Supporting Information

Naturally Occurring A51V Variant of Human Cytochrome *c* Destabilizes the Native State and Enhances Peroxidase Activity

Haotian Lei and Bruce E. Bowler*

Department of Chemistry and Biochemistry, Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT 59812

Supporting Figures

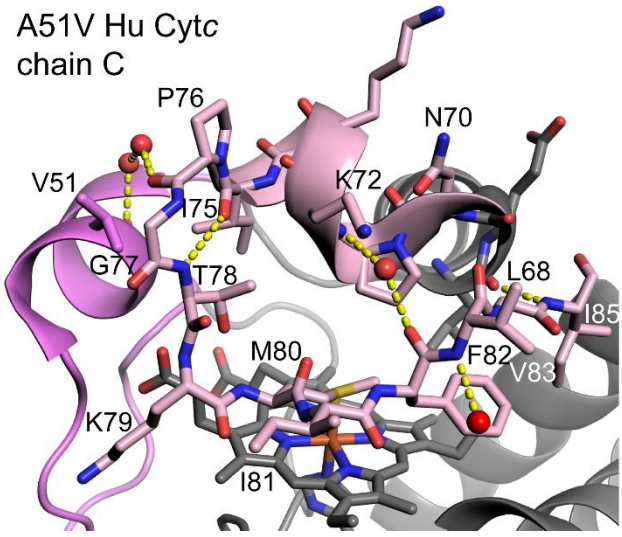

Figure S1. Hydrogen bond network across Ω-loop D for chain C of the A51V (PDB entry: 6DUJ) variant of Hu Cyt*c*. Ω-loops C and D are shown in light purple and light pink, respectively. Ω-loop D is shown with stick models. Waters are shown as red spheres. Hydrogen bonds are shown in yellow.

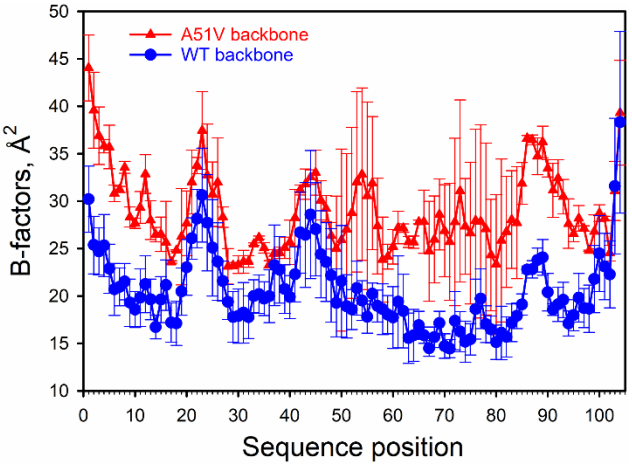

Figure S2. Backbone B-factors for the structures of A51V (PDB entry: 6DUJ) and WT (PDB entry: 3ZCF) Hu Cyt*c*. The error bars are the standard deviation of the B-factors for the 2 and 4 molecules in the asymmetric unit, respectively.

