Are plastocyanin and ferredoxin specific electron carriers or generic redox capacitors? Classical and murburn perspectives on two chloroplast proteins

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ABSTRACT: Within the context of light reaction of photosynthesis, the structure-function correlations of the chloroplast proteins of plastocyanin and ferredoxins (Fd) are analyzed via two perspectives: 1) The Z-scheme, which considers PC/Fd as specific affinity binding-based electron-relay agents, thereby deterministically linking the functions of Cytochrome *b₆f* (Cyt. *b₆f*) and Photosystem I (PS I) to NADP⁺ reduction by Fd:NADPH oxidoreductase (FNR) via protein-protein contacts and 2) The murburn explanation for oxygenic photophosphorylation, which deems PC/Fd as generic ‘redox capacitors’, temporally accepting and releasing one-electron equivalents in reaction milieu. Amino acid residues located on the surface loci of key patches of PC/Fd vary in electrostatic/contour (topography) signatures. Crystal structures of four different complexes each of cyt.*f*-PC and Fd-FNR show little conservation in the contact-surfaces, thereby discrediting ‘affinity binding-based electron transfers (ET)’ as an evolutionary logic. Further, thermodynamic and kinetic data on wildtype and mutant proteins interactions do not align well with model 1. Furthermore, micromolar physiological concentrations of PC (when *K_d* values 100 μ M) and the non-conductive architecture of chloroplasts render the classical model untenable. In the 2nd model, PC is optional and higher concentrations of PC (sought by model 1) could inhibit ET, quite like the role of cytochrome *c* of mitochondria and cytochrome *b₅* of cytoplasmic microsomes. Also, PC is found in both lumen and stroma, and plants lacking PC survive and grow. Thus, evidence from structure, interactive dynamics with redox partners and physiological implications of PC/Fd supports the murburn perspective that these proteins serve as generic redox-capacitors in chloroplasts.

Keywords: *plastocyanin; ferredoxin; Z scheme, murburn concept; oxygenic photosynthesis; light reaction; Q cycle; electron transport chain; chloroplast;*

INTRODUCTION

Both ferredoxin (Fd) and plastocyanin (PC) are soluble, monomeric, globular, redox-active proteins involved in the light reaction of photosynthesis (1, 2). PC is a ~10 KDa blue coloured Cu-protein of 97-104 amino acids, with linear dimensions of ~3 to 4 nm. It shows an antiparallel fl-barrel structure containing 8 beta sheets and one Cu atom (3-5) (Figure 1 and Figure S1, Supplementary Information). Since crystal structures of PC from at least 15 organisms (cyanobacteria, algae, pteridophytes, angiosperms, etc.) have been solved using XRD and NMR, PC is one of the most well-studied plant/photosynthetic proteins. Plant type ferredoxins (Fds) are and several crystal structures of diverse Fds from myriad phototroph sources is available. Fds are [2Fe2S] cluster iron-sulphur proteins (~11 KDa, 94-108 amino acids) located on the stromal side of the thylakoid membrane (Figure 2) and they mediate electron transfers between reduced PS I to oxidized FNR (Fd-NADP⁺ reductase). Several Fds' crystal structures are revealed and they also interact with other redox proteins (such as thioredoxins and ferredoxin:thioredoxin reductase, FTR; apart from PS I and FNR) (6).

Figure 1: Surface view of spinach plastocyanin (1AG6). *Left:* H87 and Cu are highlighted. PC looks like a pseudo-cylinder with the Cu atom towards the north side. The aminoacids of the hydrophobic patch (L12, A33, G34, F35, P86 & A90) of the north side are coloured red. Y83 is coloured magenta and the D (42, 44 & 61 - green) and E residues (bottom, 43, 45, 60 & 61 - blue) of the eastern/acidic patch are shown. *Right top panel:* A schematic of the structure shown in the left is presented for spatial comprehension. *Right bottom panel:* In the north patch, H37, C84, H87 and M92 form a 'quasi-tetrahedral' or 'distorted trigonal bipyramidal' coordination sphere with the central Cu atom. The conserved β -barrel structure is shown in Supplementary Information (SI), Figure S1.

Figure 2: Structure of spinach Fd (1A70). *Left:* The [2Fe₂S] cluster and aminoacid residues which are deemed to be necessary for binding to FNR are highlighted. Fd is globular, with 4 conserved Cys residues that hold the FeS cluster in place towards one end of the protein. When binding to FNR and other redox proteins, this side of the cluster is generally known to interact. Key residues on the surface such as D26, E29, E30, D34, D65 and D66 were found to be crucial for interaction with FNR. D residues are shaded green and E residues are coloured blue. *Right:* The coordination sphere of the [2Fe₂S] cluster is shown, with iron atoms (brown spheres) and sulphur (yellow spheres) of the cluster bound by the four Cys residues of varying positions in Fds.

PROBLEM STATEMENT AND METHODOLOGY

In spite of PC/Fd being very well-studied proteins, significant confusions and discordance exist regarding their functionality (7-12). We believe that this is so because the perceptions on PC/Fd were “concretized” before the structures and functions of other components of the photosynthetic system were known and the information available at later timeframes were either overlooked or interpreted to fit the acclaimed perspective. Therefore, the current study investigates the structure-function correlations of PC/Fd within the light reaction of photosynthesis (also called oxygenic photolysis-photophosphorylation or PI-Pp) from a skeptic’s perspective, prioritizing several newly revealed information and concepts.

There are essentially two mechanistic proposals regarding biological electron transfers (7), as shown in Figure 3. The classical explanation (depicted in left panel of Figure 3) requires a high affinity binding (lasting millisecond timescales) between the original donor and final acceptor proteins, and the electron transfer occurs as a result of tunneling (Marcus’ outer sphere mechanism) between the redox centers, with the electron following a definite route from the donor to the acceptor. This view would necessitate: i) high mobility and concentrations of both donor and acceptor, & ii) for mutual affinity-based identification and deterministic electron relay with the donor-acceptor complex, both the ligand sphere of redox-metal centers (Cu & 2Fe) and the surface residues of PC/Fd that interact with their

respective redox partners must be evolutionarily conserved. On the other hand, murburn theory (13-21) (shown in the right panel of Figure 3) does not refute the classical concept, but is a larger set of events that encompasses the classical theory. It sees PC/Fd as generic redox capacitors that could accept and give electrons from/to a wide bevy of species, as governed by stochastic interactions and thermodynamic equilibriums. This purview does not mandate a 1:1 long-term complex between the primary e⁻-donor and final e⁻-acceptor, as the protein-complex formation is considered a low-probability outcome in physiology. Several faster one-electron transfers could occur in milieu and the outcomes that lead to effective one-electron stabilization or two-electron sinks are ultimately favored in this scheme. In short: affinity binding-based topographical recognition guides the deterministic classical ET, whereas mobility/proximity/stability, redox potentials and several other factors dictate outcomes in the stochastic murburn ET. To better demarcate the roles of PC/Fd within chloroplasts, we undertake a comprehensive analysis of the data available on these proteins to study: i) evolutionary conservations and changes in structure using modern alignment and visualization tools, ii) use fundamental thermodynamics/kinetics arguments to correlate reported in vitro data to known physiological outcomes of the system, and iii) assess the overall role of PC/Fd with respect to new data and other aspects of protein structure and chloroplast architecture.

Figure 3: The classical and murburn theories of biological electron transfers. The classical ET theory mandates a high affinity binding of suitably juxtaposed proteins forming a complex, stabilized for prolonged lifetimes (running into milliseconds), followed by a deterministic and directional electron transfer via a thermodynamic push (from the donor to the acceptor, owing to a potential gradient). The definition of classical constants are: k_{on} is the second order rate constant (Units = $M^{-1}s^{-1}$) for the formation of initial Donor-Acceptor complex; K_d is the dissociation constant of this initially formed Donor-Acceptor complex (Units = M); k_{ET} is generally a pseudo-first order approximation of the number of transfers in a given time within the bi- or ter-molecular complex (Units = s^{-1}); k_2 is the overall second order rate constant (Units = $M^{-1}s^{-1}$) derived and K_{eq} is the overall equilibrium constant (Units = dimensionless) that governs the reaction, also determining the overall energetic yield (Units = kJ/mol) given by the equation, $\Delta G^\circ = -RT \cdot \ln(K_{eq})$ [which could also approximate $\sim nF \cdot \Delta(E_m^\circ)$]. Murburn ET does not refute the classical theory but the overall outcome is seen as a result of several stochastic interactions, and not just from the binding of the primary donor with the final acceptor proteins.

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Figure 4: Analysis of residue conservation in 13 different PCs using JalView 2.11.1.0. The red boxes represent the general areas where the aminoacids of the northern side of PC are found. It is important to note that the entire range (borders) of these residues is presented. (The reader may check specifically for conservation of L12, A33, G34, F35, P86, G89 and A90). The blue boxes denote the regions of the eastern (acidic) patch, in which, D42, E43, D44, D53 and either D/E in positions 59-61 have been considered to be important for PC's interaction with its redox partners.

Figure 5: Sequence comparison of various Fds using JalView. Red boxes show residues which were reported to be necessary for interaction of Fd with PS I and the blue box contains residues which interact with FNR.

Table 1: Pair-wise alignment of diverse PCs, with spinach (1AG6) as reference: EMBOSS-Needle for global alignment and EMBOSS-Water for local alignment; Rice et al., 2000) and the % identity, similarity and gap scores are shown.

