Supplementary Material for Michel & Bren, "Submolecular Unfolding Units in *Pseudomonas aeruginosa* Cytochrome c_{551}"

Table S1 ΔG_{HX} values (kJ/mol) for residues in *Pa* cyt c_{551} in the presence of GuHCl. Blank boxes represent prolines or residues which exchanged too quickly to measure an exchange rate.

	[GuHCl]:	0 M	0.2 M	0.4 M	0.6 M	0.8 M	1.0 M	1.2 M	1.4 M
3	**Pro**								
4	**Glu**	17.6	5.4						
5	**Val**								
6	**Leu**								
7	**Phe**	13.9	9.6			15.4		15.0	
8	**Lys**	16.4	18.3						
9	**Asn**								
10	**Lys**	19.5	16.5	18.3	18.5				
11	**Gly**								
12	**Cys**								
13	**Val**	16.0	16.9	17.2	16.0	15.2	16.8	17.4	14.2
14	**Ala**	15.3	16.2	18.3	16.4	17.1	16.1	17.3	15.2
15	**Cys**	25.4	25.0	24.9	24.5	24.3	22.8	22.1	19.9
16	**His**	33.7	31.8	32.7	30.6	28.7	26.1	22.0	20.3
17	**Ala**	26.0	24.4	23.2	22.1	21.6	19.8	19.3	
18	**Ile**								
19	**Asp**								
20	**Thr**								
21	**Lys**								
22	**Met**								
23	**Val**								
24	**Gly**	23.8	22.6	21.5	20.5	19.5	18.5	16.4	15.8
25	**Pro**								
26	**Ala**	17.3	17.0	17.4	14.9	15.1	15.5	17.0	
27	**Tyr**	15.4	20.2	16.1	15.9	18.4	19.6	18.4	
28	**Lys**	17.6	18.0	17.7	17.1	17.0	16.9	18.5	
29	**Asp**	21.3	18.8	18.1	16.6	15.5	14.8	12.4	
30	**Val**	22.4	18.7	17.6	16.1	15.0	14.0	12.0	10.1
31	**Ala**	24.6	22.3	20.5	18.3	17.4	16.5	15.5	14.9
32	**Ala**	23.7	22.8	20.1	19.2	18.4	17.1	15.2	15.7
33	**Lys**	22.5	20.9	19.8	18.1	18.2	16.0	15.9	18.3
34	**Phe**	21.0	19.2	18.5	16.8	16.8	17.1	15.7	
35	**Ala**	16.7	16.3						
36	**Gly**								
37	**Gln**	19.8	23.0	21.6		18.7	19.0	19.8	18.8
38	**Ala**								
39	**Gly**								
40	**Ala**	16.8	12.0						

