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

Supplementary Information

Unusual Domain Response in a Multidomain Protein in Presence of Macromolecular Crowders

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Table 1: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 25 g/L of macromolecular crowder.

Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.75	23.4	0.77	22.7	0.65	25.3
1	0.66	25.1	0.77	23.0	0.78	22.3	0.64	25.3
2	0.63	25.5	0.75	23.3	0.77	22.5	0.64	25.2
3	0.58	26.5	0.74	23.7	0.74	22.9	0.64	25.1
4	0.51	27.4	0.73	25.6	0.63	24.8	0.61	25.5
5	0.43	28.9	0.58	26.9	0.55	26.1	0.59	25.7
6	0.33	30.9	0.40	29.4	0.41	28.5	0.56	26.1
7	0.31	31.4	0.24	30.7	0.36	29.7	0.53	26.5
8	0.29	31.8	0.45	31.2	0.33	30.1	0.49	27.3

Table 2: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 50 g/L of macromolecular crowder.

Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.85	21.0	0.78	22.5	0.57	26.7
1	0.66	25.1	0.86	20.6	0.79	22.1	0.56	26.7
2	0.63	25.5	0.84	21.0	0.77	22.4	0.56	26.6
3	0.58	26.5	0.81	21.6	0.75	22.8	0.56	26.4
4	0.51	27.4	0.71	23.7	0.64	24.6	0.52	27.1
5	0.43	28.9	0.62	25.1	0.56	26.0	0.53	26.8
6	0.33	30.9	0.49	27.4	0.42	28.4	0.51	26.9
7	0.31	31.4	0.43	28.3	0.36	29.5	0.49	27.3
8	0.29	31.8	0.41	28.7	0.34	29.9	0.46	27.8

Table 3: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 75 g/L of macromolecular crowder.

Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.85	20.7	0.81	21.9	0.55	27.1
1	0.66	25.1	0.86	20.3	0.83	21.3	0.53	27.3
2	0.63	25.5	0.85	20.8	0.82	21.3	0.53	27.2
3	0.58	26.5	0.82	21.4	0.78	22.2	0.49	27.7
4	0.51	27.4	0.71	23.5	0.71	23.3	0.48	27.8
5	0.43	28.9	0.63	24.8	0.58	25.5	0.47	27.8
6	0.33	30.9	0.49	27.1	0.47	27.4	0.44	28.2
7	0.31	31.4	0.43	28.6	0.37	29.4	0.45	28.1
8	0.29	31.8	0.41	28.2	0.33	30.2	0.45	28.0

Table 4: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 100 g/L of macromolecular crowder.

Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.86	20.6	0.85	20.8	0.55	28.3
1	0.66	25.1	0.88	20.0	0.86	20.4	0.53	28.1
2	0.63	25.5	0.87	20.0	0.84	20.7	0.53	27.9
3	0.58	26.5	0.83	21.2	0.82	21.3	0.49	28.0
4	0.51	27.4	0.76	22.5	0.71	23.4	0.48	28.4
5	0.43	28.9	0.63	24.7	0.63	24.8	0.47	28.9
6	0.33	30.9	0.52	26.6	0.49	27.1	0.44	29.1
7	0.31	31.4	0.42	28.5	0.43	28.1	0.45	29.1
8	0.29	31.8	0.38	29.3	0.41	28.4	0.45	29.6

Table 5: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 125 g/L of macromolecular crowder.

