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The orientations of cytochrome *c* in the highly dynamic complex with cytochrome b_5 visualized by NMR and docking using HADDOCK

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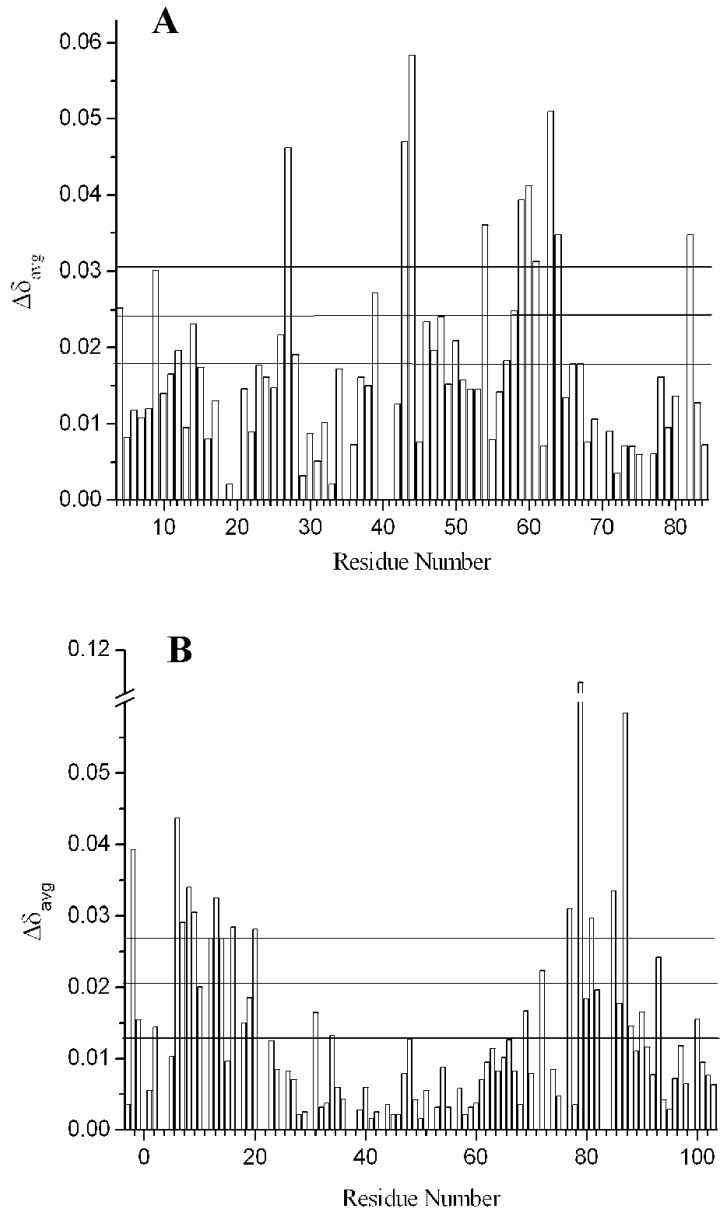

Figure S1. Averaged amide chemical shift perturbations ($\Delta\delta_{avg}$) plotted as a function of the residue number. (A) ^{15}N ferricyt b_5 in the presence of 2.5 molar equivalents of ferrocyt *c*. (B) ^{15}N ferrocyt *c* in the presence of 2.5 molar equivalents of ferricyt b_5. The lines denote the significance levels of the mean $\Delta\delta_{avg}$ value, the mean plus one half of the standard deviation, and the mean plus one standard deviation.

