Explanations for the enhancement of oxygenic photosynthesis by bicarbonate and diverse additives: Affinity-driven binding with photosystems versus murburn model

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Abstract: The classical explanation of photosynthesis starts with the abstraction of electrons from water molecules bound to the MnComplex of Photosystem II. These electrons are then passed/transported via sequentially ordered redox centers of various biomolecules, before finally being used for the reduction of NADP⁺ bound to flavin-enzyme reductase. The finding by several research groups that additives like bicarbonate/chloride ions enhance the light reaction cannot be satisfactorily explained by affinity-based binding of such species to Photosystems and the research community is divided on this topic. Recently, we had demonstrated that the classical deterministic/serial ETC perception was flawed and applied murburn bioenergetics (a stochastic/parallel paradigm of ion-radical equilibriums) to explain the physiological Emerson effect and overall phenomenology of photophosphorylation (Manoj et al., *J. Biomol. Str. Dyn.* 2021). Herein, we elucidate that bicarbonate could enhance the light reaction by virtue of aiding murburn interactive equilibriums in milieu. Our accurate thermodynamic calculations show that the one-electron abstraction from bicarbonate [$\Delta_r G^\circ \approx 491$ kJ/mol] is comparable with water [$\Delta_r G^\circ \approx 527$ kJ/mol]. With structural analysis of photosystems and their interactions with known electron donors/systemic components, we chart out the thermodynamics of probabilistic reactions that explain delocalized O₂-evolution. Copious thioredoxin enables the capture of

photoactivated electrons, which reduce the nicotinamide nucleotides whereas the diffusible reactive species (DRS) generated in milieu sponsor phosphorylations and thermogenic/oxygenic reactions. These considerations provide a satisfactory resolution of outstanding observations reported in literature, e.g. Stemler & Radmer, *Science*, 1977; Radmer & Ollinger, *FEBS Lett.*, 1980; Clausen et al., *Plant Physiol.*, 2005.

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

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/QH₂: quinones/quinols; RC-Chl: Reaction Center chlorophyll; TMP: trans-membrane potential; WSC: water splitting complex (also called OEC, oxygen evolving complex or MnComplex);

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1. Introduction to the additive (bicarbonate) sponsored effects

From fossil records, scientists estimate that earth has witnessed about three gigayears of biological photosynthetic activity (Nowicka and Kruk, 2016). Historical documents indicate that by 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: $\text{CO}_2 + 2\text{H}_2\text{A} + h\nu \rightarrow \text{CH}_2\text{O} + 2\text{A} + \text{H}_2\text{O}$, wherein 'A' denotes an oxygen or sulfur atom (Whitmarsh & Govindjee, 1995). 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 (Ruben et al., 1941). Since the latter half of 20th 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 of 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 ion served to enhance oxygen evolution in the light reaction (Warburg and Krippahl, 1958), 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) (Baranov et al., 2000; van Rensen, 2002), some have advocated a more direct, i.e. substrate like role of bicarbonate (Stemler & Govindjee, 1973; Stemler, 2002). In later times, as some researchers emphatically claimed that there was no evidence for the direct roles of bicarbonate (Clausen et al., 2005; Hillier et al., 2006; Aoyama et al., 2008; Ulas et al., 2008), the general opinion has 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 (Shevela et al., 2012), a few others have campaigned steadfast for bicarbonate playing more direct roles (Wu et al., 2015; Wu, 2021a,b). 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 (Manoj et al., 2020; Gideon et al., 2020; Manoj & Manekkathodi, 2021; Manoj et al., 2021a-d) 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/analyses, 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 (Manoj & Bazhin, 2021; Manoj et al., 2021e). 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) (Suga et al., 2019) (and other proteins/biomolecules) was analyzed by visualization software like Chimera 1.12 (Pettersen et al., 2004) and PyMOL (Schrodinger & Delano, 2020). Cavities of proteins were analyzed by POCASA (Yu et al., 2010). Small molecular/ionic properties were availed from ChemSpider/PubChem registry and ACDLabs/ChemAxon predictions.

