



Protonic Capacitor Cell Energetics: Serious Problems to Consider

Todd P Silverstein**Chemistry Department (Emeritus), Willamette University, Salem, OR 97301, USA*

***Corresponding Author:** Todd P Silverstein, Chemistry Department (Emeritus), Willamette University, Salem, OR 97301, USA.

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Abstract

In a recent Landmark Review (Amer. J. Physiol.-Cell Physiol. (2025) 329: C1944-1984), James Lee described his body of published work employing the transmembrane-electrostatically localized protons/cations charges (TELPs/TELCs) hypothesis. In addition to enumerating the usefulness of his model in explaining basic biological processes like oxidative phosphorylation and neurotransmission, and in re-interpreting experimental results reported by others, Lee strove to express and support his conclusion that “the putative potential well/barrier model is not needed to explain... protonic bioenergetics”. In this paper I critique this unwarranted conclusion, and also enumerate other serious problems with the equations, calculations, conclusions, and presentation of the scientific literature found in Lee’s Landmark Review.

Keywords: Capacitor; Protons

Introduction

Recently, James Lee published a Landmark Review in this journal [1], describing his body of published work employing his transmembrane-electrostatically localized protons/cations charges (TELPs/TELCs) hypothesis. In addition to enumerating the usefulness of his model in explaining basic biological processes like oxidative phosphorylation and neurotransmission, and in re-interpreting experimental results reported by others, Lee strove to express and support his conclusion that “the putative potential well/barrier model is not needed to explain... protonic bioenergetics” [1]. In this Introduction I will critique this unwarranted conclusion. Afterward, I will enumerate other serious problems with the equations, calculations, conclusions, and presentation of the scientific literature found in Lee’s review.

In 1961, both Peter Mitchell [2] and RJP Williams [3] published landmark papers in which they identified the importance of proton electrochemistry in linking spontaneous substrate oxidation to nonspontaneous ATP synthesis in oxidative phosphorylation and chloroplast photophosphorylation. In Mitchell’s “chemiosmotic” hypothesis, redox-driven proton pumping released excess protons into the bulk aqueous phase on the positive (P) side of the bioenergetic membrane; these protons then spontaneously flowed through the ATP synthase, from the P-side back to the N (negative)-side, driving ATP synthesis. Mitchell’s chemiosmotic hypothesis, which was easily testable, accrued much experimental support over the next decade and a half, resulting in his Nobel Prize award in 1978. In Williams’s hypothesis, key proton transfer steps occurred either within the nonpolar interior core of the bioenergetic membrane, or at the membrane surface. Because intramembrane

and membrane-surface probes were not available at the time, William's hypothesis languished in the 1960s and 1970s, and he was not chosen to share Mitchell's Nobel Prize.

One of the strong points of Mitchell's hypothesis was that he derived a thermodynamic equation allowing calculation of the transmembrane proton electrochemical potential, $\Delta\mu_{H^+}$. (Equation 1)

$$\text{Equation 1: } \Delta\mu_{H^+} = F\Delta\psi + RT\ln\left(\frac{[H^+]_{P,bulk}}{[H^+]_{N,bulk}}\right)$$

F is the Faraday constant (kJ/mol/V), R is the gas law constant (kJ/mol/K), $\Delta\psi$ is the transmembrane electrical potential, in V, and the proton concentrations in the second term are measured in the bulk aqueous phase on the P- and N-sides of the membrane. $\Delta\mu_{H^+}$, the transmembrane proton electrochemical potential, is expressed in units of kJ/mol (or kcal/mol).

The vectorial proton transport reaction envisioned in Equation 1 is stipulated from the N-side to the P-side, and $\Delta\psi$, then measured relative to the N-side, i.e. $\Delta\psi = \psi_p - \psi_N$, is positive in sign; furthermore, because proton concentration is higher on the P-side, $\Delta\mu_{H^+}$ must be positive. Thus, redox-driven proton pumping from the N- to P-side is nonspontaneous, while proton flow in the opposite direction (through the ATP synthase) is spontaneous. Mitchell's Equation 1 afforded a quantitative thermodynamic calculation of the driving force for ATP synthesis.

The electrochemical potential difference in Equation 1 can also be expressed in units of volts, as the protonmotive force, pmf:

$$\text{Equation 2: } \frac{\Delta\mu_{H^+}}{F} = \text{pmf} = \Delta\psi + (RT/F)\ln\left(\frac{[H^+]_{P,bulk}}{[H^+]_{N,bulk}}\right) = \Delta\psi - 2.3(RT/F)\Delta\text{pH}$$

$$\text{where } \Delta\text{pH} = \text{pH}_{P,bulk} - \text{pH}_{N,bulk}$$

Regarding the two "sources" of free energy in Equation 1 and Equation 2, $\Delta\psi$ and ΔpH , Lee wrote about belief

"in a notion [that] $\Delta\psi$ and ΔpH are independent thermodynamic parameters. This notion is inaccurate since it vaguely treats $\Delta\psi$ as an independent parameter and does not explain what really makes the voltage of the transmembrane potential, $\Delta\psi$ " [1].

Lee's first criticism is correct: $\Delta\psi$ and ΔpH are not in fact independent thermodynamic parameters. As noted by Lee and

many others, due to the low capacitance of the nonpolar membrane interior (with low permittivity), electrogenic proton pumping yields a significant $\Delta\psi$ after the pumping of only a small number of protons. Thus, it is not surprising that mitochondria feature large $\Delta\psi$ and a small ΔpH . On the other hand, chloroplasts feature the opposite: a small $\Delta\psi$ and a large ΔpH . Mitchell and many others explained this by recognizing the effect of electrogenic transport of other ions (e.g., K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , $H_2PO_4^-$, etc.), which served to decrease $\Delta\psi$ while building up ΔpH [4-9]. Thus, $\Delta\psi$ and ΔpH are indeed interrelated: electrogenic proton pumping alone causes a positive $\Delta\psi$ (P relative to N) and a small negative ΔpH ; added electrogenic counterion transport serves to decrease $\Delta\psi$ and make ΔpH more negative. From these processes we see that Mitchell and others certainly did explain "what really makes the voltage of the transmembrane potential, $\Delta\psi$," so Lee's second criticism is unfounded.

Lee remarked later in his Landmark Review that "Perry, *et al.* [10] have wonderfully provided direct experimental evidence that $\Delta\psi$ does not always mirror changes in mitochondrial [bulk-liquid phase] pH, which is also expected by the TELPs model that shows $\Delta\psi$ as a function of the TELCs density but not the bulk-liquid phase pH". What Perry, *et al.* actually reported was that "changes in $\Delta\psi$ may often but do not necessarily follow changes in ΔpH " [10]. In their Box 2, they described studies on mitochondria in stressed rodent cortical neurons, in which $\Delta\psi$ across the mitochondrial inner membrane was larger (hyperpolarized) while ΔpH was smaller than mitochondria in control neurons. However, they determined that these changes were due to "dumping of Ca^{2+}_{mito} [i.e., from the mitochondrial matrix] ... into the cytoplasm" [10]. Thus, Perry concluded that electrogenic Ca^{2+} transport from the mitochondrial matrix (N side) into the intermembrane space (P side, and then into the cytoplasm) caused an increase in $\Delta\psi$ (hyperpolarization) by adding positive Ca ions to the P side. Furthermore, if this Ca^{2+} transport occurred via a Ca^{2+}/H^+ antiporter, then this would also account for the decrease in ΔpH (less negative). In other words, the results from Perry, *et al.* are well-explained by Mitchell's chemiosmotic theory.

By the late 1970s, problems with Mitchell's chemiosmotic theory arose. Primarily, examples of ATP synthesis in the absence of sufficient $\Delta\mu_{H^+}$ driving force began appearing in the bioenergetic literature [11-13]. In response to these difficulties, Mitchell's theory

was modified to distinguish between $\Delta\mu_{H^+}$ measured between the two bulk aqueous phases (the “delocalized” or “classical” Mitchellian chemiosmotic theory), and $\Delta\mu_{H^+}$ measured between the surfaces of the two sides of the membrane (localized chemiosmotic theory). Note that the notion of localized chemiosmosis, distinguishing between the bulk aqueous and membrane surface phases, arose from experimental measurements that challenged the prevailing “classical” chemiosmotic theory.

In the literature, three hypotheses have been proposed to explain localized chemiosmosis, i.e., the significantly higher proton concentration in the P surface phase relative to the adjacent bulk phase: (i) The binding of surface protons to negative surface charge, especially anionic lipid head groups; (ii) Lee’s TELP protonic biocapacitor; and (iii) the existence of a potential well and/or kinetic barrier between the two phases, as proposed by Junge/Mulkidjanian/Cherepanov. As noted by Lee [1] including both delocalized and localized protonic coupling. According to the TELCs model, the excess positive charges of TELCs at one side of the membrane are balanced by the excess negative charges of transmembrane-electrostatically localized hydroxide anions (TELAs and others [14], there is a fair amount of evidence that localized chemiosmosis exists in the absence of negative surface charge, thus hypothesis (i) is not supported. This leaves two seemingly valid hypotheses; because these hypotheses make different predictions as to the source of localized chemiosmosis, it should be possible to devise experiments that allow researchers to choose one over the other.

A detailed presentation of the TELP and potential well/barrier hypotheses is beyond the scope of this review, but here we will present a brief summary. The TELP hypothesis [1], posits that electrogenic proton pumping establishes a transmembrane potential across the mitochondrial inner membrane. This in turn sets up a “protonic capacitor,” governed by Gauss’s law and featuring excess protons electrostatically localized at the P-side membrane surface, along with excess hydroxide anions at the opposite surface. A schematic diagram is presented in Figure 1.

Using Gauss’s law and electrostatics equations, and defining a specific thickness for the interfacial surface layer containing the localized excess ions, Lee devised equations allowing him to predict a supplementary that adds to the delocalized from

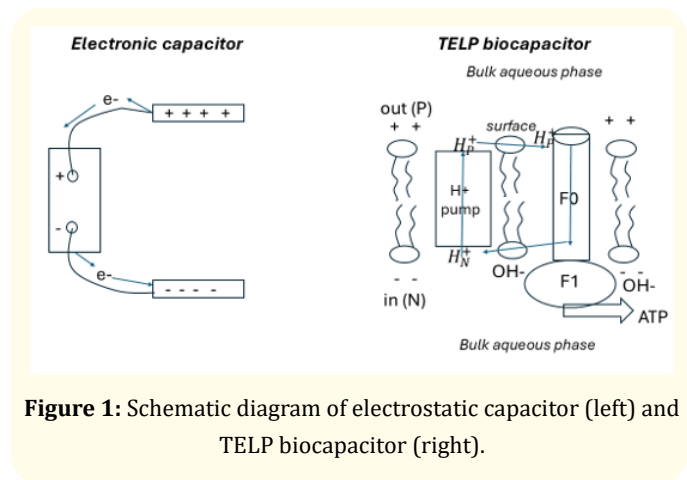


Figure 1: Schematic diagram of electrostatic capacitor (left) and TELP biocapacitor (right).

Equation 1 to drive ATP synthesis. To date, experiments employing biological membranes have not been able to corroborate these TELP predictions [15-17].

In their potential well/barrier model, Cherepanov, Mulkidjanian and Junge proposed the existence of a kinetic barrier that retards the release of protons released at the P-surface of the membrane [18-23]. Most of these surface protons flow back across the membrane through the F_1F_0 -ATP synthase before they can scale the kinetic barrier and be released into the bulk aqueous phase. This sets up a steady state disequilibrium in which the P-surface proton concentration is higher than in the bulk phase. This model envisions P surface protons sitting in a potential well, separated from the bulk phase protons by a kinetic barrier that retards mixing.

In his Landmark Review, Lee voiced two major criticisms of the potential well/kinetic barrier hypothesis: (i) that it disallows any exchange of protons between the bulk and surface phases; and (ii) that it is not “necessary”. Criticism (i) is based on Lee’s lack of understanding that experiments support the existence of a finite energy barrier, which merely slows down a reaction, rather than an infinite barrier, which disallows the reaction entirely.

Regarding criticism (ii), several times in his Landmark Review Lee claimed to “explain why the putative potential well/barrier model is not needed to explain the TELPs formation and protonic bioenergetics” [1]. including both delocalized and localized protonic coupling. According to the TELCs model, the excess

positive charges of TELCs at one side of the membrane are balanced by the excess negative charges of transmembrane-electrostatically localized hydroxide anions (TELAsFirst, the potential well/barrier hypothesis explains localized chemiosmosis, not “the TELPs formation”. Thus, one would not expect the potential well/barrier model to have any relationship to “the formation of TELPs”.

Second, throughout his Landmark Review, as well as in all of his other TELP papers, Lee assumes that his TELP protonic biocapacitor actually exists, even in the absence of any biologically relevant experimental support. Thus, he states that the potential well/barrier model “is not needed” because his TELP hypothesis already explains localized chemiosmosis. But that is not how the scientific method works! Just because you have devised a hypothesis that explains the data does not mean that other hypotheses are “not needed”.

