Analysis of IgG kinetic stability by differential scanning calorimetry, probe fluorescence and light scattering

by

Michal Nemergut, Gabriel Žoldák, Jonas V. Schaefer, Florian Kast, Pavol Miškovský, Andreas Plückthun, and Erik Sedlák

Figure S1

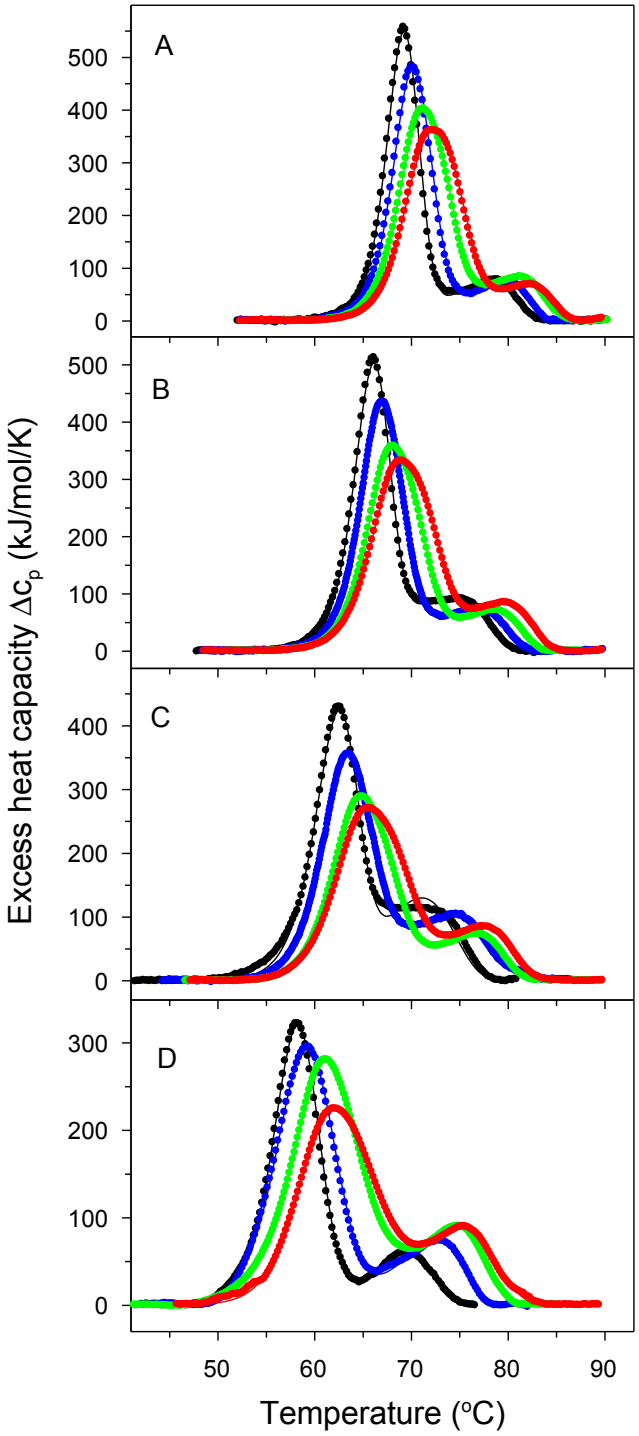

Figure S1. Effect of scan rate on DSC profile at increasing urea concentrations. Urea concentrations: 0 M (A), 1 M (B), 2 M (C), and 3 M (D). The used scan rates were 0.25 K/min (black), 0.5 K/min (blue), 1.0 K/min (green), and 1.5 K/min (red), respectively. Experimental data are shown as circles. The fits according our model (Eq. 4 and Eq. 5) are shown as solid lines in corresponding colors. IgG concentrations were 5 µM in all measurements.