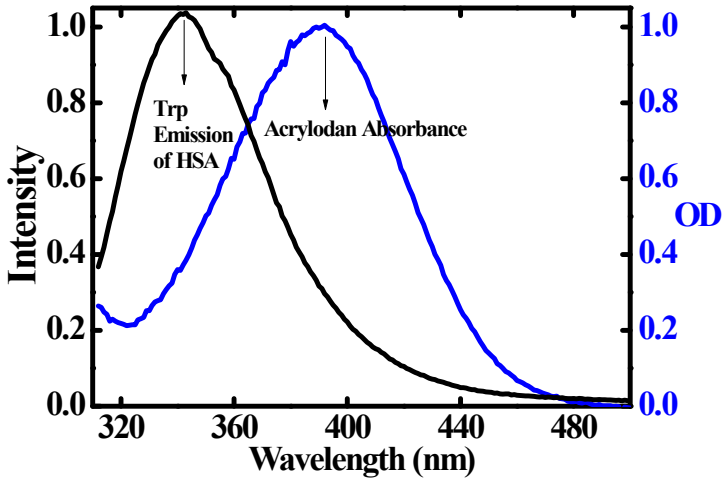
Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.86	20.5	0.89	19.7	0.48	28.4
1	0.66	25.1	0.87	20.1	0.90	19.1	0.48	28.3
2	0.63	25.5	0.85	20.5	0.88	19.6	0.46	28.4
3	0.58	26.5	0.83	21.2	0.85	20.3	0.44	28.7
4	0.51	27.4	0.72	23.3	0.75	22.6	0.43	28.8
5	0.43	28.9	0.64	24.8	0.66	24.1	0.40	29.1
6	0.33	30.9	0.50	27.1	0.53	26.4	0.38	29.5
7	0.31	31.4	0.44	28.2	0.47	27.4	0.35	30.1
8	0.29	31.8	0.42	28.6	0.45	27.7	0.33	30.5

Table 6: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 150 g/L of macromolecular crowder.

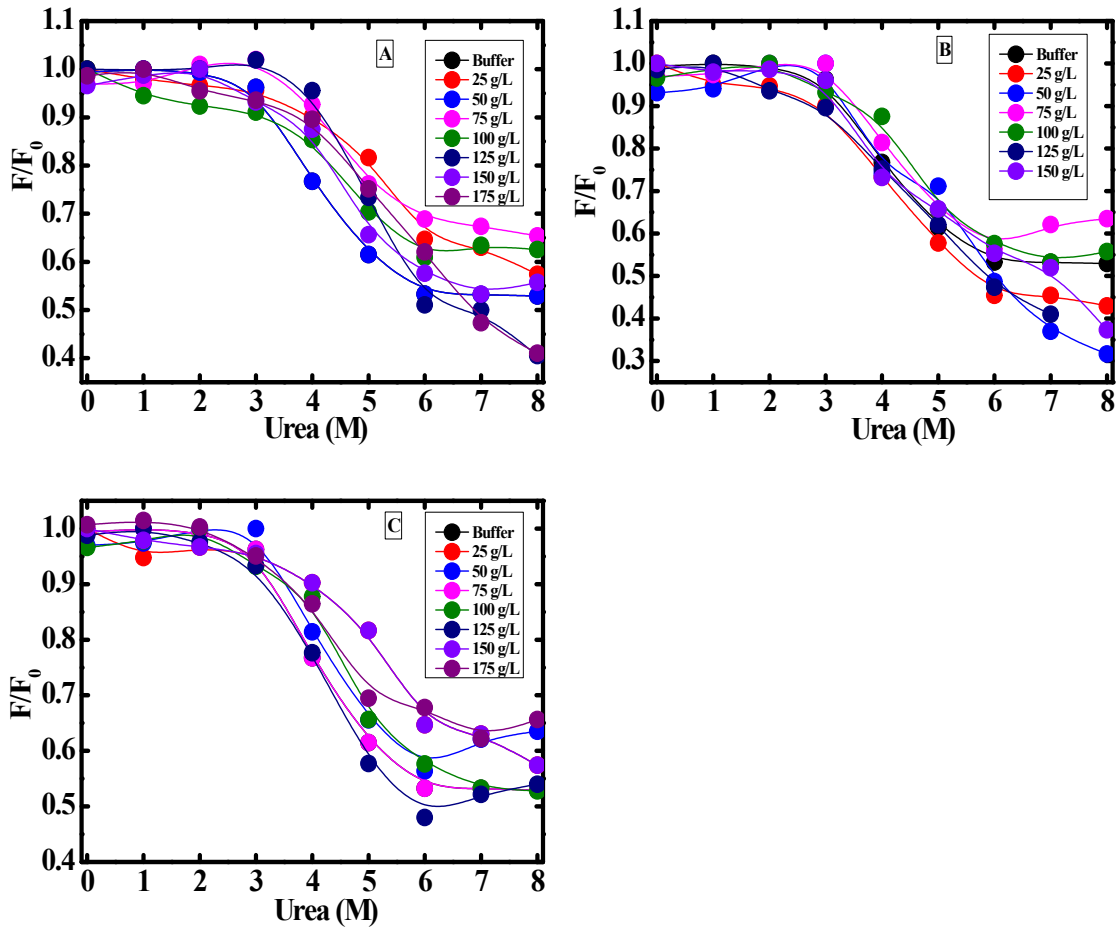
Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.86	20.3	0.90	19.3	0.49	28.5
1	0.66	25.1	0.88	19.9	0.91	18.8	0.45	28.8
2	0.63	25.5	0.86	20.3	0.89	19.3	0.43	29.1
3	0.58	26.5	0.83	21.0	0.87	20.0	0.42	29.1
4	0.51	27.4	0.73	23.2	0.76	22.4	0.39	29.7
5	0.43	28.9	0.64	24.7	0.68	23.9	0.36	30.1
6	0.33	30.9	0.51	27.0	0.54	26.2	0.30	31.3
7	0.31	31.4	0.45	28.0	0.48	27.2	0.29	31.6
8	0.29	31.8	0.43	28.5	0.46	27.5	0.28	31.8