No.	Source of PC	Global alignment (%)			Local alignment (%)		
		identity	similarity	gaps	identity	similarity	gaps
1	1BXU (<i>Synechococcus</i>)	39.6	58.4	11.9	40.8	60.2	10.2
2	1KDI (<i>Adiantum capillus-veneris</i>)	35.2	53.3	8.6	35.9	54.4	8.7
3	2BZC (<i>Dryopteris crassirhizoma</i>)	36.2	54.3	8.6	36.9	55.3	8.7
4	2Q5B (<i>Phormidium</i>)	38.1	59	5.7	40.4	62.6	3.0
5	7PCY (<i>Enteromorpha</i>)	58.0	68.0	3.0	60.4	70.8	2.1
6	1IUZ (<i>Ulva</i>)	58.0	67.0	3.0	60.4	69.8	2.1
7	2CJ3 (<i>Anabaena</i>)	43.4	54.7	7.5	46.0	58.0	5.0
8	1TU2 (<i>Nostoc</i>)	43.4	54.7	7.5	46.0	58.0	5.0
9	9PCY (French bean)	82.8	88.9	0.0	82.8	88.9	0.0
10	2PCY (Poplar)	78.8	90.9	0.0	78.8	90.9	0.0
11	1PLA (parsley)	70.7	79.8	2.0	71.4	80.6	2.0
12	3B3I (<i>Prochlorothrix</i>)	70.7	79.8	2.0	42.9	62.2	4.1

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Our findings on PC can be compared to Guss & Freeman et al.'s results (24). They found that 52 residues were conserved and 11 were substituted conservatively among a total of 99 amino acid residues and observed an identity score of 62% between algal (*Chlamydomonas*) and plant (poplar; *Populus nigra*) PCs. When comparing the evolution of PCs (listed herein) with a higher plant PC (spinach, 1AG6), we can see lower identity scores for fern protein than for algal or cyanobacterial proteins. Interestingly, PC of *Prochlorothrix*, a photosynthetic prokaryote (2B3I), had better scores than fern, some algal and cyanobacterial varieties. In toto, the identity scores were lower than expected, showing that PC protein sequence is not highly conserved across species. A similar profile was observed when a phylogenetic tree was drawn (Figure S2, SI). In toto, 14 residues were conserved globally (as found with ClustalW alignment; Figure S3, SI). Among them (G6/8, P16/18, G24/26, H37/38/39, N38/41, G78/80, Y80/82, Y83/85, C84/86, P86/88, H87/88/89, A90/92, M92/94, V98/100), the residue numbers are different in various PCs (with gaps in alignment) and only H87/89, P86/88 and A90/92 are part of the hydrophobic patch. Another interesting observation is that while the core region (shape and fold) is almost identical in the different PCs which were

superimposed using Chimera MatchMaker (25), some regions (eg. eastern patch loop) were dissimilar.

Acidic residues on Fd confer a negative charge to the protein, and this is considered to be responsible for interaction with positively charged aminoacid residues of Fd's redox partners (6). The variations in all surface aminoacids of the diverse Fds, the residue position as well as the number of times it is present on the surface is presented in Table S2, SI. It can be seen that both the positions as well as number of times the residues have been recorded on the surface were found to vary. Therefore, even though Fds have relatively high identity% and similarity% scores, the 'evolutionary conservation' logic here is not applicable. For example, while 11 acidic residues (D/E) are spread all over the protein for spinach and *Synechocystis*, the numbers of D/E residues and their relative locations differ significantly with the other Fds (totally 8-12 D residues and 6-13 E residues, Table S3, SI). Such findings can be seen for other aminoacid residues also. Therefore, recognitions based on specific surface aminoacid residue(s) would not be expected to work efficiently, as indicated by the sequence analysis of Fds. From Table 2 (1-10 cyanobacteria, 11-12 algae, and 13-17 plants), it can be seen that Fds from the same organism (*T. elongatus*) were remarkably different (1 & 2, 5AUI and 6IRI), thereby downplaying binding-based recognitions mechanisms.

Table 2: Local and global alignment of diverse Fds from photoautotrophs (with respect to 1A70 as the reference Fd)

No.	Source of Fd	Global Alignment (%)			Local Alignment (%)		
		Identity	Similarity	Gap	Identity	Similarity	Gap
1	5AUI (<i>Thermosynechococcus elongatus</i>)	62.2	77.6	2.0	63.5	79.2	1.0
2	6IRI (<i>Thermosynechococcus elongatus</i>)	45.4	59.3	10.2	51.6	67.4	2.1
3	6L7O (<i>Thermosynechococcus elongatus</i>)	61.6	76.8	3.0	63.5	79.2	1.0
4	3B2G (<i>Leptolyngbya boryana</i>)	62.6	77.8	3.0	64.6	80.2	2.1
5	1ROE (<i>Synechococcus elongatus</i>)	62.2	77.6	2.0	63.5	79.2	1.0
6	1OFF (<i>Synechocystis sp.6803</i>)	66.3	79.6	2.0	68.4	82.1	0.0
7	1QT9 (<i>Nostoc sp. PCC 7119</i>)	62.6	78.8	3.0	63.9	80.4	2.1
8	4FXC (<i>Arthrospira platensis</i>)	61.6	75.8	3.0	62.9	77.3	2.1
9	1RFK	66.7	77.8	3.0	68.0	79.4	2.1

	(<i>Mastigocladus laminosus</i>)						
10	3P63 (<i>Mastigocladus laminosus</i>)	71.1	82.5	1.0	72.6	84.2	0.0
11	1AWD (<i>Chlorella fusca</i>)	63.9	79.4	3.1	66.7	82.8	0.0
12	3AV8 (<i>Aphanothece sacrum</i>)	64.3	76.5	2.0	65.6	78.1	1.0
13	1FRR (<i>Equisetum arvense</i>)	57.7	73.2	2.1	59.6	75.5	0.0
14	3B2F (<i>Zea Mays</i>)	73.5	82.7	1.0	75.0	84.4	0.0
15	5H57 (<i>Zea mays</i>)	61.2	75.5	2.0	62.5	77.1	1.0
16	4ZHO (<i>Arabidopsis thaliana</i>)	66.7	78.1	7.6	73.7	86.3	0.0
17	4ZHP (<i>Solanum tuberosum</i>)	67.0	76.4	8.5	73.2	83.5	0.0

Cu-coordination sphere in PCs: Two histidine nitrogen atoms and a cysteine sulphur atom are conserved, whereas the fourth ligand could be a sulphur atom from methionine or nitrogen from glutamine. The exact locations of the histidines, cysteine and methionine/glutamine are found to vary slightly within the amino acid sequence, and their immediate neighbours also change among the proteins (see Table 3). This suggests that while there is some conservation of the coordination environment, the ambience near the Cu atom varies across the proteins. The Cu-N bond lengths were 2.10 and 2.04 Å, the Cu-S(Cys) bond was 2.52 Å and the Cu-S(Met) bond was slightly longer (~2.9 Å), as measured from the crystal structure of the Poplar PC (2PCY) (24). These bond lengths, when measured for all the proteins' coordination spheres, were slightly different. For example, in *Phormidium* PC (2Q5B), the Cu-N bond lengths were 2.05 and 2.08 Å and the Cu(Cys) and Cu(Met) bonds were 2.24 and 2.60 Å, respectively. Alterations in these bond lengths can result from differences in the relative positions of the residues involved in copper ligation. The copper site was shown to be more flexible and the H87-M92 loop is considered to regulate electron transfer between Cyt.*f* and PC (26). The Cu coordination sphere supposedly undergoes modifications to facilitate electron transfer when PC binds to Cyt.*f*. Counter-intuitively, such a change was minimal when PC bound to PS I. The same authors showed that both reduced and oxidized PC showed no drastic changes in the Cu coordination sphere (27). In short, our analysis shows that the residue numbers, local ambience and the bond lengths of the Cu ligands were found to vary; and this scenario is not expected for a conserved routing of electrons to and from the central copper atom.

Fe-coordination sphere in Fds: Four Cys residues are involved in tethering the [2Fe2S] cluster to the Fds and the variations in the exact locations are given in Table S3, SI. The average S-Fe bonds within the cluster and Fe-S bonds between the cluster and coordination cysteine sulphur atoms lie anywhere between 2.19-2.37 Å. In the diverse Fds, alterations in the bond lengths occur minimally when the redox state of the cluster changes from [2Fe2S]³⁺ to [2Fe2S]²⁺.

Key surface residues in various patches of PC (including Tyr): Cyt. *f* and PS I are deemed to interact via hydrophobic residues-sponsored protein-protein contact (28). The north side of the protein supposedly has conserved residues L12, A33, G34 F35, P86, G89 and A90. An eastern (acidic) patch formed by a type-I β-turn (residues 59-61) containing D and E residues (29) are believed to create a negative charge around Y83 residue, which is also thought to be crucial for electron transfer (30). Numerous studies have emphasized that these residues are crucial for PC binding to its redox partners (Cyt. *f* and PS I) through hydrophobic and electrostatic interactions which facilitates subsequent electron transfer (31-37).

Table 3 presents the amino acids from the hydrophobic patch of various PCs, which one would expect to play important roles for binding and electron transfer reactions. We present the data in two other perspectives of non-aligned amino acids sequence (Figure 4) and topographical (space-filling) view (Figure 6). PCs' north side view (Figure 6) shows that the entire north side is not conserved and the topologies of this north side are not identical. In our analysis of the eastern patch, the Asp residue at this position is not always present and the amino acid residues of this turn are not necessarily conserved. Except for a few instances, where the residue stretch of DEDE (42-45) was present, the other eastern patch residues varied among the PCs. If the eastern patch is critical for PC interaction with its redox partners, then the lack of the negatively charged residues of the important β-turn (59-61- either E or D) in 2B3I, 2Q5B and some other PCs seriously questions the idea that these residues form the electron transfer route in concert with Y83. Another fact is that the 'critical' Y83 is not present in all of the PCs compared herein (magenta highlights), and at times, there are more than one Y residue (see Figures 4 & 6 for more details). Therefore, there is hardly any conservation logic evident for the topographical (surface) signature of the protein.

Table 3: Coordination sphere and surface residues of PCs from various phototrophs: Surface amino acids were identified with NetSurf P-2.0 and physically verified individually using UCSF Chimera 1.14 by colouring each residue and marking its location. The total number of surface residues is around 75% of the total protein length (data shown in Table S1, SI for 5 different PCs).

