Supporting Information

Correlated and Anti-correlated Domain Movement of Human Serum Albumin: A Peek into the Complexity of the Crowded Milieu

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Table S1: Variation of efficiency (E) and interdomain distance *r* (Å) in various pH solutions with increasing concentration of Dextran 6 (g/L).

Ac HSA					**NPA HSA**				
pH 1.9	Crowder (g/L)	R_0	r	e	pH 1.9	Crowder (g/L)	R_0	r	e
	0	25.00	27.03	0.38		0.00	24.01	26.81	0.34
	25	25.17	27.54	0.37		25.00	23.85	27.24	0.31
	50	25.31	28.04	0.35		50.00	23.85	27.70	0.29
	75	25.35	28.73	0.32		75.00	23.85	28.19	0.27
	100	25.41	29.28	0.30		100.00	23.86	29.08	0.23
	125	25.47	29.94	0.27		125.00	23.95	29.43	0.22
	150	25.48	30.25	0.26		150.00	23.85	29.88	0.21
	175	25.26	30.87	0.23		175.00	23.87	30.16	0.20
	200	25.19	31.53	0.21		200.00	23.87	30.54	0.19
pH 3.6					pH 3.6				
	0	25.30	26.71	0.42		0.00	23.81	25.25	0.41
	25	25.42	27.25	0.40		25.00	23.78	25.50	0.40
	50	25.61	27.93	0.37		50.00	23.70	26.28	0.35
	75	25.67	28.31	0.36		75.00	23.77	26.85	0.32
	100	25.71	29.18	0.32		100.00	23.49	27.27	0.29
	125	25.74	29.57	0.30		125.00	23.53	27.70	0.27
	150	25.78	29.95	0.29		150.00	23.62	28.14	0.26
	175	25.62	30.32	0.27		175.00	24.12	28.50	0.27
	200	25.49	31.02	0.24		200.00	24.42	29.10	0.26
pH 5.5					pH 5.5				
	0	25.20	26.14	0.45		0.00	23.45	24.77	0.42
	25	24.90	26.48	0.41		25.00	23.45	25.02	0.40
	50	24.90	27.18	0.37		50.00	23.45	25.04	0.40
	75	25.10	27.61	0.36		75.00	23.46	25.09	0.40
	100	25.06	28.66	0.31		100.00	23.46	25.21	0.39
	125	24.72	29.05	0.28		125.00	23.46	25.69	0.37
	150	24.98	29.30	0.28		150.00	23.47	26.20	0.34
	175	24.80	29.75	0.25		175.00	23.47	26.72	0.31
	200	24.73	30.50	0.22		200.00	23.47	27.25	0.29

pH 7.4					pH 7.4				
	0	25.24	25.40	0.49		0.00	23.45	23.66	0.49
	25	25.12	25.30	0.49		25.00	23.46	23.50	0.50
	50	24.89	26.70	0.40		50.00	23.46	23.66	0.49
	75	24.92	27.10	0.38		75.00	23.45	23.29	0.51
	100	25.20	28.30	0.33		100.00	23.47	23.10	0.52
	125	25.14	28.40	0.32		125.00	23.47	22.69	0.55
	150	25.29	28.50	0.33		150.00	23.47	22.34	0.57
	175	25.25	28.70	0.32		175.00	23.47	21.77	0.61
	200	25.21	29.20	0.29		200.00	23.48	21.17	0.65
pH 9.15					pH 9.15				
	0	25.48	24.76	0.54		0.00	23.45	23.13	0.52
	25	25.62	24.66	0.56		25.00	23.47	22.97	0.53
	50	25.70	26.06	0.48		50.00	23.82	23.05	0.55
	75	25.74	26.46	0.46		75.00	23.53	22.21	0.59
	100	25.85	27.66	0.40		100.00	23.53	21.80	0.61
	125	26.08	27.76	0.41		125.00	23.63	21.21	0.66
	150	26.22	27.86	0.41		150.00	23.65	20.60	0.70
	175	26.18	28.06	0.40		175.00	23.67	20.10	0.73
	200	26.27	28.56	0.38		200.00	23.69	19.76	0.75
pH 10.5					pH 10.5				
	0	25.80	24.28	0.59		0.00	23.37	22.88	0.53
	25	25.82	24.18	0.60		25.00	23.46	22.72	0.55
	50	25.93	25.58	0.52		50.00	23.46	22.80	0.54
	75	26.07	25.98	0.51		75.00	22.47	21.96	0.53
	100	26.18	27.18	0.44		100.00	22.42	21.64	0.55
	125	26.21	27.28	0.44		125.00	22.17	20.87	0.59
	150	26.47	27.38	0.45		150.00	21.88	20.37	0.61
	175	26.47	27.58	0.44		175.00	21.59	19.80	0.63
	200	26.51	28.08	0.41		200.00	21.30	19.54	0.63

Table S2: Variation of efficiency (E) and interdomain distance *r* (Å) in various pH solutions with increasing concentration of Dextran 40 (g/L).