Table 7: Efficiency and interdomain (domains I and II) distances during chemical denaturation of HSA in absence of any crowding agents and 175 g/L of macromolecular crowder.

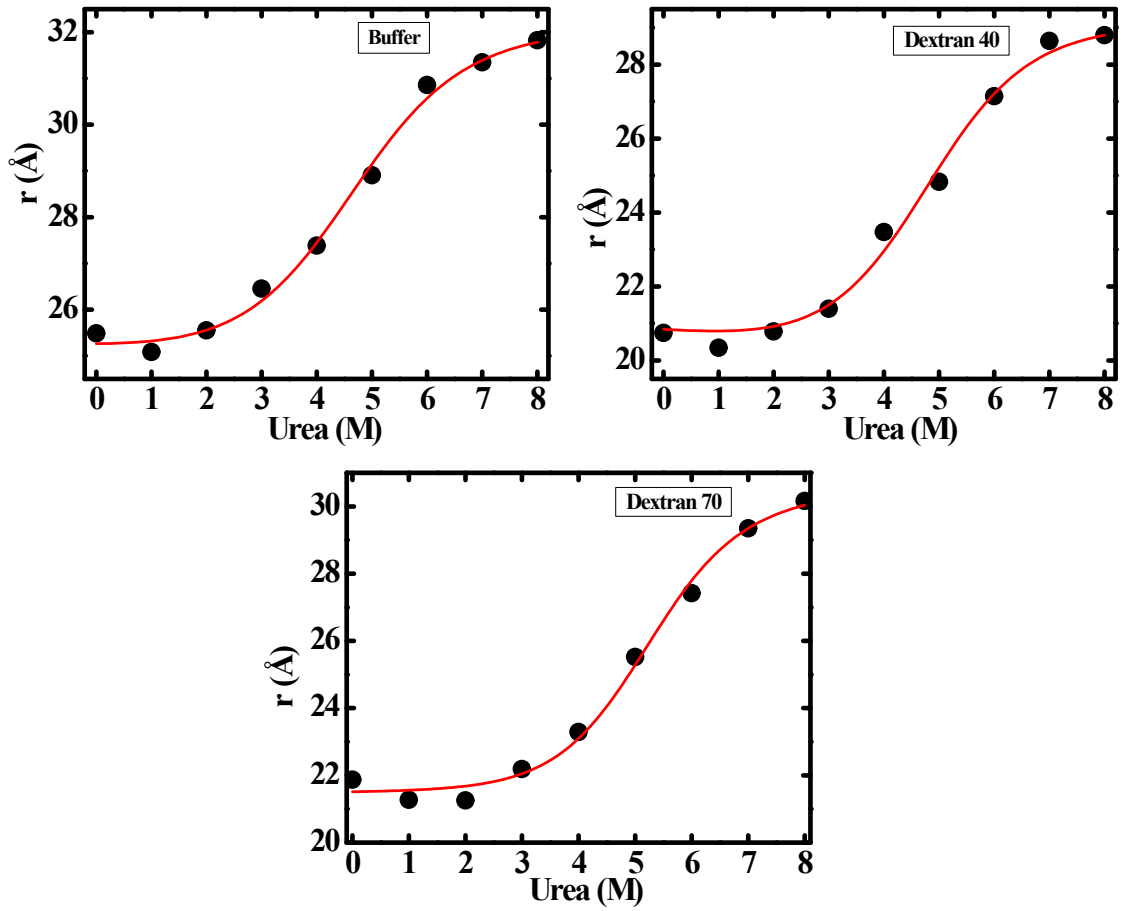
Urea (M)	**Buffer**		**Dextran 40**		**Dextran 70**		**Dextran 6**	
	E (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)	*E* (±0.02)	*r* (Å) (±0.3)
0	0.65	25.5	0.87	20.0	0.92	18.5	0.46	28.7
1	0.66	25.1	0.89	19.5	0.93	17.8	0.42	29.3
2	0.63	25.5	0.87	20.0	0.91	18.5	0.40	29.6
3	0.58	26.5	0.84	20.7	0.89	19.3	0.40	29.5
4	0.51	27.4	0.74	23.0	0.78	21.9	0.37	30.2
5	0.43	28.9	0.65	24.6	0.70	23.5	0.34	30.6
6	0.33	30.9	0.52	26.9	0.56	25.8	0.28	31.8
7	0.31	31.4	0.46	28.0	0.50	26.8	0.27	32.1
8	0.29	31.8	0.44	28.4	0.48	27.1	0.25	32.8



