Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for:

Thermal Stability Modulation of Native and Chemically-Unfolded State of Bovine Serum Albumin by Amino Acids

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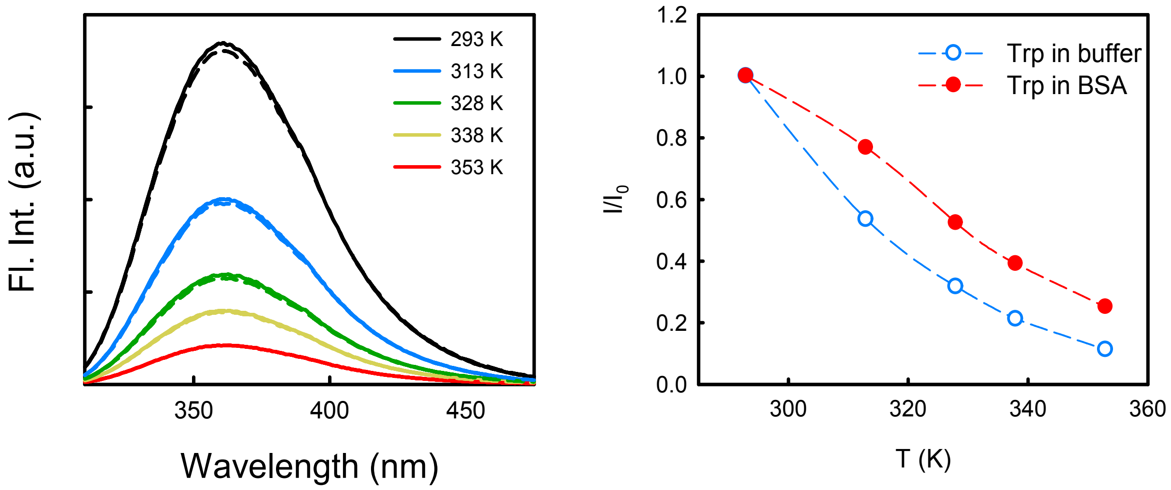

Figure S1. (a) Emission spectrum of Trp (0.08 M) in buffer at different temperatures. The solid lines represent heating while the dotted lines represent re-cooled Trp in buffer. (b) I/I0 (at the peak) vs. temperature profile of Trp emission in buffer (blue hollow symbol) and in BSA (red filled symbol).

