

Tables and figure captions

Table S1.

Thermodynamic parameters of thermal unfolding of $0.32 \times 10^{-3} \text{ mol dm}^{-3}$ cytochrome c in presence of 4-chlorobutan-1-ol at different pH (Scan Rate 0.5 K min^{-1}).

Alcohol [†]	$t_{1/2}/^{\circ}\text{C}$	ΔH_{cal} (kJ.mol^{-1})	ΔC_p ($\text{kJ.K}^{-1}.\text{mol}^{-1}$)	ΔS ($\text{kJ.K}^{-1}.\text{mol}^{-1}$)	β ($\Delta H_{\text{VH}}/\Delta H_{\text{cal}}$)	σ^*
pH=4.0						
0	71.6	360	7.4	0.92	0.97	0.839
0	71.6	352	7.1	1.02	1.02	0.583
0	71.7	369	6.9	1.07	0.98	0.982
0	71.8	362	6.1	1.05	1.04	0.846
Average	71.6	360	6.9	1.05	1.00	
	± 0.1	± 7.0	± 0.6	± 0.07	± 0.03	
5	70.9	391	5.3	1.00	1.03	0.535
5	70.1	381	4.4	1.11	1.00	0.835
5	70.2	391	5.4	1.14	1.00	0.901
Average	70.4	387	5.0	1.13	1.01	
	± 0.4	± 6.0	± 0.6	± 0.07	± 0.02	
25 ^a	67.7	412	5.3	1.21	0.98	1.450
25 ^a	67.6	404	4.4	1.19	1.00	1.390
25 ^a	67.5	406	5.4	1.19	1.06	0.845
Average	67.6	407	5.3	1.20	1.01	
	± 0.1	± 4.0	± 0.1	± 0.01	± 0.04	
50 ^a	64.9	372	6.3	-	-	0.875
50 ^a	64.4	379	8.1	-	-	0.731
50 ^a	64.2	380	7.2	-	-	0.754
Average	64.5	377	7.2			
	± 0.4	± 4.0	± 0.9			
75	60.6	364	5.9	-	-	1.490
75	60.5	367	3.1	-	-	1.460
75	60.1	370	4.5	-	-	0.868
Average	60.4	367	4.5			
	± 0.3	± 3.0	± 1.4			

Alcohol [†]	$t_{1/2}/^{\circ}\text{C}$	ΔH_{cal} (kJ.mol ⁻¹)	ΔC_P (kJ.K ⁻¹ .mol ⁻¹)	ΔS (kJ.K ⁻¹ .mol ⁻¹)	σ^*
100	55.7	372	6.5	-	0.872
100	55.7	378	9.7	-	1.120
100	55.6	373	8.1	-	0.882
100	55.7	376	6.0	-	0.876
Average	55.7 ±0.1	375 ±3.0	7.6 ±1.7		
125	53.2	348	5.8	-	0.885
125	53.1	337	4.8	-	0.842
125	53.2	343	6.0	-	0.776
Average	53.1 ±0.1	343 ±6.0	5.5 ±0.6		
150	48.3	265	5.8	-	1.060
150	48.6	259	4.0	-	0.738
150	48.5	263	4.5	-	0.952
Average	48.5 ±0.2	262 ±3.0	4.8 ±0.9		
175	42.9	206	3.6	-	0.663
175	43.4	203	3.9	-	0.724
175	43.0	215	3.1	-	0.697
Average	43.1 ±0.3	208 ±6.0	3.5 ±0.4		
200	35.3	134	3.6	-	0.449
200	36.0	143	4.0	-	0.398
200	35.0	137	3.8	-	0.441
Average	35.4 ±0.5	138 ±5.0	3.8 ±0.20		
pH=6.0					
25	71.7	359	7.6	-	1.130
25	71.2	360	4.5	-	0.977
25	71.4	367	6.1	-	0.956
Average	71.4 ±0.3	362 ±4.0	6.1 ±1.6		