An example of this type of single-hypothesis bias can be found in Lee’s presentation of the usefulness of his model: “The TELP [hypothesis]... has already been useful in elucidating real-world bioenergetic systems... [including] ATP synthesis in alkalophilic bacteria... [and] bioenergetics in mitochondria...” [1]. But recall that it is localized chemiosmosis that is the key to “elucidating” these important bioenergetic systems, and TELPs is just one of two seemingly valid hypotheses that explain localized chemiosmosis.

Lastly, throughout his Landmark Review Lee mistakes evidence and statements supporting the existence of localized chemiosmosis with support for his TELP hypothesis. In other words, he assumes that his hypothesis is the only valid one, ignoring (or denigrating) the potential well/barrier model, which itself has garnered significant experimental support [15]. For example, Lee cited a critic’s statement “the significance of the localized protonmotive force is now widely appreciated,” opining that this statement “seems to be somewhat in line with the TELPs model”. Later in his review Lee discussed results supporting localized chemiosmosis in melibiose transporter (melB) function. He quoted the conclusion of Hariharan., *et al.*: “The data support a recent novel TELPs theory over Peter Mitchell’s [delocalized] chemiosmosis theory” [24]. But the Hariharan., *et al.* data support the existence of localized chemiosmosis; they do not allow one to choose between the two hypotheses that explain this model. Here and elsewhere, Lee has conflated his hypothesis with the experimentally-supported localized chemiosmosis model. As noted above, in order to favor one hypothesis over another, one must determine predictions that would differ between the two hypotheses, and then devise experiments to test these predictions. Neither Lee nor any

other researchers have (yet?) published the results of any such experiments.

Results and Discussion

Problems with the theory underlying the TELP hypothesis

Electronic vs. protonic capacitor

Regarding the theory underlying TELP, Lee wrote that “the model was developed with the application of Gauss’s law based on the understanding of liquid water as a protonic conductor” [1]. In other words, Lee has drawn a direct analogy between an electrical circuit featuring a capacitor charged with electrons vs. bioenergetic membranes energized by proton pumping. These two systems are depicted side-by-side in Figure 1.

Lee relies on the analogy between these two systems, one of which is macroscopic and the other nanoscopic. However, besides this obvious size difference, one must also consider the distinctions between an electronic capacitor featuring charged electrons moving within a solid metallic conductor vs. a protonic biocapacitor featuring charged hydrated protons moving within a liquid water conductor. Some of the implications of these distinctions are listed in Table 1. Note that in the TELP protonic biocapacitor, relative to the electronic capacitor, charges are a billion times larger and move a thousand times more slowly in a medium whose conductivity is ten million times lower.

	Electrostatic capacitor	TELP Bio-capacitor
Moving charge:	electron	H ⁺ (aq)
Size of moving charge:	< 10 ⁻¹⁸ m	≈ 0.6 nm; (10 ⁹ -fold larger)
Stationary excess charge:	“dry” electron	H ⁺ hexa-aquo complex with ionic double layer
Subject to Brownian motion?	no	yes
Conductive medium:	Solid metal	Liquid water
Charge velocity* (m/s):	10 ⁶	≈800; (10 ³ -fold slower)
Medium conductivity† (S/m):	(1 – 6) 10 ⁷	1 – 6; (10 ⁷ -fold less)

Table 1: Differences between electrostatic capacitor and TELP biocapacitor circuits.

* random thermal velocity; † range for various metals, and pure water to seawater.

But perhaps most importantly, interface-localized protons in the TELP model are subject to Brownian motion and the lower entropy of protons in this high-concentration phase relative to the adjacent bulk phase. Such is not the case for electrons in a capacitor. This brings up another important distinction between the two capacitors: The equivalent of the bulk aqueous phase in the TELP protonic biocapacitor is the power supply (or battery) in the electronic capacitor. Because the power supply drives electrons onto the negative capacitor plate, electrons experience no back-force away from the plate. On the other hand, the combination of Brownian motion and entropy provide a back-force driving protons localized at the interface of the TELP protonic biocapacitor into the adjacent bulk aqueous phase, where the proton concentration is lower.

Problems with the TELP thermodynamic equation

Lee has modified Mitchell's pmf equation (Equation 2) to account for an electrostatically-localized proton layer (L) at the P surface of the membrane, i.e., P,L. (We note here that he also posits a similar hydroxide layer at the N surface (N,L), but for reasons discussed below, this layer does not appear in his modified equation.)

$$\text{Equation 3: pmf} = \Delta\psi + (RT/F)\ln\left(\frac{[H^+]_{P,L} + [H^+]_{P,bulk}}{[H^+]_{N,bulk}}\right)$$

Note that the pmf in Equation 3 applies to proton translocation from the N-side bulk phase to the P-side L phase. If we multiply the fractional log term by $[H^+]_{P,bulk}/[H^+]_{P,bulk}$ we get

$$\text{Equation 4: pmf} = \Delta\psi + (RT/F)\ln\left(\frac{[H^+]_{P,bulk}}{[H^+]_{N,bulk}}\right) + (RT/F)\ln\left(\frac{[H^+]_{P,L} + [H^+]_{P,bulk}}{[H^+]_{P,bulk}}\right)$$

The first two terms on the right side of the equation are the Mitchellian bulk-to-bulk pmf (Equation 2), while the last term represents the added force due to the electrostatically localized proton layer (a.k.a. the "local pmf").

We can use Equation 4 to calculate pmf for a low-potential mitochondrion, $\Delta\psi = 120$ mV, given pH = 7.4 (N, bulk) vs. 7.3 (P, bulk), and $\text{pH}_{P,L} = 1.9$ (calculated from Lee's TELP capacitance equations). The Mitchellian pmf is only 126 mV, which is just short of the 130 ± 8 mV required to drive ATP synthesis [16]; on the other hand, the "local pmf" is 459 mV, or 78% of the total (585 mV). Thus, Lee's TELP equations explain how a mitochondrion with insufficient Mitchellian pmf can nonetheless drive ATP synthesis when local pmf is added in.

There are, however, three important flaws in Lee's pmf equations above. First, the equations assume that protons flow through F_0 (the proton channel component of the F_1F_0 ATP synthase) from the P-side L layer into the N-side bulk layer. There is currently no evidence regarding the location of the F_0 proton outlet site on the N-side; if proton release turns out to be into Lee's putative localized OH^- layer instead, then the proton concentration difference ends up being astoundingly high [17]: $\Delta\text{pH}_L = 12.27 + 2 \cdot \log(\Delta\psi)$. In fact, this is likely to be an underestimate, as it uses the K_w value for bulk phase water, 10^{-14} ; this value is likely to be smaller at the membrane surface, where water is structured differently and is less polar (dielectric constant $\approx 10 - 30$). Lee dubbed this carefully derived [17] ΔpH_L equation "overly simplified," but neglected to specify any flaws in the derivation.

Second, Lee has assumed that protons in the P-side electrostatically localized L layer can reach the F_0 proton inlet site that protrudes 1 nm from the nonpolar membrane interface. As discussed in the next section, the localization of the P,L protons to a layer only 0.3 nm from the nonpolar membrane interface should preclude this. Thus, Lee has assumed in Equation 3 and Equation 4 that protons enter F_0 from the P,L phase and exit into the N,bulk phase. The former assumption is almost certainly wrong, and there is currently no evidence to support the latter assumption.

Lastly, Lee has ignored proton activity coefficients (γ_{H^+}) in his equations. We show in the Supporting Information (section I) that in the bulk aqueous phases, $\gamma_{H^+} \approx 1$. However, in the L layer, γ_{H^+} is likely to be quite different from 1, both because the TELP-predicted proton concentration exceeds 0.01 M, and because the structure and polarity of water differ significantly from the bulk phase [19,44]. Thus, γ_{H^+} in the P,L phase is unknown and Equation 3 and Equation 4 cannot be used to calculate accurate pmf values in the L phase.

Problems with membrane topology of the TELP protonic biocapacitor

In his Figure 2, Lee presented a schematic diagram of a phosphatidyl choline lipid bilayer that he adapted slightly from the Wikimedia Commons website (which is not peer-reviewed) [25]. This diagram depicts a bilayer whose nonpolar interior core is 2.5 – 3.5 nm thick, and whose headgroup region is split into two distinct regions, a 0.3 nm thick "intermediate" region at the

nonpolar interface that is partially hydrated, and the remainder of the headgroup region (0.7 – 1.0 nm thick) that is fully hydrated. Regarding the 0.3 nm partial hydration region (corresponding to a single monolayer of water, whose diameter is 0.28 nm), Lee opined that “the TELP [H⁺] are located likely within the 0.3 nm ‘intermediate’ region with partial hydration...” as are the TELA (OH⁻) on the other side of the membrane.

In his Figure 2 Lee did not cite any literature supporting this diagram, but in a previous paper [26] he cited Nagle and Tristram-Nagle’s (2000) BBA paper [27]. But in their Figure 2C, they only showed that hydration is higher in the area from the center of the headgroup region out to the bulk phase (6 – 35 H₂O/PL), compared to the interior headgroup region stretching from the center to the nonpolar interface surface (2 – 11 H₂O/PL); this “intermediate hydration region” appeared to be about 0.5 nm thick. However, there is no evidence in their Figure 2C of a clear boundary between the two regions. Instead, water hydration declined smoothly from the negatively charged phosphate group at the center of the headgroup region, down to the surface of the nonpolar phase.

A more detailed presentation of the literature on bilayer membrane hydration can be found in the Supporting Information, section III. Summarizing here: lipid hydration decreases smoothly

from the water/headgroup interface to the headgroup/fatty acid interface. Furthermore, water penetrates into two regions of the nonpolar interior core of the bilayer: 0.3 nm below the headgroup/fatty acid interface, and also into the central region, between the two lipid leaflets of the bilayer. Finally, dielectric constant also decreases smoothly from the bulk aqueous phase ($\epsilon = 78$) down to the headgroup/fatty acid interface ($\epsilon = 10 - 30$; see Figure S1 for a schematic diagram). This analysis allows us to identify six regions of the lipid bilayer (Table 2, Figure S1) which feature different hydration levels, and water layers that feature different dielectric constants and most likely different physical structures and properties (e.g., proton diffusion rates). Three water layers of particular interest are: the two water monolayers that lie between the outer edge of the headgroup and the bulk aqueous phase (HG surface layer 1 and 2 in Table 2 - these are often referred to as the “surface” layer); and the water monolayer at the headgroup/fatty acid interface (HG layer 1 in Table 2 - this is where Lee posits his TELP proton layer to lie). One important conclusion that we can draw from the bilayer hydration literature is that Lee’s invocation of a distinct headgroup partial hydration layer to support the localization of TELP protons (and TELA hydroxides) is not supported by the literature.

Type of water*	Distance (nm)**	Dielectric constant	# H ₂ O/PL
Bulk	> 1.35	78	-----
HG surface layer 2	1.05 – 1.35	66 – 78	50 – 70
HG surface layer 1	0.75 – 1.05	55 – 66	50 – 70
HG layer 2	0.3 – 0.75	30 – 55	11 – 35
HG layer 1	0 – 0.3	10 – 30	2 – 6
Sub-HG layer	-0.3 to 0	2 – 10	0 – 2
Interleaflet layer	-1.65 to -1.35	40	0 – 1

Table 2: Layer locations and physical properties of types of water associated with a DOPC lipid bilayer membrane. Data are collected from these references: [28-32].

* key: HG surface layer 1 is the water monolayer in direct contact with the choline edge of the DOPC lipid headgroup (HG); HG surface layer 2 is the water monolayer between the bulk water and HG surface layer 1; HG layer 1 is the water monolayer in direct contact with the fatty acid nonpolar interface; HG layer 2 is the water monolayer between HG layer 1 and HG surface layer 1; sub-HG layer is the water monolayer within the fatty acid nonpolar region that is closest to the HG layer; the interleaflet layer lies in the center of the bilayer, at the methyl ends of the fatty acid chains in each leaflet.

** Distance from the HG/fatty acid interface: Positive distances are up toward bulk water, while negative distances are below the interface into the nonpolar interior.

This conclusion is important because later in his paper, Lee wrote that “the protonic inlet of the ATP synthase (Complex V) located rightly [sic] at the TELPs layer in flush [sic] with the membrane surface, enables the effective utilization of TELPs...” [1]. The topology is depicted in Lee’s Figure 7, which was first published by Dudkina, *et al.* [33]. This figure shows the F_0 proton inlet protruding about 1 nm from the surface of the P side of the mitochondrial inner membrane, which matches recent published structures of F_0 : ref. [34]- Figure 4; ref. [35] - Figure 5; ref. [36] - Figure 5. Because Lee stated that his putative TELP layer rests within 0.3 nm of the edge of the nonpolar inner core of the membrane, TELP protons localized within this region are not free to enter the F_0 proton inlet located 1 nm away from the membrane surface. On the other hand, if TELP protons are not strictly localized within this 0.3 nm layer, then what does “localized” mean? If they are free to drift out of this layer, then where is the line separating the electrostatically-localized L layer, and the adjacent bulk phase?

