Refuting the ideas advocated by Yuly et al. (PNAS, Sep. 2020): ‘Universal free energy landscapes’ and ‘deterministic electron-relay circuitry’ are unsustainable within membrane-embedded cytochrome *b* protein complexes involved in bioenergetic routines
(A letter to the Editor)

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Contextual background: This communication discusses the interactions/outcomes of isoprenoid quinones/quinols (Q/QH₂) and membrane-bound cytochromes involved in bioenergetic routines. Particularly, we use qualitative and quantitative arguments to counter the idea that highly deterministic electron relays are triggered within Complex III of mitochondria, resulting from the donation of two electrons by QH₂ (Yuly et al., 2020). In this regard, at least two hypotheses claim to explain the role(s) of membrane-embedded Q/QH₂ in vital metabolic processes such as mitochondrial oxidative phosphorylation (OxPhos) and chloroplastid/cyanobacterial photophosphorylations (PhotoPhos).

[i] In the classical 2e⁻ or proton-centric ‘chemiosmotic rotary ATP synthesis’ (CRAS model, driven by trans-membrane potential or TMP) (Boyer, 1997; Mitchell and Moyle, 1967), quinones are seen as highly mobile 2e⁻-transporting agents from Complexes I/II to Complex III of mitochondria or from Photosystem II to Cytochrome *b₆f* in chloroplasts. Q-cycle is a multi-step and multi-molecular sequential reaction in which two molecules each of quinol (QH₂) and cytochrome *c* (Cyt. *c*) /plastocyanin (PC) interact with one molecule of cytochrome *b* complex (CBC = cytochrome *bc₁* or Complex III in mitochondria / cytochrome *b₆f* in chloroplasts) bound to one molecule of Q (ubiquinone or CoQ in mitochondria and plastoquinone or PQ in chloroplasts) (as shown in Figure 1, for mitochondria). The balanced equation and thermodynamic yield of reactions classically prescribed for CBCs and

alternate/terminal oxidases (which also oxidize quinols) are shown in the bottom of Table 1. This elaborate set of interactions at CBC produces two molecules each of Q and reduced Cyt. *c* plus one molecule of QH₂. Besides serving as a mechanism for the regeneration of oxidized Q and reduced Cyt. *c** from QH₂, 8 protons are simultaneously pumped out from the matrix into the inter-membrane space (IMS) (or from stroma into lumen in chloroplasts), per four electrons going through the Q-cycle. These pumped-out protons (PoPs) are supposed to aid ATP-synthesis, upon their return to the matrix via Complex V. Therefore, Q-cycle caters to the electron transport chain (ETC) and PoPs facets of the CRAS model of bioenergetics. For orchestrating the multi-molecular Q-cycle across the three phases of the organelle (IMS, inner phospholipid membrane or IPLM and matrix in mitochondria; stroma, thylakoid membrane and lumen in chloroplasts), highly sophisticated electronic circuitry (EC) and electron bifurcation (EB) switches are supposedly operative within the CBC. In this regard, within each half of the purported Q-cycle, EB ensures the deterministic changing the course of the two electrons donated by QH₂, one each to Cyt. *c* (bound at the IMS side) and Q (bound at CBC within the trans-membrane region). Therefore, within one leg of a given half of the Q-cycle, EB enables a lower mid-point potential center to receive electrons from a higher mid-point potential center (the downward dotted curve in the trans-membrane phase of the CBC, left panel of Figure 1).

(ii) In the new 1e or oxygen-centric murburn model (driven by diffusible reactive species, DRS), Q/QH₂ are transient one- or two- electron sinks in the membrane phase (Manoj, 2017; Manoj, 2018; Manoj et al., 2019a,b; Manoj, 2020; Manoj et al., 2020a). Further, Q/QH₂-CBC-O₂-DRS interactions reset one-electron reactions in milieu. Thus, they help in recycling electrons lost to the membrane phase, generating DROS (diffusible reactive oxygen species) in the matrix or reduced cytochrome *c* in the IMS. Examples of mass-charge balanced equations and schematics of murburn interactions are shown in top parts of Table 1 and right panel of Figure 1. DROS in the context above imply various diffusible protonated or deprotonated species of oxygen-centered ions and radicals, also including singlet oxygen. So, oxygen can gain an electron to become a DROS like superoxide, which could react with another molecule or ion in milieu, to gain an electron in phosphorylation reactions (or lose the electron to Q in the membrane) and become yet another DROS like peroxide in matrix. In turn, this can be broken into more DROS like hydroxyl radical and hydroxide ion. The term murburn connotes 'mured burning' (or closed burning); involving interactive equilibria among molecules, unbound ions and radicals (DROS); signifying a process of "mild/moderated unrestricted redox catalysis" by various proteins in and around the membrane. Murburn schemes might invoke an enzyme-substrate complementation (like classical enzymes), but this aspect is not obligatory. With respect to CBC, this mechanism involves fast bi-

