Chapter

Comprehensive Analyses of the Enhancement of Oxygenesis in Photosynthesis by Bicarbonate and Effects of Diverse Additives: Z-scheme Explanation Versus Murburn Model

Kelath Murali Manoj, Nikolai Bazhin, Abhinav Parashar, Afsal Manekkathodi and Yanyou Wu

Abstract

The Z-scheme electron transport chain (ETC) explanation for photosynthesis starts with the serial/sequential transfer of electrons sourced from water molecules bound at Photosystem II via a deterministic array of redox centers (of various stationary/mobile proteins), before "sinking" via the reduction of $NADP^+$ bound at flavin-enzyme reductase. Several research groups' finding that additives (like bicarbonate) enhance the light reaction had divided the research community because it violated the Z-scheme. The untenable aspects of the Z-scheme perception were demonstrated earlier and a murburn bioenergetics (a stochastic/parallel paradigm of ion-radical equilibriums) model was proposed to explain photophosphorylation and Emerson effect. Herein, we further support the murburn model with accurate thermodynamic calculations, which show that the cost of one-electron abstraction from bicarbonate [491 kJ/mol] is lower than water [527 kJ/mol]. Further, copious thioredoxin enables the capture of photoactivated electrons in milieu, which aid in the reduction of nicotinamide nucleotides. The diffusible reactive species (DRS) generated in milieu sponsor phosphorylations and oxygenic reactions. With structural analysis of Photosystems and interacting molecules, we chart out the equations of reactions that explain the loss of labeled O-atom traces in delocalized oxygenesis. Thus, this essay discredits the Z-scheme and explains key outstanding observations in the field.

Keywords: murburn concept, light reaction, photosynthesis, bicarbonate, photolysis, oxygenesis

1. Introduction to the additive (bicarbonate) sponsored effects

From fossil records, scientists estimate that earth has witnessed about three gigayears of biological photosynthetic activity [1]. Historical documents indicate that by the mid-nineteenth century, the global scientific community was aware that green plants could use sunlight to convert carbon dioxide to carbohydrates, using water and liberating oxygen. By 1930s, Cornelius van Niel had put together a tangible/generic stoichiometry for photosynthesis in diverse organisms utilizing different precursors: $CO2+2H2A+h\nu \rightarrow CH2O+2A+H2O$, wherein "A" denotes an oxygen or sulfur atom [2]. Although in the early phases of photosynthetic research, it was perceived that O_2 formed originated from CO_2, the use of radioactive isotopes led to the conclusion that water was the source of oxygen [3]. Since the latter half of twentieth century, photosynthesis was traditionally investigated as two separate components: the light and dark reaction. The first part includes NADPH/ATP synthesis and oxygen evolution, whereas the second part comprises the steps that lead to CO_2 fixation. None contend these days that CO_2 serves as the direct substrate in the dark reaction of oxygenic photosynthesis. However, ever since it was reported that bicarbonate ions served to enhance oxygen evolution in the light reaction [4], the photosynthetic research community has been divided on the role(s) of this seemingly innocuous species. While some believe that the stimulatory outcome is purely due to indirect effects on Photosystem II functioning (where bicarbonate has been reported to bind at different loci) [5, 6], some have advocated a more direct, that is, substrate role of bicarbonate [7, 8]. In later times, as some researchers emphatically claimed that there was no evidence for the direct roles of bicarbonate [9–12], the general opinion shifted against the "PS II-binding based effects" school of thought. In response, while a few of the original advocates toned down their support for bicarbonate being crucial [13], a few others have campaigned steadfastly for bicarbonate to play more direct roles [14–16]. In the context of the conundrum prevailing on bicarbonate (as well as several other additive molecules'/ions' ability to impact the photosynthetic process), we apply the murburn model of photosynthesis [17–25] to clarify upon several other associated facts from the literature and reason out the structure–function correlations of proteins and dynamics of molecular interactions in milieu.

2. Theoretical approach to a convoluted problem

In this theoretical exploration/analysis, we survey the potential reactions occurring in milieu with a probabilistic approach. We theorize that if affinity-based interactions drive electron transfers in physiology, the interaction dynamics between donor-acceptor pair must be governed by the various known molecular descriptors of affinity. Further, in continuance with our earlier works in the field of bioenergetics, we aim to understand and predict the feasibility of electron/group transfer processes based on the reactant's/reaction's thermodynamic profiles and known aspects of kinetics. Details on the various modalities of thermodynamic calculations are reported elsewhere [26, 27]. These insights are then applied to study the structure–function correlations of Photosystem II and other biomolecules of the photosynthetic reaction system in thylakoids. The known structure of cyanobacterial Photosystem II (pdb:6JLJ) [28] (and other proteins/biomolecules) was analyzed by visualization software like Chimera 1.12 [29] and PyMOL [30]. Cavities of proteins were analyzed by

POCASA [31]. Small molecular/ionic properties were availed from ChemSpider/PubChem registry and ACDLabs/ChemAxon predictions.

3. Addressing experimental observations with the classical Z-scheme and murburn models

3.1 Evident flaws in photosynthetic "electron flow" and their explanations

From the earlier perceptions that photo-assisted reactions directly resulted in complex group transfers, the consensus had set in by the mid-later part of twentieth century that light reaction led to a deterministic series of transfers of electrons derived from water bound at Mn-complex of PS II (along intermittent pit-stops at the redox centers of multiple proteins/biomolecules), leading ultimately to the reduction of $NADP^+$ bound at flavoenzyme reductase. This gradual outer-sphere electron transfer [32] process was supposedly synchronous with "pumping of protons," whose energy was subsequently harnessed for making ATP. The ability to fix CO_2 in the dark reaction rested on the redox/phosphorylation power of NADPH/ATP formed in the light reaction.

One crucial oversight made in the earlier times was that the photosynthetic product of molecular oxygen (an omnipresent molecule in thylakoids) could also serve as an electron acceptor in the Hill (or light) reaction [33]! The diradical of oxygen is both 1e and 2e active and it is difficult to imagine any deterministic regulatory mechanism that could override shunting by oxygen. Further, it was/is common knowledge that several ions and natural/synthetic redox-active molecules could affect or donate/accept electrons with respect to the photosynthetic ETC [34–41]. In the context of the current discussion, this important aspect is investigated further. A compilation (from the publications referenced above and the citations mentioned therein) of the affinity descriptors of some of the spectrum of ions/molecules known to shunt/affect the photosynthetic pathway by serving as electron *donors or donors and acceptors (both)* to

No.	Name (projection radius)	Molecular Formula	Mass (g/mol)	To PSI/PSII	MV (cm^3)	PSA ($Å^2$)	Log P	H-Bonds	Rotating bonds
1	Tetraphenylboron (tetraphenylborate)	$C_{24}H_{20}B$	319	I/II	na	na	na	0.0	4
2	Diphenylcarbazide	$C_{13}H_{14}N_4O$	242	II	187	65	2.24	5.4	4
3	Ferrocyanide	C_6FeN_6	212	II	na	143	na	6.0	0
4	2-ketogluconate	$C_6H_9O_7$	193	II	na	138	−2.82	7.5	5
5	Benzidine	$C_{12}H_{12}N_2$	184	II	159	52	1.5	2.4	1
6	Hydrazobenzene	$C_{12}H_{12}N_2$	184	II	156	24	2.94	2.2	3
7	Ascorbate (3.9–5.4)	$C_6H_7O_6$	175	I/II		110	−2.41 (−1.26)	6.4	2
8	TMPD, Wurster's blue (3.5–5.9)	$C_{10}H_{16}N_2$	164	II	165	6 (6.5)	2.08	2.0	2
9	Iodide	I^-	127	II	na	na	na	na	na
10	Cysteine (3.0–4.5)	$C_3H_7NO_2S$	121	II	91	102 (63)	0.23 (−2.79)	3.3	2

No.	Name (projection radius)	Molecular Formula	Mass (g/mol)	To PSI/PSII	MV (cm^3)	PSA ($Å^2$)	Log P	H-Bonds	Rotating bonds
11	Hydroquinone (3.4–4.3)	$C_6H_6O_2$	110	I/II	86	40	0.64 (1.37)	2.2	0
12	Phenylenediamine (3.4–4.6)	$C_6H_8N_2$	108	I/II	94	52	0.05 (0.32)	2.4	0
14	Semicarbazide (2.8–3.7)	CH_5N_3O	75	II	43	82	−1.61	4.5	1
15	Hydrogen peroxide (2.0–2.3)	H_2O_2	34	II	24	40	−0.43	2.2	1
16	Hydroxylamine (2.0–2.6)	H_3NO	33	I/II	30	46	−0.81	2.3	1
17	Hydrazine (2.1–2.6)	H_4N_2	32	I/II	36	52	−1.2	2.4	1
18	Water (1.7–2.0)	H_2O	18	II	18	(25.3)	−1.38 (−0.65)	1.2	0
19	*Bicarbonate (2.6–3.0)*	*CHO_3*	*61*	*??*	*na*	*60*	*−0.81 (0.25)*	*3.2*	*0*

For smaller molecules, values in braces are from ChemAxon (given when significant differences exist with ACDLabs). H-bond entries are the number of acceptor atoms and donor atoms, respectively. MV and PSA stand for molar volume and polar surface area, respectively.

Table 1.
Affinity descriptors of various known electron donors to Photosystem I and/or Photosystem II.

No.	Name	Molecular Formula	Mass (g/mol)	To/From PSI/PS II	MV (cm^3)	PSA ($Å^2$)	Log P	H-Bonds	Rotating bonds
1	TMPD (3.5–5.9)	$C_{10}H_{16}N_2$	164	II	165	6	2.08	2.0	2
2	Menadione	$C_{11}H_8O_2$	172	I	141	34	2.38	2.0	0
3	Phenazine	$C_{12}H_8N_2$	180	I	144	26	2.84	2.0	0
4	Diquat	$C_{12}H_{12}N_2$	184	I	na	8	−4.71	2.0	0
5	Pyocyanine	$C_{13}H_{10}N_2O$	210	I	na	40	na	3.1	1
6	Diaminobenzidine	$C_{12}H_{14}N_4$	214	I/II	164	104	−0.95	4.8	1
7	Paraquat (methyl viologen)	$C_{12}H_{14}Cl_2N_2$	257	I	na	7.8	1.7	0.0	1
8	Dichlorophenol indophenol (DCPIP)	$C_{12}H_7NCl_2O_2$	268	I	187	50	1.91	3.1	1
9	Flavin mononucleotide (FMN)	$C_{17}H_{22}N_4NaO_{10}P$	496	I/II	na	214	−1.7	10.3	6
10	Plastoquinone (PQ)	$C_{53}H_{80}O_2$	749	I/II	808	34	20.18	2.0	26

Table 2.
Some affinity descriptors of various known electron donors and acceptors (both!) to PS I and/or II.

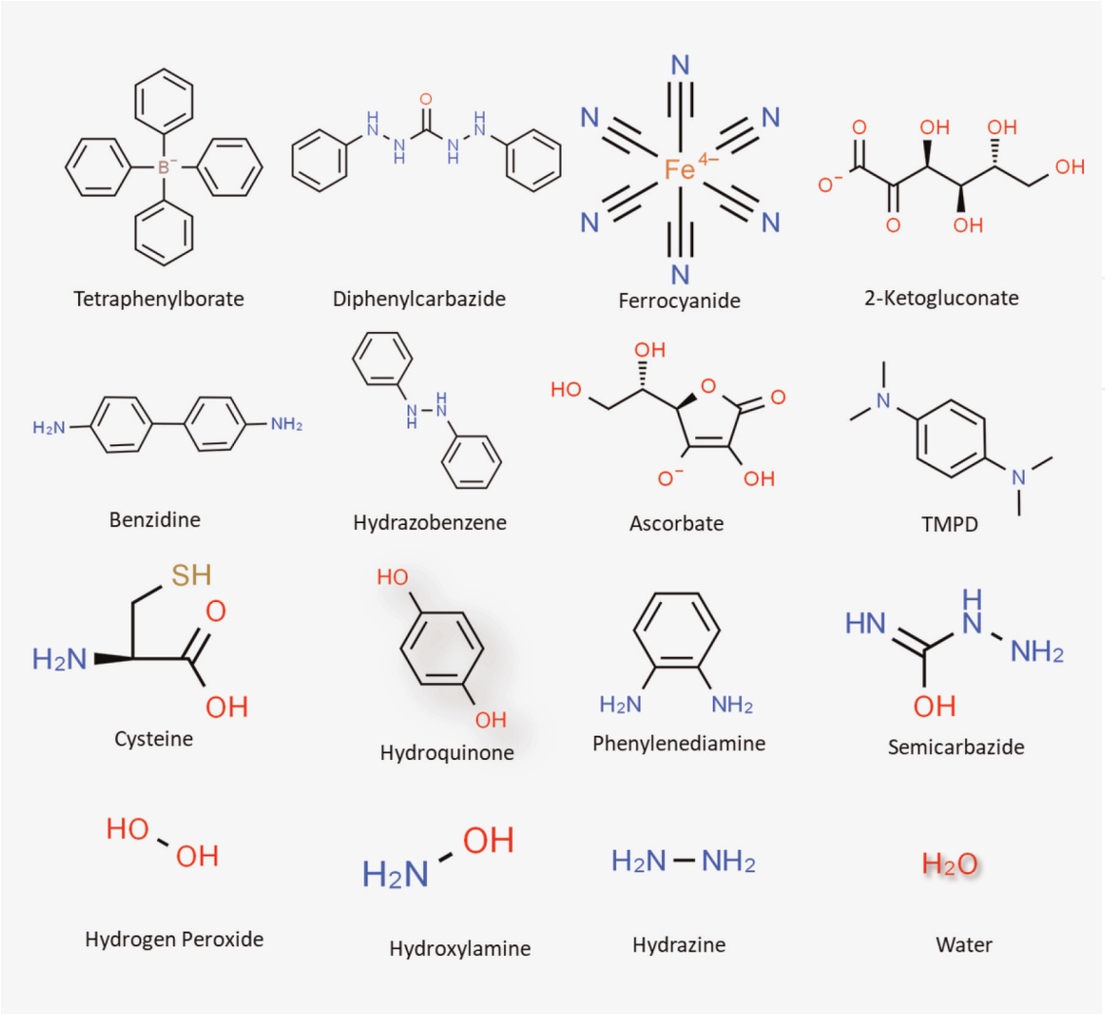


Figure 1.
Structures of molecules and ions serving as electron donors to PS I/II.

the two photosystems is presented in **Tables 1** and **2** and **Figures 1** and **2**, respectively. (In continuum, **Table A1** and **Figure A1** of Item 1 of Appendix lists a compilation of some electron acceptors from PS I and II).

It can be noted and inferred that if such a diverse group of chemical species could serve as source of electrons to PS I/II, it is quite probable that bicarbonate could also serve in this role. Clearly, there is little selectivity/specificity in the overall e-transfer mechanism, as these molecules differ in drastic ways. Also, it is difficult to accept that such diverse molecules posing discrete descriptors of affinity/reactivity (varying topographies, geometries/projection radius/surface area/volume, electrostatic signatures, redox potentials, partition coefficients, hydrogen bonds, rotatable bonds, etc.) could proffer impacting outcomes, if the physiological electron transfer mechanism were to be based on affinity-based interactions between the so-called donor-acceptor pairs, at defined loci. Some molecules are seen to donate and accept electrons to the same photosystem or to both photosystems; this would surely not afford any directionality. Therefore, the findings clearly suggest that deterministic ETC (such as Z-scheme) must be discounted; as any such serial electronic circuitry would not work sustainably in physiology. For this to happen in some miraculous ways, oxygen and several other reaction components must somehow not behave in their "natural" way! In this regard, we have pointed out that the same crucial oversight was made in

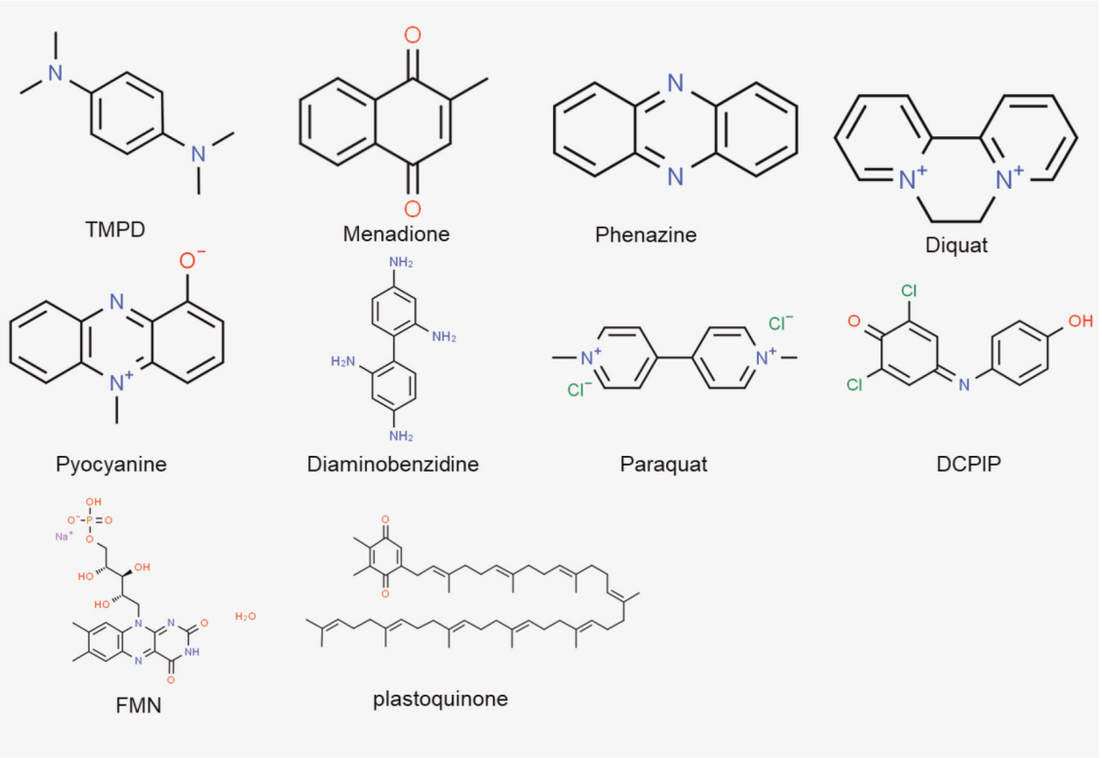


Figure 2.
Structures of molecules and ions serving as electron donors and acceptors (both!) to PS I/II.

respiratory physiology. In the mitochondrial and molecular respiratory system of various cells, several molecules and ions of diverse geometries, dimensions, and redox potentials could also serve as electron donors/acceptors [26]. We had reasoned this fact with the spontaneous murburn equilibriums occurring in milieu.

