Validating the predictions of murburn model for oxygenic photosynthesis: Analyses of ligand-binding to protein complexes and cross-system comparisons

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1. Introduction

In the first part of our write-up, we had questioned the classical explanation for photolysis-photophosphorylation (PI-Pp) comprising of cyclic oxygenesis at MnComplex (Kok-Joliot cycle) and electron transport chain (Z-scheme ETC). Also, in lieu of the long-standing explanations, we had proposed a detailed murburn model for PI-Pp. This development, in turn, was based on our earlier works with heme/flavin enzyme reductionist systems (Manoj et al., 2010a, 2010b), which relies on the catalytic process involving diffusible reactive species (DRS) and their interactive equilibrium with diverse components of the reaction milieu (Manoj, 2018a, 2018b, 2020). A simplified scheme of murburn interactions within the metabolic perspective is shown in Figure 1, which advocates that O2-DROS-H2O equilibrium serves as a primary electron/moiety transfer catalytic agent in routine redox metabolism.

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Abstract: In this second half of our treatise on oxygenic photosynthesis, we provide support for the murburn model of the light reaction of photosynthesis and ratify key predictions made in the first part. Molecular docking and visualization of various ligands of quinones/quinols (and their derivatives) with PS II/Cytchrome b6f complexes did not support chartered 2e-transport role of quinols. A broad variety of herbicides did not show any affinity/binding-based rationales for inhibition of photosynthesis. We substantiate the proposal that disubstituted phenolics (perceived as protonophores/uncouplers or affinity-based inhibitors in the classical purview) serve as interfacial modulators of diffusible reactive (oxygen) species or DRS. The DRS-based murburn model is evidenced by the identification of multiple ADP-binding sites on the extra-membrane projection of protein complexes and structure/distribution of the photo/redox catalysts. With a panoramic comparison of the redox metabolic machinery across diverse organellar/cellular systems, we highlight the ubiquitous one-electron murburn facets (cofactors of porphyrin, flavin, FeS, other metal centers and photo/redox active pigments) that enable a facile harnessing of the utility of DRS. In the summative analyses, it is demonstrated that the murburn model of light reaction explains the structures of membrane supercomplexes recently observed in thylakoids and also accounts for several photodynamic experimental observations and evolutionary considerations. In toto, the work provides a new orientation and impetus to photosynthesis research.

Abbreviations: ADPOH/ADP: adenosine diphosphate; ADPOP/ATP: adenosine triphosphate; CBP: chlorophyll binding protein; CN: cyanide; Comp. I-V: Complexes I-V, CoQ: coenzyme Q or ubiquinone; Cyt.: cytochrome; CRAS: chemiosmotic rotary ATP synthesis; CYP: Cytochrome P450; DCBQ: 2,6-Dichloro-1,4-benzoquinone; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP: dichlorophenol indophenol; DMQO: 2,6-dimethoxybenzoquinone; DNP: dinitrophenol; DNP-INT: 2,6-iodo-6-isopropyl-3-methyl-2'4,4'-trinitrodiphenyl ether; (D)R(O)S: (diffusible) reactive (oxygen) species; DBMIB: dibromomethoxyquinone; ECS: effective charge separation; ETC: electron transport/transfer chain; Fd: Ferredoxin; FNR: Ferredoxin-NADP reductase; KC: Kok-Joliot cycle, K-J cycle; LHC: light harvesting complex; LLLT: low-level laser therapy; mETC: mitochondrial electron transport chain; mOXPhos: mitochondrial oxidative phosphorylation; murburn: mured burning; murzone: zone where murburn occurs; murzyme: enzyme that mediates catalysis via murburn mechanism; mXM: microsomal xenobiotic metabolism; MBH: Hydrogen gas-evolving membrane-bound hydrogenase; MBS: Elemental sulfur (S0)-reducing reductase; NDH: NADPH dehydrogenase; NQNO: 2-Nonyl-4-hydroxyquinoline N-oxide; PC: plastocyanin; PBQ: p-benzoquinone; PPBQ: p-phenyl benzoquinone; Pi/POH: inorganic phosphate; pmf: proton motive force; PI-Pp: photolysis-photophosphorylation; PQ/PQH2: plastoquinone/plastoquinol; PS: photosystem; RC-Chl: Reaction Center chlorophyll; Q SAR: quantitative structure-activity relationship; TBHQ: tert-butyl hydroquinone; TMP: trans-membrane potential; WSC: water splitting complex (also called OEC, oxygen evolving complex or MnComplex); UCP: uncoupler protein; ZS (ETC): Z-scheme (electron transport chain)
This new perspective of electron/moiety transfer (murburn concept) has revamped key mechanistic underpinnings in heme/flavin enzymology. Some of the salient examples are: (i) Redox proteins could recycle at high amounts of DROS (Manoj & Hager, 2001). (ii) The diversity of substrates, the multi-phasic inhibitions seen at higher concentration of substrates, and unusual activations/inhibitions by various additives were reasoned out (Andrew et al., 2011; Gade et al., 2012; Manoj, 2006; 2018b; Manoj et al., 2010a; 2016c; Manoj & Hager, 2008; Parashar et al., 2014b; Parashar & Manoj, 2012). (iii) While the active site reaction was highly sensitive to substrate concentration, some of the diffusible species-mediated reactions (with substrate dimensions larger than active-site) afforded practically zeroth order kinetic profiles even when substrate was in the range of tens of micromolar concentrations (Manoj, 2006; 2018b; Manoj et al., 1999; Manoj & Hager, 2001; 2006). (iv) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006). (v) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006). (vi) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006). (vii) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006). (viii) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006). (ix) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006). (x) Reactions mediated by diffusible species occurring outside the active site afforded selectivity (which is the ability to differentiate between substrates, say A, B & C) and specificity (say, ability to attack the substrate only at the para or beta substitution) (Hager, 2010; Manoj, 2006, 2018b; Manoj et al., 2020a). Such unusual findings and explanations enabled us to resolve mechanistic aspects of mixed function oxidase system of liver microsomal xenobiotic metabolism (or mXM) (Gideon et al., 2012; Manoj, 2018b, 2018c; Manoj & Hager, 2001; 2006).
2. Methodology

Molecular docking of the photosynthesis inhibitors was performed using a protocol similar to the protocol followed in Manoj et al., 2019a. The inhibitor molecules were downloaded from PubChem, or obtained by drawing using ChemDraw Ultra 8.0. The receptor proteins, 5XNL (PS-II) and 5L8R (PS I) were downloaded from PDB and converted to pdbqt. Using AutoDock Tools (ADT) (Morris et al., 2001), the pdbqt files of ligands and the receptor proteins were prepared. The Gridmap was generated using the AutoGrid. The gridbox parameters were as follows – $72 \times 74 \times 72$ xyz points (grid spacing value 0.375 Å) and the grid center $x$, $y$, and $z$ were set as 0.728, 0.918 and 0.860 respectively. The most widely known photosynthesis inhibitors (particularly, of PSII and PSI) were docked to the subunits reported in literature, where these inhibitors are known to bind. Assessment of ligand binding or inhibitory constant ($K_i$) values were derived from best-docked poses. While the absolute value of binding energy (or $K_i$) calculated from in silico docking may not be a good marker for the absolute/realistic value, the value can definitely be used as a comparative marker to correlate the various docks amongst similar classes of molecules. UCSF Chimera and PyMoL were used for viewing molecules. Discovery Studio viewer was used for viewing amino acid interactions with docked compounds.
3. Results & discussion

Before proceeding with the analyses presented below, the reader may peruse a brief review of the currently available information on the chloroplast photo-redox machinery, i.e. architecture of chloroplast, structure of protein complexes, LHC, etc. (Item 1, Supplementary Information).

3.1. Interaction of natural quinones/quinols and their analogs with protein complexes

We analyzed if the structural features support the classical purview wherein membrane quinones are supposed to serve as chartered electron transport agents from PS II to Cytochrome b₆f.

3.1.1. Cytochrome b₆f

Cytochrome b₆f has two quinone binding sites – Qp and Qn; while Qp is the PQH₂ oxidation site which is close to FeS protein and cytochrome f; Qn is the quinone reduction site (Cramer et al., 2006). First, we analyzed the various aspects involved in the binding of the natural quinols and quinones with the two purported binding sites (Psites and Nsites) on Cytochrome b₆f, as shown in Figure 3. As can be seen, the sites exist at the cusp of three peptide chains and are present at the opposite sides of the trans-membrane portion of the protein complex. It is very difficult to conceive that a quinone/quinol site could evolve and be conserved for a hydrophobic ligand in the trans-membrane region of three different peptide chains. This is when the various quinones/quinols have differing tail lengths and each one would be expected to be positioned differently, as the hydrophobic interactions are expected to be predominant with longer isoprenoid tails. The results of docking of quinones and quinols of various lengths (Figure 4) at the two purported sites of the trans-membrane region of the protein are given in Table 1.

The difference between quinones and quinols lies in the head group oxo- and hydroxyl- groups, which could potentially be differentiated by suitable H-bonding residues in the binding site (if any). We would expect a higher binding efficiency for quinols at Psites (for oxidation) and for quinones at the Nsites (for reduction). The binding-based e-transfer theory was significantly supported only for the Psite binding of quinol/quinone pairs of longer isoprenoid chain lengths. For all others, as a trend, increase in log P correspondingly increased the binding energy, indicating that hydrophobic interactions were primarily responsible for determining interactions. Based on the docking energies and Kᵢ values, we found that neither the Psites nor the Nsites gave any consistent or significant rationale for differentiating the headgroup quinones or quinols. What this leads to is a very undesirable dynamics of interactions for quinone/quinol with the respective binding sites. For example: the longer chain quinol would always out-compete the shorter chain quinol for binding at the Nsite. So, within the ETC purview, there is no advantage in having a distribution of quinones of different chain lengths. In contrast, the murburn explanation does not need the existence of specific binding sites for quinones/quinols on the protein complex. The ETC’s Q-cycle scheme requires a tetramolecular scheme coordinated across three phases and seeks the concomitant binding of quinol at Psites, quinone at Nsites, and plastocyanin at cytochrome f; all this for the sole purpose of pumping non-existent protons across the membrane phase! For example, PQ₉ has a convoluted snake-like energy-minimized binding pose that involves interactions of the methyl groups of the tail with several amino acids on several chains of the b₆f complex. Moreover, the head ought to go in first and then the tail must interact with the quinone-binding pockets in the Q₉ and Q₈ sites and one can only imagine what would happen if the more energetically favourable interactions of hydrophobic and van der Waal’s forces would stabilize the tail and prevent the head from entering into the the binding pocket, much like a snake enters head first into its crevice. In comparison, the murburn explanation involves the highly mobile diffusible reactive species-centered regeneration of one-electron equivalents and does not necessitate such docking scheme (Manoj & Bazhin, 2021; Manoj et al., 2021).

3.1.2. Photosystem II

X-ray diffraction studies of PS II had revealed that the QA and QB sites had dimensions of 12.0 Å² and 25 Å², respectively and the “replaceable” QB site is known to have greater flexibility or is only partly occupied (Dudekula & Fragata, 2006). The QA and QB sites are located deep within the interior on D2 (chain D)/D1 (chain A) proteins respectively, with the quinone supposedly binding stronger at QA than at QB (Lambreva et al., 2014). Interestingly, researchers opined that the QC site may not even exist; to quote them in their own words, “However, the third plastoquinone molecule observed in the structure solved by Guskov and coworkers, the so called QC has not been observed in this last structure, casting some doubts on the real physiological function of QC.” (Lambreva et al., 2014). It is only forthright to infer that quinones of the QA and QB sites would have to be incorporated within the bulk protein during the assembly phase of the protein complex, as there are no free channels for the quinones to access this location post-assembly. The dislodgeable quinone-binding site (QC) adjacent to the peripheral surface is supposed to serve as the mobile e-transport PQ docking site (Figure 5). Quite like the Cyt. b₆f scenario, the mobile electron transporter QC site is located at the junction of three distinct peptide chains (formed with the union of peptide chains E, F and J). We opine that the two-electron agents of quinones cannot conveniently relay one electron equivalents between QA-Q₈ and QB-Q₉, that too, across distances approximating 2 nm. If they do, then why should QC be a two-electron transfer agent? Also, we do not believe that there is any evolutionarily valid affinity-based rationale for the conservation or functioning of the QC site. We had earlier pointed out that: i) the PS II complexes located at high densities within the granal stacks cannot relay electrons via PQ to the Cyt. b₆f located at the peripheral regions of thylakoids (Manoj et al., 2021) and (ii) the low mobility of plastoquinones in the lipid phase and the higher distribution of the longer-chained
quinones (when compared to the smaller isoprenoid chain quinones) is antithetical to the requisites of the ETC.

