Rewiring photosynthesis: engineering wrong-way electron transfer in the purple bacterial reaction centre

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Abstract
The purple bacterial reaction centre uses light energy to separate charge across the cytoplasmic membrane, reducing ubiquinone and oxidizing a c-type cytochrome. The protein possesses a macroscopic structural two-fold symmetry but displays a strong functional asymmetry, with only one of two available membrane-spanning branches of cofactors (the so-called A-branch) being used to catalyse photochemical charge separation. The factors underlying this functional asymmetry have been the subject of study for many years but are still not fully understood. Site-directed mutagenesis has been partially successful in rerouting electron transfer along the normally inactive B-branch, allowing comparison of the kinetics of equivalent electron transfer reactions on the two branches. Both the primary and secondary electron transfer steps on the B-branch appear to be considerably slower than their A-branch counterparts. The effectiveness of different mutations in rerouting electron transfer along the B-branch of cofactors is discussed.

Introduction
Publication of the X-ray crystal structure of the Rhodopseudomonas viridis reaction centre in the mid-1980s revealed the beautiful symmetry at the heart of the photosynthetic process [1–4]. Reaction centres were shown to contain two membrane-spanning cofactor branches arranged around an axis of two-fold pseudosymmetry (Figure 1). Subsequent structures for the Rhodobacter sphaeroides [5–8] and Thermochromatium tepidum [9] reaction centres have shown that this arrangement is common to all photosynthetic purple bacteria, and the same basic structural blueprint has also been seen in the Photosystem I and II reaction centres of oxygenic photosynthesis [10,11].

Despite the structural symmetry seen in purple bacterial reaction centres, spectroscopic studies have shown that only one of the two potential electron transfer chains is active in catalysing membrane-spanning electron transfer – the so-called A-branch (sometimes termed the L-branch) [12–15]. The remaining B-branch (or M-branch) does not appear to play a significant role. The exact ratio of A-branch/B-branch electron transfer at room temperature in membrane-bound reaction centres is not known, but estimates span the range from 30:1 [16] to 200:1 [17]. A detailed picture of the mechanism of light-driven charge separation and ubiquinol formation has been built up (see [18–23] for reviews), which is summarized in the legend to Figure 1.

In Type II reaction centres such as the purple bacterial complex and Photosystem II, the two cofactor branches have specialist roles, with the A-branch catalysing a ps time-scale separation of charge across the membrane dielectric, and the B-branch catalysing the double reduction and protonation of ubiquinone at the ubiquinol reductase (Qb) site. Type I reaction centres also have two cofactor branches, but here the need for functional asymmetry is less obvious as the quinone acceptor passes electrons onto an iron–sulphur centre located on the symmetry axis. Spectroscopic evidence has been presented that suggests that both cofactor branches catalyse membrane-spanning electron transfer in Type I reaction centres, although this may not universally be the case (see [24–27] and references therein).

The striking contrast between the structural symmetry and functional asymmetry displayed by the purple bacterial reaction centre has raised a number of interesting questions. Perhaps the most obvious of these, addressed mainly through theoretical and computational studies, is ‘what are the factors that determine the asymmetry of the primary charge separation reaction?’ A number of contributory factors have been proposed, including asymmetry in electronic coupling between cofactors on the A- and B-branches, attributed in some studies to small differences in the spacing of cofactors on the two branches [28–36], asymmetry in the dielectric environment on the two branches [37,38] and asymmetry in static intraprotein electric fields [39]. A much discussed idea is that asymmetric electron transfer is largely attributable to a difference in free energy between the first product of

Key words: bacteriochlorophyll, electron transfer, membrane protein, photosynthetic bacteria, protein engineering, reaction centre

Abbreviations used: BCH, bacteriochlorophyll; BChl, bacteriochlorophyll; B, A- and B-branch accessory BChl; BPhe, bacterioopheophytin; QA, QB, A- and B-branch ubiquinone; MChl, M-component; C, A- and B-branch BPhe; PO, POH, A- and B-branch ubiquinone; A, B, M-polypeptide; L-polypeptide; L, M-component; M212, M214H, L328 F, M35, M105; β, for the beta mutation in the two species).
Protein scaffold and enlarged view of cofactor organization in the *Rb. sphaeroides* reaction centre