<i>PDB Organism</i>	<i>Cu coordination sphere and Y83</i>	<i>North side hydrophobic patch residues</i>
1AG6 <i>Spinacia oleracea</i> (Spinach, plant)	H37,H87,C84,M92 Y83 present	D8,D9,G10,S11,L12,A13,N32,A33,G34,F35, P36,L62,L63,N64,A65,P66,G67,S85, P86,H87,Q88,G89,A90,G91 (24 residues)
1BXU <i>Synechococcus elongatus PCC</i> 7942 (Cyanobacterium)	H37,H87,C84,M92 may also be involved Y82,83 present	G6,A7,D8,N9,G10,M11,L12,A13,N32,K33, L34,A35,P36,L62,A63,F64,S65,P66,E85, P86,H87,G89,A90,G91 (24 residues)
1KDI <i>Adiantum capillus-veneris</i> (Fern, plant)	H37,H90,C87,M95 Y86 present	G6,D7,E8,V9,G10,N11,K13,F12,V32,G33, E34,T35,G36,D61,E62,N63,D64,L65,L66, S67,E68,D69,T88,P89,H90,K91,S92,A93 (28 residues)
2BZC <i>Dryopteris crassirhizoma</i> (Mutant PC from Fern, plant)	H37,H90,C87,M95 Y86 present	E8,V9,G10,N11,F12,E34,T35,P36,E62,N63, L65,S67,E68,D69,T88,P89,H90,K91,S92,A93 (20 residues)
2Q5B <i>Phormidium laminosum</i> (Algae)	H37,H90,C87,M95 Y87 present	A9,D10,S11,G12,N34,K35,L36,P37,P38,F66, S67,P68,P91,H92,A95,G96 (16 residues)
7PCY <i>Enteromorpha prolifera</i> (Algae)	H37,H87,C84,M92 Y83 present	G7,D8,D9,G10,S11,L12,A33,G34,F35,P36,N 38,Y62,L63,N64,S65,K66,D85,P86,H87,S88, G89,A90,G91 (23 residues)
1IUZ <i>Ulva pertusa</i> (Algae)	H37,H87,C84,M92 Y83 present	D8,D9,G10,S11,L12,A33,G34,F35,P36,Y62, N64,S65,K66,P86,A88,G89,A90,G91 (18 residues)
2CJ3 <i>Anabaena variabilis</i> (Cyanobacterium)	H39,H92,C89,M97 Y88 present	D10,K11,G12,L13,L14,K35,V36,P37,P38, L63,L64,M66,S67,P68,Q70,P91,H92, G94,A95 (19 residues)
1TU2 <i>Nostoc sp. PCC 7119</i> (Cyanobacterium)	H39,H92,C89, M97 Y88 present, but far away from heme f in the complex	D10,K11,G12,L13,L14,K35,V36,P37,P38, Q63,M66,S67,G69,E90,P91,H92,R93, G94,A95 (19 residues)
9PCY <i>Phaseolus vulgaris</i> (French bean, plant)	H37,H87,C84,M92 Y83 present	D9,G10,S11,L12,A33,G34,F35,P36,N38,E59, E60,E61,L62,N64,A65,P66,S85,P86,H87, Q88,A90,G91 (22 residues)
2PCY <i>Populus nigra</i> (Poplar, tree)	H37, H87, C84,M92 Y83 present	D8,D9,G10,S11,L12,N32,A33,G34,F35, P36,S85,P86,H87,Q88,G89,A90 (16 residues)
1PLA <i>Petroselinum crispum</i> (Parsley, plant)	H37, H85, C82, M90 Y81 present	G6,S7,D8,D9,G10,L12,V13,N31,N32,A33, G34,F35,P36,Y60,L61,N62,G63,A64,G65, E66,E83,H85,A86,A88,G89 (25 residues)
2B3I <i>Prochlorothrix hollandica</i> (photosynthetic prokaryote)	H39, H85, C82, M90 Y81 present	D10,K11,Y12,A13,P14,M33,N34,K35, V36,G37,P38,L60,A61,I62,A63,P64,T83,P84, H85,R86,G87,A88,G89,M90 (24 residues)

Figure 6: Top-down view of the north side of PC: In this view, one of the His residues of the Cu coordination sphere which is located on the top (while another one is almost always a little buried) has been positioned in the centre (cyan colour). P is coloured magenta, F-silver, L-forest green, I-light green, V-hot pink, G-black, Y-red/light pink, R-red (if present), A-orange, K-brown, S-cream/light yellow, M-yellow, D-dark blue, E-grape (purple blue) and N-purple.

Key surface residues of Fds: From Figure 5 and Table S2 (SI), it can be noted that except for E30, D65 and D66, the other residues are not conserved. These E/D residues are not present on the surface of Fd at the same positions or surfaces either. Chloroplast Fds have been known to interact with diverse proteins like– hydrogenase (HydA1/2), bilin reductases, FTR, glutamate synthase, sulphite reductase, nitrate reductase, nitrite reductase, PS I and FNR (38). This probably makes Fd one of the most promiscuous redox proteins and such a protein with diverse partners surely must have different surface aminoacid residues/binding sites. Unlike the hydrophobic patch of PC, electrostatic interactions have been considered as the driving force in orchestrating protein-protein interactions between Fd and other proteins (39). It is very rare for any protein to have/evolve such affinities for nearly 10 different proteins which are structurally and functionally diverse. Moreover, taking into consideration the fact that the key acidic aminoacid residues are not conserved across species, it is difficult to envisage high affinity driven protein-protein complexes between Fd and its partners.

2. Analyzing electron donor-acceptor (protein-protein) complexes' crystal structures:

Cyt. *f*-PC complexes: When the surface residues (northern and eastern patch, in particular) were analyzed for their interactions with Cyt. *f* in crystal structures of the four reported PC-Cyt. *f* complexes- 1TU2 (40), 2PCF (41), 2JXM (42) and 1TKW (43), not all of the purported PC residues which are deemed mandatory for interaction were found to be involved in the actual complex formation with Cyt. *f* (Table 4). Supplementary Figure S4 shows that PC binds with different orientations to Cyt. *f*. Sometimes, while only the north side is bound (2JXM and 1TU2), the north side as well as eastern patch were found to interact with Cyt. *f* in 1TKW. In only 1TKW and 2PCF, D/E residues of the eastern patch of PC featured in the interaction. The distance between Fe (Cyt. *f*) and Cu (PC) was closer than the distance between Fe (the electron donor) and Y83 (the electron relay). If the electron transfer occurs via relay through critical aminoacids (F and Y), what is the purpose of large cavities and the solvent accessibility of the heme residue? Moreover, the distance between Cu (in PC) and iron in heme *f* is much lesser in these complexes (11-17 Å) than the alleged round-about and long route involving the F/Y residues in Cyt. *f* and Y83 of PC surrounded by an acidic patch. That is, why would electrons flow through a prolonged path, when the interatomic distances between the two key metal ions is much shorter? In Cyt.*f* as well, barring Y1 -which was found in all 4 complexes, the other interacting residues were not the same in all the complexes). Only in 1TU2 and 2JXM, four residues that were involved in direct contact in the crystal structures were conserved in complex formation. In 1TU2, 1TKW and 2PCF, large channels in Cyt. *f* expose the heme residue, which is highly accessible to the solvent environment. Fedorov et al. concluded from their molecular dynamics studies that the crucial loop of G188, E189 and D190 in Cyt. *f* was involved in stabilization of the complex (44). While some presume that low salt concentrations facilitate protein-protein associations of these proteins, Ueda et al. showed that the acidic patch residues of PC were not involved in salt bridge formation with either PS I or Cyt. *b₆f* in the electron transfer complexes at lower ionic strengths (45). An important point to consider is that crystallographic complexes are derived by using very high concentrations of proteins, which could give non-specific binding (particularly, with hydrophobic patches), and such interactions could have little physiological significance. Given such a background and the non-positive results, we infer that there is no 'conserved aminoacid stretch' or motif which is responsible for the PC binding to its redox partners.

288 **Table 4. Interacting residues in Cyt. *f* and plastocyanin complexes**

PDB ID Organism	Surface aminoacid residues involved in contact between the two proteins		Residues of the hydrophobic patch
	Plastocyanin	Cytochrome <i>f</i>	
1TU2 <i>Nostoc sp. PCC 7119</i>	K35,V36,P37,P38,Q63, L64,M66,P91,H92 (9/105 total AAs)	Y1,F3,W4,Q6,Q7,V68,N71, D100,V101,Y102,Q104, G117,P118,P120,D189 (15/254 total AAs)	Totally different from that of PC in 2PCF (even if there are hydrophobic contacts)
2PCF <i>Spinacia oleracea</i> (PC), <i>Brassica rapa</i> (Cyt. <i>f</i>)	PC Cu atom to heme <i>f</i> Fe atom: 11.03 Å PC H92 to heme <i>f</i> Fe atom: 13.3 Å PC Y88 to heme <i>f</i> Fe atom: 17.70 Å		L12,F35,A90 (only these 3 of PC interact with Cyt. <i>f</i>)
	L12,F35,P36,S58,L62, N64,S85,H87,Q88,A90 (10/99 total AAs)	Y1,I3,F4,Q59,L61,N63, K65,G67,A68,N70,S101, Q103,G116,P117,V118, P119,N167,D186,K187, R209 (20/250 total AAs)	
	PC Cu atom to heme <i>f</i> Fe atom: 16.75 Å PC H87 to heme <i>f</i> Fe atom: 6.67 Å PC Y83 to heme <i>f</i> Fe atom: 15.33 Å		
2JXM <i>Prochlorothrix hollandica</i>	Y12,K35,V36,G37,P38, T58,L60,I62,P64,H85, R86, G87,A88 (12/97 total AAs)	Y1,F3,Y4,Q6,Y7,Q61,D63, S67,N70,Y102,L103,T105, L116,G118,P119, L120, P121,Y162,P163,T164 (20/249 total AAs)	Totally different from that of PC in 2PCF (even if there are hydrophobic contacts)
1TKW <i>Populus nigra</i> , <i>Brassica rapa</i>	PC Cu atom to heme <i>f</i> Fe atom: 12.69 Å PC H87 to heme <i>f</i> Fe atom: 8.57 Å PC Y81 to heme <i>f</i> Fe atom: 17.5 Å		Only L12 and A90 interact with Cyt. <i>f</i>
	D1,G10,S11,L12,D42, D44,E59, Y83,P86,H87,Q88,A90 (12/99 total AAs)	Y1,I3,F4,Q7,N8,L61,A62,N63, K65,P117,R156,Y160,P161,D16 2,K185,E186,K187,G188,R209 (19/249 total AAs)	
	PC Cu atom to heme <i>f</i> Fe atom: 13.74 Å PC H87 to heme <i>f</i> Fe atom: 8.58 Å PC Y81 to heme <i>f</i> Fe atom: 14.73 Å		

