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Supporting Material

The Binding Interface of Cytochrome *c* and Cytochrome c_1 in the bc_1 Complex: Rationalizing the Role of Key Residues

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Supplemental Material

Figure S1. Cytochrome *c* (mature protein) sequence alignment

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                -5    1         10         20         30         40
                      |         |          |          |          |
CYC_YEAST     ---TEFKAGSAKKGATLFKTRCLQCHTVEKGGPHKVGPNLHGIFGRHSGQAEGYSYT
CYC_NEUCR     ----GFSAGDSKKGANLFKTRCAQCHTLEEGGGNKIGPALHGLFGRKTGSVDGYAYT
CYC_CHIMP     --------GDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGYSYT
CYC_HUMAN     --------GDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGYSYT
CYC_BOVIN     --------GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFSYT
CYC_HORSE     --------GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFTYT
CYC_CHICK     --------GDIEKGKKIFVQKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAEGFSYT
CYC_WHEAT     ASFSEAPPGNPDAGAKIFKTKCAQCHTVDAGAGHKQGPNLHGLFGRQSGTTAGYSYS
CYC_CELEG     ---SDIPAGDYEKGKKVYKQRCLQCHVVDS-TATKTGPTLHGVIGRTSGTVSGFDYS

                50        60        70        80        90        100
                          |         |         |         |         |
CYC_YEAST     DANIKKNVLWDENNMSEYLTNPKKYIPGTKMAFGGLKKEKDRNDLITYLKKASE---
CYC_NEUCR     DANKQKGITWDENTLFEYLENPKKYIPGTKMAFGGLKKDKDRNDIITFMKEATA---
CYC_CHIMP     AANKNKGIIWGEDTLMEYLENPKKYIPGTKMIFVGIKKKEERADLIAYLKKATNE--
CYC_HUMAN     AANKNKGIIWGEDTLMEYLENPKKYIPGTKMIFVGIKKKEERADLIAYLKKATNE--
CYC_BOVIN     DANKNKGITWGEETLMEYLENPKKYIPGTKMIFAGIKKKGEREDLIAYLKKATNE--
CYC_HORSE     DANKNKGITWKEETLMEYLENPKKYIPGTKMIFAGIKKKTEREDLIAYLKKATNE--
CYC_CHICK     DANKNKGITWGEDTLMEYLENPKKYIPGTKMIFAGIKKKSERVDLIAYLKDATSK--
CYC_WHEAT     AANKNKAVEWEENTLYDYLLNPKKYIPGTKMVFPGLKKPQDRADLIAYLKKATSS--
CYC_CELEG     AANKNKGVVWTKETLFEYLLNPKKYIPGTKMVFAGLKKADERADLIKYIEVESAKSL
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CYC_YEAST – *Saccharomyces cerevisiae* (Baker's yeast); CYC_NEUCR – *Neurospora crassa* (bread mould); CYC_CHIMP – *Pan troglodytes* (chimpanzee); CYC_HUMAN – *Homo sapiens* (human); CYC_BOVIN – *Bos taurus* (cow); CYC_HORSE – *Equus caballus* (horse); CYC_CHICK – *Gallus gallus* (chicken); CYC_WHEAT – *Triticum aestivum* (bread wheat); CYC_CELEG – *Caenorhabditis elegans* (nematode). Highlighted: *c*-heme binding motif (yellow), conserved basic residues of cyt *c* in the *c*-c_1 interface (cyan), conserved glutamine (green).

Table S1. Summary of MD simulations

Simulation	Time, ns	Remarks
freeA	19.84	276,149 atoms; NPT; bc_1 dimer with cyt *c* bound
freeB	16.12	
freeC	10.75	
freeD	9.92	
freeE	7.97	
fixA	10.0	276,145 atoms; NVT; bc_1 dimer with cyt *c* bound; only atoms < 15 Å from cyt *c*-c_1 free to move; 2Fe2S clusters removed
fixB	10.0	
cc_1A	88.48	40,233 atoms; NPT; cyt *c* bound to water-soluble domain of cyt c_1
cc_1B	83.17	
cc_1C	93.62	

Table S2. Contact times (%)[1] in the Cyt *c*-Cyt c_1 binding interface involving at least one atom pair between Cyt *c* and Cyt c_1

