive force drives other reactions of transport or ATP synthesis. The photograph of Mitchell in the lab was provided by Peter Rich from his personal collection

up in very large and complicated membrane molecules in the mitochondrial membrane or, as we will see, in the cell membrane of bacteria.

As the electrons pass through this chain, various steps of the reactions are coupled to proton translocation—across the membrane—so that the free energy in the reducing power—that originated with food—is converted into a gradient of protons, an electrochemical gradient, and that is the delocalized source of free energy which can be converted into the other forms, for example, transport or ATP synthesis. So we actually now know the structures of most of the major molecules involved, for example, in the mitochondrial electron transport chain (Fig. 3) starting with the NADH dehydrogenase of which we know the portion that is outside of the membrane, but not that of membrane-embedded portion. For succinic dehydrogenase/oxidase, cytochrome bc_1 complex, and cytochrome oxidase, where complete structures are known, we can trace the path by which electrons are passed down in a sequence of reactions and various steps so as to pump or translocate or react in a variety of mechanistic ways to move protons from the inside of the mitochondria to the outside, generating a gradient which can then be used to drive protons through other work—conserving membrane structures like [that of] the ATP synthase.

Now this hypothesis is elevated to the level of a theory called the chemiosmotic theory that is universal (Berg et al. 2002); it is found in all the major energy conserving forms of metabolism and in all forms of life,—in both aerobic and anaerobic situations, in respiration and methanogenesis, and in photosynthesis,—and in all the life forms within the archaea, eukarya, and bacteria. Just to remind you that in the case of microbes or the prokarya, the electron transport chain is found in the cell membrane, which is sort of the analog of the mitochondrial membrane. So my own interest and work in this area comes from my beginnings—we have this sort of historical perspective—some of us, the older members of this group here, that comes from starting off in the area of photosynthesis (Fig. 4).

So I work with photosynthetic organisms and in particular with photosynthetic bacteria and there the electron transport chain is pared down to rather a convenient minimum. The energetic electrons, which go into the electron transport chain, are actually obtained by the input of light. So, low energy electrons are elevated to a high energy level and two turnovers of a protein known as the photosynthetic reaction center produces a quinol, i.e., it reduces a quinone to a quinol. The quinol comes out and diffuses in the membrane to meet up with cytochrome bc_1 complex, which is completely equivalent to the one found in mitochondria. There, the quinol is oxidized to quinone, which then cycles back to the reaction center. The electrons go through a rather complicated pathway in the cyt bc_1 complex, come out to a cytochrome *c* and then are donated back to the reaction center. So the actual electron transport reaction here is cyclical and the only net gain of free energy is the gradient of protons, which is generated by a couple of steps within the cyclic pathway. So reaction centers or photosynthetic systems in general convert light energy into two basic forms. One is the reducing power which is distributed to other membrane complexes like the [cyt] bc_1 complex and the other is an electrochemical gradient which includes both a true electrical potential because electrons are moved across the membrane, the insulating layer of the membrane (a process I will show you in a moment) and also protons that are taken up …as part of the electrochemical energy conversion.

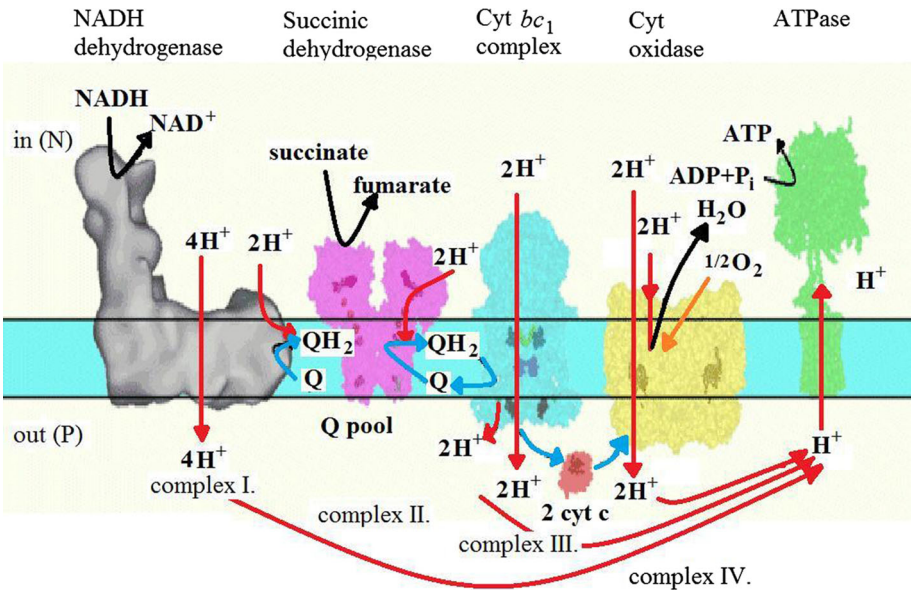

Fig. 3 Mitochondrial electron transport chain (ETC). In mitochondria, the ETC involves the participation of four large membrane proteins in the inner membrane: *Complexes I–IV* are connected by mobile carriers ubiquinone (Q) and cytochrome *c* (cyt *c*). Several steps in the ETC drive protons across the membrane. These are then used by ATP-synthase. [Adapted from a figure in Biophysics 354 (~2004), Lecture 8, at the University of Illinois at Urbana-Champaign, by A. R. Crofts; note that the complete structure if *Complex I* is now known]

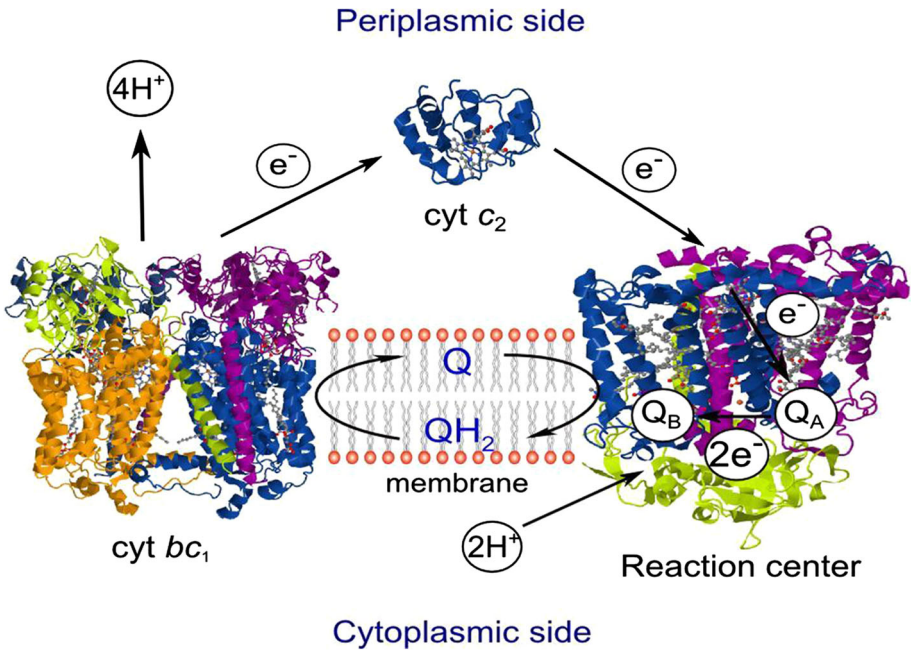

Fig. 4 Cyclic electron flow of bacterial photosynthesis that involves cytochrome bc_1 oxidoreductase (cyt bc_1) and reaction center (RC) complex; it is mediated by mobile electron carrier ubiquinone (Q) in the membrane and cytochrome c_2 (cyt c_2) in the periplasmic (aqueous) region. In photosynthetic systems, "reducing" electrons from ETC are produced by photochemistry in the RC. The photosynthetic RC converts light energy into electrochemical form of proton electrochemical gradient and quinol (reducing power). [Adapted from Fig. 1 in Kis et al. (2014)]

So what I want to emphasize at this point is that for a variety of reasons, which I hope will become quickly apparent, bioenergetic proteins are really, I believe, an ideal system for studying the true essence of how proteins work in a very general sense. So what I am interested in is how proteins work; it turns out that bioenergetic proteins, in particular photosynthetic ones, have attributes which allow you to ask very detailed but very general questions about how proteins do essentially everything including [to] be enzymes, [to] be antibodies, [to] be signaling cofactors et cetera. The reason we can ask such detailed questions is that the essential centers where electron transfer takes place in the redox enzymes of bioenergetics,—the quintessential feature of these proteins,—are cofactors which have phenomenally detailed binding environments leading to very striking differences in properties, and they have very intense spectroscopic properties which makes them easily observable.

You can't learn much without being able to observe something. So we know that we learn as soon as we enter into the field of biology ……the proteins really seem able to do anything,—an extraordinary varied range of reactions catalyzed. Whether they do anything, of course, depends on what is useful evolutionarily speaking, but it seems they can do anything!