Further, the classical ETC-CRAS explanation for photosynthesis would also mandate the following insurmountable premises:

i. Several dozens of deterministic e-transfer steps must occur before the release of an oxygen molecule at the WSC. {For example, a dozen e-transfer steps must occur in a single Q-cycle of four electrons- $[(QH_2 - FeS - Heme - PC = 3)\ x\ 2] + [(QH_2 - Heme - Heme - Q = 3)\ x\ 2] = 12$. As only four electrons reach PC for every 8 electrons going through Q cycle, the Q-cycle component alone would take two dozen e-transfer steps for the release of an oxygen molecule!}. It is impossible to imagine the orchestration of such a fastidious outcome, given that several components are distributed at unfavorable ratios and not arranged in the sequence or locations where mandated.

ii. Non-existent/unavailable protons are needed to build the proton motive force for serving the endergonic ATP synthesis {For example, a cyanobacterium of 0.5-micron dimension has a volume of $\sim 0.125 \times 10^{-15}$ liters. The usual functioning of these cells occurs at a pH of 8, wherein protons are at 10^{-8} M concentration. Since a liter of pH 8 solution has $6.023 \times 10^{23} \times 10^{-8}$ protons ($= \sim 6 \times 10^{15}$ protons), the small volume of a cyanobacterium has only $0.125 \times 10^{-15} \times 6 \times 10^{15} = 0.75$ protons! This is when there are tens of thousands of protein complexes (Complexes I–V) in a cell or bioenergetic organelle.

iii. Even if protons are made available through some miraculous means, how is the directionality of ATP synthesis by complex V ascertained? As per the Boyer model, proton moving in or out via the c-ring determines the hydrolysis or synthesis at the alpha-beta subunits. Since there is little proton gradient in physiology, how does a proton-based rationale give ATP synthesis when protons are not used at the active site and when protons are present in both in- and out- phases [20]?

iv. Perhaps, the arrangements of components/pigments in the photosynthetic structures in earlier revealed bacterial systems could have indicated an ordered mechanism [42]. However, the structural distribution of plant pigments, photosystems, and LHC arrangement in chloroplasts revealed later show very little order [43]. Therefore, energy transfer between the various light-absorbing photo-active pigments scattered around in the thylakoid membranes and the redox-centers of photosynthetic proteins must occur via stochastic measures [23].

v. The equations prescribed for the overall process must violate the fundamental laws of thermodynamics for attaining viability. {$2NADP^+ + 3ADPOH + 3POH \rightarrow O_2 + 2NADPH + 2H^+ + 3ADPOP + H_2O$; Overall $\Delta_r G'^{o}_{aq} = 1463.8$ kJ/mol) is the prescribed equation. The input for the overall process is 4 einsteins of 680 nm photons (4×175.9 = 703.6 kJ/mol) and 4 einsteins of 700 nm photons (4×170.9 kJ/mol = 683.6 kJ/mol), giving a total of only 1387.2 kJ/mol, which means that there is a significant shortage of 76.6 kJ/mol to even things out energetically!}

Daniel Arnon, the pioneer who laid the foundation of cyclic/acyclic photosynthesis and made key contributions that led to the establishment of Z-scheme [44–46] changed his perception and tried to "wade against the current" by quoting several arguments and demonstrating NADPH formation in ways other than the Z-scheme format ([47–49] and several citations from his group mentioned therein, starting from 1980). Z-scheme's steadfast adherents chose to sidetrack Arnon and the undeniable evidence which showed that crucial components (such as plastocyanin and cytochrome b_6f) of the deterministic e-transfer scheme were in fact, completely optional in physiology [50–52]. Therefore, such conclusive evidence dictates that the classical ETC-CRAS model for light reaction of photosynthesis should be jettisoned, and it is in this context that murburn model presents a viable alternative [19, 22].

3.2 Asking the right question: How do the photosystems work?

Since the electron flow is deterministic in Z-scheme, Photosystem II (PS II) and Photosystem I (PS I) must have a primary donor and acceptor site each, called D2-A2 and D1-A1, respectively. That is, Z-scheme dictates that the small molecule of water donates electron at D2 and the biomolecule of quinone accepts at A2, whereas the protein plastocyanin donates at D1 and the protein ferredoxin accepts at A1 (**Figures 3** and **4**). In this regard, the information that photosystems can give/accept electrons to/from a wide variety of redox-active small molecules and ions (as shown in the earlier section) is very crucial. From a survey carried out in our study of available literature and seconded by the earlier insightful assertions of Hauska [35], a statistical criterion is

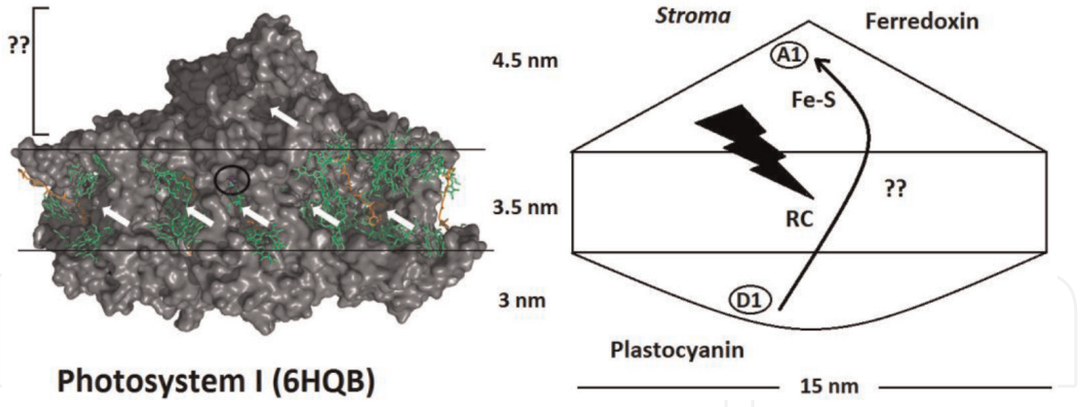


Figure 3.
A schematic representation of the Z-scheme functionality of Photosystem I, drawn to approximate scale. *The left panel shows the structure of PS I and the right panel shows the essential schematic representation of the same. As seen, there are several chlorophylls and carotenoids scattered in the membrane-phase of PS I (marked out with the horizontal straight lines) and the stromal phase apoprotein has Fe-S centers. The white arrows mark grooves/cavities in the protein, enabling diffusible species dynamics. It is not clear how the small amount of plastocyanin could provide electrons to the electron-deficient RC, post photo-activation induced e-transfer processes. Also, some of the FeS centers and the overall structure of stromal part of PS I are not functionally accounted by Z-scheme perceptions [19, 22]. The question mark in the left and right panels pose queries on the roles of the large extra-membrane apoprotein and the mechanism of deterministic electron relays, respectively. The circled spot in the left panel is the reaction center (RC).*

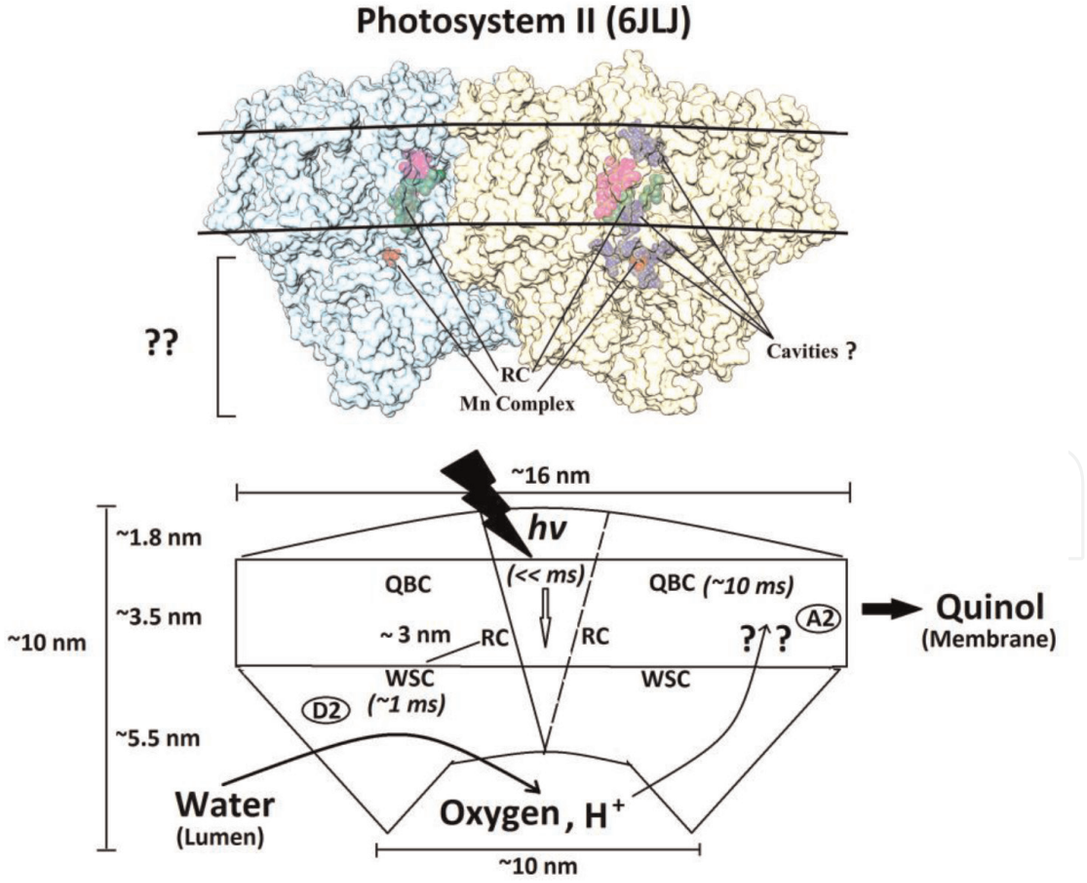


Figure 4.
A schematic representation of Z-scheme functionality of PS II, drawn to approximate 2D-scale. *(Please refer text for discussion.) The purported binding site of bicarbonate is the non-heme iron center (located near the pointed arrow of the lightening sign in the bottom panel image. The big question marks on the left of the top panel ask- why should PS II have such bulbous extensions? The cavities in the PS II structures are inexplicable in the classical purview.*

evident. Hydrophilic ions or molecules (such as ferricyanide or benzoquinone-2-sulfonate) are served at A1, whereas hydrophobic molecules (such as benzoquinone or p-phenylenediamine) are served at A2. Although the diversity of acceptors is not agreeable to the affinity-based binding rationale (deemed necessary for selective electron transfers), the location of the acceptor sites apparently seems to be in alignment with the Z-scheme. It is crucial to note that while lipophilic quinones could serve at D1 (and D2), water-soluble compounds (like sulfonate derivatives of phenazine or DCPIP) could not. This finding directly goes against the Z-scheme layout, because in both these photosystems, electrons are supposed to be availed from the soluble phase (plastocyanin in PS I and water in PS II). It can be seen from the earlier section that synthetic/natural molecules like TMPD/plastoquinone are known to give and accept electrons to/from both photosystems! How is it determined in physiological premises whether a molecule could serve as a donor or acceptor, and at which port? Particularly, while some researchers disown that bicarbonate has any binding-based physiological role in PS II function at one hand [12] (which others quote to argue that the stimulation by bicarbonate is an artifact!), yet others claim that bicarbonate is an essential binding-based cofactor for PS II [53]. How can the feud be settled? The answer is unavailable in the classical perspective, which perceives only binding-based effects as important criteria. Therefore, the right question to be asked is- what is the role of the photosystems in the light reaction? Or, how do the diverse molecules impact the photosystems' functioning?

3.3 Murburn model for the light reaction of photosynthesis

Today, we know that Z-scheme is unsuitable for explaining the synergy Emerson observed between Photosystems I and II [54–56]. The fatal logical flaw is that a serial arrangement of components would surely lower e-flow/reaction rates (thereby negating the logic of Z-scheme conception!) and only a parallel functioning of components could explain the synergy of photosystems [19]. Therefore, the imperative mandate for venturing beyond the classical paradigm of "Kok-Joliot cycle (KJC), Z-scheme ETC, Q-cycle and chemiosmotic rotary ATP synthesis (CRAS)" must be registered [18]. In this regard, we have conclusively critiqued the classical view [18, 57], rendering it incapable of redemption. In lieu, we have proposed a murburn concept based explanation for oxygenic photosynthesis (**Figure 5**), which is based on DR(O)S mediated catalytic outcomes.