While the structural aspects of PS II and Cyt. b$_{6}$f and the motility/distribution of quinones/quinols do not support the Z-scheme ETC, the realities are well explained by the murburn model. In the murburn model, DR(O)S serve as electron transfer agents in lipid phase. The photoactive pigments and redox centers within the proteins merely achieve photoelectronic emissions and the photosystem RC brings about effective charge separation (ECS), thereafter setting a stochastic reaction scheme. In this purview, quinones and quinols are considered as one or two electron reservoirs in the membrane phase, which exist in equilibrium with oxygen/DRoS. As a result, quinones serve as 2e-sink, enabling ECS.
and thereafter, they are once again recycled for one-electron equivalents in milieu. This scheme does not necessitate binding of quinones and squeezing out of quinols from PS II, as required/suggested by the ETC mandate (Zobnina et al., 2017). Also, based on our simple findings and deductions, we question the suggestion made by some researchers that...
the tail groups can enable differentiation of quinones and quinols at the various Q-sites of PS II (Lambreva et al., 2014).

3.2. Inhibition of the function of photosynthetic proteins by diverse molecules

Mechanistic insights are derived by tracing the effects of inhibitors on catalytic phenomena. We first probed the outcomes of how various molecules (belonging to several categories) could potentially affect the photosynthetic proteins by exploring their binding through in silico docking studies, along the ideas reported in literature. The molecules belonged to structurally diverse classes like - quinone/quinol derivatives, substituted phenolics and other miscellaneous herbicides like triazines, phenylureas, bipyrimidines, etc. Functional disruption can result owing to reversible or irreversible modes when: (i) reversible and competitive binding of a molecule to the locus where a substrate binds, thereby preventing substrate interaction and product formation, (ii) a molecule reversibly and non-competitively/allosterically altering the contour of the locus where a substrate binds, thereby preventing substrate interaction and product formation, (iii) a molecule reversibly and uncompetitively preventing the release of a substrate that has already bound, thereby preventing substrate/product release, (iv) a molecule that irreversibly leads to mechanism-based inhibition - say reacts with a reaction intermediate and covalently modifies the catalytic surface, thereby taking the catalysis out of interactive equilibriums, and (v) a molecule that catalytically converts a diffusible intermediate necessary for the overall reaction. These scenarios are captured in Figure 6, which also shows the murbumb/murcat model of enzyme function on the right. We explored the logic of these outcomes in the three key photosynthetic protein complexes- PS I & II and Cytochrome b$_6$f.

Herbicides like atrazine, ametryn, diuron, hexazone and tebuthiuron are known to inhibit NADPH and ATP synthesis by binding to the "inhibitor binding cleft" in D1 protein in PSII (Devine et al., 1992). By serving as ligands of the exchangeable quinone site (Q$_b$) in PSII reaction center and inhibitor binding-based blockade of electron transfer to plastiquinone (Rutherford & Krieger-Liszkay, 2001), these molecules are considered to elicit their herbicidal activities (Nirusimhan et al., 2021). Non-covalent interactions between the PSII reaction center in D1 subunit (which contains the RC) and these photosynthesis inhibitors have been reported widely in literature (Bowyer et al., 1990). Moreover, the toxicity caused by these herbicides was found to be light dependent, through chlorophyll (triplet state)-mediated generation of singlet oxygen, leading to protein damage. When a perpetual triplet state of Chl is maintained, the propensity for formation of ROS such as $^1$O$_2$ from O$_2$ is higher and this accounts for photodamage mediated by the photosynthesis inhibitors. As per the conventional theory, these molecules cause acceptor side photoinhibition because the binding of PQ is considered to occur at the stromal side of the PSII complex and the Q$_a$, Q$_b$ and PQ binding sites are located at the acceptor side (Krieger-Liszkay et al., 2005).

3.2.1. Quinone/quinol derivatives

The basic structural frameworks of quinone derivatives we explored are shown in Figure 7 and the results of their binding with various proteins are given in Table 2. It is quite interesting to note that researchers have considered the D1 binding (Q$_b$ site occupancy) by various quinone derivatives as crucial to the ETC (De Causmaecker et al., 2019; Husu et al., 2015; Kale et al., 2017). Inhibitory molecules like DBMIB have traditionally been used to effectively serve as electron relays in Q$_a$-Q$_b$ ETs. Such outcomes speak for the murbumb model ET, rather than the currently held deterministic ETC. We have pointed out similar outcomes in the drug metabolism field wherein two different drugs could have both inhibitory and enhancing effects in reactions (Manoj et al., 2016b; Venkatachalam et al., 2016). Generally, the quinone analogs gave significantly lesser affinity than PQ9 (by about one or two orders, when seen in terms of the equilibrium constants), the most preponderant quinone on the chloroplast membranes. Does this mean that the purported competitive inhibitors have lower affinity of binding to the quinone sites when compared to the natural quinone substrates? Also, clear cut logic for binding energy variations was not seen upon changing the various substitutions on the parent quinone. This shows that the inhibitions observed at PS II or cyt. b$_6$f with these chemicals is not an outcome of binding based effects. Quite inexplicably, the EC$_{50}$ values reported in literature for different photosynthetic systems with a variety of inhibitors were generally much lower than the respective binding constants K$_b$/K$_{eq}$. We have accounted for such outcomes in xenobiotic metabolism field using murbumb concept and it is only natural that such general pan-system observations have simple explanations.

In the current context, it is difficult to understand the dual nature of the purported Q$_b$ site. While researchers opine that this is the site where majority of quinone analogues and several other types of inhibitors bind and inhibit electron transfer process, it is also believed that the quinone is tightly bound at this very site (which cannot be accessed from the outside in a facile manner)! When considering that Q$_a$ and Q$_b$ sites are deep-located and have a surface area of only 12-25 Å$^2$, it can be clearly envisaged that the access of the larger molecules would be kinetically limited and only the head of a benzoquinone or phenyl moiety (~5 Å in linear dimensions) can interact at this site (if at all the molecule gains dynamic access). Even if we consider that all the molecules can easily access the Q$_a$ site, the docking energy terms of quinones are higher than that of the inhibitors, at several instances, with the affinity being higher by one or two orders! Then, it is inexplicable how the molecules could inhibit, particularly with an EC$_{50}$ even lower than the physiological K$_B$ or in silico K$_B$. The Cytochrome b$_6$f binding sites of NQNO, DBMIB, DNP-INT and the other molecules like UHDBT, DCMU and bromanil are known to define the two binding sites of the quin(ones/ols) (PQ and PQH$_2$) (Hasan et al., 2013). As per Oettmeier et al. (1978), entries 2,3,4,5, 8, 9 and 10 in Table 2 are specific inhibitors of PSII (D1). A brief recapitulation of some key available literature on the quinone analogs explored in this work is presented first.
Specifically, 1 (DCBQ) is supposedly an artificial e’ acceptor of PSII and is employed with FeCN (which aids in keeping the quinones in oxidized form) (Longatte et al., 2017). Nixon et al. (1995) stated that DCBQ accepts e’ from Q₈ inactive site, while 5 (DMBQ) accepts e’ only from the Q₈ active site. Dudekula and Fragata (2006) opined that DCBQ accepts e’ from the non-heme iron between QA and Q₈ and thus, inhibits photosynthesis. DCBQ is known to efficiently replace the Q₈ quinone and enhance the rate of oxygen evolution in the Hill reaction (Srivastava & Strasser, 1995). One report says that DCBQ accepts electrons from the QA site, while DMBQ accepts electrons from the Q₈ site (Vass et al., 1995). DMBQ is known to bind and accept electrons at the Q₈ site of D1 in PSII and induce degradation of the D1 sub-unit in the presence of UV-B radiation. The order of D1 breakdown by various quinone analogs is – DBMIB (Dibromothymoquinone) > DMBQ > PBQ > DCBQ > dihydroxy BQ (Vass et al., 1995). DMBQ is often used as a mediator of ET between photosynthetic complexes (Baillieul et al., 2008) and also used as a probe to check direct ET from H₂O in water splitting complex (OEC) to DMBQ (Berkowitz & Gibbs, 1982). Such facts are better explained in the non-specific murburn model whereas the deterministic ETC scheme is inadequate in the context. Chloranil is a strong oxidizing agent and inhibits several key plant functions (Saini et al., 2011). The presence of a radical anion of chloranil was reported by Horke & Verlet, 2011. Halogenated 1,4-benzoquinones are known to act as irreversible inhibitors of photosynthetic ET (Oettmeier et al., 1987). This is because they are vinylogous acid halides with ability to react with nucleophiles in addition/elimination reactions to form covalent bonds with targets. When it comes to extent of inhibitions caused by halogenated 1,4-benzoquinones, the order of inhibition is chlorinated < brominated < iodinated compounds (Oettmeier et al., 1978). This order is in exact agreement with the benzobromarone analogues-mediated inhibition of Cytochrome P450 systems, confirmed to result owing to DROS modulations (Parashar et al., 2014a). These compounds appear to have pi-donor substituent effects. Bromanol is also a good oxidizing agent and it also supposedly binds to D1 at Q₈ site (Dudekula &
### 3.2.2. Disubstituted phenolics

Disubstituted phenolics like dinitrophenol and benz bromarone are classical inhibitors (or uncouplers) of the mitochondrial and endoplasmic reticulum redox machinery. Some such molecules are known to efficiently relay electrons and act as “synthetic ET relay agents”. This aspect is akin to the activations of peroxidase and P450 reactions that we have observed in our work (Manoj et al., 2016b). Most disubstituted phenols are also perceived to bind to cytochrome \( b_f \) (Dostatni et al., 1987; Oettmeier et al., 1987) and disrupt Q-cycle or ETC process. The structure of molecules studied herein are given in Figure 8 and the data for binding to the sites on cytochrome \( b_f \) are given in Table 3. Once again, the binding efficacy of these molecules was poorer than the binding constants of the natural substrates of plastoquinone and plastoquinol. We have demonstrated that dihalophenolics are interfacial DROS modulators (using up protons in the process) and therefore, serve as efficient uncouplers. This activity was earlier misconstrued as protonophores or proton-shuttling activity of DNP in mitochondria (Geisler, 2019). The protonophore role of the disubstituted phenolics can be dismissed because if a proton cannot traverse across the membrane, it is highly energetically unfeasible that a molecule like dinitrophenol (which is much larger/massive and has multiple charged) can flip-flop on the membrane, only to afford protons ‘piggy rides across membranes’. Therefore, the Mitchellian explanation for the uncoupling effects of disubstituted phenolics was a mere mirage.

On one hand, di-substituted phenolics are supposed to function as generic uncouplers, and on the other hand, they are also supposed to bind to the photosynthetic proteins (Escher et al., 1999). Bromoxynil is supposed to bind and compete with other inhibitors like triazine for binding with the \( Q_b \) site (Vermaas et al., 1984; Vermass, 1984). Such aspects are better explained with DROS mediated effects, which we had shown in P450 system (Venkatachalam et al., 2016). Researchers have inferred that ‘bromoxynil does not bind to \( Q_b \) when it is in semiquinone state’ (Nixon et al., 1984).

#### Table 2. Binding of quinone analogs to cytochrome \( b_f \) and PS II.

| No. | Name / (R,X,Y,Z) | Log P | \( \text{Psite}, K_i \) (M) | \( \text{Nsite}, K_i \) (M) | \( D_1, K_i \) (M) \(^{(ref)} \) | Experimental \( EC_{50} \) (M) \(^{(ref)} \) |
|-----|------------------|-------|-----------------|-----------------|------------------|------------------|
| 1   | 2,6-dichlorobenzoquinone / (Cl,Cl,H,H) | 2.24  | 6.3 x 10^{-4}  | 3.7 x 10^{-4}  | 1.0 x 10^{-4}  | N.A.             |
| 2   | Tetrachloro-1,4-benzoquinone (Chloranil) / (Cl,Cl,Cl,Cl) | 3.34  | 1.4 x 10^{-3}  | 7.0 x 10^{-3}  | 2.3 x 10^{-3}  | 6.6 x 10^{-3}  |
| 3   | Tetrafluoro-1,4-benzoquinone (Fluoranil) / (F,F,F,F) | 3.07  | 2.8 x 10^{-6}  | 2.7 x 10^{-6}  | 3.4 x 10^{-6}  | 4.5 x 10^{-6}  |
| 4   | Tetrabromo-1,4-benzoquinone (Bromanil) / (Br,Br,Br,Br) | 2.63  | 5.8 x 10^{-10} | 3.7 x 10^{-10} | 1.0 x 10^{-10} | N.A.             |
| 5   | Dibromothymoquinone / (Br,CH(Me)2,Br,H) | 3.05  | 1.9 x 10^{-6}  | 1.8 x 10^{-5}  | 3.7 x 10^{-5}  | 6.7 x 10^{-6}  |
| 6   | Phenyl p-benzoquinone / (H,Phenyl,H,H) | 2.15  | 2.9 x 10^{-5}  | 3.8 x 10^{-5}  | 5.0 x 10^{-5}  | 5.0 x 10^{-6}  |
| 7   | 2,6-dimethyl p-benzoquinone / (Me,Me,H,H) | 3.68  | 3.2 x 10^{-8}  | 2.5 x 10^{-8}  | 3.4 x 10^{-8}  | 4.7 x 10^{-9}  |
| 8   | Tetr-butylhydroquinone / (H,C(Me)3,H,H) | 3.56  | 3.2 x 10^{-6}  | 1.2 x 10^{-5}  | 2.0 x 10^{-5}  | 2.0 x 10^{-5}  |
| 9   | Piperidine-A / (O,Me-O,Me,C3H7,O,O-Me) | 4.87  | 6.7 x 10^{-10} | 4.6 x 10^{-10} | 8.6 x 10^{-10} | 8.0 x 10^{-10} |

Compounds in entries 1-9 are benzoquinones; those in entries 10 and 11 belong to hydroxyquinone and monohydroxypyridine class, respectively. NR- not relevant; N.A.- Not available.

