Electronic Supplementary Information

Analysis of bimodal thermally-induced denaturation of type I collagen extracted from calfskin

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Table S1. Peak temperatures, relative peak areas and enthalpy changes of the endothermic denaturation process experienced by CI in acidic solutions

Concentration, % (w/w)	**Heating rate, Kmin^{-1}**	**Peak 1**	**Peak 2**	**Determination coefficient (R^2)**	**ΔH,* kJmol^{-1}**
		Peak temperature (T_1, T_2), ^{0}C/ relative peak area, %			
0.075	0.1	31.06/19.8	35.43/80.2	0.98953	9.87
	0.2	31.86/21.9	36.23/78.1	0.99580	10.00
	0.3	32.46/20.1	36.93/79.9	0.99670	9.87
	0.4	33.08/16.9	37.59/83.1	0.99683	9.73
	0.5	33.59/18.9	38.05/81.1	0.99824	10.00
		Equilibrium peak temperature (T_{1eq}, T_{2eq}), ^{0}C/ correlation coefficient (R)			ΔH_{mean}, kJmol^{-1}
		30.53±0.10/ 0.99119	34.87±0.12/ 0.99559		9.89±0.11
0.100		(T_1, T_2), ^{0}C/ relative peak area, %		R^2	ΔH, kJmol^{-1}
	0.1	31.13/19.1	35.45/80.9	0.99340	6.70
	0.2	31.82/21.1	36.27/78.9	0.99424	7.22
	0.3	31.98/22.6	36.89/77.4	0.98978	7.90
	0.4	32.90/22.6	37.52/77.4	0.99016	9.00
	0.5	33.55/20.4	38.11/79.6	0.99272	9.30
		(T_{1eq}, T_{2eq}), ^{0}C/R			ΔH_{mean}, kJmol^{-1}
		30.51±0.21/ 0.98287	34.88±0.10/ 0.99792		8.02±1.12
0.220		(T_1, T_2), ^{0}C/ relative peak area, %		R^2	ΔH, kJmol^{-1}
	0.1	31.39/19.2	35.36/80.8	0.99632	5.14
	0.2	32.14/16.1	36.22/83.9	0.99860	5.18
	0.3	32.63/17.0	36.87/83.0	0.99853	5.14
	0.4	33.22/16.9	37.45/83.1	0.99872	5.00
	0.5	33.30/8.7	37.99/91.3	0.99734	5.00
		(T_{1eq}, T_{2eq}), ^{0}C/R			ΔH_{mean}, kJmol^{-1}
		31.07±0.20/ 0.97407	34.83±0.12/ 0.99539	80.2±11.2	5.09±0.09
0.290		(T_1, T_2), ^{0}C/ relative peak area, %		R^2	ΔH, kJmol^{-1}
	0.1	31.12/17.3	35.47/82.7	0.99883	7.41
	0.2	32.08/18.5	36.25/81.5	0.99786	7.24
	0.3	32.69/17.2	36.98/82.8	0.99804	7.31
	0.4	32.96/19.0	37.42/81.0	0.99849	7.55
	0.5	33.48/18.3	37.95/81.7	0.99928	7.24
		(T_{1eq}, T_{2eq}), ^{0}C/R			ΔH_{mean}, kJmol^{-1}
		30.79±0.23/ 0.97734	34.98±0.14/ 0.99315	50.9±0.9	7.35±0.13
		Average relative peak area, %			
		18.6±3.0	81.4±3.0		

*All enthalpies are considered in kJ per mole of amino acid residue whose mean molar mass was taken as 100 g mol^{-1}

Table S2. Hypothetical polydispersity of CI aggregates in acidic solutions. A Gaussian distribution was considered with $M_0 = 360$ kDa and $\sigma^2 = 300$

weight fractions, w_i	molecular masses of aggregates, M_i (kDa)	$\overline{M}_w^*$ (kDa)	$\overline{M}_v$ (kDa)
0.00066	300	360	360.3≈360
0.00397	310		
0.01712	320		
0.05222	330		
0.11871	340		
0.19342	350		
0.22760	**360**		
0.19342	370		
0.11871	380		
0.05222	390		
0.01712	400		
0.00397	410		
0.00066	420		

* $\overline{M}_w = \sum_i w_i M_i$

Table S3. Theoretical compositions of a CI solution containing collagen in monomeric, dimeric and trimeric form ($\overline{M}_v = 359\,kDa$)

weight fraction of collagen, %		
w_1 (monomer)	**w_2 (dimer)**	**w_3 (trimer)**
85.00	14.76	0.24
86.00	13.09	0.91
87.00	11.43	1.57
88.00	9.77	2.33
89.00	8.10	2.90
90.00	6.44	3.56
91.00	4.78	4.22
92.00	3.11	4.89
93.00	1.45	5.55
93.80	0.12	6.08