The new model (which need not depend crucially on binding-based outcomes but is more an interactive dynamics of molecules, unbound ions, and reactive radicals in milieu) justifies the structure of proteins, architecture/distribution of components and organelles, overall thermodynamics, kinetics, probability considerations and affords a globally valid/tangible mechanistic explanation for the light reaction [17, 19, 21–26, 58]. Under this scheme, the photosystems enable ECS (effective charge separation) and facile charge replenishment (**Figure 6**). Further, electron transfers in this scheme need not be based solely on donor-acceptor affinity binding-based interactions (**Figure 7**). It is in this context of murburn model that we address the long-standing debate on the role (s) of bicarbonate ions (and other non-specific agents) on the dynamics and efficacy of the light reaction. From the two **Figures 6** and 7, it can be seen that the stochastic murburn model permits electron inputs/withdrawals via the involvement of unbound ions and oxygen. This is enabled by the generation of transient electron-charged or electron-deficient species from these agents, which generate a pool of redox relays. The

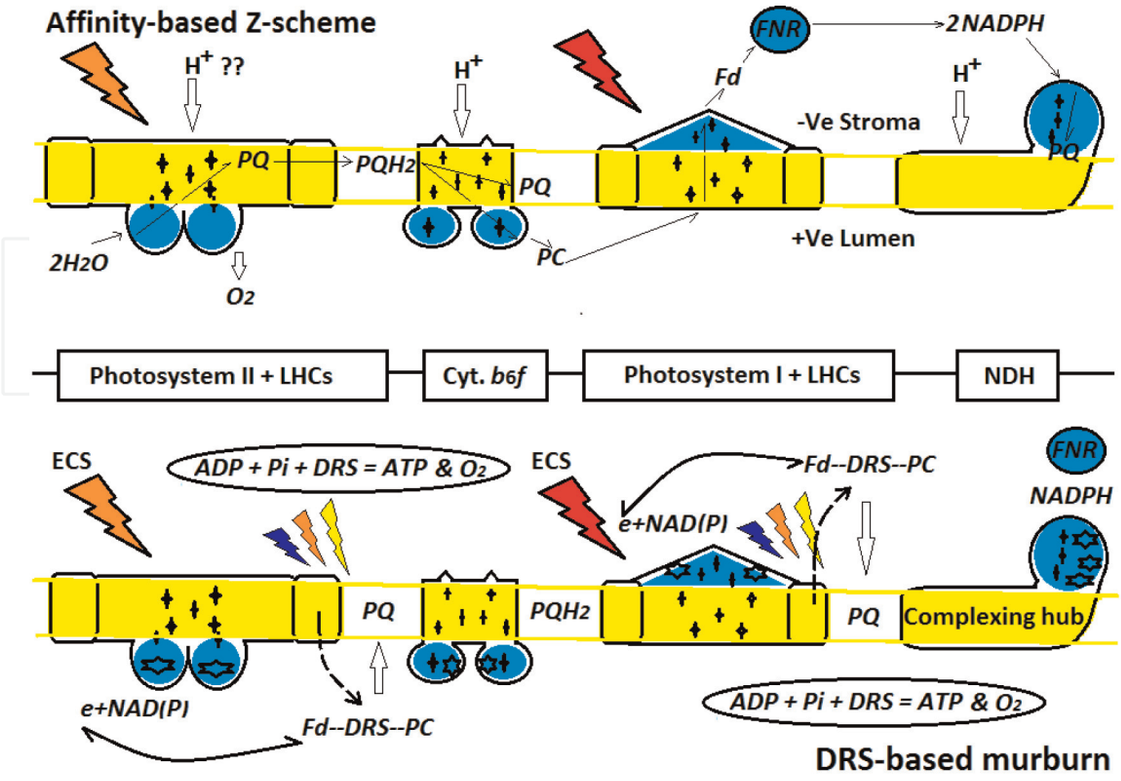


Figure 5.
Structure–function correlations of chloroplast/thylakoid membrane-embedded redox proteins, under Z-scheme and murburn models. *The redox centers (porphyrins) in protein complexes are designated with '┼' sign. In the Z-scheme shown on top, LHCs are exciton relay agents, conveying photons of two specific wavelengths to the two Photosystems II & I, which generate the mobile reducing equivalents of plastoquinol (PQH2) and reduced ferredoxin (Fd) from water and reduced plastocyanin (PC) respectively. This is when Cytochrome b_6f and NADPH-dehydrogenase (NDH) serve the role of proton pumps, to generate a proton motive force or trans-membrane potential (TMP), which is harnessed by F_oF_1ATPase to make ATP by the CRAS mechanism. Only PS II utilizes water and produces oxygen in this scheme and NADPH is produced by FNR at the end of a multi-dozen step e-transfer process. All components are mandated to have a definite order of electron donor and acceptor function, arranged in a series without any role for oxygen or DRS. Stoichiometry is depicted only for the formation of one molecule of oxygen from two molecules of water, giving rise to two molecules of NADPH. The stroichiometic requirement/involvemnt of other intermediates and formation of ATP are not shown. In the murburn model shown on the bottom, all photo-active pigments are involved in redox reactions, whereas only PS II & I can bring about effective charge separation (ECS). Due to photo-activated liberation of electrons in the form of diffusible reactive species (DRS), soluble proteins Fd and PC are involved in redox equilibriums with DRS. Similarly, the binding sites for ADP (shown has six-cornered stars) on the membrane protein complexes enable effective activation of ADP/Pi the photophosphorylation process. Nicotinamide reduction is via simpler bimolecular processes requiring the buffering of Fd. Oxygen involvement and evolution in the murburn model is delocalized, although PS II's Mn-complex can serve as an effective peroxidase, driving higher oxygen evolution. Since the murburn model is inherently a stochastic/statistical model (as a result of an aggregate of several parallel competing reactions), stoichiometry is variable and non-integral. For the facts/arguments which establish conclusively that Z-scheme CRAS is untenable (besides the ones presented in the paragraph leading to the introduction of this figure) and for a greater clarity on the murburn model for light reaction, please refer our recent publications [19, 22].*

formation of stable 2e products and their utilization and/or porting thereafter determines the reaction dynamics in milieu.

3.4 Thermodynamics and kinetics of physiological bicarbonate reactions

In plant and animal cells, the hydration/dissociation equilibrium of CO_2-bicarbonate system is of immense physiological significance and therefore, well-studied. When gaseous CO_2 mixes with water, there are four particles/species formed that co-exist in a complex interactive equilibrium: CO_{2aq} (a, dissolved/aquated molecule), H_2CO_3

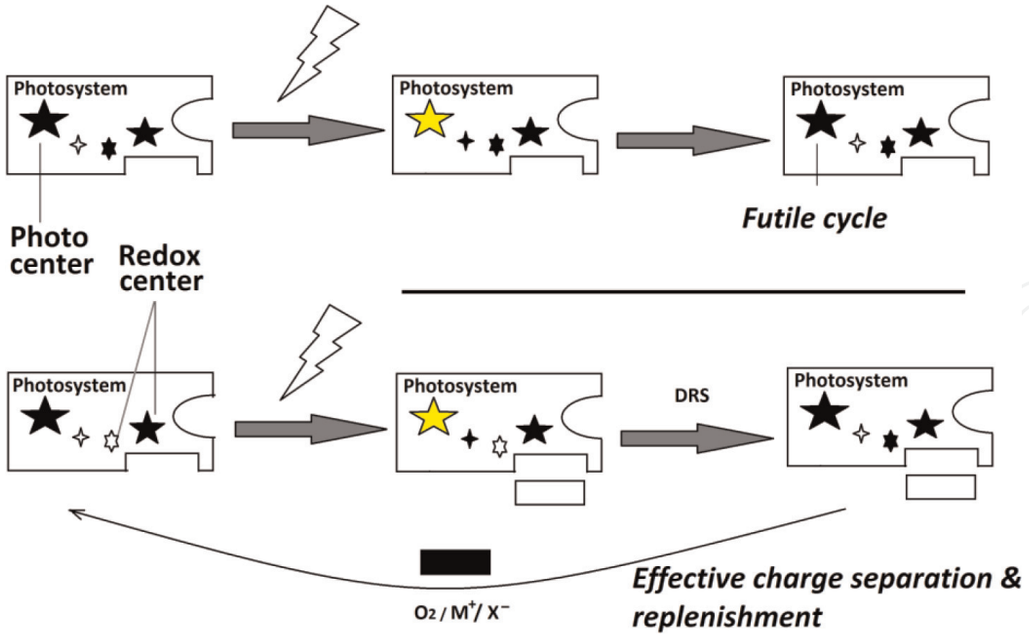


Figure 6.
A schematic to explain the effective charge separation principle of murburn concept. *A futile cycle is shown in the top panel wherein photoactivation (thunderbolt sign) of a center releases an electron which is retrieved at the same center, without any other event occurring in the milieu. In the lower panel, the arrangement of suitable redox centers in proper redox states and the involvement of DRS ensure effective charge separation, wherein the photoelectric electron does not go back to its original source. The ECS at a photosystem enables NADP reduction and photophosphorylation thereafter. Here, DRS are shown to replenish the electron-deficient photo-active center. This outcome could also occur through tunneling, if a suitable redox center is available nearby. M and X stand for cation and anion species, respectively. Blackened stars or rectangles represent reduced (e-replenished) states whereas yellow stars represent photoactivated center that has lost an electron.*

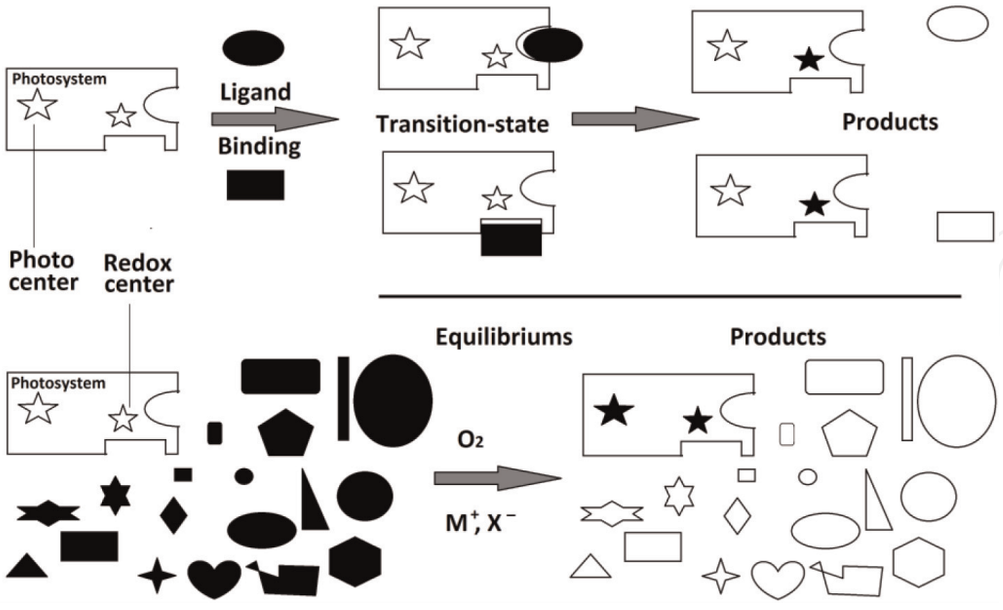


Figure 7.
The murburn modes of electron transfer: *While the classical scheme (top panel) only entails affinity driven ET between donor-acceptor complementation (allowing receipt of electrons at the redox center close to the binding site), the murburn model does not negate this possibility but also endorses interactive equilibriums involving diffusible reactive species (DRS). It can be noted that the involvement of DRS enables the electron transfers between remote redox centers with photo-active centers and molecules/ions of diverse topography and other features. Blackened shapes represent reduced states whereas unfilled shapes represent oxidized states.*

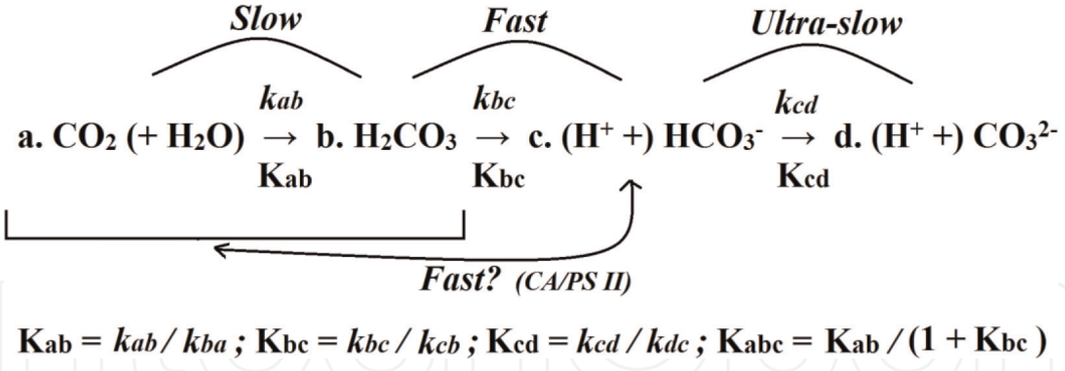


Figure 8.
Simplified representation of the highly complex "carbon dioxide-carbonic acid-bicarbonate-carbonate" interactive equilibriums and the relevant kinetic/thermodynamic constants. *The direct a-c equilibrium is too slow and c-d equilibrium is relevant only at very high pH. (The constants involved for a-b are secondary derivations. Only the forward rate constants are depicted with italicized "k" on the upper side of the directional arrow, whereas the equilibrium constant is depicted with capitalized "K" on the lower side.) The relations between kinetic and thermodynamic constants are given below the equations. (https://www.aqion.de/site/carbonic-acid-kinetics#fnref:1).*

(b, uncharged carbonic acid), HCO_3^- (c, monovalent bicarbonate anion) and CO_3^{2-} (d, divalent carbonate anion). The scheme of these species and the equilibrium/kinetic constants governing their interactions is presented in **Figure 8**. Although the enzyme carbonic anhydrase (CA, a zinc cofactor containing enzyme present at high copy number in chloroplasts) has been extensively studied and reviewed periodically [59–61], it is not really clear as to how these interactive equilibriums operate in physiological dynamics. Also, it has been a general understanding that Photosystem II (PS II) has some CA-type activity [62, 63]. The theoretical investigation bears relevance in light of renewed interest in the roles of bicarbonate and CA [64]. In this context, we would like to point out that perceptions involving the roles of protons in overall thermodynamic treatments were misplaced earlier [27]. Therefore, a revisit to the pertinent treatment is mandated in the new light of awareness.

3.4.1 Well-studied 2e reactions and their equilibriums

The overall interactive process and equilibriums can be understood by considering formative steps for the three soluble ingredients: carbonic acid, bicarbonate ion, and carbonate ion. We consider the theoretical and experimental information on individual reactions from the complex equilibriums. Please consult Item 2, Appendix, for the details of calculation of the empirical $\Delta_r G°$ (standard free energy change of reaction) from the $\Delta_f G°$ (standard free energy change of formation) values, which are given within braces in the pertinent equations. The values determined in this study are given in the first line of the equation and those seen/sourced from literature (as listed in Item 2, Appendix) are in the next line. The pitfalls of experimentally determined equilibrium constants are discussed elsewhere [27].

3.4.1.1 Formation of carbonic acid

$$CO_2\ (-386) + H_2O\ (-237.2) \rightarrow H_2CO_3\ (-623.1); \Delta_r G° = 0.1\text{kJ/mol}; \approx K_{eq} = 0.96\text{M}^{-1}$$

$$\text{Exp.}K_{eq} = [H_2CO_3]/[CO_2] \times\ [H_2O] = 1.3 \text{ x } 10^{-3} \text{ or } 5.4 \text{ x } 10^{-5}\ (\approx 16.5\text{kJ/mol or } 24.3\text{kJ/mol})$$

The rate constants were 0.039 s^{-1} for the forward reaction and 23 s^{-1} for the reverse reaction [65], which gives K_{eq} to be 1.7×10^{-3}. In another study, the rate constants were 0.04 (or 0.025 to 0.04) s^{-1} for the forward and 18 (or 10 to 20) s^{-1} for the backward reaction [66]. The second order forward rate constant is 0.0027 M^{-1} s^{-1} (which multiplied by 55.5, the molarity of water, gives the first order reaction rate constant as 0.15 s^{-1}) whereas the reverse reaction is 50 s^{-1}. Thereby the equilibrium constant is 0.0027/50 = 5.4×10^{-5} M^{-1} [67] or the dimensionless constant would be 3×10^{-3} (= 0.15/50). Therefore, the major amount of carbon-dioxide remains as soluble gas and does not get hydrated to become an acid. In physiological ranges of pH and temperature, the ratio is ~340 CO_2: 1 H_2CO_3. Though there is a considerable spread in the literature regarding the experimental kinetics and equilibrium values, the consensus understanding is that this equilibration process is relatively slow, and the hydration/dissociation process can go both ways, with hydration being less preferred. Very importantly, although the overall directional trend predicated by empirical/theoretical energetics and experimental considerations are in agreement, the value of $\Delta_r G°$ does not correspond accurately to experimental K_{eq} (as expected from the equation: $\Delta G = -RT\ ln.K_{eq}$) in this CA mediated primary reaction (which is theoretically independent of pH, as evident in the equation).

3.4.1.2 Formation of bicarbonate ion

As per **Figure 8**, we can envisage three interconnected ways, 2p, 2q, and 2r.

$$CO_2\ (-386) + H_2O\ (-237.2) \rightarrow H^+\ (412.5) + HCO_3^-\ (-999.8);$$
$$\Delta_r G° = 35.9\ \text{kJ/mol};\ \approx K_{eq} = 5.1\ \text{x}\ 10^{-7}\ (2p)$$

$$\text{Exp.}K_{eq} = [H^+] \times\ [H_2CO_3]/[CO_2] \times\ [H_2O] = 1.5\ \text{x}\ 10^{-8};\ (\log\ K = -7.82)$$

$$\text{Exp.}K_{eq} = [H^+] \times\ [HCO_3^-]/[CO_2] = 4.5\ \text{x}\ 10^{-7}\ \left(\log\ K = -6.35 = -\text{apparent}\ pK_a\right)$$

While the forward reaction is slow, the reverse of this reaction is catalyzed by the proficient enzyme, carbonic anhydrase (CA), and this reaction is fast and practically diffusion limited. The empirical and experimental values of energetics and kinetics seem to agree.

$$CO_2\ (-386) + OH^-\ (-569.7) \rightarrow HCO_3^-\ (-999.8); \Delta_r G° = -44.1\text{kJ/mol};\ \approx K_{eq} = 5.4\ \text{x}\ 10^7 M^{-1}\ (2q)$$
$$\text{Exp.}K_{eq} = [HCO_3^-]/[CO_2] \times\ [OH^-] = 4.6\ \text{x}\ 10^7 M^{-1}$$

Rate constants of the forward and reverse reaction are 8.5×10^3 M^{-1} s^{-1} and 2×10^{-4} s^{-1}, respectively [68]. The forward reaction may be catalyzed by CA, and this is fast and practically diffusion limited. There is a fair agreement on empirical calculation and experimental equilibrium constant; energetics and kinetics are also in agreement.

$$H_2CO_3\ (-623.1) \rightarrow H^+\ (412.5) + HCO_3^-\ (-999.8);$$
$$\Delta_r G° = 35.8\text{kJ/mol};\ \approx K_{eq} = 5.3\ \text{x}\ 10^{-7} M\ (2r)$$

$$\text{Exp.}K_{eq} = [H^+] \times\ [HCO_3^-]/[H_2CO_3] = 2\ \text{x}\ 10^{-4}\ \text{OR}\ \left(\text{empirical theory}\ 6.0\ \text{x}\ 10^{-7}\right)$$
$$\left(\log\ K = -3.69 = -\text{true}\ pK_a\right)$$

The forward reaction's rate constant is $1\times10^{7}\ s^{-1}$ whereas the reverse as $5\times10^{10}\ M^{-1}\ s^{-1}$ [69]. Ultrafast reactions observed both ways. Emperical energetics favors the reverse reaction. The direction of experimental K_{eq} and overall kinetics agrees with energetics.