Citations for experimental determination of binding of ligands: (1) Dudekula & Fragata, 2006; (2) Oettmeier et al., 1978; (3) Bailleul et al., 2008; (4) Belatik et al., 2013; (5) Roberts et al., 2004; (6) Jegerschoeld et al., 1990; (7) Ikezawa et al., 2002.

Citations for \( EC_{50} \) (1) - Yruela et al., 1991; (2) Oettmeier et al., 1978; (3) Trebst et al., 1979; (4) Ikezawa et al., 2002; (5) Urbach et al., 1979.
1995) and this merely connotes that the quinone cytoles via one-electron process (as the muburn model predicts). The ability of such molecules to generate singlet oxygen (Krieger-Liszkay, 2005) is also better explained by the muburn model (Manoj et al., 2021). While dinoseb has relatively low affinity for the D1 site in silico (Table 3), researchers have reported a $K_d$ of $3 \times 10^{-8}$ M for its effect on D1 (Snel & van Rensen, 1983) whereas $K_d$ was $7 \times 10^{-8}$ M (Oettmeier & Masson, 1980). Kaminskaya et al. (2007) provide direct support for our inference that molecules like dinoseb are interfacial DROS modulators when they reported that the inhibitor could alter the redox potential of cytochrome $b_{559}$. This cytochrome is at least 25 Å away from the $Q_b$ site in PSII (Müh & Zouni, 2016) and such statements do not make any concrete sense in the classical paradigm.

As the quinone/quinol head is the key determinant for binding with the respective sites on D1 ($Q_A/Q_B/Q_C$ on PSII) or Cytochrome $b_{56}$ ($Q_P/Q_n$ or Psite/Nsite), we do not see any tangible rationale for the different binding site residue combinations to discriminate the moieties, irrespective of which of these three sites within the two complexes are compared. We have compared the binding effects of diverse substituted phenolics with D1 and Cytochrome $b_{56}$. We see clearly that the $K_i$ values obtained using docking approach are sometimes equal to, higher, or lower for the different binding sites. Since the inhibitors are supposedly competitors of $PQ/PQH_2$, they must be more effective at binding to these sites. Why then do we have substituted phenols binding only to the $Q_b$ site of D1 (PS II), as per literature, and there are no significant reports of binding of the very same compounds to Cytochrome $b_{56}$? If very long isoprenoid chain-containing quinones can bind to the various sites on D1 protein and Cytochrome $b_{56}$, it would only be natural for such inhibitors to bind Cyt. $b_{56}$. Clearly, the binding-based approach is limited in its potential to explain outcomes.

### 3.2.3. Diverse inhibitor molecules

Approximately, 30% of the commercial herbicides fall under the category of PI-Pp inhibitors and they are supposed to work by binding to specific loci of photosynthetic proteins, thereby preventing crucial electron transfers. Although inhibitors of Cyt. $b_{56}$ are known, the majority of commercial inhibitors can be broadly divided into two categories - those that affect PSI and those that affect PSII. In both cases, the physiological symptoms are only seen if plants are illuminated (that is- the inhibitors disrupt the ETC and this process damages the plant) and is found to lead to chlorosis and necrosis, ultimately resulting in plant death. The carotenoids and xanthophylls present in the chloroplasts are also supposed to protect chlorophylls from photo-oxidation (Ahrens, 1994; Andersson, 1996; Niyogi et al., 1997; Ross & Lembi, 1999). The PS I inhibitors (called membrane disruptors, electron interceptors, contact herbicides, etc.) supposedly perturb

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**Table 3. Binding of disubstituted phenolics and their derivatives.**

| Item | Ligand                  | Log $P$ | $K_a$ $Q_p$ (M) | $K_a$ $Q_n$ (M) | $K_a$ $D_1$ (M) | $K_d$ $D_1$ (M)$_{\text{Ref.}}$ | $EC_{50}$ (Ref.) |
|------|-------------------------|---------|-----------------|-----------------|-----------------|-------------------------------|-----------------|
| 1    | Bromoxynil              | 3.11    | $1.1 \times 10^{-4}$ | $9.4 \times 10^{-4}$ | $1.0 \times 10^{-4}$ | $7.0 \times 10^{-4}$ (1) | $9 \times 10^{-4}$ (1) |
| 2    | Ioxynil                 | 3.76    | $1.0 \times 10^{-5}$ | $3.8 \times 10^{-5}$ | $5.7 \times 10^{-5}$ | $2.0 \times 10^{-6}$ (2) | $2.7 \times 10^{-6}$ (2) |
| 3    | DCPIP                   | 2.58    | $2.5 \times 10^{-6}$ | $2.7 \times 10^{-6}$ | $1.7 \times 10^{-5}$ | N.A.                           | N.A.            |
| 4    | PY35                    | 6.64    | $2.0 \times 10^{-7}$ | $1.0 \times 10^{-6}$ | $7.0 \times 10^{-7}$ | $Q_b$ site (2)                | N.A.            |
| 5    | Benzobromarone          | 5.15    | $1.0 \times 10^{-7}$ | $1.5 \times 10^{-6}$ | $1 \times 10^{-6}$  | N.A.                           | N.A.            |
| 6    | Dinitrophenol (DNP)     | 1.80    | $2.9 \times 10^{-5}$ | $2.8 \times 10^{-4}$ | $6.0 \times 10^{-3}$ | N.A.                           | N.A.            |
| 7    | Dinoseb                 | 3.37    | $2.3 \times 10^{-6}$ | $4.7 \times 10^{-5}$ | $8.0 \times 10^{-4}$ | $2.5 \times 10^{-5}$ (3)      | $6.9 \times 10^{-7}$ (3)  |
|      |                         |         |                 |                 |                 | $K_i = 3.1 \times 10^{-8}$ (4) |                 |
| 8    | DNP-INT                 | 5.02    | $1.8 \times 10^{-6}$ | $1.3 \times 10^{-5}$ | $1.2 \times 10^{-4}$ | N.A.                           | $1 \times 10^{-7}$ (7)  |
| 9    | PNO8                    | 4.96    | $3.4 \times 10^{-5}$ | $1.3 \times 10^{-4}$ | $5.2 \times 10^{-4}$ | $Q_b$ site (2)                | $7.9 \times 10^{-8}$ (8)  |

N.A.- not available/not applicable.

Citations for $K_a$: (1) Zimmermann et al., 2006; (2) Nakajima et al., 1996; (3) Giardi et al., 1992; (4) Snel & van Rensen, 1983.

Citations for $EC_{50}$: (1) Bettiol et al., 2016; (2) Juneau et al., 2007; (3) Nakajima et al., 1996; (4) Madhavi et al., 1995; (5) Umamaheswari & Venkateswarlu, 2004; (6) Oettmeier et al. (1980); (7) Vidal et al., 2009; (8) Nakajima et al., 1995.
the ETC by diverting electrons from key Fe-S proteins (Fuerst & Norman, 1991) and are represented by bipyridylidum like paraquat (methyl viologen). The molecule per se is not deemed toxic but it is supposed to lead to the production of DROS, which are deemed to cause cellular disruption. The PSII inhibitors (represented by triazines, substituted ureas and uracils, phenylcarbamates, nitriles, pyridazines, pyridazinones, acid amides, benzothiadiazoles, etc.) are supposed to bind to the thylakoid D1 Qb protein (Jansen et al., 1993), thereby blocking electron transfers to the mobile plastocyanine pool. (There is yet another physical mode of inhibition, called photoinhibition, which usually results from blue light-induced damage to oxygen-evolving complex accompanying PSII. This is not focused herein.) Besides these well-studied inhibitions of PI-Pp, there are also reports of inhibitions sponsored by histones and polycations, reminiscent of the inhibition of mOxPhos (Brand et al., 1972).

For select inhibitors (with molecular structures depicted in Figure 9), we present a compilation of reported indicators of activity and affinity, and compare them with their blind-docking energies shown in Table 4.

The docked small molecules showed modest affinities, as the Kd ranged from 10^{-4} to 10^{-6} M, for the diverse classes of inhibitors. This is when the EC_{50} values ranged from 10^{-4} to 10^{-5} M and when the experimentally determined Kd values were 1-100 M for the four molecules: atrazine, diuron, bromacil and bromoxynil (Zimmermann et al., 2006). Restated, when making a comparison for EC_{50} values, some of the molecules effectively inhibited the reactions at significantly lower concentration ranges than the in silico Kd or experimental Kd values. We have solved such conundrums in heme enzymology, mXM and mOxPhos systems by attributing these outcomes to the involvement of DROS-inhibitor interactions (Manoj, 2018a; Manoj et al., 2016b, 2016c, 2016d, 2020a; Parashar et al., 2014a; Parashar & Manoj, 2021; Venkatachalam et al., 2016), and the same mechanistic implications are evident in the PI-Pp system. Here, we come across curious examples, such as: though atrazine had slightly better binding terms over diuron (in silico Kd of 100 M and 200 M respectively; experimental Kd of 600 nM and 800 nM respectively) with PSII, there were significant differences in EC_{50} values: 200 nM and 20 nM, respectively. Further, molecules containing the sulfur lone pair conjugated to the triazine ring gave better inhibitions (say, S-Me compared to O-Me) within the same class of triazines, signifying that the key reaction involves an electrophilic species. If binding were to be the most important criterion, the carbamates or pyridate should have given high inhibitions, but such expectations were seen to be unmet by experimental findings. Very importantly, it was seen that the EC_{50} (or experimental Kd values) reported for the same molecule varied significantly across research groups and target organisms (Wilkinson et al., 2015; Wilski et al., 2006). This is not expected in a binding-based scenario because the proteins and small molecules are supposedly conserved across species. These observations strongly suggest that it is not binding-based outcomes that determine inhibitions, but it is reaction-mechanism based events that lead to inhibitions by these potent agents. This is corroborated by the fact that inhibitions are noted only with photosynthetic physiology (at the impingement of light, and not otherwise); a clear signal that supports the murburn proposal that DROS are the obligatory agents involved in the interactive scheme with these molecules. In this regard, the reactions of molecules like diuron and paraquat are documented with DROS like hydroxyl or superoxide radicals (da Silva Simões et al., 2017; Manonmani et al., 2020). Therefore, this is strong evidence in support of the murburn perspective of PI-Pp.

With the classical view, it is inexplicable as to why cyanide could serve as a toxic principle in the PI-Pp process. Both Photosystems I and II (whose functioning is supposedly inhibited by cyanide) are not known to be inhibited owing to any hemeFe binding-based effects, either. Emerson showed that the dark process is temperature sensitive but the light process is not (with respect to oxygen yield). At low temperature, 10 μM cyanide gave higher inhibition at short duration of darkness or continuous light, whereas it gave no inhibitory effect at longer dark periods. This could signify that light stimulation is associated with DROS production and DROS has utility in the photosynthetic process, which is subverted by CN. Such outcomes can be reasoned out within the murburn scheme, as cyanide modulates DROS (Manoj et al., 2020a). It is also important to note that most of the photosynthesis inhibitors are not just toxic to plants, but are toxic to aquatic life and animals also. Therefore, we may not reckon that these molecules recognize specific shapes/loci and mediate their effects, because these substances are environmental toxins and have biohazardous attributes. Almost all of these substances are ecotoxicological agents – especially, ametryn, metribuzin, bromacil, prometryn and fluometuron (Wilkinson et al., 2015). What is more perplexing is that despite the very apparent structural differences of molecules within a given herbicide class (and when comparing other classes), almost all PSII inhibitors allegedly bind to the very same site. The two points support the relevance of murburn explanation. A 3D-QSAR study showed that the log Kow (LogP) values of 18 different triazines does not play any significant role and that the specific chemical groups on the triazines compared in the study would account for specific modes of action of these compounds (Gramatica et al., 2001). In literature, there are no marked distinctions in the binding sites of various triazines mentioned in this study. Indeed, all of the literature reports show that these molecules specifically bind to the Qb site of the D1 subunit of PSII. In the PDB ID: 5XNL complex of C2S2M2 of PSII-LHCII that was chosen for the docking studies herein, one can view the large size of the entire complex. We deem that it is highly likely for these small molecules which have small van der Waal’s radii to percolate into almost any possible site on the photosynthetic complexes, the chlorophyll binding proteins, or the other several proteins of the rather massive plant proteome. For example, dicyclohexylcarbodiimide was found to bind to light harvesting complexes of LHC-II and quench chlorophyll fluorescence (Ruban et al., 1998; Xu et al., 2012). A report shows that these molecules bind to the same location on D1 and that their binding affinity (as well
Figure 9. Backbones and structures of some inhibitors of Pl-Pp surveyed in the current study.

