

Table S1: Heme methyl ^1H chemical shifts for *Ht* cyt *c*₅₅₂ in parts per million (ppm) at pH 7

Protein	^1H 1-CH ₃	^1H 3-CH ₃	^1H 5-CH ₃	^1H 8-CH ₃	average
wild-type	18.1	22.2	22.8	24.2	21.8
M13V	17.9	20.7	22.8	23.8	21.3
K22M	18.4	21.3	23.2	24.0	21.7
M13V/K22M	18.0	19.7	23.1	23.3	21.0

Table S2: Chemical shifts of His nuclei for *Ht* cyt *c*₅₅₂ as a function of pH

pH	wild-type ¹ H	wild-type ¹⁵ N	pH	M13V ¹ H	M13V ¹⁵ N	pH	K22M ¹ H	K22M ¹⁵ N	pH	M13V/K22M ¹ H	M13V/K22M ¹⁵ N
5.60	13.2	183.1	6.97	11.83	170.6	6.55	12.62	180.6	5.30	11.17	167.6
6.25	13.2	183.0	7.00	11.83	170.5	7.03	12.63	180.8	5.85	11.18	167.7
6.70	13.2	182.9	7.56	11.84	170.5	7.50	12.64	180.6	6.70	11.19	167.7
6.95	13.2	182.9	8.01	11.85	170.7	7.96	12.66	180.6	7.05	11.20	167.7
6.97	13.2	182.9	8.53	11.87	170.7	8.21	12.66	180.8	7.52	11.20	167.7
7.40	13.2	182.5	9.03	11.92	170.9	8.22	12.66	180.7	8.05	11.23	167.7
7.94	13.2	182.8	9.42	11.98	171.3	8.67	12.69	181.0	8.65	11.25	167.8
8.55	13.3	183.1	9.78	12.05	171.7	8.95	12.73	180.9	9.10	11.29	167.9
9.10	13.4	183.4	9.84	12.15	171.90	9.43	12.80	181.4	9.47	11.34	167.9
9.67	13.5	184.3	10.23	12.17	172.4	9.97	13.05	182.6	9.93	11.45	168.5
9.96	13.6	185.0	10.61	12.37	173.2	10.01	13.06	183.0	10.10	11.51	168.2
10.38	13.8	186.8	11.07	12.50	173.9	10.40	13.21	183.8	10.45	11.65	169.4
10.53	14.0	187.6	11.16	12.54	174.1	10.80	13.45	185.1	10.92	11.87	170.7
10.95	14.0	189.1	11.34	12.59	174.4	11.49	13.46	185.2	11.08	12.17	172.3
11.11	14.1	188.7	11.48	12.6	174.5	11.94	13.93	188.4	11.42	12.17	172.3
-	-	-	11.57	12.58	174.5	-	-	-	12.15	12.30	173.2

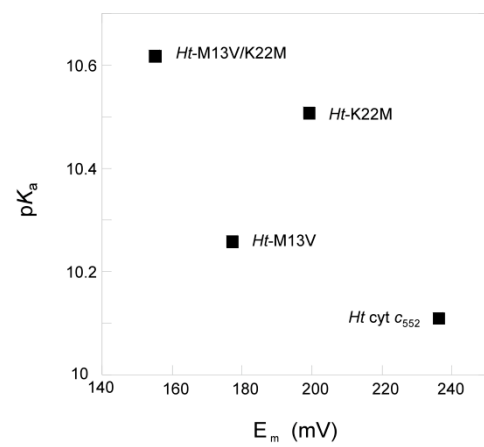


Figure S1: Plot of His 16 $pK_{a(2)}$ vs. E_m

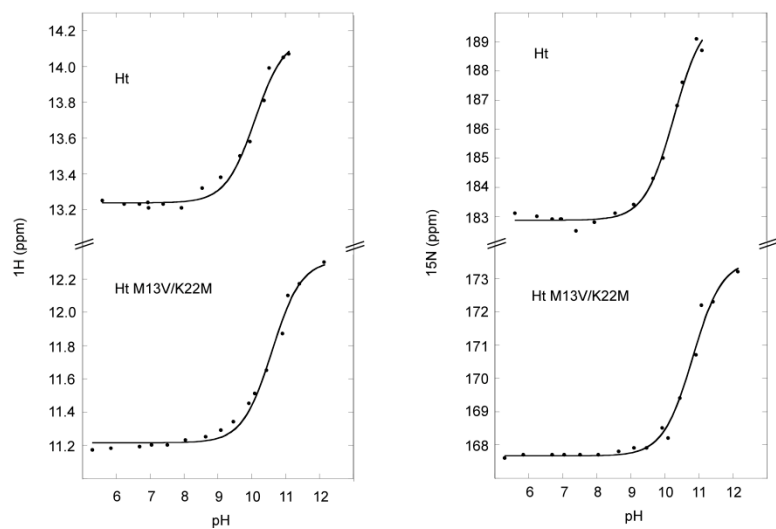


Figure S2: Representative fits of chemical shift vs. pH to determine $\text{p}K_a$ for *Ht* cyt c_{552} wild-type and M13V/K22M. At pH values higher than those shown the cross-peak loses intensity and is difficult to observe.