molecular collisions (singlet oxygen + quinol; superoxide + quinone; oxygen + semiquinone; etc.) and unordered interactive electronic equilibriums of O₂/DROS/Cyt. c with the redox centers of CBCs in all three phases. The murburn model for mitochondrial OxPhos or chloroplastid PhotoPhos is a simple stochastic model, which does not seek that CBC must possess specific Q/QH₂ binding sites or any deterministic/sophisticated ETC layouts or EB switches.

Figure 1: Classical (left) and murburn (right) purviews for the roles of Complex III, quinones and cytochrome c. In the classical scheme, the outer and inner phospholipid membranes (OPLM and IPLM, respectively) are restrictive with respect to oxygen and protons. Binding of one molecule each of QH₂ & Q at two designated loci (+,-) within the IPLM and a molecule of oxidized cytochrome c at the IMS regions are mandatory for enabling the deterministic EC/EB (shown by dotted lines) and generating PoPs (shown by upward arrows). The matrix-ward bulbous domain (shown in orange) has no function whatsoever. In the murburn scheme, binding of quinones/quinols or cytochrome c to CBC is not mandatory, but their interactions with O₂/DROS is the highlight in this scheme. Within this purview, the membranes are not restrictive with respect to protons/O₂ and the matrix-ward extension has ADP-binding sites which enable efficient presentation of the same to DROS or DROS-activated phosphate ions. In this context, the arrows show the mobility/reaction of electrons, protons, oxygen and DROS.

Table 1: Comparison of the classical and murburn (involving Q, electrons, protons, oxygen and DROS) models (Manoj and Bazhin, 2019). (Q stands for ubiquinone or CoQ₁₀ and values in bold connote nons-spontaneity.)

No.	Reactions in physiological conditions					$\Delta_r G'^0$ (kJ/mol)
	Examples of Q-murburn equilibriums					
1	Q	+	e_{aq}^-	$\rightarrow$	$Q^{\bullet-}$	-247
2	$^*Q^-$	+	$^*O_2^- (+2H^+)$	$\rightarrow$	QH_2 + O_2	-118
3	$^*Q^-$	+	$^*O_2^- (+xH^+)$	$\rightarrow$	Q + HO_2^-/H_2O_2	-114 / -179
4	$^*Q^-$	+	$^*O_2H(+H^+)$	$\rightarrow$	QH_2 + O_2	-93
5	$^*Q^-$	+	$^*O_2H(+H^+)$	$\rightarrow$	Q + HO_2^-/H_2O_2	-89 / -153
6	$^*Q^-$	+	$^3O_2 / ^1O_2$	$\rightarrow$	Q + $^*O_2^-$	-2.9 / -96
7	QH_2	+	1O_2	$\rightarrow$	$Q^{\bullet-}$ + $^*O_2H (+H^+)$	-0.5
8	QH_2	+	$^3O_2 / ^1O_2$	$\rightarrow$	Q + H_2O_2	-61 / -154
	Examples of H-murburn equilibriums					
9	*H	+	Cyt.c _{ox}	$\rightarrow$	Cyt.c _{red} H	-285

10	*H	+	$^3O_2 / ^1O_2$	$\rightarrow$	H^+	+	$^*O_2^-$	-211/-304
11	H^+	+	OH^-	$\rightarrow$	H_2O			-79
12	H^+	+	HO_2^-	$\rightarrow$	H_2O_2			-65
13	H^+	+	$^*O^-$	$\rightarrow$	*OH			-64
14	H^+	+	e_{aq}^-	$\rightarrow$	*H			-39
15	H^+	+	$^*O_2^-$	$\rightarrow$	*O_2H			-26
Classical or CRAS reactions								
A	$2QH_2(+Q)$	+	$2Cyt.c_{ox}(+8_iH^+)$	$\rightarrow$	$2Q+QH_2$	+	$2Cyt.c_{red}H(+8_oH^+)$	-33 (+160)= 127
B	$2QH_2$	+	$^3O_2(+8_iH^+)$	$\rightarrow$	$2Q$	+	$2H_2O(+8_oH^+)$	-156 (+160)= 4