(A) The electron transfer cofactors are encased in a protein scaffold formed by the L (blue), M (green) and H (violet) polypeptides. The cofactors are arranged around an axis of two-fold symmetry (- - - - -). The route of light-driven electron transfer is shown by the arrows. The primary electron donor (P), is a pair of BChls located close to the periplasmic face of the membrane. Photoexcitation of the primary electron donor to form the first excited singlet state \( P^* \) triggers electron transfer across the membrane to the QA ubiquinone, by a monomeric BChl (BA) and a Bphe (HA). BChl and Bphe are identical molecules with the exception that the central magnesium atom of the former is replaced by two hydrogen atoms in the latter, a difference that raises the reduction potential of Bphe by 200–300 mV. The transferred electron is then passed to the ubiquinol reductase (QB) site, where two sequential electron transfers result in the reduction of ubiquinone to ubiquinol, two protons being taken up from the cytoplasm. The oxidized \( P^+ \) is rereduced by a \( c^- \)-type cytochrome that transiently binds to the periplasmic surface of the reaction centre. Electrons are returned from ubiquinol to the \( c^- \)-type cytochrome by the cytochrome bc1 complex, with proton release on the periplasmic side of the membrane.

Substitution of BChl for Bphe at the H\(_A\) (A-branch Bphe) site: mutation Leu-M214 to His

Almost all of the mutant reaction centres that have been used to study B-branch electron transfer have contained the so-called ‘beta mutation’, in which the residue Leu-M214 has been replaced by His (Table 1). This includes a series of mutants constructed by Kirmaier, Holten and co-workers, who have carried out the most extensive study of B-branch electron transfer. The beta mutation, which was first constructed in *Rb. capsulatus* (where the equivalent residue is Leu-M212; see the nomenclature footnote on the title page for an explanation of the style of presentation) causes the reaction centre to assemble with a BChl rather than Bphe at the H\(_A\) position [48].

The new A-branch BChl, termed \( \beta_A \) (where \( \beta \) stands for BChl replacing a Bphe), has a long wavelength (Q\(_A\)) absorbance band significantly towards the red of that of the A-branch electron transfer, the \( P^+\)BA\textsuperscript{−} radical pair state [where P stands for a primary electron donor and BA for the A-branch accessory BCHl (bacteriochlorophyll)], and the analogous \( P^+\)Bphe\textsuperscript{−} state. The latter is proposed to have a significantly higher free energy than either \( P^+\)BA\textsuperscript{−} or \( P^* \) (the first singlet excited state of P that drives membrane-spanning electron transfer) [40–44]. To date, the underlying physical basis for asymmetric primary electron transfer has not been proven, and the experimentally inconvenient answer may be that a combination of the above factors determines near-exclusive use of the A-branch.

A second question is ‘can membrane-spanning electron transfer be rerouted along the B-branch of cofactors?’ To address this, site-directed mutagenesis has been used to make electron transfer along the A-branch less efficient, and to encourage electron flow along the B-branch. One of the challenges of this work is discriminating between A- and B-branch electron transfer in a structure where the cofactors at each symmetrical position along the A- and B-branches are chemically identical, spectroscopically highly similar, and are separated by similar distances. The two reaction centre BPhes (bacteriopheophytins), for example, have almost identical absorbance spectra at room temperature, as do the two monomeric BChls. As a result, some mutations designed to inhibit electron transfer along the A-branch have had a dual purpose of also changing the spectroscopic properties of the A-branch cofactors, or removing them altogether by excluding the cofactor from the complex.

The purpose of this short review is to look at the effectiveness of individual mutations in rerouting electron transfer along the B-branch, and to summarize the progress made in understanding the kinetics of B-branch electron transfer and properties of the radical pair states formed. Data from the literature regarding relative yields of A- and B-branch electron transfer after the formation of \( P^+ \) in wild-type and mutant reaction centres are summarized in Table 1. Due to limited space, B-branch electron transfer elicited by 390 nm excitation will not be considered [45–47].