Ac HSA					**NPA HSA**				
pH 1.9	Crowder (g/L)	R_0	r	e	pH 1.9	Crowder (g/L)	R_0	r	e
	0	25.00	27.03	0.38		0.00	24.01	26.81	0.34
	25	25.75	25.04	0.54		25.00	23.85	25.58	0.40
	50	26.33	23.12	0.69		50.00	23.85	24.60	0.45
	75	25.75	22.17	0.71		75.00	23.85	23.71	0.51
	100	25.04	22.28	0.67		100.00	23.86	23.02	0.55
	125	25.60	22.18	0.70		125.00	23.95	22.75	0.58
	150	25.61	21.98	0.71		150.00	23.85	22.28	0.60
	175	25.62	21.68	0.73		175.00	23.87	22.02	0.62
	200	25.63	21.38	0.75		200.00	23.87	21.52	0.65
pH 3.6					pH 3.6				
	0	25.30	26.71	0.42		0.00	23.81	25.95	0.37
	25	25.09	24.08	0.56		25.00	23.78	24.16	0.48
	50	25.20	22.16	0.68		50.00	23.70	22.86	0.55
	75	25.13	21.21	0.73		75.00	23.77	22.10	0.61
	100	25.04	21.32	0.72		100.00	23.49	21.48	0.63
	125	25.01	21.22	0.73		125.00	23.53	21.16	0.65
	150	24.96	21.02	0.74		150.00	23.62	21.07	0.66
	175	24.91	20.72	0.75		175.00	24.12	20.79	0.71
	200	24.86	20.42	0.77		200.00	24.42	20.98	0.71
pH 5.5					pH 5.5				
	0	25.20	26.14	0.45		0.00	23.45	24.77	0.42
	25	25.16	23.78	0.58		25.00	23.45	23.28	0.51
	50	24.98	21.86	0.69		50.00	23.45	22.17	0.58
	75	24.93	20.91	0.74		75.00	23.46	20.80	0.67
	100	24.89	21.02	0.73		100.00	23.46	20.42	0.70
	125	24.78	20.92	0.73		125.00	23.46	20.05	0.72
	150	24.69	20.72	0.74		150.00	23.47	19.71	0.74
	175	24.61	20.42	0.75		175.00	23.47	19.59	0.75
	200	24.52	20.12	0.77		200.00	23.47	19.31	0.76
pH 7.4					pH 7.4				
	0	25.40	25.40	0.50		0.00	23.45	23.66	0.49
	25	25.74	23.36	0.64		25.00	23.46	22.57	0.56
	50	25.82	21.44	0.75		50.00	23.46	21.59	0.62

	75	25.94	20.49	0.80		75.00	23.45	20.25	0.71
	100	25.98	20.60	0.80		100.00	23.47	20.02	0.72
	125	26.18	20.50	0.81		125.00	23.47	19.69	0.74
	150	26.31	20.30	0.83		150.00	23.47	19.27	0.77
	175	26.45	20.00	0.84		175.00	23.47	19.14	0.77
	200	26.58	19.70	0.86		200.00	23.48	18.82	0.79
pH 9.15					pH 9.15				
	0	25.84	24.76	0.56		0.00	23.45	23.13	0.52
	25	26.02	23.01	0.68		25.00	23.47	22.13	0.59
	50	25.90	21.09	0.77		50.00	23.82	20.72	0.70
	75	25.80	20.14	0.82		75.00	23.53	20.04	0.72
	100	25.82	20.25	0.81		100.00	23.53	19.46	0.76
	125	25.80	20.15	0.81		125.00	23.63	19.25	0.77
	150	25.77	19.95	0.82		150.00	23.65	19.08	0.78
	175	25.75	19.65	0.83		175.00	23.67	18.81	0.80
	200	25.72	19.35	0.85		200.00	23.69	18.54	0.81
pH 10.5					pH 10.5				
	0	25.80	24.28	0.59		0.00	23.37	22.88	0.53
	25	25.75	22.83	0.67		25.00	23.46	21.82	0.61
	50	25.79	20.91	0.78		50.00	23.46	20.05	0.72
	75	25.83	19.96	0.82		75.00	22.47	19.31	0.71
	100	25.67	20.07	0.81		100.00	22.42	19.07	0.73
	125	25.71	19.97	0.82		125.00	22.17	18.80	0.73
	150	25.69	19.77	0.83		150.00	21.88	18.51	0.73
	175	25.67	19.47	0.84		175.00	21.59	18.47	0.72
	200	25.65	19.17	0.85		200.00	21.30	18.40	0.71

Table S3: Variation of efficiency (E) and interdomain distance *r* (Å) in various pH solutions with increasing concentration of Dextran 70 (g/L).

pH 1.9	Ac HSA				pH 1.9	NPA HSA			
	Crowder (g/L)	R_0	r	e		Crowder (g/L)	R_0	r	e
	0	25.00	27.03	0.38		0.00	23.26	26.81	0.30
	25	25.53	25.33	0.51		25.00	23.47	26.20	0.34
	50	25.74	24.43	0.58		50.00	23.47	25.39	0.38
	75	25.36	23.75	0.60		75.00	23.47	24.69	0.42
	100	24.60	22.84	0.61		100.00	23.47	23.43	0.50
	125	24.96	21.53	0.71		125.00	23.55	22.51	0.57
	150	24.86	20.93	0.74		150.00	23.60	21.80	0.62
	175	24.76	20.13	0.78		175.00	23.64	21.08	0.67
	200	24.67	19.53	0.80		200.00	23.68	20.05	0.73
pH 3.6					pH 3.6				
	0	25.30	26.71	0.42		0.00	23.43	25.95	0.35
	25	25.45	24.70	0.54		25.00	23.43	25.79	0.36
	50	25.31	23.81	0.59		50.00	23.43	25.18	0.39
	75	25.56	23.27	0.64		75.00	23.26	24.32	0.43
	100	25.31	22.17	0.69		100.00	23.26	22.98	0.52
	125	25.42	21.01	0.76		125.00	23.21	22.20	0.57
	150	25.44	20.67	0.78		150.00	23.16	21.41	0.62
	175	25.45	19.81	0.82		175.00	23.11	20.64	0.66
	200	25.46	19.11	0.85		200.00	23.06	19.60	0.73
pH 5.5					pH 5.5				
	0	25.20	26.14	0.45		0.00	23.45	24.77	0.42
	25	25.27	23.45	0.61		25.00	23.45	24.38	0.44
	50	25.35	23.24	0.63		50.00	23.45	24.07	0.46
	75	25.36	22.66	0.66		75.00	23.46	23.17	0.52
	100	25.51	21.57	0.73		100.00	23.46	22.09	0.59
	125	25.55	20.37	0.80		125.00	23.46	21.48	0.63
	150	25.62	20.05	0.81		150.00	23.47	20.75	0.68
	175	25.69	19.33	0.85		175.00	23.47	19.89	0.73
	200	25.76	18.54	0.88		200.00	23.47	18.82	0.79
pH 7.4					pH 7.4				
	0	25.40	25.40	0.50		0.00	23.45	23.66	0.49
	25	25.45	22.70	0.67		25.00	23.46	23.26	0.51
	50	25.47	22.50	0.68		50.00	23.46	23.01	0.53
	75	25.63	21.90	0.72		75.00	23.45	22.11	0.59