Figure S2

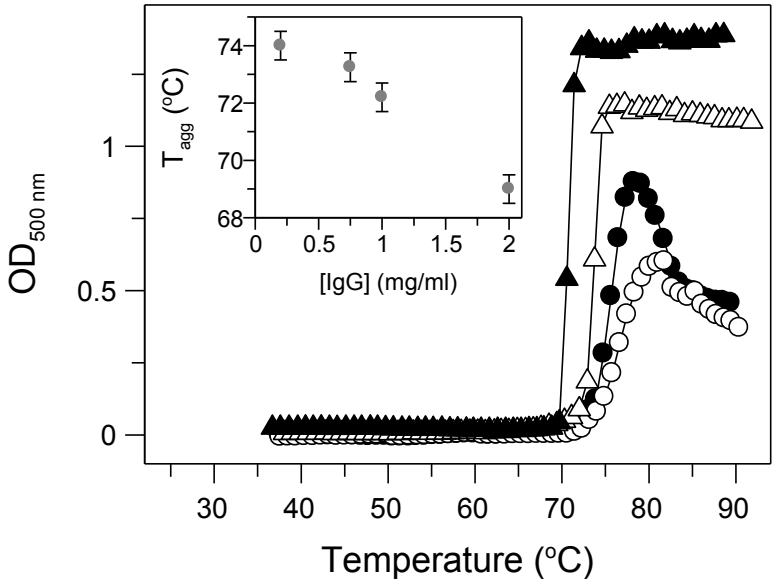

Figure S2. Effect of IgG concentration on its temperature-induced aggregation. IgG concentrations tested were: 0.2 mg/ml (white circles), 0.75 mg/ml (black circles), 1.0 mg/ml (white triangles), and 2.0 mg/ml (black triangles). The protein aggregation was monitored as absorbance at 500 nm. The scan rate was 1.0 K/min in all measurements. Inset: T_{agg}, defined as the temperature at which $OD_{500\ nm}$ reaches the value 0.1, as a function of IgG concentration.

Figure S3

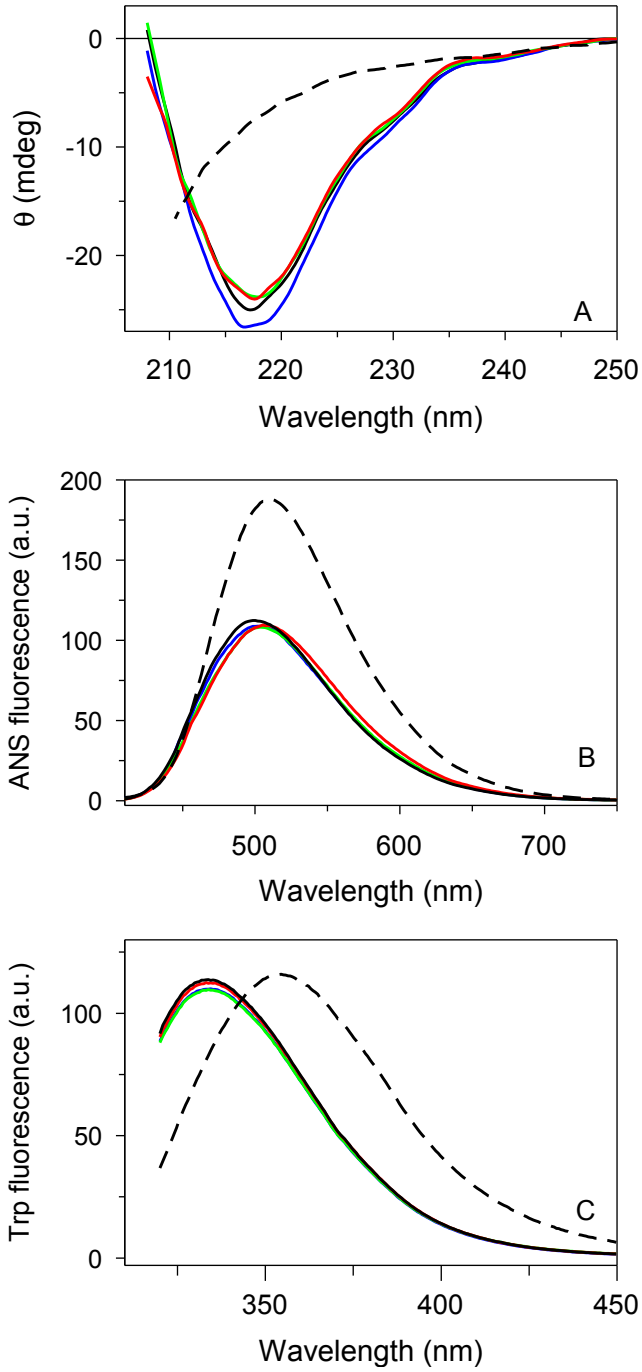

Figure S3. Increasing urea concentrations (0 M - black solid line, 1 M - blue solid line, 2 M - green solid line, and 3 M - red solid line) do not affect ellipticity (A), ANS fluorescence (B), and intrinsic tryptophan fluorescence (C). For comparison, the effect of 8.5 M urea, i.e. fully denaturing urea concentration, on the IgG6B3 properties is shown in dashed lines. The protein concentrations were 5 μM in all measurements.