Substitution of BChl for Bphe at the H\(_A\) (A-branch Bphe) site: mutation Leu-M214 to His

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The new A-branch BChl, termed \( \beta_A \) (where \( \beta \) stands for BChl replacing a Bphe), has a long wavelength (Q\(_A\)) absorbance band significantly towards the red of that of the
Table 1 | The fate of P* in wild-type and mutant reaction centres

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Primary ET A-branch*</th>
<th>P* to ground state†</th>
<th>Primary ET B-branch‡</th>
<th>Increase of B-branch ET due to the mutation in boldface§</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rb. Capsulatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>∼100%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LM212H</td>
<td>94%</td>
<td>–</td>
<td>6%</td>
<td>6.0-fold</td>
<td>[62]</td>
</tr>
<tr>
<td>LM212H→SL178K</td>
<td>90%</td>
<td>–</td>
<td>10%</td>
<td>1.67-fold</td>
<td>[62]</td>
</tr>
<tr>
<td>GM201D/LM212H</td>
<td>70%</td>
<td>15%</td>
<td>15%</td>
<td>2.5-fold</td>
<td>[16,49,62]</td>
</tr>
<tr>
<td>GM201D/LM212H→SL178K</td>
<td>62%</td>
<td>15%</td>
<td>23%</td>
<td>1.53-fold</td>
<td>[62]</td>
</tr>
<tr>
<td>LM212H→FL197V/FL121D</td>
<td>83%</td>
<td>5%</td>
<td>12%</td>
<td>2.0-fold</td>
<td>[63]</td>
</tr>
<tr>
<td>GM201D/LM212H→FL197V/FL121D</td>
<td>67%</td>
<td>15%</td>
<td>18%</td>
<td>1.2-fold</td>
<td>[63]</td>
</tr>
<tr>
<td>GM201D/LM212H→VM131D</td>
<td>70%</td>
<td>15%</td>
<td>15%</td>
<td>0.0-fold</td>
<td>[64]</td>
</tr>
<tr>
<td>YM2120F→FL181Y</td>
<td>75%</td>
<td>10%</td>
<td>15%</td>
<td>&gt;15-fold</td>
<td>[66]</td>
</tr>
<tr>
<td>YM2120F/LM212H→FL181Y</td>
<td>60%</td>
<td>10%</td>
<td>30%</td>
<td>5.0-fold</td>
<td>[58,65]</td>
</tr>
<tr>
<td>YM2120F/LM212H→WM250V→FL181Y</td>
<td>60%</td>
<td>10%</td>
<td>30%</td>
<td>5.0-fold</td>
<td>[58]</td>
</tr>
<tr>
<td><strong>Rb. Sphaeroides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>∼100%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>LM214H</td>
<td>95%</td>
<td>&lt;5%</td>
<td>∼3%</td>
<td>3.0-fold</td>
<td>[49]</td>
</tr>
<tr>
<td>GM203D/LM214H</td>
<td>83%</td>
<td>10%</td>
<td>7%</td>
<td>2.3-fold</td>
<td>[49]</td>
</tr>
<tr>
<td>YM210W/LM214H</td>
<td>n.r.</td>
<td>n.r.</td>
<td>&lt;10%</td>
<td>Uncertain</td>
<td>[73]</td>
</tr>
<tr>
<td>GM203D/YM210W/LM214H</td>
<td>n.r.</td>
<td>n.r.</td>
<td>&lt;10%</td>
<td>Uncertain</td>
<td>[73]</td>
</tr>
<tr>
<td>GM203D/LM214H→AM260W</td>
<td>n.r.</td>
<td>n.r.</td>
<td>&lt;10%</td>
<td>Uncertain</td>
<td>[73]</td>
</tr>
<tr>
<td>HM182L</td>
<td>65%</td>
<td>–</td>
<td>35%</td>
<td>Major increase, but ET is blocked</td>
<td>[58,68]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>at the first radical pair, either P+βB− for HM182E or P+ΦB− for HM182L</td>
<td></td>
</tr>
<tr>
<td>HM182E</td>
<td>65%</td>
<td>–</td>
<td>35%</td>
<td></td>
<td>[72]</td>
</tr>
<tr>
<td>HM182E→ML174D</td>
<td>72%</td>
<td>–</td>
<td>28%</td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>TM1350/HM182L</td>
<td>67%</td>
<td>–</td>
<td>33%</td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>VM1750/HM182L</td>
<td>75%</td>
<td>–</td>
<td>25%</td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>HM182L/GM203D/LM214H→AM260W</td>
<td>55-65%</td>
<td>–</td>
<td>35-45%</td>
<td></td>
<td>[71]</td>
</tr>
</tbody>
</table>

*Percentage yield of the reaction P*→P+βH−, or P+ΦB− where the LM214H or LM212H mutations are present.
†Percentage yield of decay of P* to the ground state by fluorescence.
‡Percentage yield of the reaction P*→P+βA− (unless indicated otherwise).
§Increase in B-branch electron transfer caused by the mutation indicated in boldface in column 1.