	100	25.85	20.80	0.79		100.00	23.47	21.07	0.66
	125	25.84	19.70	0.84		125.00	23.47	20.60	0.69
	150	25.93	19.30	0.85		150.00	23.47	19.87	0.73
	175	26.03	18.50	0.89		175.00	23.47	19.02	0.78
	200	26.12	17.80	0.91		200.00	23.48	18.00	0.83
pH 9.15					pH 9.15				
	0	25.48	24.76	0.54		0	23.45	23.13	0.52
	25	25.49	22.16	0.70		25.00	23.47	22.84	0.54
	50	25.50	21.88	0.71		50.00	23.82	22.42	0.59
	75	25.47	21.36	0.74		75.00	23.53	21.62	0.62
	100	25.62	20.21	0.81		100.00	23.53	20.41	0.70
	125	25.65	19.08	0.86		125.00	23.63	20.01	0.73
	150	25.61	18.69	0.87		150.00	23.65	19.04	0.79
	175	25.82	17.98	0.90		175.00	23.67	18.57	0.81
	200	25.91	17.26	0.92		200.00	23.69	17.81	0.85
pH 10.5					pH 10.5				
	0	25.80	24.28	0.59		0.00	23.37	22.88	0.53
	25	26.04	21.68	0.75		25.00	23.46	22.69	0.55
	50	25.89	21.41	0.76		50.00	23.46	22.38	0.57
	75	25.89	20.87	0.78		75.00	22.47	21.49	0.57
	100	25.74	19.80	0.83		100.00	22.42	20.39	0.64
	125	25.79	18.38	0.88		125.00	22.17	19.79	0.66
	150	25.76	17.81	0.90		150.00	21.88	18.82	0.71
	175	25.74	17.38	0.91		175.00	21.59	18.34	0.73
	200	25.71	16.81	0.93		200.00	21.30	17.69	0.75

Table S4: Variation of efficiency (E) and interdomain distance *r* (Å) in various pH solutions with increasing concentration of Ficoll 70 (g/L).

pH 1.9	Ac HSA				pH 1.9	NPA HSA			
	Crowder (g/L)	R_0	r	e		Crowder (g/L)	R_0	r	e
	0	25.00	27.03	0.38		0.00	23.26	26.81	0.30
	25	25.53	24.86	0.54		25.00	23.26	27.21	0.28
	50	25.74	24.10	0.60		50.00	23.26	27.97	0.25
	75	25.36	23.60	0.61		75.00	23.45	28.65	0.23
	100	24.60	22.61	0.62		100.00	23.45	29.02	0.22
	125	24.96	21.15	0.73		125.00	23.51	29.47	0.20
	150	24.86	20.43	0.76		150.00	23.56	29.82	0.20
	175	24.76	18.80	0.84		175.00	23.62	30.58	0.18
	200	24.67	17.23	0.90		200.00	23.68	31.05	0.16
pH 3.6					pH 3.6				
	0	25.30	26.71	0.42		0.00	23.43	25.95	0.35
	25	25.45	24.50	0.56		25.00	23.37	26.84	0.30
	50	25.31	23.77	0.59		50.00	23.26	27.63	0.26
	75	25.56	23.09	0.65		75.00	23.37	28.38	0.24
	100	25.31	22.32	0.68		100.00	23.37	28.76	0.22
	125	25.42	20.77	0.77		125.00	23.32	29.14	0.21
	150	25.44	19.88	0.81		150.00	23.31	29.48	0.20
	175	25.45	18.50	0.87		175.00	23.30	30.25	0.17
	200	25.46	16.92	0.92		200.00	23.29	30.73	0.16
pH 5.5					pH 5.5				
	0	25.20	26.14	0.45		0.00	23.45	24.77	0.42
	25	25.27	23.25	0.62		25.00	23.45	25.92	0.35
	50	25.35	22.78	0.66		50.00	23.46	26.71	0.31
	75	25.36	22.11	0.70		75.00	23.47	27.46	0.28
	100	25.51	21.48	0.74		100.00	23.46	27.81	0.26
	125	25.55	19.77	0.82		125.00	23.47	28.22	0.25
	150	25.62	18.62	0.87		150.00	23.47	28.56	0.24
	175	25.69	17.41	0.91		175.00	23.48	29.33	0.21
	200	25.76	15.97	0.95		200.00	23.48	29.81	0.19
pH 7.4					pH 7.4				
	0	25.40	25.40	0.50		0.00	23.37	23.66	0.48
	25	25.45	22.65	0.67		25.00	23.45	25.15	0.40
	50	25.47	22.14	0.70		50.00	23.45	25.94	0.35

	75	25.63	21.28	0.75		75.00	23.45	26.69	0.32
	100	25.85	20.60	0.80		100.00	23.46	27.04	0.30
	125	25.84	18.79	0.87		125.00	23.49	27.45	0.28
	150	25.93	17.82	0.90		150.00	23.51	27.79	0.27
	175	26.03	16.75	0.93		175.00	23.53	28.56	0.24
	200	26.12	15.24	0.96		200.00	23.54	29.04	0.22
pH 9.15					pH 9.15				
	0	25.48	24.76	0.54		0.00	23.45	23.13	0.52
	25	25.49	22.48	0.68		25.00	23.45	24.78	0.42
	50	25.50	22.07	0.70		50.00	23.46	25.57	0.37
	75	25.47	20.95	0.76		75.00	23.46	26.32	0.33
	100	25.62	20.42	0.80		100.00	23.45	26.67	0.32
	125	25.65	18.46	0.88		125.00	23.46	27.08	0.30
	150	25.61	17.67	0.90		150.00	23.46	27.42	0.28
	175	25.82	16.50	0.94		175.00	23.46	28.19	0.25
	200	25.91	14.80	0.97		200.00	23.46	28.67	0.23
pH 10.5					pH 10.5				
	0	25.80	24.28	0.59		0.00	23.37	22.88	0.53
	25	26.04	22.35	0.71		25.00	23.45	24.72	0.42
	50	25.89	21.97	0.73		50.00	23.45	25.51	0.38
	75	25.89	20.67	0.79		75.00	23.45	26.26	0.34
	100	25.74	20.34	0.80		100.00	23.46	26.61	0.32
	125	25.79	18.56	0.88		125.00	23.49	27.02	0.30
	150	25.76	17.62	0.91		150.00	23.51	27.36	0.29
	175	25.74	16.42	0.94		175.00	23.53	28.13	0.25
	200	25.71	14.78	0.97		200.00	23.54	28.61	0.24