3. Addressing experimental observations with classical and murburn models

3.1. 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 20th 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 electron transfer process was supposedly synchronous with “pumping of protons”, whose energy was

subsequently harnessed for making ATP. The ability to fix CO₂ 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 (Mehler, 1951)! 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 (Izawa et al., 1969; Hauska et al., 1975; Hauska, 1977; Kelley & Izawa, 1978; Maslenkova & Zeinalov, 1995; Magnuson, 1998; Kana & Govindjee, 2016; Tschortner et al., 2019). In the context of this 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 the two photosystems is presented in Tables 1/2 and Figures 1/2, respectively. (In continuum, Table S1 and Figure S1 of Supplementary Information lists a compilation of some electron acceptors from PS I and II.)

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

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	Diphenylcarbazine	C ₁₃ H ₁₄ N ₄ O	242	II	187	65	2.24	5,4	4
3	Ferrocyanide	C ₆ FeN ₆	212	II	na	143	na	6,0	0
4	2-ketogluconate	C ₆ H ₉ O 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 ₆ H ₇ O 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 ₃ H ₇ NO ₂ S	121	II	91	102 (63)	0.23 (-2.79)	3,3	2

11	Hydroquinone (3.4-4.3)	C ₆ H ₆ O ₂	110	I/II	86	40	0.64 (1.37)	2,2	0
12	Phenylenediamine (3.4-4.6)	C ₆ H ₈ N ₂	108	I/II	94	52	0.05 (0.32)	2,4	0
14	Semicarbazide (2.8-3.7)	CH ₅ N ₃ O	75	II	43	82	-1.61	4,5	1
15	Hydrogen peroxide (2.0-2.3)	H ₂ O ₂	34	II	24	40	-0.43	2,2	1
16	Hydroxylamine (2.0-2.6)	H ₃ NO	33	I/II	30	46	-0.81	2,3	1
17	Hydrazine (2.1-2.6)	H ₄ N ₂	32	I/II	36	52	-1.2	2,4	1
18	Water (1.7-2.0)	H ₂ O	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 ACD/Labs). 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.

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

Table 2: Some affinity descriptors of various known electron donors and acceptors (both!) to PS I and/or 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 ₈ O ₂	172	I	141	34	2.38	2,0	0
3	Phenazine	C ₁₂ H ₈ N 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 ₂ O	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 ₂ N ₂	257	I	na	7.8	1.7	0,0	1
8	Dichlorophenol indophenol (DCPIP)	C ₁₂ H ₇ NCl ₂ O ₂	268	I	187	50	1.91	3,1	1
9	Flavin mononucleotide (FMN)	C ₁₇ H ₂₂ N ₄ NaO ₁₀ P	496	I/II		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

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

It can be noted and inferred that if such 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 no 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 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 (Manoj & Bazhin, 2021). 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- $[(QH_2-FeS-Heme-PC = 3) \times 2] + [(QH_2-Heme-Heme-Q = 3) \times 2] = 12$. As only 4 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 through 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?
- (iv) Perhaps, the arrangements of components/pigments in the photosynthetic structures in earlier revealed bacterial systems could have indicated an ordered mechanism (Kühlbrandt, 1995). However, the structural distribution of plant pigments, photosystems and LHC arrangement in chloroplasts revealed later show very little order (Croce & van Amerongen, 2020). 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 unforeseen measures (Manoj & Manekkathodi, 2021).
- (v) The equations prescribed for the overall process must violate the fundamental laws of thermodynamics for attaining viability. $\{2\text{NADP}^+ + 3\text{ADPOH} + 3\text{POH} \rightarrow \text{O}_2 + 2\text{NADPH} + 2\text{H}^+ + 3\text{ADPOP} + \text{H}_2\text{O}; \text{Overall } \Delta_r G'^0_{\text{aq}} = 1463.8 \text{ kJ/mol}\}$ is the prescribed equation. The input for the overall process is 4 einsteins of 680 nm photons ($4 \times 175.9 = 703.6 \text{ kJ/mol}$) and 4 einsteins of 700 nm photons ($4 \times 170.9 \text{ kJ/mol} = 683.6 \text{ kJ/mol}$), 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 (Arnon, 1954a,b; Arnon, 1991) had 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 (Arnon et al., 1980; Arnon et al., 1981; Arnon, 1995 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 *b₆f*) of the deterministic e-transfer scheme were in fact, completely optional in physiology (Zhang et al., 1994; Fernandez-Velasco et al., 2001; Pesaresi et al., 2009). Therefore, such conclusive evidence

dictates that the classical ETC-CRAS model for light reaction of photosynthesis should be jettisoned, and we present murburn model as a viable alternative (Manoj et al., 2021a,b).