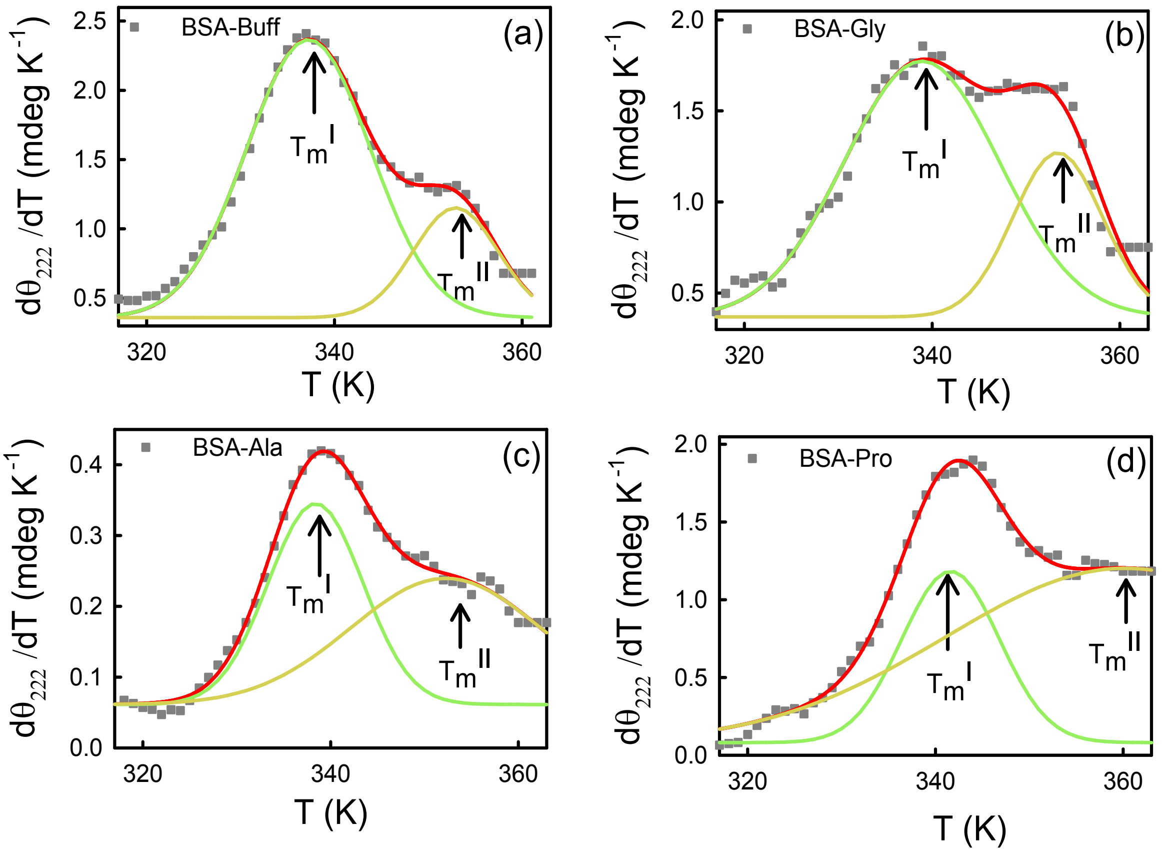

Figure S2. First derivative (followed by filtering by Savitzky-Golay least square method by Origin 8.5) of molar ellipticity at 222 nm with respect to temperature for BSA-buffer and BSA-AA systems. The experimental points (grey circles) are fitted with two Gaussian curves corresponding to two melting processes: (I) green and (II) yellow. The red broken line is the overall fit.

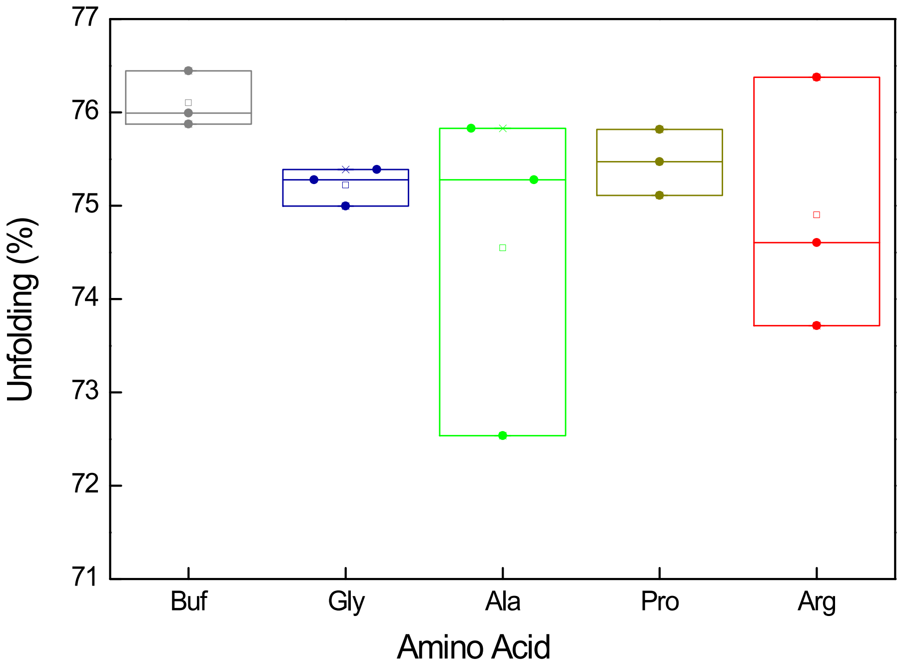

Figure S3. Significance test for unfolding (%) measured by temperature dependent fluorescence technique.