3.4.1.3 Formation of carbonate ion

We can consider two ways.

$$HCO_3^- \ (-999.8) \rightarrow H^+ \ (412.5) + CO_3^{2-} \ (-1347.7);$$
$$\Delta_r G = 65.3\text{kJ/mol}; \approx K_{eq} = 3.6 \text{ x } 10^{-12}\text{M (3m)}$$

$$\text{Exp.}K_{eq} = [H^+] \times \left[CO_3^{2-}\right]/[HCO_3^-] = 4.7 \text{ x } 10^{-11} \left(\log\ K = -pK_a = 10.33\right)$$

$$CO_2 \ (-386) + H_2O \ (-237.2) \rightarrow 2H^+ \ (412.5) + CO_3^{2-} \ (-1347.7);$$
$$\Delta_r G^\circ = 100.5\text{kJ/mol}; \approx K_{eq} = 2.4 \text{ x } 10^{-18}\text{M (3n)}$$

$$\text{Exp.}K_{eq} = [H^+]^2 \times \ [H_2CO_3]/[CO_2] \times \ [H_2O]; (\log\ K = -17.45)$$

Practically, these are the ultraslow reactions that would have little relevance in physiology. Once again, the energetics and kinetics are in agreement.

In the various steps, it is seen that kinetics aligned with thermodynamic disposition. If α is the fraction of the species of the three dissolved derivatives of CO_2 (H_2CO_3, HCO_3^- and CO_3^{2-}), the profiles of the three species can be traced with respect to pH. With simple chemical equilibrium considerations, it can be clearly seen/inferred that bicarbonate ion is the overwhelming species in the physiological conditions of chloroplast (at pH ~8), with very little carbonic acid or carbonate formation occurring from the direct dissolution or equilibration of gaseous CO_2. The point to note is that the value of the theoretical equilibrium constant for the direct hydration of CO_2 (reaction 1, mediated by CA) is close to zero and the reaction would be highly facile in physiological milieu. The detailed considerations above are presented because they are required to elucidate the interactive chemistry in dynamic/steady-state conditions (wherein a participant of equilibrium like bicarbonate could be actively formed and consumed by metabolic processes) within a plant physiological milieu.

3.4.2 Murburn 1e/2e equilibriums, with bicarbonate as electron donor

Contrary to the classical purview of affinity-based and deterministic rationales of serial electron transfers, murburn proposal is based in understanding physiological reactions in terms of stochastic/statistical events/outcomes resulting from a milieu that contains diverse molecules and ions of various activity/mobility and redox potentials. In this purview, any component can react at any juncture of the interactive electron/moiety transfer equilibrium; provided that they are present in appropriate concentrations and presented in the precise locus, with a favorable orientation. Continuing from the earlier section's discussion, we can see that the classical CA-catalyzed facile equilibrium favored reactions can be represented as:

$$CO_2 \ (-386) + OH^- \ (-569.7) \rightarrow HCO_3^- \ (-999.8); \Delta_r G^\circ = -44.1\text{kJ/mol} \quad (1)$$

$$H^+ \ (412.5) + HCO_3^- \ (-999.8) \rightarrow CO_2 \ (-386) + H_2O \ (-237.2); \ = \Delta_r G^\circ = -35.9\text{kJ/mol} \quad (2)$$

$$H_2CO_3 \ (-623.1) \rightarrow CO_2 \ (-386) + H_2O \ (-237.2); \Delta_r G^\circ = -0.1\text{kJ/mol} \quad (3)$$

Therefore, the CA activity would be deemed pH dependent. The first reaction occurs on the alkaline side and leads to the formation of bicarbonate. The second reaction occurs on the acidic side and results in the loss of bicarbonate. Since chloroplast physiology of pH 8 favors bicarbonate formation by CA, effective utilization of the same is a definite theoretical option. The reverse of (3) could equate to (2) in steady-state, and this reaction would be expected to be highly viable kinetically if accompanied by exergonic reactions (as envisaged during the events resulting post photo-activations). After the events of photo-activation and electron donation, we consider the premises wherein an electron can be abstracted from bicarbonate ion or carbonic acid, via murburn photolytic reactions.

$$HCO_3^- \ (-999.8) \rightarrow CO_2 \ (-386) + {}^*OH \ (25.8) + e^- \ (-148.53); \Delta_r G^\circ = 491.1 kJ/mol \quad (4)$$

$$H_2CO_3 \ (-623.1) \rightarrow CO_2 \ (-386) + {}^*OH \ (25.8) + e^- \ (-148.53) + H^+ \ (412.5); \quad \Delta_r G^\circ = 527 kJ/mol \quad (5)$$

From the above $\Delta_r G^\circ$ values of the equations, we had recently proposed for electron abstractions in transformed scale $\Delta_r G'^{o}_{aq}$ [19], are given within the parentheses after equations in bold:

$$ {}^*O_2^- \ (-381.9) \rightarrow O_2 \ (16.4) + e^- \ (-148.53); \quad \Delta_r G^\circ = 249.8 kJ/mol \ \left(\Delta_r G'^{o}_{aq} = 250 kJ/mol\right) \quad (6)$$

$$H_2O_2 \ (-134.03) \rightarrow {}^*O_2^- (-381.9) + 2H^+ \ (412.5) + e^- \ (-148.53); \quad \Delta_r G^\circ = 428.6 kJ/mol \ \left(\Delta_r G'^{o}_{aq} = 426 kJ/mol\right) \quad (7)$$

$$OH^- \ (-569.7) \rightarrow {}^*OH \ (25.8) + e^- \ (-148.53); \quad \Delta_r G^\circ = 447 kJ/mol \ \left(\Delta_r G'^{o}_{aq} = 446 kJ/mol\right) \quad (8)$$

$$H_2O \ (-237.19) \rightarrow {}^*OH \ (25.8) + H^+ \ (412.5) + e^- \ (-148.53); \quad \Delta_r G^\circ = 527 kJ/mol \ \left(\Delta_r G'^{o}_{aq} = 525 kJ/mol\right) \quad (9)$$

It can be seen that both values are in good agreement, ratifying our methods. It is forthright to deduce that if the electron can be abstracted from the water molecule (as indicated by isotope analysis of [3]), the involvement of diffusible reactive oxygen species (DROS- like $^*O_2^-$, H_2O_2, $^*OH$, OH^-, $^1O_2$, etc.) in the scheme cannot be negated due to theoretical considerations and experimental observations. Also, bicarbonate ion could serve as a direct source of electrons and oxygen atom for the photosynthetic intermediates, as it is more viable. It can be seen in either case, the electron or oxygen may be indirectly/ultimately sourced from water/hydroxide ion, via the following two considerations A & B.

(A). 1 + 2p = 2r, followed by 2r + (4)

$$(1) \ CO_2 + H_2O \rightarrow H_2CO_3; \Delta_r G^\circ = 0.1 kJ/mol$$

$$(2p) \ H_2CO_3 \rightarrow H^+ + HCO_3^-; \Delta_r G^\circ = 35.8 \ kJ/mol$$

$$(2r) \ CO_2 + H_2O \rightarrow H^+ + HCO_3^-; \Delta_r G^\circ = 35.9 kJ/mol$$
$$(4) \ HCO_3^- \rightarrow CO_2 + {}^*OH + e^-; \Delta_r G^\circ = 491.1 kJ/mol$$

$$\text{Totaled} : H_2O \rightarrow {}^*OH + H^+ + e^-; \Delta_r G^\circ = 527 kJ/mol \approx \text{Rxn.}(9)$$

(B). 2q + (4)

$$(2q)\ CO_2 + OH^- \rightarrow HCO_3^-; \Delta_r G° = -44.1 kJ/mol$$
$$(4)\ HCO_3^- \rightarrow CO_2 + {}^*OH + e^-; \Delta_r G° = 491.1 kJ/mol$$
$$\text{Totaled}: OH^- \rightarrow {}^*OH + e^-; \Delta_r G° = 447 kJ/mol \approx \text{Rxn.}(8)$$

Now, any electron-deficient photo/redox system which has the following features (ECS modalities) can avail electron from various species to regenerate their native state, and it can be seen that the value for bicarbonate lies in between the ones of hydroxide ion and water molecule.

$$PS^+ + OH^- \rightarrow PS + {}^*OH \text{ or } \left\{\Delta_f G°(PS) — \Delta_f G°(PS^+) = -596 kJ/mol\right\} \quad (10)$$

$$PS^+ + HCO_3^- \rightarrow PS + CO_2 + {}^*OH \text{ or } \left\{\Delta_f G°(PS) — \Delta_f G°(PS^+) = \sim -612 kJ/mol\right\} \quad (11)$$

$$PS^+ + H_2O \rightarrow PS + {}^*OH + H^+ \text{ or } \left\{\Delta_f G°(PS) — \Delta_f G°(PS^+) = \sim -676 kJ/mol\right\} \quad (12)$$

After electron abstraction and formation of hydroxyl radical, oxygen evolution is a discretized or delocalized process (and not localized at MnComplex alone!). The overall energetics would be as shown below for single oxygen molecule evolution process:

$$4OH^- (-569.7) \rightarrow O_2 (16.4) + 2H_2O (-237.19) + 4e^- (-148.53); \Delta_r G° = 1227 kJ/mol \quad (13)$$

$$2HCO_3^- (-999.8) \rightarrow O_2 (16.4) + 2CO_2 (-386) + 2H^+ (412.5) + 4e^- (-148.53); \Delta_r G° = 1475 kJ/mol \quad (14)$$

$$2H_2O (-237.19) \rightarrow O_2 (16.4) + 4H^+ (412.5) + 4e^- (-148.53); \Delta_r G° = 1547 kJ/mol \quad (15)$$

It can be seen from reactions (4), (5), (A) & (B) that all reactions occurring via bicarbonate amount to ~527 kJ/mol or lower energy terms (since heterolysis of water incurs ~79 kJ/mol, which is the difference between reactions A & B), making it a viable option with respect to water (which requires the same energy). Clearly, the energetics is favorable with bicarbonate and this consideration explains the enhanced oxygen evolution (or electron transfer processes) in the presence of this ion! Therefore, reaction (14) is quite viable in physiology. The last two reactions above can be rewritten for starting from a single moiety, to enable comparison with earlier equations given in literature:

$$H^+ (412.5) + HCO_3^- (-999.8) \rightarrow ½\ O_2 (16.4) + 2e^- (-148.53) + 2H^+(412.5) + CO_2 (-386); \Delta_r G° = 737 kJ/mol. \quad (16)$$

$$H_2O (-237.1) \rightarrow ½\ O_2 (16.4) + 2e^- (-148.53) + 2H^+ (412.5); \Delta_r G° = 773 kJ/mol \quad (17)$$

We would like to point out that the accurate Gibbs free energy calculations we report here are much higher than the misplaced earlier values reported in literature [14, 70], which give 103.8 and 156.1 kJ/mol respectively for reactions (14) and (15), for the same ½O_2 stoichiometry. The difference is also seen in relative terms (for bicarbonate versus water); our calculations showing a difference of ~36 kJ/mol compared to Dismukes' differential of ~52.3 kJ/mol. We have discussed the source of such errors and disagreements (as also seen in section II, Eq. (1)) in energetics/equilibriums in recent communications [24, 27].

3.4.3 Bicarbonate could also catalyze generic murburn processes

Beside the core logic described above (where bicarbonate gets consumed in the reaction), the enhancement proffered by bicarbonate can also result due to catalytic role of bicarbonate. Bicarbonate is an effective activator of peroxide, giving peroxymonocarbonate ion [71, 72], a process also facilitated by CA. Research conducted in the last decade shows that carbonate/bicarbonate ions (erstwhile taken as innocent buffer participants) can serve as catalytic agents in oxidative reactions [73], potentially aiding several 1e and 2e murburn equilibriums in milieu. Specifically, bicarbonate ion has been known to enhance photolysis of water by aluminum porphyrins, which are known to proceed via 1e mechanism [74]. This observation is directly relatable to the chemistry of chloroplast reactions. Such positive effects (quite similar to how the addition of some ash to common sugar can enhance its burning in air; another example of a non-deterministic oxidative reaction!) could also explain the enhancement of oxygenic photosynthesis by bicarbonate. In essence, this results because of the lowering of activation energy by the catalyst and by virtue of molecule-unbound ion-radical equilibriums' interactive dynamics, leading to facile sinking of electron pairs into products.

3.5 Explaining discretized oxygenesis, NADP reduction, and ADP phosphorylation

In the Z-scheme, two molecules of water must stay bound to the Mn-complex until several rounds of electrons are transferred through the Z-scheme; DROS production is considered futile and physiological aberrations. In the murburn model, the *OH and other DROS like superoxide formed in the mileu could react/collapse with another similar molecule (or dismutate or cross-react) to form 2e stabilized products of hydrogen peroxide, water, and oxygen molecules. A DROS product like peroxide would also react with the originally formed radicals, further propagating the highly spontaneous and fast binary reactions. Some examples are shown below:

$$^{*}\mathrm{OH}\ (25.8) + {}^{*}\mathrm{OH}\ (25.8) \rightarrow \mathrm{H_2O_2}\ (-134.03); \Delta_r G^{\circ} = -185.6\mathrm{kJ/mol} \tag{18}$$

$$^{*}\mathrm{O_2}^{-}\ (-381.9) + {}^{*}\mathrm{O_2}^{-}\ (-381.9) + 2\mathrm{H}^{+}\ (412.5) \rightarrow \mathrm{H_2O_2}\ (-134.03) + \mathrm{O_2}\ (16.4); \Delta_r G^{\circ} = -158.8\mathrm{kJ/mol} \tag{19}$$

$$^{*}\mathrm{OH}\ (25.8) + {}^{*}\mathrm{O_2}^{-}\ (-381.9) + \mathrm{H}^{+}\ (412.5) \rightarrow \mathrm{H_2O}\ (-237.19) + \mathrm{O_2}\ (16.4); \Delta_r G^{\circ} = -277.4\mathrm{kJ/mol} \tag{20}$$

$$^{*}\mathrm{O_2}^{-}\ (-381.9) + \mathrm{H_2O_2}\ (-134.03) + \mathrm{H}^{+}\ (412.5) \rightarrow {}^{*}\mathrm{OH} + \mathrm{H_2O} + \mathrm{O_2}; \Delta_r G^{\circ} = -91.8\mathrm{kJ/mol} \tag{21}$$

$$^{*}OH\ (25.8) + H_2O_2\ (-134.03) \rightarrow {}^{*}O_2^{-}\ (-381.9) + H^{+}\ (412.5) + H_2O\ (-237.19); \Delta_r G^{\circ} = -98.4 kJ/mol \quad (22)$$

Thereafter, peroxide could also serve as an electron source to the photo-activated electron-discharged photocatalysts in thylakoids (including photosystems or LHC species), thereby giving rise to superoxide radical [eq. (7)]. Such peroxidase/dismutase reactions could be efficiently carried out by an agent like Mn-Complex of PS II or any other heme in milieu could also serve this role or even CA like enzymes. The reactions proposed herein [particularly, such as (7) and (16)] are supported by the demonstration that Mn-substituted CA works as a peroxidase (in assistance with bicarbonate) and the inference made therein that the overall process could involve radical chemistry [75].