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 290 **PS I-Fd complex:** To our knowledge, there is only one crystal structure of a complex
 291 between PS I and Fd (5ZF0), and this is not available for analysis. However, literature reports
 292 are available for PS I and Fd interactions. The D subunit of PS I appears to be necessary for
 293 binding and reduction of Fd by PS I (46, 47). The complex is deemed to be stabilized by
 294 interaction between E93 of Fd and K106 of PS I-D subunit (48). Interestingly, the E subunit
 295 of PS I is known to bind FNR directly (49) and ternary complexes of PS I, FNR and Fd have
 296 also been reported (50). In the Z-scheme, PS I is known to reduce Fd, a mobile electron
 297 carrier, which then binds to FNR, which in turn reduces NADP⁺ to form NADPH. If this is

the path of electron transfer, why would FNR need to bind directly to PS I? Also, what is the need for two different isoforms of Fds (51) and also FNR (12)? Flavodoxins (Flds) are known to be alternative electron carriers in cyanobacteria and algae (but not in higher plants), which perform the function of Fds in iron-limiting conditions (52). Flds are also known to exchange electrons with Fds and also partner redox reactions with both FNR and PS I (53). The structure of Fds and Flds are quite different and it is improbable that both these proteins would have similar binding affinities to the redox partners PS I and FNR. When the two proteins (2V5V-Fld and 1QT9-Fd) from *Nostoc sp.* PCC 7119 were aligned using ClustalW, only 19 amino acid residues were conserved between them. Analysis using EMBOSS-Needle (global alignment) gave identity and similarity scores of 12.7 and 19.8%, respectively. How could Flds (an FMN containing redox protein of ~170 amino acids) with remarkably different topology and surface electrostatics substitute for Fds? Such outcomes can effectively be addressed by non-specific interactions espoused by the murburn paradigm.

PC-PS I complex: To the best of our knowledge, there is no crystal structure of PC-PS I complexes reported till date. However, both northern and eastern patch residues of PC have been reckoned to be involved in PC-PS I interactions. PsaB D612 and D613 of PS I were proposed to be necessary for docking of PC, which also is through hydrophobic contact (37). When we explored the crystal structures of PS I, we found that the residues W622, D619 and E611 (*Synechocystis* PS I; PDB ID: 5OY0) were far apart from each other and it is not clear which of these residues are deemed to interact with PC. Also, the exact role of PsaF is not clear, because the distance from PsaF to the P700 center is roughly >40 Å in the crystal structures 5OY0 (& 4XK8 of *Pisum sativum*) and ~35 Å in 6JO6 (*C. reinhardtii* PS I). In the 6JO6 structure, the Y626, D623 and E612 residues are spaced apart from the P700 center (by 11, 16 and 24 Å, respectively) and the PsaF chain is slightly elevated above the contour of PsaB. While the Y, D and E residues, although jutting out of the PsaB protein- are still below the PsaF region, which would mean that if PC were to bind to this region spanning over ~35-40 Å from PsaF to the P700 center, it would be bound to a slightly elevated PsaF on one side and much lower Y residue on the other side. Besides, in those regions, there are many other Y, D and E residues which could also interact. We wonder why these specific residues have been reckoned to act as electron transfer conduits. PS I-N subunit, which is present only in the luminal side of PS I in higher plants alone, was shown to be important for PS I-PC interactions. However, even though the ET rates were reduced, the authors admit that photoautotrophic growth remained unaffected (54). These reports show that the interaction

modality of PC and PS I is obscure and there is little consensus among the researchers on the topic. PS I has more than a 2 nm extra-membrane structural extension into the lumen side (55). This would mean that the mean distance of copper atom to the nearest redox center in PS I would be at least 2.5-3 nm.

Fd-FNR complexes: In the crystal structure of Fd-FNR (56), the redox centers of FNR and Fd are bound to each other in close proximity (in a 1:1ratio) and binding of these two proteins allegedly brings the two redox cofactors (FAD in FNR and FeS cluster of Fd) to roughly 6.0 Å close to each other. Several residues of these two proteins are supposed to form the intermolecular interface of the complex, which is formed by five key residues in Fd- Y37, C39, A41, C44 and Y63 plus four key residues of FNR- V92, L94, V151 and V313. We analyzed the interacting/contact residues of three different Fd-FNR complexes (see Table 5) and found no evidence for a conservation of binding logic within these three protein-protein complexes. It can be seen that the number of interacting residues, their nature and their loci varied. Once again, we also point out that such complexes are derived and studied at very high concentrations, which could give non-specific binding outcomes. Further, it is intriguing to see that though Y83 is neither located near the FeS cluster nor in the path of electron transfer between the two cofactors, it is known to affect ET rates by an unknown mechanism (56). Such observations could be explained by the non-specific relay scheme offered by murburn perspective. In Anabaena Fd, a highly conserved F65 is known to interact with several aminoacids of FNR (L76, L78 and V36); also, R40 and E29 of Fd were found to interact with E154 of FNR (57).

Table 5: Residues involved in contact between Fd and FNR

No.	Source of Fd	Fd	FNR
1	1GAQ	E29, E30, D34, L35, Y37, S38, C39, R40, A41, G42, C44, S45, S46, Q61, S62, Y63, L64, D65, D66, H78, E92, E94 and A98 (23 residues)	T29, N30, K33, P34, K35, K85, N86, K88, V91, V92, L94, K153, E154, E278, F297, D298, K301, K304, R305, V313 (20 residues)
2	3W5U	E29, Y37, S38, C39, R40, A41, G42, C44, S45, S62, Y63, D65, Q68, L95 (14 residues)	N30, K33, K35, L88, K91, L94, K153, E154, K275, E278, K301, K304, R305, V313 (14 residues)
3	3W5V	S55, D57, S59, C70, T82, S83 (6 residues)	C19, D160, N162, D265, N266 (5 residues); S-S linkage between C79 of Fd and C19 of FNR
4	5H5J	Y24, E30, V34, E35, L36, P37, Y38, S39, C40, R41, A42, G43, A44, S60, D61, G62, S63, P64, D67, Y81 (20 residues)	E23, L26, N27, K30, P31, K32, K84, N90, V91, R92, L93, K156, I157, K310, V314, E315, V316 (17 residues)

3. Thermodynamic and kinetic data for binding and electron transfers of wildtype/mutant PCs/Fds with their redox partners:

PC as electron acceptor/donor: The overall experimental equilibrium constant of Cyt. *f* to PC electron transfer reaction is only 2 (58), and this is because the redox potential gradient is very low. The redox potential gradient from cytochrome *c* to PC is greater (250 mV to 380 mV; when compared to the practically non-existent gradient between cytochrome *f* and PC), which dictates that thermodynamically, the free energy change ($\Delta G^\circ = n.F.\Delta E^\circ_m$) would be higher for the former reaction. Yet, the overall calculated reaction rate of k_2 [as defined by $(1/k_2) = (1/k_{on}) + (K_d/k_{ET})$] is higher for the Cyt. *f* – PC reaction. Now, the relevant question: Is it the higher intrinsic ET rate within the Cyt. *f* – PC complex and/or the lower K_d of the Cyt. *f*-PC interaction that determines the favourable Cyt. *f* – PC reaction kinetics? To answer this question, we must understand the protocol adopted for determining the binding constants and ET rates. While the binding assay is done at several tens of micromolar proteins, the ET reaction stopped flow measurements are done at about two orders lower concentrations. At higher concentrations, the binding agent may also sponsor redox changes, which gets registered into the heme charge transfer Soret band absorption range, where heme *f* is monitored. It can be noted that though oxidized PC has negligible absorbance at 410 nm (or at 422 nm, wherein binding and reaction rate are measured) (59), reduced copper's spectral signature is significantly high at 410 nm (60). Since Cyt. *f* and PC have comparative redox potentials, reduction of PC could also lead to a 410 nm signature. We have pointed out such procedural influences in similar issues faced in other heme enzymes and P450 redox research (14, 15, 61, 62). (However, the following discussion is made over-sighting such issues.)

The most important point to remember is that the physiological concentration of PC in chloroplasts is around $\sim 1 \mu\text{M}$ (63), and at such low concentrations, the major population of PC would be unbound. Therefore, the overall outcomes of kinetics at lower concentrations of the proteins cannot be explained by the 'erroneous' binding constants (which are anyway insignificant with respect to the reaction assay concentrations). It is important to point out that even the reaction assay employed is unrealistic: 10X of oxidized PC is taken with respect to reduced Cyt. *f* (59). The idea that intrinsic ET is preferred over certain routes is disclaimed by the overall conformity of rates in mutants within the Cyt. *c*₆ and PC transfers. Even in Cyt. *f* – PC transfers, the value changes are only an order lesser or higher than the wildtype. That

is- if the positive control with wildtype shows $10^7 \text{ M}^{-1}\text{s}^{-1}$ and the test reaction with mutant shows $10^6 \text{ M}^{-1}\text{s}^{-1}$, the mutant still gives ~10% rate of the wildtype reaction. In kinetics and equilibria, this value is NOT mechanistically insignificant. If the value of mutant reaction is several orders lower, then the mechanism would be deemed to be more attributed to the ‘crucially required factor’ (say, via an obligatory tyrosine). In other words, substituting a tyrosine with another amino acid residue should not give a functional ET between the Cyt. *f* and mutant PC, whereas we do observe significant ET rates even in this system. In the absence of oxidized plastocyanin, the negative control of reduced Cyt. *f* taken alone is relatively stable and the absorbance change in the cytochrome Soret is not seen to occur significantly within the reaction times. That is- the mutant still could give 10^5 to 10^6 times the rates of the negative control and this is mechanistically significant. However, the fact that addition of PC alters absorbance profiles of Cyt. *f* has been taken as an indication of ONLY direct interactions of PC and Cyt. *f*. We have corrected such assumptions with our works in heme enzymology and explained the kinetic outcomes for such proteins with murburn concept (13-15, 64, 65).