With the sort of insight that we get from choosing the right system we can ask how they do it. Just some examples,—and what I really want to focus on,—is that we know that different proteins can take the same chemical and make it do entirely different things. So heme, [of] which there are actually several—but if you just take particular heme, a *b* heme,—the same kind of heme that you find carrying O_2 in hemoglobin and myoglobin,—you find exactly the same heme in b-type cytochromes doing something different. For example, the cytochromes, which are involved in the burst of leukocytes that dispenses with infections by spraying hydrogen peroxide all over the place. It's also found in certain cytochrome oxidases and within these different proteins the same heme has exceedingly different properties, which we characterize as being redox potential. That redox potential can vary as much as the equivalent acid-base property of a p*K* is different in, say, sodium hydroxide compared to hydrochloric acid. So the protein environment of these cofactors makes them just entirely different. We also conceive this kind of diversity of effect as the proteins have when we think about the (visual) spectrum of red, blue and green rhodopsins. You have a very wide range of color and that color is imposed on the same chemical, which is retinal, by the environment of the protein (Kochendoerfer et al. 1999).

Similarly, we see it in photosynthetic structures where the pigment, which is being imposed upon is chlorophyll or bacteriochlorophyll. So it turns out then that the reaction center,—and this happens to be the reaction center from a bacterium,—has a remarkable property, which allows you to ask how on earth the proteins do this.

So this is the structure of the reaction center from a particular bacterium that I work on (Fig. 5). It consists of three protein subunits—two of them are in the membranes (so the membrane is along the middle here) and the subunits in the membrane are highly homologous and are called the L subunit and the M subunit. They bind all of the

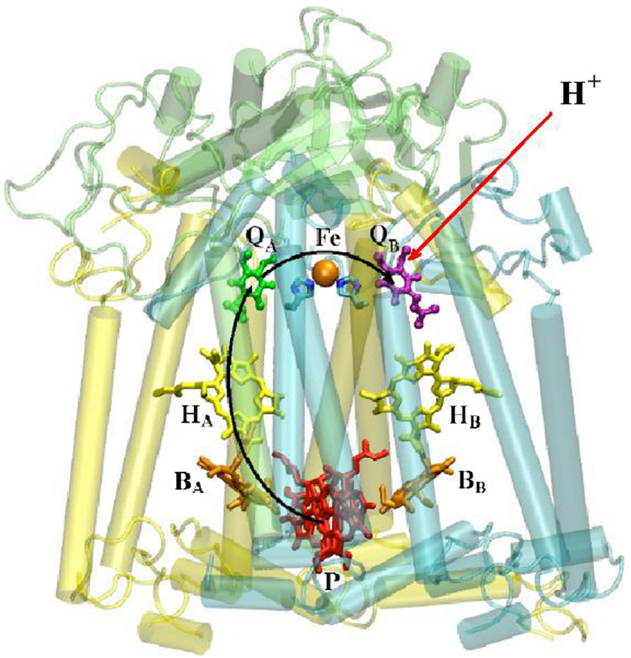

Fig. 5 The reaction center (RC) complex from photosynthetic purple bacterium *Rhodobacter sphaeroides.* The membrane plane runs from *left* to *right*, with the cytoplasmic phase at the *top* and the extracellular (periplasmic) space at the *bottom*. The polyisoprene groups of all cofactors have been truncated (Q_A and Q_B) or removed (chlorins). The RC comprises three subunits. The L and M subunits bind all the cofactors which are arranged around a quasi-2-fold *rotational symmetry axis*, normal to the plane of the membrane and passing through the primary (electron) donor (P), the special pair dimer of bacteriochlorophylls (BChl), and a ferrous (Fe^{2+}) iron midway between the two quinones. The subunit H stabilizes the structure, caps significantly the structure over Q_A, while Q_B is much less protected and is involved in H^+-ion uptake and transfer associated with electron transfer to the quinones. The active electron transfer path is indicated by *arrows*. It proceeds from the excited singlet state of the primary donor (P*), via the A-branch of cofactors—monomer BChl (B_A) and BPhe (H_A), bound to the L subunit—to the primary quinone, Q_A, which is bound in a fold of the M subunit. From Q_A^- the electron crosses the *symmetry axis* to the secondary quinone, Q_B, bound in a similar fold in the L subunit. [Modified from Fig. 1 in Wraight and Gunner (2009)]

cofactors that are involved in light energy conversion and these cofactors amount to four bacteriochorophylls, two bacteriopheophytins, which are bacteriochlorophylls without magnesium, and two quinones.

You can see here the symmetrical arrangement of the cofactors in the protein, but it also turns out that the arrangement of the proteins is also very highly symmetrical. If we drew a line from here [the dimer P] to here [iron atom Fe] then there would be a two-fold rotational axis so we have these cofactors, which are present in chemically identical pairs. There are two bacteriochlorophylls down here which are actually a dimer [P] and that's where the light energy is first taken and converted into redox energy. There are two more bacteriochlorophylls here, 2 bacteriopheophytins and then 2 quinones, but the electron transfer only occurs down this [one] pathway [A branch]. So that the two sides are chemically identical seemingly, and yet functionally exceedingly asymmetrical! So we have the ideal situation where we can say what is the difference between the environment of this [bacterio] pheophytin and that [bacterio] pheophytin …which makes this one [H_A] active and this one [H_B] not active. Or in the case of the majority of the work that we carry out, although these two quinones [Q_A and Q_B] are chemically identical—they both are ubiquinones, the same as found in mitochondria—, yet functionally quite distinct (Wraight and Gunner 2009).

This is just to emphasize the spectroscopy that's available with the system like this, and that makes so much of our so very detailed, but I hope highly generalizable, conclusions possible. This is the spectrum of the reaction center (Fig. 6). This peak out here is at about 860 nm [and] is predominantly associated with the two bacteriochlorophylls, the special pair, that form(s) what's called the primary photopigment or primary donor [P]. This peak here [at about 800 nm] comes from these two bacteriochlorophylls. This peak here [at about 760 nm] comes from these two bacteriopheophytins [H_A and H_B]. [*portion deleted*]. But the point I wanted to make is that [they have] very intense absorbance bands. These reaction centers are of beautiful sky blue color, and the spectrum also emphasizes that these bacteriochlorophylls absorb at a very different place from these two bacteriochlorophylls because the protein environment around them is very different. But because of this kind of spectroscopy we know that electron transfer starts from an excited state of this primary donor—it proceeds across—the electron is "ejected" and proceeds

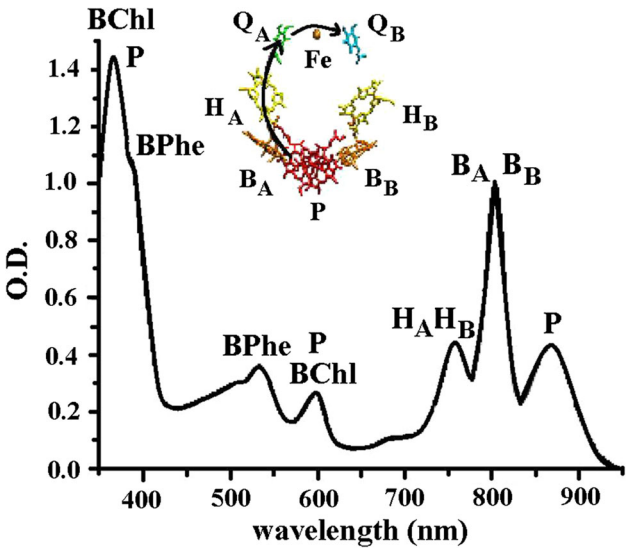

Fig. 6 Steady state optical absorption spectrum of RC from *Rhodobacter sphaeroides*. The different bands of the spectrum can be attributed to specific cofactors of the RC protein. [This figure is reproduced from the lecture by Colin Wraight]

across the membrane—it reaches this point [H_A] in about three picoseconds. It goes from here [H_A] to here [Q_A] in about two hundred picoseconds and from here [Q_A] to here [Q_B] in about 10 microseconds. It turns out [that] measuring picosecond kinetics—if you have the right spectroscopy—is really easy so long, of course, you have a half a million dollars worth of spectroscopic equipment, but technically speaking [it] is actually very straightforward. So I'm going to focus on the reactions at this last part here with the electron arriving across the membrane to this primary quinone called Q_A and [then it] is transferred to the secondary quinone Q_B and then when the second turnover [takes place] this quinone is fully reduced to a quinol—it comes out of the reaction center like a converted substrate and is replaced by a quinone molecule from the membrane.

So this just summarizes what happens on the first flash (see Fig. 7). We reduced the primary quinone and that [reduced] quinone is then able to transfer its electron in a favorable forward direction to the secondary quinone and the electron just hangs around there waiting for the next turnover. So we form a stable anionic semiquinone.