After ECS, at the two photosystems (as detailed in **Figure 9**), electrons can be taken up Fd, which aids $NAD(P)^{+}$ reduction and this proposal is supported by Arnon's group works through 1980s [49]. It can now be understood that that at high interfacial area permitted by the stacking of thylakoids and low water activity (practically aprotic conditions), the DROS radicals are stable and effectively drive phosphorylations [76]. Quinols in the memebrane merely aid these processes by serving as 1e/2e pitstops [25]. This consideration explains how/why quinones/quinols serve as donor/acceptors of both PS I and II (as discussed in Section 3.1, **Figure 2**). Besides the Eqs. (17)–(19), oxygenesis could also accompany photosphorylation steps, which are aided by the various membrane protein complexes that bind ADP (**Figure 9**). Therefore,

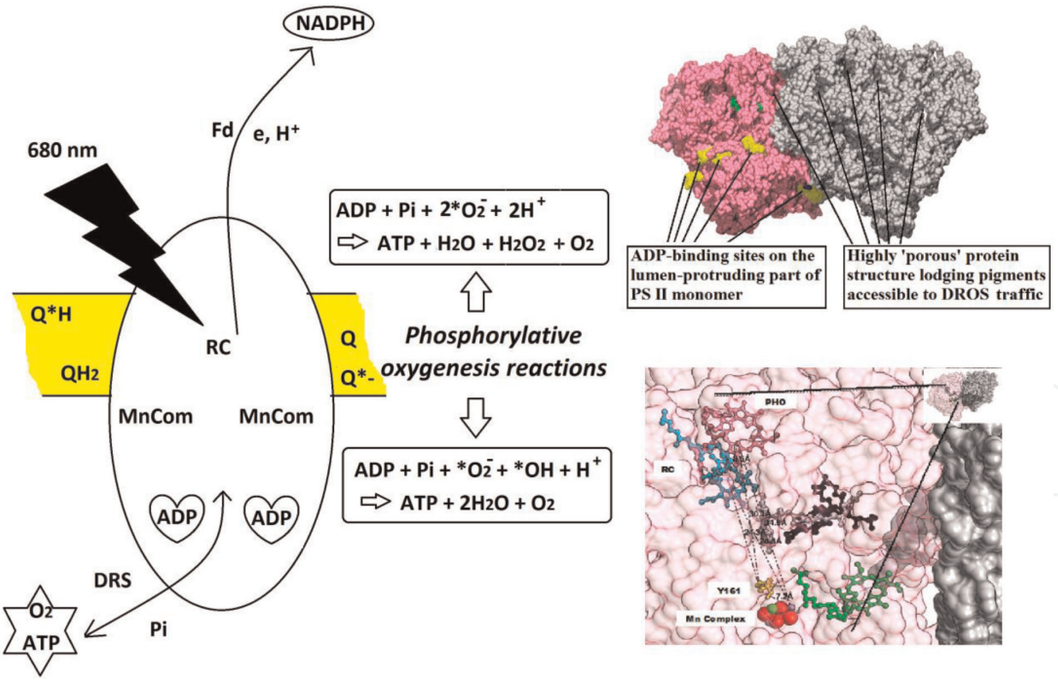


Figure 9.
The murburn explanation for a photosystem (e.g., PS II) and its justification in known structural details: *After excitation at 680 nm, RC loses an electron by ECS, which is taken up by ferredoxin, which subsequently reduces NADP, directly or indirectly. The electron-deficient RC can be served by a multitude of species, including DROS like superoxide or peroxide or hydroxide and other species like bicarbonate or water. Quinones in the membrane help stabilize the ECS and also serve as transient pit-stops for the electrons, before they are recycled. The DROS species that could be formed in the meanwhile carries out ADP phosphorylation (as per [19, 22, 26]), aided by the multitude ADP site on the non-membrane portion (right top image). This reaction may also lead to oxygenesis (two equations shown in boxes are thermodynamically facile). The structural constituents (panels on the right) and observed distributions agree well with the murburn model.*

oxygenesis is aided by chloroplast-membrane proteins (like PS II/NDH/cytochromes) or soluble proteins (CA/peroxidase) or it could also result due to DROS-cross reactions in milieu. For other evidence, arguments and equations of murburn phosphorylations and NAD(P) reductions, please refer our earlier works [19, 21, 26] (**Figure 9**). The presence of bicarbonate serves as an effective ionic conduit/catalyst/substrate in the 1e and 2e murburn equilibriums occurring in the vicinity of thylakoid membranes.

It can be seen by the analyses of the known structures of Photosystem II that neither TMPD nor PQ (synthetic/natural molecules that can give and receive electrons to/from both photosystems, **Tables 1** and **2**) can reach purported binding sites of PS II (like Mn-complex or non-heme Fe). We do not envisage how even much smaller molecules, such as water or peroxide, could reach the Mn-complex at a steady rate. In this regard, we have shown that redox proteins like peroxidase could abstract electrons via interactive equilibriums in milieu, without the final donor actually getting into the heme active site [77]. Just as water formation was mistakenly considered to happen only at Complex IV (by oxygen staying bound, waiting for 4e and $4H^+$) of the respiratory energetic scheme, oxygen formation/liberation is erroneously perceived to occur only at PS II's Mn-complex (by binding two water molecules, liberating 4e, $4H^+$ and one oxygen molecule). While Complex IV and Mn-complex may be major peroxidase-type murzymes that enable a strong displacement of the equilibrium by effective consumption of accumulated peroxide (forming water and oxygen, respectively), oxygen formation at multiple loci within milieu would also be facile [as shown in the equations above and via Eqs. (17)–(19)]. This consideration also explains the experimental observation of oxygenesis even with 700 nm light, in the Emerson experiment. We have already demonstrated and explained the enhancement of one-electron reactions by several agents like chloride ions in simple peroxidase reactions using murburn concept [77]. The same enhancement effect is also observed in the light reaction of oxygenic photosynthesis [36, 37], thereby confirming the relevance of 1e/2e murburn equilibriums in milieu. The effect can be perceived as akin to the phenomenon wherein distilled water (with miniscule amounts of H^+/OH^- ions) does not conduct electricity but when salts of diverse ions are dissolved in water, it shows better conductivity. Therefore, murburn concept explains the outcomes seen with the non-specific agents in a simpler way, rather than the assumption that all such diverse species have multiple binding sites on different proteins and that such binding could afford allosteric regulatory effects based on conformation changes. Besides being justified in the structure–function correlations and the distributional/experimental facts reported, the bimolecular reactions detailed herein add up thermodynamically (to explain NADP reduction, oxygenesis, thermogenesis and ATP synthesis) [19, 20] and it is also well known that such radical mediated reactions also have high kinetic viability [78, 79].

3.6 Reasoning long-standing observations/conundrums with the murburn model

a. In the murburn model, since electrons can be given by multiple agents and received by diverse species, there is considerable flexibility for probabilistic fecundity. Since all the protein components work independently and in parallel in the murburn model, the Emerson enhancement effect (a few mainstream media perceptions can be seen from the two internet links made by professionals in the field: https://www.maximumyield.com/photosynthesis-maximized/2/924; https://www.youtube.com/watch?v=AJZXFP8ynGA) of oxygenenesis or phosphorylation is explained adequately. Owing to the

chemical disposition of the participants (oxygen is a "free to roam and react species"!), the formation of DROS is inevitable and the spontaneous bimolecular oxygenesis reactions discussed above cannot be 'prevented' by preset deterministic mandates that Z-scheme imposes. There exists adequate scope for bicarbonate to effectively serve a constructive role in oxygenesis [say, via (4) and (18) or (14)+(19)].

b. The requirement of the external protons (on the left side of the equations) and their ability to displace the oxygenesis equilibriums [(17)–(19)], reduction of nicotinamide nucleotide (via $NADP^+ + H^+ + 2e^- \rightarrow NADPH$) and phosphorylations (via $ADP + Pi + H^+ \rightarrow ATP + H_2O$ or through one-electron murburn equilibriums leading to superoxide generation in situ) also explain the enhancement of photosynthesis in the Jagendorf experiment [80]. Therefore, the pmf-based CRAS was erroneously assumed to be a valid proposal.

c. CA is copious in chloroplasts and it is a highly efficient enzyme. It catalyzes the spontaneous/facile proton-utilizing formation of CO_2 (the physiological substrate of RUBISCO), the hydroxide-utilizing formation of bicarbonate and the slightly endergonic hydration of CO_2. (the latter two reactions giving murburn substrates for oxidized photosystems/pigments). Therefore, the presence or the preponderance of CA (and CA-like activity of Photosystem II or any other agent thereof) in chloroplasts can be deemed relevant. However, the CO_2 production by CA may not be obligatorily required physiologically in C3 plants [64] because the CA-type activity of Photosystem II may substitute in lieu. Since CA's absence leads to altered pH and DROS dynamics, it is projected that CA could serve as a murzyme (with the zinc atom cycling via a transient one-electron reduced state). Such a mechanism could explain this enzyme's practically diffusion-limited turnovers and the requirement of the metal cofactor. Since CA and Photosystem II are present at high copy numbers in chloroplasts, they have the ability to influence the in situ bicarbonate-involving equilibriums discussed in section 2. In this regard, it is essential to consider the arguments of Warburg [81] and the unexplained data several groups presented thereafter [10, 11, 82, 83]. For the readers' convenience, the major experimental dat profiles of cited above are reproduced from the original sources in Item 3 of the Appendix. The following are direct explanations of and deductions from those works:

    1. Our re-interpretations above are supported by the sole figure of Stemler & Radmer's [83] paper in *Science*, dealing with the provision of ^{18}O-labeled bicarbonate to disrupted chloroplasts depleted of bicarbonate/CO_2 and lacking the enzyme CA. (This is shown as panel (A) in Item 3 of Appendix.) They observed that: (a) the immediate/instantaneous formation of heavy-atom labeled dissolved CO_2 upon the provision of labeled bicarbonate, (b) the evolution of unlabeled dissolved CO_2 in milieu occurs in parallel to the evolution of unlabeled oxygen, and (c) though delayed by a few minutes; there is a small amount of label found in oxygen evolved. The first and second observations show that even in CA/CO_2 depleted systems, the HCO_3^-- CO_2 dynamics is instantaneously facile/

operative in physiology. If bicarbonate is not involved in photosynthesis, we would not expect any label in evolved CO_2 at all, and this consideration is violated by observation (c). The low yield of label in evolved oxygen can be explained considering that the maximal concentration of (the labeled oxygen containing) bicarbonate in physiological milieu would only be at a few mM levels, (the unlabeled oxygen containing) water concentration is at 55.6 M (which is a conservative excess of $>10^4$!). Therefore, labeled bicarbonate could instantly undergo the facile reverse of 2p and 2q. Thereby, the heavy oxygen atom label in bicarbonate would be reduced to a minuscule fraction. However, provision of heavy label in water cannot drive up the label into bicarbonate, owing to equilibrium considerations. Thus, the vast majority of label in evolved oxygen would always be seen as sourced from water. On the other hand, the presence of some label in the evolved oxygen is supportive of bicarbonate's involvement, and cannot be reasoned in any other way! Therefore, it is now deemed inappropriate to argue whether the electrons or oxygen come from water or bicarbonate, as bicarbonate and water are intricately connected via a network of 1e/2e equilibriums in physiology (discussed in sections 2-4).

2. **Figures 1**–3 of Radmer-Ollinger's [82] *FEBS Lett.* paper is shown as panel (B) in Item 3 of Appendix. **Figure 1** clearly establishes the controls that practically, little labeled oxygen is seen when the system is presented with labeled oxygen-containing bicarbonate. The authors' data analyses reveal that the amount of labeled oxygen given is a fraction of the total bicarbonate (3 and 32% of oxygen atoms of CO_2 being doubly and singly labeled, respectively!). If we consider radical-rebound reactions being operative (leading to the carbonic anhydrase type outcomes), the kinetic isotope effects would dictate that the heavy atom is not dislodged statistically, making only the lighter atoms of the bicarbonate involve in the reaction. Therefore, the initial timeframes O_2 evolved might not show any label at all, due to low availability at one hand, and low reactivity at the other! The authors correctly reason and correlate with earlier studies that the amplitude of oxygen yields go up with the provision of bicarbonate, an important aspect which is conveniently sidelined by Z-scheme advocates (who just focus only on the negative oxygen-label data!). One crucial finding that everyone misses is that there is significant labeled oxygen evolution in the 2nd flash also, which is accentuated by the addition of bicarbonate. **Figure 2** of their paper is the exploration of oxygen evolution with the provision of labeled and unlabeled oxygen-containing water. The first observation is that the labeled water signals for labeled oxygen was lower by at least eight folds (in comparison to unlabeled oxygen production with unlabeled water), confirming the inference of kinetic isotope effects lowering rates (i.e., radical rebound mechanism being operative). Once again, the larger amplitudes obtained in these traces confirm that oxygen is evolved even after second light flash (equivalent to the first flash of Kok-Joliot experiments, as a priming flash is unaccounted in their protocols!). In the labeled water experiment, this result is inexplicable with the classical Mn-complex centered Kok-Joliot cycle, which would require prior-bound ^{18}O intermediates that must undergo a mechanistically impossible "sequential double-hits" at a non-photo-excitable center [19]. This earlier reported finding conclusively disclaims the classical Kok-Joliot model

and provides strong support for the murburn oxygenesis mechanism. **Figure 3** of the same paper shows that bicarbonate significantly enhances this second flash's yields whereas a control like sodium chloride does not give similar outcomes! Surely, this necessitates that the "double-hit" argument must be jettisoned, as there is no foreseeable way in which bicarbonate could bring this effect. This finding suggests a rapid equilibrium based effect, wherein bicarbonate's presence impacts oxygen evolution, an outcome which is permitted within the murburn radical interactions purview. Further, in the murburn model, the oxygen evolved in the first few pulses could also get consumed internally for competing reactions. The presence of additives, such as the reducible ferricyanide only affects this internal competition, thereby influencing the yields in the second light pulse. The stochastic murburn model allows for multiple such discrete equilibriums (which convincingly explain the outcomes!) whereas the deterministic Z-scheme does not. Also, the quartet periodicity accentuated at the third flash is not a conserved observation (which we had pointed out earlier!), thereby disclaiming the Kok-Joliot cycle.

3. The so-called decisive works in this field by Clausen et al. [10] and Hillier et al. [11] that downplay the roles of bicarbonate do not take into account the observations reported earlier and inferences we made above, and continue to misinterpret the isotope findings, overlooking the major aspects (like kinetic isotope effects and the factual observation of heavy atom trace in oxygen from bicarbonate, albeit at later times!). Hilliers et al. work was done primarily with PS II core-containing membrane fractions, and that too, incorporating CA inhibitors like ethoxyzolamide and high concentration of the oxidizer, ferricyanide. Such a single-turnover experiment has little relevance to physiological steady-state conditions wherein multiple photosystems, LHCs and ample oxygen (and no ferricyanide!) + bicarbonate production mechanism would be present. Showing that oxygen can be produced even in the absence of the physiological ambiance and without labeled isotope containing oxygen is not any evidence to elucidate the actual physiological process (involving bicarbonate)! Even in such reductionist/unrealistic experimental work (which was designed to show that bicarbonate cannot have physiological role; and not designed to investigate the physiological role of bicarbonate!), the authors have themselves admitted that there is significant (although not accounting for the major process!) label in O_2 produced by labeling bicarbonate! **Figure 2** of Clausen et al. paper is presented in Item 3 of Appendix as panel (C). Clearly, it can be seen that even in this experimental work, oxygen evolution is noted after the second flash and once again, the "third peak maximal quartet" paradigm is not reproducible (when compared to a similar experiment in **Figure 2** of Radmer-Ollinger paper). Even in this work, it was seen that labeled oxygen given in water also equilibrates with CO_2 to some extent, and the decay of such heavy labeled CO_2 is attributed to physiological photosynthetic activity. The fact that label is seen in oxygen in later time frames (in Stemler-Radmer paper) and lower yield seen in labeled water (in Radmer-Ollinger paper) shows the kinetic isotope effects

$$HCO_3^- + PS^{+*} \rightarrow CO_2 + {}^*OH + PS$$

$${}^*OH + {}^*OH \rightarrow H_2O_2$$

$${}^*OH + OH^- \rightarrow H_2O + {}^*O^-$$

$${}^*OH + H_2O_2 \rightarrow {}^*O\text{-}O^- + H_2O + H^+ \text{ or } {}^*O\text{-}O^- + H_2O + H^+$$

$${}^*OH + {}^*O\text{-}O^- + H^+ \rightarrow H_2O + O_2$$

$${}^*OH + {}^*H \rightarrow H_2O$$

$$2HCO_3^- + 2{}^*OH + 2H^+ \rightarrow 2CO_2 + H_2O_2 + 2H_2O$$

Figure 10.
Murburn reaction for bicarbonate activation and steps for loss of heavy atom trace in evolved oxygen. *As per the first equation given above, bicarbonate gives the electron-deficient photoactive molecule an electron. (The label O atom is in bold colored font.) This equation is evidenced by Stemler & Radmer paper [83], which shows that as soon as labeled bicarbonate HCO_3^- was presented, labeled CO_2 was produced. This data has to be interpreted in conjunction with the middle image of* ***Figure 3*** *of Radmer-Ollinger paper [82], which clearly shows enhanced oxygen peak with second light pulse (upon presenting non-labeled bicarbonate). Further, the sole image in the former paper also shows that oxygen evolved does have label when presented with bicarbonate, albeit the label comes only after some time. This is because of kinetic isotope effects (evident in the labeled water data* ***Figure 2*** *of Radmer-Ollinger paper), as ^{18}O is knocked out at approximately an order lesser (~1/8) than the ^{16}O atom. Here, it cannot be argued that the oxygen label is from labeled water formed from the bicarbonate-evolved hydroxyl radical reactions. In which case, it must be accepted that bicarbonate does serve as a source of electrons! In turn, this would lead to the theoretical imperative that it could also be a source of oxygen, albeit to low extents! Therefore, the hydroxyl O-label derived from bicarbonate label must go into water or peroxide or other species. The rest of the murburn equations [26] that follow (after the photosystem mediated electron-abstraction step) explain how the heavy label in $^*OH$ goes into non-O_2 species.*

involved in radical rebounds. As per the murburn model, CA type activity and multiple other components interact (see discussion below) to contribute the O atom into molecular/gaseous oxygen or electrons in NADPH. Besides our theoretical treatments presented herein, our inference of bicarbonate interacting with DROS is supported by findings of Warburg's original observations and several scientists' data [4, 8, 13–16, 81, 83–87]. To sum up, bicarbonate could potentially serve as a catalyst or a source of electrons and/or oxygen in photosynthesis, and the observations noted in connection could be explained by the equations given in **Figure 10**.