remaining H8 BPhe on the B-branch, and is expected to have a significantly lower redox potential (200–300 mV) than the BPhe it has replaced [48]. The LM212H (Leu→His) single mutant reaction centre has a slower rate of primary electron transfer to a state that has mixed P+βA−/P+βB− character (referred to henceforth as P+Iβ−) [48]. It also has a slower rate of secondary electron transfer from P+βA− to P+Q8− (where Q8 stands for A-branch ubiquinone; from 200 to 600 ps−1), and a decreased yield of electron transfer to Qβ (from approx. 100% to approx. 60%) [48]. The 40% decrease in the yield of the P+Q8− state is due to competition from an accelerated rate of decay of the mixed P+Iβ− state to the ground state (0.9 ns−1 compared with 20 ns−1 for decay of P+HA− in the wild-type) [48].

In addition to slowing the A-branch electron transfer and lowering the yield of P+Q8− the mutation allows absorbance changes associated with reduction of the H8 cofactor to be discriminated, as this is now the only BPhe in the complex [16,48]. It has been proposed that the LM212H mutant forms the P+H8− state with a yield of approx. 6% in Rb. capsulatus, and 3% for the equivalent LM214H mutation in Rb. sphaeroides [16,49]. As the yield of P+H8− in wild-type reaction centres is not known with precision, other than that it is very small (<1%), the exact extent to which the beta mutation increases the yield of B-branch electron transfer is not clear, but it is probably at least 10-fold.

Exclusion of the Qβ ubiquinone: mutation Ala-M260 to Trp

A second strategy that has been used to disrupt the A-branch is steric-exclusion of the Qβ ubiquinone from the Rb. sphaeroides reaction centre through mutation of Ala-M260 to Trp (AM260W). A combination of spectroscopy and X-ray crystallography has shown that this mutation prevents incorporation of the Qβ ubiquinone during assembly through
some minor protein structural changes, with the result that electron transfer along the A-branch is blocked beyond the H_{A,0} cofactor [50,51]. The AM260W mutation isolates the QA cofactor as the only reaction centre ubiquinone, and drastically shortens the lifetime of the radical pair that is the end-product of A-branch electron transfer, from approx. 100 ms for P^+Q_{A}^- in the wild-type complex to approx. 20 ns for P^+H_{B}^- in the AM260W mutant [50]. The principal advantage of this mutation is that it allows formation of Q_{A}^- through B-branch electron transfer to be detected in the absence of competing spectroscopic changes arising from the reduction of QA [52–55].

Laible and co-workers have used the mutation Trp-M250 to Val (WM250V) to destabilize the binding of the QA ubiquinone, and hence allow the purification of QA-deficient reaction centres. These reaction centres have then been incubated with 30–40 molar equivalents of ubiquinone-6 in order to selectively reconstitute the QA ubiquinone [56–58], or purified using the detergent Deriphat 160-C that allows retention of the QA ubiquinone [57,58].

**Acids and bases**

Kirmaier, Holten and co-workers have published a series of reports looking at the effects of acidic or basic residues on the yield of P^+H_{B}^- in the *R. capsulatus* reaction centre (Table 1). The best characterized of these is Gly-M201 to Asp (GM201D), a mutation first constructed in *R. sphaeroides* [59]. This mutation is thought to destabilize B_{A}^−, either by placing a negative charge close to the B_{A} macrocycle [60], or by removing a water molecule that interacts with B_{A} [61]. Recent findings from the authors’ laboratory suggest that the latter explanation is the more likely, as a Gly to Leu mutation has similar effects to the Asp change [J.A. Potter, P.K. Fyfe, D. Frolov, M.C. Wakeham, R. van Grondelle, B. Robert and M.R. Jones, unpublished work]. In *R. capsulatus* the GM201D mutation increased the yield of P^+H_{B}^- by 2.5-fold when added to the LM212H change [16,62], whereas in *R. sphaeroides* the equivalent GM203D mutation increased this yield by over 2-fold when added to the LM214H change [49].

The LM212H and GM201D/LM212H mutations have also been combined with the double change FL97V–FL121D [63]. The FL121D mutation places an acidic amino acid adjacent to the H_{A} BPhe in order to destabilize H_{A}^−, whereas the FL97V mutation makes the mutant reaction centre more structurally stable. The FL121D mutation increased the yield of P^+H_{B}^- by 2-fold in the LM212H background but by only 1.2-fold in the GM201D/LM212H background.