Table S5: Variation of efficiency (E) and interdomain distance *r* (Å) in various pH solutions with increasing concentration of PEG8 (g/L).

pH 1.9	Ac HSA				pH 1.9	NPA HSA			
	Crowder (g/L)	R_0	r	e		Crowder (g/L)	R_0	r	e
	0	25.00	27.03	0.38		0.00	23.26	26.81	0.30
	25	25.17	26.32	0.43		25.00	23.35	27.21	0.29
	50	25.31	25.93	0.46		50.00	23.43	27.80	0.26
	75	25.35	25.20	0.51		75.00	23.40	28.40	0.24
	100	25.41	27.70	0.37		100.00	23.43	27.02	0.30
	125	25.47	28.78	0.32		125.00	23.41	26.62	0.32
	150	25.48	29.38	0.30		150.00	23.37	26.04	0.34
	175	25.26	30.02	0.26		175.00	23.40	25.32	0.38
	200	25.19	31.67	0.20		200.00	23.45	24.80	0.42
pH 3.6					pH 3.6				
	0	25.30	26.71	0.42		0.00	23.43	25.95	0.35
	25	25.42	25.91	0.47		25.00	23.42	27.47	0.28
	50	25.61	25.54	0.50		50.00	23.26	28.24	0.24
	75	25.67	24.80	0.55		75.00	23.38	29.02	0.21
	100	25.71	27.41	0.41		100.00	23.26	27.10	0.29
	125	25.74	28.42	0.36		125.00	23.26	26.67	0.31
	150	25.78	28.87	0.34		150.00	23.26	26.26	0.33
	175	25.62	29.68	0.29		175.00	23.38	25.41	0.38
	200	25.49	31.17	0.23		200.00	23.45	24.95	0.41
pH 5.5					pH 5.5				
	0	25.20	26.14	0.45		0.00	23.45	24.77	0.42
	25	24.90	25.21	0.48		25.00	23.46	28.01	0.26
	50	24.90	24.78	0.51		50.00	23.46	28.92	0.22
	75	25.10	23.85	0.58		75.00	23.46	29.75	0.19
	100	25.06	26.93	0.39		100.00	23.46	27.40	0.28
	125	24.72	27.91	0.33		125.00	23.46	27.15	0.29
	150	24.98	28.25	0.32		150.00	23.47	26.92	0.30
	175	24.80	28.92	0.28		175.00	23.47	26.07	0.35
	200	24.73	29.61	0.25		200.00	23.47	25.60	0.37
pH 7.4					pH 7.4				
	0	25.24	25.40	0.49		0.00	23.45	23.66	0.49
	25	25.12	24.60	0.53		25.00	23.46	28.58	0.23
	50	24.89	24.06	0.55		50.00	23.46	29.41	0.20

	75	24.92	22.98	0.62		75.00	23.46	30.21	0.18
	100	25.20	25.89	0.46		100.00	23.46	27.92	0.26
	125	25.14	26.87	0.40		125.00	23.46	27.70	0.27
	150	25.29	27.17	0.39		150.00	23.47	27.41	0.28
	175	25.25	27.81	0.36		175.00	23.47	26.60	0.32
	200	25.21	28.74	0.31		200.00	23.47	26.10	0.35
pH 9.15					pH 9.15				
	0	25.48	24.76	0.54		0.00	23.45	23.13	0.52
	25	25.62	24.21	0.58		25.00	23.46	28.81	0.23
	50	25.70	23.69	0.62		50.00	23.46	29.57	0.20
	75	25.74	22.72	0.68		75.00	23.46	30.47	0.17
	100	25.85	25.17	0.54		100.00	23.46	28.35	0.24
	125	26.08	26.41	0.48		125.00	23.46	28.14	0.25
	150	26.22	26.68	0.47		150.00	23.47	27.52	0.28
	175	26.18	27.41	0.43		175.00	23.47	26.68	0.32
	200	26.27	28.46	0.38		200.00	23.47	26.25	0.34
pH 10.5					pH 10.5				
	0	25.80	24.28	0.59		0.00	23.37	22.88	0.53
	25	25.82	24.16	0.60		25.00	23.45	28.90	0.22
	50	25.93	23.58	0.64		50.00	23.37	29.60	0.19
	75	26.07	22.58	0.70		75.00	23.37	30.51	0.17
	100	26.18	24.95	0.57		100.00	23.46	28.40	0.24
	125	26.21	26.17	0.50		125.00	23.43	28.25	0.25
	150	26.47	26.37	0.51		150.00	23.44	27.60	0.27
	175	26.47	27.10	0.46		175.00	23.45	26.72	0.31
	200	26.51	28.31	0.40		200.00	23.46	26.31	0.33