3.7 Is the light reaction of photosynthetic plants a deterministic or stochastic process?

The classical sequence or order of the equations/processes occurring via Z-scheme and Mn-complex based oxygen evolution cannot have bicarbonate binding at the WSC/OEC and the PS II must bind bicarbonate elsewhere, like the non-heme iron center of PS II [88]. A study of the Mncomplex containing extra-membrane region does not show major channels connecting to the exterior bulk phase. It is not clear how water molecules could channel into the Mn-center (and likewise, the larger bicarbonate ion would find it even more difficult!). The large bulbous protrusions of the PS II and porous/cavity-ridden nature of the protein at its various loci are inexplicable in the classical purview (**Figures 4** and **9**).

The classical perspective sticks to affinity-driven binding-based causatives alone and since it sees oxygen evolution occurring only at PS II's Mn-complex, researchers

have stuck to probing the effects of bicarbonate resulting only from its binding to PS II. Since the non-heme Fe center is not a "route" charted out in the Z-scheme, the outcome can only attributed to (non-traceable or unaccountable) allosteric effects. Regardless, assuming that bicarbonate can and does bind anywhere at PS II (as perceived above by some researchers who tried to explain the bicarbonate enhancement within an overzealous extension of the discredited Z-scheme), the following set of equations formed the basis for the interpretations. That is, bicarbonate splitting gave $4e^-$ and 2 CO_2 molecules, which could be recycled via CA type activity. It can be seen that the sum total energetic yield of this bicarbonate mediated process is equal to simple water splitting in this classical scheme too (refer earlier equation xv), as given below.

$$2HCO_3^- + 2H^+ \rightarrow O_2 + 2CO_2 + 4H^+ + 4e^-$$
$$2CO_2 + 2H_2O \rightarrow 2HCO_3^- + 2H^+$$

———————————————————————————————————————

.

$$2H_2O + 4\,h\nu \rightarrow O_2 + 4H^+ + 4e^- \ (\Delta_r G° = 1547\ \text{kJ/mol} – [4\,h\nu \approx 704\ \text{kJ/mol}] = 843\ \text{kJ/mol}) \quad (23)$$

The other half-reaction occurring at a disconnected locus of PS II would be:

$$2Q + 4H^+ + 4e^- \rightarrow 2QH_2 \ (\Delta_r G° = -1229.34\ \text{kJ/mol}) \quad (24)$$

While this reaction's mechanistic approach could perhaps enable the justification or the formation of labeled oxygen atoms with the provision of labeled bicarbonate or water, it cannot explain the enhancement of oxygen yields with bicarbonate. Also, it cannot reason the reduction of $NADP^+$ or phosphorylation of ADP independently by this complex. The overall requirement at PS II is 1547 kJ/mol and this is apparently offset by the 704 kJ/mol contribution of four photons and 1229 kJ/mol derived from two quinone molecules' reduction (exceeding the required amount by a value of ~386 kJ/mol). **Figure 4** shows the spatio-temporal overview of the processes that transpires at PS II, as per Z-scheme. It can be seen that the yield obtained for the temporally and spatially disconnected quinone reduction (which completes at 10^{-2} seconds) occurring nanometers away from both the RC (where the energy or electron transfer events occur at 10^{-12} to 10^{-5} second time-frames) and OEC/WSC (where the purported water-lysis occurs in 10^{-3} seconds) cannot be coupled with any known mechanism. This makes the overall process unviable via all considerations (thermodynamic, kinetic, mechanistic and probabilistic). That is, since there is nothing called "half a molecule of O_2" in reality, Z-cheme dictates that only after two $NADP^+$ molecules are completely reduced at the end of multiple ETC cycles can a molecule of oxygen evolve at OEC. Therefore, for one molecule of oxygen to be released by the WSC, PS II must make two QH_2 molecules. The second molecule of Q cannot bind until the first formed QH_2 detaches, and each such quinone must deterministically home its way to QBC. These considerations mean that with 1 *hv*: 1e stoichiometry, PS II should orchestrate multiple events at different loci independently! It should split one O-H bond (of ~460 kJ/mol) releasing one electron (± proton), using the insufficient energy provided by a photon of 176 kJ/mol (assuming absolutely efficient energy conservation/coupling between RC and WSC). Further, if we consider that the proton formation/release is not energetically favored and that WSC must have deterministic proton relay

networks to enable the reduction of quinone at its binding site (QBC), the ETC-CRAS mechanism appears improbable. (Then again, the membrane is also supposed to be impermeable to proton fluxes, at the same time, to build *pmf*!) Such deterministic perceptions do not limit the stochastic scheme of murburn model, which has ion-radical equilibriums. For greater insights, please refer our conclusive discussions discrediting the various aspects of "Kok-Joliot cycle–Z-scheme–CRAS" explicatory paradigm presented in our recent publications [17, 19, 21, 23, 25, 26, 58]. While quinols are practically immobile in the reaction time frames and cannot move deterministically in membranes, DRS like superoxide can freely move, relaying electrons. Also, DROS formed in milieu can attack ADP bound to the protein or phosphate found in milieu, thus aiding photophosphorylation. For the details of murburn model of NADP reduction and ADP phosphorylation, please refer the murburn precepts paper [19].

In the murburn purview, we can envisage the following set of reactions for PS II + LHC:

$$n\ \mathrm{LHC}/n\ \mathrm{PS} + n\ \mathrm{DS} + n\ h\nu \rightarrow n\ \mathrm{LHC}^{+\,*}/n\ \mathrm{PS}^{+\,*} + n\ \mathrm{DRS}^{-\,*}\ (\text{e.g. } {}^{*}\mathrm{H},\ \mathrm{Mg}^{+},\ {}^{*}\mathrm{O_2}^{-}) \tag{25}$$

Under such high potentials generated by multiple membrne-embedded species donating electrons and these electrostatics stabilized transidently via effective charge separation afforded by the various redox centers of PS II, the membrane becomes highly positively charged. Meanwhile, protons keep coming in through the membrane at millisecond timescales and DRS (like superoxide) can take up these protons and undergo dismutations to give peroxide and other DRS. This peroxide can be easily used by Mn-complex to further liberate electrons/O_2 and DRS. Furthermore, the stochastic interactive networks of "membrane complexes and soluble proteins (like Fd/PC) + DRS + water + hydroxide ion + bicarbonate ion + ADP/Pi + NAD(P)" thereafter can also liberate oxygen and make the other products (NADPH and ATP) in the vicinity of PS II. Further, the large lumenal protrusions bearing ADP sites and the distribution of Fd/PC in both stroma/lumen and the findings of Arnon (reduction of NADP even at PS II) support the murburn model of ADP phosphorylation and NADP reduction. In reductionist systems lacking CA or anoxic initial conditions also, oxygen formation would be possible wherein PS II could abstract an electron directly from a starting species like hydroxide ion. Such murburn processes would also not have energetic or kinetic or mechanistic or probabilistic limitations, as imposed by the erstwhile explicatory paradigm. In such a purview, other reactions of bicarbonate (an example is given below) would also be highly feasible, enabling rapid loss of isotope traces:

$$2\mathrm{HCO_3}^{-} + 2{}^{*}\mathrm{OH} \rightarrow 2{}^{*}\mathrm{CO_3}^{-} + 2\mathrm{H_2O}$$
$$2{}^{*}\mathrm{CO_3}^{-} + 2\mathrm{H}^{+} \rightarrow 2\mathrm{CO_2} + \mathrm{H_2O_2}$$

———————————————————————————————————.

$$2\mathrm{HCO_3}^{-}\ (-999.8) + 2{}^{*}\mathrm{OH}\ (25.8) + 2\mathrm{H}^{+}\ (412.5) \rightarrow 2\mathrm{CO_2}\ (-386) + \mathrm{H_2O_2}\ (-134.03) + 2\mathrm{H_2O}\ (-237.19);\ \Delta_r G^{\circ} = -257.41\ \mathrm{kJ/mol} \tag{26}$$

$$2\mathrm{H_2CO_3}\ (-623.1) + 2{}^{*}\mathrm{OH}\ (25.8) \rightarrow 2\mathrm{CO_2}\ (-386) + \mathrm{H_2O_2}\ (-134.03) + 2\mathrm{H_2O}\ (-237.19);\ \Delta_r G^{\circ} = -185.81\ \mathrm{kJ/mol} \tag{27}$$

The above "simple chemical engine" (SCE) murburn model for chloroplast resting on "effective charge separation" (ECS) principles is also justified by the underlying stochastic mechanistic principles, as observed in phosphorylation chemistry. In mid-twentieth century, Mildred Cohn had done pioneering experiments with oxygen-labeling in mitochondrial physiological phosphorylations [89, 90]. She had found that oxygen atom labels incorporated into phosphate or ATP were too quick and too numerous to be accounted for by classical enzyme reactions like substrate level phosphorylations. Reinterpreted in the current awareness, this experiment had originally showed that it was the DRS-activity that resulted in the physiological interactive equilibriums. The pioneer's original findings need to be understood and exalted, to really appreciate the dynamics of DR(O)S in bioenergetics. It can be clearly seen that only bimolecular fast radical reactions (as proposed in the current manuscript, via the murburn model) can afford meaningful explanations to the effects observed in bioenergetic chemistry.

4. Summation

By definition, since the Z-scheme sees electron flow from water to NADP (via a defined set of redox centers present on various proteins/biomolecules) as the chartered route, bicarbonate's involvement cannot be explained in the classical purview. This is because each set of electron transfer reaction in Z-scheme is governed by affinity-based logic and tuned to evolutionary perfection. Also, if electrons came in and went out at multiple entry and exit points, there is no point in having the terminology and consideration of a Z-scheme model anyway. In other words, the mandate of the Z-scheme cannot accommodate multiple e-donors or acceptors within physiology. Therefore, its advocates were disinclined to consider data/evidence that violates the ordered sequence of processes that they perceive to be physiologically operative. This was the primary reason that most classical researchers were reluctant to accept the effect of bicarbonate ions (by say, binding on to the non-heme Fe of PS II), in the first place.

We would reiterate that it is inappropriate to view any physiological redox reactions (incorporating multiple redox-active components within milieu) in a deterministic perspective. Within the murburn purview, a given component could have multiple interactive roles, as expected in a probabilistic scheme. This statement is particularly valid when evolutionary mandates may not dictate topographical identification/demarcation of substrates or when the molecules are too small to enable such topological differentiations. We demonstrated this fact with simple reductionist systems, wherein it was shown that classical treatments are inadequate to capture the outcomes observed [77, 91]. It is an evidence driven conclusion that photosynthetic electron transfer processes are not affinity-driven [17, 19, 21, 22, 35, 41] and the overall event is one-electron punctuated processes with a two-electron endings (akin to a paragraph formation using commas and periods) [18]. Murburn model is a stochastic scheme, with no "prescribed route" for reaction outcomes (although preferred routes may exist). The murburn precepts only necessitate an efficient charge separation process at the photosystem so that the photo-ejected electron does not get reabsorbed at the same electron-discharged chlorophyll. This perspective explains the random distribution of the multitudes of pigments and redox-active components in chloroplasts. The murburn purview also supports the evidence-backed proposal that

several ions, such as bicarbonate or chloride, may enhance reaction outcomes in photosynthesis [7, 36, 37]. We have demonstrated herein that this effect is owing to thermodynamic and kinetic reasons, wherein such species play favorable roles in murburn ion-radical equilibriums, and they need not directly bind to any particular locus of the photosystems. In this context, it is inappropriate to continue to consider DR(O)S as a mere manifestation of pathophysiology. Evolution should have done away with the multitudes of 1e centers if DRS were merely deleterious. We also know that all molecular species have contextual relevance. That is: the right amounts of a given DRS at the right places could be useful to physiology. Wrong amounts of an unwanted DRS at the inappropriate place would surely be unproductive to life order. The inferences presented herein and the relevance of DRS in routine physiology is further corroborated by the ubiquitous nature and pan-systemic appeal of murburn concept. The murburn explanation for photo-transduction in vision [92] and the relevance of murzyme functioning for explaining observations and theoretical premises in diverse phospholipid-laden systems of endoplasmic reticula (microsomes), mitochondria, rod cell outer segments, etc. [76] are supportive testaments to this claim. Given that this macroscopic awareness affords a more comprehensive and tangible picture than deeming localized oxygen evolution at Mn-Complex alone, the findings on structures of PS II need to be re-interpreted. Although we could not find water-access modalities to the Mn-center in our analyses, some reports from other groups demarcate several channels [93]. If this were in fact the case, how is it physically possible to restrict two specific water molecules at the active complex, simultaneously preventing the formation/release of DRS at/from PS II [94]? This query would still remain inexplicable in the classical perspective, seeking more and more intangible apologetics of the classical view. Given the fact that the Z-scheme-KokJoliot cycle modality of photosynthesis (which was incorporated in the mega ETC-CRAS paradigm of bioenergetics) has been theoretically dissected and demonstrated to be infeasible, and also the premise that the classical perspectives have been experimentally nullified by multitudes of observations [18–20, 22, 25], the classical perspective should be deemed a redundant aspect of the past. In the least, an honest discussion should be initiated between the workers, mediated by biochemists and biophysicists that do not pose conflict of interests. Else, the future generations would continue to be taught redundant ideas [95, 96], limiting critical thought and potentially waylaying the outlooks availing more efficient green methodologies for harnessing the potential of photosynthesis.

To ratify the murburn chemistry in oxygenic photosynthesis, one of the easiest ways is to add bicarbonate at various concentration ranges and note the outcomes on oxygenensis, nicotinamide reduction and ADP phosphorylation. Since these processes are intricately woven into murburn chemistry, we predict that the stoichiometry of observed would be uncertain/variable, and might show unusual concentration-based effects. Also, this effect is not expected only at PS II, as the outcome is based on multiple reaction equilibriums in milieu (and not necessarily binding to a particular site on a protein!). At this level of awareness, although it is evident that the photosynthetic chemistry would show a variable/uncertain stoichiometry owing to murburn operational principles, the overall process can now be minimally represented as: $2AH/2H_2A + n h\nu \rightarrow A_2 + 2H^+/4H^+ + 2e^-/4e^-$ for simple substrate precursor molecules. Since the murburn model proposes that proton consumption is more favored in bioenergetic routines, the latter route appears more probable. The insights herein could be availed for designing murburn-based photosynthetic (bio)reactors.

Acknowledgements

The work was powered by Satyamjayatu: The Science & Ethics Foundation.

Author contributions

KMM conceived the problems, solutions and wrote the first draft of the paper. YW presented crucial arguments and literature, pointed out key theoretical aspects involved and helped in rewriting the paper. NMB calculated the free energy of formation of carbon dioxide in water and cross-checked the equations. AM provided inputs and helped in shaping the paper. AP made some images of photosystems and analyzed structures.

Funding statement

The work was not funded by any specific agency/body.

Conflict statement

The authors have no conflict of interests to declare.

Data statement

All data needed for the perusal of this document are presented within the manuscript text/supplementary information or duly cited therein.