Figure S4

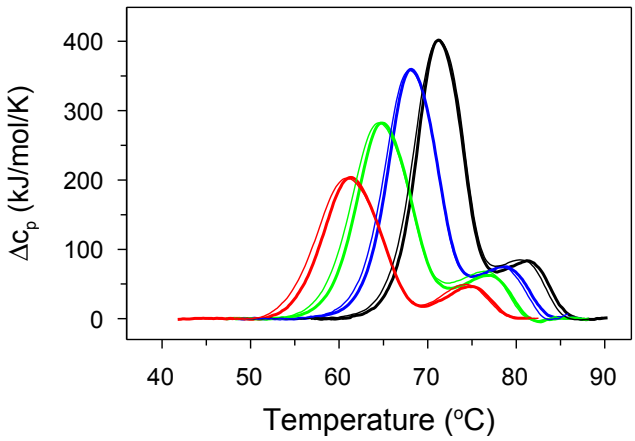

Figure S4. The presence of ANS does not affect DSC transitions of IgG6B3 in different urea concentrations (0 M - black solid line, 1 M - blue solid line, 2 M - green solid line, and 3 M - red solid line). DSC transitions of IgG6B3 in the presence of ANS (500 µM, thin lines) and in the absence of ANS (thick lines). DSC scans were performed with a protein concentration of 0.75 mg/ml (5 µM) in PBS pH 7.4 and a scan rate of 1.0 K/min.

Figure S4

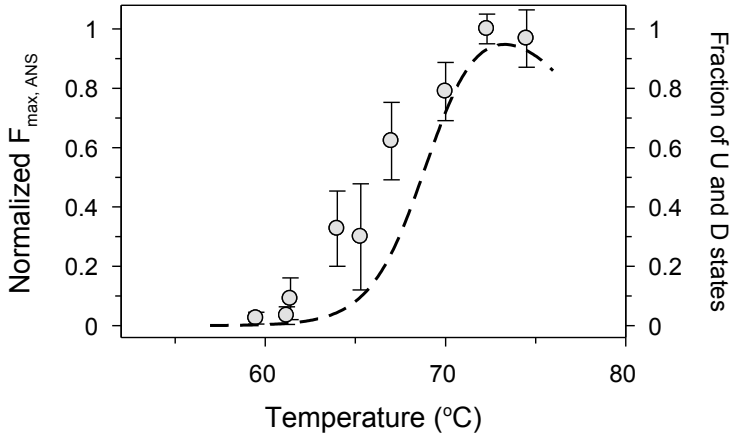

Figure S4. Combined molar fraction of states U and D as a function of temperature (dashed line), derived from Equation 5, and the normalized amplitudes of the ANS fluorescence (grey circles) obtained from the kinetic measurements shown in Figure 4. The ANS fluorescence measurements were performed with a protein concentration of 0.75 mg/ml (5 μM) and ANS concentration of 500 μM.

Figure S5

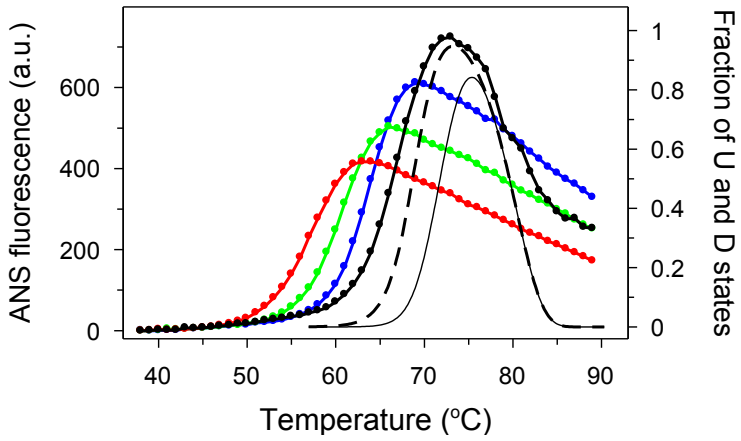

Figure S5. ANS fluorescence as a function of temperature at increasing urea concentrations (0 M - black solid line, 1 M - blue solid line, 2 M - green solid line, and 3 M - red solid line). The molar fraction of the states U and D (dashed line) and of the state D (thin solid line) as a function of temperature are also shown. The ANS fluorescence measurements were performed with a protein concentration of 0.75 mg/ml (5 µM) and ANS concentration of 500 µM at a scan rate of 1.0 K/min. The buffer was PBS at pH 7.4.

Table S1. Parameters describing individual steps of the thermal denaturation of IgG in the presence and in the absence of ANS at different urea concentrations analyzed according the kinetic model as shown in Equation 4. The protein concentration was 3.3 μM. The scan rate of the measurements were 1.0 K/min.