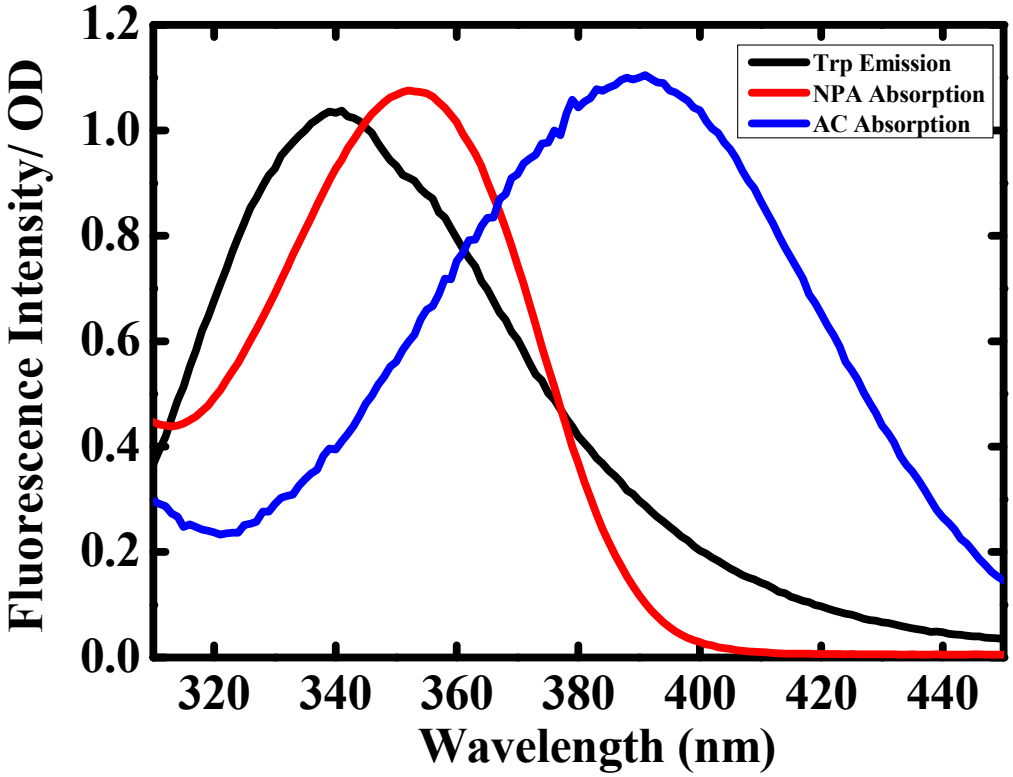

Figure S1: Normalised absorption spectra of the acceptors, acrylodan (Ac) and p-nitro-phenyl anthranilate (NPA), and emission spectrum of donor (Trp-214) in phosphate buffer.

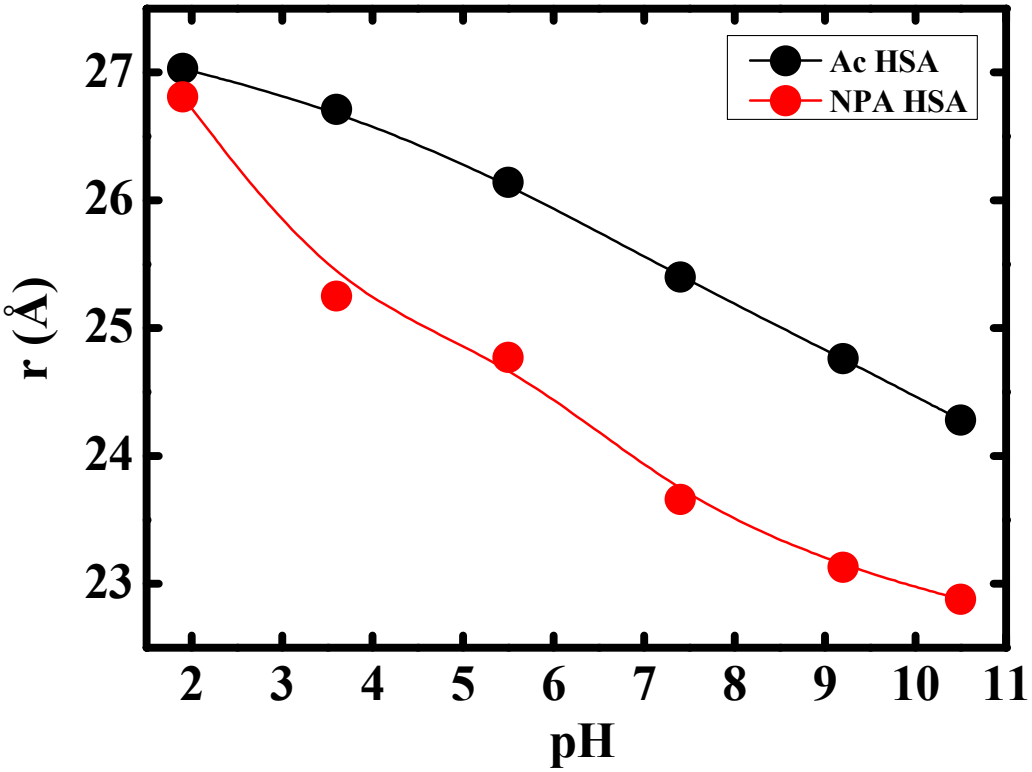

Figure S2: Interdomain distance changes between domains I-II (Ac-HSA) and domains II-III (NPA-HSA) with increasing pH.