Alcohol [†]	$t_{1/2}/^{\circ}\text{C}$	ΔH_{cal} (kJ.mol ⁻¹)	ΔC_p (kJ.K ⁻¹ .mol ⁻¹)	ΔS (kJ.K ⁻¹ .mol ⁻¹)	σ^*
50	62.2	331	4.7	-	1.680
50	61.8	335	4.9	-	1.430
50	61.8	331	5.7	-	1.470
Average	61.9 ±0.3	332 ±0.2	5.1 ±0.5		
75	54.5	225	6.5	-	0.665
75	54.9	232	7.1	-	1.220
75	54.8	220	4.7	-	0.949
Average	54.7 ±0.2	225 ±6.0	6.1 ±1.2		
100	48.6	204	4.2	-	0.647
100	48.6	196	5.1	-	0.757
100	48.8	199	6.0	-	0.704
Average	48.7 ±0.1	200 ±4.0	5.1 ±0.9		
125	41.4	112	5.7	-	0.410
125	41.0	109	4.6	-	0.256
125	41.8	115	5.2	-	0.463
Average	41.4 ±0.4	112 ±3.0	5.2 ±0.6		
pH=7.0					
25	73.9	356	1.8	-	2.970
25	73.8	348	1.6	-	3.030
25	73.7	354	3.8	-	2.280
25	73.5	339	1.8	-	2.390
Average	±0.2	349 ±8.0	2.3 ±1.0		
50	64.8	336	8.9	-	0.886
50	64.5	334	4.9	-	0.999
50	64.7	353	7.7	-	0.771
Average	64.7 ±0.2	341 ±10.0	7.2 ±2.1		

Alcohol [†]	$t_{1/2}/^{\circ}\text{C}$	ΔH_{cal} (kJ.mol ⁻¹)	ΔC_p (kJ.K ⁻¹ .mol ⁻¹)	ΔS (kJ.K ⁻¹ .mol ⁻¹)	σ^*
75	58.6	314	4.6	-	1.510
75	58.0	311	4.8	-	1.530
75	58.4	305	5.4	-	1.460
Average	58.3 ±0.3	310 ±5.0	4.9 ±0.40		
100	52.4	256	5.2	-	0.986
100	52.1	244	4.4	-	0.937
100	51.9	245	1.9	-	0.950
Average	52.1 ±0.3	248 ±7.0	3.8 ±1.7		
125	43.1	181	2.4	-	0.479
125	43.6	177	3.2	-	0.457
125	43.4	177	3.9	-	0.387
Average	43.4 ±0.3	178 ±2.0	3.2 ±0.8		
150	35.9	156	4.0	-	0.379
150	35.5	144	3.8	-	0.347
150	36.0	152	2.3	-	0.383
Average	35.8 ±0.3	151 ±0.6	3.4 ±0.9	-	

a σ is standard deviation of the fit in kJ.K⁻¹.mol⁻¹

[†] The concentration of alcohol and protein is in 10⁻³ mol dm⁻³

Table S2.

Thermodynamic parameters of unfolding of $0.32 \times 10^{-3} \text{ mol dm}^{-3}$ cytochrome c in presence of 4-chlorobutan-1-ol at different pH (Scan rate dependence)

Scan Rate (K min ⁻¹)	Alcohol [†]	$t_{1/2}/^{\circ}\text{C}$	ΔH_{cal} (kJ.mol ⁻¹)	ΔC_p (kJ.K ⁻¹ .mol ⁻¹)	σ^*
pH=4.0					
0.1	100	46.9	252	5.4	1.47
0.1	100	46.3	249	3.9	1.54
0.1	100	46.3	248	3.9	1.11
Average	100	46.5	250	4.4	
	100	±0.3	±2.0	±0.9	
0.3	100	53.6	318	3.6	0.688
0.3	100	53.9	330	5.1	0.455
0.3	100	54.0	318	3.7	0.681
Average	100	53.8	322	4.1	
		±0.2	±7.0	±0.8	
0.5	100	55.7	372	6.5	0.872
0.5	100	55.7	378	7.0	1.120
0.5	100	55.6	373	8.1	0.882
0.5	100	55.7	376	6.0	0.876
Average	100	55.7	375	6.9	
		±0.1	±3.0	±0.9	
0.7	100	58.8	330	7.4	0.561
0.7	100	58.7	329	7.2	0.532
0.7	100	58.5	327	7.1	0.520
Average	100	58.7	329	7.2	
		±0.2	±2.0	±0.2	
1.0	100	61.0	303	6.4	0.866
1.0	100	60.8	292	6.6	0.923
1.0	100	61.7	305	4.0	0.332
Average	100	61.2	300	5.7	
		±0.5	±7.0	±1.4	
pH=6.0					
0.1	75	44.4	168	8.1	0.700
0.1	75	44.7	181	6.4	0.884
0.1	75	44.8	178	7.3	0.658
Average	75	44.6	175	7.3	
		±0.1	±7.0	±0.9	