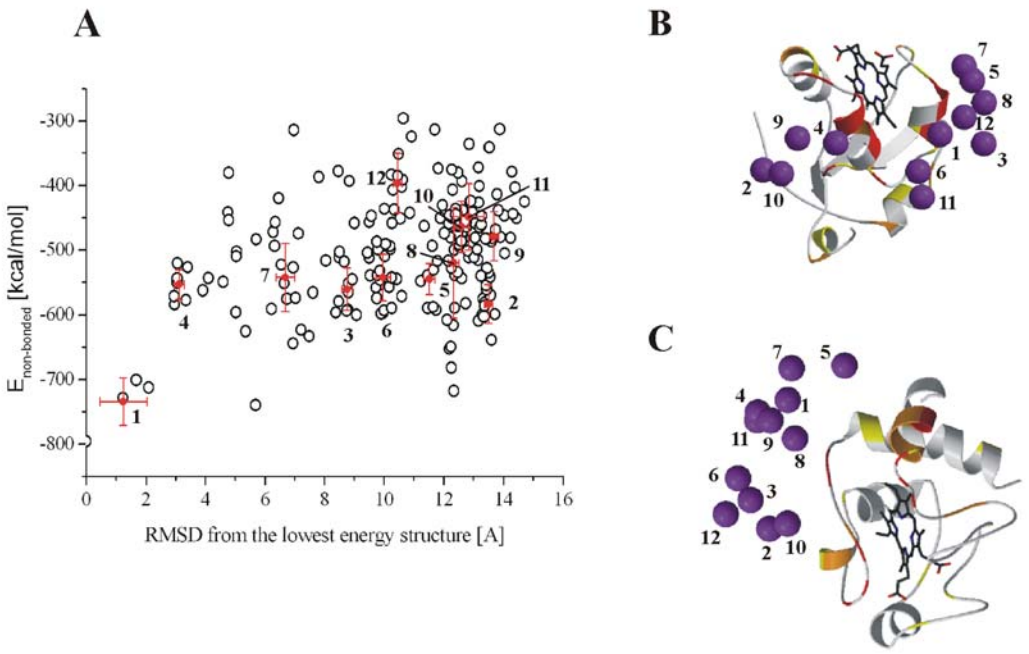

Figure S2. HADDOCK docking solutions for the binary complex of cyt b_5 with ferrocyt *c*. (A) Intermolecular non-bonded energy as a function of the backbone RMSD from the lowest energy structure for the docked structures. The clusters averages are indicated by red circles with error bars, which represent the standard deviation from the mean for the five lowest-energy structures of each cluster; the clusters are numbered according to the increasing energy. Centres of mass of (B) cyt *c* from each cluster (purple spheres) superimposed onto the ribbon representation of cyt b_5, and (C) cyt b_5 from each cluster (purple spheres) superimposed onto the ribbon representation of cyt *c*. The ribbon representations of the proteins in (B) and (C) are colored according to the averaged amide chemical shift changes ($\Delta\delta_{avg} \geq 0.029$ ppm in red, $\Delta\delta_{avg} \geq 0.023$ ppm in orange, $\Delta\delta_{avg} \geq 0.017$ ppm in yellow for cyt b_5; $\Delta\delta_{avg} \geq 0.030$ ppm red, $\Delta\delta_{avg} \geq 0.022$ ppm orange, $\Delta\delta_{avg} \geq 0.014$ ppm in yellow for ferrocyt *c*). The haem group is shown in sticks. The centres of mass were calculated for the lowest energy structure of each cluster. The numbering of the clusters corresponds to that in (A).

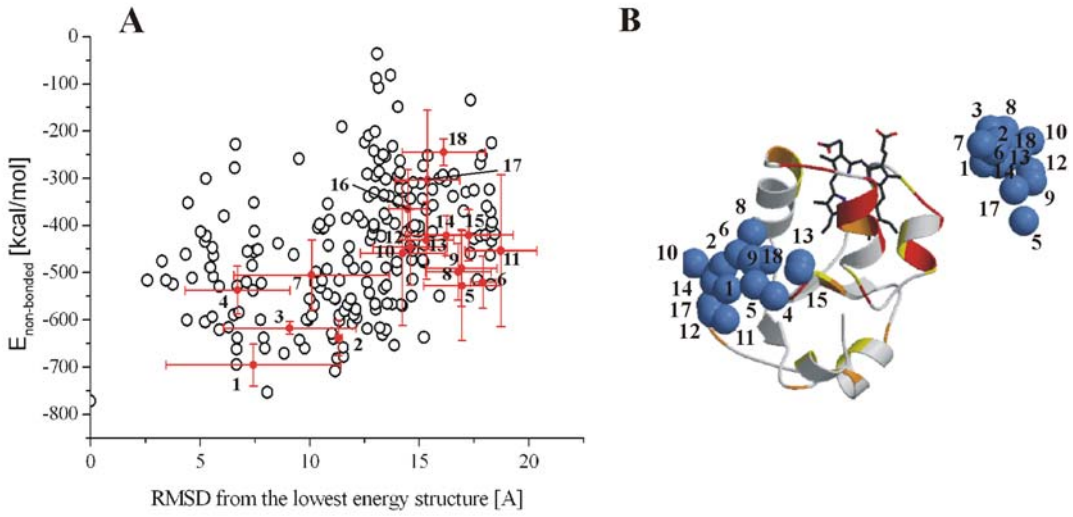

Figure S3. HADDOCK docking solutions for the ternary complex of cyt b_5 with two ferrocyt *c* molecules. (A) Intermolecular non-bonded energy as a function of the backbone RMSD from the lowest energy structure for the docked structures. The clusters averages are indicated by red circles with error bars, which represent the standard deviation from the mean for the five lowest-energy structures of each cluster. (B) Centres of mass of ferrocyt *c* from each cluster (blue spheres) superimposed onto the ribbon representation of cyt b_5. The centres of mass were calculated for the lowest energy structure of each cluster. The numbering of the clusters corresponds to that in (A). The ribbon representation of cyt b_5 in (B) is colored according to the chemical shift changes as in Figure S2, and the haem group is shown in sticks.