The second turnover starts off with the reaction center in a slightly different state. The secondary quinone (Q_B) is already singly reduced. …….[The next "light reaction"] reduces the primary quinone, again and now that second electron is transferred to the secondary quinone [already in semiquinone form],—the protons are taken up, the quinol is formed [and] comes out, etc. So there are two important things to note here. First of all, although these quinones are chemically identical, the environment around them makes it possible for the first electron to be favorably transferred

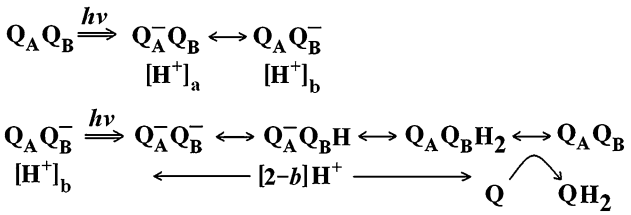

Fig. 7 Quinone reduction cycle of the reaction center (RC). Following the first photoactivation, the first electron is shared between the two quinones. The negative charges of the anionic semiquinones induce proton uptake to the protein, contributing to the partial shielding and stabilization of the semiquinones. After the second excitation, proton activated second interquinone electron transfer takes place that leads to full reduction of Q_B coupled with the delivery of two protons to the quinone head group. The QH_2 leaves the RC and is replaced by an oxidized quinone from the membrane pool. This returns the acceptor quinones to their original state and allows RC turnover to proceed under multiple-flash activation. Under such conditions, binary oscillations can be observed in the formation and disappearance of a semiquinone and in the uptake of protons from the medium [$b < 1$ on the first flash and $(2 - b)$ on the second]. [Adapted from Wraight (2004)]

to the secondary quinone. So there's a change or difference in the electron affinity or the redox potential of the second quinone compared to the first quinone so that the electron always goes forward. Also even on the second electron transfer there is a forward electron transfer equilibrium which favors the right hand side. And the other thing to note is that on the second electron transfer here both protons are taken up—so this is the same sequence here starting off with the second electron activation to reduce Q_A. In fact before that electron is transferred, this semiquinone [Q_B^-] picks up a proton… forms a neutral semiquinone [Q_BH], and then the second electron is transferred and then the second proton is taken up. So there's a lot of specialized equipment around the secondary quinone that makes these issues possible. So the challenge is to design,—or imagine how the protein is designed,—this secondary quinone region that allows first of all the electron transfers to be favorable in both cases between two otherwise seemingly identical chemicals but also how this arrangement can be designed to favor electron transfer to Q_B. And electrons—remember—are negatively charged. But the site is also designed to allow for protons to be transferred,—and protons are positively charged. It turns out that almost everything that proteins do is based on electrostatics. So this is a little bit of a conundrum. So all of this activity—this design activity—takes place in this region, which I call the Q_B acidic cluster because it turns out that there are [is] an enormous number of acidic residues here. It has the highest density of ionizable residues known in any protein that has so far been identified.

So here is the secondary quinone [Q_B] down here (Fig. 8). If you're able to do cross eyed stereo that's what this figure is; if you can't—don't worry—it's probably not very important, and I have only labeled one side anyway which means that if you can do it—you'll [or can] get a headache after a few minutes. So this is the acidic cluster this is the surface of the protein out here the cluster of ionizable residue on the surface.

It turns out that because of the density of ionizable residues here, it is a very, very complicated interplay of electrostatic interactions which makes it both—complicated to understand but also very interesting to try to understand. So, in addition to that being a high-density of acidic residues here, it turns out that most of them are ionized. These are buried residues so you might think that it would be difficult to bury a charge and in general that's kind of a nice way to start thinking but it turns out that almost all of these residues are either ionized or partially ionized and since they are mostly negative,—almost never should be. So there must be some kind of counterbalance of pluses [positive charges]. So there are a few pluses around here. Lysine here and an arginine there but the vast majority of the counter charge to

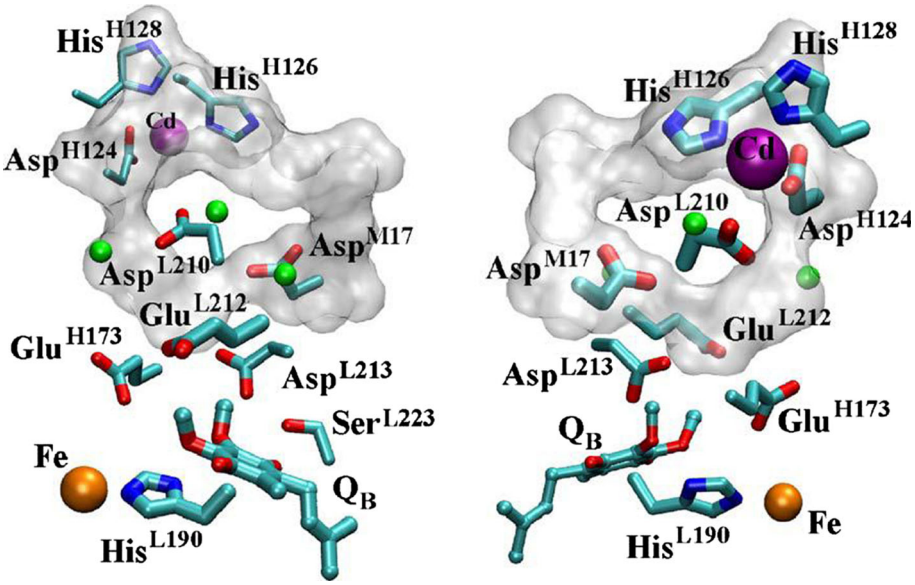

Fig. 8 Stereo view of the Q_B acidic cluster in the reaction center from *Rba. sphaeroides*. *Left* view is from the inside looking towards the protein surface. *Right* view is looking down on the entrance from the outside. The *white contoured surface* encloses surface residues: Asp-H124, His-H126, His-H128 and Asp-M17. All other residues are buried. The bound cadmium (Cd^{2+}) is an inhibitor of proton entry—it lowers the pK_as and deprotonates the histidine residues. Residues are sized to indicate depth of field. [Adapted from Fig. 6 in Wraight (2006)]

the negative charges we see on these acidic residues comes from a generalized positive potential inside the protein which arises from the dipoles of the peptides; in other words it's a backbone potential. Now we don't—sort of attend to—pay much attention to it. Nevertheless so there is an underlying positive potential here and that allows most of these ionizable acidic residues to be actually negatively charged (Wraight 2004).

I don't know whether it is right to say "it allows it" or "it is required" because you can't have one without the other. Now when we look at the properties of the secondary quinone it turns out that the major contributors to the favorable reduction of Q_B comes from or is controlled by the overall electrostatics, but with particular contributions from these two residues: a glutamic acid which is residue E212 in the L subunit and aspartic acid which is residue D213 in the L subunit. So this is how we identify the beginnings of how we identify that these residues are important.

…[Let us] look at the one-electron equilibrium constant between the first quinone and the second quinone and we look at it as a function of pH (Fig. 9). As you change the pH of the medium then you're going to protonate or deprotonate different groups you are gonna change the net charge of the protein in a fairly progressive fashion as we go up in pH. Things will become de-protonated or more negative. So we look at the black line here the value of this equilibrium constant and note—this is logarithmic so it's changing rather dramatically. Low pH has a value on the order [of] say fifty, that [for] the equilibrium [constant] between the left and the right hand side.

As we raise the pH, the equilibrium constant gets smaller and that's associated with the ionization of this aspartic acid. As it becomes ionized, its negative charge inhibits the transfer of the negative electron to Q_B. Then in

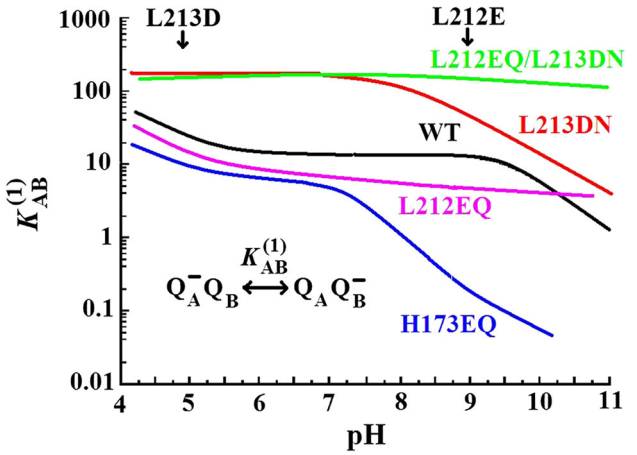

Fig. 9 Roles of Glu^{L212} and Asp^{L213} in the first electron transfer equilibrium. Mutating Glu^{L212} (L202EQ single and L212EQ/L213DN double mutants) takes away a negative charge near Q_B and enhances the first electron transfer equilibrium in the alkaline pH range. Mutating Asp^{L213} (L213DN single and L212EQ/L213DN double mutants) takes away a negative charge near Q_B and enhances the first electron transfer equilibrium in the acidic pH range where it would normally control it. Mutating Glu^{H173} (mutant H173EQ) takes away a negative charge but other acids (Asp^{L213} and Glu^{L212}) ionize more and are closer to Q_B, so the first electron transfer equilibrium is decreased. [Adapted from Fig. 3 in Takahashi and Wraight (1992), and from Fig. 2 in Takahashi and Wraight (1996)]