Table 4. Comparison of in silico binding constant (K) values and experimental EC_50 of some inhibitors of the chloroplast protein complexes. (Item 3 Supplementary Information presents data on several other molecules.)

| No. | Class        | Name / Substitutions (R, X, Y) | Log P | Binding energy (kcal/mol) | Predicted K_50 (M) | Experimental EC_50 (M), (ref.) |
|-----|--------------|-------------------------------|-------|--------------------------|-------------------|-------------------------------|
| 01  | Triazines    | Simazine (H, Cl, H)           | 2.35  | -4.7                     | 4 × 10^{-4}       | 1 × 10^{-7} (1)              |
| 02  | Triazines    | Atrazine (Me, Cl, H)          | 2.81  | -5.1                     | 2 × 10^{-4}       | 2 × 10^{-7} (1)              |
| 03  | Triazines    | Propazine (Me, Cl, Me)        | 3.18  | -5.5                     | 1 × 10^{-4}       | 3 × 10^{-7} (2)              |
| 04  | Triazines    | Prometin (Me, OMe, Me)        | 2.90  | -5.1                     | 2 × 10^{-4}       | 5 × 10^{-7} (1)              |
| 05  | Triazines    | Ametryn (H, SMe, Me)          | 3.04  | -5.0                     | 2 × 10^{-4}       | 2 × 10^{-7} (1)              |
| 06  | Triazines    | Prometin (Me, SMe, Me)        | 3.41  | -5.3                     | 1 × 10^{-4}       | 5 × 10^{-7} (1)              |
| 07  | Carbamates   | Desmedipham (H, Me)           | 3.46  | -6.2                     | 3 × 10^{-5}       | 1 × 10^{-6} (3)              |
| 08  | Carbamates   | Phenmedipham (Me, Et)         | 3.45  | -6.6                     | 1 × 10^{-5}       | 1 × 10^{-6} (3)              |
| 09  | Pyrimidines  | Bromacil (s-Bu, Me, Br)       | 1.35  | -5.7                     | 6 × 10^{-5}       | 1 × 10^{-7} (1)              |
| 10  | Pyrimidines  | Terbacil (t-Bu, Me, Cl)       | 1.35  | -5.8                     | 5 × 10^{-5}       | 6 × 10^{-7} (4)              |
| 11  | Phenyl ureas | Propanil (Et)                 | 3.09  | -5.7                     | 6 × 10^{-5}       | 2 × 10^{-9} (6)              |
| 12  | Phenyl ureas | Diuron/DCMU (N(Me)2)          | 2.81  | -5.4                     | 1 × 10^{-4}       | 2 × 10^{-8} (1)              |
| 13  | Phenyl ureas | Linuron (N(Me)(OMe))          | 2.70  | -5.3                     | 1 × 10^{-4}       | 5 × 10^{-8} (6)              |
| 14  | Phenyl ureas | Chlorotoluron (N(Me)2)        | 2.47  | -5.6                     | 7 × 10^{-5}       | 4 × 10^{-9} (7)              |
| 15  | Other ureas  | Fluometuron                   | 2.57  | -5.7                     | 2 × 10^{-4}       | 6 × 10^{-7} (1)              |
| 16  | Other ureas  | Tebuthiuron                   | 1.84  | -5.7                     | 7 × 10^{-5}       | 1 × 10^{-7} (1)              |
| 17  | Miscellaneous| Bentazon                      | 1.56  | -6.0                     | 4 × 10^{-5}       | 1 × 10^{-6} (9)              |
| 18  | Miscellaneous| Pyridate (Oct)               | 6.27  | -7.5                     | 3 × 10^{-6}       | 1 × 10^{-6} (9)              |
| 19  | Miscellaneous| Hexazine                      | 1.41  | -6.6                     | 1 × 10^{-5}       | 6 × 10^{-8} (1)              |
| 20  | Miscellaneous| Metribuzin                    | 1.54  | -5.5                     | 9 × 10^{-5}       | 3 × 10^{-7} (1)              |
| 21  | Miscellaneous| Pyrazon                       | 1.21  | -5.5                     | 9 × 10^{-5}       | 5 × 10^{-8} (10)             |
| 22  | Bipyridinium | Diquat                        | -6.39 | -5.8                     | 5.3 × 10^{-5}     | 3 × 10^{-10} (13)            |
| 23  | Bipyridinium | Paraquat                      | -6.28 | -5.3                     | 1.3 × 10^{-4}     | 3.8 × 10^{-8} (14)           |
| 24  | Bipyridinium | Diquat                        | -6.39 | -5.0                     | 2 × 10^{-4}       | 3 × 10^{-5} (11)             |
| 25  | Bipyridinium | Paraquat                      | -6.28 | -4.5                     | 5 × 10^{-4}       | 8 × 10^{-9} (12)             |
| 26  | Phenylurea   | Diuron (DCMU)                 | 2.81  | -4.8                     | 2.5 × 10^{-4}     | 3.8 × 10^{-5} (15)           |
| 27  | Bipyridinium | Diquat                        | -6.39 | -4.7                     | 3.5 × 10^{-4}     | 3 × 10^{-6} (13)             |
| 28  | Bipyridinium | Paraquat                      | -6.28 | -4.5                     | 5.2 × 10^{-4}     | 3.8 × 10^{-8} (14)           |
| 29  | Phenylurea   | Diuron (DCMU)                 | 2.81  | -5.2                     | 1.5 × 10^{-4}     | 3.8 × 10^{-5} (15)           |
| 30  | Bipyridinium | Diquat                        | -6.39 | -5.68                    | 6.8 × 10^{-5}     | 3 × 10^{-6} (13)             |
| 31  | Bipyridinium | Paraquat                      | -6.28 | -5.7                     | 6.6 × 10^{-5}     | 3.8 × 10^{-8} (14)           |
| 32  | Phenylurea   | Diuron (DCMU)                 | 2.81  | -5.76                    | 6 × 10^{-6}       | 3.8 × 10^{-5} (15)           |

*The structure is identical to diuron except that the chlorine atom at the para position is replaced by a methyl group. The EC_50 values entered in italics are from the same research group, and therefore can be good for a relative comparison within a given inhibitor class or cutting across classes. References for EC_50:
(1) Wilkinson et al., 2015; (2) Faust et al., 2001; (3) Traoré et al., 2018; (4) Peck & Chief, 2019; (5) Villarroel et al., 2003; (6) Snel et al., 1998; (7) Ma et al., 2006; (8) Michel et al., 2004; (9) Gressel, 1992; (10) Bisewska et al., 2012; (11) Malaspina et al., 2017; (12) Qian et al., 2009; (13) Peterson (1997); (14) Park et al., 2017 and (15) Kumar et al., 2010.*
as earlier reported literature $K_d$ values) are much lower than the $K_d$ values for PQ (Husu et al., 2015). The ability of these molecules, especially in the presence of light- to elicit ROS production, shows that the toxicity of these compounds is not merely due to binding and inhibition of ET from one point to another within PSII. Rather, the toxicity of these compounds is because of their non-specific ROS generating/ modulating ability, as opposed to mere site-specific binding. While chemical group differences in triazines do not majorly alter the binding site preferences, different triazines have relative variations in binding energy and $K_d$ values (in literature reports and in the docking results herein), showing that these herbicides have differential abilities to interact with ROS.

In toto, the data and analyses presented in the tables and figures of this section (and Item 3, SI), in conjunction with the earlier literature in the field, strongly suggest that the outcomes are reasonable by considering that DRS react/interact with diverse molecules leading to delocalized outcomes. The fact that diverse molecules are supposed to bind to different protein molecules of varying topographies does not make good logic. On the other hand, all photosynthetic proteins are involved with DROS production/utilization, and this view can make good sense in our attempt to explain the outcomes of inhibition in these systems.

### 3.3. Analyses of ADP-binding sites on protein complexes

The location of ADP-binding sites on the structures of chloroplast proteins is shown in Figures 10 and 11 and summed in Table 5. The two key protein complexes- PS II & Cyt. $b_{6f}$ (Figure 10) (Kurisu et al., 2003; Suga et al., 2019) show multiple grooves and channels (both in trans-membrane and lumen domains), with many redox centers that could be accessed easily by solvent/DROS. This ‘porous’ facet is not expected if the electron transfers occurred purely via Marcus model or if proton pumps were involved. Further, the presence of non-deterministically positioned and non-covalently held multitudes of pigments across the two proteins calls for a stochastic scheme. (Also, why should carotenoids and chlorophylls be present in the trans-membrane region of Cyt. $b_{6f}$?) While the extant paradigm does not offer any explanation for the presence of PS II’s extra-membrane bulbous extension into the lumen, the murburn proposal sees this structure as low-affinity binding and presentation facets for ADP. The six ADP binding sites each revealed per PS II and Cyt. $b_{6f}$ monomers (Figure 10) have dissociation constants in the range of $10^{-5}$ to $10^{-3}$ M, signifying that binding is relevant at the physiological ADP concentration of $\sim 2.5 \times 10^{-4}$ M (Holfgreve et al., 1997). The in silico binding methodology we adopted (Manoj et al., 2016d; 2019a; Venkatachalam et al., 2016) and data/loci obtained are validated by the non-binding of the negative control molecule of NADP with the chloroplast protein complexes even at $\Delta G \sim -2.0$ kcal/mol. With another consideration, a maximal approximate estimation of ATP in chloroplast is $\sim 1.4$ mM (Voon et al., 2018) and the physiological ratio of ATP/ADP is $\sim 2.4$ (Gardeström & Wigge, 1988), which makes the physiological ADP concentration of to be $\sim 0.6$ mM. We consider that approximately 5 times this value could give some relevant binding, which is $\sim 3$ mM; NADP (the negative control) did not afford binding with the chloroplast proteins even with a $K_d \sim 3 \times 10^{-2}$ M, which is approximately hundred times the physiological concentration of ADP. Therefore, our binding methodology is ratified, and these binding sites are physiologically relevant, with respect to the murburn theory.

In NDH, the proximal redox-active cysteine residue ligating the first Fe-S center is solvent-accessible, thereby enabling efficient DROS-dynamics (Figure 11, panels A & B), quite like what we have demonstrated for the liver microsomal CYPs (Manoj et al., 2016d; Venkatachalam et al., 2016). NDH’s aqueous-phase arm would enhance one-electron species’ lifetimes and harnessing them for phosphorylation by ‘sinking effect’ sponsored by the Fe-S centers. This is evidenced by the multitudes of ADP-binding sites around the bulbous arm. Unlike the mitochondrial system’s Complex I, NDH lacks a flavin because steady-state DROS generators such as ferredoxin-NADPH reductase (FNR), Cyt. $b_{6f}$, LHCs, etc. are already present nearby. The above deductions are supported by the crystal structure wherein the third or basal FeS center within the bulbous arm is $\sim 2$ nm away from the ‘benzoquinone’ site, which, in turn, is also a couple of nanometers from the trans-membrane region (Pan et al., 2020). It is quite unlikely that plastoquinones would diffuse into such binding sites deep into the protein and diffuse out after they get reduced. Such perceptions are merely in vogue owing to the deterministic mandate of the ETC. In the murburn purview, the trans-membrane foot of this protein serves as a ‘hub or recruiter’ (and not proton pump!), where other proteins and complexes could draw together spatially (Yadav et al., 2017) to optimize DROS dynamics, quite similar to Complex I’s foot’s role within the ‘respirasomes’ of mitochondria (Manoj et al., 2019a, 2019b). The newly proposed structure-function correlation for NDH also explains the conversion of NADPH formed (in the murburn equation for PI-Pp) into the formation of ATP within chloroplasts, via ‘chlororespiration’. In this regard, the murburn perspective considers the stromal projection of PS I with the Fe-S centers (Netzer-El et al., 2018) as facets aiding DROS-mediated phosphorylation, enabled by the presence of multiple ADP binding sites (Figure 11, panels C & D). This proposal is supported by the experimental findings (Kozuleva et al., 2014) that- (i) rate of $O_2$ reduction by PS I increases with increasing light intensity, (ii) quinone stature at the $A_1$-site affects $O_2$ reduction by PS I, and (iii) phylloquinone at the $A_1$-site reduces $O_2$. In oxygenic photosynthesis, it is unavoidable that oxygen/DROS is ‘refrained’ from accessing the redox centers or $e_{aq}$. Therefore, the murburn modality of electron transfer is facile in the chloroplast system also, quite akin to mitochondria and cytoplasmic microsomes. One of the most convincing aspects of our proposal is the comparison of the membrane-bound hydrogenase from the archaeeae, *Pyrococcus furiosus*, with the NDH of chloroplasts, both of which have three Fe-S centers (Yu et al., 2018) and the Complex I of archaea, bacteria and mammals. Though the proteins have structural differences (please refer
Supplementary Information file, Item 4, for a visual snapshot), the fundamental route of action is via murburn concept. This protein too has ADP binding sites, and in this system, ATP is formed using hydrogen atom radicals (in lieu of the oxygen-centered radicals) to enable the phospho-anhydride/ester formation from ADP and Pi. There is no tangible way that these proteins could otherwise function! The electron transfer between the FeS routes within the pendulous arm would be in microsecond time-scales, which cannot be connected to the millisecond time-scales for proton-pumping within the trans-membrane foot. Clearly, the protein structure’s evolutionary mandate is to enhance the lifetime of diffusible reactive species. Thus, the murburn model is ratified across diverse life forms, from archaea to plants.