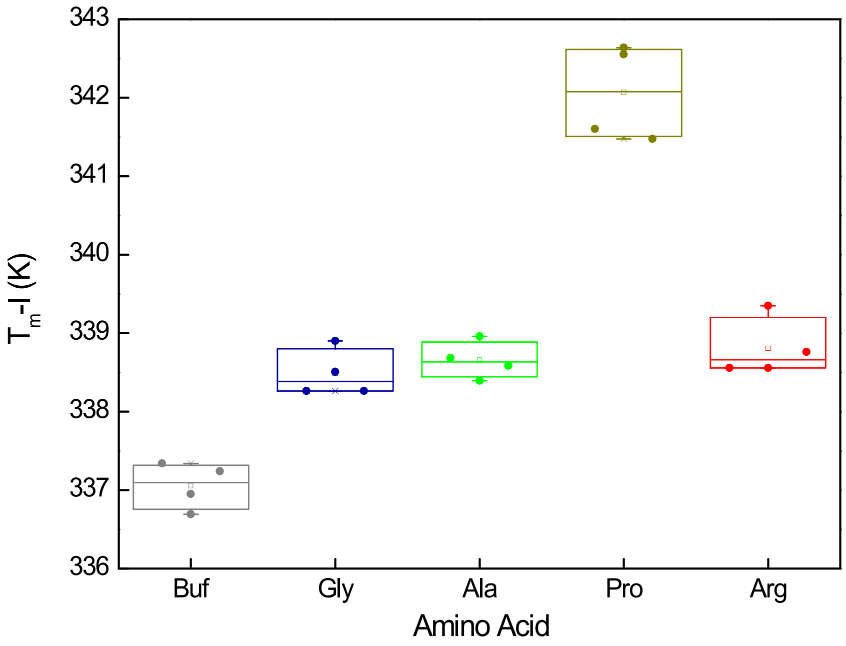

Figure S4. Significance test for T_m-I for all amino acids.

Table S1. Secondary structural parameters of BSA in buffer and in presence of different amino acids

System	**α-Helix (%)**	**β-Antiparallel (%)**	**β-Parallel (%)**	**β-Turn (%)**	**Random Coil (%)**
Buffer	61.18	3.49	4.29	12.57	18.46
Gly	68.27	2.55	3.33	11.26	14.69
Arg	68.85	2.44	3.22	11.13	14.26
Ala	76.97	1.62	2.19	9.42	9.90
Pro	77.07	1.52	2.19	9.32	9.89
4M Urea					
Buffer	47.71	5.49	6.31	14.95	25.64
Gly	49.80	5.18	5.89	14.63	24.49
Arg	48.88	5.29	6.10	14.73	25.00
Ala	52.79	4.66	5.47	14.08	22.90
Pro	53.54	4.55	5.36	13.97	22.57

Table S2. Fitting parameters for the fit of native fraction of BSA in buffer and in different amino acids by sigmoidal fitting.

System	**In buffer**			**In presence of 4M urea**		
	T_m at $\varphi=0.5$ (K)	**T_m from Sigmoidal fit (K)**	**a**	**T_m at $\varphi=0.5$ (K)**	**T_m from Sigmoidal fit (K)**	**a**
Buffer	338	338.2 ± 0.2	0.96	326	323.8 ± 0.5	1.09
Gly	339	340.3 ± 0.2	0.96	328	326.3 ± 0.4	1.05
Ala	342	342.7 ± 0.2	0.99	332	330.3 ± 0.4	0.99
Pro	344	344.7 ± 0.1	0.97	325	323.6 ± 0.5	1.02
Arg	342	342.1 ± 0.1	0.98	322	319.8 ± 0.7	1.12