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 useful. From a survey carried in our study of available literature, and seconded by the earlier insightful assertions of Hauska (1977), a statistical criteria is evident. Hydrophilic ions or molecules (such as ferricyanide or benzoquinone-2-sulfonate) served at A1 whereas hydrophobic molecules (like benzoquinone or p-phenylenediamine) 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. What is crucial to note is 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 (Ulas et al., 2008) (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 (Forman et al., 2020). 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?

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 (Manoj et al., 2021a,b). 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.

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 (Emerson, 1957; Emerson et al., 1957; Govindjee & Rabinowitch, 1969). For, 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 (Manoj et al., 2021a). 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. In this regard, we have conclusively critiqued the classical view (Manoj, 2018), 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.

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 b₆f 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 F₀F₁ATPase 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 stoichiometric requirement/involvement 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 (Manoj et al., 2021a,b).

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 (Manoj et al., 2020a,b; Gideon et al., 2020; Manoj & Manekkathodi, 2021; Manoj & Bazhin, 2021; Manoj et al., 2021a-d). 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 & 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 formation of stable 2e products and their utilization and/or porting thereafter determines the reaction dynamics in milieu.

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 equilibria 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.

3.4. Thermodynamics and kinetics of physiological bicarbonate reactions

In plant and animal cells, the hydration/dissociation equilibrium of CO₂-bicarbonate system is of immense physiological significance and therefore, well-studied. When gaseous CO₂ mixes with water, there are four particles/species formed that co-exist in a complex interactive equilibrium: CO_{2aq} (a, dissolved/aquated molecule), H₂CO₃ (b, uncharged carbonic acid), HCO₃⁻ (c, monovalent bicarbonate anion) and CO₃²⁻ (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 (Badger & Price, 1994; Moroney et al., 2001; Kupriyanova et al., 2017), it is not really clear as to how these interactive equilibria operate in physiological dynamics. Also, it has been a general understanding that Photosystem II (PS II) has some CA-type activity (Lu and Stemler, 2002; Shitov et al., 2009; Ignatova et al., 2011). The theoretical investigation bears relevance in the light of renewed interest in the roles bicarbonate and CA (Hines et al., 2021). In this context, we would like to point out that perceptions involving the roles of protons in overall thermodynamic treatments were misplaced earlier (Manoj et al., 2021e). Therefore, a revisit to the pertinent treatment is mandated in the new light of awareness.

Figure 8: Simplified representation of the highly complex ‘carbon dioxide-carbonic acid-bicarbonate-carbonate’ interactive equilibria 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>)

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, Supplementary Information, for the details of calculation of the empirical $\Delta_r G^\circ$ (standard free energy change of reaction) from the $\Delta_f G^\circ$ (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 bold and those seen/sourced from literature (as listed in Item 1, Supplementary Information) are in normal fonts. The pitfalls of experimentally determined equilibrium constants are discussed elsewhere (Manoj et al., 2021e).

1. Formation of carbonic acid

Exp. $K_{\text{eq}} = [\text{H}_2\text{CO}_3] / [\text{CO}_2] * [\text{H}_2\text{O}] = 1.3 \times 10^{-3}$ or 5.4×10^{-5} ($\approx 16.5 \text{ kJ/mol}$ or 24.3 kJ/mol)

The rate constants are 0.039 s^{-1} for the forward reaction and 23 s^{-1} for the reverse reaction (Housecroft and Sharpe, 2005) which gives K_{eq} to be 1.7×10^{-3} . In another study, the rate constants are 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 (Stumm and Morgan, 1996). The second order forward rate constant is $0.0027 \text{ M}^{-1} \text{ s}^{-1}$ (which multiplied with 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 being $0.0027/50 = 5.4 \times 10^{-5} \text{ M}^{-1}$ (Berg et al., 2002) 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 the acid. In physiological ranges of pH and temperature, the ratio is $\sim 340 \text{ CO}_2 : 1 \text{ H}_2\text{CO}_3$. 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 $\Delta_r G^\circ$ does not correspond accurately to experimental K_{eq} (as expected from the equation: $\Delta G = -$

RT $\ln K_{eq}$) this CA mediated primary reaction (which is theoretically independent of pH, as evident in the equation).

