Why the Electron Chooses One of Two Symmetry-Related Paths in the Type II Bacterial Photosynthetic Reaction Centers

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Abstract:
Photosynthetic reaction centers carry out a series of electron and proton transfer reactions that convert the energy of light into high energy reduced products and add to the proton gradient. All photosynthetic reaction centers (Photosystems I, II, and different bacterial reaction centers (bRCs)) have their charge separating cofactors arranged with two, c_2-symmetric paths from primary donor to terminal acceptor. In Type II reaction centers (PSII and bRCs), electrons utilize only one active branch. In bRCs the electron transfer occurs in the A branch from the excited state of the bacteriochlorophyll (BChl) dimer P_{860} through $BChl_A$, bacteriopheophytin$_A$ (BPh_A), to Q_A, and then to the B-branch Q_B. B-branch $BChl_B$ and BPh_B do not participate. A similar path is found in PSII. These proteins challenge our understanding of how proteins can turn on or off the activity of bound ligands. The shift of the electrochemical midpoint potential (E_m) of each cofactor by the protein environment is calculated using continuum electrostatics within the MultiConformation Continuum Electrostatic program (MCCE). In bRCs from *Rhodobacter sphaeroides,* the electrostatic potentials favor reduction of the active branch cofactors. The residues that contribute to a more positive potential on the A branch are identified. These are often distant from the cofactors. The large difference of the potential at the competing BChls may direct the electron transfer to the A branch. Residues TyrM210 and PheL181 are at symmetrical positions on the A and B branches, near P_{860}, between $BChl_A$ and $BChl_B$. Mutated bRCs that swap the Tyr and Phe (YF mutant), have been shown to support some electron transfer

to the B-branch cofactors. The calculated E_ms with the YF swap show that more positive B-branch potentials, which help explain why there is now electron transfer along the B branch.

1. Introduction

Photosynthetic organisms capture solar energy and convert it into stable chemical compounds that fuel all life on earth.[1] In the photosynthetic reaction centers, a photon initiates a series of electron transfers from one redox cofactor to another across proteins embedded in cell membranes.[2] The electron transfers between terminal donors and acceptors are often coupled to protonation changes that add to the transmembrane proton gradient.[3]

All photosynthetic reaction centers: Reaction Centers from purple non-sulfur bacterial (bRCs), photosystem II (PSII), photosystem I (PSI)), and or homodimeric reaction centers from green-sulfur Heliobacter (HbRCs) have a core with two symmetric peptide chains and embedded electron transfer cofactors (Fig 1).[4, 5] The two peptide chains – L and M in bRCs, D1 and D2 in PSII, PsaA and PsaB in PSI – are most likely the result of gene duplication.[1] In the Type-II reaction centers (PSII and bRCs), only one branch is used for electron transfer.[6, 7] In contrast in Type I reaction centers (PSI[8] and HbRCs[9]), both chains of cofactors support electron transfer to the terminal acceptor.[10, 11] A longstanding question is how the structure of Type II RCs differentiates between active and inactive branches.[6, 12, 13] Understanding how electron transfer is controlled will show how proteins can tune reactivity to differentiate function between similar structures.

1.1 Electron transfer in bRCs

The sequence of electron transfers in bRCs starts with the bacteriochlorophyll (BChl) homodimer (P_{860}) absorbing a photon, yielding P_{860}*. An electron transfers to the A-branch bacteriopheophytin (BPh_A) within 3-4 ps forming $P^+BPh_A^-$. The intervening monomeric $BChl_A$ participates in combination as a transient intermediate electron acceptor or to lower the barrier for electron tunneling via superexchange.[14] By either mechanism, the electron affinity of $BChl_A$ will affect the rate of BPh_A reduction. BPh_A^- then reduces ubiquinone$_{10}$ (Q_A) in ≈ 200 ps to yield $P^+Q_A^-$. The quantum yield for Q_A reduction is essentially 100%.[15] The B-branch Q_B accepts the electron from Q_A^-, and then binds a proton from the cytoplasm in microseconds.[16-18] Cytochrome c_2 is the electron donor needed to reduce P^+, essential for the next turnover. Following two turnovers, QH_2 is released into the intra-membrane quinol pool adding to the proton gradient.[13, 19]

Fig 1: The *R. sphaeroides* bRC cofactors (PDB ID: 1AIG). The long isoprene tails on each BChl, BPh and ubiquinone (UQ) have been removed for clarity. Yellow: P_{860}, magenta: BChl, green: BPh, and purple: Ubiquinone. Red: TyrM210 and PheL181.

The symmetrical cofactor arrangement was a surprise when revealed by the first x-ray crystal structure of the bRCs from *Blastochloris (B.) viridis*.[4, 12, 20] However, the amino acids residues surrounding the cofactors in the aligned L and M peptides in the *Rhodobacter (R.) sphaeroides* (also known as *Cereibacter*) bRCs, are only 34% identical and 17% similar residues.[21] The differences between the peptide can be a source of directionality.

The L and M peptides contact both A and B branch cofactors. In the helical intra-membrane region, the L peptide (and the D-helix of the M peptide) surrounds $BChl_A$ and BPh_A while the symmetry-related portion of the M peptide (and the L peptide D helix) surrounds $BChl_B$ and BPh_B. The L and M peptides cross over to form the P_{860} binding site on the periplasmic side. On the cytoplasmic side, Q_A, which is close to BPh_A, is surrounded by the M chain and Q_B by the L peptide.