Critiquing the work of Yuly et al. (PNAS, 2020): In their computational work, Yuly et al. assumed that Q-cycle is operative at CBC and used the Marcus outer sphere electron transfer (ET) theory to model the free energy landscape (FEL) of the ET phenomena in CBC (Yuly et al., 2020). They project that Q-cycle reactions are energetically/kinetically viable and they infer that their results connote the existence of a ‘universal FEL for efficient EB in CBCs’. Marcus theory (Marcus, 1953) is an amalgamation of thermodynamics-based derivations from electro-chemical potentials, Eyring’s transition state theory, Arrhenius rate law and Franck-Condon principle. It is often used to predict electron transfer rates in biological systems (Gray and Winkler, 1996; Page et al., 2003). Free energy landscaping (FEL) enables a topological visualization of the correlations of diverse thermodynamic/kinetic variables with respect to the various conformations existing in the system. This is carried out to afford a dynamic picture of some desired aspects (like stability, reaction rate, etc.) of the system. The methodology Yuly et al. adopted involves attributing the diverse redox centers of CBCs with various oxidation states and redox potentials at various temporal coordinates; with the redox centers given relatively constrained spatial loci (inter-center distances). To cater to their chosen mandate, they weighted their model by avoiding too high or too low energy states of intermediates. They derive results to demonstrate that a ‘universal FEL’ can exist that enables viable and bifurcated or confurcated electron transfers (computed in flux/second) in equilibrium or steady-state, without short-circuiting or energy-dissipation.

Our concerns on Yuly et al.’s assumptions/findings are: (i) While a multi-redox-centered protein (such as the CBC) can be constrained and modeled for EB in silico with a view to ensure directional ET giving the preferred end-products, this does not necessarily imply that such an ET or EB option is available or feasible in physiological ambience. (ii) If there is a ‘universal free energy landscape’ of electron transfer mechanism across the CBCs, we would expect it to reflect in some kind of structural conservation across various organisms. The *b* subunit of Complex III has two hemes, the *c* subunit has one heme and the ISP (iron-sulfur protein) subunit has one 2Fe-2S center (Xia et al., 1997). Though the bacterial cytochrome *bc*₁ complex (if rarely present!) has only 3 subunits, the vertebrate equivalent has 11 subunits (including

the three of prokaryotes). In plant chloroplasts, the equivalent of Cytochrome *b₆f* complex monomer has 8 subunits. Besides having some redox centers and similar subunits in common with the mitochondrial counterpart, the chloroplast complex has yet another heme in the trans-membrane region and also has the photo-active/redox-active molecules of chlorophyll *a* and β -carotene (Kurisu et al., 2003). The structural diversities of some of the diverse CBCs are depicted in Figure 2, and the pertinent legends further emphasize our arguments in this regard, which are strong qualitative arguments that disclaim the classical mechanism's conservation across species. Further, Complex III is expected to function in monomeric, dimeric and respirasome configurations (Crofts et al., 2008; Nałęcz, 1986). Further, the CRAS mechanism does not have any roles attributed at all for the matrix-subtending portions of Complex III. Furthermore, it is highly unlikely that the multitudes of variations within the dielectric ambiances of these proteins/subunits could ever permit the conservation of a viable ETC-EB-FEL across diverse CBCs' functioning. (iii) Electron transfers were supposed to occur by mobile functions of the Fe-S module (Zhang et al., 1998) and specific amino acid residues were also supposed to move for enabling proton gating/pumping functions (Crofts et al., 2006). We could not see how these classical perspectives are reflected and accommodated in Yuly et al.'s works. (iv) Very importantly, some redox centers (like heme *b* of Complex III) are easily solvent accessible (Hunte et al., 2000) and the CBCs are notorious for generating DROS (Drose and Brandt, 2008). Since DROS-production by mitochondrial and chloroplast CBCs is an established experimental fact (Baniulis et al., 2013; Bleier and Dröse, 2013), short-circuiting by O₂/DROS is a reality that counters the concept of deterministic ETs in CBCs. Experts have traditionally perceived short-circuits within the Marcus outer sphere model, and seldom considered the ability of O₂/DROS/ions ability to serve as electron-relays or catalytic agents. These mobile species' dimensions are less than a couple of angstroms and they can easily move several tens of Angstroms in a microsecond within the reaction zone (Manoj et al., 2020a). These were overlooked under the misperception that diffusible reactive species have little roles in productive physiology, and this perception has been abrogated with the advent of murburn concept. (v) The kinetics and energetics of individual steps do not permit the feasibility of Q-cycle at all of the three CBC-systems explored herein, as demonstrated in Table 2. It can be seen that the energy and kinetics terms offer barricades at different steps of the Q-cycle, which clearly indicate the lack of conservation mechanisms (as seen from a quantitative perspective).