Table S3. T_m values obtained from temperature dependent CD measurements

System	**Buffer**		**4M Urea**	
	T_m^{I} (K)	**T_m^{II} (K)**	**T_m^{I} (K)**	**T_m^{II} (K)**
Buffer	337.3 ± 0.1	352.9 ± 0.3	310.4 ± 0.5	333.3 ± 0.6
Gly	338.8 ± 0.6	353.4 ± 0.4	311.2 ± 0.5	335.3 ± 0.6
Ala	338.4 ± 0.2	352.3 ± 1.5	317.4 ± 1.3	340.7 ± 0.7
Pro	341.6 ± 0.2	360.2 ± 2.5	313.1 ± 0.6	336.4 ± 0.6
Arg	338.5 ± 0.2	350.1 ± 1.6	315.0 ± 0.3	336.1 ± 0.4

Table S4. Thermodynamic parameters of BSA thermal unfolding in buffer and in presence of different amino acids obtained from nonlinear equation (4)

Sample	**Buffer**			**4M Urea**		
	ΔH_{VF} (kcal mol^{-1})	**$\Delta\Delta H_{VF}$ (kcal mol^{-1})**	**ΔC_p (kcal K^{-1} mol^{-1})**	**ΔH_{VF} (kcal mol^{-1})**	**$\Delta\Delta H_{VF}$ (kcal mol^{-1})**	**ΔC_p (kcal K^{-1} mol^{-1})**
Buffer	30.8±0.6	-	1.01±0.05	7.0±1.0	-	0.91±0.10
Gly	28.3±0.6	-2.5±0.8	1.14±0.05	9.5±0.7	2.5±1.2	0.99±0.15
Ala	36.9±0.5	6.1±0.8	0.59±0.03	22.3±0.6	15.3±1.2	0.51±0.05
Pro	34.2±0.9	3.4±1.1	0.76±0.05	19.7±3.0	12.7±3.2	0.29±0.05
Arg	36.4±0.5	5.6±0.8	0.41±0.03	13.2±0.2	6.2±1.0	0.78±0.02

Table S5. Thermodynamic parameters obtained by fitting in non-two state model of C_p vs temperature curve

System	**T_m^I (K)**	**ΔH_{cal}^I (kcal mol^{-1})**	**$\Delta\Delta H_{cal}$ (kcal mol^{-1})**	**ΔH_V^I (kcal mol^{-1})**	**ΔS^I (kcal mol^{-1} K^{-1})**	**$\Delta\Delta S$ (kcal mol^{-1}K^{-1})**	**T_m^{II} (K)**	**ΔH_{cal}^{II} (kcal mol^{-1})**	**ΔH_V^{II} (kcal mol^{-1})**	**ΔS^{II} (kcal mol^{-1} K^{-1})**
Buffer	336.1 ±0.2	157±0.3	-	69.7	0.46	-	350.0 ±0.4	52.5±0.3	95.2	0.14
Gly	335.6 ±0.1	115±0.3	-42±0.4	80.2	0.33	-0.13	350.6 ±0.5	16.2±0.2	152	0.07
Ala	337.7 ±0.1	163±0.6	6±0.7	70.2	0.46	0	350.9 ±0.3	96.3±0.6	88.8	0.29
Pro	336.0 ±0.1	146±0.3	-11±0.4	77.4	0.40	-0.06	345.0 ±0.3	39.5±0.3	109	0.14
Arg	338.0 ±0.2	151±0.3	-6±0.4	87.1	0.42	-0.04	350.5 ±0.3	37.9±0.2	116	0.13

Table S6. Significance test ANOVA single factor; calculated in excel 2016 for steady state fluorescence data for the analysis of unfolding (%)

System	α-value (Significance level)	p-value (value of marginal significance) for Unfolding (%)
Buf-Gly	0.05	0.013
Buf-Ala	0.05	0.206
Buf-Pro	0.05	0.319
Buf-Arg	0.05	0.207

Table S7. Significance test ANOVA single factor; calculated in excel 2016result for temperature dependent circular dichroism data for the analysis of T_m^I

System	α-value (Significance level)	p-value for T_m^I
Buf-Gly	0.05	3.2E-06
Buf-Ala	0.05	1.4E-04
Buf-Pro	0.05	6.1E-06
Buf-Arg	0.05	3.2E-04