2. Formation of bicarbonate ion

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

Exp. $K_{eq} = [\text{H}^+] * [\text{H}_2\text{CO}_3] / [\text{CO}_2] * [\text{H}_2\text{O}] = 1.5 \times 10^{-8}$; (log K = -7.82)

Exp. $K_{eq} = [\text{H}^+] * [\text{HCO}_3^-] / [\text{CO}_2] = 4.5 \times 10^{-7}$ (log K = -6.35 = - apparent pK_a)

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.

Exp. $K_{eq} = [\text{HCO}_3^-] / [\text{CO}_2] * [\text{OH}^-] = 4.6 \times 10^7 \text{ M}^{-1}$

Rate constants of the forward and reverse reaction are $8.5 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ and $2 \times 10^{-4} \text{ s}^{-1}$ respectively (Gutknecht et al., 1977). 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 is seen; energetics and kinetics are also in agreement.

Exp. $K_{eq} = [\text{H}^+] * [\text{HCO}_3^-] / [\text{H}_2\text{CO}_3] = 2 \times 10^{-4}$ OR (empirical theory 6.0×10^{-7}) (log K = -3.69 = - true pK_a)

The forward reaction's rate constant is $1 \times 10^7 \text{ s}^{-1}$ whereas the reverse as $5 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ (Pocker and Bjorkquist, 1977). Ultrafast reactions observed both ways. Empirical energetics favors the reverse reaction. The direction of experimental K_{eq} agrees with energetic and overall kinetics agrees with energetics.

3. Formation of carbonate ion

We can consider two ways.

Exp. $K_{\text{eq}} = [\text{H}^+] * [\text{CO}_3^{2-}] / [\text{HCO}_3^-] = 4.7 \times 10^{-11}$ ($\log K = -\text{pK}_a = 10.33$)

Exp. $K_{\text{eq}} = [\text{H}^+]^2 * [\text{H}_2\text{CO}_3] / [\text{CO}_2] * [\text{H}_2\text{O}]$; ($\log K = -17.45$)

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 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:

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 (iii) could equate to (ii) 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.

The above $\Delta_r G^\circ$ values of the equations we had recently proposed for electron abstractions in transformed scale $\Delta_r G'^{\circ}_{\text{aq}}$ (Manoj et al., 2021a), are given within the parentheses after equations in bold:

(ix) $\text{H}_2\text{O} (-237.19) \rightarrow {}^*\text{OH} (25.8) + \text{H}^+ (412.5) + \text{e}^- (-148.53)$; $\Delta_r G^\circ = 527 \text{ kJ/mol}$ ($\Delta_r G'^{\circ}_{\text{aq}} = 525 \text{ kJ/mol}$)

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 Ruben et al., 1941), the involvement of diffusible reactive oxygen species (DROS-like ${}^*\text{O}_2^-$, H_2O_2 , ${}^*\text{OH}$, OH^- , ${}^1\text{O}_2$, 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 + (iv)

(1) $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3$; $\Delta_r G^\circ = 0.1 \text{ kJ/mol}$

(2p) $\text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-$; $\Delta_r G^\circ = 35.8 \text{ kJ/mol}$

(2r) $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{HCO}_3^-$; $\Delta_r G^\circ = 35.9 \text{ kJ/mol}$

(iv) $\text{HCO}_3^- \rightarrow \text{CO}_2 + {}^*\text{OH} + \text{e}^-$; $\Delta_r G^\circ = 491.1 \text{ kJ/mol}$

Totalled: $\text{H}_2\text{O} \rightarrow {}^*\text{OH} + \text{H}^+ + \text{e}^-$; $\Delta_r G^\circ = 527 \text{ kJ/mol} \approx \text{Rxn. (ix)}$