Figure 2: Perspectives of relevant redox centers of CBCs, as available from crystal structures. Redox cofactors of some CBCs from mitochondrial (top row, left to right: buffalo, chick and yeast), chloroplasts (bottom left and center, spinach and chlamydomonas) and cyanobacteria (bottom right, nostoc) are shown with the corresponding counterparts respectively color-coded. The monomers from the dimeric complexes are aligned in a similar fashion for an approximate visual comparison. Clearly, the distances, orientations, number of redox centers and the binding sites of Q/QH₂ or inhibitors are seen/shown to vary. BCR- β -carotene, PQ- plastoquinone, Q- ubiquinone, TDS- tridecylstigmatellin, ChlA- chlorophyll A, Stg/StgA- stigmatellin(A).

Table 2: An assessment of the energetics and kinetics of individual steps of Q-cycle in mitochondria, chloroplasts and cyanobacteria. (The unfavorable values are highlighted in bold.)

No.	Step	From (mV)	Distance (Å)	$\log k_{ET} (s^{-1})^*$	To (mV)	Yield (kJ/mol) [#]	Viable? [§]
<i>CoQ cycle at Cyt. bc_1 in mitochondria = -35.2 kJ/mol</i>							
1a	CoQH ₂ to FeS	45	7	10.6	280	-45.4	Yes
2a	FeS to Heme c_1	280	27	-3.8	215	6.3	No
3a	Heme c_1 to Cyt. c	215	4	10.6	235	-1.9	Yes
4a	CoQH ₂ to Heme b_{lp}	45	12.4	4.4	-30	14.5	No
5a	Heme b_{lp} to Heme b_{hn}	-30	20.5	0.9	30	-5.8	No
6a	Heme b_{hn} to CoQ	30	4	10.6	45	-2.9	Yes

<i>PQ cycle at Cyt. b_6f complex in chloroplasts = -43.5 kJ/mol</i>							
1b	PQH ₂ to FeS	80	26.4	-1.0	335	-49.2	No
2b	FeS to Heme <i>f</i>	335	26.9	-3.1	365	-2.9	No
3b	Heme <i>f</i> to PC	365	16.7	2.9	375	-1.0	No
4b	PQH ₂ to Heme b_{lp}	80	16.8	-1.0	-150	44.4	No
5b	Heme b_{lp} to Heme b_{hn}	-150	20.3	1.2	-50	-9.7	No
6b	Heme b_{hn} to PQ	-50	27.1	-2.1	80	-25.1	No
<i>PQ cycle at Cyt. b_6f complex in cyanobacteria = -13.5 kJ/mol</i>							
1c	PQH ₂ to FeS	85	19.8	2.8	310	-43.4	No
2c	FeS to Heme <i>f</i>	310	26.8	-2.9	365	-5.3	No
3c	Heme <i>f</i> to Cyt. c_6	365	15.5	3.4	340	2.4	No
4c	PQH ₂ to Heme b_{lp}	85	19	-1.0	-75	30.9	No
5c	Heme b_{lp} to Heme b_{hn}	-100	20.4	0.9	-50	-4.8	No
6c	Heme b_{hn} to PQ	-50	12.7	4.8	-85	6.8	No

*Assuming ρ (packing density) as 0.76 and λ (reorganization energy in eV) for proteins 0.7, the electron transfer kinetics (as seen from a transformed Marcus equation) is dependent on inter-center distances and driving force of electron transfer (Moser et al., BBA 2006): $\log k_{ET} = 15 - 0.6 R - 4.4 (\Delta G^\circ + 0.7)^2$. Here, k_{ET} is the number of electrons transferred per second; usually perceived to be anywhere between 10^3 to $10^{12} s^{-1}$; R is the distance between the redox centers in Angstroms; ΔG° is the driving energy in eV. Though the constants and variables in the equation cannot be ascertained as experimental or theoretical truths/facts, the relation gives an 'expert consensus' to correlate observations and study the phenomena of ET in biological systems. A calculation by another group assuming different criteria for Marcus equations had shown that practically all steps of the ET at CBC were unviable (Xia et al., 2013). Earlier publications show the details of distances and potentials in the CBCs (Crofts, 2004; Govindjee and Björn, 2017; Manoj et al., 2020a; Tschörtner et al., 2019)

#Yield = $-n.F.\Delta E_{m,7}$; where n is the number of electrons, F is Faraday's constant and $E_{m,7}$ is the standard midpoint potential.