CRAS-ZS-KJC paradigm was mooted when the structures of membrane complexes were not known and when it was believed that DROS were purely ‘agents of chaos and toxic wastes’. With the currently available structural insights, we can see that the assumptions/explanations of double hits/misses and the constants used to fit the periodicity simulation (Forbush et al., 1971), as MnComplex is not photoactive and is located far away from the RC of PS II. Also, the multimeric Q-cycle (Manoj et al., 2021), the protrusions of proteins, etc. do not offer relevant mechanistic correlations within the CRAS-ZS-KJC perspective. It is questionable that water participates in the reaction only at MnComplex of PS II, particularly when the crystal structure shows that the MnComplex is located just outside the trans-membrane region and is well-buried within the protein. It cannot be envisaged how this catalyst would access a steady supply of water molecules and hold on to them. It is known from in vitro studies that species that cannot be foreseen to bind
two molecules of H₂O like ZnO/WSe₂ (Hu et al., 2019) or simple Mn-porphyrin based systems (Willner & Calvin, 1981) could also mediate photocatalytic water-splitting to release O₂. Plants can grow, photosynthesize and evolve oxygen in manganese-deficient stature (Janpen et al., 2019), albeit inefficiently. The common “money plants/pothos/devil’s vine” (genus Epipremnum) are typical examples. Murburn perspective considers the MnComplex of PS II as a feature that could enable efficient stabilization or equilibration of excess aquated electrons (eₐq) and/or DROS. Though MnComplex could enhance oxygenesis, the discrete and delocalized DROS chemistry could also result in oxygen formation, which could explain Emerson’s observation. A structure-function analogy could be found in the fungal enzyme chloroperoxidase (CPO), which works via diffusible reactive species/radicals (Manoj, 2016; Manoj et al., 2020a). CPO also has a manganese ion, besides the heme-thiolate cofactor. When the fungus is grown in Mn-deficient medium, the CPO

### Table 5. Docking of ADP with various chloroplast protein complexes show multiple low-affinity binding sites, ratifying the murburn model of phosphorylation. (The archaic MBH also shows ADP-binding site.)

| S.No. | Protein complex | Binding energy (kcal/mol) | Binding constant (mM) | No. of distinct sites |
|-------|----------------|----------------------------|----------------------|----------------------|
|       | ADP            | NADP                       | ADP                  | NADP                  | ADP                  | NADP                  |
| 1     | PS II (6JLJ)   | −6.01 to −3.5              | > −0.77              | 0.04-2.6             | NR                   | 6                     | 0                     |
| 2     | Cyt. b₆f (1VF5) | −5.17 to −3.53             | > −1.35              | 0.16-1.5             | NR                   | 6                     | 0                     |
| 3     | PS I (6HQB)    | −5.29 to −3.6              | > +1.31              | 0.1-2.0              | NR                   | 10                    | 0                     |
| 4     | NDH (6KHJ)     | −4.45 to −3.45             | > +4.1               | 0.5-3.0              | NR                   | 12                    | 0                     |
| 5     | MBH (6CFW)     | −4.14 to −3.53             | > −2.04              | 0.9-2.6              | NR (32)              | 2                     | 0                     |

NADP was taken as a negative control; MBH = Membrane bound hydrogenase from Pyrococcus.
formed is active (Wang et al., 2003), although turnovers are lowered because the enzyme deactivates quicker. Manganese has a highly variable valency (a theoretical range of +7 to −3) and it could ‘save’ PS II Complex from the highly variable redox potentials of the ambience. Several reactions (as discussed in the first part of this essay) could be a source of peroxide, and this molecule could be in dynamic equilibrium with the MnComplex. Therefore, MnComplex could be an efficient oxidase-peroxidase (akin to the oxidase-peroxidase ‘turbo logic of Complex IV’ in mOxPhos), which could explain the enhanced oxygen production at this site and/or it could shift the equilibrium to photolysis by enabling effective NADP⁺ reduction (as suggested by Daniel Arnon’s works of 1980s (Arnon et al., 1981), which is discussed in another section of this manuscript).

### 3.4. Cross-system comparisons

#### 3.4.1. Comparison of mOxPhos and Pl-Pp

In Section 4 (critique of Z-scheme), we have already demonstrated that several specific observations that were incompatible with the Z-scheme are explained and accommodated within the murburn model. Herein, we employ pan-systemic approaches first in order to highlight the involvement of DROS (murburn concept) in cellular physiology and draw comparative inferences. Then, we come to specific evidences that endorse the murburn model for chloroplast mediated light reaction of photosynthesis.

It is now opportune to see some perceived similarities in oxidative- and photo-phosphorylation schemes, toward which we present Figure 12, inspired from (Berg et al., 2007). The mOxPhos reactions occur at the inner membrane of mitochondria and are driven majorly by the chemical energy of NADH oxidation. Similarly, Pl-Pp occurs around the inner membrane of chloroplasts and is driven by radiant energy derived from sunlight. A quick survey of the elements of these two systems (as discernible in the publications cited above) allows us to infer that mOxPhos and Pl-Pp could be deemed similar or anti-/quasi-parallel, because:

i. In both systems, micron-scaled organelles employ electron transfer phenomena at their inner phospholipid membrane interface, with ETs across a total distance of ~10^3 Å within time-frames of 10^3 s⁻¹ per cycle. Also, both processes employ a series of multi-protein complexes with diverse redox centers (hemes, Fe-S proteins, flavins, etc.); generating a trans-membrane potential (TMP) or proton motive force (pmf) and producing DROS and liberating heat during their functioning.

ii. Both paradigms avail diverse redox systems for (supposedly) forming intricate electron transfer/transport chains (ETCs). The systems incorporate the common elements of proton pumps, membrane-based organo-quinone 2-electron translocator and aqueous-phase based metallo-protein 1-electron translocator (Cyt. c and PC/Fd, ferredoxin).

iii. Overall, the ETC reaction schemes in both systems require the input of a total of 4-electrons from two substrate molecules (two NADH/succinate molecules in mOxPhos and two water molecules in Pl-Pp) to give an output of two reduced molecules (two water molecules in mOxPhos and two NADPH molecules in photophos). But before the transfer of the two-electron equivalents to the membrane-harbored quinones in the next step, several one-electron relays are supposedly involved in these highly complicated systems (e.g. Complex I in mOxPhos and Photosystem II in Pl-Pp).

iv. Very strikingly, the element of trans-membrane electron conveyor (called Complex III in mitochondria and Cyt. b₆f in chloroplasts) is common, receiving 2-electron equivalents from membrane-quinones and giving away 1-electron equivalents to an aqueous phase.

Earlier, we revamped the mechanism of mOxPhos system based on its mechanistic similarities with the mXM system (Manoj, 2018a, 2018b). Herein, we intend to question the perceptions on Pl-Pp system based on the corrections we have already made within the mXM and mOxPhos systems. Since it is beyond reasonable doubt that the hemeproteins use diverse DROS for catalysis and we have consolidated the murburn theory in mXM and mOxPhos systems, it is forthright to deduce that the presence of similar redox proteins in chloroplasts is owing to the evolutionary significance of DROS-based metabolism across diverse organelles and organisms. If the fundamental photo-electric charging of the Pl-Pp system with photons is discounted, the reaction paradigms involve DROS and are essentially downhill (exothermic), falling within the purview of the stochastic and bimolecular murburn scheme. In this regard, experimental results show direct evidence that ATP synthesis is correlated to DROS concentration (Nicholls, 2004; van Hameren et al., 2019).

#### 3.4.2. Cyanobacterial system

Unlike the eukaryotic plants that have both mitochondria and chloroplasts (two distinct organelles to carry out the separate electron flows of OxPhos and PI-Pp respectively) within their cells, cyanobacteria conduct both photosynthesis (Pl-Pp) and respiration (OxPhos) within their thylakoids. They do so using the common machineries of Cyt. b₆f, plastoquinone and Cyt. c₆. In Figure 13, the simplified architecture of a cyanobacterium and the extent understanding of cyanobacterial respiratory and photosynthetic ETC are shown. It can be seen that maintaining the directionality of uphill and downhill electron transport in the two counter-directional ETCs is not possible simultaneously, as this would lead to futile cycles.

Figure 14A depicts the essential requirements of a controllable working model, which includes the efficient coupling of uphill and downhill machineries. In such a system, the elements of the uphill and downhill reactions are distinct and there are separate (inter-connected) reservoirs of the “starting fuel” synthesized or used. In Figure 14B, the currently prevailing mechanistic impression of the cyanobacterial metabolism is shown. As seen, this model has a susceptibility to drain (wastefully) with the given
configuration. As soon as the light illumination stops, catabolic activities would drain the redox equivalents, killing the cell. This is better explained with an analogy from day to day life. A battery that is put on a load of “x” watts cannot get simultaneously charged by another capacitor of “y” watts (y ≈ x), using the same electro-motive force conduits. An ordinary automobile’s battery gets charged and takes up load at the same time owing to a modular setup where the power loop logics are distinctly demarcated. Even in these setups, as soon as charging alternator is taken off, the system would be drained off all power, if the load remains connected.

Under the prevailing perspective, it is difficult to see an unintelligent Cyt. c₆ deciding on imparting electrons to PS I or Complex IV. This “decision making” is crucial because it could lead to an “active synthesis” of NADPH or depletion of the same. That is- let us imagine that the bacterium is availing sunlight. Cyt. c₆ would more probably give electrons to Complex IV (rather than a transient electron deficient PS I); and this Complex I would keep breaking down any NADPH that might ever form in the cytoplasm. The result would be that photoexcitation would only lead to a “cycling” of protons/water from thylakoid to cytoplasm. It is unlikely that a protein like Cyt. c₆ can be instructed intelligently to switch between the OxPhos and Pl-Pp cycles, based on fluctuating cellular demands. The current mechanism seeks Cyt. c₆ to “drive up and down the road at the same time”, around the membrane within anti-parallel traffics of OxPhos and Pl-Pp. Such a mechanistic demand of a deterministic ETC is destined to fail. Further, several non-conventional but proven and accepted electron transfer possibilities exist in the Pl-Pp system, of which three putative interactions mediated by PQ (plastoquinone), Fd (ferredoxin) and FNR (ferredoxin-NADP reductase) are denoted by dashed connectives in Figure 13. Other components of the Pl-Pp system, namely- PS II, PS I, etc. could also give electron(s) to the omnipresent O₂ and

Figure 12. A comparison of the perceived structure-function correlations and mechanistic aspects of mOxPhos and PI-Pp systems (Berg et al., 2007). The vectorial orientations of proton-pumps/Complex V and electron transport chain are depicted. The contents within the brackets signify the electron acceptance capacity and/or transferability of the given component. The membrane-embedded proteins were considered as proton pumps, a perception that was questioned and superseded by murnbarn explanations.
DROS. If so, the currently perceived Pl-Pp ETC would be just "a deterministic scheme", and less of a probabilistically relevant pathway. This statement would be further explicated in the section below.

With the best possible orientations and juxtaposition of the proteins, there is yet a stark problem with the prevailing explanations. That is- if proton pumps and a deterministic ETC were the key operatives, it is not clear how this feat

Figure 13. Schematic of cell structure and ETC proteins' distribution of thylakoid membranes of cyanobacteria. Though there are little deterministic ways that the elements of the Pl-Pp or OxPhos ETC can be selectively or preferentially placed adjacently on a given membrane, we have presented the two systems' perceptions on the top and bottom membranes respectively.