Site directed mutations were proposed to turn off A-branch electron transfer and coax electrons down the alternative B branch.[22-27];Magdaong, 2022 #22882;Hanson, 2025 #22944] Despite the similarity of the L and M peptides and identity of A- and B-branch cofactors, it has proved to be surprisingly difficult to fully activate B-branch electron transfer in either *R. sphaeroides* or related *R. capsulatus*.[6, 7, 26, 28-30]

Many bRC mutants have been made and the electron transfer to B-branch cofactors measured. In one group of mutants the occupant of the BPh_A side was changed by replacing LeuM212 in *R. capsulatus* bRCs (M214 in *R. sphaeroides*) by histidine.[31, 32] This provides an axial ligand, so a BChl (denoted β_A) replaces BPh_A. The result is denoted the ß mutant. BChl has a redox potential 260 mV lower than BPh *in vitro* making it harder to reduce.[33, 34] This E_m shift appears to be retained in the protein. However, in the ß mutant, only ≈ 6% of the electrons go from the excited P_{860} to BPh_B.[24, 29]

It was noted early on that the symmetry of the cofactor environment in the two branches is broken around the BChl, with a Tyr on the A side (M208 in *R. capsulatus* bRCs, M210 in *R. sphaeroides*) and a Phe in the symmetrical position on the B side (L181 in both species) (Fig 1).[24, 35] It was suggested that the different polarity of Tyr vs. Phe could influence the properties of the BChl in the A and B branch to help steer electron transfer along the A branch. Indeed, the double mutant where the Tyr and Phe are swapped (YF swap) in *R. capsulatus* bRCs led to reduction of 15% of BPh_B.[24] A triple mutant, named YFß here, that swapped the Tyr and Phe and includes the ß mutation, yielded ~25% reduction of BPh_B in *R. capsulatus* and ~15% in *R. sphaeroides* with the remainder giving reduction of $ß_A$ plus decay of P_{860}* to the ground state. [24, 29, 35] Thus, the YF swap leads to significant electron transfer to the B branch. This is in line with simulations that suggested that the position of the Tyr dipole could significantly affect the free energy difference between the A- and B-branch electron transfers.[36, 37] Here we will explore the free energy of electron transfer in the YF and YFß mutants more thoroughly.

Here we present the E_ms for reduction of the A- and B-branch BChl and BPh employing two crystal structures of *R. sphaeroides* strain R-26 (1AIG, 2GNU) and carotenoid sphaeroidene containing structure 2J8C. Additionally, YF mutant proteins are made *in silico*: within the MultiConformation Continuum Electrostatic (MCCE) program and using the CHARMM-GUI[38]

followed by relaxation with Molecular Dynamics (MD). All three parent structures show more positive potentials on the A-side BChl and BPh favoring their reduction. We identify the amino acids that contribute significantly to the asymmetry and their conservation is examined. The YF-swap mutations are shown to make $BChl_B$ more positive than $BChl_A$, but the A-side BPh_A is still preferred. However, exchanging BChl for BPh_A in a YFß bRC favors reduction of BPh_B rather than $ß_A$.

2. Methods

2.1 Input structures:

The coordinates from two crystal structures of *R. sphaeroides* R-26 bRCs (1AIG[39] and 2GNU[40]) that are carotenoidless and the structure 2J8C[41] that contains the carotenoid sphaeroidene are used. The structures differ in the position of Q_B. In the proximal position, assumed to be the active site, the quinone makes a hydrogen bond to HisL190.[39, 42] In the distal position, the head group has moved out along the channel normally occupied by its isoprene tail. The 1AIG (2.6 Å resolution) structure has Q_B in the proximal position,[43] 2GNU (2.2Å) finds Q_B between proximal and distal sites[40] and 2J8C (1.87Å) has Q_B in a distal position.[44] Glycerol (GOL), Lauryl Dimethylamine-n-oxide (LDA), Phosphate (PO4), Heptane-1,2,3-Triol (HTO), 1,2-Diacyld-Sn-Glycero-3-Phosphocholine (PC1) and GGD) are removed from the three structures. Cardiolipin (CDL) and sphaeroidene (SPO), found only in 2J8C, were retained. A 31.5 Å slab is added around the hydrophobic region with the IPECE program[45] to model the region of the membrane. All water molecules in the structures are removed and replaced with implicit solvent.

2.2 Multi-Conformational Continuum Electrostatics (MCCE)

The Multi-Conformational Continuum Electrostatics (MCCE) program, brings the cofactor redox states, residue protonation states, and chosen positions to come to equilibrium at a given solution pH and E_h.[46] The protein backbone is fixed. Calculations with multiple input Protein Databank structures or MD snapshots provide a measure of how much the results depend on the initial structure. A microstate is a one choice for unique charge and position for each side chain and ligand.[47] Electrostatic interactions are calculated with continuum electrostatics, while van der Waals and torsion energies use the Amber force field. The microstate energy is described fully in SI Part 1. Monte Carlo (MC) sampling yields a Boltzmann ensemble of microstates.

2.2.1 Conformer choices. In the calculations reported here side chains sample 'isosteric conformers', which leave most heavy atoms fixed but allow polar hydrogen atoms to move. Thus, Asp, Glu, Arg, Lys, His, Cys, Tyr and chain termini have neutral and charged alternatives (conformers); all hydroxyls have conformers in different torsion minima; conformers are made for both neutral His tautomers and for Asn and Gln swapping the O and N of their amide side chain; the redox cofactors have oxidized and reduced conformers.