(B). 2q + (iv)

(2q) $\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$; $\Delta_r G^\circ = -44.1 \text{ kJ/mol}$

(iv) $\text{HCO}_3^- \rightarrow \text{CO}_2 + {}^*\text{OH} + \text{e}^-$; $\Delta_r G^\circ = 491.1 \text{ kJ/mol}$

Totalled: $\text{OH}^- \rightarrow {}^*\text{OH} + \text{e}^-$; $\Delta_r G^\circ = 447 \text{ kJ/mol} \approx \text{Rxn. (viii)}$

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.

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:

It can be seen from reactions (iv), (v), (A) & (B) that all reactions occurring via bicarbonate amount to $\sim 527 \text{ kJ/mol}$ or lower energy terms (since heterolysis of water incurs $\sim 79 \text{ kJ/mol}$, 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 (xiv) 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:

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 (Dismukes et al., 2001; Wu, 2021a), which give 103.8 kJ/mol and 156.1 kJ/mol respectively for reactions (xiv.a) and (xv.a), for the same $\frac{1}{2}\text{O}_2$ stoichiometry. The difference is also seen in relative terms (for bicarbonate versus water); our calculations showing a difference of $\sim 36 \text{ kJ/mol}$ compared to Dismukes' differential of $\sim 52.3 \text{ kJ/mol}$. We have discussed the source of such errors and disagreements (as

also seen in section II, equation 1) in energetic/equilibrium in a recent communication elsewhere (Manoj et al., 2021c).

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 (Richardson et al., 2000; Bakhmutova-Albert et al., 2010), 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 (Patra et al, 2020), potentially aiding several 1e and 2e murburn equilibriums in milieu. Specifically, bicarbonate ion has been known to enhance photolysis of water by aluminium porphyrins, which are known to proceed via 1e mechanism (Kuttassery et al., 2019). 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 milieu 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:

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

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

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

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

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 [equation (vii)]. 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 (vii) and (xvi)] 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 (Okrasa & Kazlauskas, 2006).

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's works through 1980s (Arnon, 1995). 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 (Manoj et al., 2021f). Quinols in the membrane merely aid these processes by serving as $1e/2e$ pitstops (Manoj et al., 2021g). This consideration explains how/why quinones/quinols serve as donor/acceptors of both PS I and II (as discussed in Section III A, figure 2). Besides the equations (xvii) – (xix), oxygenesis could also accompany photosynthesis steps, which are aided by the various membrane protein complexes that bind ADP (Figure 9). Therefore, 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 (Manoj et al., 2020; Manoj & Bazhin, 2021; Manoj et al., 2021a). (Figure 9). The presence of bicarbonate

serves as an effective ionic conduit/catalyst in the 1e and 2e murburn equilibriums occurring in the vicinity of thylakoid membranes.

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 Manoj & Bazhin, 2021; Manoj et al, 2021a,b), 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. Boxed equations are examples wherein phosphorylations could also result in oxygenesis (For mass-balancing treatments, ADP and Pi stand for ADPOH and POH, respectively).

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 & 2) can reach purported binding sites of PS II (like MnComplex or non-heme Fe). We do not envisage how even much smaller molecules such as water or peroxide could reach the MnComplex 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 (Manoj et al., 2016). 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 MnComplex (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 equations (xvii)-(xix)]. 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 (Manoj et al., 2016). The same enhancement effect is also observed in the light reaction of oxygenic photosynthesis (Izawa et al., 1969; Kana & Govindjee, 2016), 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) and it is also well known that such radical mediated reactions also have high kinetic viability (Buxton et al., 1988; Bielski & Cabelli, 1995).

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 oxygenesis or phosphorylation is explained adequately. Owing to the chemical disposition of the participant species (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 (iv) and (xviii) or (xvi)+(xix)].