Figure 14. Comparison of controlled working model (A) and spontaneously drained model (B) schemes for cyanobacterial redox metabolism.
could be achieved for the PI-Pp cycle. This is because while the OxPhos cycle would have three proton pumping Complexes (Complexes I & IV beside Cyt. b_{6f}), the PI-Pp machinery has only Cyt. b_{6f} serving the role of proton pump. Most PI-Pp researchers no longer consider PS II a proton pump, as can be seen from later texts and publications! This lack of consensus shows clearly that the theories and protocols adopted for establishing ‘proton pump’ concepts are faulty (Manoj et al., 2020b). We have pointed out that this proton-pumping cannot be managed by the ‘energy packet’ available to Complex III or Cyt. b_{6f} (Manoj, 2018a; Manoj et al., 2021). Further, we see no justifiable purpose served by having a trans-membrane “electron-routing” step and self-reduction of quinones (via the Q-cycle), all of which would only render the “ETC” kinetically non-viable with respect to the overall electron transfer rates. Recent developments in respirasome structures confirm this deduction (Manoj et al., 2019a), rendering the “Q cycle” as yet another redundant feature of the Mitchellian paradigm. For an exhaustive critique on the concept of ETC, please refer our recent works already cited (Manoj, 2018a; and the first part of this work).

Very importantly, the smallest photosynthetic cyanobacteria belong to the Prochlorococcus types (Partensky et al., 1999), which have an average dimension of half a micron. Such a small organism would have practically no “free protons” within the cell to serve any proton pumps’ requirements at physiological pH (Manoj, 2018a, 2018b). Therefore, under the premise that proton pumping in micron-dimensioned organisms is questionable, the current understanding would leave little modalities for ATP synthesis within the PI-Pp paradigm and cannot explain why the electron needs to take such a roundabout route from water to NADPH either. Therefore, a study of the cyanobacterial mOxPhos & PI-Pp ETC shows that the extant explanations for electron transfers and driving mechanisms for ATP synthesis are non-viable. On the other hand, existing features afford ample scope for the application of the non-deterministic murburn scheme in PI-Pp, which uses DR(OS) and dynamic inlet of protons.

### 3.4.3. Effects of miscellaneous additives and proton dynamics

Uncoupling is a term used in redox enzymology when the enzymes use up the redox equivalents, but do not give physiologically relevant or fruitful reactions. For example, in the mXM system, NADPH (the source of redox equivalents) could get used up without hydroxylating the xenobiotic substrate. Instead, DROS accumulation or water formation may happen. Similarly, in the mOxPhos system, NADPH gets used for heat/DROS/water formation, sans ATP synthesis.

Uncoupling proteins (UCP) in mitochondria cause heat generation and floral thermogenesis is well known in plants. Particularly, the skunk cabbage is famous for its ability to achieve even a 25°C higher differential from a near-freezing ambiance (Ito-Inaba, 2014). (A chloroplast-based heat generation process is also known, and this is attributed to non-photochemical quenching (NPQ) at higher light radiance. However, this is not the topic of discussion here.) The thermogenesis phenomenon was believed to result from a UCP-mediated uncoupling phenomenon, which supposedly led to the dissipation of trans-membrane proton potential (TMP) into heat. Based on new awareness, we have recently shown that UCP-mediated uncoupling and thermogenesis resulted due to its ability to sponsor DROS-based reaction chemistry around the inner mitochondrial membrane (Manoj et al., 2018). The extant TMP-based explanation (Sweetlove et al., 2006) cannot clarify the beneficiary effect of UCP in photosynthesis whereas murburn concept posits explanations that agree with thermodynamic principles.

In liver microsomes, the N-term peptide sequence of a flavoenzyme, cytochrome P450 reductase (CPR) was supposed to be involved in essential redox partnering with a heme-enzyme, cytochrome P450 (CYP) (Gideon et al., 2012). It was noted that when the N-term segment was truncated and left within the reaction medium, significant “uncoupling” resulted. We had shown that the uncoupling mediated by the N-term segment (deemed to be functionally analogous to UCP/dinitrophenol of mitochondria) resulted owing to its ability to modulate DROS. The reaction schemes of mXM and mOxPhos are compared with PI-Pp in Table 6.

It is seen that quite similar to the microsomal and mitochondrial systems, the chloroplast system also shows uncoupling reactions (giving heat and DROS), which were perceived to be due to “faulty electron transfers”. Some molecules like disubstituted phenolics (like dinitrophenol or DNP, which is known to uncouple oxidative reactions with ATP-synthesis), are also known to cause uncoupling in mXM and PI-Pp systems, implying that the uncoupling mechanism is common across these membrane-embedded redox systems. Since the mXM system has no relevance for proton-pumps and since the proton-potential based explanation for mOxPhos system is untenable, the DROS-based murburn scheme is the potential candidate to explain the affects/effects observed across the systems. The new rationale proposes that DROS modulation at the membrane interface by molecules like DNP is facile due to interfacial catalysis mediated by extra-organellar protons (and not owing to dissipation of proton-gradients by uncoupling molecules serving as trans-membrane proton-shuttlers, as believed earlier).

Further, all three systems (mXM, mOxPhos & PI-Pp) are simple distributions of protein complexes on lipid membranes and Ockham’s razor suggests that a simple stochastic theory is more plausible than a sophisticated deterministic theory. This inference is further corroborated with the results obtained by the incorporation of additives as probe molecules within the reaction milieu. In such reductionist experimental strategies, the following queries/results stand out: (i) If PI-Pp ETC only employs Marcus’ outer sphere model of electron transfer, how can several man-made dyes and redox active molecules put in and draw out electrons from the purportedly deterministic ETC (Hauska, 1977)? (ii) The prevailing mechanistic understanding cannot account for the unusual concentration-dependent effects brought about by certain additives in PI-Pp systems (Avron & Shavit, 1965; Watling-Payne & Selwyn, 1974). Such outcomes are typical manifestations of the operational relevance of murburn scheme involving radicals, which show concentration-dependent
stabilization/activity profiles (Parashar et al., 2018). (iii) Prasanna Mohanty’s works had shown that certain cations adversely affect the photosynthetic outcomes and we have argued that such outcomes result in redox metabolic schemes because the negatively charged superoxide would be liable to modulation at the phospholipid interface (Manoj et al., 2019a, 2019b).

Since cytoplasm is practically an open source of water (as plasma membrane is not impervious to protons), protons are available for the membrane proteins (like cytochrome P450s) lodged on cytoplasmic reticulum. However, 3-dimensionally constrained micro-domains like mitochondria and chloroplasts are different. We present some simple calculations in the context. Gauging from electron micrographs and literature available, an average thylakoid is ~0.5 micron in linear dimension, and the lumen is ~0.05 micron thick (e.g. text-books cited earlier). Therefore, assuming a flattened (cubic or cylindrical) vesicular structure, the maximal volume of a thylakoid would be ~10^-17 liters. As a result, in the starting or resting phase, the crude calculations for protons within the lumen of thylakoid a cellular physiological pH of 7 would equal 6.023 x 10^23 x 10^-7 x 10^-17 ≈ 0.6. More precisely, assuming that chloroplast have an average resting pH of 8 and thylakoids are stacked cuboids with a dimension of (1 x 0.015 x 0.233) microns (Austin & Staehelin, 2011), the number of protons per lumen of an average thylakoid is = 6.023 x 10^23 x 10^-8 x (1 x 0.015 x 0.233) x 10^-15 = 0.02. Therefore, thylakoids are practically aprotic and this could enhance the stability of radicals significantly. (This does not mean that protons are not available for catalysis. Grothuss mechanism would ensure catalytic process but pumping protons out into another and discontinuous macrosystem is a totally different thermodynamic process.) The stroma would have a much higher volume (say, 5 μm x 5 μm x 5 μm) of ~10^-13 liters, and it would have protons approaching = 6.023 x 10^23 x 10^-7 x 10^-13 ≤ 10^8. (It should be borne in mind that these differences in numbers cannot lead to gradients. This is because the proton concentration in the two phases are still the same, and therefore, a pH gradient cannot exist.) Assuming an initial closed system of 100 thylakoids within a chloroplast (at say, time t1), only if the protein complexes efficiently pumped >90% of the stromal protons into the thylakoids (at say, time t2), the gradient across the thylakoid membrane would approach the requirement for ATP synthesis. This is given by the relation: 61 log [90/(0.5 x 0.5 x 0.05)]/[1000/(5 x 5 x 5)] = 180 mV. Here, the volume term of a thylakoid is ~0.01% of the stroma. Even if the thylakoids are connected, we would have the same calculation because while the total lumen volume increases by 100 folds, the proton amount in the lumen also gets multiplied by 100. It is known that 180 – 200 mV (correlating to a proton concentration gradient of ~10^5) is the minimal theoretical or practical requirement for deriving or observing ATP synthesis, per the “transmembrane pump” mechanistic purview (Nicholls, 2004). The calculation above is from an initial state to a final state and it cannot explain the steady state functioning of the chloroplast, quite like the scenario demonstrated for mitochondria (Manoj et al., 2018, 2018b). Since the pumps are not synchronized and there are no modularities (that is- a protein pump is not associated with its own “outer covering shell”, and several such shells are not triggered simultaneously with some phase transition operation), the proton concentration would rather equilibrate in steady state, than create a gradient. Such simple considerations discredit the “proton pump” concept for the thylakoid membrane and generate even more questions. Why should there be thousands of proteins to deal with a small number of protons? Also, how can the proteins (or uncoupler molecules like DNP) flip back and forth into different conformations under a steady state trans-membrane potential? How can the proteins react to such a TMP which they themselves set up? Quite simply, in the absence of any direct evidence for proton pumps, all these impossible requirements seek too much intelligence at the proteins’ ends. A survey of text-books and reviews shows various mechanisms and citing different numbers of protons per each of the complexes. Also, historically, the roles of various complexes as proton pumps have been a matter of intense debate, and a consensus is not yet reached amongst the researchers on this contentious issue till date (Manoj et al., 2020b, 2021). Such a scenario questions the fundamental crux of the CRAS concept. These issues are effectively addressed by the murburn model of PI-Pp, which attributes functional roles for DROS and interfacial protons in multiple reaction equilibriums (Manoj et al., 2018).

### 3.4.4. Perspectives panning diverse systems

We present a reductionist analysis of some redox metabolic systems from various cellular loci and elucidate the commonalities of reaction components of select redox catalytic
Compositional attributes of redox enzymatic systems. The various systems show significant similarities in the formative elements of the reaction systems. It is likely that systems with common components abound by similar mechanisms. Text in large braces (within items 1 & 2 below) are examples cited for the proteins.

| Item | Extracellular | Peroxosomal | Mitochondrial | Cyanobacterial | Chloroplastid |
|------|---------------|-------------|---------------|---------------|--------------|
| 1. Primary coenzyme | Porphyrin | [CPR] | Comp. I, II | Porphyrin | Comp. IV/PS II |
| 2. Secondary coenzyme | [CPR] | Comp. I/CBP | LHC, CBP | Flavins | PS II |
| 3. Tertiary component | Nil | Glutathione, b | One, open | Ascorbate | Two, closed |
| 4. Membrane | nil | Glutathione, b | Several, closed | Glutathione, b | Several, closed |
| 5. Activators | H2O2/+ | DROS | DROS | NAD(P)H/+ | DROS |
| 6. Substrates | RH | ROH | RH | RH | ATP + H2O + H2O2 + NADPH + O2 + Heat |
| 7. Mediators | Heat | Heat | Heat | Heat | Heat |
| 8. Products | H2O + Heat | H2O + Heat | H2O + Heat | H2O + Heat | H2O + Heat |

systems therein (Table 7), as we go from simple to more complex systems, from left to right.

