Supplementary material to:

Hybrid fusions show that inter-monomer electron transfer robustly supports cytochrome bc_1 function in vivo.

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Materials and methods

Construction of mutants

In the present work plasmid pMTS1 [1] (carries a *petABC* operon coding for three subunits of *R. capsulatus* cytochrome bc_1) and its derivatives were used as the expression vectors. The plasmid pMTS1-BS [2] was used for expression of hybrid B_SB_S complex (cytochrome bc_1 containing cytochrome c_1 and FeS subunits from *R. capsulatus* and cytochrome *b* from *R. sphaeroides*) and plasmid pMTS1-BSBST [2] – for expression of hybrid fusion B_S-B complex (cytochrome bc_1-like complex in which two separate cytochrome *b* subunits in the dimer were replaced with a hybrid cytochrome b_Sb built of *R. sphaeroides* and *R. capsulatus* cytochromes *b* fused together).

The mutated derivatives of pMTS1-BS and pMTS1-BSBST were created following general strategies described in [2]. Specifically, to create derivatives of pMTS1-BS, the desired point mutations (H212N, H217W and H212N/H217W in cytochrome *b* gene) were first introduced into pPET1-BS plasmid using PCR-based QuikChange Site-directed Mutagenesis (Stratagene) and oligonucleotide primers pairs listed in Table 1. Subsequently, the appropriate fragments of mutated pPET1-BS plasmids were cloned to expression vector pMTS1 as described in [2]. The so constructed derivatives of pMTS1-BS were used to express $^{N}B_SB_S^{N}$, $^{W}B_SB_S^{W}$, and $^{N/W}B_SB_S^{N/W}$ complexes.

Table 1. Oligonucleotide primers used for mutagenesis and PCR.

Name of the primer	5' - 3' DNA sequence
S_H212N_F	CTCGTGGCCATC*AAC*ATCTGGGCCTTCC
S_H212N_R	GAAGGCCCAGAT*GTT*GATGGCCACGAGGGC
S_H217W_F	CATCTGGGCCTTC*TGG*TCGACGGGCAACAAC
S_H217W_R	GTTGCCCGTCGA*CCA*GAAGGCCCAGATGTGG
SW_H212N_F	CTCGTGGCCATC*AAC*ATCTGGGCCTTC*TGG*
SW_H212N_R	*CA*GAAGGCCCAGAT*GTT*GATGGCCACGAGGG
H217W_F	CATCTGGGCCTTC*TGG*ACCACCGGCAACAAC
H217W_R	GTTGCCGGTGGT*CCA*GAAGGCCCAGATGTGG

"S" in the name of the primer denotes sequence designed to bind to *R. sphaeroides* cytochrome *b* gene; "SW" indicates primers designed to bind to *R. sphaeroides* cytochrome *b* gene with H217W mutation. Remaining primers bind to *R. capsulatus* genes. The mutated triplets are italicized.

To create derivatives of pMTS1-BSBST that were used to express $^{W}B_{S}$-B^{W}, $^{W}B_{S}$-B_{W} and $^{N/W}B_{S}$-B_{W} the desired mutations were first introduced into genes for *R. sphaeroides* cytochrome *b* on pPET1-BS-Not or for *R. capsulatus* cytochrome *b* on pUC-BLST plasmids using QuikChange Site-directed Mutagenesis and oligonucleotide primers pairs (Table 1). The mutated genes were assembled in appropriate combinations using cloning strategy described in [2]. All newly constructed expression vectors were introduced to *R. capsulatus* MT-RBC1 via triparental crosses as described in [1]. Construction of other mutants used in this work was described in [2]. DNA sequence and correct size of all constructs were verified both at the stage of preparing the constructs and after re-isolation of plasmids from *R. capsulatus* strains.

References

[1] E. Atta-Asafo-Adjei, F. Daldal, Size of the amino acid side chain at position 158 of cytochrome *b* is critical for an active cytochrome bc_1 complex and for photosynthetic growth of *Rhodobacter capsulatus*, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 492–496.

[2] M. Czapla, E. Cieluch, A. Borek, M. Sarewicz, A. Osyczka, Catalytically-relevant electron transfer between two hemes b_L in the hybrid cytochrome bc_1-like complex containing a fusion of *Rhodobacter sphaeroides* and *capsulatus* cytochromes *b*, Biochim. Biophys. Acta. 1827 (2013) 751–760.