[the] neutral pH region, the equilibrium region is fairly constant [pH independent] and then as we go to higher pH, we find [that] it becomes more and more unfavorable. It turns out [that] as this glutamic acid ionizes… so yet another negative charge is put near the secondary quinone, which inhibits the transfer of the negative electron. This is a fairly typical pK for a carboxylate –around five say. This is an unusually high pK for carboxylate and that arises both

from the fact that it's buried but also it is interacting with a number of other groups. So now the way part of how we understand what's going on here is to [make] site-directed mutations. So we've done a lot of mutational work on this acid cluster (Takahashi and Wraight 2006). Let's just imagine what might happen. Since the ionization of acidic residues is dependent to a large extent on there being a positive charge to allow them to be ionized, so in other words we have more or less electrostatic neutrality. If we take away one of these negative charges we can expect that some of the others will further ionize and try to compensate for that loss of negative charge because there is more positive charge out there still to be compensated—gained more positive potential.

So if we target one—and that is target this residue here—aspartic acid, the L213, [and] if we now mutate [it] to asparagine, which is neutral at all pHs, then it would be interesting to see what happens. So we mutated [it] okay! (Takahashi and Wraight 1992). Taking away that negative charge allows other charges to become more predominant. So this residue, which was only partially ionized now becomes fully ionized and this one which was partially ionized has become more but still partially ionized. So we take away one negative charge and there's a tendency for this whole kind of network from compensating [the] system to make up for the negative charge we've taken away. But the charge that we've taken away is very close to Q_B, which is where all the actions are taking place. So even though we have compensated [it] by increasing the negative charge elsewhere we still get a loss of negative potential in the region of the secondary quinone or, in other words, a more positive potential than we had before. So when we look at the effect of this mutant we find [that] the more positive potential in the region of Q_B allows the electron transfer to be more favorable. Positive potential or less negative potential allows more negative charge of the electron. So that's quite consistent with what we would expect. Something to do with electrostatic environment of $Q_{B...}$ [it] is important for determining how well it can take a negative electron. But now let's look at another one.

Let's mutate this residue [H173E], the glutamic acid in the H subunit, which is another subunit that caps the whole structure (Takahashi and Wraight 1996). Taking away this negative charge will also allow other residues to become more ionized. So there will be some compensation of the loss of that negative charge. Let's see which ones can ionize further. Well it turns out that these ones, which are very close to the quinone can ionize more and since they are closer to the quinone their influence is greater than the one we have taken away. So it turns out… [when] we take away a negative charge, and we actually make the environment around Q_B more negative, which is obviously, at first sight, counter-intuitive—but when we think of how proteins really work and how complicated they are and the nature of electrostatic energies then it's not too surprising. In fact if we look at this mutant which is down here we find that taking away the negative charge here has made the equilibrium constant less favorable because it's made the potential near Q_B actually more negative by virtue of this reshuffling of the ionization states [in] this complicated region.

What it has actually done—it [has] downshifted the p*K* of the glutamic acid into a region where it influences the equilibrium constant at lower pH. We can do this with a variety of other residues and get some idea of how this complicated electrostatic interplay really takes place. Now this region of the protein is not only involved in setting the sort of equilibrium properties of the secondary quinone, it is also responsible for delivering the protons that must …..[get] to the quinone when we reduce it finally to the quinol because reducing it to the quinol takes two electrons but also takes two protons. So that the same region here is responsible for actually providing, in some constructive way, a pathway which allows protons to come from the solution through about 15 angstroms. This is a long distance proton transfer event—to be delivered to the secondary quinone. You can see this sort of range of the scales here. The electron is transferred from the primary (electron) donor [P] to the quinones over about twenty-five angstroms that actually acts to charge up the membrane electrically. But also the protons must come in from [the] solution over a somewhat equivalent distance, which also contributes to the net charging of the electric potential across the membrane. So the same sort of thing happens here and in some of the work that I've been talking about is being done in my own lab.

There is another [research] group at the University of California San Diego [George Feher and Mel Okamura and their coworkers] where a lot of the stuff that I've been talking about has been done. In a particular very nice work in that group, Pia Ädelroth and Mark Paddock have identified the somewhat unexpected role of some surface histidines to be very important for delivering protons to the secondary quinone (Ädelroth et al. 2001; Paddock et al. 2003). In fact they represent the entry site of the protons into the protein (Fig. 10). The reason why these are important actually comes down to something we tend to forget about and that is that the physiological environment is actually not terribly "healthy" for reactions which involve protons because there are damn few of them. At pH 7, the concentration of protons is 0.1 micromolar [μM]. So if you [are] especially in a situation where you consider that there may be competition between a cation like a proton and other cations around, there are a hundred mM sodium or 200 mM potassium out there. So there's a huge disparity between cations that might be involved in various kinds of charge neutralization process. To compensate for

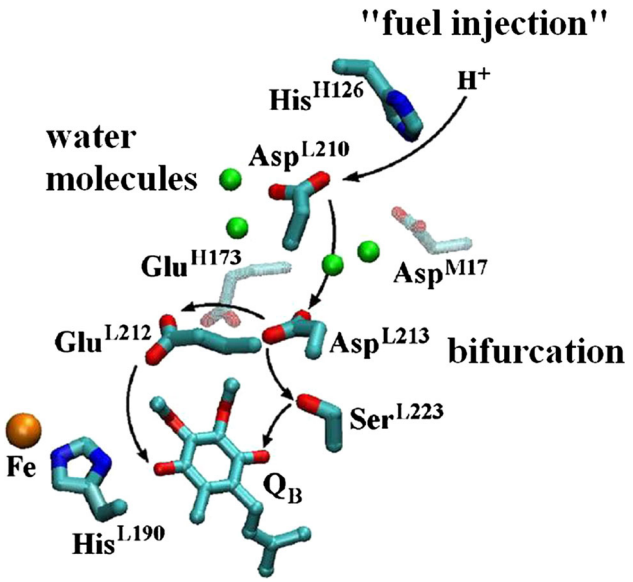

Fig. 10 The Q_B site acid cluster and proton transfer pathway to Q_B. Only *side chains* are shown, and the prenyl tail of Q_B has been truncated. Proton enters at surface histidines ("fuel injection") and follows a certain pathway to Q_B, via several carboxylates, labeled by *residue number* and structural water molecules. Water molecules (*green*) fill some but not all gaps in the putative H^+ pathway. The path bifurcates at or after L213 to deliver H^+ to the two carbonyls of the secondary quinone. [Adapted from Fig. 5 in Wraight (2006)]

that there are a number of devices in proteins which actually facilitate working at high turnover rates and also against a very unfavorable competition with a cation. One of these things is something I call *fuel injection* (Fig. 10). Which is that these histidines are partially protonated at neutral pH, which provides effectively a much higher concentration of protons locally than just the average appearance or disappearance or comings and goings of protons in a solution of 0.1 microM. So we take these histidines away, [and] 0.1 microM protons just do not do it. They get in and arrive much too slowly to really contribute to the possible turnover times of the correct reactions going on—here in the secondary quinone.

Other residues which when mutated kill off proton delivery… [these] include the two that we've been talking about so far because they are right next to the quinone and they are actually involved in sort of the last step. They are the things that actually hand off the protons to the secondary quinone and a few other residues. Not all of the acidic residues selected [are important] but a few of the acidic residues are specifically involved in delivering protons. It turns out that if you mutate this same aspartic acidic residue as before [L213] we find that the first proton [H(1)], which has to get to the secondary quinone, in order to allow the second electron to be delivered is blocked. Whereas if we mutate the glutamic acid [L212], again the same glutamic acid that was responsible for the pH dependence that we were talking about before, [we observe] that [it] delivers the second proton [H(2)]. So after the second electron has been transferred, we have a quinol and then a proton is taken up in—it comes from this glutamic acid. So the pathway apparently bifurcates and with a number of other studies that we've done over the last few years this is [an] overall idea as to how the proton transfer pathway works. The protons enter this surface site which consists of the histidines—comes through the protein although quite possibly also involves buried water molecules. There are water molecules in here, which can be very vaguely seen or if you can't see if you have a space in a protein in an x-ray structure—the chances are it's not empty. If it's big enough to have a water molecule it probably will—but if that water molecule is very mobile you can see it. So there's a lot of space in here, which can contain water molecules.