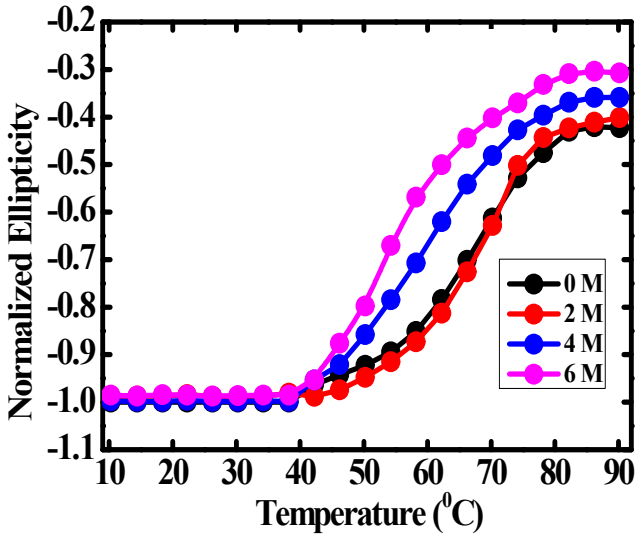
Supplementary Figure 1: Normalised absorption spectrum of acceptor (Ac) and emission spectrum of donor (Trp) in phosphate buffer.



Supplementary Figure 2: Normalised emission spectral trends of Trp in (A) Dextran 40, (B) Dextran 70 and (C) Dextran 6 with increasing concentration (as mentioned in legend). F_0 is the fluorescence intensity at a particular crowder concentration and 0 M urea while F is the same but in presence of increasing concentrations of the denaturant.



Supplementary Figure 3: The representative thermodynamic fits of chemical denaturation (as a function of Trp-Ac distance) of HSA in buffer and various crowded media.



Supplementary Figure 4: Normalized thermal denaturation profile of HSA as monitored using CD at different urea concentrations in Buffer.