Table S1. Active and passive residues used in the definition of the ambiguous distance restraints (AIRs) and flexible segments for docking of cyt b_5 and cyt *c*.

cyt b_5 in complex with ferricyt *c*	
Active	A4, H26-Y27, E43-E44, D53, N57, E59-V61, S64, D66, D82, HEM89†
Passive	K2-A3, Y6, K28, E37, G42, V45, E48-Q49, E56, G62, T65, R68-L70, P81, R84, S85, K86, T88, HEM89
Flexible segments	S1-T8, I24-Y30, F35-K72, L79-HEM89

cyt b_5 in complex with ferrocyt *c*	
Active	A4, Y27, E43-E44, 48, E59-V61, S64, D82, HEM89†
Passive	A3, K5-Y6, T8, E10-E11, Q13, N16, H26, K28, P40, G42, V45, R47, Q49, E56-N57, G62, R68, L70, H80-P81, R84
Flexible segments	A1-S18, I24-Y30, E38-G51, A54-S64, D66-K72, E78-K86

ferricyt *c*	
Active	T8, K11-T12, Q16, T69, K72-K73, G77, K79, A81, K86
Passive	K4-K5, R13, L15, K27-V28, Y46-T49, A51, E66, N70, Y74, P76, T78, G83, K87, D90-R91, Y97, HEM104
Flexible segments	G1-H18, P25-P30, E44-A51, M64-K89, HEM104

ferrocyt *c*	
Active	T8, T12-R13, Q16, K72, G77, K79, A81, K86-K87
Passive	K4-K5, K11, L15, V28, S47, T69-N70, K73, P76, G83, E88-K89
Flexible segments	S2-H18, H26-P30, G45-T49, Y67-K89

† The solvent-accessible haem atoms CMD (5-CH_3) and CAD (6-α-Pr CH_2), the 1D resonances of which are affected by the binding of cyt *c*, are defined as active.

Table S2. Structural statistics of the five best docked structures (lowest E_{nb}) for each cluster of (A) cyt b_5 – ferricyt *c*, (B) cyt b_5 – ferrocyt *c*, (C) (cyt b_5) – (ferricyt *c*)$_2$, and (D) (cyt b_5) – (ferrocyt *c*)$_2$. The clusters are sorted according to the increasing intermolecular non-bonded energy (E_{nb}).

A **cyt b_5 – ferricyt *c***

	N[1]	RMSD[2]	sd[3]	E_{tot}[4]	sd[3]	E_{nb}[5]	sd[3]	E_{vdw}[6]	sd[3]	E_{elec}[7]	sd_3	E_{AIR}[8]	sd[3]	BSA[9]	sd[3]
1	2	1.3	1.3	-559	5	-769	8	-62	11	-706	19	209	3	1631	156
2	45	13.7	0.1	-471	36	-678	40	-56	4	-622	42	206	8	1523	21
3	40	13.7	0.1	-422	41	-634	37	-38	5	-597	37	212	6	1276	39
4	6	6.0	0.2	-354	48	-571	40	-34	8	-537	40	217	12	1203	69
5	11	8.8	0.1	-345	27	-559	20	-34	6	-525	22	213	15	1254	52
6	5	11.0	0.2	-334	53	-545	59	-55	5	-490	63	210	10	1575	78
7	25	13.8	0.1	-339	18	-544	21	-47	6	-497	23	204	6	1334	79
8	5	7.4	0.1	-219	34	-463	34	-23	8	-441	32	244	7	930	39
9	10	6.9	0.2	-209	33	-440	31	-33	3	-407	30	230	10	1085	36
10	5	9.9	0.2	-172	34	-412	35	-42	6	-370	38	239	6	1263	35

B **cyt b_5 – ferrocyt *c***

	N[1]	RMSD[2]	sd[3]	E_{tot}[4]	sd[3]	E_{nb}[5]	sd[3]	E_{vdw}[6]	sd[3]	E_{elec}[7]	sd[3]	E_{AIR}[8]	sd[3]	BSA[9]	sd[3]
1	4	1.3	0.8	-527	30	-734	37	-395	6	-696	37	195	5	1249	48
2	23	12.2	0.1	-456	37	-662	37	-47	5	-615	37	195	4	1466	51
3	8	13.5	0.2	-400	20	-602	22	-40	1	-562	23	193	6	1174	46
4	19	10.0	0.2	-374	12	-590	9	-46	6	-544	12	205	4	998	36
5	10	6.7	0.3	-377	35	-587	31	-37	5	-550	31	202	2	1217	70
6	9	8. 8	0.2	-384	15	-586	12	-37	3	-549	15	190	6	1221	36
7	7	3.1	0.1	-354	17	-565	15	-38	4	-527	16	203	3	1157	32
8	5	11.5	0.2	-340	31	-545	24	-45	4	-499	22	194	4	1263	46
9	18	12.9	0.7	-286	22	-502	22	-49	5	-454	22	208	3	1145	58
10	8	13.6	0.2	-295	29	-502	24	-51	4	-451	26	197	3	1437	73
11	9	12.5	0.2	-277	20	-491	22	-32	1	-458	22	205	6	1002	21
12	10	10.4	0.1	-223	21	-431	23	-35	2	-396	21	198	6	1080	75