So the protons have conducted through water molecules but especially the acidic residues to this aspartic acid [L213] and then this either hands off a proton to this carbonyl group of the quinone, or it goes to glutamic acid and is then handed off to this carbonyl group. So the work that we're doing now, which is sort of predicted from this—much of this work has been going on for the last ten years but some of the conclusions that I have given you are quite recent. We are interested in the detailed mechanisms as to what are the properties of a residue in here, which is functional as a proton carrier? Is the specific p*K* critical? How far can you take the p*K* of a group away from what we think of as being ionizable before it becomes unuseful (?). In some cases you can convert these into say a threonine or a serine not something you would think of as being ionizable but protonatable transiently. So there's a wide range of possibilities as to what really is critical as to whether these residents can work the way they appear to.

Acknowledgment: I will acknowledge that most of the experimental work-up I have talked about has been done by a fellow who started off as a graduate student but he refused to leave—he became a Post Doc and then a senior scientist—he has been with me since 1986—I think: *Eiji Takahashi* has done a lot of this. A dear colleague of mine, *Péter Maróti*, a Hungarian, who visits me quite regularly, has done some of this, and *Vladimir Shinkarev* who "escaped" from Russia in 1989 when the wall came down—never went home either. Then a longtime friend colleague and it turns out my brother-in-law *Les Dutton* who was in a very similar area. So thank you very much for your attention.

Epilogue by Maróti and Govindjee

For fun and a bit of frolic, credits were listed, at one place, as: *Leading man*: Eiji Takahashi; *Gaffer*: Péter Maróti; *Key Grip*: Vladimir Shinkarev; *Best Boy (man)*: Les Dutton;

Director: Colin Wraight; *Screen Play*: *Rba. sphaeroides*; Executive Producer(s): National Science Foundation, NSF (USA); and National Institutes of Health, NIH (USA).

We note that Takahashi; Maróti; and Shinkarev have presented their own remembrances (see Govindjee et al. 2015).

II. The poster

On the design features of long distance proton transport in biological systems, with focus on photosynthetic bacteria (C. A. Wraight)

Since the poster had very few words, we have added text, in italics, to make the poster understandable to the readers.

The poster started with its **abstract**:

The design criteria employed by Nature are wholly determined by evolutionary pressures acting upon many functions, simultaneously. At the molecular level, this has been well described for biological *electron* transport. It is now also becoming evident in biological *proton* transport, where internal waters play a key role.

This was followed by ***introductory remarks***:

Proton transfer (PT) is of major importance in two fields of biology: acid-base catalysis in enzyme activity and transport in bioenergetics, usually coupled to electron transfer. The primary purpose of proton transport is to translocate H^+ ions into and across the membrane. The PT in biology is fast, specific and robust, and can be over long distances. The PT involves many elementary proton transfer steps and is exquisitely dependent on structure including (permanent or transient) delivery pathways.

The major challenges are:

1) High specificity at low ion availability *($[H^+] \approx 10^{-7}$ M at neutral pH compared to cation concentrations of $[K^+]$ and $[Na^+] \approx 0.1$ M in surrounding solutions)*

2) High throughput at low concentration and

3) Evolutionary robustness, not durability.

How are these addressed?

The elementary acts of PT are fast in water

The proton in water is generally considered to be present as hydronium, H_3O^+, which is similar to Na^+ in size and solvation characteristics. It can therefore be expected to exhibit diffusive properties similar to Na^+. However, the rapid exchange of H^+ between H_3O^+ and H_2O allows for a unique transport process known as the Grotthuss mechanism, which transfers protonic charge without diffusive movement of either an individual H^+ ion or oxygen atom (Fig. 11; figures 1-10 are in section I, above). Due to the hydrogen bonding between water molecules, the Grotthuss process (Eigen's structural diffusion) is fast and efficient (Wraight 2005). The Grotthuss mechanism contributes at least 85% of the measured transfer number of hydrogen ions, and the ionic mobility of H^+ is about 7 times that of Na^+.

The protons have the potential for this unique mode of transport not only in water but in other highly connected hydrogen bonding systems, as well. The Grotthuss-like mechanism can be envisioned for any chain of hydrogen bonded acid–base groups with an appropriate geometry for continuous hydrogen bonding—each hydrogen bonded atom must act simultaneously as donor and acceptor. This could be fulfilled by several O and N functional groups, and especially –OH. The potential for hydrogen-bonded chains (HBC) to facilitate fast proton transfer in biology was proposed by Onsager (1967, 1969) and developed by Nagle and coworkers *(Nagle and Morowicz 1978; Nagle and Nagle 1983) and gave rise to the term "proton wire".*

However, this mechanism could be hard-wired in proteins as HBCs of amino acids. However, an HBC consisting only of protein groups would be a fairly rigid structure, which would be counter-productive, especially for rapid, reversible PT. Instead, natural designs incorporate rotationally highly mobile water molecules that facilitate PT at rates similar to that in the bulk phase.

Specific proton transport proteins

Gramicidin A (gA) : *The peptide consists of 15-residues and, together with a second associated molecule, forms a head-to-head dimer making a complete, but transient transmembrane ion channel (Fig. 12). The lifetime of the head-to-head dimer is on the order of 0.1–1 s, depending on pH, temperature, and membrane lipid type.*

The gramicidin channel is readily permeable to monovalent cations and protons. The water file allows, but is not designed for, rapid Grotthuss-type proton transport. Because the H^+ and water mobility are almost as high as in bulk water, fast H^+ throughput, i.e., maximum current of about 10^9 ions/s can be observed.

The proton selectivity is moderate. The effective diffusion coefficient for H^+ is 20–30 times larger than for any other ionic species (e.g. K^+), or for net flux of water. *It is due to two effects. 1) The large protonic conductance of gramicidin is a consequence of the Grotthuss-type mechanism that proceeds independently of molecular diffusion, at a rate quite similar to that in bulk water. 2) The alkali cations move in a single file with the water column as a whole that provides an upper limit for cation diffusion in the channel. Relative to bulk solution, the mobility of Cs^+ ion in the channel decreases at least 14-fold compared to 3-fold, or less, for H^+.*

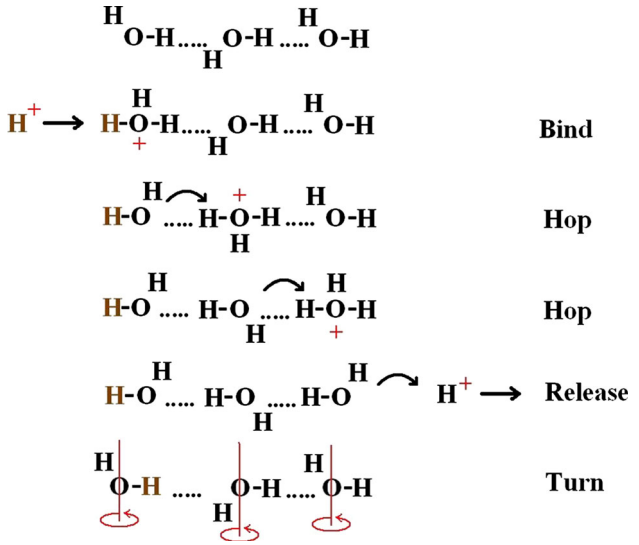

Fig. 11 Demonstration of Grotthuss mechanism of proton transport along a *chain* of three hydrogen bonded water molecules without diffusive movement of the H^+ ion. The proton transfer occurs in a stepwise manner of binding, hoping and release of the H^+ ion—completed by 180° turn of the water molecules to return to the original position. [Modified from the unpublished poster by Colin Wraight]

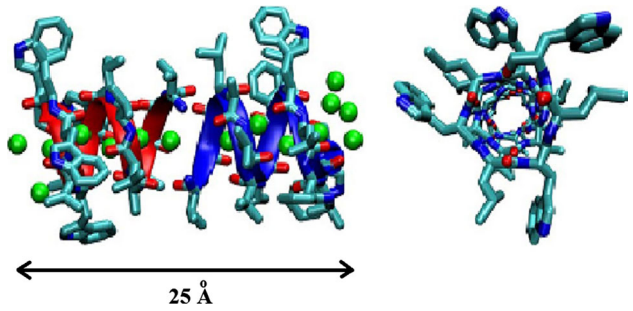

Fig. 12 Formation of a transient transmembrane channel by two gramicidin A molecules associated by hydrogen bonding. *Longitudinal* view of the head-to-head dimer with water molecules (*green*, *left*) and *view down* the central pore with no water molecules (*right*). [Adapted from Fig. 1 in Wraight (2006)]

High selectivity and throughput *can be* achieved with a number of special designs that enable high fluxes at physiological pH (very low H^+ activity), with very high specificity—proton specific gates, proton antennae, proton injectors, surface trapping, 2-D diffusion, buffer transport. The selectivity can be increased significantly by the incorporation of acid/base-type H^+ selective components or "gates," e.g., amino acids. The number of modifying elements should be limited to one or a few, so as to retain the high mobility of the water (Fig. 13).