(b) The requirement of the external protons (on the left side of the equations) and their ability to displace the oxygenesis equilibria [(xvii) – (xix)], reduction of nicotinamide nucleotide (via $\text{NADP}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADPH}$) and phosphorylations (via $\text{ADP} + \text{Pi} + \text{H}^+ \rightarrow \text{ATP} + \text{H}_2\text{O}$ or through one-electron murburn equilibria leading to superoxide generation in situ) also explain the enhancement of photosynthesis in the Jagendorf experiment (Jagendorf & Uribe, 1966). 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 (Hines et al., 2021) 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 equilibria discussed in section II. In this regard, it is essential to consider the arguments of Warburg (Warburg, 1961) and the unexplained data several groups presented thereafter (Stemler & Radmer, 1975; Radmer & Ollinger, 1980; Clausen et al., 2005; Hillier et al., 2006). For the readers’ convenience, the major observations of some publications cited above are reproduced from the original sources in Item 3 of the

Supplementary Information file. 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 (1975) 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 (i) in Supplementary Information.) 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 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 II-IV).

2. Figures 1-3 of Radmer-Ollinger's (1980) *FEBS Lett.* paper is shown as panel (ii) in Supplementary Information. 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 8 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-excitabile center (Manoj et al., 2021a). 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 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 3rd 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. (2005) and Hillier et al. (2006) 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 ethoxzolamide 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 ambience 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 O₂ produced by labeling bicarbonate! Figure 2 of Clausen et al. paper is presented in Supplementary Information as panel (iii). 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 CO₂ to some extent, and the decay of such heavy labeled CO₂ 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 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 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 (Warburg & Krippahl, 1958; Warburg, 1964; Metzner, 1975; Stemler & Radmer, 1975; Radmer & Ollinger, 1979; van Rensen et al., 1999; Stemler, 2002; Shevela et al., 2012, 2013; Koroidov et al., 2014; Wu et al., 2015; Wu, 2021a,b). To sum up, bicarbonate could potentially serve as 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.

Figure 10: Murburn reaction for bicarbonate activation and steps for loss of heavy atom trace in evolved oxygen.

As per the first equation given in Figure 4 below, 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 (1975), 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 (1980), which clearly shows enhanced oxygen peak with 2nd 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 ($\sim 1/8$) 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 (Manoj & Bazhin, 2021) that follow (after the photosystem abstraction step in Figure 4) explain how the heavy label in ${}^*\text{OH}$ goes into non- O_2 species:

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 MnComplex 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 (Barber, 2013). A study of the MnComplex containing extra-membrane region does not show any channel 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 MnComplex, 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.

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

While this reaction 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 2 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 O₂” 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 QH₂ molecules. The second molecule of Q cannot bind until the first formed QH₂ detaches, and each such quinone must deterministically home its way to QBC. These considerations mean that with 1 *hν* : 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 aspects do not limit the stochastic scheme of murburn model, which has ion-radical equilibria. 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 (Manoj et al., 2020a,b; Gideon et al., 2020; Manoj & Manekkathodi, 2021; Manoj & Bazhin, 2021; Manoj et al., 2021a-d). 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 (Manoj et al., 2021a).

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

Under such high potentials generated by multiple membrane-embedded species donating electrons and these electrostatics stabilized transiently 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/O₂ 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 (Figure 1 and point b above). 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:

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 (Cohn, 1953; Cohn & Drysdale, 1955). 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 set of physiological redox reactions (incorporating multiple redox-active components within milieu) in a purely deterministic perspective because within the murburn purview, a given component could have multiple interactive roles. 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 (Manoj, 2006; Manoj et al., 2016). It is an evidence driven conclusion that photosynthetic electron transfer processes are not affinity-driven (Hauska, 1977; Tschortner et al., 2019; Gideon et al., 2020; Manoj et al., 2020; Manoj et al., 2021a,b) and the overall event is one-electron punctuated processes with a two-electron endings (akin to a paragraph formation using commas and periods). 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 the light reaction of photosynthesis (Izawa et al., 1969; Stemler & Govindjee, 1973; Kana & Govindjee, 2016). 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.

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: $2\text{AH}/2\text{H}_2\text{A} + n\text{h}\nu \rightarrow \text{A}_2 + 2\text{H}^+/4\text{H}^+ + 2\text{e}^-/4\text{e}^-$ 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.