Problems with the stability of the TELP protonic biocapacitor (and Table 1)

Concerning the stability of the putative TELP protonic biocapacitor, Lee wrote: “In a TELPs-membrane-TELA capacitor, the transmembrane attractive force is likely to be stronger than that of a single charge-pair calculation, since a [TELP H^+] may interact with multiple [TELA hydroxide] anions across the membrane”. To calculate the full attractive force, Lee then proposed his Equation (5):

$$F = \frac{qq'}{(4\pi\epsilon_0)d^2} + \gamma \sum_{i=1}^n \frac{qq'}{(4\pi\epsilon_0)((ib)^2 + (d^2))} \sin(a_i)$$

Lee’s equation (5)

The first term in this equation accounts for the Coulombic attractive force between the central charge pair, and the second term sums the adjacent pair attractions. Lee presented no derivation of this equation, nor did he cite any reference supporting it. Regarding the parameter gamma in the second term, he stated that it is a “dimensional factor: = 2 for a single 2-dimensional cross section system; and may be about 8 (or larger than 8 if membrane thickness d is larger than that of a typical biological membrane) for a 3-dimensional membrane protonic capacitor. a_i represents an angle between a membrane-liquid interface line and an electrostatic interaction line between a TELP (H^+) and [the i^{th}] TEA (OH^-)”. Nowhere did Lee explain where the value of 8 came from, nor how much larger than 8 could be, nor what the relationship

between and the membrane thickness is. Figure 2 below, a version of Lee’s Figure 3, explains the geometric relationship between the

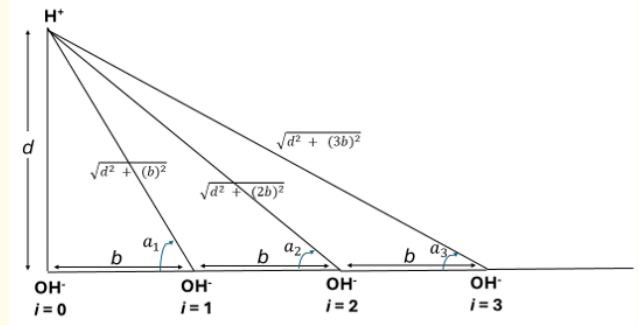


Figure 2: Geometric relationship between a single TELP proton and nearby hydroxide anions on the N side of the membrane. d is the thickness of the nonpolar core of the membrane, and b is the distance between adjacent hydroxide anions at the N-side interface. a_i angles for the $i = 1, 2$, and 3 hydroxides are depicted.

TELP proton and the interacting TEA hydroxide anions across a membrane of thickness d .

In addition to the problems with the parameter γ , the squared distance parameter in the denominator of the second term of Lee’s Equation 5, $((ib)^2 + d^2)/\sin(a_i)$, makes no sense. As shown in Figure 2, this should be simply the squared distance between the interacting charges, namely the square of the hypotenuse: $(ib)^2 + d^2$. Thus, Lee’s Equation (5) is incorrect, as are all of the calculations in the three right-most columns in his Table 1, which employed this incorrect equation.

Furthermore, for the two left-most columns in his Table 1 (TELC/ μm^2 and μm^2 /TELC), Lee claimed to have used $C/S = 9.2$ mF/ m^2 , when in fact he used the value 9.0 mF/ m^2 . And for the dielectric constant of the nonpolar phase, he used 1.88, the dielectric constant of hexane. In fact, as pointed out in a previous Lee critique [15], due to slight water penetration into the nonpolar core, the average dielectric constant in this region is about 70% higher than that of pure hexane, namely, ≈ 3 [37,38]. Thus, the calculated values in all five columns of Lee’s Table 1 are incorrect.

Finally, Lee supported his contention that the TELP protonic biocapacitor is stable: “For a transmembrane potential of 100 mV, we calculated its associated TELCs surface density of 5620 charges/ μm^2 , which gives a mean separation distance of ≈ 13.3 nm between adjacent TEA OH^- anions. Using this separation

distance... [b] in Equation (5) (with $\gamma = 8$ and $i = 1$ to 12) we calculated the transmembrane attractive force (F) of an individual TELP (H^+) interacting with multiple TELAs to be in the range from (2.08 to 1.16) 10^{-11} N for a typical lipid bilayer... with its alkane core membrane thickness [ranging] from 2.5 to 3.5 nm. Accordingly, to move such a TELP away from the membrane-liquid interface by 1 nm (say, 2.08 10^{-11} N of [sic] 2.5 nm to 1.16 10^{-11} N of [sic] 3.5 nm), it would require 1.62 10^{-20} J of energy ($= 10^{-9}$ m $\frac{1}{2}$ (2.08 10^{-11} + 1.16 10^{-11} J/m)), which is equivalent to 3.8 times as much as the Boltzmann kT thermal kinetic energy at a physiological temperature of 310 K. These results again indicate that a TELPs-membrane-TELAs capacitor can be quite stable” [1].

There are two problems with these conclusions, both of which have been pointed out repeatedly in prior Lee critiques. First, the energy calculation above assumes that Coulombic force declines linearly with distance, when in fact, force is a reciprocal function of distance ($F \propto 1/r$). But more importantly, in comparing the Coulombic energy of attraction to the Boltzmann kinetic thermal energy, Lee considers only enthalpy and ignores entropy. Because proton concentration is much higher within the putative localized proton layer at the membrane surface compared to the adjacent bulk phase, there is a strong entropic force impelling the release of surface protons into the bulk. For example, water evaporates fairly readily at room temperature, which is 75 °C below its boiling point, because the activity of water in the atmosphere is much lower than its activity of 1 in the liquid phase. This occurs even though

available thermal energy $3/2 RT = 3.7$ kJ/mol is nearly 12-fold less than the $\Delta H^\circ_{\text{vap}}(H_2O)$ of 44 kJ/mol. Thus, Lee’s calculated 3.8-fold difference between kT and the TELP (H^+)—TELA (OH^-) attractive force in no way demonstrates that the “TELPs-membrane-TELAs capacitor can be quite stable”.

Lack of experimental support for the TELP protonic biocapacitor

Lee wrote “TELPs are likely to stay within the first layer of water molecules on the hydrophobic core membrane surface [i.e., in the interfacial 0.3 nm partially hydrated “intermediate” layer]... So far, there are no special artificial pH sensors that could be used to directly measure TELPs in in vivo biomembrane systems” [1]. The history behind this claim is of interest here.

Before 2015, Lee considered the thickness of his electrostatically localized interfacial layer could be up to 1.5 nm thick. From 2015 onward, his calculations only used a 1.0 nm thickness. In 2022, Silverstein [17] tabulated literature pH measurements within this 1.0 nm layer, and found that all reported proton concentrations were 1 – 6 orders of magnitude less than those predicted by TELP equations. In addition to critiquing these literature values (see excerpt below), Lee has now narrowed the location of his localized proton layer (within 0.3 nm of the nonpolar interface) to a region for which “there are no special artificial pH sensors that could be used to directly measure TELPs in in vivo biomembrane systems”. Thus, the latest iteration of his TELP hypothesis is now experimentally untestable.

System	Method	pH _{surf}	ΔpH_{b-s}^*	ΔG_{b-s}^* [x RT]	Ref.
lipid bilayer	lipid fluorophore ^a	5.0 ± 0.2	2.0	4.6	[21]
lipid bilayer	electrostatics calc. ^b	4.5	3.0	6.9	[32]
lipid bilayer	lipid fluorophore	5.5	2.0	4.6	[40]
lipid bilayer	molecular dynamics ^b	4.7	2.3	5.3	[41]
lipid bilayer	molecular dynamics	3.3	3.7	8.5	[42]
ΔpH_{b-s} range:	2.0 to 3.7	avg. ± s.d.:	2.6 ± 0.7		
Mitochondria	lipid fluorophore	6.2 – 6.6	0.5 – 0.9	1.6	[43]
Bioenergetic Membrane	electrostatics calc. ^b	6.0 ± 0.5	≥ 1.0	≥ 2.3	[19]
Bioenergetic Membrane	electrostatics calc.	6.1 ± 0.2	0.9 – 1.4	2.1-3.2	[44]

Bioenergetic	electrostatics calc.	≤ 6	≥ 1.0	≥ 2.3	[18]
Membrane					
$\Delta\text{pH}_{\text{b-s}}$ range:	0.7 to 1.2	avg. $\pm$ s.d.:	1.04 ± 0.23		
water/decane	molecular dynamics	4.4	2.6	6 ± 2	[20]
interface					
Water/nonpolar interface	molecular dynamics	5.2	1.8	4.1	[45]
Water/ chcl_3	molecular dynamics	5.8	1.2	2.8	[45]
Interface					
Water/air interface	molecular dynamics	5.7	1.3	3.0	[45]
Water/air interface	molecular dynamics	4.8	2.2	5.1	[46]
Water/air interface	molecular dynamics	5.2	1.8	4.1	[47]
Water/air interface	molecular dynamics	6.0	1.0	2.3	[48]
Water/air interface	molecular dynamics	6.5	0.5	1.2	[49]
Water/air interface	molecular dynamics	6.6	0.4	0.9	[50]
$\Delta\text{pH}_{\text{b-s}}$ range:	0.4 to 2.6	avg. $\pm$ s.d.:	1.4 ± 0.7		

Table 3: Experimental measurements of pH at the water/nonpolar interface; “Surface” pH values (pH_{surf}) reported within 1 nm of a water/hydrophobic interface, either measured experimentally with head group-attached lipid-fluorophore proton sensors, or calculated from molecular dynamics simulations or electrostatics. First published in the Supporting Information (section VI) for ref. [16], and in Appendix II of ref. [39].

* $\Delta\text{pH}_{\text{b-s}} = \text{pH}_{\text{bulk}} - \text{pH}_{\text{surf}}$; pH_{bulk} was generally 7.0, but was up to 7.5 in a few studies.
 $\Delta G_{\text{b-s}} = 2.3\Delta\text{pH}_{\text{b-s}}$, in units of RT

- ^a The proton-sensing fluorescein was attached to the amine of the lipid head group ethanolamine moiety; this aromatic molecule is expected to sample environments ranging from the middle of the headgroup region out to adjacent water layers.
- ^b The protons visualized by molecular dynamics simulations and electrostatics calculations were localized within 0.5 nm of the water/non-polar interface.

In his Landmark Review, Lee criticized an early version of the published surface pH measurements tabulated by Silverstein [17]: “Silverstein’s Table 1 of his critique [17] [updated version reproduced above, Table 3] lists ‘surface pH (pH_{surf})’ [values]..., however, none of them has any relevance to TELPs. For instance, some of the [pH_{surf} values] were from molecular dynamics simulations at the liquid/[nonpolar] interface... without any transmembrane potential... [These] so-called ‘enriched protons’ ... at the liquid/[nonpolar] interface are irrelevant to the protonmotive force since they are not TELPs... Those non-TELP protons would have to be ‘enriched’ by a type of fixed interface property... which

(even if exist [sic]) would occur before the membrane system is energized through TELPs formation...”

“The measured mitochondrial [pH_{surf} values]... are obviously the bulk-phase pH values, not really surface pH as he seems to be [sic] claimed. Therefore, the ‘critique’ based on his Table 1 again seems to reflect certain misunderstandings or errors in his analysis around the TELPs [hypothesis]... An independent researcher has now also pointed out that ‘Silverstein’s critiques are untenable’ [1,51]”. including both delocalized and localized protonic coupling. According to the TELCs model, the excess positive charges of TELCs

¹We pointed out above that the existence of a distinct 0.3 nm partially hydrated later at the nonpolar-headgroup interface has no experimental support. Even so, the idea that the electrostatically localized proton layer, if it exists, would lie within the water monolayer adjacent to the nonpolar interface lines up well with electrostatic capacitors, where excess electrons occupy the surface of the negative metal plate closest to the positive plate. (And similarly for the positive holes on the surface of the positive plate.) At the same time, we note that later in his review, Lee wrote “TELPs... typically stay within a 1-nm thin layer on the membrane surface.” This contradicts his earlier statements placing the TELP layer within 0.3 nm of the nonpolar interface.

at one side of the membrane are balanced by the excess negative charges of transmembrane-electrostatically localized hydroxide anions (TELAs)

Before we discuss Lee's criticisms above, it is important to recall that the localized chemiosmosis model envisions a layer of fast-diffusing enriched protons at the membrane surface. However, authors define the location of this surface layer differently (see Figure S1 in section III of the Supporting Information for a schematic diagram). As noted above, for years, Lee specified the location of his putative TELP layer as within 1 nm of the edge of the nonpolar fatty acid region, which includes the entirety of the headgroup region. In his Landmark Review, Lee has narrowed the location to just 0.3 nm from the nonpolar interface, i.e., the water monolayer adjacent to the edge of the fatty acid region. Meanwhile, Zhang, *et al.* presented evidence that at the simple decane/water interface, their hypothetical barrier lay about 0.6 nm from the decane layer, thus surface proton diffusion occurred within the first two water monolayers at the interface [20]. Similarly, Yamashita and Voth [42] found a layer of "hydrated excess protons ... [in which the] distorted Zundel-like cation is extensively formed in the deep interface region for [three different lipid bilayer] systems... The Zundel-like cation bridges the negative sites of the phosphate groups and the carbonyl groups..." This would place this proton layer within 0.6 nm of the fatty acid (nonpolar) interface. On the contrary, in their work on lipid bilayers, Pohl's group placed surface proton diffusion 1 nm from the center of the headgroup region out toward the water phase; this would include the two water monolayers adjacent to the headgroup region [21,23].