Another flaw is that some workers have reported overall k_2 ET rates as high as $2 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ (33), which approach diffusion controlled rates. This is because collision rates of 10^8 - $10^9 \text{ M}^{-1}\text{s}^{-1}$ have been assumed (66). Such high values for collision and protein-protein interaction-mediated electron transfer must be questioned, because we are dealing with proteins such as PS I and Cyt. *b₆f* which are stationary and much larger than the 10 KDa PC. When PC (Cu center) reduction was monitored, lower rates of $10^2 \text{ M}^{-1} \text{ s}^{-1}$ were obtained, while inter-protein ET rates (between horse heart Cyt. *c* and PC) were found to be higher, at 10^4 - $10^5 \text{ M}^{-1} \text{ s}^{-1}$ (67). Moreover, binding in biology would involve collisions and dependence on the ionic strength of the medium for ‘optimal’ interactions and electron relays. Kovalenko et al. simulated the interactions of PC and ferredoxin with PS I and concluded that there was a “non-monotonic dependence of the complex formation at low ionic strength” which was due to “long-range electrostatic interactions” (39). In their analysis, the complex formation depended on ionic strength in order to be successful. In a similar redox metabolic system occurring in cytoplasmic microsomes, we had that protein-protein complexation between cytochrome P450 (CYP) and cytochrome P450 reductase (CPR) is a low probability event (68, 13, 14, 19) and explained outcomes with the application of murburn concept. We had shown that increasing the molarity of phosphate buffer increased the reduction rate of Cyt. *c* (13). Also, even when CYP and CPR were physically separated using a membrane (in other words, no

donor-acceptor binding occurred), CYP was still able to oxidize its substrate. Further, copper ions are one-electron redox cyclers which can modulate the DROS dynamics in the reaction milieu and DROS serve as effective redox relay triggers/agents (13). In toto, the derivable inference is that electron reception from any donor by wildtype and mutant PC proteins are not majorly affected by changing residues that are touted to be pivotal for binding or ET. This inference is also endorsed further by the structural analyses of the protein partners and their known complexes. The larger picture appears to be that a multitude of parameters govern the overall outcome, as endorsed by the more inclusive murburn model (Figure 3).

With respect to PC-PS I interaction, three residues of PS I (PsaB) that were deemed to be important were a critical tryptophan residue (W627 and W651 in *C. reinhardtii*), one E and one D residue (69). Moreover, PsaF, a much smaller protein, was also deemed crucial for PC binding to PS I and for higher ET rates (70). The same group showed that a K23Q mutation of PsaF affected ET from PC/Cyt. c_6 to PS I and also crosslinking of these proteins with PS I (Table 6) (71). A positively charged PC from *Anabaena* sp. PCC 7119 was investigated using site-directed mutagenesis and the authors revealed that replacement of R88 (which is adjacent to His87 of the Cu ligation site) is required for efficient reduction of PS I and mutation of this arginyl residue (and R64, an equivalent in Cyt. c_6 which is close to heme group). More importantly, they reported that mutation of the critical Y83 with either A or F residues did not alter the kinetics of PS I reduction (72). Despite replacement of the purported key residues using site-directed mutagenesis, electron transfer is not altogether abrogated by mutation of these critical residues allegedly involved in protein-protein contact and the redox potentials are also not drastically altered. R88 is found only in the 1BXU sequence in our analysis and the other R (R64) residue is not present in any of the PCs which we have analyzed.

In the spinach PC system, changing one to three key residues from G8, L12, F35, D42, E43, D44, E59, E60, Y83 (thereby bring about a charge difference of +1 to +4) gave mutant proteins that showed varied pI (3.82 of the wildtype compared and 3.64 to 6.2 of the mutants) and midpoint redox potential ($E^{\circ}m = \sim 380$ mV for the wildtype, ± 25 mV for the mutants, in the pH range of 6.0 to 9.0). However, the mutants gave comparable functionality for electron transfers with PS I with electron transfer kinetic constants like k_{ET} ($\sim 7 \times 10^4$ s⁻¹ for the wildtype, whereas ~ 3 to 6×10^4 s⁻¹ for the various mutants) (29, 32, 36, 73). The redox potential of PS I was found to be 475 – 500 mV (32), K_d of PC's and Cyt. c_6 's interaction with PS I was determined to be in the range of 10^{-4} M (32, 37) and the overall electron

transfer rate to PS I was $\sim 10^7 \text{ M}^{-1}\text{s}^{-1}$ for both PC and Cyt. c_6 (37, 70). Since Cyt. c_6 ($\sim 3 \text{ nm}$ in linear dimensions, 9 KDa of <90 residues, $E_m^\circ = 365 \text{ mV}$) is a structurally distinct and divergent protein from PC (74, 75), the binding-based mechanism fails to explain the parallels observed here. We also must recall that R88 (or R64) is not conserved, and therefore, is not essential for electron transfer from PC to PS I (72).

Table 6: Values of kinetics and thermodynamic constants for PC's interaction with other proteins in the role of an electron-acceptor.

Reaction	k_{on} ($\text{M}^{-1}\text{s}^{-1}$)	K_d (M)	k_{ET} (s^{-1})	k_2 (calc.) ($\text{M}^{-1}\text{s}^{-1}$)
PC – redox partners				
<i>Cyt. f</i> - <i>Wt. PC</i>	4.5×10^7	10^{-4}	6×10^4	4.2×10^7
<i>Cyt. f</i> - <i>Mut. PC</i> [#]	$10^6 - 10^8$	$10^{-4} - 10^{-3}$	$4 \times 10^3 - 6 \times 10^4$	$10^6 - 10^8$
<i>Cyt. c</i> - <i>Wt. PC</i>	2.3×10^7	10^{-3}	3×10^3	3.5×10^6
<i>Cyt. c</i> - <i>Mut. PC</i> [¶]	2.2×10^7	10^{-3}	3×10^3	$2.1-3.5 \times 10^6$
Fd – redox partners				
<i>Wt. PS I-Fd</i>	na	$\sim 10^{-5}$	na	2.3×10^8
<i>Mut. PS I-Fd</i> ^ψ	na	$\sim 10^{-4}$	na	4.5×10^7
<i>Mut. PS I-Fd</i> ^{&}	na	$\sim 10^{-4}$	na	6.3×10^6
<i>Mut. PS I-Fd</i> [†]	na	$\sim 10^{-4}$	na	2.8×10^6

[#] - Y83F, Y83L (34); L12N, L12E, L12A, D42N and F35Y (59)

[¶] - L12N, L12D, L12E, Y83F, D42N and F35Y (59)

^{ψ, &, †} - K35T, K35D and K35E, respectively (76)

Fd as electron acceptor/donor: K35 of the PsaI subunit of PS I is involved in interaction with Fd, as reported by Fischer et al. (76). Mutation of this residue to K, D, or E was found to deleteriously affect electron transfer rates between PS I and Fd. The details of rate constants and ET rates between WT PS I as well as mutant PS I with Fd are provided in Table 6. Mutation of non-canonical residues in both proteins was found to lower the ET rates between Fd and FNR; Examples are– the highly conserved S47, F65 and E94 residues (in Fd) and K75, L76 and E301 (in FNR). Also, other residues such as D67 in Fd; E139, L78, K72, and R16 in FNR caused minor alterations in kinetic parameters (57). The critical arguments of protocols adopted and using appropriate references (positive/negative controls) we presented earlier for interpreting such data (in case of PC) are relevant here too. An array of Fd and FNR mutants had been characterized kinetically (using transient and steady state spectroscopy) by Hurley et al. (57, 77) and again, the electron transfer reaction between Fd and FNR was not lost due to the mutations.

4. Architectural aspects of chloroplasts:

In the Z-scheme, there are three legs of downhill electron transfers, viz. water to RC^+ , Pheophytin to PC and Phylloquinone to NADP^+ . PC is supposed to ferry electrons from the

photo-activated PS II sponsored second downhill phase to the photo-activated PS I sponsored third downhill phase. The problem is that PS II are primarily found at packing densities in the appressed grana region. That is, PS II complexes are located deep within the interior thylakoids of the grana whereas Cyt. *b₆f* and PS II are located in the peripheral thylakoids and stromal lamellae (78, 79). It is an enigma how PQ would undertake a journey from the appressed thylakoid membranes to the peripheral regions to serve the Q Cycle at Cyt. *b₆f*, to reduce PC. Further, it is also an enigma as to why PC must deterministically undertake journeys from such a Cyt. *b₆f* molecule to a remote PS I, when there are no specific binding or other thermodynamic logic to demand such a motion. Further, PC is distributed across the lumen of thylakoids and stroma, more or less evenly (63). If we accept the Z-scheme as the binding logic, then it is a sheer waste of resources to have the limiting reaction (Q-cycle) component in the stromal phase. If we understand PC/Fd as redox buffers for discrete ‘thermodynamic windows’, there are no such incongruities and the chloroplast architecture and relative distributions fit into the overall picture well.

5. Overall physiology:

Although the ratio of PS I:PC is 1:2-3, such stoichiometries are not a prerequisite for efficient photosynthesis because even a decrease of 20-40% PC levels did not significantly affect the ET rates (9). Angiosperms express two isoforms of PC as a recent evolutionary adaptation (80). Cytochrome *c₆* is a functional analog of PC, which is deemed to exchange electrons with PC and also transfer electrons from Cyt.*b₆f* to PS I (75). Weigel et al. mutated 2 PETE genes in *Arabidopsis thaliana* (it has 2 PCs) and found that increasing the dose of the gene encoding for Cyt.*c₆* did not substitute for PC and then affirmed that due to lack of phototrophic growth in the PC knockout, PC is indispensable for photosynthetic electron flow (81). Some have shown that PC is not the only electron transfer protein in plants and that Cyt.*c₆* can act as its substitute (82). Also, these two proteins were found to differ strongly in their global electrostatic charge and interestingly, are acidic proteins in eukaryotes and either basic or neutral in cyanobacteria, requiring different purification strategies (83). Very crucially, as the thylakoid lumen is purportedly kept at a positive polarity with respect to the stroma, there is little drive for Fd to carry away electrons from PS I for a deterministic journey to FNR.