Though there are notable differences in composition, it can also be seen that there is a fundamental framework of similarity across the systems. The electronic pit-stops belong to various classes like quinones, porphyrins and Fe-S proteins; and the systems have the commonality of \( \text{NAD(P)+O}_2+\text{DROS} + \text{Heat} \). Further, DROS-utilizing CYPs are not just found within cytoplasmic endoplasmic reticulum (microsomes), they are also localized in mitochondria (Sangar et al., 2010) and chloroplasts (Warzecha, 2016). Similarly, catalase/peroxidases are not only found in vacuoles, cytoplasm or periplasm; their presence was also demonstrated in mitochondria and chloroplasts (Arita et al., 2006; Heinze & Gerhardt, 2002; Prasad et al., 1995). Since mXM and mOxPhos systems use DROS-based strategies, it is natural to deduce that the PI-Pp system also could fall within the murburn logic. The production of DROS was sidelin earlier as side-reactions or pathophysologies but we have recently shown that ATP synthesis via DROS is a viable physiological route (Manoj et al., 2019a, 2020a, 2021). This assertion is also supported by the demonstration of DROS-assisted ATP synthesis in mitochondria (Mailer, 1990), chloroplasts (Tyszkiwicz & Roux, 1987) and erythrocytes (Ellsworth et al., 2006). Since ATP synthesis is correlated to DROS concentration (Nicholls, 2004; van Hameren et al., 2019), it is a misplaced idea now to consider that DROS are purely deleterious. Several direct beneficial evidence of DROS-assisted metabolism and signaling has begun to accrue since the last couple of decades (Heidler et al., 2010; Mittler, 2017; Schulz et al., 2007). Yet, a discerning individual may ask isn’t murburn concept a rather chaotic reaction mechanism, as it uses diffusible radicals? To this, we say that murburn concept is best illustrated via a simple analogy: A wet cloth daubed in oil later can be set on fire. Though this is a highly chaotic exothermic reaction, the fabric does not get charred significantly so long as there is oil to burn. Similarly, as long as there is a sustained supply of metabolic substrates present that are dynamically reacted and removed as products, the scaffold of lipid membrane is left more or less intact. (Of course, the membrane does get affected deleteriously cumulatively, and this is one of the reasons for aging and death.) In this regard, we would like to reproduce (verbatim) the concluding section of an article from our group (Jacob & Manoj, 2019)-“Just because a cut-injury in the kitchen most probably results from knife-abuse, one does not infer that knives do not have any positive role in the kitchen! Also, one may find gloves and cutting-boards to hold and handle knives in the kitchen. Their presence too does not imply that knives have no constructive culinary roles! (In this analogy- knives, gloves and cutting boards are equivalent to DROS, redox enzymes and antioxidants respectively.)”

We present Table 8 (a continuation of our analysis presented in Table 7), which demarcates the perceptual change from classical views to murburn concept. The comparison within this table also highlights the inability of classical view to- (i) address key aspects of the mechanistic
chemistry and (ii) capture the holistic picture of the discussions made with greater details and clarity. It must be noted that while the murburn explanation is consistent throughout the systems, the classical purviews invoke different principles across the diverse systems. The final queries of assessment (A-E, at the bottom of Table 8) enables the discerning reader to select the more suitable hypothesis. The extant paradigm of PI-Pp requires the membranes to be highly regulatory and also seeks the membrane-embedded proteins to change conformation and make phospho-ester bonds based on mechanical cues (which is unheard and unseen, other than in CRAS mechanism). The murburn explanation for photosynthesis is a simple scheme of bimolecular chemical reactions that does not seek electro-mechanical intelligence from the chloroplast/thylakoid membranes. We have demonstrated the experimental viability for DROS to make the phospho-ester bond and also provided thermodynamic and kinetic foundations to our proposals (Manoj et al., 2019a, 2020a, 2021). Also, our theory is the only existing proposal that justifies the obligatory requirement and role for oxygen in diverse life forms and explains cyanide toxicity (Manoj & Soman, 2020). Further, with respect to the functioning of redox proteins in various loci and organelles of a cell (Item 1, Supplementary Information), it is unlikely that the metabolic logic and principles change deterministically or intelligently, on a case to case basis. Therefore, the ubiquitously applicable bimolecular reactions of murburn scheme is a viable model for PI-Pp.

3.5. Consolidated view

3.5.1. Supercomplexes (LHCs, CBP, PS, etc.)

In the xenobiotic metabolic machinery embedded in ER and the oxidative phosphorylation proteins in mitochondrial membranes, we have explained the logic of why membrane redox proteins are often clumped and make super or mega complexes (Manoj et al., 2019a; Parashar & Manoj, 2021). The packing enables better utilization of DRS species amongst the proteins. If complexation based chemistry was the mechanistic route, such a packing would be disadvantageous as it blocks sides of proteins altogether. Figure 15 shows examples of various super and mega complexes known on chloroplast membranes. This is yet another testimony for the evolutionary significance of the murburn model of function of such systems. Such higher order associations of protein systems occur in various permutations and combinations based on fluctuations in environmental conditions such as light and these structural adaptations are inexplicable when going by the conventional theories of structure-function in photosynthesis. Some other examples are PSI-PSII megacomplex (seen at high light), PSI-LHCl-LHCCI supercomplex (at low light), PSI-LHCl-LHCCI-FNR-Cyt. b6f-PGLR1 supercomplex, PSI-LHCl-LHCCI supercomplex, C2M2S2 supercomplex of PSI-LHCCI, C2M2S2N2 type supercomplex of PSII-LHCCI, PSII-C2S2, PSI-LHCl complexes (Järvi et al., 2011).

3.5.2. Correlation of findings from other systems

Photodynamic processes and murburn explanations: Low-level laser therapy (LLLT) is a non-invasive physiotherapy treatment for musculoskeletal conditions. Such photo-therapy in medicine is explained by several theoretical assumptions (Castano et al., 2005; de Freitas & Hamblin, 2016; Sommer, 2019; Zielke, 2014). LLLT is known to enhance mitochondrial ATP generation. This, in turn, is also correlated to DROS production (de Freitas & Hamblin, 2016; Sommer et al., 2015; Xu et al., 2008). Photobiomodulation was shown to enhance mitochondrial TMP and ATP synthesis in auditory cell lines (Chang et al., 2019). We believe that the most chemico-physically sound and parsimonious mechanism for these observations invokes obligatory roles for DROS; which can explain the enhanced TMP and ATP synthesis. Photosensitive molecules of our body (like hemoglobin and melatonin) can easily avail light in the red spectrum (Castano et al., 2005; Farivar et al., 2014) and undergo excitation and react with singlet or triplet oxygen (after inter-system cross-over) to generate DROS (Manoj & Manekkathodi, 2021). Support for our theory is available by a work which shows that incorporation of light-harvesting chlorophyll pigments also allow mitochondria of animals such as Caenorhabditis and mammals to produce ATP upon photo-activation cues (Xu et al., 2014). Further, researchers have used chlorophyll-enriched polymeric nano-preparations for photothermal therapy for cancer (Pemmaraju et al., 2018). The photodynamic therapy (for cancer) using methylene blue is known to incur the production of DROS (Tardivo et al., 2005). Porphyrin-loaded encapsulations have been employed for photo-induced generation of DROS for therapeutic purposes (Tsubone et al., 2020). Such outcomes are directly correlated to facile generation of photo-induced DROS and their favorable roles in diverse loci, in variable physiological ambiance. Therefore, considering these outcomes as irrelevant chemistry with respect to photosynthesis in plants is disregarding chemical evidence.

Physiology of vision (photoreception) and murburn explanation: The physiology of vision is very similar under the consideration that in both photosynthesis and vision, the photon needs to be transduced into a tangible electrochemical signal. In this regard, we explained the functioning of rhodopsin, which incorporates retinal (a carotenoid, quite akin to the carotenoids found in thylakoid membranes), and also uses oxygen to tap the incident light ray to generate a signal. The electron replenishment in the system is powered by the rod and cone cells (Manoj & Jacob, 2020), whose structures are somewhat similar to the stacked grana of chloroplasts. Such a similarity in components and compartmentalization shows the commonality of murburn principles operating in both systems.

3.5.3. Summatting the murburn scheme for PI-Pp

It is evident that DROS are the common elements across these diverse systems and the presence of one-electron redox-active centers enable an efficient stabilization/utilization of radical species like superoxide. As in other reaction systems where murburn concept was demonstrated, the
Table 8: Erstwhile consensus perceptions vs. recent murburn attributions and other agendas of comparison: For a given criterion, the upper (shaded) row depicts the classical view and the lower row is the murburn perspective.

| Criteria | Extracellular | Peroxisomal | Mitochondrial | Cyanobacterial | Chloroplastid |
|----------|---------------|-------------|---------------|----------------|---------------|
| 1. Energetic drive | Redox gradient | Redox gradient | Redox gradient | Redox gradient | Redox gradient |
|          | Thermodynamic pull | Thermodynamic pull | Thermodynamic pull | Thermodynamic pull | Thermodynamic pull |
| 2. Reaction site | Enzyme pocket | Enzyme pocket | Enzyme pocket | Enzyme pocket | Enzyme pocket |
|          | Delocalized | Delocalized | Delocalized | Delocalized | Delocalized |
| 3. Key species | Comp. 1 | Comp. 1 | Comp. 1 | Comp. 1 | Comp. 1 |
|          | Diverse/DROS | Diverse/DROS | Diverse/DROS | Diverse/DROS | Diverse/DROS |
| 4. Electron Transfers | Specific and affinity-based | Specific and affinity-based | Specific and affinity-based | Specific and affinity-based | Specific and affinity-based |
|          | Non-specific, equilibrium-based | Non-specific, equilibrium-based | Non-specific, equilibrium-based | Non-specific, equilibrium-based | Non-specific, equilibrium-based |
| 5. Primary e-Source | O-H bond | O-H bond | O-H bond | O-H bond | O-H bond |
|          | NADPH | NADPH | NADPH | NADPH | NADPH |
| 6. Final e-Sink | Active site relay | Active site relay | Active site relay | Active site relay | Active site relay |
|          | ATP | ATP | ATP | ATP | ATP |
| 7. Protons-Proteins | NA, soluble enzyme | NA, soluble enzyme | NA, soluble enzyme | NA, soluble enzyme | NA, soluble enzyme |
|          | Required to make CYP + CPR complex | Required to make CYP + CPR complex | Required to make CYP + CPR complex | Required to make CYP + CPR complex | Required to make CYP + CPR complex |
| 8. Membrane | NA | NA | NA | NA | NA |
|          | Not needed, soluble enzyme | Not needed, soluble enzyme | Not needed, soluble enzyme | Not needed, soluble enzyme | Not needed, soluble enzyme |
| 9. TMP | NA | NA | NA | NA | NA |
|          | NA | NA | NA | NA | NA |
| 10. Kinetics (limitation) | Classical (E-S interactions) | Classical (E-S interactions) | Classical (E-S interactions) | Classical (E-S interactions) | Classical (E-S interactions) |
|          | Atypical (Conditional) | Atypical (Conditional) | Atypical (Conditional) | Atypical (Conditional) | Atypical (Conditional) |
| 11. Q/PC/Cyt. | NA | NA | NA | NA | NA |
|          | Spec. relay | Spec. relay | Spec. relay | Spec. relay | Spec. relay |
| 12. DROS/ Radicals | NA | NA | NA | NA | NA |
|          | NA | NA | NA | NA | NA |
|          | Generic capacitor | Generic capacitor | Generic capacitor | Generic capacitor | Generic capacitor |
| 13. Inhibitions | NA | NA | NA | NA | NA |
|          | Promiscuous mainstay | Promiscuous mainstay | Promiscuous mainstay | Promiscuous mainstay | Promiscuous mainstay |
|          | Block ETC or Proton pumps | Block ETC or Proton pumps | Block ETC or Proton pumps | Block ETC or Proton pumps | Block ETC or Proton pumps |
| 14. Uncoupling | Active site or Milieu based | Active site or Milieu based | Active site or Milieu based | Active site or Milieu based | Active site or Milieu based |
|          | Active site | Active site | Active site | Active site | Active site |
| 15. Dose response | Simple | Simple | Simple | Simple | Simple |
|          | Complex | Complex | Complex | Complex | Complex |
| 16. Heat prodn. | Water formation | Water formation | Water formation | Water formation | Water formation |
|          | DROS quenching | DROS quenching | DROS quenching | DROS quenching | DROS quenching |
| 17. Transition state | Multimolecular | Multimolecular | Multimolecular | Multimolecular | Multimolecular |
|          | Bimolecular | Bimolecular | Bimolecular | Bimolecular | Bimolecular |
| 18. Networking of components | Serial, ordered | Serial, ordered | Serial, ordered | Serial, ordered | Serial, ordered |
|          | Parallel, unordered | Parallel, unordered | Parallel, unordered | Parallel, unordered | Parallel, unordered |
| 19. Stoichiometry | Defined | Defined | Defined | Defined | Defined |
|          | Variable, non-integral | Variable, non-integral | Variable, non-integral | Variable, non-integral | Variable, non-integral |
| 20. Overall scheme | Deterministic | Deterministic | Deterministic | Deterministic | Deterministic |
|          | Stochastic/ Statistical | Stochastic/ Statistical | Stochastic/ Statistical | Stochastic/ Statistical | Stochastic/ Statistical |
| 21. A. Structures? | Yes | Yes | Yes | Yes | Yes |
reaction components have multiple roles (Manoj, 2006; Manoj et al., 2016c); water, oxygen and DROS are produced and utilized at multiple sites. For example- (i) water is used by PS II, and produced in ATP synthesis and by DROS reacting among themselves. (ii) superoxide could be produced and used at multiple loci like FNR, NDH (NADPH dehydrogenase), CBP/LHC, PS I & II, etc. Since DROS are produced and released in the vicinity of membrane-embedded proteins containing redox centers, it makes bioenergetic sense to have a clumping of such complexes, to better harness the released species. If protein-protein binding was the actual mechanistic route for electron transfer, such homo and hetero complexes would only minimize the partner recognition ability. This was one of the major rationales that helped explain the existence of supercomplexes (respirasomes) in mitochondria (Manoj et al., 2019a) and the same logic holds good for chloroplasts too. Our proposal is supported by the fact that photo-chemically released electrons around the thylakoid membranes would react instantly with oxygen to give superoxide. This reaction has a transformed Gibb’s energy term (aq), $\Delta G^{\circ} = -250$ kJ/mol (Manoj & Bazhin, 2021; Manoj & Manekkathodi, 2021). Therefore, this reaction’s high equilibrium constant, $K_{eq} = 10^{44}$ and high rate constant, $k = 2 \times 10^{19} \text{M}^{-1}\text{s}^{-1}$ supports the murnburn model of PI-Pp (Takahashi et al., 1988). As only the electron-projecting light-aided reaction requires the input of energy from photons, the rest of the reactions in the murnburn scheme are dictated by stochastics (probability- without any need for a multi-molecular serial cascades) are all thermodynamically and kinetically facile. The minimalistic steady-state murnburn reaction model of PI-Pp can be represented by five statements (and the process summary is shown in Figure 16), for the directly coupled ‘electron transfer + group transfer’ equation:

\[
\begin{align*}
\left\{ \text{NADP}^+ + \text{OH}^- \right\} + \left\{ \text{ADP} + \text{Pi} \right\} \\
\rightarrow \left\{ \text{NADPH} + [\text{DROS/H}_2\text{O/O}_2] + \text{ATP} \right\}
\end{align*}
\]