Lee is correct that the two molecular dynamics simulations of the lipid bilayer cited in Table 3 were carried out using non-energized ($\Delta\psi = 0$) membranes [41,42]. However, four of the measurements reported in Table 3 were carried out on energized bioenergetic membranes. Lee's belief that "the measured mitochondrial $[pH_{surf}]$ values... are obviously the bulk-phase pH values" is clearly incorrect. For example, the $pH_{surf} = 6.2 - 6.6$ reported by Xiong, *et al.* [43] in energized mitochondria (Table 3), utilized a lipid headgroup attached fluorescein that is likely to sample environments ranging from the headgroup region to out to adjacent water layers. But importantly, the difference between the bulk and surface pH (ΔpH_{b-s}) of 0.5 - 0.9 was significantly greater than the uncertainty

in the two measurements (pH_{bulk} and pH_{surf}). Thus, Lee's contention that these lipid fluorophores report the bulk pH is clearly wrong.

Finally, the electrostatics calculations published by the Mulikidjanian/Cherepanov/Junge group also reported pH_{surf} in the headgroup region, within 0.5 nm of the edge of the nonpolar inner core of the energized bioenergetic membrane [18,19,44]. Here again, the ΔpH_{b-s} values of 0.9 - 1.4 were significantly larger than the uncertainty (e.g., ± 0.5). All four of these studies employed energized bioenergetic membranes, featuring biologically relevant non-zero transmembrane potentials.

Even though, as Lee pointed out, several pH_{surf} reports studied non-energized water/nonpolar interfaces, with no transmembrane potential, the differences between these reported values are nevertheless of interest: (i) The average bulk vs. surface pH difference (ΔpH_{b-s}) for the non-energized water/nonpolar interface (1.4 ± 0.7) is less than that for the non-energized bulk water/lipid bilayer interface (2.6 ± 0.7 ; $P = 0.015$, Table 3). Thus, the amphiphilic nature of the lipid bilayer serves to enhance H^+ enrichment at the (non-energized) membrane surface by more than an order of magnitude compared to the water/ nonpolar interface. This interesting result has not yet been reported in the literature. (ii) The depth of the potential well supporting higher proton concentration at the lipid bilayer surface ranges from 4.6 to 8.5 RT (Table 3), which is similar to the 6 - 9 RT well reported by Zhang, *et al.* at the decane/water interface [20]. (iii) ΔpH_{b-s} for the non-energized water/nonpolar interface (1.4 ± 0.7) is greater than that for the energized bulk water/bioenergetic membrane interface (1.04 ± 0.23 ; Table S3), but the difference is not statistically significant ($P = 0.3$). One would in fact expect energized bioenergetic membranes to have a lower ΔpH_{b-s} , because the working bioenergetic membrane includes proton sinks (i.e., the F_1F_0 ATP synthase) that consume protons, thus raising pH_{surf} and lowering ΔpH_{b-s} .

Lee was also correct in stating that the excess protons at non-energized water/nonpolar interfaces are distinct from TELPs, because the TELP equation predicts zero electrostatically localized protons when the transmembrane potential is zero. However, Lee displayed his single-hypothesis bias in this excerpt, implying that any enriched surface protons that "are not TELPs... are irrelevant

to the protonmotive force" [1]. In other words, even in the absence of biologically relevant experimental support for the existence of his TELP protonic biocapacitor, Lee believes that this is the only model that is "relevant to the protonmotive force".

In fact, all protons within 1 nm of the membrane surface can feed into the F_1F_0 complex to drive ATP synthesis (see below). Hence, the fact that over a dozen publications have reported surface proton enrichment in the absence of any membrane potential suggests that a TELP protonic biocapacitor, if it exists at all, is certainly not the only source of interfacial protons driving localized chemiosmosis.

This misunderstanding arises again in Lee's discussion of the Heberle, *et al.* demonstration of ATP synthesis by bacteriorhodopsin purple membrane fragments [53]. Citing the Yaguzhinsky, *et al.* result showing ATP synthesis at the water/octane interface [54], the 2022 critique pointed to the "enriched surface concentrations of protons in systems where $\Delta\psi = 0$, e.g., at the water/nonpolar interface..." [17] Lee claimed "this argument is moot since it seems mistakenly regarding [sic] TELPs as any 'enriched protons' [at] the membrane surface" [1]. But the Yaguzhinsky, *et al.* result concerns proton-driven ATP synthesis, NOT TELPs. They measured ATP synthesis in the absence of $\Delta\psi$, thus, the only thing that can drive the reaction is localized chemiosmosis via enriched proton concentrations at the water/octane interface. Lee is correct that this has nothing to do with TELPs (because $\Delta\psi = 0$); nevertheless, even in the absence of TELPs, ATP synthesis occurs. Thus, the Yaguzhinsky, *et al.* result shows, yet again, that a TELP protonic biocapacitor, if it exists at all, is certainly not the only source of interfacial protons driving localized chemiosmosis.

Before we leave this excerpt, it is important to point out that the final passage ("an independent researcher... pointed out that Silverstein's critiques are untenable" [51]) is taken completely out of context. Tamagawa's judgment in ref. [51] referred to a limited number of statements in a paper discussing neuronal transmembrane potentials. It had absolutely nothing to do with the measured surface pH values in Table 3. Lee's willful distortion of the literature is regrettable.

Misrepresentation of Wolf, *et al.* results using lipophilic cationic fluorophores to measure $\Delta\psi$ across mitochondrial membranes

In his section "TELCs model explaining why lipophilic cationic dyes can detect transmembrane potential," Lee wrote: "In addition

to the Nernstian accumulation into the mitochondrial matrix space, some of the lipophilic cationic dyes [employed by Wolf, *et al.* [55] in their study of mitochondrial transmembrane $\Delta\psi$] could distribute themselves to the TELCs layer... on the P-side of the mitochondrial inner membrane through the cation-proton exchange process... That is, some of the non-Nernstian TMRE fluorescence in the mitochondria [sic] membrane may represent the transmembrane-electrostatic partitioning... of the dye molecules to... the TELC layer... This could help to explain some of their differential staining to the different segments of the membrane... We would predict that the cationic dyes could stain more at the cristae..."

Unfortunately, these excerpts contain a number of confusing statements and misrepresentations of the cited literature. First, potential-sensing ionic fluorophores do not "stain" the membrane; they report transmembrane potential by changes in their emitted fluorescence. Secondly, lipophilic ions partition across the membrane in response to the transmembrane potential; thus, cationic fluorophores accumulate on the negative side of the membrane. Why then would the positively charged dyes partition themselves into the TELC layer on the positive (P) side of the membrane? But most importantly, Wolf, *et al.* specifically ruled out any fluorophore "partitioning... based on non-Nernstian binding..." [55].

Lee closed this section thusly: "If cristae junctions could somehow be closed, the TELCs model would predict that each individual crista could act like an independent bioenergetic unit... [Wolf, *et al.*] further demonstrated that the inhibition of ATP synthase proton translocation (TELPs consumption), with oligomycin, induced local hyperpolarization of the mitochondrial membrane potential... Therefore, the work of Wolf, *et al.* appears to be also well in line with the TELCs (TELPs) model" [1].

Here Lee imputes to his TELC hypothesis conclusions that actually derive from basic physics and biochemistry. If apposing membranes at a crista junction pinch together and close off the crista, then membrane topology requires that such an isolated crista will act as "an independent bioenergetic unit". This prediction stems from a knowledge of membrane topology and basic electrostatics, with no need to invoke the TELC hypothesis. Similarly, because spontaneous proton flow through the F_1F_0 ATP synthase is electrogenic, it drains the transmembrane $\Delta\psi$ (as well as ΔpH) as ATP is synthesized. Thus, inhibiting the ATP synthase

must hyperpolarize the membrane, as reported by Wolf, *et al.* This conclusion is drawn from a knowledge of the mechanism of the proton flow-driven F_1F_0 ATP synthase, with again no need to invoke the TELC hypothesis. Finally, the same argument applies to protonophores like CCCP: Because they are electrogenic, as they transport protons across the membrane toward the low potential (negative) side, they collapse $\Delta\psi$. This effect on $\Delta\psi$ stems from the simple electrostatics of electrogenic ion transport, with again no need to invoke TELC.

Problems applying TELPs to bacteriorhodopsin purple membrane fragments

In a now classic demonstration of localized chemiosmosis, Heberle, *et al.* [53] energized bacteriorhodopsin-containing purple membrane fragments with laser flashes, and observed three different proton transport processes: (i) initial proton pumping from the N (inner/cytoplasmic) side to the P side (outer/extracellular) side through light-driven bacteriorhodopsin; this was followed by two simultaneous proton passive diffusion processes: (ii) proton translocation along the surface of the purple membrane disk, from the P side around the rim to the N side; and (iii) proton release from the P surface into the P (extracellular) adjacent bulk phase. The correlation times following laser flash for the three proton transport steps were quite different: 76, 228, and 1290 μ s, respectively. Furthermore, Heberle, *et al.* published an Arrhenius plot ($\ln k$ vs. $1/T$) of these three processes, reporting their activation energies: 40, 88, and 54 kJ/mol, respectively. (Note that E_a for proton release to bulk (step iii) = 54 kJ/mol corresponds to 22 RT at 20 °C, which matches the barrier for proton surface to bulk release reported by Weichselbaum, *et al.* [21] of 20 – 30 RT.)

Lee rejected the conclusion of Heberle, *et al.* of a 22 RT barrier for proton translocation between the surface and bulk phases. Instead, he hypothesized that the delay in proton release is due to a stable biocapacitor across the purple membrane fragment:

“When a transmembrane proton pumping process which is an electrogenic charge transport across a bacteriorhodopsin purple membrane open flat sheet is energized by a single-turnover laser flashlight [sic], a transient transmembrane potential ($\Delta\psi$) will form and then decay as the pumped excess charges returning [sic] to the other sides [sic] of the membrane. The formation of a transient transmembrane potential ($\Delta\psi$) will result from a protonic

capacitor formation with TELPs on the positive (extracellular) side and with TELAs on the negative (cytoplasmic) side along a bacteriorhodopsin purple membrane open flat sheet, at least transiently” [1].

Thus, Lee’s proposed protonic biocapacitor comprises electrostatically localized protons at the P_L nonpolar interface, and localized hydroxides at the N_L interface. This transient protonic capacitor establishes a non-zero $\Delta\psi$ across the purple membrane that decays to zero as protons diffuse along the P_L surface, around the membrane fragment rim to the N surface, where they are “extinguished” by combining with hydroxides at the N_L surface. The decay is enhanced by hydroxide diffusion in the opposite direction. Thus, Lee calculated that the transient non-zero $\Delta\psi$ would decay with a lifetime of 152 μ s, that is, the difference between the correlation time for process (ii), surface proton translocation from P to N, and process (i), proton pumping from N surface to P surface (228 – 76 μ s). Unfortunately, no one has (yet?) reported any measurements of transient non-zero $\Delta\psi$ across energized purple membrane fragments. Lee concluded “these TELPs-model-predicted features were exactly shown up [sic] in the [Heberle, *et al.*] experiment...” [1].

There are a number of problems with Lee’s conclusions. First, the evidence provided by the Heberle, *et al.* experiments is explained by localized chemiosmosis; it does NOT favor any particular hypothesis explaining localized chemiosmosis. In other words, their results support the potential well/barrier hypothesis just as much as the TELP hypothesis. In fact, as noted above, Heberle, *et al.* measured the height of the kinetic potential barrier (22 RT) between surface and bulk protons, thus they employed this hypothesis to explain their results. Furthermore, while positing that there is “no need” to consider the existence of a potential well or barrier between the two phases, Lee neglected to explain the 20 -30 RT potential barrier that both Heberle, *et al.* and Weichselbaum, *et al.* actually measured from their Arrhenius/Eyring plot experiments.

Second, Lee’s explanation considers only interface-localized protons and hydroxides as the source of transmembrane potential. Heberle, *et al.* showed that within 76 μ s of the laser flash, an excess proton showed up at the P surface. Lee concluded that for the next 152 μ s, as the proton diffused along the P surface and around the membrane fragment rim to the N surface, a transient non-zero $\Delta\psi$

²Regarding these proton-sensing lipid fluorophores Lee has opined that their “active site... is expected to stay in the bulk liquid phase... at a position above the lipid headgroup, likely more than 1.5 nm away from the first layer of water molecules (where most of the TELPs reside)...” (52) This incorrect conclusion is apparently based on his belief that the two-dimensional line-drawing chemical structure of the lipid represents its actual conformation when incorporated into the membrane. In fact, the eight C-C, C-O, P-O, and C-N freely rotating single bonds between the lipid head group and the aromatic fluorescein allow for dozens (hundreds?) of conformations that would place the proton-sensing group both within the headgroup layer and into adjacent water layers. The aromaticity of the fluorescein group is expected to favor partitioning into the lower dielectric headgroup region, i.e., less than 0.7 nm from the first water monolayer at the nonpolar/fatty acid interface.