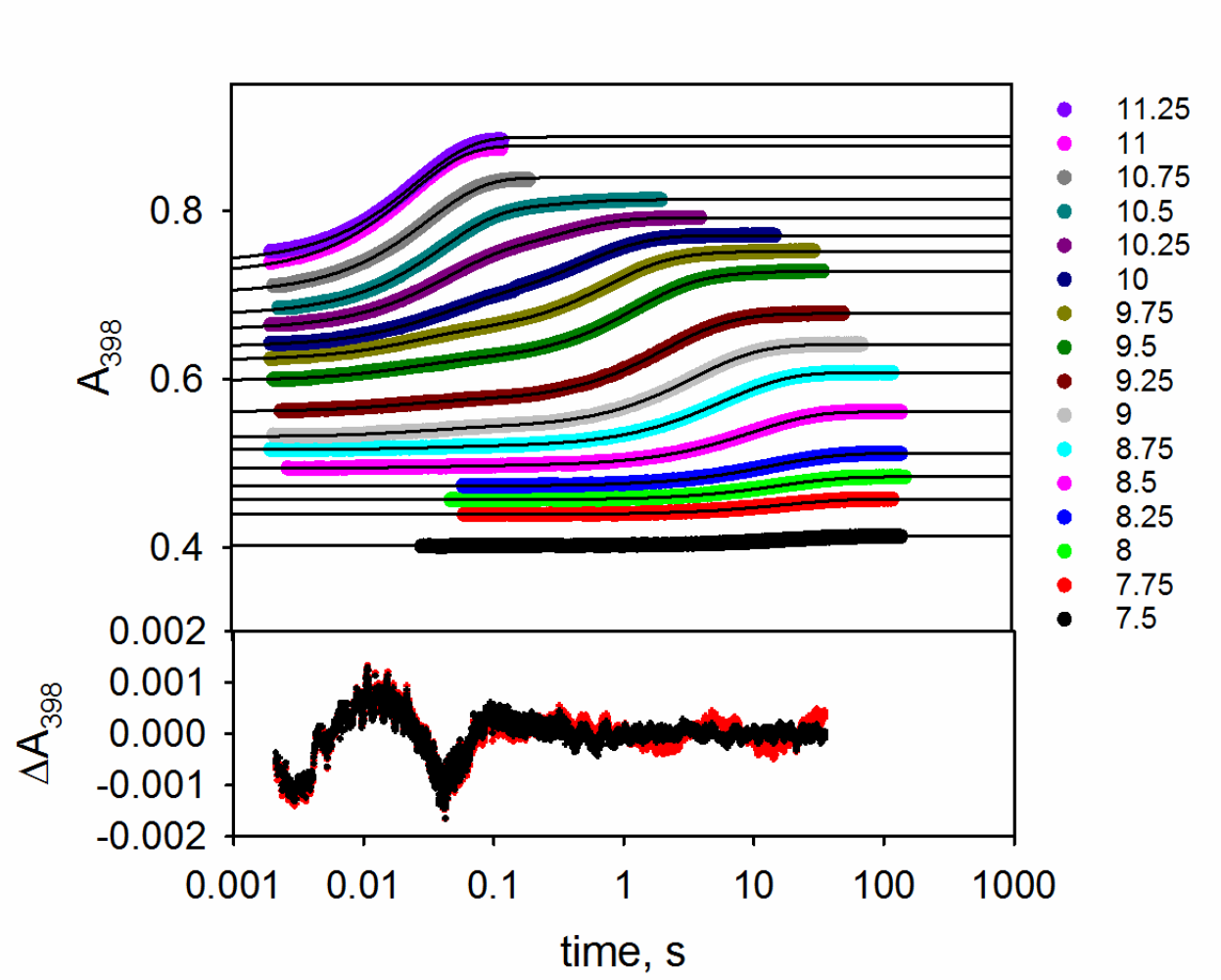

Figure S3. Absorbance at 398 nm, A_{398}, vs time stopped-flow data for pH jump experiments for A51V Hu Cyt*c* from pH 6 to ending pH values of 7.50-11.25. All data were collected at 25 °C. To allow the data at different pH values to be overlaid effectively, the observed A_{398} was adjusted at some pH values by a constant amount at every time point. The solid curves from pH 7.50-8.25 are fits to a two-exponential equation, from pH 8.50-10.00 are fits to a three-exponential equation, from pH 10.25-10.50 are fits to a two-exponential equation, and from pH 10.75-11.25 are fits to a single-exponential equation. The bottom panel compares residuals for the fit to a two-exponential equation (red) vs three-exponential equation (black) at pH 9.50.