Turning to the B-branch, the *R. capsulatus* mutation SL178K places a basic residue near the B_{B} BChl, which conceivably could stabilize B_{B}^−. When added to the LM212H mutation it increased the yield of P^+H_{B}^- by 1.67-fold, and when added to the GM201D/LM212H pair it increased the yield by approximately 1.5-fold [62].

Finally, the *R. capsulatus* mutation VM131D introduces an Asp residue at a symmetrical position to Glu-L104, which is known to hydrogen bond to the keto carboxyl of H_{A}. Formation of a hydrogen bond with the keto carboxyl of H_{A} could stabilize H_{A}^− and encourage electron flow from B_{B}^−. When added to the GM201D/LM212H pair, the VM131D mutation had no effect on the yield of P^+H_{B}^- [64], which could indicate that the yield of the native B_{B}^− to H_{B} reaction is already maximal, or that the VM131D mutation has no effect on H_{B}.

**Swapping the M208/L181 pair**

The highest reported yield of P^+H_{B}^- to date is 30%, in a *Rb. capsulatus* mutant with the changes YM208F/LM212H/FL181Y [58,65]. Residue Tyr-M208 has a very strong influence on the rate of A-branch electron transfer, and is absolutely conserved among purple bacterial reaction centres (see [22] for a review). The symmetry related Phe-L181 was also thought to be absolutely conserved, but in a recently deposited sequence for the L-polypeptide from *Roseococcus thiosulfatophilus* this residue is reported as being a leucine (GenBank accession number AAL57745). The yield of P^+H_{B}^- has been estimated at 15% in a YM208F/FL181Y double mutant [66], and introduction of this pair into the LM212H background gave a yield of 30% [58,65], a 5-fold increase over that obtained with just the LM212H change. Residue Tyr-M208 is thought to influence the rate of primary charge separation principally through a stabilization of the P^+B_{A}^- radical pair, due to a favourable interaction of B_{A}^- with the OH group of the Tyr side chain [19,39,40,67]. Accordingly, the partial activation of B-branch electron transfer in the YM208F/FL181Y double mutant may come about through simultaneous destabilization of B_{A}^- and stabilization of B_{B}^-.

It has also been proposed that Tyr-M208 enhances the electronic coupling between P^+ and P^+B_{A}^- [28], and this may be another relevant factor.

**Mutations that change the cofactors on the B-branch**

Mutation of His-M182 to Leu causes replacement of the B_{B} BChl with a BPhe (denoted Φ_{B}, where Φ stands for BPhe replacing an accessory BChl) [68]. This *R. sphaeroides* reaction centre showed 65% A-branch electron transfer to P^+H_{A}^- and 35% B-branch electron transfer to P^+Φ_{B}^-; but there was no evidence for further electron transfer from Φ_{B}^- to H_{B}. The likely explanation for the 2:1 branching ratio in the HM182L mutant is the lower reduction potential of the new Φ_{B} co-factor, which would lower the free energy of P^+Φ_{B}^-.

The lack of forward electron transfer could mean that the free energy of P^+Φ_{B}^- is lower than that of P^+H_{B}^- [68,69].

A number of additional mutations have been made into the HM182L background in order to encourage electron flow from P^+Φ_{B}^- to P^+H_{B}^- [70]. Asp substitutions were introduced at positions Met-L174 and Val-M175 in an attempt to raise the free energy of P^+Φ_{B}^−, and Thr-M133 was mutated to Asp in order to lower the free energy of P^+H_{B}^- through donation of a hydrogen bond to the keto carboxyl of H_{B}. As might be expected, the first two of these Asp
Evidence of reduction of Q₈

A number of studies have exploited mutations that prevent or disrupt binding of Q₈ to look for evidence of reduction of Q₈ by the B-branch. In *Rb. capsulatus*, the GM201D/LM212H pair has been added to the Q₈-disrupting WM250V mutation (see above). This triple mutant showed a 11% yield of P⁺Q₈⁻ from an undetectable level to 35%. Again, there was no evidence for forward B-branch electron transfer to P⁺HB⁻, with instead approx. 30% of this population decaying by the repopulation of P⁺ followed by A-branch charge separation and the remaining 70% decaying by recombination of P⁺B⁻ to the ground state [72]. The result suggested that the mutation had caused lowering of the free energy of P⁺B⁻ to a value equal to or below that of P⁺HB⁻, leading to a slow rate of forward electron transfer compared with the rate of charge recombination or repopulation of P⁺ [72].