³Lee’s assumption that his TELP model is the only valid hypothesis explaining localized chemiosmosis appears several times in his Landmark Review, e.g., “biomembrane energization is typically through the formation of a TELPs-membrane-TELAs capacitor...”

existed across the membrane. But Lee ignored the fact that any excess charge on one side of the membrane will affect $\Delta\psi$. As the buffer employed by Heberle, *et al.* included 0.15 M KCl, one must also consider the vectorial electrophoretic diffusion of K^+ and Cl^- ions when predicting $\Delta\psi$.

Even though protons diffuse more quickly than other bulk phase aqueous ions ($D_{H^+} = 9.3 \text{ nm}^2/\text{ns}$; [56]), K^+ and Cl^- also diffuse readily, with $D = 2 \text{ nm}^2/\text{ns}$ [56]. Let us break down proton pumping into two half-steps, moving the excess proton halfway across the membrane (2.5 nm) in the first 38 μs after the flash, and then the remaining distance in the next 38 μs . Considering only this pumped proton, a non-zero $\Delta\psi$ will indeed develop during the first 38 μs . But since K^+ and Cl^- also diffuse readily in the bulk phases, they must respond to this developing $\Delta\psi$, with K^+ moving from the P to the N side, and Cl^- moving in the opposite direction. From Fick's law of diffusion, translation distance varies with time according to Equation 5:

$$\text{Equation 5: } \langle d \rangle^2 = q_i D_i t$$

Where $\langle d \rangle$ is the average distance traveled in time t , D_i is the diffusion coefficient for i -dimensional translation, and $q_i = 2$ (for 1-dimensional translation), $q_2 = 4$, and $q_3 = 6$.

Now consider a Cl^- anion in the N-side bulk phase at the purple membrane fragment rim. In response to the developing $\Delta\psi$, a Cl^- ion will diffuse toward the P-side proton release site at the center of the purple membrane disk ($r \approx 300 \text{ nm}$), a total distance of 307 nm (= 1 nm from N bulk to disk rim + 5 nm across the rim + 1 nm into the P bulk + 300 nm over to the center of the membrane fragment). From Equation 5 (using $q_3 = 6$ for 3-dimensional diffusion of the anion) we can calculate this time to be 7854 ns = 7.9 μs . In other words, 30 μs before the pumped proton is even halfway across the membrane, a Cl^- counterion can travel the distance from the N-side bulk phase to the proton release site on the P-side of the membrane fragment. (And the same is true of a K^+ cation diffusing in the opposite direction.) Thus, the electrogenicity of the bacteriorhodopsin proton pump will be neutralized by the nearly simultaneous vectorial bulk phase electrophoretic diffusion of K^+ and Cl^- , and the transient non-zero $\Delta\psi$ envisioned by Lee will not have a chance to develop. In the Supporting Information (section IV.B), we calculate that the number of available K^+ and Cl^- far exceeds the number of protons pumped across a purple membrane fragment.

Lee argued in his Landmark Review that "there is no evidence for [the] proposed vectorial ion flow: K^+ mov[ing] from the P side toward the N side, and Cl^- mov[ing] in the opposite direction" [1]. This is an odd claim, for two reasons: First, electrophoresis of electrolyte ions within an electric field is a common phenomenon explained by basic electrostatics. Lee's doubt that this would occur is puzzling, and he presents no argument as to why he believes that this would not occur. Second, Lee's application of his TELP model to the Heberle, *et al.* demonstration of localized chemiosmosis on energized purple membrane fragments includes no actual "evidence" of the existence of his electrostatically localized P_L proton layer and N_L hydroxide layer. It thus seems odd to complain that the critique also presented no "evidence" for the resulting electrophoresis of bulk phase electrolyte ions, a process that is taken for granted in electric fields.

Lee argued further that because light-driven proton pumping by bacteriorhodopsin is the initial step in the process, consideration of its slowness is an "argument [that] is misconceived and irrelevant since the formation of transient [non-zero] transmembrane potential is the first step... The other steps happen later..." [1]. What Lee seems to be ignoring here is that when subsequent steps in a cycle are much faster than the initial step, subsequent steps can reverse the effects of the initial step so that its products never accumulate at all. Consider turning on a bathtub water faucet, followed by opening up the drain: If one is able to open the drain before the first drops of water (from prior opening of the faucet) reach the tub, then water never accumulates.

Other problems with statements made by Lee in this section of his Landmark Review are dealt with in the Supporting Information (section IV). The bottom line here is that when one considers the vectorial electrophoretic diffusion of Cl^- and K^+ counterions, the purple membrane fragments employed by Heberle, *et al.* would not be expected to support even a transient non-zero $\Delta\psi$. In the absence of any contradictory experimental measurements of $\Delta\psi$ across the membrane fragments, one would have to conclude that the TELP hypothesis cannot be used to explain the localized chemiosmosis reported by Heberle, *et al.*

Problems applying Fick's law to the decane/water drop experiment

In an effort to study a simplified model of localized chemiosmosis, Zhang, *et al.* employed a single nonpolar/polar interface, namely, a water droplet (140 μL) underneath a layer of decane (280 μL)

that was doped with a proton-sensing lipid fluorophore localized to the decane/water interface. They were able to measure both the diffusion coefficient of proton flow along the interface, as well as the rate constant of proton escape into the bulk aqueous phase. They found that interfacial protons diffused at roughly the same enhanced rate as protons along the surface of lipid membranes ($D = 5.1 \text{ nm}^2/\text{ns}$). From an Arrhenius plot of temperature-dependent proton escape rate constants, they calculated a free energy well of 8.7 RT at the interface. This potential well presented a kinetic barrier to the release of interfacial protons into the bulk aqueous phase, and explained the transient high concentration of interfacial protons. There is much literature support for the existence of a proton potential well at the nonpolar/water interface, as noted in Table 3. For a more detailed explanation of the experimental details, along with an analysis of misleading literature statements made by Lee in his introduction to this section of his review, please see the Supporting Information, section V.

Lee rejected the potential well demonstrated by Zhang, *et al.* and proposed instead an alternative explanation that relied on a simple application of Fick's law (Equation 5). Unfortunately, Lee used $q_1 = 2$ in his statement of Fick's law, his Equation (7); for proton diffusion at the 2-dimensional decane/water interface, he should have used $q_2 = 4$. Here is Lee's hypothesis explaining the transient protonic wave demonstrated by Zhang, *et al.*: "... the spread of protons from the HCl [injected into] the liquid is much faster than that of the chloride anions... Because the protons can spread far ahead of the slow diffusing chloride anions, this temporarily created a transient effect of excess protons [that]... spread from the bulk liquid phase toward the liquid-oil interface... When the chloride anions finally catch up with the protons so that all the protonic charges will be balanced with the chloride anions... the effect of the transient excess protons will end so that the protons that have reached the liquid-oil interface will finally move out ... and return to the bulk liquid phase" [1].

There are two problems with Lee's explanation. First, Zhang, *et al.* injected the HCl aliquot through a micropipet ($\approx 1 \mu\text{m}$ diameter) right at the decane/water interface ("at a distance of $< 1 \mu\text{m}$ from the interface" [20]). If the protons had to reach the interface by diffusing first into the bulk liquid and then to the interface, as suggested by Lee, there would be a delay in fluorescence detection of protons even at the shortest distance from the injection site ($45 \mu\text{m}$). Such a delay was not observed. Furthermore, the proton

diffusion coefficient measured by Zhang, *et al.* should have been that of bulk aqueous phase diffusion, $9.31 \text{ nm}^2/\text{ns}$; instead, Zhang, *et al.* reported $D_{\text{H}^+} = 5.1 \text{ nm}^2/\text{ns}$.

More importantly, the diffusion process proposed by Lee would lead to a permanent wave of protons, not a transient wave. As protons diffuse in the water droplet bulk phase by Brownian motion, they spread randomly and evenly, everywhere in the droplet, including at the decane/water interface. When the chloride anions "catch up" to the protons, they would simply insert themselves between protons. At this point, equilibrium is reached with a homogeneous solution of HCl everywhere within the water droplet. There will be no force causing the interfacial protons to leave the interface and "return to the bulk phase". Thus, the final interfacial proton concentration will reach an equilibrium maximum value and there will be no transient protonic wave.

Lee also erred in his explanation of the observation of Zhang, *et al.* that added NaCl increases the speed of the interfacial protonic wave. Lee wrote "the higher NaCl concentration could slightly impede the spread of the Cl⁻ from the injected HCl [site] relatively [sic] to the H⁺ conduction" [1], including both delocalized and localized protonic coupling. According to the TELCs model, the excess positive charges of TELCs at one side of the membrane are balanced by the excess negative charges of transmembrane-electrostatically localized hydroxide anions (TELAs). In fact, the exact opposite is true: It is common knowledge that electrolyte conductivity increases linearly with ionic strength. A schematic drawing (Figure S1) explaining this problem can be found in the Supporting Information, section V.

In rejecting the potential well model put forward by Zhang, *et al.* on the basis of their Arrhenius plots, Lee put forward this argument: "If the potential [well]... as proposed by Junge and Mulikidjanian [and Zhang, *et al.*] had really existed, then the protons of HCl injected into the bulk liquid phase would not have been able to enter from the bulk liquid phase into the liquid-oil interface. Furthermore, if somehow the protons once get [sic] into the liquid-oil interface, then the protons would not have been able to get out of the liquid-oil interface to return into the bulk liquid phase..." [1].

Lee has made this claim repeatedly in his recent papers, and it has been repeatedly shown to be false. In order for a proton to be permanently trapped at the interface, as put forth by Lee, the

⁴Lee made one further assumption in his previous paper on this topic (26), namely, that injected protons moving as a fast front ahead of slower Cl⁻ counterions, are kept out of the bulk phase (and restricted to the water/decane interface) due to Gauss's law. However, Gauss's law applies to electrons moving within a solid metal conductor. Aqueous ions (which are much larger than electrons) that move within liquid water (a poorer conductor than metal) are subject to Brownian motion and entropy; their diffusion would NOT be expected to be limited by Gauss's law.

barrier would have to be infinitely high. The finite potential barrier (20 – 30 RT) reported by Weichselbaum, *et al.* [21], as well as the 6 – 9 RT potential well reported by Zhang, *et al.* merely slows down the release of interfacial protons, it does not disallow it entirely. It is dispiriting that Lee continues to make this debunked claim, and it continues to make its way into the peer-reviewed literature.

Later in his Landmark Review, Lee presented a second argument against the potential well/barrier model: “At the experimental temperature of 20 °C, Silverstein’s claimed $\Delta G^\ddagger \approx 20 - 30$ RT in the liquid water phase at the location of 0.4 nm away from the decane surface ... is likely to be questionable since he has never explained how could it [sic] be possible for water molecules to form such a high activation barrier that is far much [sic] higher than their hydrogen bond energy... $\Delta G^\circ = 2.7$ kJ/mol (with $\Delta H^\circ = 7.9$ kJ/mol). For the mechanism of proton mobility, the activation energy is known to be 11.3 kJ/mol, in contrast to Silverstein’s claimed $\Delta G^\ddagger \approx 20 - 30$ RT” [1].

Aside from Lee’s misattribution to Silverstein conclusions that were in fact published by Pohl’s group [20,21], there are many problems with this claim. First, Pohl’s group employed Arrhenius and Eyring plots to determine the depths of their potential well and barrier. If these do not in fact exist, and their results are instead explained by Lee’s Fick’s law explanation, then how can one explain the activation barriers calculated from Pohl’s Arrhenius/Eyring plots?

Second, the hydrogen bond free energy in water reported by Markovitch and Agmon [57] (2.7 kJ/mol) is far below H-bond energies of 3 – 5 kcal/mol (12 -21 kJ/mol) that are generally found in the literature; a good example is the value of 3.6 – 3.7 kcal/mol (15.1 – 15.5 kJ/mol) tabulated in Table 8.1, p. 142 of ref. [58]. This is close to the estimated value obtained by simply dividing $\Delta H^\circ_{\text{vap}}(\text{H}_2\text{O}) = 44.03$ kJ/mol by 3.5 H-bonds per H_2O molecule = 13 kJ/mol. Part of the reason for this discrepancy is that reported hydrogen bond energies are generally enthalpies, rather than free energies. The water H-bond enthalpy reported by Markovitch/Agmon was 7.9 kJ/mol. Thus, the enthalpy they obtained from their multistate empirical valence-bond calculations seems to be too low by a factor of nearly 2-fold (= 15.3/7.9).

Third, when considering the activation barrier for proton transfer, one must deal with the hydrogen bond energy of a

hydrated proton, not the H-bond between neutral water molecules. Markovitch and Agmon [57] reported the former enthalpy (for their “D0” H-bond) to be 18.3 kJ/mol. Adjusting for the factor of 2, this becomes 37 kJ/mol, or 15 RT at 298 K. The energy of the charge dipole $\text{H}^+ \dots \text{O}$ hydrogen bond in the $\text{OH}_3^+ \dots \text{OH}_2$ Zundel complex is generally cited as 20 – 50 kJ/mol (8 – 20 RT), but the value cited by Jeffrey [58] is much higher: 32 kcal/mol = 54 RT.