A dielectric constant of 80 is used for the surrounding water and interior cavities with a radius ≥ 1.4Å. For the fast, charge-separation electron-transfer reactions from P* to P^+BChl^- and to P^+BPh^- a dielectric constant of 2, providing only electronic polarizability, is used for the protein and the surrounding IPECE membrane slab.[37] The surrounding water retains the higher dielectric constant of 80. This choice provides additional relaxation of the reduced states. For slower reactions, MCCE uses a dielectric constant of 4 or even 8 for the cofactor environment, to include additional dielectric relaxation caused by slower protein motions.[48]

2.2.2 Force field. The PARSE charge set is used for all amino acids.[49] This has been benchmarked for residue pK_as[50, 51] and cofactor E_ms.[42, 52, 53] In the PARSE charge set, the atoms in non-polar side chains often have zero charge. Thus, they will not influence the calculated

cofactor redox potentials. Amber van der Waals and torsion energies are used. The atomic partial charges for neutral, ground state and reduced forms of BChl and BPh are taken from Ishikita.[54] We reduce the long tail of BChl and BPh to a methyl group and distributed the charge of the phytol group evenly to the methyl group atoms. The charge of CDL (Cardiolipin) and SPO (Sphaeroidene) in the 2J8C structure are obtained with the atomic charge calculator.[55] The MCCE parameters for these are available at https://github.com/judywei2333/charge_topology.git. See S.I. Eqn1 for a more complete description of the energy calculation.

2.2.3 BChl and BPh $E_{m,sol}$ MCCE calculates how the cofactor midpoint potential (E_m) is shifted by the protein from the value it has in water ($E_{m,sol}$).[56] E_ms, versus the Standard Hydrogen Electrode (SHE), measured in CH_2Cl_2 are: 640 mV for $BChl^+$, -860 mV for $BChl^-$, -580 mV for BPh^-.[33] The shift from CH_2Cl_2 to water will always stabilize the charged state. Here, we estimated this shift to be 200 mV which is in line with earlier calculations of Ishikita.[57, 58] Thus, an $E_{m,sol}$ for $BChl^-$ of -660 mV and -380mV for BPh^- are used here.

2.2.4 Equilibrium residue protonation states. In MCCE, Monte Carlo (MC) sampling yields the Boltzmann distribution of microstates at a defined system pH and electrochemical potential (E_h). Table S.I.1 shows the residues that are not fully in their expected protonation state at pH 8 with all cofactors in their neutral ground state. For example, a neutral, protonated GluL104 makes a hydrogen bond to BPh_A. All of the other residues with perturbed protonation states are on the quinone side, often near the surface.

2.2.5 Redox states. To determine the E_m for each cofactor, the system is brought to equilibrium with E_h changing. The probability of the reduced cofactor being found is fit to the Nernst equation to determine E_m, i.e. the E_h where the ground and reduced states have equal energy (S.I. Eqn 1). Each cofactor is titrated independently with all others fixed in their neutral forms. Thus, only one electron is added to the system at a time. The free energy of reduction is shifted by the protein by $-nF\Delta E_m$, where ΔE_m is the E_m shift from $E_{m,sol}$, n is the number of electron(s), and F is the Faraday constant. Following MC sampling, the individual cofactor-residue interactions are estimated using the conformer probabilities obtained by MC sampling with a mean field approximation (S.I. Eqn. 2).

2.2.6 Sign convention. A positive shift in E_m favors reduction. For free energy calculations, the neutral cofactor is the reference, reactant, state. Thus, a negative ΔG favors the product $BChl^-$, BPh^-, as well as P^+ and Q^-. In the comparison between the B- and A-branches, the B branch is the reference. Thus, positive ΔE_{A-B} and negative ΔG_{A-B} favor reduction of the A-branch cofactor.

2.3 Preparation of bRC YF mutants

The YF swap mutations, replaces PheL181 and TyrM210 with TyrL181 and PheM210. The mutation is made in two ways. MCCE can repair side chains. The L181 residue is renamed Tyr and the program adds the hydroxyl to the original Phe ring. The Phe is 'built' by replacing the Tyr hydroxyl with a hydrogen and renaming the side chain to Phe. No relaxation of the structure is carried out in MCCE beyond sampling of the surrounding side-chain conformers. This provides the change due to these two residues alone with minimal changes to other residues.

The YF swap is also made *in silico* in the 1AIG crystal structure using the CHARMM-GUI[38] and then subjecting the structure to Molecular Dynamics (MD). The MD simulation is

carried out for 200 ns as described previously.[16, 17] The MCCE protonation states calculated in the parent 1AIG structure are used in MD. MCCE calculations of the cofactor E_ms are carried out on six snapshots in time at 80ns, 86ns, 128ns, 180ns and 186ns.

2.4 BLAST

Of particular interest is how the differences between the L and M peptides change the electrostatic potential on the two cofactor branches. BLAST protein alignment (BLASTp)[59] of the L and M chains shows that out of the 254 residues, 86 are identical (34%) and 45 (17%) are similar. Thus, 51% of the residues are similar or identical.

2.5 Residue conservation

Multiple sequence alignment was carried out on sequences of L and M bRC peptides from 250 species of photosynthetic bacteria using the BLAST experimental database. The conservation and alternative residues that align with the residues that influence the cofactor E_ms are identified.