What has been found out about the B-branch?

The spectroscopic analysis of ‘B-branch active’ mutants has provided insights into key differences between the A- and B-branches. The first is that the reaction P⁺ → P⁺HB⁻ proceeds with a time constant of 100 ps (*Rb. capsulatus*) or 200 ps (*Rb. sphaeroides*), as opposed to 3 ps for the equivalent A-branch P⁺ → P⁺H⁺ reaction [16,49]. This is consistent with a predominance of P⁺ decay by electron transfer along the A-branch. Once formed, the P⁺HB⁻ state recombines to the ground state with a time constant of 1–4 ns [58], as opposed to 10–20 ns for recombination of P⁺H⁺. Finally, the time constant for the P⁺HB⁻ → P⁺Q⁺ reaction has been estimated as 2–12 ns [57,58] and approx. 10 ns [71], at least an order of magnitude slower than the 200 ps P⁺H⁺ → P⁺Q⁺ reaction on the A-branch. The picture that emerges therefore is the one in which forward B-branch electron transfer is much slower than A-branch electron transfer, and where the efficiency of the 2–12 ns P⁺HB⁻ → P⁺Q⁺ step may be adversely affected by competition from the 1–2 ns P⁺H⁺ recombination. Indeed, the greatest uncertainty seems to involve the yield of the P⁺HB⁻ → P⁺Q⁺ step, with estimates ranging from 100% to approx. 1%. This uncertainty may be a reflection of the fact that the occupancy of the Q₈ site is likely to be highly preparation-dependent.

The key to the rewiring of light-driven electron transfer seems to involve changing the properties of the accessory BCHls. The highest yields of P⁺HB⁻ have been achieved with mutations that have the potential to destabilize B₇₅⁺, such as YM208F and GM201D, and which have the potential to stabilize B₇₅⁺, such as FL181Y and SL178K. These effects are usually discussed in terms of changes in the free energies of the P⁺B₇₅⁻ and P⁺B₇₅⁻ states, but it is not yet clear how changes in electronic coupling or reorganization energy

mutations decreased the yield of P⁺ΦB⁺, but they did not activate forward electron transfer to H⁺. The TM133D mutation had almost no effect on the yield of P⁺ΦB⁺, and also did not activate onward electron transfer to H₂ [70]. The lack of an effect of the TM133D mutation matches the result obtained with the equivalent VM131D mutation in *Rb. capsulatus*, outlined above. The HM182L mutation has been combined with the triple change GM203D/LM214H/AM260W by de Boer et al. [71]; this ΦB⁺-containing mutant also showed a 35–45% yield of P⁺ΦB⁺, but no evidence of forward electron transfer to Q₈ [71].

The M182 residue has also been changed to Glu (HM182E) [72]. This mutation does not cause replacement of the B₈ BChl by BPhe, but does increase the yield of B-branch electron transfer to P⁺B⁻ from an undetectable level to 35%. Again, there was no evidence for forward B-branch electron transfer to P⁺HB⁻, with instead approx. 30% of this population decaying by the repopulation of P⁺ followed by A-branch charge separation and the remaining 70% decaying by recombination of P⁺B⁻ to the ground state [72]. The result suggested that the mutation had caused lowering of the free energy of P⁺B⁻ to a value equal to or below that of P⁺HB⁻, leading to a slow rate of forward electron transfer compared with the rate of charge recombination or repopulation of P⁺ [72].
also contribute. One thing that has become apparent is that effective re-routing of electron transfer will probably require a combination of several fairly subtle changes, as major changes to the energetics of B-branch electron transfer, such as replacement of the B₈ BChl by BPhe (Φ₈) for example, seem to have adverse effects on forward electron transfer to H₈ despite activating up to 45% reduction of Φ₈.

Finally, one wonders why an intact B-branch of cofactors has been retained in Type II reaction centres, given the effectively exclusive use of the A-branch for transmembrane electron transfer. In particular, the H₈ BPhe does not seem particularly optimized for efficient bacterial reaction centre remains unclear. The reasons for the retention of this apparent redundant electron transfer pathway in the purple bacterio reaction centre need further investigation (unpublished work). The reasons for the retention of this apparently redundant electron transfer pathway in the purple bacterial reaction centre remain unclear.

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