Finally, the activation energy for proton transfer cited by Lee (from Markovitch & Agmon), 11.3 kJ/mol (4.6 RT), is measured for proton transfer in bulk water. However, the 20 – 30 RT barrier calculated by Weichselbaum, *et al.* was for proton release from the water/decane interface into the bulk water phase. The latter transition state is thus influenced not only by the structure, dynamics, and mechanism of proton transfer in bulk water, but also at the interface.

Many papers have reported that water adjacent to the nonpolar interface has very different physical properties than bulk water [19,44] and references therein]: In addition to fast proton transfer at the interface, the dielectric constant is much lower (about 10 – 20, cf. 78 for bulk water). This 4- to 8-fold lower dielectric constant suggests that the proton-water Coulombic attraction will be much stronger at the interface cf. the bulk water phase, thus supporting a higher barrier ($18 - 36 \text{ RT} = 4.6 \text{ RT} \times (4 - 8)$) for releasing aqueous protons from the interface into the bulk water phase. In addition, Weichselbaum, *et al.* reported that the 20 – 30 RT barrier was primarily entropic, with “only a minor enthalpic contribution that roughly corresponds to the breakage of a single hydrogen bond” [21]. Thus, the barrier is mainly due to loss of entropy as the proton moves from the interface to its transition state on the way to the bulk water phase; this has more to do with water reorganization than it does with hydrogen bond energies. Suffice it to say that the activation barrier of 20 - 30 RT reported by Weichselbaum, *et al.* is not unreasonable.

In a 3-page section of his Landmark Review subtitled “The putative potential well/barrier model not needed,” Lee discussed a free energy profile showing the change in free energy with distance from the decane/water interface, based on the potential well/barrier results from Pohl’s group. Lee concluded that “even if assuming [sic] the putative potential well/barrier model would be true, it still could not explain the relevant bioenergetics [of the bacteriorhodopsin purple membrane and the mitochondrial inner

membrane... Furthermore,]... the potential well/barrier model would predict that all the Al films placed in the anodic water chamber [of the electrolysis apparatus] would trap the excess protons..." [1].

However, as noted by Lee, the profile, including the free energy values on the y-axis and the distance values on the x-axis, characterizes the decane/water interface. There is no reason to expect that these values will be exactly the same for a much more complex biological membrane, nor for a solid aluminum film. In addition, Lee's claim regarding the latter electrolysis system is problematic. He has claimed that the detection limit for his Al films is $\text{pH} < 3.8$, whereas Pohl's group reported interfacial surface pH values of 4.4 – 5.2 (Table 3). Hence, Lee's conclusion "if the potential well/barrier model were true, all the Al films in contact with the anode water would have displayed protonic Al corrosion by the excess protons" is false.

Finally, we note that the Pohl group published a response to Lee's reinterpretation of their results; in this paper they argued against Lee's "new theory about interfacial proton diffusion" and reinforced their potential well model, concluding that "the commonly accepted laws of electrostatics and diffusion prevail" [23]. In his Landmark Review, Lee took issue with a single argument in the Pohl response, their use of the Bjerrum length in judging protonic capacitor stability. Lee did not address any of their arguments supporting the existence of the potential well, nor their arguments rejecting the validity of Lee's experimental "demonstration" of the existence of the protonic capacitor in a macroscopic electrolysis cell. We will deal with these issues now.

Problems with the water electrolysis TELPs demonstration

Lee opined that "protonic (TELPs) membrane capacitors have been experimentally demonstrated well beyond any reasonable doubt... We employed a biomimetic anode water-membrane-water cathode system where excess protons and excess hydroxide anions were generated by utilizing a... water electrolysis system, and their distributions were evaluated using a proton-sensing aluminum (Al) film..." [1].

First, it seems odd to call a macroscopic solid 75 μm thick Teflon disk plus Al film "biomimetic", considering that biological membranes are pseudo-liquid (gel phase), only a few nanometers thick, and comprised of amphiphilic lipids and proteins. This is

especially true when one considers that Lee rejected the liquid decane/water interfacial system studied by Zhang, *et al.* thusly: "The experimental system of Pohl's group strictly does not qualify to be considered as any reasonable biomimetic system... It is questionable whether they could have any substantial relevance to cell energetics... [and] could even cause unnecessary confusions [sic] in the scientific community... [Pohl's group made] passionate claims... in a manner similar to the parable with one of the blind men holding an elephant's tail and passionately claiming 'elephant is a rope'" [1].

Nevertheless, Lee's results using this water electrolysis apparatus were intriguing. Based on the current flow he calculated the amount of protons released at the anode. As the pH in the anodic chamber did not change much during the course of the 10 h electrolysis, he assumed that the protons were electrostatically localized in the L layer adjacent to the 2.55 cm^2 Teflon disk situated between the anodic and cathodic chambers. Assuming a 1 nm thickness of the L layer (not the 0.3 nm layer assumed for bilayer membranes), he calculated a predicted pH of 2.92 in this layer. Presumably, the L layer on the cathodic side of the Teflon disk would have a pH of 11.08.

Next, Lee placed a "proton-sensing aluminum (Al) film" on the anode-facing surface of the Teflon disk, concluding that the film "displayed dramatic TELPs (protonic corrosion) activity". The photograph in his Figure 12(P) shows the entire Al film stained a grey-black color with a light grey or white rim around much of the rim.

Thus, Lee has assumed that his Al films sense protons by showing "protonic corrosion" that produces black stains on the film. In his Landmark Review, Lee does not identify the source of the black color, but in previous papers he cited $\text{Al}(\text{OH})_3$ precipitates, even though these are white. (Also, $\text{Al}(\text{OH})_3$ could not form in the acidic L layer, $\text{pH} \approx 3$, but would have to form in the bulk phase, $\text{pH} \approx 6$, then diffuse through the acidic L layer without being dissolved by the acid.) He also never weighed these films: If they were corroded by acid oxidation ($2 \text{Al}(\text{s}) + 6 \text{H}^+(\text{aq}) \rightarrow 2 \text{Al}^{3+}(\text{aq}) + 3 \text{H}_2(\text{g})$), then he should be able to correlate a weight decrease with the number of anodic protons supposedly produced. But most importantly, Lee never actually tested the acid-sensitivity of his Al films, exposing them to buffers of pH 1 – 5 to see if the black stains were produced and determine the detection limit. Finally, he never tried any

standard method of pH detection, for example by placing pH strips on the Teflon surface. Suffice it to say that Lee's assumption that the black stains on his Al films represent proton sensing seems like a leap of faith rather than a confirmed scientific conclusion.

Lee also added sodium and potassium salts to the anodic side, showing a decrease in black staining of the Al films. By qualitative visual estimation of the decreased staining, he claimed to be able to calculate equilibrium constants for cation/proton exchange: $(5.1 \pm 0.5) \cdot 10^{-8}$ for Na^+ and $(6.9 \pm 0.9) \cdot 10^{-8}$ for K^+ . There are two problems with these values. First, it seems unexpected that qualitative visual estimation of film staining can actually produce an accurate value for an equilibrium constant. In fact, Silverstein showed [59] that these equilibrium constants yield staining predictions that disagree with the photographic results reported by Lee. Second, the extremely low exchange equilibrium constants (10^{-8}) are hard to explain considering the physical chemistry involved. Firstly, the radii of these three aqueous cations are all roughly the same (0.28 nm for $\text{H}^+(\text{aq})$, 0.36 nm for $\text{Na}^+(\text{aq})$, 0.33 for $\text{K}^+(\text{aq})$), thus, it is hard to imagine what physical force could account for an added stability of 41 kJ/mol for protons over Na^+ and K^+ in the L layer. Secondly, the higher charge density of the smaller H^+ cation makes its hydration free energy much higher: -1102 kJ/mol for H^+ vs. -397 kJ/mol for Na^+ and -314 kJ/mol for K^+ . It seems likely that ions moving from the bulk aqueous phase into the L layer adjacent to the nonpolar interface would have to become at least partially dehydrated, which should be much more unfavorable for $\text{H}^+(\text{aq})$, compared to $\text{Na}^+(\text{aq})$ and $\text{K}^+(\text{aq})$. This too would argue against a low proton/cation exchange K_{eq} .

In his Landmark Review Lee attempted to answer the criticism that TELP protonic capacitor layers would not be stable across a macroscopic 75 μm thick Teflon disk [23]. In order to calculate the number of localized hydroxide anions across the "75 μm thick Teflon membrane... [that] each proton will be able to electrostatically 'see,'" [1]. Lee calculated the area occupied by these hydroxides as $18,000 \mu\text{m}^2 = \pi \times (75 \mu\text{m})^2$. Using his TELC number density (at 100 mV) of 5600 charges per μm^2 , he estimated the number of hydroxides attracted to each proton as $10^8 = 5600 \times 18000$.

Using this calculated number, Lee concluded "the numbers of TELA OH^- anions that can together electrostatically attract a TELP H^+ can be as large as 10^8 . Consequently, the second term of the

protonic transmembrane attractive force equation [Lee's Equation (5) above] becomes far more important than the first term..." From this Lee opined that the conclusion of Knyazev, *et al.* that the TELP/TELA capacitor across the water electrolysis 75 μm membrane was unstable was "just completely groundless or misconceived..." [26]. Instead, Lee stated that the 10^8 interacting hydroxides were sufficient to stabilize this TELP/TELA capacitor.

There are two problems with Lee's calculation of 10^8 interacting hydroxide anions. First, he did not explain why the interacting hydroxides would occupy the area of a circle whose radius equals the thickness of the Teflon membrane, 75 μm . There does not seem to be any clear reason why these two distances (membrane thickness, and radius of interacting circle) must be the same.

Second, although his Equation 5 purportedly allows one to calculate the Coulombic force between a TELP proton and all interacting TELA hydroxides at the membrane's opposite surface, Lee neglected to employ this equation to actually calculate the attractive force across the 75 μm disk. In fact, this equation allows one to determine the distance beyond which surface hydroxides do not add appreciably to the attractive force. Accordingly, we calculated the value of the second term in Lee's Coulombic equation for $i = 1$ to 200,000 at a 100 mV transmembrane potential, and the forces were summed, according to the equation (Supporting Information, section VII).

Because the distance $i b$ increases with i , the Coulombic force decreases, and the net attractive force reaches its asymptotic maximum above $i \approx 50,000$, at a value of -1.02×10^{-15} J/m (Figure 3). Thus, the energy required to move a localized proton 1 nm away (this is Lee's stipulated Teflon disk L layer thickness) from the membrane surface would be 1×10^{-24} J. This is over 4000-fold less than the available thermal energy at 310 K, $k_B T = 4.3 \times 10^{-21}$ J. Even if one doubles the "dimensional factor" γ in Lee's Equation 5, this merely doubles the maximum attractive force, leaving the proton release energy well below thermal energy. Thus, the putative localized proton capacitor across this macroscopic membrane is NOT stable, in agreement with the judgment of Knyazev, *et al.* [23].

For a more detailed critique of Lee's purported demonstration of a TELP protonic capacitor in his water electrolysis experiments, please see the Supporting Information, section VI.

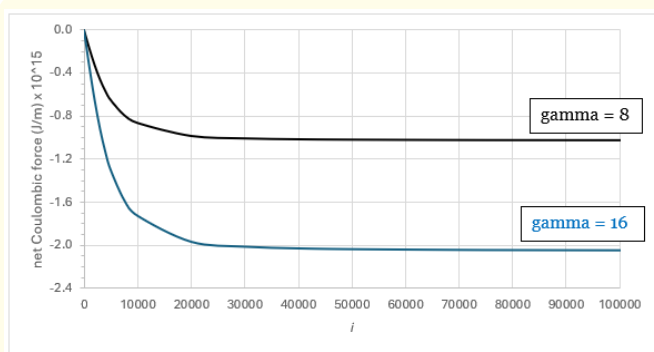


Figure 3: Net Coulombic force of attraction between a single L layer proton and multiple hydroxide counterions across a 75 μm thick membrane. Calculations can be found in the Supporting Information, section VII.

Problems with TELPs isothermal heat driving ATP synthesis

In his Landmark Review, Lee described his “discovery” that mitochondria and bacteria (especially alkaliphilic bacteria) harness environmental heat to help drive ATP synthesis in what he has dubbed an isothermal “type-B energetic process”. For example, Lee reported [60] the pmf available from redox-driven proton pumping as 228 mV, and the pmf required for ATP synthesis as 115 mV; alkaliphilic bacteria present a particular problem because the proton electrochemical pmf available in their delocalized (bulk to bulk) pmf is only about 50 mV at an external pH of 10. Thus, Lee used his TELP equations to calculate a total proton pmf (bulk + TELP) of 350 mV at pH 10 (and 470 mV at pH 7 – 8.5). This 350 mV proton pmf at pH 10 solved the problem of supplying sufficient proton flow energy to drive ATP synthesis (115 mV), but it raised the problem of supplying more proton flow energy than was available from the energy input, redox-driven proton pumping (228 mV).