Cyt *c* residue	fixA		fixB		freeA		freeB		freeC		freeD		freeE		Mean±SD[2]	
	Noh[3]	EN[4]	Noh	EN	Noh	EN	Noh	EN	Noh	EN	Noh	EN	Noh	EN	Noh	EN
Thr-12	94	0	64	0	95	0	93	17	88	0	60	0	93	0	85±6	0
Arg-13	97	1	100	15	79	12	93	1.0	97	0	96	9	98	5	91±4	8±2
Gln-16	98	95	97	92	100	89	78	64.0	92	59	100	98	100	98	98±1	88±6
Lys-27	31	30	91	56	76	60	64	27.0	82	60	83	56	76	43	74±8	53±5
Val-28	93	43	99	71	69	27	83	11.0	97	48	91	21	88	32	86±6	38±7
M3Lys-72[5]	98	95	82	62	96	93	11	10.6	88	72	77	23	94	84	90±3	76±11
Lys-79	85	40	83	40	66	56	0	0.0	99	63	58	31	99	94	78±7	53±8
Ala-81	97	2	97	1	96	0	5	0.0	97	4	98	6	61	0	93±5	2±1
Lys-86	63	61	100	100	84	81	87	80.0	99	99	97	97	85	81	88±5	86±6
Lys-87	88	85	93	84	55	54	82	73.0	59	58	56	48	98	98	70±8	67±8

[1] Percent contact times are averages for each trajectory, omitting the first 3 ns, during which time cyt *c* reached an equilibrium bound state

[2] The mean omits freeB (cyt *c* never fully bound)

[3] Noh = any heavy (non-hydrogen) atom

[4] EN = pairwise interactions between any electronegative atoms with a potentially favorable polar/electrostatic bond, e.g., omitting O..O with no available proton for H-bonding, and NH..NH

[5] For M3Lys-72, the EN column lists contact between any headgroup methyl and any O atom of cyt c_1

Un-highlighted residues – participants in (apolar) bonds identified in the 1KYO.pdb crystal structure (1)

Yellow highlighted residues – conserved polar resides seen to provide additional, major electrostatic interactions in MD simulations.

Table S3. Contact times (%)[1] with at least one atom pair between Cyt *c* and the finger of the non-binding Cyt c_1

Cyt c_1 atom		Cyt *c* atom	fixA	fixB	freeA	freeB	freeC	freeD	freeE	Mean[2]	SD
Noh[3]		Noh	77.7	87.4	83.7	11.4	76.3	67.2	92.8	80.9	3.3
EN[4]		EN	64.3	73.3	44.4	8.5	70.1	55.9	91.3	61.3	6.6
Glu-140	OE1/2	EN	25	27	16.8	2.2	5.4	2	0.8	14.0	4.3
Gln-141	OE1	EN	27.2	28.2	15	2.3	54.6	32.1	67.4	32.1	7.8
"	NE2	EN	38.1	59.3	21.1	2.9	60.5	41.6	85.7	44.0	9.2
"	O/N[5]	EN	52.2	61.3	31.9	5.7	69.5	53.9	91.3	53.4	8.3

[1] Percent contact times are averages for each trajectory, omitting the first 3 ns, during which time cyt *c* reached an equilibrium bound state

[2] The mean omits freeB (cyt *c* never fully bound)

[3] Noh = any heavy (non-hydrogen) atom

[4] EN = any electronegative atom with potentially favorable polar/electrostatic interaction, e.g., omitting O..O with no available proton for H-bonding, and NH..NH

[5] O/N = OE1 *or* NE2 of Gln-141

Major partners (present in at least 3 trajectories, and 15-60% in at least one) are:

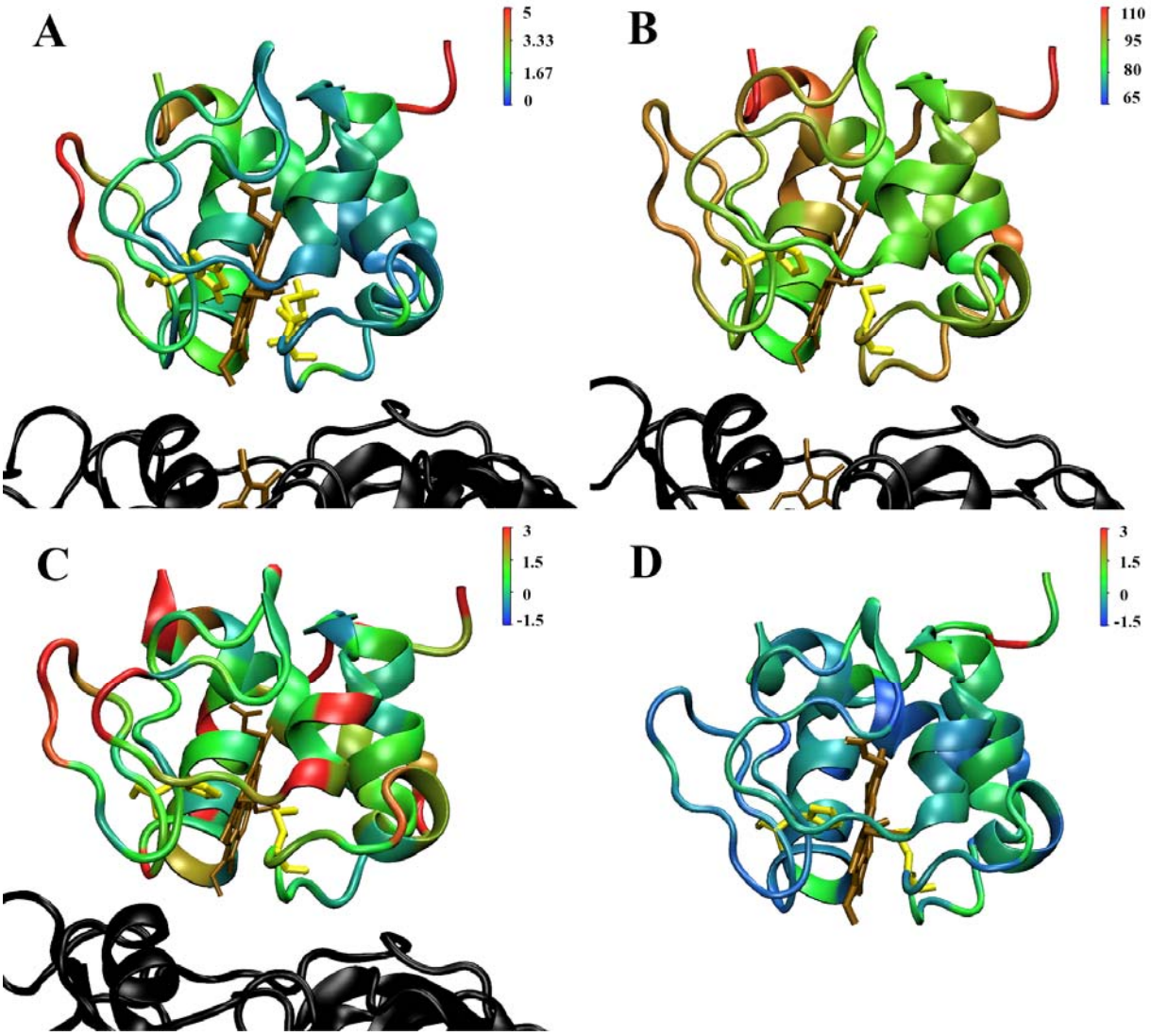
Glu-140:OE1/2 .. Ser-47:OG, Lys-79:NZ

Gln-141:OE1 .. Ser-47:OG, Asp-50:N, Ala-51:N, Gly-77:N, Lys-79:NZ

Gln-141:NE2 .. Ser-47:O/OG, Tyr-48:O, Asp-50:OD1/2

Asn-143 also contributed a small fraction of polar contacts with Asp-50:OD1/2 of cyt *c*

Figure S2. Mobility and resolution of yeast iso-1-cytochrome *c* bound to cyt c_1 and unbound



Cyt *c* structures are colored according to: Panel A - average displacement (RMSD, Å) of non-hydrogen atoms of the cyt *c* backbone with respect to the initial structure (1KYO.pdb (1)) observed in trajectory freeA. Panel B - the B-factors of backbone non-hydrogen atoms in 1KYO. Panel C - z-scores for cyt *c* bound to bc_1 complex from 3CX5.pdb (2). Panel D - z-scores for unbound cyt *c*, from 1CRH.pdb (3). Cyt c_1 is shown in black. Hemes are shown in ochre and heme ligands in yellow. (Note that z-scores are for the whole residue, not just backbone atoms.)