Declarations

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Supplementary Information

Explanations for the enhancement of oxygenic photosynthesis by bicarbonate and diverse additives: Affinity-driven binding with photosystems versus murburn model

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Item 1: Survey of electron acceptors from photosystems.

Table S1: A compilation of electron acceptor from the two 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 ₂ N ₈ O ₂	727.6	I	na	87.6		8,0	9
2	DCPIP	C ₁₂ H ₇ NCl ₂ O ₂	268.0	I	186.7	50	1.91	3,1	1
3	Benzoquinone	C ₆ H ₄ O ₂	108.0	I/II	86.0	34.1	0.26	2,0	0
4	Flavin mononucleotide	C ₁₇ H ₂₂ N ₄ NaO ₁₀ P	496.3	I/II	na	214	-1.7	10,3	6
5	Ferrioxalate	C ₆ FeO ₁₂	319.9	I	na	na	na	na	na
6	HgCl ₂	HgCl ₂	271.4	I/II	na	0		0,0	0
7	Methyl red	C ₁₅ H ₁₅ N ₃ O ₂	269.3	I	230.1	52	4.91	5,1	4
8	Paraquat	C ₁₂ H ₁₄ Cl ₂ N ₂	257.1	I	na	7.8	1.7	0,0	1
9	Ferricyanide	C ₆ FeN ₆	211.9	I/II	na	143		12,0	0
10	Anthraquinone	C ₁₄ H ₈ O 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 ₆ O ₂	158.2	I	122.6	34	1.79	2,0	0
13	Silicotungstate	H ₇₂ Na ₄ O ₄₀ SiW ₁₂	2970.1	II	na	657	na	40,4	8

Figure S1: Structures of various electron donors listed in Table S1.

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>).

(<http://ion.chem.usu.edu/~sbialkow/Classes/3650/Carbonate/Carbonic%20Acid.html>)

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

1. $\text{CO}_{2\text{gas}}$

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

2. $\text{CO}_{2\text{aq}}$

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

Thus, CO_2 in the gas phase is in equilibrium simultaneously with two particles $\text{CO}_{2\text{aq}}$ and H_2CO_3

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

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

3. H_2CO_3

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

$$\text{Then } \Delta_f(\text{H}_2\text{CO}_3) = \Delta_r G(1) + \Delta_f(\text{CO}_{2\text{aq}}) + \Delta_f(\text{H}_2\text{O}) = 0 - 386 - 237.1 = -623.1 \text{ kJ/mol}$$

4. HCO_3^-

HCO_3^- arises from acid dissociation

The dissociation constant is $4,47 \cdot 10^{-7}$, and $\Delta_r G^0 = 36.78 \text{ kJ/mol}$.

Hence,

$$\Delta_f G^0(\text{HCO}_3^-) = \Delta_f G^0 + \Delta_f G^0(\text{H}_2\text{CO}_3) - \Delta_f G^0(\text{H}^+) = 36.78 - 623.1 - 412.5 = -999.82$$

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

$$\Delta_r G^0 = 64.56 \text{ kJ/mol}$$

Hence ,

$$\Delta_f G^0(\text{CO}_3^{2-}) = -1347.7 \text{ kJ/mol}$$

Standard thermodynamic parameters of particles, arising from the dissolution of CO_2 in water.

Molecule	$\Delta_f H^0$, kJ/mol	$\Delta_f G^0$, kJ/mol
$\text{H}_2\text{O}_{\text{aq}}$	-285.8	-237.1
$\text{CO}_{2\text{gas}}$	-393.5	-394.4
$\text{CO}_{2,\text{aq}}$	-413.8	-386.0
H_2CO_3	-699.2	-623.1
HCO_3^-	-1078.25	-999.8
CO_3^{2-}	-1449.6	-1347.7

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

[Panel (A): Figure 1 of Stemler A, Radmer R. (1975). *Science* 190:457-458. Panel (B): Figures 1 to 3 of Radmer R, Ollinger O. (1980). *FEBS Lett.* 110(1):57-61. Panel (C): Figure 2 of Clausen J, Beckmann K, Junge W, Messinger J. (2005). *Plant Physiol.* 139(3):1444-1450.] 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.