Most scientists would take this as a warning that perhaps the theory underlying the TELP hypothesis was wrong. For example, Lee wrote: “Thermotrophy of cells utilizing environmental heat energy was previously thought to be impossible for centuries” [1]. Nevertheless, Lee doubled down, developing his claim that oxidative phosphorylation is a “type-B energetic process” that is allowed to harness environmental heat to violate the second law of thermodynamics. Most of these claims were shown to be untenable in a recent critique [16]. Here I will respond only to major points in

the analysis that Lee presented in his Landmark Review. Problems with minor points are elucidated in the Supporting Information, section IXA and IXB.

In explaining his contention, Lee wrote: “Type B energetic processes, such as the newly discovered thermotropic [sic] function that isothermally utilizes environmental heat energy to do useful work in driving ATP synthesis, follow the first law of thermodynamics... but do not necessarily have to be constrained by the second law, owing [sic] to their special asymmetric functions. For example, in mitochondria, their special asymmetric functions ... comprise: (1) transmembrane-electrostatic proton localization; (2) the transmembrane asymmetry of inner mitochondrial membrane protein structures with the protonic outlets of redox-driven proton-pumping protein complexes protruded away from the membrane surface by 1 – 3 nm into the bulk liquid P-phase, whereas the protonic inlet of the F_1F_0 ATP synthase located rightly [sic] at the TELPs layer; and (3) the lateral asymmetry of mitochondrial cristae with an ellipsoidal shape that enhances the density of TELPs at the cristae tips where the F_1F_0 ATP synthase enzymes are located... [This] ellipsoidal shape (instead of a spherical shape) without any additional chemical energy input would appear to violate the second law of thermodynamics, but perfectly support the identification of the TELPs-associated Type-B energetic process” [1].

Lee has claimed here and elsewhere that he has “discovered” a biological system that obeys the first law of thermodynamics, but violates (and is allowed to violate) the second law. We show in section IXC of the Supporting Information that in order to have oxidative phosphorylation violate the second law, one must define an artificially “narrow universe” that turns out to be not isolated, thus, the first law also does NOT apply. If, on the other hand, one defines the universe normally (so the first law does apply), then the second law is NOT violated. Thus, Lee’s claim that oxidative phosphorylation “follows the first law of thermodynamics but not the second law” is false.

Regarding claim (3), the ellipsoidal shape of mitochondrial cristae does not support a Type-B designation for oxidative phosphorylation because the shape does in fact require “additional chemical energy input”. Specifically, the biogenesis of mitochondria requires lipid and protein synthesis, and both of these anabolic processes involve energy input. Thus, the cristae shape does not violate the second law of thermodynamics.

⁵Unfortunately, Lee calculated this value from standard redox potentials, so it only applies to standard conditions, e.g., all concentrations 1 M. The value under typical biological conditions has been calculated to be substantially less: 193 mV.(61) See also the Supporting Information, section IIA for ref. (16).

Regarding Lee's asymmetry claim (2) above, note that it stems from a schematic topological diagram originally published by Dudkina, *et al.* [33], that Lee reproduced as his Figure 7. In the Dudkina, *et al.* figure, proton extrusion on the P side is designated with arrows emerging from the bottom of the shapes representing the three proton pumps. These arrows are not intended to designate the specific location of the actual proton release sites, thus Lee presented no evidence to back his claim that protons are released into the bulk phase. On the contrary, we presented evidence [16] from recent published structures solved for the three mitochondrial inner membrane proton pumps: For Complex I, "the actual proton release sites lie almost flush with the membrane surface;" for Complex III, "extended H-bonded water chains have been found connecting each UQ binding site to the nearest membrane surface water layer; thus, protons are likely to be released into the surface layer, not the bulk phase;" and for Complex IV, "the proton inlet and outlet sites are generally within 0.1 – 0.5 nm of the lipid headgroup surface; thus, they lie within the two water monolayers adjacent to the membrane surface" [16]. (Further discussion of these findings, along with the appropriate references, can be found in section VII of the Supporting Information for ref. [16].) Lee's claim that pumped protons are released into the bulk phase on the P side of the membrane is thus not supported.

We have discussed above the structural evidence concerning the proton inlet site in the F_1F_0 ATP synthase. The Dudkina, *et al.* figure shows the inlet flush with a line representing the middle of the lipid headgroup layer (4 nm across the bilayer). However, this inlet is surrounded by protein "hills" on all sides that protrude about 1 nm from the lipid headgroups. If the TELP layer is localized within 0.3 nm of the fatty acid surface, then how can these protons climb the 1 nm "hill" to get into the inlet site?

Thus, membrane protein structure literature does not support Lee's asymmetry claim (2). If this claim is false, then oxidative phosphorylation cannot be a "type-B energetic process". Note that we have not ruled out the possibility that type-B processes exist; in our critique we explained the arguments put forward by both the advocates and the critics of type-B processes, concluding that "this is a fascinating controversy, but its details lie well beyond the scope of this paper. Here we wish only to examine the evidence in support of Lee's contention that bioenergetic membrane asymmetry supports a type-B process" [16].

Thus, it was disappointing to find the following rather strident passage in Lee's Landmark Review: "Blind faith in the second law of thermodynamics could be such an inhibitory factor that might prevent certain people from discovering or understanding any Type-B energetic processes. For example, in his recent article [16], with little scientific evidence, Silverstein not only blindly denies the existence of asymmetric biomembrane [sic]... but also blindly assumes that the total pmf energy would precisely equal to the NADH redox chemical energy and to the ATP product energy. In his article, Silverstein did not provide any credible evidence to support his proposal... Furthermore, even if assuming his proposal would be true, then a mitochondrion with Silverstein's 'the total pmf energy would precisely equal to both the NADH redox chemical energy and the ATP product energy' would be in a thermodynamically equilibrated 'dead' state that could not really make any net ATP to support cell growth. From here readers may also be able to understand that Silverstein's article may represent an excellent example of a blind faith in the second law of thermodynamics with a self-prophezing and self-fulfilling approach to fit with his blind faith of [sic] oxidative phosphorylation does not violate the second law of thermodynamics" [1].

First we note that our denial of the asymmetry of bioenergetic membranes was not "blind", but supported by structural information cited from the peer-reviewed literature, as discussed above. Second, our conclusion that the chemical potential from spontaneous redox reactions ($\Delta\mu_{H^+} = -4.6$ kcal/mol H^+) turned out to be identical to that for ATP synthesis in the mitochondrial matrix (and more than that for ATP that is exported to the cytoplasm: $\Delta\mu_{H^+} = -4.2$ kcal/mol H^+) was also not "blind". It was supported by "credible evidence" that can be found in the meta-analysis presented in tables throughout section II of the Supporting Information [16].

Lee's claim of a "thermodynamically equilibrated dead state that could not make ATP" shows a lack of understanding of non-equilibrium thermodynamics. If a spontaneous process with $\Delta G_s = -x$ is coupled with a nonspontaneous process with $\Delta G_{ns} = y$, and $y/|x| < 1$, then the nonspontaneous process is driven forward with a thermodynamic efficiency of 100% $\cdot y/|x|$. However, even if the free energy difference for the nonspontaneous process is x , then the reaction still runs forward, except that it has the unusual thermodynamic efficiency of 100%. This is an unexpectedly high efficiency for a macroscopic machine, but has been shown to occur

for nanoscopic machines like actomyosin muscle contraction and ATP-driven rotary motion in the F_1F_0 complex. The fact that net ΔG is zero does not mean that the cell is “dead” as stated by Lee; it just means that after one accounts for the energy put in to make ATP, there is no free energy left to drive any other processes, and there is no waste heat given off. However, ATP is still made!

Perhaps the most problematic of Lee’s contentions is his claim that RT represents thermal energy in thermodynamic equations, and this kinetic energy can be used to drive ATP synthesis. He expressed his claim thusly: “In the local pmf equation, the isothermal utilization of proton thermal kinetic energy (kT) is inherently expressed as RT... This equation implies that the isothermal utilization of TELPs thermal kinetic energy (kT) must be a generic phenomenon in biological systems... That is, protonic isothermal utilization of environmental heat utilization [sic] is possible as long as there are transmembrane potential-associated TELPs”.

While it is true that one can calculate the translational kinetic energy of a mole of molecules as $3/2 RT$, the RT term in thermodynamic equations like Equations 1 – 4 does NOT signify kinetic energy, but rather chemical potential or free energy. The basis for this lies in the fundamental thermodynamic equation describing the dependence of chemical potential of solute i on its activity or concentration:

$$\text{Equation 6: } \mu_i = \mu_i^\circ + RT\ln(a_i) = \mu_i^\circ + RT\ln(\gamma_i c_i)$$

where μ_i° is the chemical potential (in kJ/mol) of solute i under standard conditions, a = activity, c = molar concentration, and γ = activity coefficient.

Because the log term is unitless, one can see from Equation 6 that the energy term RT (in kJ/mol) represents chemical potential (μ) rather than kinetic energy. Equation 6 is the basis of the equation describing the change in chemical potential when neutral solute i moves from phase 1 to phase 2:

$$\text{Equation 7: } \Delta\mu_i(1 \rightarrow 2) = \Delta\mu_i^\circ(1 \rightarrow 2) + RT\ln\left(\frac{[i]_2}{[i]_1}\right)$$

where we have assumed that $\gamma_i = 1$ in both phases.

And also the equation describing the dependence of a reaction’s standard free energy on equilibrium concentrations and temperature:

$$\text{Equation 8: } \Delta G_{\text{rxn}}^\circ = -RT\ln(K_{\text{eq}}) = -RT\ln()$$

The RT energy term in Equation 7 represents chemical potential, while in Equation 8 it represents free energy. Thus, we have shown that the RT energy term in all of these thermodynamic equations, including the chemiosmosis equations of Mitchell and Lee, represents chemical potential or free energy, and NOT, as claimed by Lee, thermal kinetic energy.

Applying the TELPs/TELCs hypothesis to neuronal transmembrane potential

The firing cycle of the neuron (Table 4) has been studied since the early 20th century. By the 1940s-50s, Hodgkin and Huxley had developed their cable theory along with the Goldman-Hodgkin-Katz (GHK) equation to explain the changes in transmembrane potential. This theory is widely accepted, resulting in Nobel Prizes for Hodgkin and Huxley (1963), and for Katz and Axelrod (1970). The theory explains neuronal changes in membrane potential as resulting from the activities of ion pumps and gated and ungated ion channels.

Lee’s application of his TELC hypothesis to neuronal membrane potentials has been extensively critiqued elsewhere [39,62]. Here I will only deal with issues raised in his new Landmark Review. The first problem is that by stipulating $E = 0$ in the neuronal cytoplasm in his Figures 8 and 10, Lee seems to be invoking Gauss’s law. However, as we discussed above, Gauss’s law applies to electrons moving within a solid metal conductor. Aqueous ions (which are much larger than electrons) moving within liquid water (a poorer conductor than metal) are subject to Brownian motion and entropy. Their diffusion would NOT be expected to be limited by Gauss’s law, and thus one would not expect their location to be limited to the surface of the water “conductor”.

But the main problem with the TELC hypothesis as it applied to neuronal potentials is that it was developed after the discovery of the importance of neuronal ion pumps and channels. Hence, it explains the same phenomena that cable theory and the GHK equation explain, as summarized in Table 4.

The neuronal firing cycle, with the timing of membrane potential changes (Table 4, first three columns) were reported in the literature in the 1930s and 1940s. Hodgkin and Huxley developed

Cycle time ^a (ms)	Firing phase	$\Delta\psi$ (mV)		Ion transport	GHK eqn. ^c $P_{K^+} P_{Na^+}$	TELC ^d (#/μm ²)
1	Resting	-70		Na/K pump	1 0.025	-3900
2	Threshold	-55		Open NT-gated cation channel	1 0.12	-3100
2.5	Action pot'l	+30		Open V-gated Na ⁺ channel	1 6.5	+1700
2.75	Recovery	-18		Open V-gated K ⁺ channel	10 6.5	-1000
3.5	Hyperpol.	-90		Close V-gated Na ⁺ channel	10 0.025	-5100
5	Refractory ^b	-70		Close V-gated K ⁺ channel	1 0.025	-3900

Table 4: Neuronal firing cycle explained by cable theory/GHK equation and by Lee's TELC hypothesis.

^a cycle times are from Lee's Figure 9

^b The refractory period lasts several ms, until the inactivated V-gated channels are reset. Only then can the neuron be re-excited by neurotransmitter (NT) binding to receptor.

^c Permeability coefficients are relative to the resting K⁺ permeability. $P_{Cl^-} = 0.45$ throughout the cycle.

^d number of excess charges per μm² localized at the cytoplasmic membrane surface; negative numbers signify anions (especially OH⁻, but also Cl⁻, H₂PO₄⁻, HCO₃⁻); positive numbers signify cations (especially H⁺, but also Na⁺, K⁺, ½ Ca²⁺, ½ Mg²⁺); an equal number of opposite charges are localized at the extracellular membrane surface, according to the hypothesis.

their cable theory in the 1940s and 1950s (Table 4, columns 4 and 5) to explain the firing cycle. Since the 1960s, much evidence has accrued in identifying and characterizing the ion pumps and channels specified. In 2020 Lee first proposed his application of the TELC hypothesis to explain the neuronal firing cycle. The upshot of this sequence of events is that both cable theory and the TELC hypothesis explain the same set of events in the neuronal firing cycle, using the same ion pumps and channels. This makes it difficult, at first glance, to devise experiments that allow one to support one model over the other.