	[GuHCl]:	0 M	0.2 M	0.4 M	0.6 M	0.8 M	1.0 M	1.2 M	1.4 M
41	**Glu**								
42	**Ala**	15.3	12.8	13.9	14.5	13.2	18.0	15.9	17.1
43	**Glu**								
44	**Leu**	20.7	19.5	18.9	18.0	17.2	15.4	12.9	11.0
45	**Ala**	22.6	21.8	23.8	23.8	21.0	18.3	15.6	13.2
46	**Gln**	23.0	22.4	22.1	21.2	20.8	18.7	16.5	15.2
47	**Arg**	30.4	28.4	27.6	25.6	23.1	19.4	17.5	
48	**Ile**	34.2	28.8	27.3	23.2	21.5	18.6	14.8	12.2
49	**Lys**	31.9	29.0	27.2	23.3	21.5	16.9	16.6	13.4
50	**Asn**	30.8	28.3	26.7	24.1	22.4	20.0	18.1	
51	**Gly**	33.9	30.3	28.3	24.9	22.4	20.9	17.1	
52	**Ser**	33.4	30.0	27.8	25.4	22.3	19.4	18.1	
53	**Gln**								
54	**Gly**	16.9	15.3	20.1	18.1				
55	**Val**	15.8	15.9	15.9	13.5	15.1	13.2	14.3	12.2
56	**Trp**	20.5	19.5	18.8	18.9	17.5	15.7	16.1	11.6
57	**Gly**	19.4	19.4	18.3	18.1	17.5	18.1	17.7	16.4
58	**Pro**								
59	**Ile**	11.3	8.7	11.9	10.8	8.2	10.7	10.6	10.4
60	**Pro**								
61	**Met**	28.6	25.2	24.8	23.4	22.1	19.3	17.1	14.1
62	**Pro**								
63	**Pro**								
64	**Asn**	24.2	20.7	20.4	19.5	19.7	19.1	20.3	18.7
65	**Ala**								
66	**Val**	14.3	14.4	14.3	13.0	13.1	12.2	13.9	11.6
67	**Ser**	20.5	20.8	20.8	19.1	18.7	18.6	19.5	20.1
68	**Asp**								
69	**Asp**								
70	**Glu**	13.2	10.0	15.5	14.4	15.4			
71	**Ala**	26.6	26.2	26.6	23.8	21.6	19.5	17.7	13.7
72	**Gln**	27.4	25.8	25.5	23.6	22.0	18.9	16.9	16.5
73	**Thr**	23.8	24.5	24.0	22.9	20.6	18.5	18.9	
74	**Leu**	33.5	28.5	25.0	22.2	20.9	16.4	14.4	12.4
75	**Ala**	35.3	28.9	28.6	22.7	21.1	18.5	15.5	14.3
76	**Lys**	35.0	29.9	29.2	25.2	22.8	19.1	16.7	14.0
77	**Trp**	32.3	30.0	28.6	24.2	22.0	17.7	16.0	12.3
78	**Val**	32.7	26.4	24.5	21.3	20.4	16.9	14.0	11.2
79	**Leu**	32.1	27.6	26.0	21.3	20.3	16.3	15.0	10.8
80	**Ser**	28.6	27.6	26.1	24.0	21.3	18.8	18.7	
81	**Gln**								
82	**Lys**								

Table S2 To confirm the HX experiments in GuHCl were within the EX2 limit, the observed exchange rates for *Pa* cyt c_{551} in 1.4 M GuHCl at pH 5 and 1.4 M GuHCl at pH 6 were compared. The log(k_{obs}) values are ~10 fold higher at pH 5 compared to the values at pH 6, as expected for a protein exchanging within the EX2 limit.

Residue	AA	log(k_{obs}) pH 5	log(k_{obs}) pH 6
13	**Val**	-3.9	-2.9
14	**Ala**	-3.8	-3.0
24	**Gly**	-3.6	-2.8
30	**Val**	-3.9	-2.8
31	**Ala**	-3.5	-2.9
44	**Leu**	-3.9	-2.8
45	**Ala**	-3.8	-2.7
46	**Gln**	-3.7	-2.8
48	**Ile**	-3.9	-2.8
49	**Lys**	-3.4	-2.8
55	**Val**	-3.5	-2.8
56	**Trp**	-3.8	-2.7
57	**Gly**	-3.6	-2.9
59	**Ile**	-3.8	-3.0
61	**Met**	-3.9	-2.9
66	**Val**	-3.6	-2.9
71	**Ala**	-3.7	-2.7
72	**Gln**	-3.8	-3.0
74	**Leu**	-3.7	-2.7
75	**Ala**	-3.8	-2.9
76	**Lys**	-3.8	-2.7
77	**Trp**	-3.8	-2.6
78	**Val**	-4.1	-2.9
79	**Leu**	-3.9	-2.8

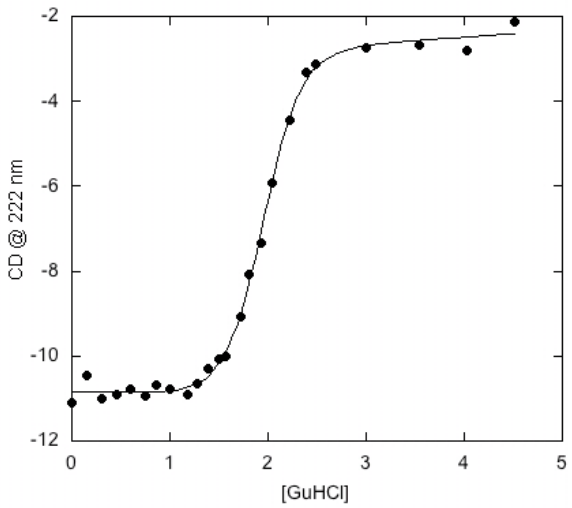

Fig. S1 GuHCl denaturation curve monitored by circular dichroism spectroscopy for oxidized *Pa*-cyt *c*. The fit shown is to a two-state model, using the equation:

$y = [(y_f + m_f[\text{GuHCl}]) + (y_u + m_u[\text{GuHCl}])(e^{-\{(\Delta G(H_2O) - m[\text{GuHCl}])/RT\}})]/(1 + e^{-\{(\Delta G(H_2O) - m[\underline{\text{GuHCl}}])/RT\}})$

where "*y*" refers to the measured CD signal, and subscripts "u" and "f" refer to the unfolded and folded states (denatured and native conditions, respectively), and $\Delta G(H_2O)$ is the free energy of unfolding in the absence of denaturant.

The fitted parameters are: y_f = -10.8 ± 0.37 mdeg; y_u = -3.13 ± 1.48 mdeg; m_f = -0.02 ± 0.47 J/mol M; m_u = -0.16 ± 0.40 J/mol M: $\Delta G(H_2O)$ = 25 ± 6 kJ/mol; m = 13 ± 3 kJ/mol M.

The concentrations of GuHCl used in this study, 0 to 1.4 M, correspond to 100 to 95% folded protein according to these data.

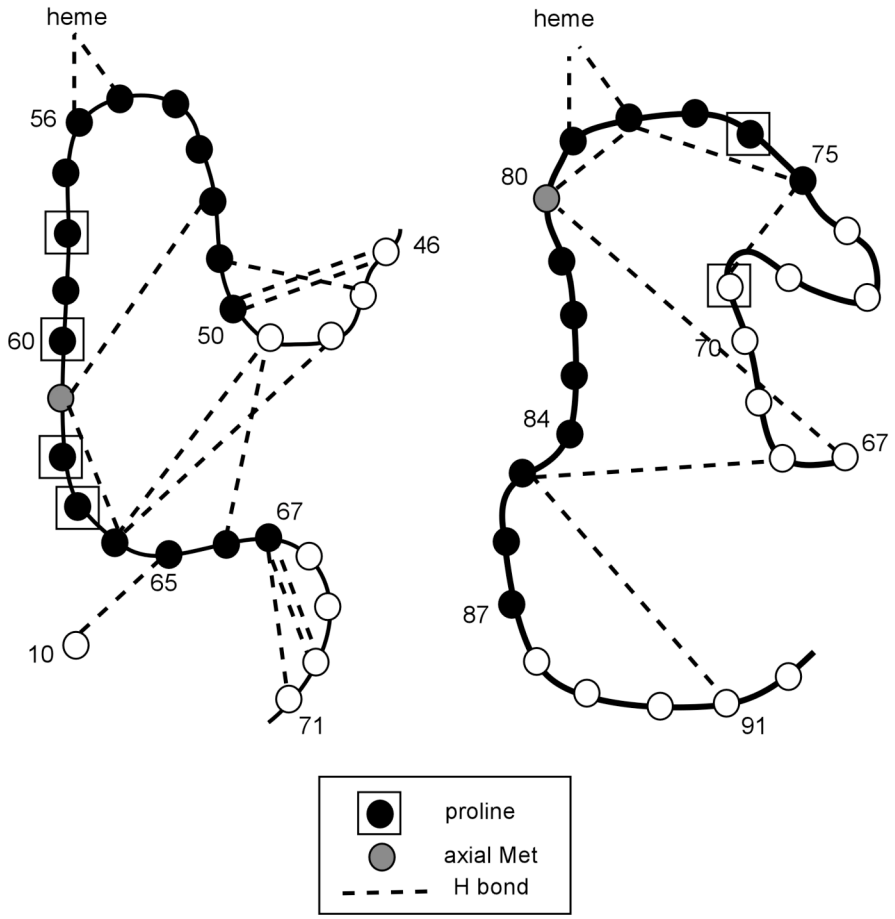

Fig. S2 Hydrogen bonds with loop 3 residues, determined from crystal structures of *Pa* cyt c_{551} (351C; left) and horse cyt *c* (1HRC; right). Filled circles represent residues with loop 3, and open circles non-loop 3 residues. Prolines are indicated with a box, and the heme axial Met with a gray circle. The geometric parameters used to determine the presence of a hydrogen bond are: r(D-A) < 3.5 and θ(D-H-A) > 150˚ where D and A are the heavy-atom hydrogen bond donor and acceptor.