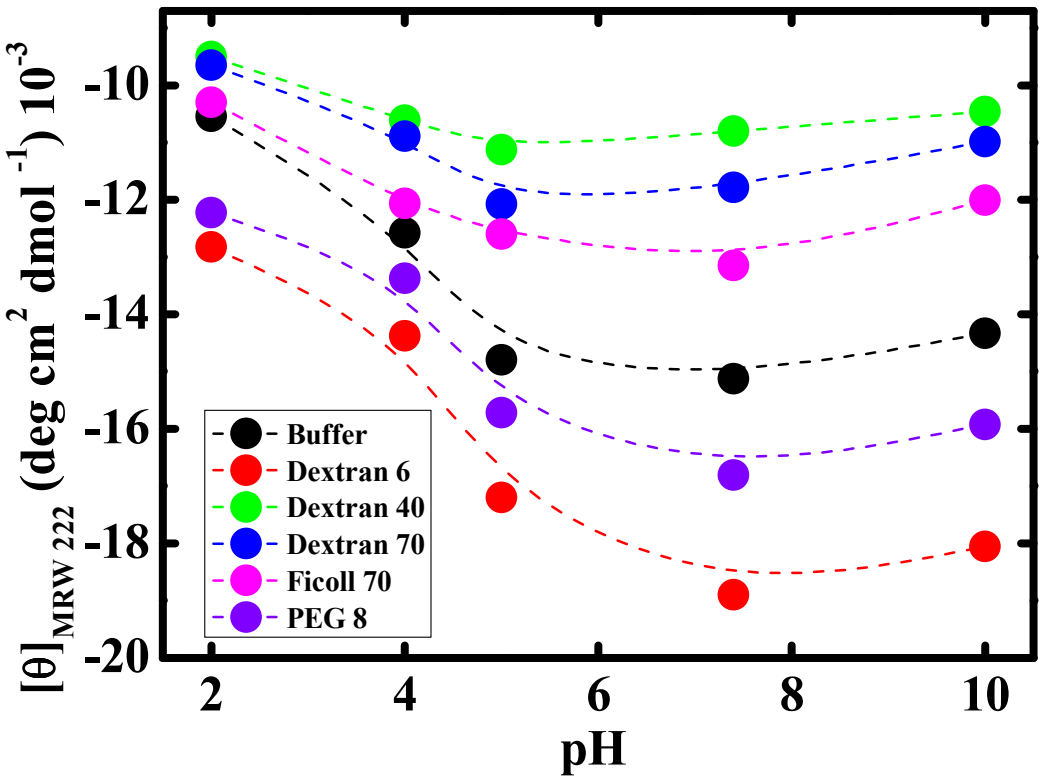

Figure S3: Effect of pH on the far-UV CD signal of HSA monitored at 222 nm with increasing pH in buffer and in presence of different crowders (200 g/L) (as mentioned in the legend) [θ_{MRW} is expressed in deg cm^2 dmol^{-1} and is calculated based on the equation, $\theta_{MRW} = \frac{MRW\theta_{obs}}{10lc}$, where θ_{obs} is the observed ellipticity in degrees, c is the concentration in moles per liter, and l is the optical path in centimeters. In the above equation MRW is the mean residue weight and is given by $\frac{M}{N-1}$, where M is the molar mass of the protein and N is the number of amino acids.]

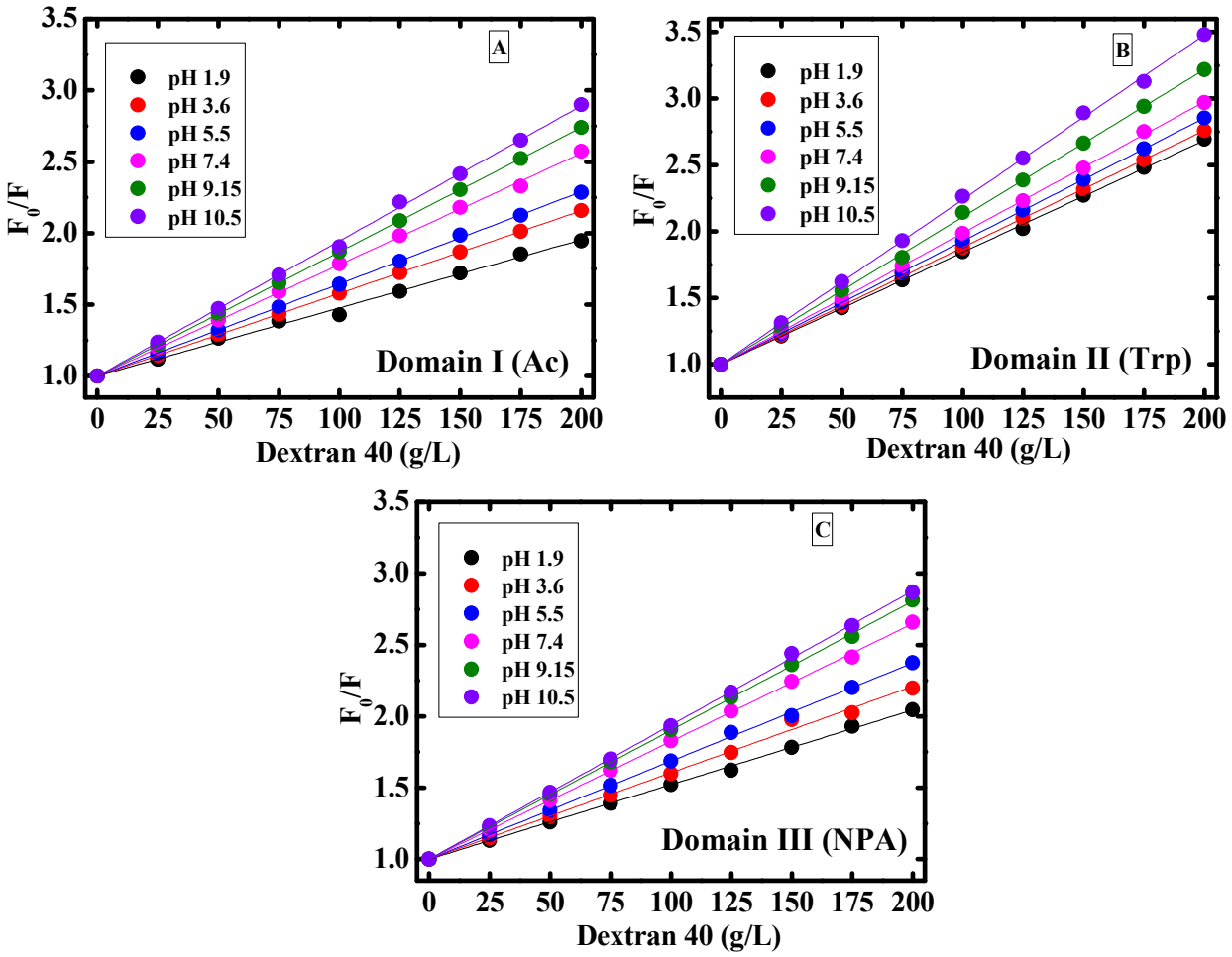

Figure S4: Stern−Volmer plots for the different domains of HSA as a function of Dextran 40 concentration at various pH values as mentioned in the legend