As one example of the difficulty of distinguishing predictions from cable theory vs. the TELC hypothesis, we note that Lee presented time-integrated equations for transmembrane potential (his Equations 12 and 13), but he neglected to mention that these equations were in fact used first by Hodgkin and Huxley in their cable theory. That is not necessarily a problem, but Lee has presented no evidence that allows one to choose between cable theory and his TELC hypothesis. In fact, there is (as of yet) no published experimental work at all that measures the predicted

TELC charges localized at the surface of the neuronal plasma membrane.

At least four times in his Landmark Review [1] including both delocalized and localized protonic coupling. According to the TELCs model, the excess positive charges of TELCs at one side of the membrane are balanced by the excess negative charges of transmembrane-electrostatically localized hydroxide anions (TELAs Lee elucidates a "prediction" regarding the neuronal firing cycle that is "exactly" observed in experimental results:

- "If the V-gated sodium channel activity is not separated from the V-gated potassium channel activity, their effect [together] to drive the rising and falling phases of an action potential may cancel each other..."
- "the voltage-gated Na⁺ channels are required to fire action potential spikes..."
- "genetic knockout or mutation of critical ion channels such as the V-gated Na⁺ or K⁺ channels would abolish action potential firing..."

- "... clustering of ion channels... at the axonal initial segment [i.e., terminal] and nodes of Ranvier ... [underlie] the ability to fire action potential spikes and saltatory conduction along a myelinated axon".

The problem with these "predictions" is that they were reported in the literature decades ago, and have long been key components of our understanding of neuronal excitability. Thus, these are not "predictions" at all, but rather prior experimental observations that both the cable theory and the TELC hypothesis were built around. It is thus not surprising that "all these 'predictions' from the TELCs [and cable] model have been experimentally observed exactly in many well-established electrophysiological phenomena..." [1].

As noted above, it has so far been difficult to identify bona fide predictions from the cable theory and TELC hypothesis that would allow one to distinguish between the two models. As one final example of this problem, Lee wrote that "... the GHK [cable] model appears to require continuous ion pumping across membrane [sic] which would be energy intensive. The TELCs model appears to be energy-efficient owing to TELCs-membrane-TELAs-capacitor energy storage, without requiring continuous ions pumping [sic] across the membrane". However, in actuality there is little difference between these two models in terms of the energy efficiency of the Na/K ATPase. Cable theory requires Na⁺/K⁺ pumping until the bulk ion gradients are established, after which pumping is only required after several dozen action potentials, in order to replenish the gradients. TELC requires Na⁺/K⁺ pumping until the protonic/cationic capacitor is charged, after which pumping is only required after several dozen action potentials, in order to recharge the capacitor. So what is the difference?

On the other hand, the role of the Na/K ATPase does present an opportunity to distinguish between predictions from the two theories. According to cable theory, the only role of the Na/K ATPase is as an antiporter ($3 \text{ Na}^+_{\text{out}}/2 \text{ K}^+_{\text{in}}/1 \text{ ATP}$) that maintains bulk phase ion gradients of Na⁺ high outside, K⁺ high inside, and Cl⁻ high outside via leak channels. The electrogenicity of this pump plays no role at all in the establishment of the polarized, inside-negative transmembrane potential. For example, Chrysafides, *et al.* wrote "We must stress that while [the electrogenicity of] this pump does not make a significant contribution to the charge of the membrane potential, it is crucial in maintaining the ionic gradients of Na⁺ and K⁺ [and Cl⁻] across the membrane. What generates the

resting membrane potential is the K⁺ that leaks from the inside of the cell to the outside [and Cl⁻ from out to in] via leak channels, generat[ing] a negative charge in the inside of the membrane vs. the outside" [63]. According to the TELC hypothesis, the electrogenicity of the Na/K ATPase is a key component of the polarized neuronal resting potential. Lee wrote: "this $3\text{Na}^+/2\text{K}^+$ transport process results in a net translocation of one positive charge... from inside the neuronal cell to the outside... result[ing] in the accumulation of excess Na⁺ cations (positive charges) outside the neuron..." These "excess Na⁺ cations" are electrostatically localized at the surface of the neuronal cell membrane, forming a biocapacitor (along with anions at the opposing surface).

Thus, one way to distinguish between these two models would be to measure the neuronal transmembrane potential in the presence of appropriate bulk phase ion gradients but in the absence of a functioning Na/K ATPase (i.e., ouabain-inhibited, or ATP-depleted). Cable theory would predict a normal, polarized resting potential of -70 mV in these neurons, whereas the TELC hypothesis would (presumably) predict depolarized resting potential of 0 mV. In fact, these experiments were some of the very first ones that Hodgkin, *et al.* performed and published [64,65], see also [66]; their finding of a normal resting potential in the absence of any Na/K pumping activity was a key part of their cable theory. On the contrary, these classic results argue against trying to explain the neuronal resting potential using the TELC hypothesis.

Summing up this final section, Lee stated repeatedly in his Landmark Review that TELC provides a better, clearer, or more "exact" explanation of neuronal potentials, but in fact, his hypothesis simply presents an alternative explanation. His hypothesis makes predictions about: (i) the number density of localized excess charges at each membrane potential, but there are (as of yet), no experimental measurements to support or challenge these predictions; and (b) the role of Na/K ATPase electrogenicity that has been disproven in the literature. Thus, what we have at this point is the cable theory/GHK equation, with its 70 years of experimental support, and the TELC hypothesis, with (as of yet) no neuronal experimental support at all. As we wrote in our previous critique: "For Lee's TELC equations to be considered a reasonable alternative to the GHK equation, experimental measurements or molecular dynamics simulations of surface concentrations of key ions (e.g., H⁺, OH⁻, Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻) that agree with TELC

predictions must be reported, peer-reviewed, and published. Short of that, scientists are well-served continuing to employ the GHK equation" [39].

Conclusions

Regarding the potential well/kinetic barrier hypothesis, Lee has claimed that (i) it is not "needed" because his TELP hypothesis explains localized chemiosmosis; and (ii) a barrier would disallow any exchange between surface and bulk protons.

Claim (i), runs counter to the scientific method: If one hypothesis explains the data, that does not mean that a second hypothesis is "not needed". One must devise experiments to choose between two valid hypotheses. On a related note, Lee has often conflated support for localized chemiosmosis with support for his TELP hypothesis.

Claim (ii) is based on a misunderstanding: While an infinitely high potential barrier would disallow proton exchange, the barrier proposed by Junge/Mulkidhanian/ Cherepanov is finite; it merely slows mixing.

Lee's TELP hypothesis is based on applying the physics of electronic capacitors to the biochemistry of a putative protonic capacitor. But differences between electrons traveling in a solid metal conductor and hydrated protons traveling in liquid water are substantial. Unlike electrons, aqueous protons are subject to Brownian motion and entropy, which would destabilize any membrane surface protonic capacitor.

In his modified protonmotive force (pmf) equation, Lee assumes that protons driving ATP synthesis flow from the P-side L phase to the N-side bulk phase, but there is currently no evidence that the proton channel outlet of the F_0 protein extends into the N-side bulk phase. Furthermore, the F_0 proton inlet protrudes at least 1 nm from the membrane surface, but the P_L protons are localized in a layer that is only 0.3 nm thick.

In his modified protonmotive force (pmf) equation, proton activity coefficients do not appear; thus are assumed to be ≈ 1 . However, L layer proton concentrations exceed 0.01 M, and the structure of water in the headgroup region differs from bulk water (dielectric constant= 10 -30 vs. 78), thus, the proton activity coefficient in the L layer is likely to differ greatly from unity. Hence, Lee's equation cannot be used to calculate an accurate value of "local" pmf.

There is no evidence supporting the existence of two distinct hydration layers within the lipid bilayer headgroup region: a partial hydration layer 0.3 nm from the nonpolar interface, and a full hydration layer elsewhere. Experimental measurements show both water concentration and dielectric constant in the headgroup region decline smoothly from the bulk phase down to just past the nonpolar interface.

Lee proposed his equation (5) to calculate the full attractive force between an L layer proton and all interacting anions on the membrane's opposite surface. This equation includes ambiguities and errors, including

- A parameter, γ , whose value is not clearly explained
- A $\sin(a)$ term that should not be present
- An incorrect value of the dielectric constant (1.88 vs. 3)

Lee's argument supporting the stability of the putative protonic capacitor is that the calculated attractive force is 3.8-fold stronger than kT . However, this argument considers only enthalpy. The negative entropic force between an L layer whose proton concentration is 10^5 -fold higher (pH 2 vs. pH 7) than the bulk phase is more than enough to destabilize the L layer.

Lee's claim that cited literature measurements of pH at the bioenergetic membrane surface are actually bulk phase values is false. The reported surface pH values are substantially lower than the bulk phase values, and the difference is statistically significant.

Proton enrichment at the lipid bilayer surface (pH 3.3 – 5.5) is about an order of magnitude greater than at the water/nonpolar interface (pH 4.4 – 6.6). This enhanced effect of the amphiphilic nature of the lipid headgroup region has not been reported previously.

From the meta-analysis of reported literature measurements of pH at the membrane surface (Table 3), the depth of the potential well supporting this surface proton enrichment is 4.6 – 8.5 RT.

Lee's claim that any enriched surface protons that "are not TELPs... are irrelevant to the protonmotive force" [1], runs counter to a report [54] that ATP synthesis was catalyzed by F_1F_0 ATP synthase localized at the octane/water interface ($\Delta\psi = 0$). This shows that that a TELP protonic biocapacitor, if it exists at all, cannot be the only source of interfacial protons driving localized chemiosmosis.

Regarding the $\Delta\psi$ measurements reported by Wolf, *et al.* Lee opined that “non-Nernstian TMRE fluorescence in the mitochondria [sic] membrane may represent the transmembrane-electrostatic partitioning”; however, Wolf, *et al.* specifically ruled out dye “partitioning... based on non-Nernstian binding...” [55].

Lee imputed to his TELC hypothesis conclusions that derive from basic physics and biochemistry: If apposing membranes at a cristae junction pinch together and close off the crista, then membrane topology (not the TELC hypothesis) requires that such an isolated crista will act as “an independent bioenergetic unit”.

Using Arrhenius/Eyring plots of proton diffusion rate constants along their bacteriorhodopsin purple membrane fragments Heberle, *et al.* reported a potential barrier between surface and bulk of 22 RT. This matches the barrier of 20 – 30 RT reported by Weichselbaum, *et al.* Lee has not attempted to explain these results.

In arguing for the existence of transient non-zero transmembrane potentials across these purple membrane fragments (due to electrogenic proton pumping), Lee neglected to consider the fast diffusion of Cl^- counterions (as well as K^+), present as 0.15 M KCl in the buffer.

Lee’s explanation of the water/decane transient proton wave reported by Zhang, *et al.* using Fick’s law, instead predicts a permanent wave (i.e., asymptotic maximum concentration). His prediction of the effect of added NaCl runs counter to the fact that electrolyte conductivity increases with ionic strength.

Lee’s arguments questioning the height of the 20 -30 RT potential barrier employed hydrogen bonds between neutral bulk phase water molecules, rather than the relevant H-bonds between hydrated protons at the membrane surface (where the dielectric constant is much lower).

Lee claims that the black stains on the Al films in his water electrolysis system are due to proton-induced corrosion, but he never confirmed this by exposing the films to acidic buffers, and thus presented no proof that the black stains signify the presence of protons.

The proton-cation exchange equilibrium constants of 10^{-8} suggest a much stronger affinity for protons over Na^+ or K^+ in the surface-localized L layer. Based on the similar sizes of these ions,

and the much higher hydration free energy for H^+ , this is hard to rationalize.

Lee’s estimation of the attractive forces stabilizing a protonic capacitor across the 75 μm electrolysis membrane contained a number of errors. Using his own Equation (5), we calculated an attractive force over 4000-fold less than $k_{\text{B}}T$. Thus, a protonic capacitor across this macroscopic membrane would not be stable.

Lee claimed that oxidative phosphorylation is a “type-B energetic process” that is allowed to violate the second law of thermodynamics due to its membrane asymmetry. Based on recent structural reports, the transmembrane asymmetry does not in fact exist; and based on a meta-analysis of reported $\Delta\psi$ and ΔpH values, it turns out that oxidative phosphorylation does not violate the second law.

Lee claimed that the RT energy parameter in pmf equations represents thermal energy that is available to isothermally drive ATP synthesis in a type-B energetic process. In fact, RT in the relevant thermodynamic equations signifies chemical potential energy or free energy, not heat.

Lee has claimed that his TELC hypothesis provides a better explanation of neuronal transmembrane potential than Hodgkin-Huxley cable theory and its attendant Goldman/Hodgkin/Katz equation. In fact, both models explain the same set of experimental results, hence the examples cited by Lee do not allow one to choose between them. On the other hand, the TELC hypothesis invokes the electrogenicity of the Na/K ATPase as a source of the polarized neuronal transmembrane potential, but early published results showing the effects of bulk phase ion concentration on transmembrane potential in the absence of a functioning Na/K ATPase support cable theory and argue against the applicability of the TELC hypothesis to this system.

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