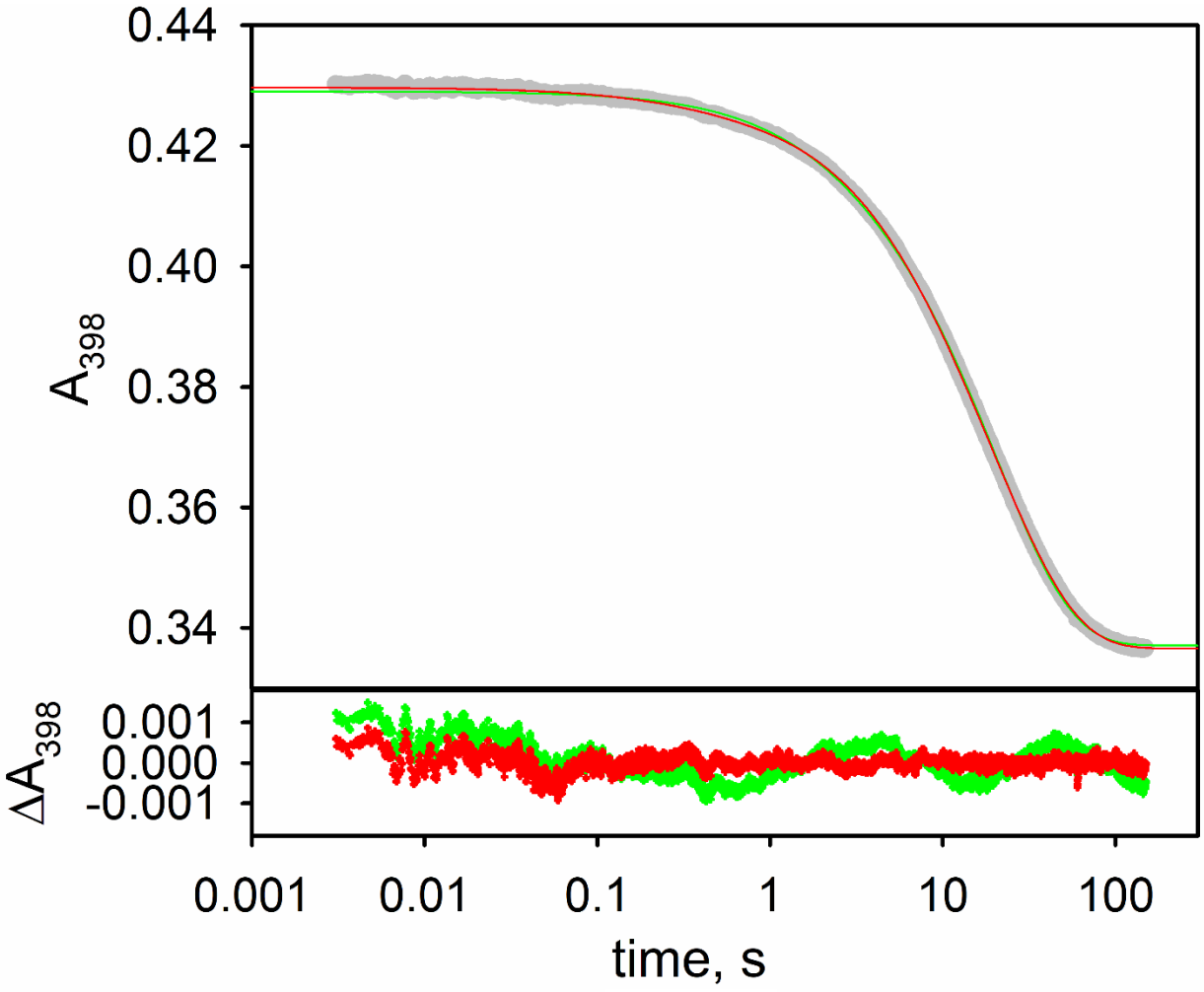

Figure S4. Representative fits of downward pH jump data (pH 10.5 to 7.75) to a three-exponential equation (red solid line) versus a two-exponential (green solid line) equation for A51V Hu Cyt*c* (upper panel). The bottom panel shows the residuals for two- (green dots) versus three- (red dots) exponential fits.