The murburn explanation is ratified by experiments which show that PC is not absolutely necessary for plant growth under optimal conditions. Zhang et al. showed that neither Cyt.*c₅₅₃*

nor PC was absolutely necessary for optimal photoautotrophic plant growth and dark respiration in *Synechocystis* sp. PCC 6803 (8). Pesaresi et al. (9) mutated both isoforms of PC in *Arabidopsis thaliana* which were encoded by genes PETE1 and PETE2 and opined that PC as well as its ‘analog’ (Cyt.*c*₆) may have other possible physiological roles, such as thylakoid redox reactions. They also found no evidence for physical interaction between PC and Cyt.*c*₆ using interactions in yeast. Complementation of the PCs by overexpression of the knocked out genes yielded essentially the same photosynthetic performance as the WT plants. With these astonishing studies, we can ascertain that PC is not indispensable for photophosphorylation and plant growth and may act as a generic redox capacitor, rather than a dedicated, binding-based and specific electron relay agent which links Cyt.*b*₆*f* and PS I in oxygenic photosynthesis. Copper is a transition metal which participates in the dynamics of Diffusible Reactive Oxygen Species (DROS, like superoxide and peroxide) and the protein environment of PC governs the redox potential of PC. DROS are indispensable for life and are routinely produced; while most of the biologists deem that these species are undesirable and toxic waste products, we have shown for more two decades that these entities occupy a central role in the chemistry of heme and flavoprotein-mediated reactions (84, 85, 13, 17). DROS are routinely produced during metabolism in aerobic respiration. Plants utilize superoxide dismutase to scavenge superoxide to form H₂O₂. Takahashi et al. reported the superoxide-mediated reduction of plastocyanin with a rate constant of $\sim 1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (86). Also, they showed that reduced PC reacted with H₂O₂ through the peroxidase-like reaction of Cu/Zn-SOD. Fenton chemistry of copper in biological systems is well-established (87). Given the simple structure of PC, there is little way it could dictate specific redox transfers alone and hence, its electron transfer ability is dictated by copper's redox state switch between only cupric (Cu²⁺) and cuprous (Cu⁺).

CONCLUSIONS

From an evolutionary perspective, it is clear that the surface residues of PCs/Fds are not conserved. Crystal structures of binary complexes do not provide any conserved binding logic either. Thermodynamics and kinetics data of WT vs. mutant these redox proteins have shown that electron transfer still occurs and there are no drastic changes in the redox potential of the mutants. Even knocking out the PC genes did not lead to changes in photoautotrophic plant growth. PCs/Fds are one of the most promiscuous redox proteins known, quite like the flavo-reductases and cytochrome *b*₅ interactions with cytochrome P450s of liver microsomal system and such functionalities are better explicated with murburn concept (13-15).

Therefore, upon examination of the data pertaining to the chloroplast reaction system and in the light of our group's findings over the last two decades, we conclude that PC/Fd are not specific electron transfer agents, but they acts as generic redox capacitors.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests to disclose.

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SUPPLEMENTARY INFORMATION

Are plastocyanin and ferredoxin specific electron carriers or generic redox capacitors? Classical and murburn perspectives on two chloroplast proteins

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Figure S1. The fl-barrel structure of PC (1AG6) and its key residues: The hydrophobic residues of the north side are coloured red, while residues of the eastern acidic patch are coloured blue (D residues) and green (E residues), respectively. The Y83 residue is coloured magenta and the Cu (brown sphere) atom's coordination sphere comprising of two H (cyan), 1C and 1M residues (yellow) is also shown.

Figure S2A. Simple phylogenetic tree of the 13 different PCs analyzed in this work: 1AG6 is similar to other higher plant PCs (9PCY and 2PCY), while the more primitive ones are far away.

Figure S2B. Phylogenetic tree of the various Fd sequences analyzed in this work: 1A70 (Spinach Fd) is compared to other Fds from various sources (cyanobacteria, algae and higher plants). From this image, it is evident that higher plant Fds (spinach-1A70, maize-1GAQ, Arabidopsis-4ZHO and potato-4ZHP) are different from other clades such as maize (5H57) to *Leptolyngbya boryana* (3B2G); (lower part) and other sequences from *Azotobacter* (1F5C) to *T.elongatus* (6L7O).

2B3I_1	Chain	ASVQIKMGTDKYAPLYEPKALSSISAGDTEFVMNKVGPNNVIFDKVP----	AGE---	SAP	53
1BXU_1	Chain	QTVAIKMGADNGMLAFEPSTIEIQAGDTVQWNNKLAPHNVVVEGQ-----	----	P	47
2Q5B_1	Chains	ETFTVKMGADSGLLQFEPANVTVHPGDTVQWNNKLPPHNILFDDKQVPGASKE---	----	LAD	57
2CJ3_1	Chains	ETTYTVKLGSDDGSLAFVPPNITVAGGESIEFINNAGFPHNVVFDAAALNPAKSAD---	----	LAK	57
1TU2_1	Chain	ETTYTVKLGSDDGSLAFVPAKLTIKPGDTEFLNNKVPPHNVVFDAALNPAKSAD---	----	LAK	57
1KDI_1	Chain	--AKVEVGDEVGNFKFYDSTITVSAGEAVEFTLVGETGHNIVFDIPAGAPGTVASELKAA			58
2BZC_1	Chain	--AKVEVGDEVGNFKFYDSTITVSAGEAVEFTLVGETPHNIVFDIPAGAPGTVASELKAA			58
7PCY_1	Chain	--AAIVKLGGDDGSLAFVPPNITVAGGESIEFINNAGFPHNVVFDAAALNPAKSAD---	----	DAI	56
1IUZ_1	Chain	--AQIVKLGGDDGSLAFVPSKISVAAGEAIEFVNNAAGFPHNVVFDAAALNPAKSAD---	----	DAI	56
1PLA_1	Chain	--AEVKLGSDGGLVFSFSSFTVAAGEKITFKNNAGFPHNVVFDAAALNPAKSAD---	----	EKI	55
1AG6_1	Chain	--VEVLLGGDDGSLAFVPSKISVAAGEAIEFVNNAAGFPHNVVFDAAALNPAKSAD---	----	AKI	55
9PCY_1	Chain	--LEVLLGGDDGSLVFPSEFVSPSGEKIVFKNNAGFPHNVVFDAAALNPAKSAD---	----	VKI	55
2PCY_1	Chain	--IDVLLGADDGSLAFVPSKISVAAGEAIEFVNNAAGFPHNVVFDAAALNPAKSAD---	----	SKI	55
		: : *	: * . : * : : :	**:::	

2B3I_1	Chain	ALSNTKLAIPAGSFYSVTLGT---	PGTYSFYCTPHRGAGMVGITITVE-	97
1BXU_1	Chain	ELSHKDLAFSPGETFEATFSE---	PGTYTYCYCEPHRGAGMVGKIVVQ-	91
2Q5B_1	Chains	KLSSHQLMFSPGESYEITFSSDFPAGTYTYCCAPHRGAGMVGKITVEG		105
2CJ3_1	Chains	SLSHKQLLMSPGQSTSTTFPADAPAGEYTFYCEPHRGAGMVGKITVAG		105
1TU2_1	Chain	SLSHKQLLMSPGQSTSTTFPADAPAGEYTFYCEPHRGAGMVGKITVAG		105
1KDI_1	Chain	SMDENDLLSEDEPSFKAKVST---	PGTYTFYCTPHKSANMKGTLTVK-	102
2BZC_1	Chain	SMDENDLLSEDEPSFKAKVST---	PGTYTFYCTPHKSANMKGTLTVK-	102
7PCY_1	Chain	SA--EDYLNSKGGTVVRKLTT---	PGTYGVYCDPHSGAGMKMTITVQ-	98
1IUZ_1	Chain	SY--DDYLNSKGGTVVRKLST---	PGVYGVYCEPHAGAGMKMTITVQ-	98
1PLA_1	Chain	SQP--EYLNAGETYEVTLT---	KGTYSFYCEPHAGAGMKGEVTVN-	97
1AG6_1	Chain	SMSEEDLLNAPGETYKVTLT---	KGTYSFYCEPHAGAGMKGEVTVN-	99
9PCY_1	Chain	SMPEEEELNAPGETYVVTLD---	KGTYSFYCEPHAGAGMKGEVTVN-	99
2PCY_1	Chain	SMSEEDLLNAPGETYKVTLT---	KGTYSFYCEPHAGAGMKGEVTVN-	99
		.	* * * * *	: *

Figure S3. ClustalW multiple sequence alignment of the PCs: The residues in asterisk are conserved after alignment (not so without alignment).

Figure S4. Surface view of PC-Cyt.f complexes: Analysis of the north side residues which purportedly interact with the hydrophobic region on Cyt.f (of Cyt.b6f) reveals that the residues are not conserved. If the pair of Cyt.f and PC (for each species, if crystal structures are available), would show what is the extent/affinity of binding. If PC of one species is put into the chloroplast of another plant, the strength of interaction could change, based on the results obtained in this analysis.

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861

862 **Figure S5. Orientations of Fd-FNR interactions in various complexes:** The FeS cluster in Fd is coloured
 863 based on atom (Fe-brown and S-yellow). The FAD in FNR is coloured based on atom (C-grey, N-blue, O-red
 864 and P-orange). In 1GAQ, the contact between the two proteins appears to be tighter (that what is observed in
 865 5H5J). The cleft of FNR (a major groove with a cavity) serves as the docking site for FAD and the FeS side of
 866 the Fd appears to dock to FNR. To obtain these structures, several μM concentrations of the two proteins were
 867 used in order to co-crystallize them. In 3W5U, a structure similar to 5H5J is seen. The distance between the two
 868 redox factors (FAD in FNR and FeS cluster of Fd is around 6-7 Å)
 869

870 **Table S1. Analysis of all surface residues of 5 different PCs:** Five PCs were analyzed for their surface
 871 residues and the residue numbers and location (north/eastern patch/other part of the protein) is indicated. Hp
 872 patch – hydrophobic patch.