Cytochrome c oxidase : *The protonation pathways (K and D) deliver protons consumed or transferred in the full turnover of cytochrome c oxidase:* $4e^- + 8H^+ (in) + O_2 \rightarrow 2H_2O + 4H^+$ *(out) (Fig. 14). The enzyme utilizes a long file of protonatable amino acids and mobile waters. The D-pathway is marked by an eponymous aspartic acid (D) at the entrance to the channel and ends at a highly conserved glutamic acid near the center of the membrane span. Since the mutation of either of these residues essentially shuts down proton delivery, they must act as effective and proton specific gates*. The very high selectivity is provided by one or two acid groups.

The D channel provides an example of a well-defined and highly connected PT pathway, where functional connectivity can also be achieved transiently with highly mobile water molecules. This could also provide a mechanistic component of gating or catalysis. The idea that a PT pathway of water molecules can be transiently organized at a specific place and point in time is inherent to proposed gating mechanisms of proton pumping in the central cavity of cytochrome oxidase (Wikström et al. 2005).

H^+-ATP synthase *The F_oF_1 ATP synthase conducts protons across the membrane while drawing energy from this flow to synthesize ATP (Fig. 15). The translocation occurs via proton wire [Junge 2013]. The series of conformational changes, channeled through the subunits of the F_0 subunit, drives a series of additional conformational changes in the stalk connecting the F_0 to the F_1 subunit. This process couples the translocation of protons to the mechanical motion (loose, tight and open states) of F_1 necessary to phosphorylate ADP to ATP.*

The translocation of H^+ ions is [of] very high selectivity and *yields* very high throughput. The H^+/Na^+ selectivity is greater than 10^7. Although the nature of the proton conductor is not known (is it a channel?), but specificity is provided at a single location.

The maximum proton transport rate is 6000 s^{-1} (the conductivity is 10 fS at pH 8) that requires H^+ collection from a large membrane surface area by lateral diffusion.

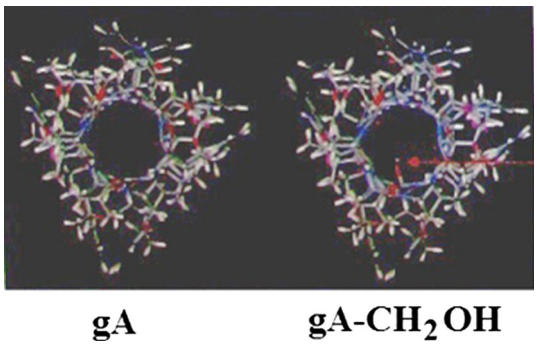

Fig. 13 Selectivity of the ionic conductance of the gramicidin A (gA) channel. The H^+/K^+ selectivity is much higher for the succinyl-gA covalent dimer (gA–CH_2–OH) than for gA. The CH_3–OH group acts as a block to H_2O diffusion but allows H^+ to pass by proton hopping (Grotthuss mechanism). [Courtesy of Samual Cukierman]

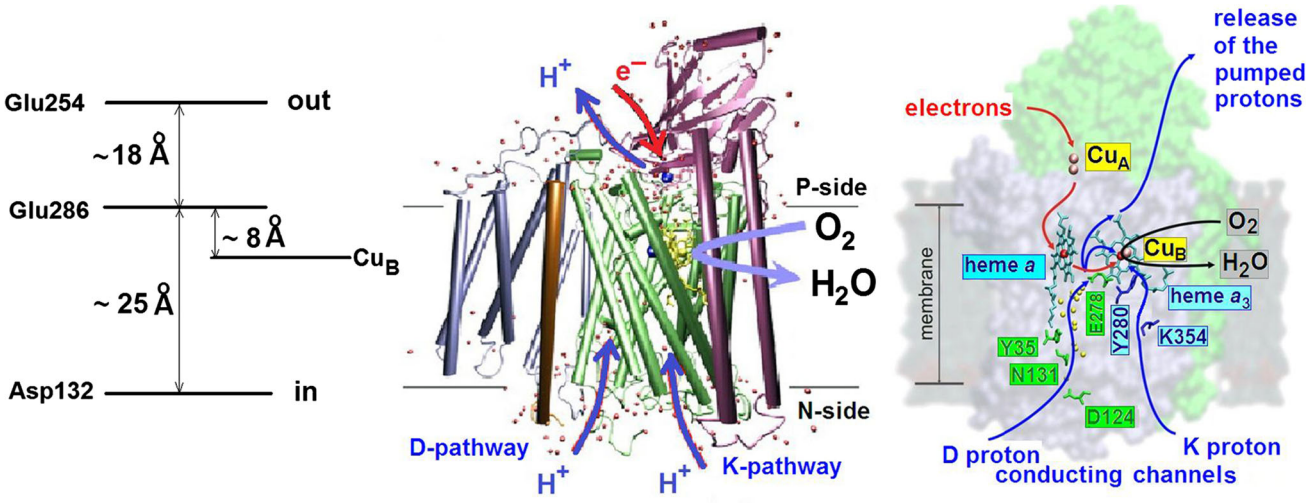

Fig. 14 Coupling of electron and proton pathways in cytochrome oxidase. The main part of the D proton channels is lined up by several polar amino acid residues and several well-defined crystallographic water molecules which effectively fill the channel up to the entrance to the central cavity between heme *a* and the binuclear center (heme a_3-Cu_B). [Modified from the unpublished poster of Colin Wraight]

The diameter of the "proton antenna" amounts [to] about 80 Å.

Photosynthetic reaction centers : *The reaction center (RC) from photosynthetic bacteria provides an excellent system for studying long distance PT, although* the H^+ uptake to the secondary quinone Q_B (>15 Å) is non-vectorial [and, thus, not electrogenic] *(Fig. 16). In RC, the transfer of electrons in the primary photochemical events generates most of the electrical component, while electron-coupled proton uptake and release accompanying the redox reactions of secondary acceptors and donors is largely responsible for the proton concentration gradient (ΔpH). The reduction of quinone (Q) to quinol (QH_2) represents the initial step of a chemiosmotic "proton pumping redox loop".*

"Fuel—Injection" *and bifurcation*: Surface histidine(s) act as local buffers and provide Hs^+ at much higher effective concentration than indicated by the bulk pH. When mutated, *cationic* acids and buffers (e.g., imidazole) bind weakly at the surface and recover this activity (*chemical rescue of proton uptake*, *Paddock et al.* 2002*; Takahashi and Wraight* 2006). Asp^{L210} and Asp^{M17} provide proton specificity in the inner path, but when mutated, small weak acids can recover activity. *The proton delivery pathway bifurcates at* Asp^{L213}*, and the two carbonyl oxygens of* Q_B *are protonated via* Glu^{L212} *and (probably)* Ser^{L223}*. These groups are the key amino acids in the proton pathway and offer the proton specificity of the deepest path.*

Key question: If soluble buffers could work as H^+ donors, why is Q_B so buried that the RC requires long distance PT and a specialized H^+ injection device (the surface His)?

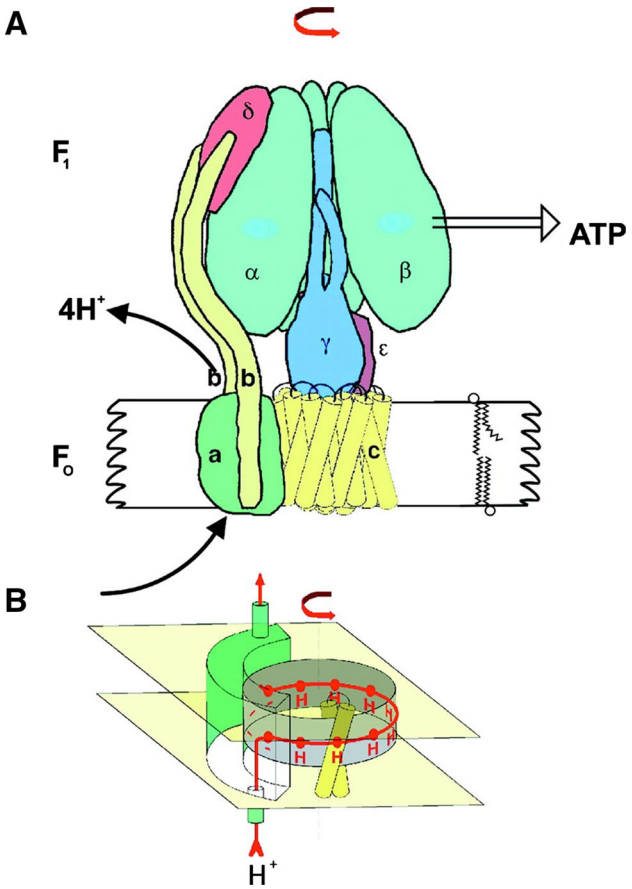

Fig. 15 Brownian ratchet mechanism of ATPase: at the expense of proton electromotive force, ATP is produced from ADP and inorganic phosphate. The H^+ ions are translocated in the membrane through the stator part of the membrane bound F_0 subunit while the other parts of the F_0 and F_1 subunits of the protein carry out stepwise rotation. [Courtesy of Wolfgang Junge]