Table S2 (continued)

C **(cyt b_5) – (ferricyt c)$_2$**

	N[1]	RMSD[2]	sd[3]	E_{tot}[4]	sd[3]	E_{nb}[5]	sd[3]	E_{vdw}[6]	sd[3]	E_{elec}[7]	sd[3]	E_{AIR}[8]	sd[3]	BSA[9]	sd[3]
1	8	5.8	4.1	-677	146	-1006	143	-66	14	-940	138	329	11	2530	336
2	14	15.2	0.2	-634	39	-978	39	-84	10	-894	40	344	4	2814	152
3	17	4.5	3.2	-582	118	-917	124	-68	4	-849	124	335	7	2851	276
4	8	11.0	3.5	-576	77	-903	77	-72	10	-830	77	327	3	2640	170
5	6	14.9	1.8	-514	118	-848	110	-75	14	-773	108	333	12	2616	265
6	14	17.8	0.5	-500	25	-841	24	-79	11	-762	21	340	15	2556	272
7	11	18.9	2.0	-435	73	-774	77	-58	13	-716	86	338	7	2285	140
8	4	18.2	2.3	-425	64	-757	59	-80	11	-678	50	332	8	2469	280
9	6	14.4	1.8	-326	101	-666	98	-60	3	-605	99	339	8	2303	36
10	8	14.7	2.7	-293	36	-622	39	-83	13	-539	50	329	10	2506	86

D **(cyt b_5) – (ferrocyt c)$_2$**

	N[1]	RMSD[2]	sd[3]	E_{tot}[4]	sd[3]	E_{nb}[5]	sd[3]	E_{vdw}[6]	sd[3]	E_{elec}[7]	sd[3]	E_{AIR}[8]	sd[3]	BSA[9]	sd[3]
1	7	7.4	4.0	-695	45	-1003	46	-80	7	-923	42	308	4	2745	110
2	8	11.3	0.2	-638	38	-943	34	-65	9	-878	40	305	9	2532	127
3	13	9.1	3.0	-617	13	-924	18	-65	7	-859	20	307	12	2318	181
4	6	6.7	2.4	-537	51	-841	47	-79	9	-763	43	304	9	2389	151
5	4	17.9	0.6	-520	56	-828	52	-50	7	-777	46	308	9	2349	250
6	5	17.0	1.8	-527	116	-827	115	-73	5	-754	113	299	7	2459	186
7	5	10.1	3.6	-505	74	-814	76	-56	10	-759	72	309	2	2221	180
8	5	16.8	1.5	-498	60	-810	62	-77	10	-733	59	312	4	2707	134
9	10	17.0	1.6	-490	81	-788	79	-87	12	-701	78	298	2	2861	149
10	4	14.3	1.9	-459	153	-764	154	-59	14	-705	145	305	7	2210	248
11	5	14.6	1.7	-446	79	-762	81	-67	10	-695	83	315	3	2329	126
12	4	18.7	1.6	-454	161	-760	165	-70	10	-690	159	306	5	2399	140
13	6	15.3	1.3	-432	82	-733	83	-84	8	-649	85	301	6	2411	135
14	6	16.3	1.9	-422	41	-723	49	-82	8	-641	50	301	10	2715	132
15	6	17.3	2.0	-421	54	-722	57	-77	11	-645	53	301	7	2558	248
16	8	14.5	0.9	-365	83	-665	79	-68	8	-597	72	299	8	2248	199
17	4	15.4	1.5	-303	148	-601	146	-63	7	-538	141	297	2	2339	274
18	5	16.1	1.9	-245	28	-547	39	-69	4	-478	40	301	11	2225	99

[1] Number of structures per cluster; [2] Backbone RMSD (Å) from the lowest-energy structure; [3] Standard deviation; [4] E_{tot} (kcal/mol) – intermolecular total energy (E_{vdw}+E_{elec}+E_{AIR}); [5] E_{nb} (kcal/mol) – intermolecular non-bonded energy; [6] E_{vdw} (kcal/mol) – intermolecular Van der Waals energy; [7] E_{elec} (kcal/mol) – intermolecular electrostatic energy; [8] E_{AIR} (kcal/mol) – ambiguous intermolecular restraints (AIRs) energy; [9] BSA – buried surface area (Å^2)