§ If $\log k_{ET} < 3$ or if yield > 0 , then the step is deemed unviable. Since the overall pseudo-first order turnover rate is in the order of milliseconds in biological systems (as measured by the uptake or release of say, oxygen molecule), the rate of ET of each step within the purported ETC must be significantly higher than this rate. Further, since the reactions are supposed to work spontaneously, the standard Gibbs free energy change should be negative. The bold 'No' indicates that the step is unviable energetically and kinetically.

In the light of the qualitative and quantitative disparities above, we doubt if the EB model for CBC would have the projected utility for explaining the physiological functioning of mitochondria or chloroplasts. We clarify our apprehensions on a larger viewpoint below.

What is our take on the subject? Our concerns are on two fronts:

(1) Is the Q-cycle a matter of necessity with respect to the OxPhos or Photophos routines? The quest for the mechanism for EB has been fueled by mandate imposed by the concepts of bioenergetic membrane proteins serving as electron transport chain (ETC) and proton pumps, which are an integral component of the CRAS scheme. Several aspects of the CRAS proposal have been questioned over decades by very seasoned scientists (Gideon, 2020). Recently, the ETC-CRAS ideas were demonstrated to be an untenable and redundant concept, as seen from a multitude of perspectives (Manoj's group's works since 2017). Advocates of CRAS have periodically changed the original proposals in the light of criticism and this cannot be a saving grace for its untenable assumptions and explanations. Even if Complexes I/II and Photosystem II can form quinols from quinones, and even if CBCs can form reduced cytochrome *c*

and reduced plastocyanin (respectively) from quinols, it does not necessarily mandate that a ‘classical bifurcation’ (electron going from quinol back to quinone) occurs at the CBC. Photosynthetic organisms can grow despite a dysfunctional CBC (Malnoe et al., 2011) and thrive even without PC (Pesaresi et al., 2009). Therefore, we need not see CBC as a sponsoring agent for PoPs or EB anymore. It must be remembered that Nalesz (a seasoned researcher on CBCs) and Mitchell (the promulgator of PoPs) did not consider Complexes III and IV as proton pumps (Moyle and Mitchell, 1978; Nałęcz, 1986). Further, there exists little consensus among the experts on these two proteins’ roles as proton pumps (Manoj et al., 2020a). Therefore, the Q-cycle is an experimentally non-demonstrable proposal, without groundings in reality. Further, the alternative and/or terminal oxidases of several bacteria are known to process diverse quinols, oxidize them and give water (plus PoPs, as per the classical scheme). We present a visual spread of these membrane-bound complexes in Figure 3. Quite evidently, there is no scope for the elaborate conservation of structural or mechanistic scope for the classical/CRAS mechanism in such proteins.

Figure 3: A comparison of redox cofactors of various quinol oxidases of diverse bacteria. The protein structures (not shown), ET redox cofactors, the distances between them and relative orientations thereof, etc. are disagreeable with the conservation of any canonical ET routes.

(2) Regardless of the reality above, is the Q-cycle (via EB) feasible at the CBCs? Feasibility of any reaction is determined by energetics, kinetics, mechanistics and overall probabilistic scope. In the fourth leg of Q-cycle, we have to consider the absolutely improbable premise that an electron gets routed from a semiquinone (bound at the QH₂ site or p-site) to another semiquinone (bound at the Q site or n-site), via the intermediacy of two hemes; that too over a conservative distance of 50 Angstroms, across unfavorable thermodynamic gradients and unfeasible kinetic frames, in the presence of blocks and leaks, and without facilitators! To drive the last nail in the coffin of Q-cycle, we present Table 3, which is a layout of some critical queries that take a stock of the necessity for conservation of Q-cycle mechanism as a fundamental component of bioenergetic routines in various life forms. If there were a common origin to prokaryotes, mitochondria and chloroplasts, and if the endosymbiosis hypothesis is true, then, since - (i) all organisms with mitochondria have a common ancestor (Martin and Mentel, 2020) and since the organisms with both mitochondria and chloroplasts clade well within this system (that is- the organisms with chloroplasts also have mitochondria but the vice versa is not true), (ii) respiration is a purely downhill catabolic process whereas photosynthesis is essentially anabolic, (iii) heterotrophy is a lesser requisite than autotrophy, and (iv) chloroplasts are relatively more complex membrane structures than mitochondria - Ockham's razor (the principle of parsimony) urges us to see prokaryotes and mitochondria preceding chloroplasts/cyanobacteria within the scheme of evolution. (The fact that anaerobic conditions prevailed earlier and photosynthesis enabled oxygen-centric life is not an anti-thesis to this deduction. Earlier mitochondrial respiration cycles need not be oxygen-centric, but some other ion or molecule with high affinity for electrons could equally suffice in lieu.)