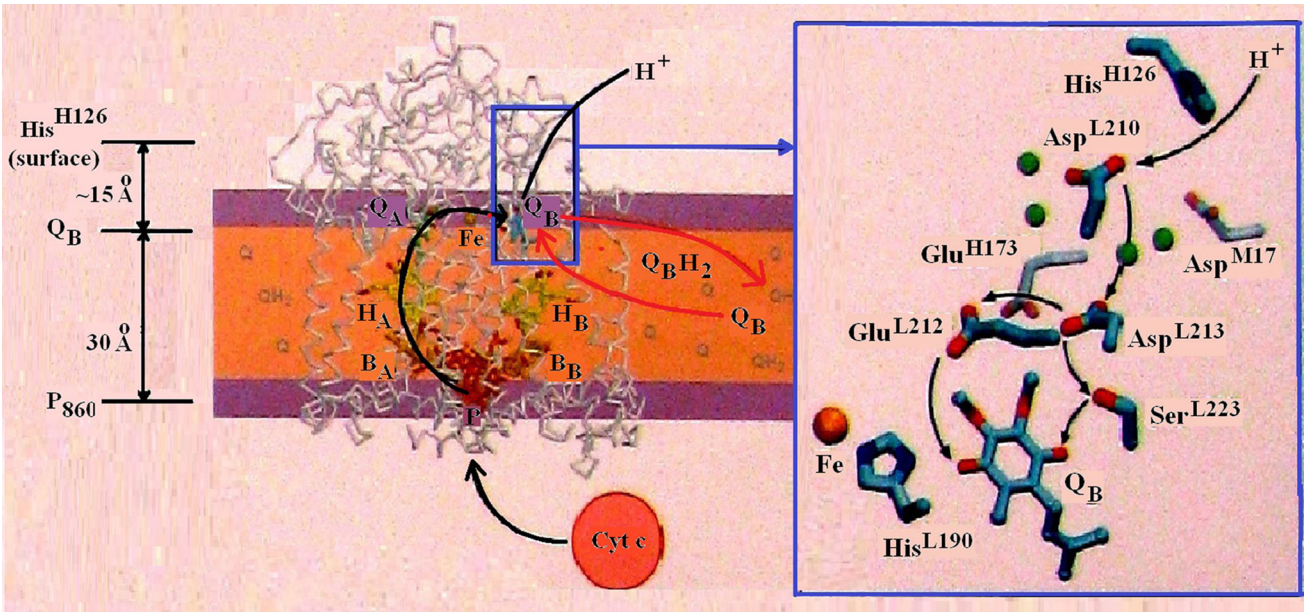

Fig. 16 Uptake of the H^+ ion from the aqueous cytoplasmic phase by reduced secondary semiquinone of the photosynthetic reaction center (RC) protein located at the interface of the photosynthetic membrane. As the RC mediated process includes the transfer of H^+ ions into but not through the membrane (in the form of QH_2 quinol), it constitutes half of a proton pump only. The proton binding is coupled to the light-induced electron transfer. The approximate positions relative to the membrane (*left*) and the key amino acids with some water molecules of the proton delivery pathway (*right*) are indicated. [Modified from the unpublished poster of Colin Wraight]

Possible answer: The redox centers involved in electron accumulation ($n \geq 2$) must be deeply buried to protect metastable intermediates from accidental reactions.

The simplest form of non-adiabatic electron transfer is [based on] the Marcus theory that defines the distance constraints of the electron transfer (ET) rate [Marcus and Sutin 1985]:

$$k_{\mathrm{ET}} = k_{\max} \cdot \exp(-\beta \cdot R) \cdot \exp\left(\frac{\Delta G^*}{k_B T}\right).$$

The ET rates are determined by the distance, R, and the free energy of reaction, ΔG^o, which modifies the activation free energy ΔG^*:

$$\Delta G^* = \frac{1}{4}\frac{(\Delta G^o + \lambda)^2}{\lambda},$$

where λ is the reorganizing energy and β is the electronic coupling constant. *An empirical equation was suggested in which the Hopfield approximation (with γ = 3.1 eV^{-1}) to the Marcus term was used to fit experimental data (Moser et al. 1992), though this has been challenged by Crofts and Rose (2007), who preferred the classical term (with γ = 4.23 eV^{-1}):*

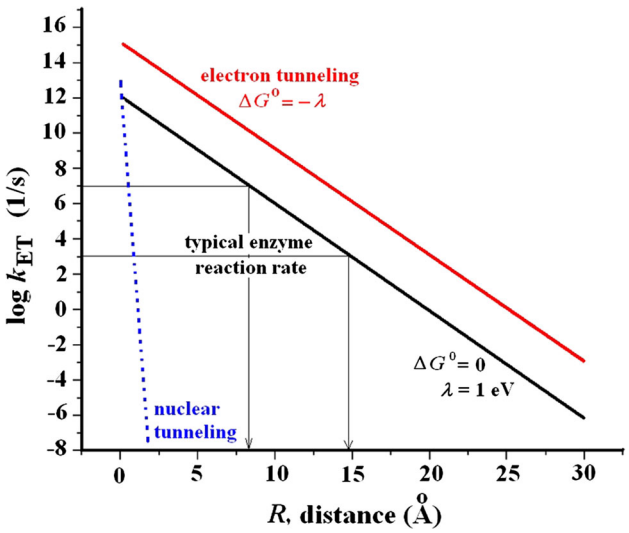

Fig. 17 Electron transfer rate (k_{ET}) versus edge-to-edge distance (R) according to the Moser–Dutton equation (see the text) in electron tunneling limit (no activation free energy, $\Delta G^* = 0$ or $\Delta G^o = -\lambda$, *red line*) and for physiologically meaningful enzymatic reaction with no free energy of reaction ($\Delta G^o = 0$) and $\lambda = 1$ eV (*black line*) together with nuclear tunneling limit (*blue line*). [Modified from the unpublished poster of Colin Wraight]

$$\log k_{\mathrm{ET}} = 13.0 - \frac{\beta}{2.303}(R - 3.6) - \gamma\frac{(\Delta G^o + \lambda)^2}{\lambda},$$

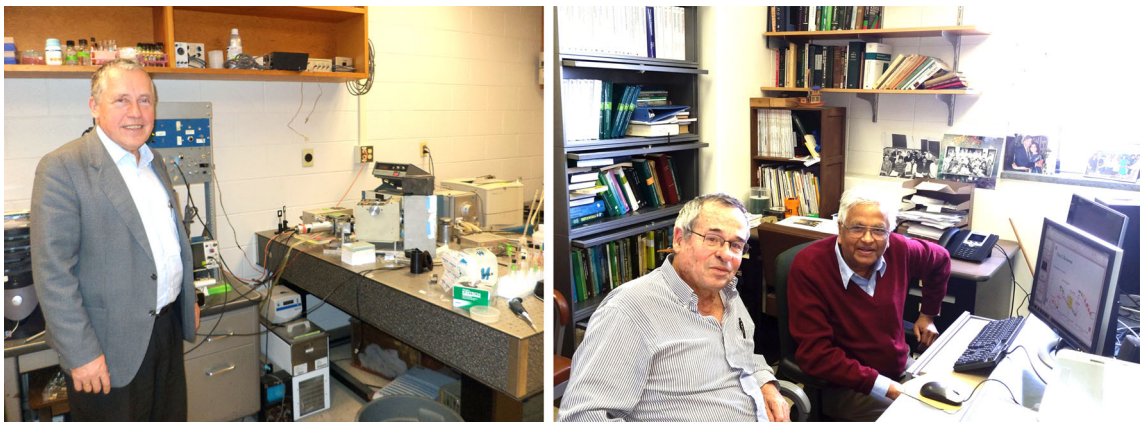

Fig. 18 Left A photograph of Péter Maróti in Colin Wraight's Laboratory at the University of Illinois at Urbana-Champaign.; this photo was taken when he was visiting the Lab sometime in 2013. **Right** A photograph of Govindjee (*right*) with Arieh Warshel, 2013 Nobel-laureate in Chemistry, while he was visiting the University of Illinois at Urbana-Champaign. Photo taken by Aditi Tiwari, 21 April 2015. Reproduced with permission

where $\beta = 1.4\ Å^{-1}$ *and* γ *as above. The plots of log* k_{ET} *vs. R (edge-to-edge) distance give a series of vertically shifted straight lines of identical slope of* $\beta/2.303$ *(Fig. 17). In electron tunneling limit, the activation free energy is zero:* $\Delta G^{*} = 0$*, i.e.* $\Delta G^{o} = -\lambda$*. Since the electron transfer is accompanied by relatively large electronic and electrostatic changes in the surroundings, the reorganization energy is on the order of 1 eV, or slightly less. Because the physiologically meaningful (enzymatic) reactions are generally faster than 1 ms (*$k_{ET} > 10^{3}\ s^{-1}$*), they occur over a distance of about 15 Å with negligible free energy of reaction (*$\Delta G^{o} \approx 0$*) (Moser et al. 2003)* (Fig. 17).