3 Results

3.1 Factors influencing cofactor reduction and how they depend on the input structures.

The E_ms for reduction of the two monomeric BChls and two BPhs on the bRC A and B branches were calculated using the coordinates from three different crystal structures, 1AIG, 2GNU and 2J8C. Reduction of $BChl_A$ is more favorable in the bRCs than in water, while $BChl_B$ reduction is disfavored (Table 1). Reduction of both BPhs is more favorable in the bRCs than in water (S.I. Tables 2 and 3).

The BChl and BPh E_ms are more positive on the A branch than on the B branch in all structures (Table 1 and S.I. Table 2). The $\Delta E_{m,BChl}$ (E_m $BChl_A$-E_m $BChl_B$) for the different structures are 164±49 mV [109 mV (1AIG), 180 mV (2GNU) and 203 mV (2J8C)], and the $\Delta E_{m,BPh}$ for the different structures are 67±20 mV (86 mV (1AIG), 68 mV (2GNU) and 46 mV (2J8C)). Thus, the shift in the electrochemical midpoint potential generated by the protein yields a robust, thermodynamic preference for the electron to go to $BChl_A$ and then to BPh_A.

Free energy terms that contribute to the calculated E_ms. In MCCE, the energy that shifts the E_m from its reference value in solution, $E_{m,sol}$, is divided here into the interactions of the cofactor: 1) with the backbone amide dipoles[60] (backbone); 2) with individual residues plus Fe^{3+}, the non-heme iron between Q_A and Q_B (side chains + Fe^{3+}); 3) with the other cofactors plus the His axial ligand to the BChl (cofactor+ligand); and 4) the loss of stabilization of the cofactor charges by the surrounding water (desolvation).[56] Table 2 provides these contributions for the 1AIG structure, and S.I. Tables 2 and 3 contain values for the other two structures.

The interactions of the cofactors with other charged or polar groups can make the electrostatic potential more positive (favoring reduction) or negative. For all four cofactors, the summed impact of the backbone amide dipoles and of the side chains favors reduction. These interactions with the protein are larger for the BPhs than for the BChls. Given the charge distribution used here, the neutral neighboring cofactors disfavor reduction of BChl but favor BPh reduction. The desolvation penalty always favors the form with the smallest net charge. This is because the reference solvent, water (ε=80), provides more intrinsic stabilization of a charge than the protein (ε=2).[61] The penalty is larger for the more deeply buried BPhs than for the BChl monomers.

Table 1. Average ΔE_m calculated in the three *R. sphaeroides* crystal structures (mV vs SHE).

	$BChl_B$	$BChl_A$	BPh_B	BPh_A
$E_{m,sol}$	-860	-860	-580	-580
E_m (all)	-984±65	-820±23	-355±16	-288±36
$\Delta E_{m,A-B}$ (all)		164±49		67±20
E_m (1AIG)	-920	-811	-336	-250
$\Delta E_{m,A-B}$ (1AIG)		109		86

Table 1: $E_{m,sol}$ is the E_m of BChl and BPh measured in CH_2Cl_2[62] shifted by 200 mV to account for the transfer to water.[57, 58] All: the average and standard deviation of the calculated E_ms in the three bRC structures. 1AIG: the values for this structure. The values for 2GNU and 2J8C are found in S.I. Table 2. Positive $\Delta E_{m,A-B}$ favors A-branch reduction (see section 2.3.6 for sign conventions used here).

3.2 Differences between the E_ms calculated in the three structures

There is modest variability in the calculated E_ms for the four cofactors in the three crystal structures with standard deviations of ≤ 50 mV. The results in 1AIG and 2GNU are more similar than that of 2J8C. S.I. Tables 2 and 3 provide the breakdown of free energy contributions to the E_m shift from solution for each cofactor in each structure. The His ligand, with its lone pair donating electron density to the central BChl Mg, destabilizes BChl reduction. The amount varies between the three structures because the His is close to the cofactor, so small changes in distance and angle affect the interaction between the BChl charge and the His dipole. The other major contribution to the differences of the E_m of $BChl_A$ is its interaction with the neighboring BPh. In 2J8C, the sum of the cofactor and ligand interactions is less favorable than in the other two structures. However, the main focus of these calculations is on the contribution of protein amino acids to the directionality of electron transfer, which are not strongly dependent on the input structure. Thus, the analysis in the main text will be restricted to the results from 1AIG. The values for the other structures can be found in the S.I. Table 2.

Table 2: Free Energy Terms Contributing to the ΔG of BChl reduction in 1AIG (meV).

	$BChl_B$	$BChl_A$	$\Delta\Delta G_{A\text{-}B,BChl}$	BPh_B	BPh_A	$\Delta\Delta G_{A\text{-}B,BPh}$
Desolvation	158	156	*-2*	475	459	*-16*
Backbone	-167	-140	*27*	-183	-192	*-9*
Side chains + Fe^{3+}	-271	-363	*-92*	-390	-579	*-189*
Background side chains	74	66	*-8*	11	126	*115*
Cof+Lig	225	224	*-1*	-188	-184	*4*
Sum	19	-57	*-76*	-275	-370	*-95*

Table 2. Negative ΔG values favor reduction; negative $\Delta\Delta G_{A\text{-}B}$ favor A-branch reduction. Desolvation: loss of solvation energy of the charge moving from the reference solvent, water, to the position within the protein; Backbone: interaction with the amide backbone dipoles; Residue interaction: Side chain + Fe^{3+}: Residues that shift the ΔG of reduction by more than ±29 meV;, Background side chains: Summed interactions with residues that interact with the cofactor by less than ±29 meV; Cofactors+Ligand: interaction with the neutral neighboring cofactors and the axial ligands to $BChl_B$ and $BChl_A$ (HisM182 and HisL153). Interactions with side chains determined with mean field energy approximation (SI Eqn 2). The differences between A- and B-branch values are shown underlined and in italic.