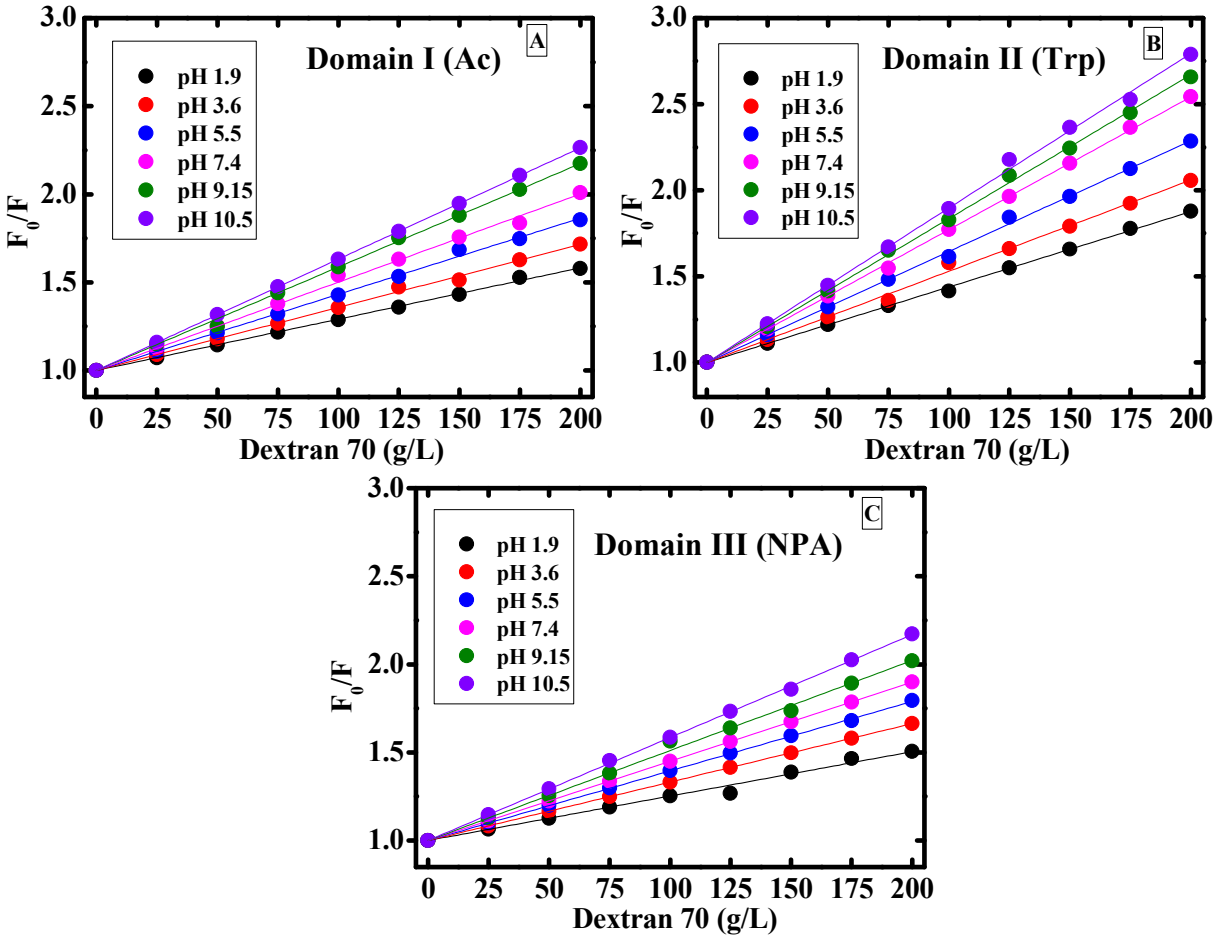

Figure S5: Stern−Volmer plots for the different domains of HSA as a function of Dextran 70 concentration at various pH values as mentioned in the legend.