Abbreviations

ANBS	adenosine nucleotide binding site
CA	carbonic anhydrase
CRAS	chemiosmotic rotary ATP synthesis
CYP	cytochrome P450
CPR	cytochrome P450 reductase
DR(O)S	diffusible reactive (oxygen) species
ECS	effective charge separation
ETC	electron transport chain
Fd	ferredoxin
FNR	ferredoxin-NADP reductase
KJC	Kok-Joliot cycle, K-J cycle
LHC	light harvesting complex
OxPhos	oxidative phosphorylation
PC	plastocyanin
PhotoPhos	photophosphorylation
pmf	proton motive force

PoPs	pumped out protons
PS	photosystem
Q/QH_2	quinones/quinols
RC-Chl	reaction center chlorophyll
TMP	trans-membrane potential
WSC	water splitting complex (also called OEC, oxygen evolving complex or Mn-complex)

A. Appendix

Item 1: Survey of electron acceptors from photosystems

No.	Name	Molecular Formula	Mass (g/mol)	From PSI/PSII	MV (cm^3)	PSA ($Å^2$)	log P	H-Bonds	Rotating bonds
1	Tetrazolium blue	$C_{40}H_{32}Cl_2N_8O_2$	727.6	I	na	87.6	na	8.0	9
2	DCPIP	$C_{12}H_7NCl_2O_2$	268.0	I	186.7	50	1.91	3.1	1
3	Benzoquinone	$C_6H_4O_2$	108.0	I/II	86.0	34.1	0.26	2.0	0
4	Flavin mononucleotide	$C_{17}H_{22}N_4NaO_{10}P$	496.3	I/II	na	214	−1.7	10.3	6
5	Ferrioxalate	C_6FeO_{12}	319.9	I	na	na	na	na	na
6	$HgCl_2$	HgCl2	271.4	I/II	na	0	na	0.0	0
7	Methyl red	$C_{15}H_{15}N_3O_2$	269.3	I	230.1	52	4.91	5.1	4
8	Paraquat	$C_{12}H_{14}Cl_2N_2$	257.1	I	na	7.8	1.7	0.0	1
9	Ferricyanide	C_6FeN_6	211.9	I/II	na	143	na	12.0	0
10	Anthraquinone	$C_{14}H_8O_2$	208.2	I	159.1	34	3.38	2.0	0
11	Diquat	$C_{12}H_{12}N_2$	184.2	I	na	8	−4.7	2.0	0
12	Napthoquinone	$C_{10}H_6O_2$	158.2	I	122.6	34	1.79	2.0	0
13	Silicotungstate	$H_{72}Na_4O_{40}SiW_{12}$	2970.1	II	na	657	na	40.4	8

Table A1.
A compilation of electron acceptors from the two photosystems.

Item 2: Thermodynamics & Kinetics

K_{eq} and log K values are from Stryer's textbook or from the following web sources:

(http://ion.chem.usu.edu/~sbialkow/Classes/3650/Carbonate/Carbonic%20Acid.html) (https://lawr.ucdavis.edu/classes/ssc102/Section5.pdf).

For calculation of thermodynamic constants, the following steps were used.

1. CO_{2gas}

In the gas phase in a standard system $\Delta_f(CO_{2gas}) = -394.4$ kJ/mol

2. CO_{2aq}

When CO_2 is dissolved in water, there are two types of particles in solution: CO_{2aq} and H_2CO_3. H_2CO_3 occurs in the equilibrium reaction

$$CO_{2aq} + H_2O = H_2CO_3 \tag{28}$$

Thus, CO_2 in the gas phase is in equilibrium simultaneously with two particles

$$CO_{2aq} = CO_{2aq} + H_2CO_3 \tag{29}$$

Solubility constant $K_H = 3{,}4 \cdot 10^{-2}$ and $\Delta_r G = 8.35$ kJ/mol. The concentration of H_2CO_3 is low and therefore the value $\Delta_r G = 8.35$ kJ/mol refers to the dissolution of CO_2. Hence,

$$\Delta_f\left(CO_{2aq}\right) = -394.4 + 8.35 = -386 \text{ kJ/mol}.$$

3. H_2CO_3

It is generally accepted that $\Delta_r G(\text{reaktion } 1) \approx 0$

Then $\Delta_f(H_2CO_3) = \Delta_r G(1) + \Delta_f\left(CO_{2aq}\right) + \Delta_f(H_2O) = 0 - 386 - 237.1 = -623.1$ kJ/mol

4. HCO_3^-

HCO_3^- arises from acid dissociation

$$H_2CO_3 = HCO_3^- + H^+$$

The dissociation constant is $4{,}47 \cdot 10^{-7}$, and $\Delta_r G^o = 36.78$ kJ/mol.

Hence,

$\Delta_f G^o\left(HCO_3^-\right) = \Delta_r G^o + \Delta_f G^o(H_2CO_3) - \Delta_f G^o(H^+) = 36.78 - 623.1 - 412.5 = -999.82$

5. Dissociation of HCO_3^- is characterized by the constant $4.68 \cdot 10^{-11} = 10^{-10.33}$.

Hence

$$\Delta_r G^o = 64.56 \text{ kJ/mol}$$

$$HCO_3^- \ (-999.8) = H^+(412.5) + CO_3^{2-} \ \Delta_r G^o = 64.56 \text{ kJ/mol}$$

Hence,

$$\Delta_f G^o\left(CO_3^{2-}\right) = -1347.7 \text{ kJ/mol}$$

A compilation of standard thermodynamic parameters of particles (arising from the dissolution of CO_2 in water) is given in **Table A2**.

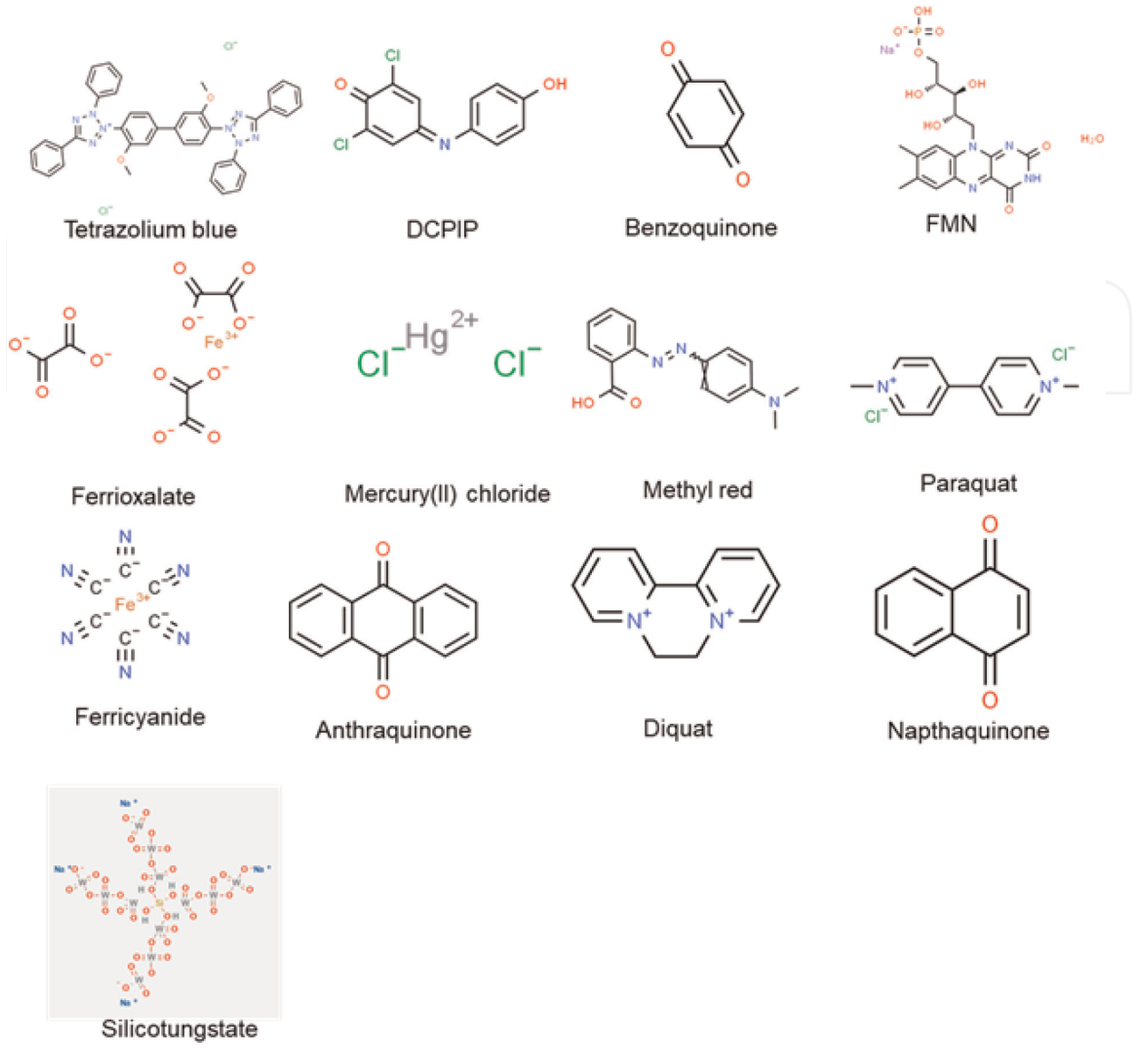


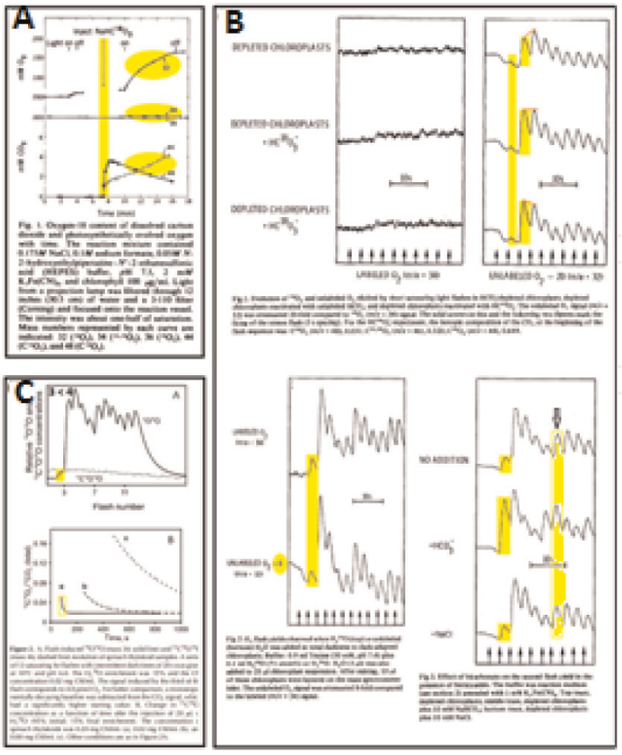
Figure A1.
Structures of various electron acceptors listed in Table A1.

Molecule	$\Delta_f H^o$, kJ/mol	$\Delta_f G^o$, kJ/mol
H_2O_{aq}	−285.8	−237.1
CO_{2gas}	−393.5	−394.4
$CO_{2,aq}$	−413.8	−386.0
H_2CO_3	−699.2	−623.1
HCO_3^-	−1078.3	−999.8
CO_3^{2-}	−1449.6	−1347.7

Table A2.
Standard thermodynamic properties of species considered in this study.

Item 3: Earlier published data on bicarbonate/water labeling and oxygenesis

[Panel (A): Stemler A, Radmer R. [83]. Panel (B): Radmer R, Ollinger O. [82]. Panel (C): Clausen et al. [10]. The resolution is deliberately reduced so as to avoid copyright issues. The readers may peruse the pertinent articles and study the areas highlighted in yellow.



Author details

Kelath Murali Manoj[1*], Nikolai Bazhin[2], Abhinav Parashar[1], Afsal Manekkathodi[3*] and Yanyou Wu[4*]

1 Satyamjayatu: The Science and Ethics Foundation, Palakkad, India

2 Institute of Chemical Kinetics and Combustion, Russian Academy of Sciences, Novosibirsk, Russia

3 Department of Physics, Sultan Qaboos University, Al-khod, Sultanate of Oman

4 State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, People's Republic of China

*Address all correspondence to: murman@satyamjayatu.com; a.manekkathodi@squ.edu.om; wuyanyou@mail.gyig.ac.cn

IntechOpen

References

[1] Nowicka B, Kruk J. Powered by light: Phototrophy and photosynthesis in prokaryotes and its evolution. Microbiological Research. 2016;**186–187**: 99-118

[2] Whitmarsh J, Govindjee G. The Photosynthetic Process. In: Singhal GS, Renger G, Sopory SK, Irrgang K-D, Govindjee, editors. Concepts in Photobiology: Photosynthesis and Photomorphogenesis. New Delhi: Narosa Publishers and Dordrecht: Kluwer Academic; 1995. pp. 11-51. Available from: https://www.life.illinois.edu/govindjee/paper/gov.html

[3] Ruben S, Randall M, Kamen M, Hyde JL. Heavy oxygen (18O) as a tracer in the study of photosynthesis. Journal of the American Chemical Society. 1941;**63**: 877-879

[4] Warburg O, Krippahl G. Hill-Reaktionen [Hill reactions]. Zeitschrift für Naturforschung. Teil B. 1958;**13B**(8): 509-514

[5] Baranov SV, Ananyev GM, Klimov VV, Dismukes GC. Bicarbonate accelerates assembly of the inorganic core of the water-oxidizing complex in manganese-depleted photosystem II: A proposed biogeochemical role for atmospheric carbon dioxide in oxygenic photosynthesis. Biochemistry. 2000;**39** (20):6060-6065

[6] van Rensen JJS. Role of bicarbonate at the acceptor side of photosystem II. Photosynthesis Research. 2002;**73**:185-192

[7] Stemler AJ, Govindjee. Bicarbonate ion as a critical factor in photosynthetic oxygen evolution. Plant Physiology. 1973;**52**:119-123

[8] Stemler AJ. The bicarbonate effect, oxygen evolution and the shadow of Otto Warburg. Photosynthesis Research. 2002;**73**:177-183

[9] Aoyama C, Suzuki H, Sugiura M, Noguchi T. Flash-induced FTIR difference spectroscopy shows no evidence for the structural coupling of bicarbonate to the oxygen-evolving Mn cluster in photosystem II. Biochemistry. 2008;**47**:2760-2765

[10] Clausen J, Beckmann K, Junge W, Messinger J. Evidence that bicarbonate is not the substrate in photosynthetic oxygen evolution. Plant Physiology. 2005;**139**(3):1444-1450

[11] Hillier W, McConnell I, Badger MR, Boussac A, Klimov VV, Dismukes GC, et al. Quantitative assessment of intrinsic carbonic anhydrase activity and the capacity for bicarbonate oxidation in photosystem II. Biochemistry. 2006;**45** (7):2094-2102

[12] Ulas G, Olack G, Brudvig GW. Evidence against bicarbonate bound in the O_2-evolving complex of Photosystem II. Biochemistry. 2008;**47**(10):3073-3075

[13] Shevela D, Eaton-Rye JJ, Shen JR, Govindjee G. Photosystem II and the unique role of bicarbonate: A historical perspective. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2012;**1817**: 1134-1151

[14] Wu Y. Is bicarbonate directly used as substrate to participate in photosynthetic oxygen evolution. Acta Geochim. 2021a; **40**:650-658. DOI: 10.1007/s11631-021-00484-0

[15] Wu Y. Bicarbonate use and carbon dioxide concentrating mechanisms in photosynthetic organisms. Acta Geochim. 2021b. DOI: 10.1007/s11631-021-00488-w

[16] Wu YY, Li HT, Xie TX. The regulation on carbon source and carbon sequestration by microalgal carbonic anhydrase, Biogeochemical Action of Microalgal Carbonic Anhydrase. Beijing: Science Press; 2015. pp. 76-111

[17] Gideon DA, Nirusimhan V, Manoj KM. Are plastocyanin and ferredoxin specific electron carriers or generic redox capacitors? Classical and murburn perspectives on two photosynthetic proteins. Journal of Biomolecular Structure and Dynamics. 2022;**40**(5): 1995-2009. DOI: 10.1080/07391102.2020.1835715

[18] Manoj KM, Bazhin N, Manekkathodi A, Wu Y. In: Ameen S et al., editors. Murburn Model of Photosynthesis: Effect of Additives like Chloride and Bicarbonate, Chlorophylls. London: Intech Open; 2022b. DOI: 10.5772/intechopen.103132

[19] Manoj KM, Bazhin NM, Jacob VD, Parashar A, Gideon DA, Manekkathodi A. Structure-function correlations and system dynamics in oxygenic photosynthesis: Classical perspectives and murburn precepts. Journal of Biomolecular Structure & Dynamics. 2021a. DOI: 10.1080/07391102.2021.1953606

[20] Manoj KM, Bazhin NM, Tamagawa H, Jaeken L, Parashar A. The physiological role of complex V in ATP synthesis: Murzyme functioning is viable whereas rotary conformation change model is untenable. Journal of Biomolecular Structure and Dynamics. 2022a. DOI: 10.1080/07391102.2022.2060307