Table 2 compares the factors that provide the difference between A- and B-branch cofactor E_ms in the 1AIG structure. Values for the two other structures are found in S.I. Table 2. Comparing the two BChls, there is no difference in the desolvation energy or interactions with the neighboring neutral cofactors. The backbone dipoles favor $BChl_B$, by only 27 meV. The most significant contribution is due to interactions with the side chains. The summed stronger individual side chain interactions (greater than our ±29 meV threshold) favor $BChl_A$ reduction by -92 meV. The individual residues that contribute will be discussed below (Table 3).

Comparing the two BPh, there are only small differences in the desolvation energy (-16 meV) and backbone dipole interactions (-9 meV), together favoring A-branch reduction by -25 meV (Table 2). The interactions with the other neutral cofactors differ by only 4 meV. The main

difference comes from the amino acid residue interactions. The summed contributions from strongly interacting residues (>±29 meV) favor BPh_A reduction by -189 meV. However, the residue interactions below 29meV, here called background residue interactions, favor BPh_B reduction leading to an overall contribution from the side chains of -74 meV for favoring BPh_A reduction (i.e.-189+115 meV).

3.3 Residues with large influences on cofactor E_ms

MCCE can identify which residues contribute the most to the midpoint electrostatic potentials that shift the *in situ* E_ms. Fig. 2 highlights the residues that interact strongly with BChl or BPh. There are residues along the symmetry axis, including Fe^{3+} and its ligand GluM234, that influence the cofactors on both sides similarly. Others like GluL212 near the Q_B site have a larger impact on BPh_B (84 meV), but still modify the E_m of BPh_A (40 meV).

Fig 2: Residues interacting with the bRC cofactors.

Fig 2. Residues that shift the free energy of cofactor reduction by greater than ±29 meV. A: $BChl_B$; B: $BChl_A$; C: BPh_B; D: BPh_A. Residues represented by green atoms favor reduction; Residues represented by magenta atoms disfavor reduction. Residues represented with sticks are aligned residues on the other chain with interaction energies below the ±29 meV threshold. These residues are listed in Table 3.

The influence of conserved and/or variable residues within the L and M polypeptide chains on their respective embedded cofactors was determined (Table 3). The A-branch cofactors are mostly within the L chain, while the B-branch cofactors are mostly in the M chain. The D-helix of each peptide and the regions on the cytoplasmic and periplasmic top and bottom of the protein also switch sides. Here the residues that influence the A-branch cofactors (mostly L chain) are compared with the aligned residues (mostly in the M chain) that influence the B-branch cofactors. Often, aligned residues in the L and M chains contribute similarly to their respective A- and B-branch cofactors. Examples include the symmetry-related pairs of ArgM164 and ArgL135, ArgM132 and ArgL103, ArgL217 and ArgM253, and the axial ligands of the BChls: HisM182 and HisL153. These do not differentiate between A- and B- branch cofactors, but are important in creating a positive potential that directs electron transfer from P_{860} to the BPhs and quinones on the acceptor side.[37]

Key residues, such as TyrM210, make it more favorable to reduce the A-branch BChl (Table 3a). The symmetry aligned PheL181 has negligible interaction with $BChl_B$. ThrM186 favors B-branch electron transfer over the aligned ValL157. AspM88 disfavors B-branch reduction while there is a gap in the residue sequence of the symmetric position on the L chain.

Residues that differentiate BPh_A and BPh_B are listed in Table 3b. GluL104, which is always calculated to be protonated *in situ*, as it makes a hydrogen bond to the ring V keto group of BPh_A favors its reduction. The aligned ThrM133 contributes negligibly to BPh_B. GluL212 disfavors BPh_B reduction more than BPh_A reduction, while its symmetry-related residue, ArgM241, has a negligible impact on both sides despite its positive charge.

Table 3 focuses on residues that interact with a cofactor by more than ±29 meV. There are many other charged and polar residues in the protein, which are categorized as background residues (Table 2). The background residues always make it hard to reduce the cofactors. For both BChls, they destabilize reduction by 60-70 meV. The background residue interactions sum to be near zero for BPh_B, while BPh_A reduction is disfavored by +115 meV indicating more interactions below our cutoff (Table 2).

Table 3: Residues shifting cofactor reduction by more than ±29 meV in the 1AIG structure.

