

Supporting information

Modulation of heme redox potential in the cytochrome c_6 family

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Materials and Methods

The sequence of the synthetic gene (including ribosomal binding site, in lower case) for the phormidium laminosum cyt c_6 was:

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aggaggtgaccATGAAAAAATTTTCTCTCTGGTGCTGCTGGGTATCGCGCTGTTCA  
CGTTCGCGTTCTCGTCCCCGGCGCTGGCGGACGCTGACCTGGCTACCGGTGCT  
AAAGTTTTCTCCGCTAACTGCGCTGCTTGCCACGCTGGTGGTATCAACCTGGT  
TAACGCTGAAAAACCCTGAAAAAGAAGCTCTGGAAAAATTCGGTATGAA  
CTCCATCGTTGCTATCACCACCCAGGTTACCAACGGTAAAGCTGGTATGCCG  
GCTTTCAAAGGTCGTCTGACCGACGACCAGATCGCTGCTGTTGCTGCTTACGT  
TCTGGACCAGGCTGAAAAAGGTTGGTGA.
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The synthetic gene was assembled from five oligonucleotides (76 - 90 bases in length, overlaps between 17 and 20 bases) using the proofreading polymerase *Psu*, and amplified using two flanking primers and Taq polymerase.

Table S1: Hydrogen bond interactions involving the heme propionates, water molecules and proteins atoms found in the heme cavity of *P. laminosum* cytochrome c_6 (chain A), *A. thaliana* cytochrome c_{6A} AA-variant (2ce0) and the V52Q-AA variant of *A. thaliana* cytochrome c_{6A} . For *P. laminosum* cytochrome c_6 the bond lengths of coordinating atoms to a zinc ion found in close proximity to the heme 6-propionate are also reported.

Atom	<i>Pl</i> cyt c_6^a	<i>At</i> cyt c_{6A} (AA)	<i>At</i> cyt c_{6A} V52Q-AA
HP 6-O2D	N27 (N $^{\delta 2}$), 3.6 Å w28, 2.4 Å	Q28 (N $^{\epsilon 2}$), 3.1 Å w44, 2.7 Å	Q28 (N $^{\epsilon 2}$), 3.1 Å w196, 3.6 Å ^d
HP 6-O1D	w26, 2.5 Å ^b K30 (N $^{\zeta}$), 2.7 Å	w131, 2.7 Å ^b K30 (N $^{\zeta}$), 2.7 Å	w19, 3.8 Å ^b
HP 7-O2A	w26, 3.8 Å ^b w17, 2.7 Å ^c	w131, 2.9 Å ^b	w19, 3.3 Å ^b w196, 3.2 Å ^d
HP 7-O1A		N41 (N $^{\delta 2}$), 3.3 Å	N41 (N $^{\delta 2}$), 3.0 Å
Q52 (51) O $^{\epsilon 1}$			w103, 3.0 Å
Q52 (51) N $^{\epsilon 2}$	w26, 2.9 Å ^b CHA, 3.0 Å ^e		w19, 2.6 Å ^b CHA, 3.4 Å ^e
Zn	N27 (O $^{\delta 1}$), 2.2 Å E29 (O $^{\epsilon 1}$), 1.9 Å w55, 2.3 Å w21, 2.3 Å		

^aChain A used. ^bConserved water molecule found in all known structures of cyts c_6 bridging the heme propionates. ^cH-bonds also to w26 not found in other cyt c_6 structures. ^dA second bridging water molecule between the heme propionates in the V52Q structure. ^eA non-hydrogen bonding interaction with the meso carbon of the porphyrin ring.