[21] Manoj KM, Gideon DA, Parashar A. What is the role of lipid membrane-embedded quinones in mitochondria and chloroplasts? Chemiosmotic Q-cycle versus murburn reaction perspective. Cell Biochemistry and Biophysics. 2020b;**79**:3-10

[22] Manoj KM, Jacob VD, Parashar A, Gideon DA, Manekkathodi A. Validating the predictions of murburn model for oxygenic photosynthesis: Analyses of ligand binding to protein complexes and cross-system comparisons. Journal of Biomolecular Structure & Dynamics. 2021b. DOI: 10.1080/07391102.2021.1953607

[23] Manoj KM, Manekkathodi A. Light's interaction with pigments in chloroplasts: The murburn perspective. J. Photochem. Photobiol. 2021;**5**:100015

[24] Manoj KM, Gideon DA, Jaeken L. Why do cells need oxygen? Insights from mitochondrial composition and function. Cell Biology International. 2021c;**46**(3): 344-358. DOI: 10.1002/cbin.11746

[25] Manoj KM, Gideon DA, Jaeken L. Interaction of membrane-embedded cytochrome b -complexes with quinols: Classical Q-cycle and murburn model. Cell Biochemistry and Function. 2021d; **40**(2):118-126. DOI: 10.1002/CBF.3682

[26] Manoj KM, Bazhin NM. Murburn precepts of aerobic respiration and homeostasis. Progress in Biophysics and Molecular Biology. 2021;**167**:104-120. DOI: 10.1016/j.pbiomolbio.2021.05.010

[27] Manoj KM et al. Murburn precepts for lactic-acidosis, Cori cycle, and Warburg effect: Interactive dynamics of dehydrogenases, protons, and oxygen. Journal of Cellular Physiology. 2021e;**237**(3):1902-1922. DOI: 10.1002/jcp.30661

[28] Suga M, Akita F, Yamashita K, Nakajima Y, Ueno G, Li H, et al. An oxyl/oxo mechanism for oxygen-oxygen coupling in PSII revealed by an x-ray free-electron laser. Science. 2019;**366**

(6463):334-338. DOI: 10.1126/science.aax6998

[29] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—A visualization system for exploratory research and analysis. Journal of Computational Chemistry. 2004;**25**(13):1605-1612

[30] Schrödinger L, DeLano W. PyMOL. 2020. Retrieved from: http://www.pymol.org/pymol

[31] Yu J, Zhou Y, Tanaka I, Yao M. Roll: A new algorithm for the detection of protein pockets and cavities with a rolling probe sphere. Bioinformatics. 2010;**26**(1):46-52

[32] Marcus RA. On the theory of oxidation-reduction reactions involving electron transfer I. The Journal of Chemical Physics. 1956;**24**:966-978

[33] Mehler AH. Studies on reactions of illuminated chloroplasts. I. Mechanisms of the reduction of oxygen and other Hill reagents. Archives of Biochemistry and Biophysics. 1951;**33**:65-77

[34] Hauska G, Oettmeier W, Reimer S, Trebst A. Shuttles of artificial electron donors for photosystem I across the thylakoid membrane. Z Naturforsch C Biosci. 1975;**30**(1):37-45. DOI: 10.1515/znc-1975-1-209

[35] Hauska G. Artificial acceptors and donors. In: Pirson A, Zimmermann MH. editors. Vol. 5. Photosynthetic Electron Transport and Photophosphorylation. Berlin-Heidelberg-New York: Springer; 1977. pp. 253-265

[36] Izawa S, Heath RL, Hind G. The role of chloride ion in photosynthesis. 3. The effect of artificial electron donors upon electron transport. Biochimica et Biophysica Acta. 1969;**180**(2):388-398. DOI: 10.1016/0005-2728(69)90123-6

[37] Kaňa R, Govindjee. Role of ions in the regulation of light-harvesting. Frontiers in Plant Science. 2016;**7**:1849. DOI: 10.3389/fpls.2016.01849

[38] Kelley PM, Izawa S. The role of chloride ion in photosystem II. I. Effects of chloride ion on photosystem II electron transport and on hydroxylamine inhibition. Biochimica et Biophysica Acta. 1978;**502**(2):198-210. DOI: 10.1016/0005-2728(78)90042-7

[39] Magnuson A. Electron donor systems in natural and artificial photosynthesis. [Doctoral Thesis]. Lund University. 1998. ISBN: 91-628-3149-6

[40] Maslenkova A, Zeilanov Y. Effect of some artificial electron donors and acceptors on the functioning of the photosynthetic oxygen evolving system. Bulgarian Journal of Plant Physiology. 1995;**21**:3-11

[41] Tschortner J, Lai B, Kromer JO. Biophotovoltaics: Green power generation from sunlight and water. Frontiers in Microbiology. 2019;**10**:866

[42] Kühlbrandt W. Structure and function of bacterial light-harvesting complexes. Structure. 1995;**3**:521-525

[43] Croce R, van Amerongen H. Light harvesting in oxygenic photosynthesis: Structural biology meets spectroscopy. Science. 2020;**369**:eaay2058

[44] Arnon DI, Allen MB, Whatley FR. Photosynthesis by isolated chloroplasts. Nature. 1954a;**174**:394-396

[45] Arnon DI, Whatley FR, Allen MB. Photosynthesis by isolated chloroplasts. II. Photosynthetic phosphorylation, the

conversion of light into phosphate bond energy. Journal of the American Chemical Society. 1954b;**76**:6324-6329

[46] Arnon DI. Photosynthetic electron transport: Emergence of a concept, 1949–59. Photosynthesis Research. 1991; **29**:117-131

[47] Arnon DI, Tsujimoto HY, Tang GM. Proton transport in photooxidation of water: A new perspective on photosynthesis. Proceedings of the National Academy of Sciences of the United States of America. 1981;**78**:2942-2946

[48] Arnon DI, Tsujimoto HY, Tang GM-S. Contrasts between oxygenic and anoxygenic photoreduction of ferredoxin: Incompatibilities with prevailing concepts of photosynthetic electron transport. Proceedings of the National Academy of Sciences USA. 1980;77:2676-2680

[49] Arnon DI. Divergent pathways of photosynthetic electron transfer: The autonomous oxygenic and anoxygenic photosystems. Photosynthesis Research. 1995;**46**:47-71

[50] Fernandez-Velasco JG, Jamshidi A, Gong XS, Zhou J, Ueng RY. Photosynthetic electron transfer through the cytochrome b6f complex can bypass cytochrome f. The Journal of Biological Chemistry. 2001;**276**:30598-30607

[51] Pesaresi P, Scharfenberg M, Weigel M, Granlund I, Schr€oder WP, Finazzi G, et al. Mutants, overexpressors, and interactors of arabidopsis plastocyanin isoforms: Revised roles of plastocyanin in photosynthetic electron flow and thylakoid redox state. Molecular Plant. 2009;**2**:236-248

[52] Zhang L, Pakrasi HB, Whitmarsh J. Photoautotrophic growth of the cyanobacterium Synechocystis sp. PCC 6803 in the absence of cytochrome c553 and plastocyanin. The Journal of Biological Chemistry. 1994;**269**: 5036-5042

[53] Forsman JA, Fagerlund RD, Biswas S, Summerfield TC, Eaton-Rye JJ. The PsbT protein modifies the bicarbonate-binding environment of Photosystem II. New Zealand Journal of Botany. 2020;**58** (4):406-421. DOI: 10.1080/ 0028825X.2020.1772320

[54] Emerson R, Chalmers R, Cederstrand C. Some factors influencing the long-wave limit of photosynthesis. Proceedings of the National Academy of Sciences. 1957;**43**:133-143

[55] Emerson R. Dependence of yield of photosynthesis in long wave red on wavelength and intensity of supplementary light. Science. 1957;**125**: 746

[56] Govindjee G, Rabinowitch E. Photosynthesis. 1st ed. New York City: John Wiley & Sons, Inc.; 1969

[57] Manoj KM. Aerobic respiration: Criticism of the proton-centric explanation involving rotary adenosine triphosphate synthesis, chemiosmosis principle, proton pumps and electron transport chain. Biochemistry Insights. 2018;**11**:1178626418818442

[58] Manoj KM, Ramasamy S, Parashar A, Gideon DA, Soman V, Jacob VD, et al. Acute toxicity of cyanide in aerobic respiration: Theoretical and experimental support for murburn explanation. Biomolecular Concepts. 2020a;**11**:32-56

[59] Badger MR, Price GD. The role of carbonic anhydrase in photosynthesis. Annual Review of Plant Physiology and

Plant Molecular Biology. 1994;**45**: 369-392

[60] Kupriyanova E, Pronina N, Los D. Carbonic anhydrase: A universal enzyme of the carbon-based life. Photosynthetica. 2017;**55**:3-19

[61] Moroney JV, Bartlett SG, Samuelsson G. Carbonic anhydrases in plants and algae. Plant, Cell & Environment. 2001;**24**:141-153

[62] Lu YK, Stemler AJ. Extrinsic photosystem II carbonic anhydrase in maize mesophyll chloroplasts. Plant Physiology. 2002;**128**(2):643-649. DOI: 10.1104/pp.010643

[63] Shitov AV, Pobeguts OV, Smolova TN, Allakhverdiev SI, Klimov VV. Manganese-dependent carboanhydrase activity of photosystem II proteins. Biochemistry (Mosc). 2009;**74**(5): 509-517. DOI: 10.1134/s00062979090 50058

[64] Hines KM, Chaudhary V, Edgeworth KN, Owens TG, Hanson MR. Absence of carbonic anhydrase in chloroplasts affects C_3 plant development but not photosynthesis. Proceedings of the National Academy of Sciences. 2021;**118**: e2107425118

[65] Housecroft CE, Sharpe AG. Inorganic Chemistry. 2nd ed. Harlow (England): Prentice-Pearson-Hall; 2005. p. 368

[66] Stumm W, Morgan JJ. Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters. 3rd ed. New York: John Wiley & Sons, Inc.; 1996

[67] Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th ed (Section 9.2). New York: W H Freeman; 2002

[68] Gutknecht J, Bisson MA, Tosteson FC. Diffusion of carbon dioxide through lipid bilayer membranes: effects of carbonic anhydrase, bicarbonate, and unstirred layers. Journal of General Physiology. 1977;**69**:779-794

[69] Pocker Y, Bjorkquist D. Stopped-flow studies of carbon dioxide hydration and bicarbonate dehydration in H_2O and D_2O acid–base and metal ion catalysis. Journal of the American Chemical Society. 1977;**99**:6537-6543

[70] Dismukes GC, Klimov VV, Baranov SV, Kozlov YN, DasGupta J, Tyryshkin A. The origin of atmospheric oxygen on Earth: The innovation of oxygenic photosynthesis. Proceedings of the National Academy of Sciences. 2001;**98**: 2170-2175

[71] Bakhmutova-Albert EV, Yao H, Denevan DE, Richardson DE. Kinetics and mechanism of peroxymonocarbonate formation. Inorganic Chemistry. 2010;**49**(24): 11287-11296

[72] Richardson DE, Yao H, Frank KM, Bennett DA. Equilibria, kinetics, and mechanism in the bicarbonate activation of hydrogen peroxide: Oxidation of sulfides by peroxymonocarbonate. Journal of the American Chemical Society. 2000;**122**(8):1729-1739

[73] Patra SG, Mizrahi A, Meyerstein D. The role of carbonate in catalytic oxidations. Accounts of Chemical Research. 2020;**53**(10):2189-2200. DOI: 10.1021/acs.accounts.0c00344

[74] Kuttassery F, Sebastian A, Mathew S, Tachibana H, Inoue H. Promotive effect of bicarbonate ion on one-electron oxidation initiated two-electron water oxidation to form hydrogen peroxide catalyzed by lluminum porphyrins.

ChemSusChem. 2019;**12**(9):1939-1948. DOI: 10.1002/cssc.201900560

[75] Okrasa K, Kazlauskas RJ. Manganese-substituted carbonic anhydrase as a new peroxidase. Chemistry - A European Journal. 2006; **12**:1587-1596

[76] Manoj KM, Gideon DA. Structural foundations for explaining the physiological roles of murzymes embedded in diverse phospholipid membranes. Biochimica et Biophysica Acta – Biomembranes. 2022c;**1864**(10): 183981. DOI: 10.1016/j.bbamem.2022.183981

[77] Manoj KM, Parashar A, Venkatachalam A, Goyal S, Satyalipsu SPG, Gade SK, et al. Atypical profiles and modulations of heme-enzymes catalyzed outcomes by low amounts of diverse additives suggest diffusible radicals' obligatory involvement in such redox reactions. Biochimie. 2016;**125**:91-111

[78] Bielski BHJ, Cabelli DE. Superoxide and hydroxyl radical chemistry in aqueous solution. In: Foote CS et al., editors. Active oxygen in chemistry. New York: Chapman & Hall; 1995. pp. 66-104

[79] Buxton GV, Greenstock CL, Helman WP, Ross AB. Critical Review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($\cdot OH/\cdot O^-$) in aqueous solution. Journal of Physical and Chemical Reference Data. 1988;**17**:513. DOI: 10.1063/1.555805

[80] Jagendorf AT, Uribe E. ATP formation caused by acid-base transition of spinach chloroplasts. Proceedings of the National Academy of Sciences of the United States of America. 1966;**55**(1): 170-177. DOI: 10.1073/pnas.55.1.170

[81] Warburg O. Annual Review of Biochemistry- Prefatory Chapter. Annual Review of Biochemistry. 1964; **33**:1-14

[82] Radmer R, Ollinger O. Isotopic composition of photosynthetic O_2 flash yields in the presence of $H_2{}^{18}O$ and $HC^{18}O_3{}^-$. FEBS Letters. 1980;**110**(1): 57-61

[83] Stemler A, Radmer R. Source of photosynthetic oxygen in bicarbonate-stimulated Hill reaction. Science. 1975; **190**:457-458

[84] Koroidov S, Shevela D, Shutova T, Samuelsson G, Messinger J. Mobile hydrogen carbonate acts as proton acceptor in photosynthetic water oxidation. Proceedings of the National Academy of Sciences. 2014;**111**: 6299-6304

[85] Metzner H. Water decomposition in photosynthesis? A critical reconsideration. Journal of Theoretical Biology. 1975;**51**:201-231

[86] Shevela D, Nöring B, Koroidov S, Shutova T, Samuelsson G, Messinger J. Efficiency of photosynthetic water oxidation at ambient and depleted levels of inorganic carbon. Photosynthesis Research. 2013;**117**:401-412

[87] Van Rensen JJS, Xu C, Govindjee. Role of bicarbonate in photosystem II, the water-plastoquinone oxido-reductase of plant photosynthesis. Physiologia Plantarum. 1999;**105**:585-592

[88] Barber J. Photosystem II: Redox and protein components. Encyclopedia of Biological Chemistry. 2013:512-519

[89] Cohn M, Drysdale GR. A study with O18 of adenosine triphosphate formation in oxidative phosphorylation. The

Journal of Biological Chemistry. 1955; **216**:831-846

[90] Cohn M. A study of oxidative phosphorylation with O-18 labeled inorganic phosphate. The Journal of Biological Chemistry. 1953;**201**:735-750

[91] Manoj KM. Chlorinations catalyzed by chloroperoxidase occur via diffusible intermediate(s) and the reaction components play multiple roles in the overall process. Biochimica et Biophysica Acta/General Subjects. 2006;**1764**: 1325-1339

[92] Manoj KM, Tamagawa H, et al. Murburn model of vision: Precepts and proof of concept. Journal of Cellular Physiology. 2022;**237**(8):333–3355. DOI: 10.1002/jcp.30786

[93] Hussein R, Ibrahim M, Bhowmick A, Simon PS, Chatterjee R, Lassalle L, et al. Structural dynamics in the water and proton channels of photosystem II during the S_2 to S_3 transition. Nature Communications. 2021;**12**(1):6531. DOI: 10.1038/s41467-021-26781-z

[94] Johnson VM, Pakrasi HB. Advances in the understanding of the lifecycle of photosystem II. Microorganisms. 2022; **10**(5):836. DOI: 10.3390/ microorganisms10050836

[95] Jaiswal S, Bansal M, Roy S, Bharati A, Padhi B. Electron flow from water to $NADP^+$ with students acting as molecules in the chain: A Z-scheme drama in a classroom. Photosynthesis Research. 2017;**131**(3):351-359. DOI: 10.1007/s11120-016-0317-z

[96] Mohapatra PK, Singh NR. Teaching the Z-Scheme of electron transport in photosynthesis: a perspective. Photosynthesis Research. 2015;**123**(1): 105-114. DOI: 10.1007/s11120-014-0034-4