Table 3a: A- and B-branch BChls					Conservation	
$BChl_B$	meV	$BChl_A$	meV	diff	$BChl_B$	$BChl_A$
Fe^{3+}	-130	Fe^{3+}	-151	-21		
GluM234	35	GluM234	39	4	1	1
ArgM164	-58	ArgL135	-60	-2	1	1
ArgL217	-24	ArgM253	-31	-7	0.99	0.99
ArgL231	-35	ArgM267	-29	6	1	
ArgM132	-69	ArgL103	-89	-20	1	1
PheL181	0	TyrM210	-75	-75	0.98	0.99
ThrM186	-54	ValL157	0	54	1	1
GluL212	32	ArgM241	-8	-40	1	1
AspM88	32	none	na	-32	0.59	
LeuM135	0	GluL106	41	41	0.36	1
Sum	-271	Sum	-363	-92		

Table 3b: A- and B-branch BPhs					Conservation	
BPh_B	meV	BPh_A	meV	diff	BPh_B	BPh_A
Fe^{3+}	-293	Fe^{3+}	-310	-17		
GluM234	78	GluM234	81	3	1	1
ArgM132	-175	ArgL103	-165	10	0.99	1
ArgM164	-45	ArgL135	-52	-7	0.99	1
ArgL217	-52	ArgM253	-58	-6	1	0.99
ArgL231	-52	ArgM267	-36	16	1	0.98
ArgM267	-48	ArgL231	-49	-1	0.99	1
ThrM133	-6	GluL104	-65	-59	0.53	0.91
AspL218	55	TrpM255	0	-55	1	0.98
LeuM135	0	GluL106	65	65	0.36	1
AlaM139	0	LysL110	-37	-37	0.38	1
AspL213	51	GlyM242	0	-51	0.82	1
GluL212	84	ArgM241	-16	-100	1	1
ArgM241	-7	GluL212	40	47	1	1
GluM232	34	none	na	-34	0.99	
gap	na	GluM232	37	37		1
gap	na	ArgM247	-35	-34		0.99
gap	na	GluL6	46	45		0.99
GluH173	39	none	na	-38		
ArgM136	-53	ArgL10	-25	28	0.59	0.99
Sum	-390	Sum	-579	-189		

Table 3: Free energy (meV): Negative values favor reduction. Free energies are the difference in interaction between ionized and neutral states, i.e. $(cofactor)^-$ - $(cofactor)^0$. Fe^{3+} and GluM234 are along the axis of twofold symmetry of the protein and their interactions with A- and B-branch cofactors are given on a single row. All other rows provide interactions with the aligned residues considering the interactions of A-branch residues with nearby A-branch cofactors or B-branch residue with B-branch cofactors. In each case at least one of the residues in the row has an interaction > ±29 meV with its cofactor. Individual residue interactions are calculated with the mean field energy approximation (S.I. Eqn 2). Conservation is the percent identity of the residue found at this position in the 250 BLASTp sequences in the multi-sequence alignment. The conservation is for the symmetry-related residues on the same row, with the left column for the residue interacting with the B-side cofactor and the right column for the residue interacting with the A-side cofactor. S.I. Table 4 provides the alternative residue found when a residue is not fully conserved.

Fig 3. Conservation of residues in the L and M peptides. The bRC L and M peptides are arranged with c_2 symmetry. The H chain caps the terminal quinone electron acceptors on the cytoplasmic side of the protein (not shown). Pink color: identical and similar residues; non-equivalent residues: Cyan: L chain; Wheat: M chain.

3.4 YF mutants

Comparison of the L and M peptides showed TyrM210 corresponds with PheL181 in the aligned sequences. The Tyr is closer to $BChl_A$ and the Phe to $BChl_B$ (Fig 1). Early studies probed P_{860}* decay upon replacement of TyrM210 in *R. sphaeroides* (M208 in *R. capsulatus*) with Phe and other amino acids and the replacement of PheL181 (both species) with Tyr and other amino acids.[63-66] Some of the first mutations that were made to try to enhance B-branch electron transfer swapped TyrM210 and PheL181.[24, 35, 66] This double mutation is denoted as the YF swap. Electron transfer to the B-branch has been studied in the *R. capsulatus* bRCs containing the YF mutant.[24] The experiments were initially carried out in *R. capsulatus* bRCs in which the YF swap was paired with the ß mutation wherein LeuM212 (M214 in *R. sphaeroides*) is replaced with a His so that there is a BChl in the BPh_A site.[35] Analogous studies more recently have been carried out in *R. sphaeroides*, and comparisons made of pairs of mutants related to YFβ for the two species.[29] Noteworthy here in comparing effects of amino acids on electron transfer yields are residues M133 and L185 near BPh_B. These residues are Val and Trp, respectively, in *R. capsulatus* and Thr and Leu, respectively, in *R. sphaeroides*. All calculations carried out here employed the native *R. sphaeroides* amino acids at the two sites. BPh_A was retained for calculations on the effects of the YF swap. The effects of replacement of BPh_A with a BChl in the β mutant were also calculated.

Table 4: Electrochemical midpoint potentials of each cofactor in the YF and beta mutants.

Average (mV)	$BChl_B$	$BChl_A$	BPh_B	BPh_A	$ß_A$
$E_{m,sol}$	-860	-860	-580	-580	-860
E_m YF_{MCCE}	-839	-911	-369	-247	-507
$\Delta E_{m,A-B}$	*-72*		*122*		*-138*
E_m YF_{MD}	-796±96	-964±118	-428±17	-389±29	-649
MD-$\Delta E_{m,A-B}$	*-169±106*		*39±14*		*-221*

Positive $E_{m,A-B}$ favor A-branch reduction. MCCE E_m: mutations prepared within MCCE by only modifying the OH and H group from TyrM210 and PheL181. MD E_m are the results of MCCE calculations on six snapshots from an MD trajectory with PheM210 and TyrL181. $ß_A$ is the results with a BChl in the position of BPh_A using the difference in $E_{m,sol}$. The differences between A- and B-branch values are shown in Italic.