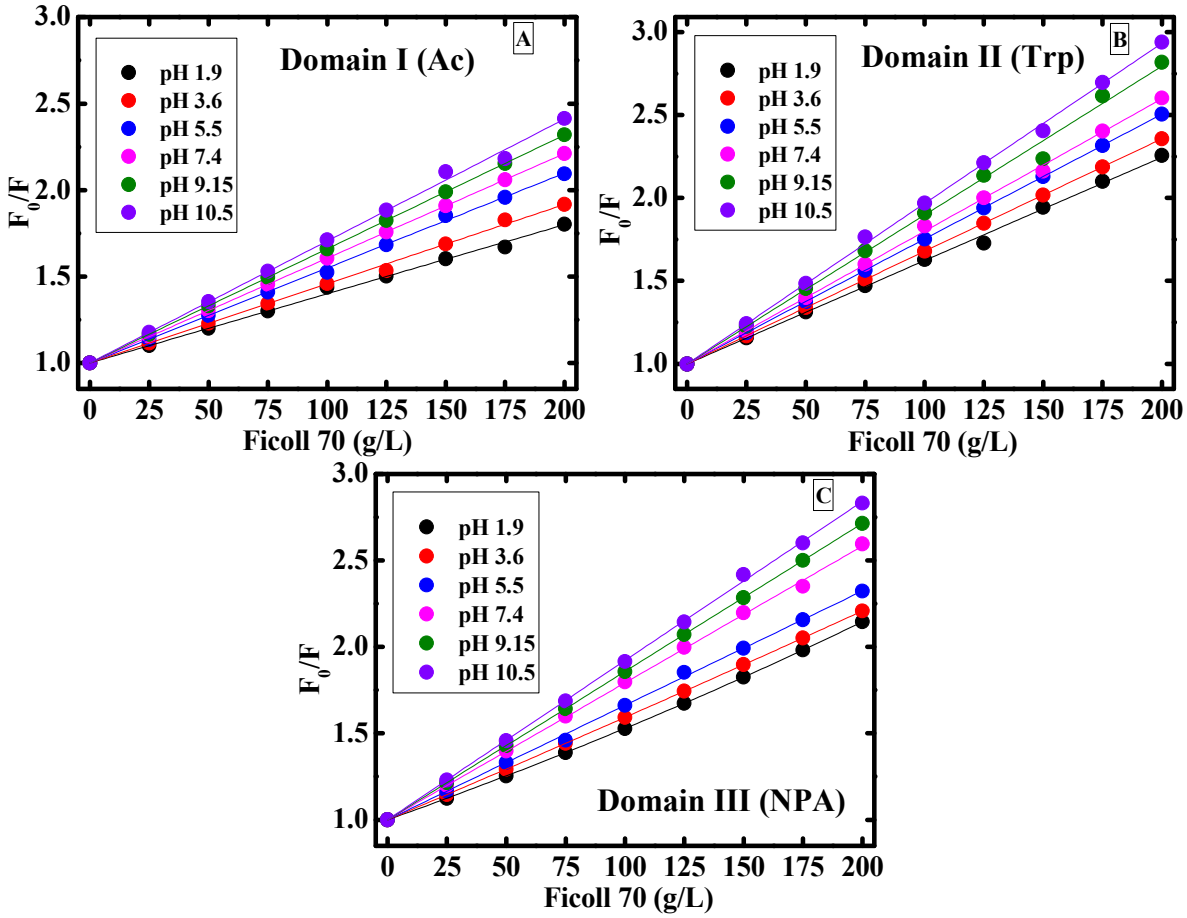

Figure S6: Stern−Volmer plots for the different domains of HSA as a function of Ficoll 70 concentration at various pH values as mentioned in the legend.

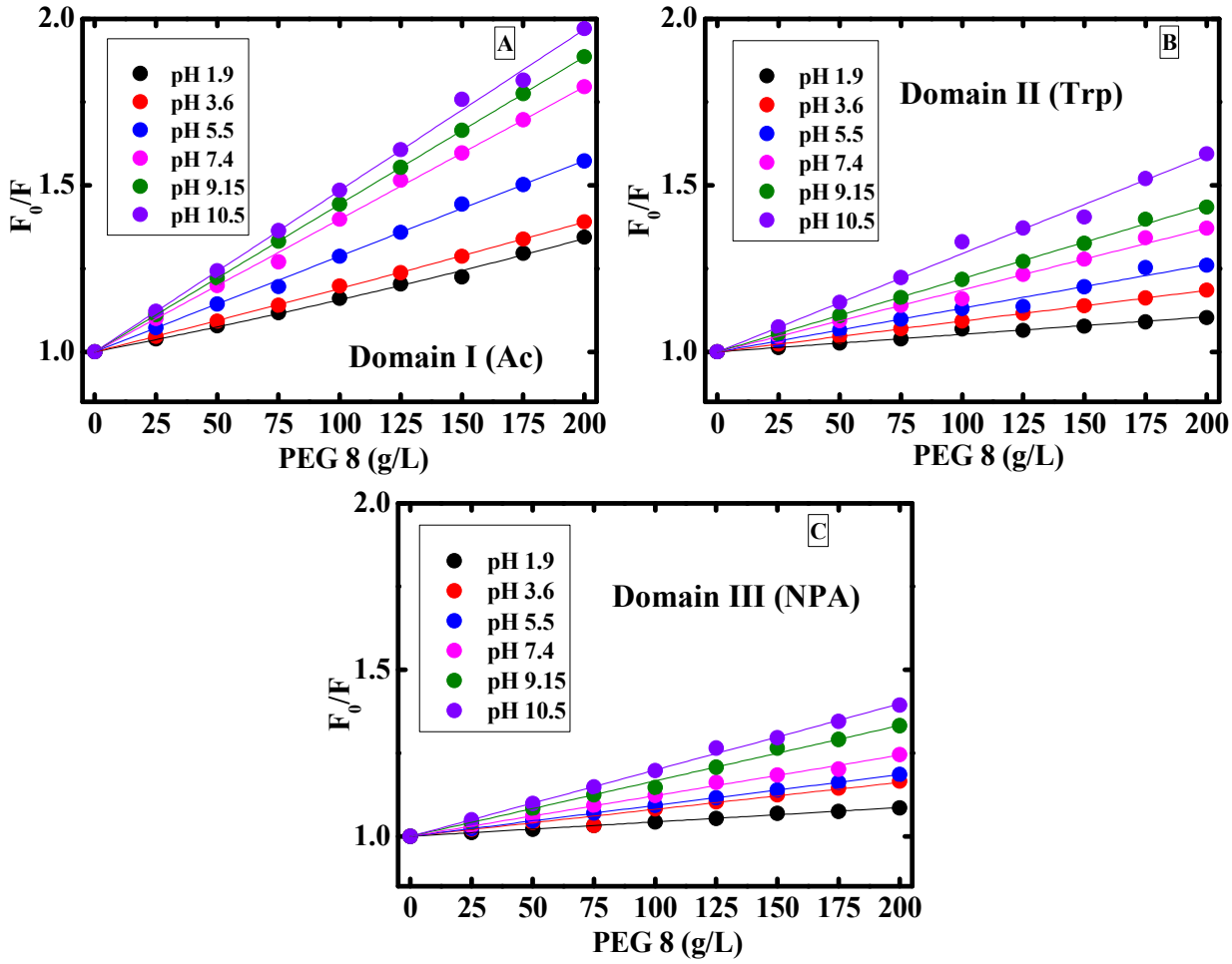

Figure S7: Stern−Volmer plots for the different domains of HSA as a function of PEG8 concentration at various pH values as mentioned in the legend.