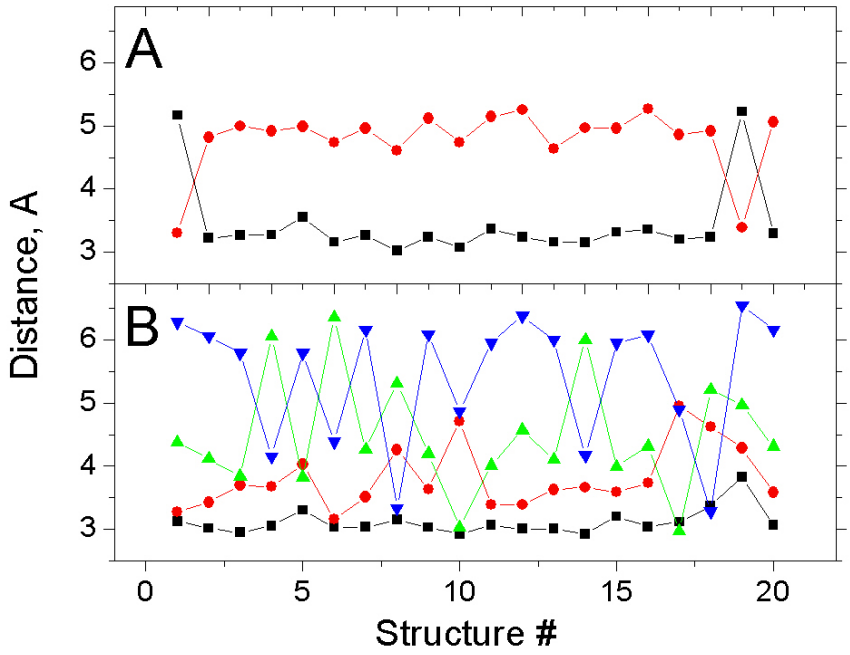
1CRH (rather 1CRG (3)) is shown in panel D for proper comparison with panel C (3CX5), as both have cyt *c* in the reduced form. However, the z-scores for oxidized and reduced cyt *c* when unbound are very similar, and both show the 21-25 loop to be better resolved than average:
1CRH (red) (1.9 Å res): Glu-21 -0.68, Lys-22 -0.56, Gly-23 -0.83, Gly-24 -0.13, Pro-25 -0.48.
1CRG (ox) (2.0 Å res): Glu-21 -0.35, Lys-22 -0.23, Gly-23 -0.15, Gly-24 -0.06, Pro-25 -0.65.

Table S4. Electrostatic interactions in the cyt *c*–CCP interface

Potential electrostatic interactions of cyt *c* (1st residue) and CCP (2nd residue)		
2GB8 (NMR structure of CCP with yeast cyt *c*)	2PCC (X-ray structure of CCP with yeast cyt *c*)	2PCB (X-ray structure of CCP with horse heart cyt *c*)
Cyt *c* CCP	Cyt *c* CCP	Cyt *c* CCP
		Lys-8:NZ..Asn-38:OD1
Lys-11:NZ..Asn-38:OD1		
Arg-13:NE..Tyr-39:O		
Arg-13:NH2..Asn-196:ND2		
Gln-16:NE2..Ala-193:O		
Gln-16:NE2..Asn-195:ND2		
Asn-70:ND2..Glu-290:OE1	Asn-70:ND2..Glu-290:OE1	
Asn-70:ND2..Glu-290:OE2		
		Lys-72:NZ..Glu-290:OE1
Lys-73:NZ..Glu-290:OE2		
Phe-82:O..Gln-120:O		
Lys-86:NZ..Glu-290:OE1		
Lys-86:NZ..Glu-290:OE2		
Lys-87:NZ..Arg-31:O		
Lys-87:NZ..Glu-32:OE2		
Lys-87:NZ..Asp-34:OD1		
Lys-87:NZ..Asp-34:OD2		
		Lys-87:NZ..Glu-35:OE2

Pairs of residues in cyt c and cyt c peroxidase (CCP) potentially forming inter-protein electrostatic interactions (<4Å distance). Left column: data derived from the 2GB8 NMR structure of the yeast cyt *c*–CCP complex in 100 mM Tris, 100 mM NaCl (4). Center column: pairs derived from X-ray structure (2PCC) of the yeast cyt *c*-CCP complex, crystallized under moderate ionic strength conditions (150 mM NaCl, trace potassium phosphate, pH 7.0 (5)). Right column: interacting residue pairs from the 2PCB X-ray structure obtained with horse heart cyt c bound to yeast CCP in low salt (5 mM potassium phosphate buffer, pH 7.0 (5).

Figure S3. Lys-86, Lys-87 interaction patterns in Cyt *c*-CCP complex



Distance between the NZ atom of Lys-86 (top panel) or Lys-87 (bottom panel) of yeast cyt *c* and potential H-bond acceptors of cyt *c* peroxidase (CCP), based on the 2GB8 NMR structure (4). Top panel: Lys-86 of cyt *c* and Glu-290:OE1 (■) and Glu-290:OE2 (●) of CCP. Bottom panel: Lys-87 of cyt *c* and Arg-31:O (■), Glu-32:OE2 (●), Asp-34:OD1 (▲), and Asp-34:OD2 (▼) of CCP.

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