The E_ms of the four cofactors were calculated in the modified structure (Fig 4). In one study the YF swap was made within MCCE keeping the rest of the structure fixed. This significantly changed the $\Delta E_{m,BChl}$ as it moves from 109 to -72, now favoring reduction of $BChl_B$ (Table 4). Much of this is due to the orientation of the Tyr OH dipole, which has two orientations in the plane of the ring. In the parent structure TyrM210 stabilizes reduction of $BChl_A$ by 80 meV. In the YF mutant TyrL181 stabilizes BPh_B reduction by 70 meV. This orientation also destabilized reduction of the other BChl by 8 meV ($BChl_B$) in the parent or 35 meV ($BChl_A$) in the YF mutant.

The structure with the mutation was also equilibrated with MD and the E_ms calculated in six snapshots. Now the $\Delta E_{m,BChl}$ is -169±106 mV. It becomes more favorable to reduce $BChl_B$ since the $BChl_B$ E_m goes up by 81 mV and the $BChl_A$ E_m goes down by 100 mV (Tables 4 and S.I. Table 5). While the E_m varies with input structure, the conclusion that reduction of $BChl_B$ is favored is robust. Results in the individual snapshots are provided in S.I. Table 6 and S.I. Table 7.

In the MCCE mutated structure of the YF mutant there is a negligible shift in the BPh E_ms. BPh E_ms calculated in the MD snapshots favors BPh_A but by less than in the parent structure. The E_m of BPh_B shifts to disfavors reduction by an additional 33 mV while the BPh_A changes by only 3 mV (S.I. Table 5). Thus, the YF swap makes the $BChl_B$ reduction significantly more favorable while moderately disfavoring BPh_B.

In the mutant YF bRCs, the electron can move downhill from P_{860}* to either BPh_A or BPh_B. In the measurements of the YF swap in *R. capsulatus*, the yield of $P^+BPh_B^-$ increases to 15% from <2% in the parent bRCs, with more P_{860}* decaying directly to the ground state in the mutant.[24] The yield of $P^+BPh_B^-$ for the YF swap in *R. sphaeroides* has not been measured, although a value of ~10% can be estimated from comparisons of related mutants from the two species.[29] Thus, the lowering of the barrier posed by the high energy, low potential $BChl_B$ has a significant influence on the yield of the B-branch.

In the YFß mutants, BPh_A is replaced by a BChl ($ß_A$). In solution, the BChl E_m is 260 mV lower than that of BPh, making the $ß_A$ more difficult to reduce. Thus, if we consider the YF mutant ΔE_m of 122 mV favoring BPh_A reduction, the YFß bRCs could disfavor $ß_A$ reduction by 138 mV relative to BPh_B (Table 4, Fig 4). Similar values are found starting with the different crystal structures (see SI. Table 5). The measurements on the YFß bRCs from *R. sphaeroides* give a yield of $P^+BPh_B^-$ of 14%.[29] The yield is 23% for YFß bRCs from *R. capsulatus*, but drops to 14% when the native Val at M133 and Trp at L185 are replaced with the Thr at M133 and Leu at L185 found in *R. sphaeroides*.[29] Reduction of either BPh_B or $ß_A$ occurs directly from P_{860}* or by the respective intervening BChl. However, although MCCE places $P^+ß_A^-$ higher in energy than $P^+BPh_B^-$, $P^+ß_A^-$ is still the predominate product in the YFß mutant. Thus, while the change in electrostatic potential plays a role, other factors contribute to the branching ratio.

Fig 4: The E_m gaps between the BChls and BPhs from the symmetric A- and B branches. The Native-FY, YF are from MCCE calculations, YFß given the assumptios that $ß_A$, replacing BPh_A with BChl, will, lower the E_m by 260 mV relative to the original BPh_A with no other changes. YF_{MD} provides the results from analysis of MD snapshots.

4. Discussion

The electron transfers occur via electron tunneling.[19, 67] The rates are dependent on the reaction free energy, on the electronic coupling between the electron donor and acceptor and on the reorganization energy for the reaction.[68-70] Differences in each of these terms contribute to the active branch for electron transfer. Measurements have been made that evaluated the reorganization energy on the A and B branches[71] and that determined how changing the free energy affects the rates of electron transfer along the A-branch.[72-76]

For example, differences in electronic coupling between P* and the A- and B-branch due to asymmetry of the charge distribution over the two BChl in P_{860} could contribute to the directionality. A similar question about the asymmetry of charge separation in PSII has been addressed computationally.[77]Two mutants were made each changing the His ligand to one BChl in the dimer to Ile. A BChl is retained on the side with the His ligand, but a BPh is now found in the site with the Ile, creating a heterodimer. The BChl:BPh pair will have a different charge distribution in the ground, excited state and product cationic states. However, irrespective of which BChl is exchanged with BPh the electron continues to go to BPh_A and Q_A.[78, 79] indicating asymmetry in the primary donor does not determine the directionality of electron transfer.