[Urea] (M)		T_{trs} (°C)	ΔH_{cal1} (kJ/mol)	T_2^* (°C)	E_{a2} (kJ/mol)	T_3^* (°C)	E_{a3} (kJ/mol)
0	- ANS	70.1	583	75.8	188	84.3	354
	+ANS	69.1	588	75.3	251	83.7	349
1	- ANS	66.7	563	73.3	190	81.9	338
	+ANS	65.9	556	73.0	216	81.3	350
2	- ANS	63.2	514	71.4	169	80.4	312
	+ANS	62.2	521	70.8	193	79.6	330
3	- ANS	59.9	460	68.6	158	77.8	280
	+ANS	58.5	507	67.5	201	77.1	370

Table S2. Parameters describing individual steps of the thermal denaturation of IgG at various scan rates and at different urea concentrations analyzed according the kinetic model as shown in Equation 4. The protein concentration was 5 μM.

[Urea] (M)	Scan rate (K/min)	T_{trs} (°C)	ΔH_{cal1} (kJ/mol)	T_2^* (°C)	E_{a2} (kJ/mol)	T_3^* (°C)	E_{a3} (kJ/mol)
0	0.25	69.5	650	76.2	193	86.7	310
	0.5	69.5	621	76.4	171	84.6	358
	1.0	70.1	583	75.8	188	84.3	354
	1.5	70.2	635	75.3	242	83.8	366
Average	-	69.9 ± 0.3	622 ± 29	75.9 ± 0.5	198 ± 30	84.9 ± 1.3	347 ± 25
1	0.25	66.6	548	73.9	166	86.4	236
	0.5	66.6	570	74.5	159	83.1	303
	1.0	66.7	563	73.3	190	81.9	338
	1.5	66.8	552	73.2	197	81.9	335
Average	-	66.7 ± 0.1	558 ± 10	73.7 ± 0.6	178 ± 18	83.3 ± 2.1	303 ± 47
2	0.25	64.0	386	70.3	151	85.4	205
	0.5	63.0	472	73.7	166	84.0	225
	1.0	63.2	514	71.4	169	80.4	312
	1.5	63.2	502	71.0	172	80.2	303
Average	-	63.2 ± 0.4	469 ± 58	71.6 ± 1.5	165 ± 9	82.5 ± 2.6	261 ± 54
3	0.25	58.3	446	70.5	138	77.7	315
	0.5	58.4	456	70.5	139	78.5	294
	1.0	59.9	460	68.6	158	77.8	280
	1.5	59.7	417	69.0	133	79.1	253
Average	-	59.1 ± 0.8	445 ± 19	69.3 ± 1.5	142 ± 11	78.3 ± 0.7	286 ± 26

Table S3. Rate constants of ANS binding to IgG at different temperatures at different urea concentrations. The rate constant were calculated according Equations 1-3.

[Urea] (M)	T (°C)	k_1 (min^{-1})	k_2 (min^{-1})	k (min^{-1})
0	59.5	0.0423	-	0.0423
	61.2	0.0347	-	0.0347
	61.4	0.0723	-	0.0723
	64.0	0.0967	1.0345	0.0884
	65.3	0.0931	1.1046	0.0859
	67.0	0.1500	1.5954	0.1371
	70.0	0.6112	0.6112	0.3056
	72.3	0.9492	0.9492	0.4746
	74.5	1.2252	1.2252	0.6126
1	56.8	0.0376	-	0.0376
	57.8	0.0341	-	0.0341
	59.3	0.0478	-	0.0478
	59.5	0.0496	-	0.0496
	60.1	0.0575	1.9822	0.0559
	60.9	0.0853	0.8431	0.0775
	62.5	0.1070	1.0911	0.0975
	63.8	0.1668	1.0208	0.1434
	65.2	0.3286	0.6807	0.2216
	67.4	0.6218	0.6218	0.3109
	70.5	0.9338	1.7036	0.6032
2	52.5	0.0235	-	0.0235
	54.0	0.0361	-	0.0361
	55.7	0.0691	-	0.0691
	58.3	0.0839	0.6742	0.0746
	60.4	0.1563	0.6128	0.1246
	62.0	0.3481	0.3481	0.1740
	63.5	0.3007	0.6539	0.2060
	65.5	0.6400	0.6400	0.3200
	69.5	2.5551	2.0052	1.1235
3	50.7	0.0352	0.1261	0.0275
	52.0	0.0450	0.3247	0.0397
	53.0	0.0563	0.1798	0.0429
	53.4	0.0610	0.1614	0.0443
	54.3	0.0731	0.1925	0.0530
	55.7	0.0967	0.5544	0.0823
	58.0	0.2824	0.5051	0.1811
	60.1	0.6537	0.6537	0.3268
	60.2	0.4325	0.4325	0.2163
	62.2	0.7784	0.7784	0.3892