873

PDB ID Organism (No. Of residues on surface/total no. Of residues)	Cu coordi- nation sphere	Residue name (No. Of times present)	Residue nos.	North end Near Cu atom	South end	Hp.patch L12,A33, G34,F35, D61,A84,P 86, G89, A90	Eastern patch D42,E43 ,D44,E4 5,D51,E 60	Other surf regions
1AG6 <i>Spinacia oleracea</i> (75/99)	H37, H87, C84, M92	A(7)	A13,22,33, 52,53,65,90	A13,33,65,90	-	A33	-	A22,52, 53
		N(3)	N32,64,99	N32,64	N99	-	-	-
		D(6)	D8,9,18,42,44,51	D8,9	-	D61	D42,44, 51	D18
		Q(1)	Q88	Q88	-	-	-	-
		E(6)	E2,26,43,45,60,76	E60,68	E76		E43,45,6 0	E2,26
		G(10)	G6,7,10,17,24,34,4 9,78,89,94	G6,7,10,34,89 ,91	G24,49,78	G34,89	-	G17,94
		H(2)	H37,87	H37,87	-	-	-	-
		I(2)	I46,55	-	I46,55	-	-	-
		L(5)	L4,12,15,62,74	L12,62	L74	L12		L4,15
		K(6)	K1,30,54,71,77,81, 95	-	K77	-	-	K30,54, 71,81,95
		M(1)	M57	-	-	-	-	M57
		F(4)	F14,19,35,41	F35	-	F35	-	F14,19,4 1
		P(5)	P16,36,47,66,86	P36,66,86	P47	-	-	P16
		S(7)	S11,20,23,48,56,58, 85	S11	S23,48			S20,56,5 8,85,
		Y(2)	70, 83 (1 other Y80 is only slightly solvent accessible)	-	-	-	-	Y70,83
		V(8)	V1,21,28,39,40,50, 72,93	-	V50	-	-	V1,21,2 8,39,40, 50,72,93
1BXU Synechococcus elongatus PCC 7942 (82/91)	H37, H87, C84, M92	A(7)	A2,7,13,23,35,72,9 0	A7,13,35,90	23	-	-	A2,72
		N(3)	N9,31,38	N9,31,38	-	-	-	-
		R(1)	R88	R88	-	-	-	-
		D(3)	D8,25,59	D8,9	-	D59?	D59	-
		Q(5)	Q2,22,28,52,99	-	Q2,22,99	-	-	28,52
		E(8)	E15,20,42,54,68,71, 76,85	E85	E20,76	E???	E???	E15,42,5 4,68,71
		G(9)	G6,10,17,24,34,49, 78,89,94	G6,10, 89	G24,49,78	G34,89	-	G17,94
		H(3)	H37,57,87	H37,87	-	-	-	H37
		I(3)	I3,19,21	-	-	-	-	I3,19,21
		L(4)	L12,34,55,62	L12,34,62	-	L12	-	L55
		K(4)	K4,33,58,95	K33	-	-	-	K4,58,9 5
		M(1)	M11	M11	-	-	-	-
		F(4)	F14,64,70,74	F64	-	-	-	F14,70,7 4
		P(6)	P16,36,53,66,77,86	P36,66,86	P77	-	-	P16,53
		S(4)	S17,56,65,75	S65	S75			S17,56
		Y(3)	Y80,82,83	-	-	-	-	Y80,82, 83
		T(7)	T- 1,18,26,69,73,79,81	-	-	-	-	T- 1,18,26, 69,73,79 ,81
		V(8)	V1,30,39,40,41,93, 97,98	-	-	-	-	V1,30,3 9,40,41, 93,97,98
1IUZ <i>Ulva pertusa</i> (90/98)	H37, H90, C84, M92	A(12)	A0,13,22,23,26,33, 45,48,52,54,88,90	A33,88,90	22,23,48	-	-	A0,13,2 6,45,52, 54
		N(3)	N32,38,64	32,64	-	-	-	38
		R(1)	R72	-	-	-	-	R72
		D(6)	D8,42,44,51,53,59	D8	-			D42,44, 51,53,59
		Q(2)	Q1,99	-	Q99	-	-	Q1
		E(5)	E25,28,43,68,85	-	-			E25,28,4 3,68,85

		G(10)	G7,10,24,34,49,67, 78,81,89,94	G10,34	G49		-	G7,24,6 7,78,81, 89,91
		H(2)	H37,87	H37,87	-	-	-	-
		I(2)	I2,19	-	-	-	-	2,19
		L(2)	L12,55	L12	-	-	-	L55
		K(5)	K4,18,66,73,93	K66	-	-	-	K4,18,7 3,93
		M(1)	M94	-	-	-	-	M94
		F(3)	F14,35,41	F35	-	-	-	F14,41
		P(5)	P16,36,47,77,86	P36,86	P47,77	-	-	P16
		S(6)	S11,17,20,56,65,75	S11,65	S75			S17,20,5 6
		Y(3)	Y57,62,,83	Y62	-	-	-	Y57,83
		T(4)	T69,76,95,97	-	T76	-	-	T69,95,9 7
		V(11)	V15,21,30,40,46,50 ,70,71,79,82,98	-	21,46,50,9 8	-	-	V15,30, 40,70,71 ,79,82
1KDI <i>Adiantum capillus- veneris</i> (Fern, plant) (90/102)	H37, H90, C87, M95	A(10)	A1,23,26,45,47,52, 57,58,75,93	A93	A23,45,47	-	-	A1,26,5 2,57,58
		N(3)	N11,63,94	N11	-	-	-	N63,94
		R(0)	-	-	-	-	-	-
		D(6)	D7,17,42,61,64,69	D69	-			D7,17,4 2,61,64
		Q(0)	-	-	-	-	-	-
		E(5)	E25,28,43,68,85	-	-	-	-	E25,28,4 3,68,85
		G(8)	G10,24,33,36,46,49 ,81,97	G10,33,36	G46,49		-	G24,81, 97
		H(3)	H	H37,87	-	-	-	-
		I(3)	I43,19	-	-	-	-	-
		L(5)	L31,99	L99	-	-	-	L31
		K(7)	K2,13, 74, 76, 91, 96, 102	K13	K102	-	-	K2,13,7 4,76,91, 96
		M(2)	M60,95	-	-	-	-	M60,95
		F(4)	F12,14,41,73	-	-	-	-	F12,14,4 1,73
		P(6)	P16,44,48,71,80,89	P89	P80	-	-	P16,44,4 8,71
		S(8)	S18,22,53,59,67,72, 78,92	S67,92	S22,78			S18,53,5 9,67,72, 92
		Y(3)	15,83,86	-	-	-	-	Y15,83, 86
		T(10)	T20,30,35,50,79,82, 84,88,98,100	T35	T50,79	-	-	T20,30,8 2,84,88, 98,100
		V(7)	V3,9,21,32,40,51,1 01	V9,32,35	V51,101	-	-	V21,40
2BZC <i>Dryopteris crassirhizoma</i> (87/102)	H37, H90, C87, M95	A(8)	A1,23,26,45,52,57, 75,93	A93	A23,52,57	-	-	A1,26,4 5,75
		N(3)	N11,63,94	N11,63	-	-	-	N94
		R(0)	-	-	-	-	-	-
		D(5)	D7,17,42,64,69	D64,69	-			D7,17,4 2
		Q(2)	Q1,99	-	Q99	-	-	Q1
		E(9)	E4,8,25,28,34,54,62 ,68,70	E8,34,62,68,7 0	-			E4,25,28 ,54
		G(8)	G6,10,24,33,46,49, 81,97	G10,33	G24,46,49 ,81		-	G6,97
		H(2)	H37,90	H37,90	-	-	-	-
		I(2)	I19,43	-	-	-	-	I19,43
		L(2)	L31,66	L31,66	-	-	-	-
		K(8)	K2,13,56,74,76,91, 96,102	K91	K102	-	-	K13,56, 74,76,96
		M(1)	M60	-	-	-	-	M60
		F(4)	F12,14,73,41	F12	-	-	-	F14,41,7 3
		P(7)	P16,36,44,48,71,80, 89	P36,89	48,80	-	-	P16,44,7 1
		S(6)	S18,22,53,59,67,72,	S67,92	S78			S18,22,5

			78,92					3,59,72, 92
		Y(3)	Y15,83,86	Y86	-	-	-	Y15,83
		T(10)	T20,30,35,50,79,82, 84,88,98,100	T35,88	T50,79	-	-	T20,30,8 2,84,98, 100
		V(7)	V3,9,21,32,40,51,1 01	V9	V51,101	-		V3,21,3 2,40

Table S2. Surface residues of 11 different Fds from various photoautotrophs: Around 11 different Fds were examined for surface residues. This was done to identify the sequence conservation of Fds.