There have been multiple calculations aimed at understanding the origins of directionality in wild-type bRCs using a variety of simulation techniques.[36, 37, 58, 80-82] Several studies have employed continuum electrostatics to determine the differences in the electrochemical midpoints (E_ms) of the A- and B-branch cofactors. Calculations using the *B. viridis* and *R. sphaeroides* bRC structures showed that the arrangement and nature of residues around the cofactors favored A-branch electron transfer, with the $BChl_A$ and BPh_A being at more positive potentials than their B-side counterparts. As here the asymmetry of E_ms was calculated to be larger for the BChls than the BPhs.[37, 58, 83]

However, comparing the calculated and experimental[35] results for the YFß mutant show that factors other than the relative E_m of the A- and B-branch cofactors contribute to the branching of the electron transfer route. Thus, in the YFß bRCs only ≈ 20% of the BPh_B and the $ß_A$ is still reduced, despite the calculations placing $P^+ß_A^-$ at higher energy than $P^+BPh_B^-$ (Fig. 4).

Many series of mutations show that other amino acids play subtle roles.[26, 27, 84, 85] For example, changing non-polar residues at positions Thr at M133 and Leu at L185 can influence the yield of BPh_B in YFß mutants. [29] Likewise, the results of the same mutations in *R. sphaeroides* and *R. capsalatus* have been compared. The L chains are 78% identical (88%simliar) and M chains 76% identical (84% similar) and each utilize only A-branch electron transfer in the wild-type protein. Parallel mutations made in the two backgrounds show similar but non-identical effects. [28, 29]

Thus, the calculations reported here provide an estimate of the importance of the electrostatic potential in setting the directionality of electron transfer. The calculations make several approximations. Thus, we are using a classical continuum electrostatics analysis with Monte Carlo sampling to calculate the relative free energy of non-equilibrium charge separated states.[86] The internal dielectric constant of 2 is appropriate as it captures the 'instantaneous' electronic polarization. However, the use of a dielectric constant of 80 for the surrounding solvent will over-stabilize the reduced products.[87] Thus, the calculated E_ms are likely too positive. Previous work has suggested techniques to provide a better estimate of the energies of non-equilibrium, which are outside of the scope of this paper.[88, 89] In considering the directionally of

electron transfer we find the contribution of the solvent to the resultant E_m is quite similar for the cofactors on the A- and B- branches (Table 2), so this excess relaxation should not affect the conclusions about the contribution of electrostatics to directionality.

Another consequence of the MCCE MC method is that the protonation states of surrounding residues are allowed to come into equilibrium with the changing redox state. No proton transfer can occur during the timescale of the reactions discussed here. There are various methods to treat this problem in the simulations.[77] Here, the few residues that show small changes in charge were identified and the titrations rerun with the protonation states of these residues fixed. The resultant E_ms of the four cofactors were lowered by between 4 and 20 mV, negligibly changing the results.

The importance of one-way electron transfer can be probed by examining the conservation of the residues that differentiate the electrostatic potential contribution to active and inactive branches (Table 3 and SI table 4). The conservation of the 13 residues that are important for setting the potential of the BChls (Table 3a) and 23 residues that influence the potential of the BPhs (Table 3b) was determined via multiple-sequence alignment. Overall, the L and M chains have similar conservation of 71.8% (L) and 69.3% (M). However, the importance of the interacting residues is seen as the important L-chain residues being 99.2% conserved while the key M-chain residues are 86% conserved. For the active $BChl_A$ and BPh_A, the key residues are greater than 99% conserved (see Fig 3). In contrast, the residues influencing $BChl_B$ are 89% conserved and those influencing BPh_B are 84% conserved. Thus, the regions around both A- and B-branch cofactors are more conserved than the protein as a whole; and the region around the active branch is more highly conserved than around the inactive path. The greater conservation of the region around $BChl_B$ than BPh_B suggests greater consequences of changes that affect the initial electron transfer direction.

5. Conclusion

Proteins create environments that direct the reactions they carry out. Type II photosynthetic reaction centers provide an example of how minor modifications in protein structure direct electron transfer to one branch of two symmetrically arranged possible paths. Thus, only $BChl_A$ and BPh_A participate in electron transfer reactions, while the B-branch cofactors do not. The study of this system provides insight into the mechanism by which proteins can tune function.

Our calculations demonstrate an important role for the electrostatic potential to help choose the path for electron transfer. The difference in potential is more pronounced at the BChls than at the BPhs. This suggests the competition between the two BChls has more impact on the direction of electron transfer than the relative favorability for reducing BPh_A and BPh_B. The residues contributing to the difference in A- and B-branch electrostatic potential around $BChl_B$ are more highly conserved than those around BPh_B supporting their greater importance in the deterring the branching ration.

Given knowledge of the bRC structure many mutations were made to help coax electrons down the B branch. This paper represents the first quantitative assessment of the YF and YFß mutations. The mutations clearly make the B-branch cofactors more electropositive. Again, the larger effect of the mutations on the relative potential at the two BChls supports their importance in defining the directionality. However, the calculations would have predicted full reduction of BPh_B, experiments show that that the higher energy However, the calculated free energies of the product states would predict that most electrons would travel down the B-branch to BPh_B, while experiments show remaining A-branch activity. This reminds us that the rates of competing reactions as well as product state free energies determine the final outcome.

Data availability

The primary data used to support the claims in this paper are included in the primary manuscript. Data for this article, including the parameters for these cofactors are available at https://github.com/judywei2333/charge_topology.git. The data supporting this article have been included as part of the Supplementary Information.

Conflicts of interest

There are no conflicts to declare.

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