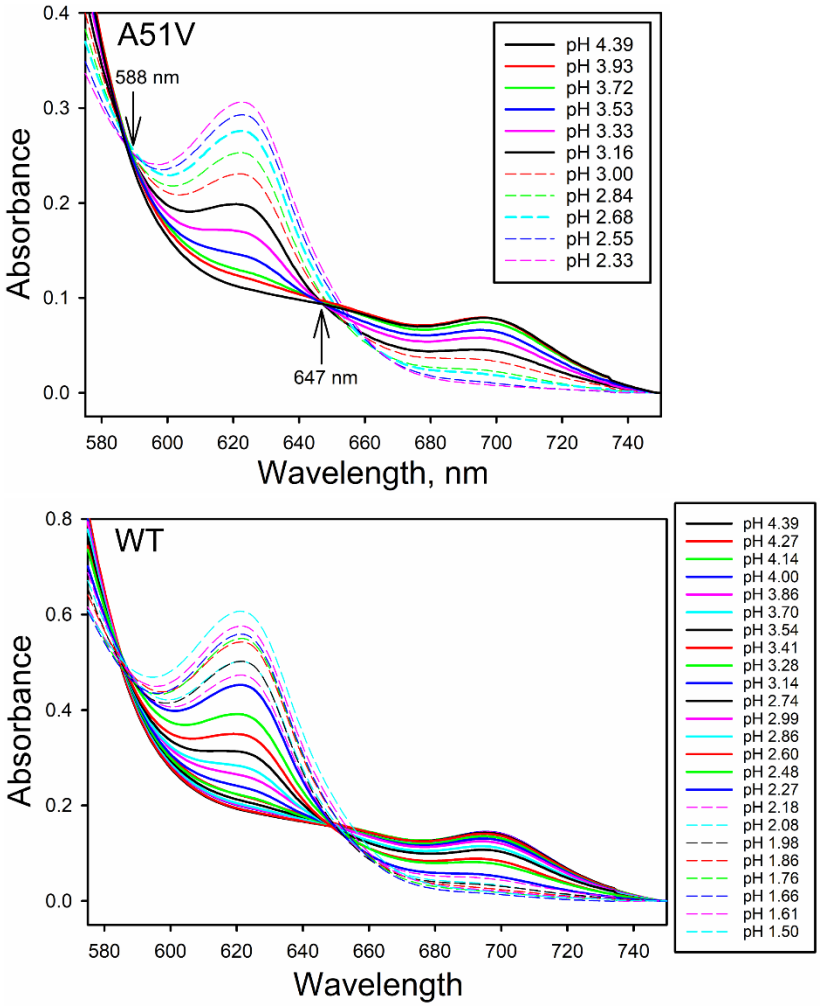

Figure S5. Spectra as a function of pH for the acid unfolding of the A51V variant (top panel) and WT (bottom panel) human cytochrome *c*. Spectra from 570 to 750 nm as a function of pH show that well-defined isosbestic points near 588 nm and 647 nm persist to near (A51V) or somewhat below (WT) the midpoint for acid unfolding (solid lines). The absorbance at 750 nm has been subtracted from the absorbance at each wavelength in each spectrum to correct for baseline drift during the titration.

Supporting Tables

Table S1. Oligonucleotide primers used for A51V site-directed mutagenesis[a]

primer	Primer sequence 5'-3'
A51V	d(tacagctacacggcg**gtg**aacaaaaacaaaggca)
A51V-r	d(tgcctttgtttttgtt**cac**cgccgtgtagctgta)

[a] Mutation sites are shown in bold and underlined

Table S2. Kinetic parameters for the alkaline transition of A51V Hu Cyt*c* obtained at 25 ºC from upward pH jump data monitored at 398 nm[a]