Aminoacid residue	Source of Fd	No. of times present on the surface	Position
A	1F5C	3	1,43,72
	1OFF	7	2,32,42,44,49,54,72
	1A70	7	1,2,27,41,78,79,97
	1AWD	8	29,25,39,41,46,65,91,92
	4ZHO	8	2,28,29,32,42,79,80,95
	4ZHP	7	2,42,49,54,71,72,98
	5AUI	3	1,42,44
	5AUK	8	1,27,28,31,41,43,53,70
	5H57	7	1,23,28,29,32,42,44
	6IRI	7	29,39,50,52,57,80,108
	3AV8	8	1,28,41,43,48,56,72,80
R	1F5C	1	106
	1OFF	1	41
	1A70	1	40
	1AWD	1	38
	4ZHO	1	41
	4ZHP	1	41
	5AUI	3	9,41,83
	5AUK	1	40
	5H57	1	41
	6IRI	5	4,19,26,49,59
	3AV8	1	40
N	1F5C	3	7,30,80
	1OFF	0	Nil
	1A70	2	13,57
	1AWD	0	Nil
	4ZHO	0	Nil
	4ZHP	0	Nil
	5AUI	1	91
	5AUK	0	Nil
	5H57	0	Nil
	6IRI	7	7,11,16,22,55,103,106
	3AV8	1	14
D	1F5C	10	15,23,37,41,58,63,86,90,93,95
	1OFF	9	12,21,22,35,58,61,67,85,95
	1A70	11	20,21,26,34,59,60,65,66,67,70,84
	1AWD	8	19,24,32,55,58,63,64,82
	4ZHO	10	20,21,22,27,35,58,61,66,67,85
	4ZHP	11	12,21,22,27,35,58,61,66,67,68,85
	5AUI	12	18,22,27,35,58,61,66,67,68,85
	5AUK	12	11,20,21,26,34,57,60,65,66,67,84,94
	5H57	10	18,21,22,27,58,61,66,67,85,95

	6IRI	8	5,30,35,69,66,79,93,102
	3AV8	12	11,13,20,21,26,34,58,61,66,67,68,85
C	1F5C	3	20,39,42
	1OFF	5	19,40,45,78,86
	1A70	4	39,44,47,77
	1AWD	3	37,42,75
	4ZHO	5	19,40,45,48,78
	4ZHP	4	40,45,78,84
	5AUI	2	40,45
	5AUK	5	18,39,44,77,85
	5H57	3	40,45,86
	6IRI	3	48,53,94
	3AV8	4	39,44,78,86
	1F5C	4	52,65,69,102
Q	1OFF	3	59,62,69
	1A70	5	17,48,58,61,68
	1AWD	4	56,59,66,89
	4ZHO	3	59,62,69
	4ZHP	3	28,59,69
	5AUI	5	32,59,62,69,92
	5AUK	3	58,61,68
	5H57	3	59,69,70
	6IRI	2	40,67
	3AV8	5	31,59,62,69,71
	1F5C	13	18,27,46,48,57,59,62,66,73,76,83,92,105
E	1OFF	8	14,18,30,31,71,89,93,94
	1A70	8	15,29,30,31,71,88,93,94
	1AWD	9	11,12,15,18,27,28,50,53,90
	4ZHO	11	8,12,14,16,31,30,68,72,89,93,94
	4ZHP	9	16,18,30,31,70,89,93,94,95
	5AUI	10	14,21,23,30,54,56,71,93,94,95
	5AUK	8	13,17,29,30,70,88,92,93
	5H57	9	11,13,14,16,30,35,53,71,72
	6IRI	6	17,18,31,38,77,101
	3AV8	6	22,29,30,89,93,94
	1F5C	2	96,99
G	1OFF	4	13,33,55,73
	1A70	4	12,32,54,72
	1AWD	5	10,30,52,68,70
	4ZHO	5	13,33,55,71,73
	4ZHP	5	13,33,55,62,73
	5AUI	4	12,33,55,73
	5AUK	4	12,32,54,72
	5H57	8	9,12,33,55,62,68,73,94
	6IRI	4	20,41,63,81
	3AV8	4	12,54,73,84
	1F5C	2	35,105
H	1OFF	1	91
	1A70	1	90
	1AWD	1	88
	4ZHO	1	91
	4ZHP	2	91,34
	5AUI	0	Nil
	5AUK	1	90
	5H57	3	15,89,91
	6IRI	4	6,28,70,99
	3AV8	1	91
	1F5C	2	40,68
I	1F5C	2	40,68

	1OFF	5	9,17,52,70,88
	1A70	2	33,69
	1AWD	1	14
	4ZHO	5	9,34,70,88,96
	4ZHP	3	9,15,88
	5AUI	2	17,70
	5AUK	4	8,16,51,69
	5H57	1	52
	6IRI	5	25,42,60,61,96
	3AV8	2	16,70
L	1F5C	6	44,70,74,88,101,104
	1OFF	5	26,34,36,65,96
	1A70	5	35,51,56,64,95
	1AWD	6	23,31,33,62,86,93
	4ZHO	5	15,26,36,65,98
	4ZHP	4	26,36,65,99
	5AUI	8	7,26,34,36,52,65,89,96
	5AUK	5	25,33,35,64,95
	5H57	5	4,6,51,83,92
	6IRI	7	21,44,73,78,104,105,107
K	3AV8	6	25,33,35,51,65,96
	1F5C	4	10,84,85,98
	1OFF	3	7,51,92
	1A70	5	4,50,52,91,92
	1AWD	4	2,6,48,69
	4ZHO	4	5,7,51,92
	4ZHP	6	5,7,51,63,83,92
	5AUI	4	4,51,72,87
	5AUK	3	6,50,91
	5H57	5	4,6,51,83,92
M	6IRI	6	3,8,23,62,74,76
	3AV8	4	4,8,50,92
	1F5C	1	64
	1OFF	0	Nil
	1A70	0	Nil
	1AWD	1	67
	4ZHO	1	97
	4ZHP	0	Nil
	5AUI	0	Nil
	5AUK	0	Nil
F	5H57	0	Nil
	6IRI	0	Nil
	3AV8	0	Nil
	1F5C	2	2,55
	1OFF	1	64
	1A70	2	16,63
	1AWD	2	61,71
	4ZHO	2	64,74
	4ZHP	3	17,64,74
	5AUI	3	38,64,74
P	5AUK	1	63
	5H57	2	17,64
	6IRI	2	72,82
	3AV8	1	64
	1F5C	0	Nil
	1OFF	3	11,37,82
	1A70	3	10,19,36
	1AWD	4	8,17,34,79

	4ZHO	3	11,37,82
	4ZHP	5	11,14,20,37,82
	5AUI	3	10,20,37
	5AUK	3	10,36,81
	5H57	4	10,20,37,82
	6IRI	4	43,45,75,90
	3AV8	6	10,19,36,55,57,82
S	1F5C	1	56
	1OFF	10	3,15,16,20,39,46,56,60,63,84
	1A70	7	38,43,45,46,55,62,83
	1AWD	8	9,36,43,44,51,57,60,81
	4ZHO	8	44,46,47,54,56,60,63,84
	4ZHP	6	3,39,44,46,47,60
	5AUI	5	13,39,60,63,84
	5AUK	10	2,14,15,19,38,45,55,59,62,83
	5H57	7	39,46,54,56,60,63,84
	6IRI	5	47,68,71,92,97
	3AV8	6	2,38,45,53,60,63
T	1F5C	2	5,82
	1OFF	6	5,10,23,53,83,87
	1A70	7	6,9,11,53,86,89,96
	1AWD	6	4,7,13,20,80,84
	4ZHO	5	3,10,83,87,90
	4ZHP	6	10,53,56,87,90,97
	5AUI	6	2,6,15,16,47,90
	5AUK	8	4,9,22,46,52,82,86,89
	5H57	3	31,47,90
	6IRI	6	2,13,24,64,91,98
	3AV8	5	6,9,17,46,83
W	1F5C	2	78,94
	1OFF	0	Nil
	1A70	0	Nil
	1AWD	0	Nil
	4ZHO	0	Nil
	4ZHP	0	Nil
	5AUI	0	Nil
	5AUK	0	Nil
	5H57	0	Nil
	6IRI	0	Nil
	3AV8	0	Nil
Y	1F5C	2	13,26
	1OFF	4	4,24,38,81
	1A70	4	3,23,37,80
	1AWD	5	1,21,35,78,94
	4ZHO	4	4,24,38,81
	4ZHP	4	4,24,38,81
	5AUI	4	3,24,81,97
	5AUK	6	3,23,37,73,80,96
	5H57	6	3,24,38,74,81,97
	6IRI	4	10,32,46,89
	3AV8	6	3,23,37,74,81,97
V	1F5C	6	17,19,22,60,77,97
	1OFF	3	57,75,79
	1A70	6	8,14,22,74,82,85
	1AWD	4	49,54,76,83
	4ZHO	6	17,23,52,53,57,86
	4ZHP	5	23,57,52,79,86
	5AUI	5	8,19,28,57,79

	5AUK	2	56,78
	5H57	7	2,5,8,34,57,79,87
	6IRI	7	9,12,15,27,54,65,95
	3AV8	5	15,27,52,79,87

Table S3: Organism details and Cys residues involved in FeS cluster coordination in Fd

No.	Fd Source	Fd designation	Cys positions
1	5AUI <i>Thermosynechococcus elongatus</i>	Fd I	40,45,48,78
2	5ZF0 <i>Thermosynechococcus elongatus</i>	Fd PS-I	Cluster absent in the crystal
3	6JO2 <i>Thermosynechococcus elongatus</i>	Fd I	41,46,49,79
4	6IRI <i>Thermosynechococcus elongatus</i>	Minor Fd	48,53,56,86
5	6L7O <i>Thermosynechococcus elongatus</i>	Fd I	Cluster absent in the crystal
6	1ROE <i>Synechococcus elongatus</i>	Fd	40,45,48,78
7	1OFF <i>Synechocystis sp</i>	Fd I	40,45,48,78
8	5AUK <i>Synechocystis sp</i>	Fd I	39,44,47,77
9	1RFK <i>Mastigocladus laminosus</i>	Fd	41,46,49,79
10	3P63 <i>Mastigocladus laminosus</i>	Fd	41,46,49,79
11	1QT9 <i>Nostoc sp</i>	Fd	41,46,49,79
12	3B2G <i>Leptolyngbya boryana</i>	Fd I	41,46,49,79
13	1F5C <i>Azotobacter vinelandii</i>	Fd I	20,39,42,45 (SF ₄) ; 8,49,16 (F ₃ S)
14	4FXC <i>Arthrospira platensis</i>	'plant type'	41,46,49,79
15	1FRR <i>Equisetum arvense</i>	Fd I	38,43,46,76
16	3AV8 <i>Aphanothece sacrum</i>	Fd I	39,44,47,78
17	1AWD <i>Chlorella fusca</i>	'plant type'	37,42,45,75
18	4ZHO <i>Arabidopsis thaliana</i>	Fd II	40,45,48,78
19	1A70 <i>Spinacia oleracea</i>	Fd I	39,44,47,77
20	4ZHP <i>Solanum tuberosum</i>	Fd I	40,45,48,78
21	5H57 <i>Zea mays</i>	Fd III	40,45,48,78
22	3B2F, 1GAQ, 2W5U and 3W5V <i>Zea mays</i>	Fd I	39,44,47,77