Scan Rate (K min ⁻¹)	Alcohol [†]	t _{1/2} /°C	ΔH _{cal} (kJ.mol ⁻¹)	ΔC _p (kJ.K ⁻¹ .mol ⁻¹)	σ*
0.3	75	51.4	202	3.7	0.859
0.3	75	51.8	204	4.1	0.823
0.3	75	52.0	200	5.9	1.090
Average	75	51.7 ±0.3	202 ±2.0	4.6 ±1.2	
0.5	75	54.5	225	6.5	0.665
0.5	75	54.9	230	7.1	1.220
0.5	75	54.8	220	4.7	0.949
Average	75	54.7 ±0.2	225 ±5.0	6.1 ±1.2	
0.7	75	57.1	246	3.8	0.733
0.7	75	56.8	250	3.2	0.753
0.7	75	57.1	254	4.9	0.547
Average	75	57.0 ±0.2	250 ±4.0	4.0 ±0.9	
1.0	75	61.1	277	4.2	0.764
1.0	75	61.5	275	4.8	1.200
1.0	75	61.2	275	4.3	1.080
1.0	75	61.2	285	3.2	0.963
Average	75	61.3 ±0.2	278 ±5.0	4.1 ±0.7	
pH=7.0					
0.1	100	43.2	170	4.3	0.571
0.1	100	42.8	180	4.1	0.702
0.1	100	42.8	181	3.9	0.792
Average	100	42.9 ±0.2	177 ±6.0	4.1 ±0.2	
0.3	100	49.0	218	1.3	0.569
0.3	100	49.4	233	3.2	0.865
0.3	100	49.4	234	2.5	0.742
Average	100	49.3 ±0.2	228 ±9.0	2.3 ±1.0	
0.5	100	52.4	256	5.2	0.986
0.5	100	52.1	244	4.4	0.937
0.5	100	51.9	245	4.8	0.950

Average **100** **52.1** **248** **4.8**
 ±0.3 **±7.0** **±0.4**

Scan Rate (K min ⁻¹)	Alcohol [†]	t _{1/2} /°C	ΔH _{cal} (kJ.mol ⁻¹)	ΔC _p (kJ.K ⁻¹ .mol ⁻¹)	σ*
0.7	100	54.7	276	1.3	0.953
0.7	100	54.8	273	1.4	0.959
0.7	100	54.6	270	0.4	0.993
Average	100	54.7 ±0.1	273 ±3.0	1.0 ±0.6	
1.0	100	56.7	233	2.1	0.893
1.0	100	57.1	239	0.6	0.976
1.0	100	56.7	234	0.0	1.010
1.0	100	57.4	229	2.2	1.090
Average	100	57.0 ±0.3	234 ±4.0	1.2 ±1.1	

Table S3.