pH	A_{1u}	$k_{obs,1}$, s^{-1}	A_{2u}	$k_{obs,2}$, s^{-1}	A_{3u}	$k_{obs,3}$, s^{-1}
7.50	0.0067 ± 0.0009	0.038 ± 0.003	0.0047 ± 0.0006	0.08 ± 0.01	-	-
7.75	0.0091 ± 0.0005	0.041 ± 0.002	0.0090 ± 0.0003	0.084 ± 0.001	-	-
8.00	0.0138 ± 0.0003	0.042 ± 0.001	0.0138 ± 0.0002	0.086 ± 0.003	-	-
8.25	0.018 ± 0.001	0.044 ± 0.001	0.0209 ± 0.0008	0.097 ± 0.005	-	-
8.50	0.053 ± 0.002	0.082 ± 0.002	0.012 ± 0.002	0.21 ± 0.02	0.0031 ± 0.0005	23 ± 6
8.75	0.057 ± 0.009	0.118 ± 0.008	0.031 ± 0.009	0.28 ± 0.05	0.0040 ± 0.0007	35 ± 12
9.00	0.052 ± 0.006	0.170 ± 0.009	0.048 ± 0.006	0.41 ± 0.04	0.008 ± 0.002	72 ± 35
9.25	0.024 ± 0.004	0.18 ± 0.02	0.081 ± 0.005	0.52 ± 0.02	0.0125 ± 0.0009	63 ± 22
9.50	0.014 ± 0.002	0.21 ± 0.02	0.091 ± 0.002	0.81 ± 0.02	0.022 ± 0.003	51 ±17
9.75	0.0101 ± 0.0009	0.23 ± 0.03	0.0885 ± 0.0009	1.29 ± 0.02	0.032 ± 0.002	39 ± 3
10.00	0.007 ± 0.002	0.34 ± 0.09	0.078 ± 0.002	1.92 ± 0.09	0.049 ± 0.003	30 ± 3
10.25			0.050 ± 0.001	2.94 ± 0.06	0.0831 ± 0.0009	24.8 ± 0.7
10.50			0.016 ± 0.002	3.6 ± 0.5	0.118 ± 0.005	25 ± 1
10.75					0.140 ± 0.003	30.4 ± 0.7
11.00					0.1517 ± 0.0008	41.4 ± 0.6
11.25					0.150 ± 0.002	38.6 ± 0.3

[a]Parameters are the average and standard deviation from a minimum of 5 trials.

Table S3. Kinetic parameters for the alkaline transition of A51V Hu Cyt*c* obtained from downward pH jump data monitored at 398 nm[a]

pH	A_{1d}	$k_{obs,1}$, s^{-1}	A_{2d}	$k_{obs,2}$, s^{-1}	A_{3d}	$k_{obs,3}$, s^{-1}
7.00	0.0794 ± 0.0008	0.0430 ± 0.0003	0.0106 ± 0.0009	0.156 ± 0.009	0.005 ± 0.002	16 ± 4
7.50	0.067 ± 0.001	0.0441 ± 0.0007	0.017 ± 0.001	0.14 ± 0.01	0.004 ± 0.002	23 ± 4
7.75	0.077 ± 0.001	0.0446 ± 0.0008	0.014 ± 0.001	0.17 ± 0.02	0.004 ± 0.001	9 ± 4
8.00	0.056 ± 0.002	0.056 ± 0.002	0.014 ± 0.002	0.19 ± 0.03	0.004 ± 0.002	11 ± 5

[a]Parameters are the average and standard deviation from a minimum of 5 trials.

Table S4. Heme distortion in WT and THC-linked variants of human Cyt*c*[a]

Variant	D_{oop}, Å	B_{2u}, Å (sad)	B_{1u}, Å (ruf)	A_{2u}, Å (dom)	$E_{g(x)}$, Å (wav(x))	$E_{g(y)}$, Å (wav(y))	A_{1u}, Å (pro)
WT (3ZCF)	0.81 ± 0.02	0.23 ± 0.04	0.76 ± 0.02	0.025 ± 0.008	0.10 ± 0.02	0.08 ± 0.01	0.02 ± 0.01
A51V (6DUJ)	0.63 ± 0.04	0.173 ± 0.002	0.58 ± 0.05	0.08 ± 0.06	0.08 ± 0.06	0.09 ± 0.07	0.029 ± 0.003
G41S (3NWV)	1.0 ± 0.1	0.274 ± 0.005	0.9 ± 0.1	0.06 ± 0.01	0.20 ± 0.03	0.17 ± 0.02	0.017 ± 0.006
Y48H (5O10)	0.79 ± 0.01	0.29 ± 0.04	0.703 ± 0.004	0.06 ± 0.01	0.162 ± 0.008	0.133 ± 0.006	0.011 ± 0.009

[a]PDB code for the structure used to determine total out-of-place distortion, D_{oop}, and the normal mode compenents of the distortion using the NSD program are given in brackets below the name of each variant. The results reported here use the complete basis set. The errors are the standard deviation for the two hemes (A51V and Y48H) and four hemes (WT and G41S) in the assymetric unit of each structure.