1. Light falling on all pigments can lead to “caged emission of electrons + production of DROS”.
2. As electrons enter NAD(P) system, they get “spin-locked, preventing futile reaction cycles”.
3. Water/OH$^-$ serve as primary e-donor at PS; DROS serve e-deficient species (including PS).
4. NADPH is used for DROS generation; DROS is used for ATP-synthesis; ATP is used by cell.
5. The overall logic is to generate and deplete NADPH/DROS/ATP, pulling the reaction to the right.

From the murnburn perspective, it is evident why provision of protons drives the reaction right, explaining Jaegendorf’s findings that were erroneously used as evidence for CRAS proposal (Manoj, 2018a; Manoj et al., 2019a, 2019b; Manoj & Bazhin, 2021; Manoj & Manekkathodi, 2021). In the murnburn model, after photon-activation of pigments, NADPH formation is a primary step. This is when NADPH formation is the terminal reaction in Z-scheme. This arrangement does not give any operational feasibility or kinetic viability for the
overall serial/sequential model of Z-scheme. Bimolecular interactions involving DROS serve as the electron relay agents and ATP-synthesizers in the murburn model, whereas multi-molecular (kinetically challenged reactions like Q-cycle) and biologically non-evolvable/irreducibly complex requirements are the mainstay of Z-scheme. In the murburn explanation, molecules like PC/Fd/PQ serve as non-specific electron buffers; Photosystems serve as effective charge separation switches and Cyt. b,f serves to recycle the electrons lost to membrane phase. Water serves the ultimate source of electrons (common to both Z-scheme and murburn model).

The introductory parts of this manuscript cite the works that refuted the mitochondrial ETC (mETC) and chemiosmotic rotary ATP synthesis (CRAS) proposals. In those publications (and herein), we have quoted unquestionable facts and experimental observations, thermodynamic yardsticks, kinetic reasoning and probabilistic analyses; and used these fabrics of scientific pursuit for advocating murburn explanations. It is obvious that if the overall downstream mETC is untenable, the overall upstream chloroplastid ETC (alias Z-scheme) should be even more so (particularly under the context that the element of CRAS is common to both Z-scheme and murburn model).

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The conclusive physiological/experimental argument comes from three independent studies and they are discussed below: As per Z-scheme, plastocyanin, the protein relaying electrons from Cyt. b,f to PS I (thus linking the two photosystems serially) is indispensable, and so is cytochrome f. However, knock-downs or mutants lacking functional plastocyanin grow and photosynthesize as efficiently as the native/wildtype variants (Pesaresi et al., 2009; Zhang et al., 1994). Even otherwise, this protein is present only at 10^(-6) M levels in the native/wildtype and that too, it is found in both grana and stroma. This cannot meet the requirement of the Z-scheme reaction, as the Kd of the protein (with Cyt. b,f/PS I) is 1000 M and the Z-scheme proposal seeks PC’s presence in thylakoid lumen (Gideon et al., 2020). However, these findings agree with the murburn proposal fully. Further, the photosynthetic system can do well even without Cyt. f (Fernández-Velasco et al., 2001), which is compatible with murburn model but which fails the Z-scheme. All reactions of the purported the chloroplast ETC (Z-scheme) are not fastidious (Barber, 1995; Arnon/Arnon et al., 1980-1990) and one can put in and draw electrons out with so many other molecules/ions/radicals of synthetic and biological origin.
Affinity-driven specific binding (like those observed in specific amino-acyl tRNAs binding to codons or avidin binding to biotin) is lacking in most of the purported ETC reaction steps. Therefore, the perception of an elaborate serial sequence of electron transport chain (such as the Z-scheme) is merely a consensus of its believers, and not mandated by observations in reality. However, it is a fact that DROS-driven ATP synthesis in diverse physiological systems (chloroplasts, mitochondria, and erythrocytes) and in vitro is observed/demonstrable (Ellsworth et al., 2006; Mailer, 1990; Manoj et al., 2019b, 2020a; Tyszkiewicz & Roux, 1987). This fact (a simple chemical reaction!) forms the basis of the murburn model of Pl-Pp. It is known that photosystems can generate superoxide and hydroxyl radicals by various mechanisms (Pospisil, 2016) and the chlorophylls/carotenoids of the various photo-active complexes have high oxidative potentials (Ishikita & Knapp, 2005) and this could lead to a concertive effect, leading to delocalized water-splitting. As DROS are also formed in the oxidation of NADPH (thereby explaining the functions of complexes like NDH), the murburn model of Pl-Pp is amply supported with theoretical and experimental grounding (Manoj & Manekkathodi, 2021). Since murburn scheme is based in simple thermodynamic principles, the evolutionary transition from anaerobic (example: sulfate or nitrate systems) to aerobic systems (Leslie, 2009) would have been facile; and this proposal is supported by our comparisons of NDH and MBH. Through in silico explorations, exhaustive analyses with novel insights and supported by findings of other groups, we have ratified most of the predictions made in the first part of this essay. Further, we present a compilation of bulleted arguments in the textbox discounting the Z-scheme and supporting the murburn model of Pl-Pp:

**Arguments supporting murburn model of Pl-Pp**

1. The involvement of DROS is a more parsimonious explanation for the generation of oxygen after 2-3

![Figure 16. A simplified breakdown of the processes in murburn model of Pl-Pp and the roles of proteins therein.](Image)
flashes. 2. In situ and in vitro, provision of a DROS like superoxide (with ADP + Pi) can give ATP formation.
2. O₂ is highly active, has variable redox potentials for different reactions, and O₂/DROS are ubiquitous.
3. Chloroplasts produce copious amounts of DRS, each protein complex (PS I/II, Cyt. b₆f, FNR) produce DRS.
4. Increase in the production of O₂ and NADPH generates more DROS in chloroplasts.
5. DROS is directly correlated to TMP and ATP synthesis, and ATP-synthesis is not noted without DROS.
6. Provision of ADP + Pi increases O₂ consumption by PS I, explicable with direct coupling of murnburn model.
7. Several solvent/O₂/DRS accessible one-electron redox/photo-active centers present in proteins/pigments.
8. Delocalization of DRS explains Emerson’s observation (O₂ production with 680 & 700 nm light).
9. Complexes PS I/II, Cyt. b₆f, NDH have ADP-binding sites, as determined in in silico exploration.
10. Chloroplasts/thylakoids have low proton availability, and flux across the membrane falls in millisecond ranges.
11. DRS-mediated reactions are bimolecular, fast and spontaneous (kinetically/thermodynamically viable).
12. Distribution of proteins agrees with murnburn model (PS II/PS I ~ 2, low amounts of PC, etc.).
13. Arnon’s works show that PS II is capable of generating NADPH; many such non-Z-scheme shunts exist.
14. Photodynamic therapy produces DRS and ATP in mitochondria.
15. Emerson enhancement effect is explained by the parallel working of components and electron reservoir concept in murnburn model.
16. The independent/fruitful working of components explains the overall molecular evolution of the system.
17. DRS is effectively known to transfer electrons to various components of the chloroplast machinery.
18. Electron transfers to and fro various components of the system are not affinity-driven, but promiscuous.
19. DRS provide a fundamental chemical connectivity to communicate across various systems of the cell.
20. Simple photocatalytic systems (lacking MnComplex + PS II) can also produce oxygen from water.
21. The similarities of various redox system components and mechanisms across the cell supports murnburn model.
22. DRS could also harness the ‘favorable’ aspects of chaos; and this is a valid and demonstrated premise!
23. DRS-involvement provides operational viability to cyanobacterial photo-respiratory system.
24. Murnburn model justifies the absorption spectrum via a vis action spectrum.
25. Minimal structure-function attributes are met at all scales; and makes Ockham’s razor cut.
26. Explains the structure-function correlation of NDH (has only Fe-S centers) as a muzyme (ATP-synthase).
27. Explains the shorter life span of leaves with higher photosynthetic rates due to the involvement of DRS.
28. Explains that the system is not miraculously efficient (as it was earlier deemed to be!).
29. Explains phase homeostasis in steady state without “intelligent” control, with mere colligative properties.
30. Explains the roles of various LHCs & CBPs (without invoking sophisticated “quantum intelligence”).
31. Explains the random distribution & non-covalently tagged photo-active pigments within the structures.
32. Explains the dosage, kinetics and panning nature of cyanide toxicity (in both chloroplasts/mitochondria).
33. Explains the inhibitory effects of diverse molecules with DROS-mechanism based inactivations/diversions.
34. Explains Mildred Cohn’s works in 1950s and Galina Mironova’s work in 1970s regarding phosphorylation.
35. Explains interfacial proton/DRS modulators’ (uncoupling small molecules) affects.
36. Explains simple chloroplast architecture with random-ized (not arranged) distribution of components.
37. Explains structural features of proteins (non-route redox centers, unfavorable redox potentials, e.g. PS II).
38. Explains the evolutionary relevance of proton deficiency in NAD(P)H.
39. Explains the stacking of thylakoids in chloroplasts as a ploy to minimize the effect of free water.
40. Explains the predominance of longer PQs over shorter ones; explains PC presence in stroma.
41. Explains oligomycin’s/venturicidin’s affect/efficacy in Complex V mediated outcomes.
42. Explains thermogenesis and outcomes mediated by uncoupling (or acyl-carrier) protein.
43. Explains variable/non-integral and anomalous kinetics or isotope effects.
44. Explains the stimulatory effects of bicarbonate on oxygenesis.
45. Explains maverick/horneric dose-response profiles of several additives in chloroplast system.

4. Conclusion
The binding of diverse natural quinones/quinols was studied with respect to the purported binding sites of PS II and and cytochrome b₆f. It was found that the classical scheme is not tenable with respect to the quinines serving as two-electron transporters from PS II to Cytochrome b₆f and it is unlikely that Q-cycle is a feasible mechanistic option. Binding studies with several derivatives of quinones/quinols re-enforce the deduction above. Further, di-substituted phenolics and diverse herbicides like triazines, carbamates, pyrimidines, phenylureas, bipyridiniums, etc. showed in silico binding profiles (with D1 protein of PSI, PSI, as well as cytochrome b₆f’s Qp/Qn sites) and their comparison with earlier reported experimental EC₅₀ (and Kᵣ/Kᵣ) values suggested that the outcomes were due to DROS mediated processes, and not affinity-binding based rationales. Furthermore, elucidation of ADP-binding sites on various photosynthetic proteins suggests strong support for the murnburn model of phosphorylation, quite akin to the earlier demonstrated mechanism in MoXPhos. This was corroborated with structural comparison
of the lesser known function of NDH with MBH/MBS protein complexes, wherein the common structural features (of FeS centers on pendulous extra-membranous apoprotein modules) lead to similar functional outcomes. Herein, we showed that since the fundamental redox metabolic logic of mXM, mOXPhos and PI-Pp have many similarities and since the first two systems (chemical reactions occurring in cytoplasm, mitochondria and periplasm) were better explained with murburn concept, we propose that the same purview can be applied in the chloroplastid processes also. The experimental outcomes observed in several other physiological systems (uncoupling by diverse phenolics, photodynamic observations of DROS/ATP generation, etc) and other structural aspects of photosynthesis machinery (occurrence of super/mega complexes, stacking of membrane vesicles) etc. also are supportive of the DROS-mediated murburn model of photosynthesis. We have thus validated several predictions made in the first part of our essay and we now invite the photosynthesis research community to further explore our proposals.

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