Table 3: A factual stock-taking of various criteria for Q-cycle mechanism of the classical (CRAS) scheme

No.	Criteria	Archaea	Eubacteria	Mitochondria	Chloroplasts	Cyanobacteria
1	<i>Can Q/QH₂ mobility meet the overall demands of kinetics?</i>	?	?	?	No	?
2	<i>Are sufficient protons available for reaction cycles in the various phases?</i>	No	No	No	No	No
3	<i>Does the proposed reaction afford optimal yields for the scheme?</i>	No	No	No	No	No

4a	<i>Is a dedicated CBC present for Q-cycling?</i>	No	No*	Yes	Yes	Yes
4b	<i>QH₂ binding site</i>	No	No	?	?	?
4c	<i>2e receiving center</i>	No	No	No	No	No
4d	<i>Bifurcation switches</i>	No	No	No	No	No
4e	<i>Q binding site</i>	No	No	?	?	?
4f	<i>Acceptor site</i>	No	No	?	?	?
4g	<i>Optimal gradients</i>	No	No	No	No	No
4h	<i>Optimal distances</i>	No	No	No	No	No
4i	<i>Are there potential blocks to smooth ET?</i>	NA	NA	No	Yes	Yes
4j	<i>Are the redox centers accessible to solvent?</i>	NA	NA	Yes	Yes	Yes
5	<i>Does the milieu have sufficient concentration and relative ratios of other components?</i>	NA	NA	No	No	No
6	<i>Are the components distributed across the phases as per the mandates of scheme?</i>	NA	NA	No	No	No
7	<i>Is the orientation of proteins and molecules crucial for outcomes?</i>	NA	NA	Yes	Yes	Yes

* There are exceptional cases like that of *Parococcus denitrificans* (PDB: 2YIU), which has a CBC.

As seen from the comprehensive analyses carried out herein, the multi-molecular and sequential Q-cycle is a low-probability scheme, disfavored by thermodynamics and kinetics and unsupported by evolutionary considerations and evidence. Therefore, the assumption that Q-cycle is operative in OxPhos or Photophos is dismissible. In stark contrast with the classical assumptions, the working principles of the murburn model for Q+CBC are shown in Table 1 and Figure 1. The disordered and bimolecular murburn scheme reactions were found to better explain the mobility and roles of oxygen, CBC and quinones. Cytochrome *c* is not just found in IMS, it is also located in the inter-cristae spaces of the matrix [Garrido et al., 2006]. This gels perfectly with our theory that cytochrome *c* is a non-specific redox buffer, equilibrating the ambience with respect to extra one-electron equivalents (Manoj, 2018; Manoj and Gideon, 2020; Manoj et al., 2019a,b). The proposals for murburn model of mOxPhos (Manoj & Bazhin, 2019) and Photophos (Manoj et al. 2020b) are agreeable with the crystal structures of various membrane proteins involved in these routines.

Conclusions: We have presented qualitative s quantitative arguments that: 1. Using only Marcus theory as the sole criterion for understanding or explaining ETs in biological systems is questionable, as murburn equilibriums also hold sway. 2. For sure, it is inferred beyond reasonable doubt that Q-cycle is neither necessary nor feasible at CBCs. It is also opportune to see that murburn model offers the scope to explain the generation of charge disparity (leading to trans-membrane potential) owing to the presence of the anionic semiquinone and explains the mobility and utility of oxygen/DROS. Further, the insights gained herein can be used to re-interpret the outcomes of interesting experiments published recently in this journal (Anand et al, 2019).

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