Thermodynamic parameters of thermal unfolding of cytochrome c in presence of 4-chlorobutan-1-ol at different pH (Scan Rate 0.5 K min⁻¹)

Protein [†]	Alcohol [†]	t _{1/2} /°C	ΔH _{cal} (kJ.mol ⁻¹)	ΔC _p (kJ.K ⁻¹ .mol ⁻¹)	σ*
pH=4.0					
0.16	100	54.1	352	6.9	0.664
0.16	100	53.6	343	5.2	0.601
0.16	100	53.8	341	7.3	0.689
Average	100	53.8 ±0.3	345 ±6.0	6.5 ±1.2	0.654 ±0.02
0.24	100	55.1	355	5.8	0.700
0.24	100	55.0	356	3.6	0.754
0.24	100	55.0	350	5.5	0.687
Average	100	55.0 ±0.1	354 ±3.0	4.9 1.2	0.714
0.32	100	55.7	372	6.5	0.872
0.32	100	55.7	378	9.7	1.120
0.32	100	55.6	373	8.1	0.882
0.32	100	55.7	376	6.0	0.876
Average	100	55.7 ±0.1	375 ±3.0	7.6 ±1.7	0.913
0.40	100	56.6	351	4.0	0.809
0.40	100	56.8	356	5.8	0.623
0.40	100	56.8	344	4.2	0.834
Average	100	56.7 ±0.1	350 ±6.0	4.7 ±1.0	0.755
0.48	100	57.6	330	6.7	0.657
0.48	100	58.1	334	4.5	0.707
0.48	100	57.4	327	4.6	0.608
Average	100	57.7 ±0.4	330 ±4.0	5.3 ±1.2	0.658
0.57	100	59.1	321	5.4	0.964
0.57	100	59.2	331	5.2	0.737
0.57	100	58.7	328	5.6	0.775
Average	100	59.0	327	5.4	0.825

		± 0.3	± 5.0	± 0.2	
Protein [†]	Alcohol [†]	$t_{1/2}/^{\circ}\text{C}$	ΔH_{cal} ($\text{kJ}\cdot\text{mol}^{-1}$)	ΔC_{P} ($\text{kJ}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$)	σ^*
pH=6.0					
0.08	75	47.8	178	5.9	0.591
0.08	75	47.8	182	3.9	0.829
0.08	75	47.8	172	4.0	0.662
Average	75	47.8 ± 0.0	177 ± 5.0	4.6 ± 1.1	
0.16	75	50.0	194	4.5	0.833
0.16	75	50.3	197	4.6	0.929
0.16	75	50.5	192	3.4	0.920
Average	75	50.3 ± 0.3	194 ± 3.0	4.2 ± 0.7	
0.24	75	52.7	205	3.9	0.994
0.24	75	52.4	208	4.3	0.779
0.24	75	52.8	213	4.1	0.941
Average	75	52.6 ± 0.2	209 ± 4.0	4.1 ± 0.2	
0.32	75	54.5	225	6.5	0.665
0.32	75	54.9	230	7.1	1.220
0.32	75	54.8	220	4.7	0.949
Average	75	54.7 ± 0.2	225 ± 5.0	6.1 ± 1.2	
0.40	75	57.0	237	5.2	0.733
0.40	75	56.1	235	6.0	0.753
0.40	75	56.9	236	5.2	0.547
Average	75	56.7 ± 0.5	236 ± 1.0	5.5 ± 0.5	
0.48	75	59.0	252	5.0	1.560
0.48	75	58.2	251	5.1	1.560
0.48	75	58.2	241	5.1	1.420
Average	75	58.5 ± 0.5	248 ± 6.0	5.1 ± 0.1	

Protein [†]	Alcohol [†]	t _{1/2} /°C	ΔH_{cal} (kJ.mol ⁻¹)	ΔC_p (kJ.K ⁻¹ .mol ⁻¹)	σ^*
0.57	75	59.6	261	4.6	1.390
0.57	75	59.7	267	4.1	1.350
0.57	75	60.1	270	6.1	1.390
Average		59.8	266	4.9	
		±0.3	±5	±1.0	

Figure Legends (in supplementary information)

Figure S1. A plot of $\Delta t_{1/2}$ versus alcohol concentration for the thermal denaturation of $0.32 \times 10^{-3} \text{ mol dm}^{-3}$ cytochrome c in presence of varying concentrations of 4-chlorobutan-1-ol, pH 4.0.