Bimolecular electron transfer: *For intermolecular ET both the binding and the dissociation between the partners are important as the rate of the turnover of the encounter complex is determined by the sum of the rates of association (*k_{on}*) and dissociation (*k_{off}*).*

For the right reaction partners, the binding is quite tight, and the dissociation constant ($K_d = k_{off}/k_{on}$) is commonly on the order of $K_d \approx 1\mu M$. For a diffusion limited reaction between medium sized, soluble proteins (50 kD, 50 Å diameter), the bimolecular rate constant is $k_{on} \approx 1{\cdot}10^{9}$ - $M^{-1}{\cdot}s^{-1}$. Thus, the dissociation rate constant is $k_{off} \approx 10^{3}$ s^{-1}, and the lifetime of the encounter complex is about 1 ms. For $\lambda \geq 1$ eV and $\Delta G^{0} \approx 0$ it allows ET up to 15 Å. This is long enough to occur over a distance of about 15 Å (Fig. 17).

Shorter complex lifetimes, however, can service reactions at shorter distances: For the *wrong* reaction partners, the binding is weak, $K_d \geq$ 10mM and $k_{off} \geq 10^{7}\ s^{-1}$ (the lifetime is about 0.1 μs) which limits the ET to $R < 9$ Å (for $\lambda \geq 1$ eV and $\Delta G^{0} \approx 0$). That might represent an undesirable reaction where a small redox-active molecule binds non-specifically to the protein with low affinity ($K_d \approx$ 10-100 mM). To limit the chance of the redox reaction during the lifetime of the complex ($\approx$ 0.1 μs), the distance to the protein cofactor should be > 8 Å).

Two statements can be drawn:

1. *Binding* controls the bimolecular ET *via* specificity.
2. The protection of redox centers from parasitic reactions imposes a requirement for long range PT. *The exchange with one-electron centers is relatively at lower risk, as the reactions are more freely reversible. For charge accumulating reactions, however, in which one-electron transfers communicate with n-electron centers, e.g. quinone (n = 2), with metastable intermediate states, such parasitic reactions are potentially highly deleterious. For example, the semiquinone lifetime in bacterial RC is tens of minutes in the dark, which makes it vulnerable to very rare redox events and very feeble reagents.*

Conclusions/speculations

(1) Biological proton transport is fast—often much faster than needed.
(2) The structural solutions are *unique* pathways—it's hard to bury charge.
(3) Rotationally mobile water is a major ingredient and is resistant to mutations = evolutionarily robust.
(4) Any path that does conduct H^{+} is likely to do it fast.
(5) A functional path is highly robust to mutations *around* it—precise pK_a values are not very critical—PT is fast over a wide range of ΔpK_a.
(6) Susceptible redox cofactors (with metastable redox states) are buried to protect them from non-specific ET reactions—necessitating long distance PT from the surface.
(7) Natural design encompasses a very broad agenda.

This ends the poster by Colin Wraight.

In the spirit of history of photosynthesis research, we end the entire Tribute to Colin Wraight by showing photographs of the authors (Fig. 18, left, of Péter Maróti; and right of Govindjee, with the 2013 Nobel laureate Arieh Warshel).

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References

Ädelroth P, Paddock ML, Tehrani A, Beatty JT, Feher G, Okamura MY (2001) Identification of the proton pathway in bacterial reaction centers: decrease of proton transfer rate by mutation of surface histidines at H126 and H128 and chemical rescue by imidazole identifies the initial proton donors. Biochemistry 40:14538–14546

Berg JM, Tymoczko JL, Stryer L (2002) Chapter 18.4: a proton gradient powers the synthesis of ATP. In: Biochemistry, 5th edn. W. H. Freeman and Company, New York

Crofts AR, Rose S (2007) Marcus treatment of endergonic reactions: a commentary. Biochim Biophys Acta 1767:1228–1232

Govindjee, Prince RC, Ort DR (2015) Colin A. Wraight (1945–2014). Photosynth Res :20. doi:10.1007/s11120-015-0174-1

Junge W (2013) Half a century of molecular bioenergetics. Biochem Soc Trans 41:1207–1218

Kis M, Asztalos E, Sipka G, Maróti P (2014) Assembly of photosynthetic apparatus in *Rhodobacter sphaeroides* as revealed by functional assessments at different growth phases and in synchronized and greening cells. Photosynth Res 122:261–273

Kochendoerfer GG, Lin SW, Sakmar TP, Mathies RA (1999) How color visual pigments are tuned? Trends Biochem Sci 24:300–305

Marcus RA, Sutin N (1985) Electron transfers in chemistry and biology. Biochim Biophys Acta 811:265–322

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:141–148

Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Glynn Research Ltd., Research Report No. 66/1. Corwell, Bodmin; also see Biochim Biophys Acta 1807:1507–1538

Mitchell P (1979) Keilin's respiratory chain concept and its chemiosmotic consequences. Science 206:1148–1159

Moser CC, Keske JM, Warncke K, Farid RS, Dutton PL (1992) Nature of biological electron transfer. Nature 355:796–802

Moser CC, Page CC, Cogdell RJ, Barber J, Wraight CA, Dutton PL (2003) Length, time and energy scales of photosystems. Adv Protein Chem 63:71–109

Nagle JF, Morowitz HJ (1978) Molecular mechanisms for proton transport in membranes. Proc Natl Acad Sci USA 75:298–302

Nagle JF, Tristram-Nagle S (1983) Hydrogen-bonded chain mechanisms for proton conduction and proton pumping. J Membr Biol 74:1–14

Onsager L (1967) Ion passages in lipid bilayers. Science 156:541–543

Onsager L (1969) The motion of ions: principles and concepts. Science 166:1359–1364

Paddock ML, Ädelroth P, Feher G, Okamura MY, Beatty JT (2002) Determination of proton transfer rates by chemical rescue: application to bacterial reaction centers. Biochemistry 41:14716–14725

Paddock ML, Sagle L, Tehrani A, Beatty JT, Feher G, Okamura MY (2003) Mechanism of proton transfer inhibition by $Cd^{(2+)}$ binding to bacterial reaction centers: determination of the p*K*(A) of functionally important histidine residues. Biochemistry 42:9626–9632

Takahashi E, Wraight CA (1992) Proton and electron transfer in the acceptor quinone complex of *Rhodobacter sphaeroides* reaction centers: characterization of site-directed mutants of the two ionizable residues, GluL212 and AspL213, in the Q_B binding site. Biochemistry 31:855–866

Takahashi E, Wraight CA (1996) Potentiation of proton transfer function by electrostatic interactions in photosynthetic reaction centers from *Rhodobacter sphaeroides*: first results from site-directed mutation of the H subunit. Proc Natl Acad Sci USA 93:2640–2645

Takahashi E, Wraight CA (2006) Small weak acids reactivate proton transfer in reaction centers from *Rhodobacter sphaeroides* mutated at AspL210 and AspM17. J Biol Chem 281:4413–4422

Wikström M, Ribacka C, Molin M, Laakkonen L, Verkhovsky M, Puustinen A (2005) Gating of proton and water transfer in the respiratory enzyme cytochrome *c* oxidase. Proc Natl Acad Sci USA 102:10478–10481

Wraight CA (2004) Proton and electron transfer in the acceptor quinone complex of photosynthetic reaction centers from *Rhodobacter sphaeroides*. Front Biosci 9:309–337

Wraight CA (2005) Chapter 12: intraprotein proton transfer—concepts and realities from the bacterial photosynthetic reaction center. In: Wïkstrom M (ed) Biophysical and structural aspects of bioenergetics. RSC biomolecular science series. Royal Society of Chemistry, Cambridge

Wraight CA (2006) Chance and design—proton transfer in water, channels and bioenergetic proteins. Biochim Biophys Acta 1757:886–912

Wraight CA, Gunner MR (2009) The acceptor quinones of purple photosynthetic bacteria—structure and spectroscopy. In: Hunter CN, Daldal F, Thurnauer M, Beatty JT (eds) Advances in photosynthesis and respiration: the purple phototrophic bacteria. Springer, Dordrecht, pp 379–405