Figure S2. A plot of ΔH_{cal} versus alcohol concentration for the thermal denaturation of $0.32 \times 10^{-3} \text{ mol dm}^{-3}$ cytochrome c in presence of varying concentrations of 4-chlorobutan-1-ol, pH 4.0.

Figure S3. A plot of $t_{1/2}$ versus protein concentration for thermal denaturation of cytochrome c in presence of $100 \times 10^{-3} \text{ mol dm}^{-3}$ 4-chlorobutan-1-ol, pH 4.0.

Figure S4. A plot of ΔH_{cal} versus protein concentration for thermal denaturation of cytochrome c in presence of $100 \times 10^{-3} \text{ mol dm}^{-3}$ 4-chlorobutan-1-ol at pH 4.0.

Figure S5. Fluorescence emission spectra of $16 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c at pH 4.0 in (B) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (C) 25 ; (D) 50 ; (E) 50 at 69.0 °C; (F) 50 at 83.0 °C; (G) 100 ; (H) 100 at 70.0 °C; (I) 200 ; and (J) $225 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S6. Stern-Volmer plot for quenching of the intrinsic fluorescence of $24 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c in presence of (■) $50 \times 10^{-3} \text{ mol dm}^{-3}$ 4-chlorobutan-1-ol at 69.0 °C; (●) $50 \times 10^{-3} \text{ mol dm}^{-3}$ 4-chlorobutan-1-ol at 83.0 °C; and (▲) $200 \times 10^{-3} \text{ mol dm}^{-3}$ 4-chlorobutan-1-ol by varying concentrations of acrylamide ($0 - 0.45 \text{ mol dm}^{-3}$) at pH 4.0.

Figure S7. A plot of $\ln(v/T_m^2)$ versus $1/T_m$ for thermal denaturation of $0.32 \times 10^{-3} \text{ mol dm}^{-3}$ cytochrome c in presence of $75 \times 10^{-3} \text{ mol dm}^{-3}$ 4-chlorobutan-1-ol at pH 6.0.

Figure S8. Near-UV CD spectra of $24 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c at pH 6.0 in (A) buffer; and in presence of varying concentrations of 4-chlorobutan-1-ol: (D) 50 and (F) $100 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S9. Far-UV CD spectra of $8 \times 10^{-3} \text{ mol dm}^{-3}$ cytochrome c at pH 6.0 in (A) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (D) 50 ; and (F) $100 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S10. Near-UV CD spectra of $24 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c at pH 7.0 in (A) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (B) 50; and (F) $125 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S11. Far-UV CD spectra of $8 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c at pH 7.0 in (A) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (D) 50 ; and (F) $125 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S12. Fluorescence emission spectra of $16 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c at pH 7.0 in (B) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (C) 25 ; (D) 100 ; (E) 100 at 70.0°C ; (F) 150 ; and (G) $175 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S13. Fluorescence emission spectra obtained from ANS ($11.6 \times 10^{-5} \text{ mol dm}^{-3}$) binding studies of $16 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c at pH 7.0 in (B) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (C) 25 ; (D) 100 ; (E) 100 at 70.0°C ; (F) 150 ; and (G) $175 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S14. Fluorescence emission spectra obtained from energy transfer between $16 \times 10^{-6} \text{ mol dm}^{-3}$ cytochrome c and ANS ($11.6 \times 10^{-5} \text{ mol dm}^{-3}$) at pH 7.0 in (B) buffer; and in the presence of varying concentrations of 4-chlorobutan-1-ol: (C) 25 ; (D) 100 ; (E) 100 at 70.0°C ; (F) 150 ; and (G) $175 \times 10^{-3} \text{ mol dm}^{-3}$.

Figure S15. Stern-Volmer plot for quenching of the intrinsic fluorescence of 24×10^{-6} mol dm⁻³ cytochrome c in presence of (■) 150×10^{-3} mol dm⁻³ 4-chlorobutan-1-ol; (●) 100×10^{-3} mol dm⁻³ 4-chlorobutan-1-ol at 70.0 °C by varying concentrations of acrylamide (0 – 0.45 mol dm⁻³) at pH 7.0.