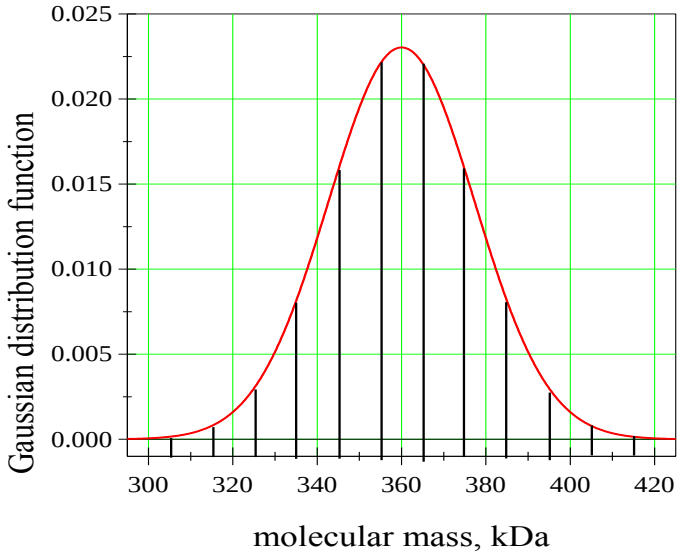

Figure S1. Gaussian distribution profile (centered at 360 kDa) of hypothetical CI aggregates in acidic solution. The area under the distribution curve and delimited by two successive vertical grids is numerically equal to the weight fraction (w_i) of the CI aggregates with molecular mass M_i (see Table S2)

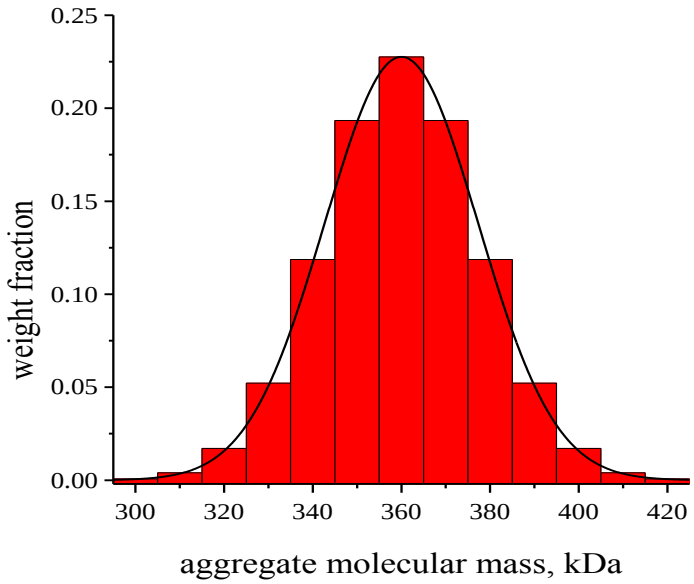

Figure S2. Histogram of polidispersity based on the Gaussian distribution plotted in Figure S1 and weight fractions shown in Table S2

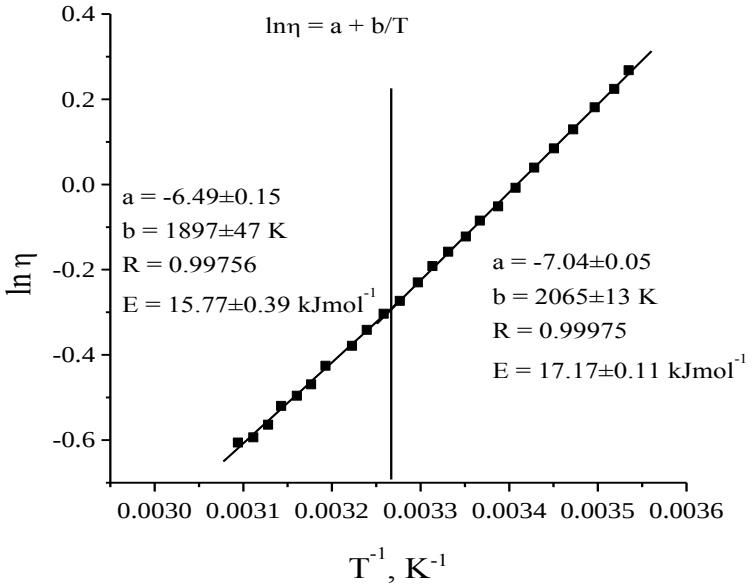

Figure S3. Viscosity vs. inverse absolute temperature as semilogarithmic plot for aqueous solution of 10^{-2}M HCl

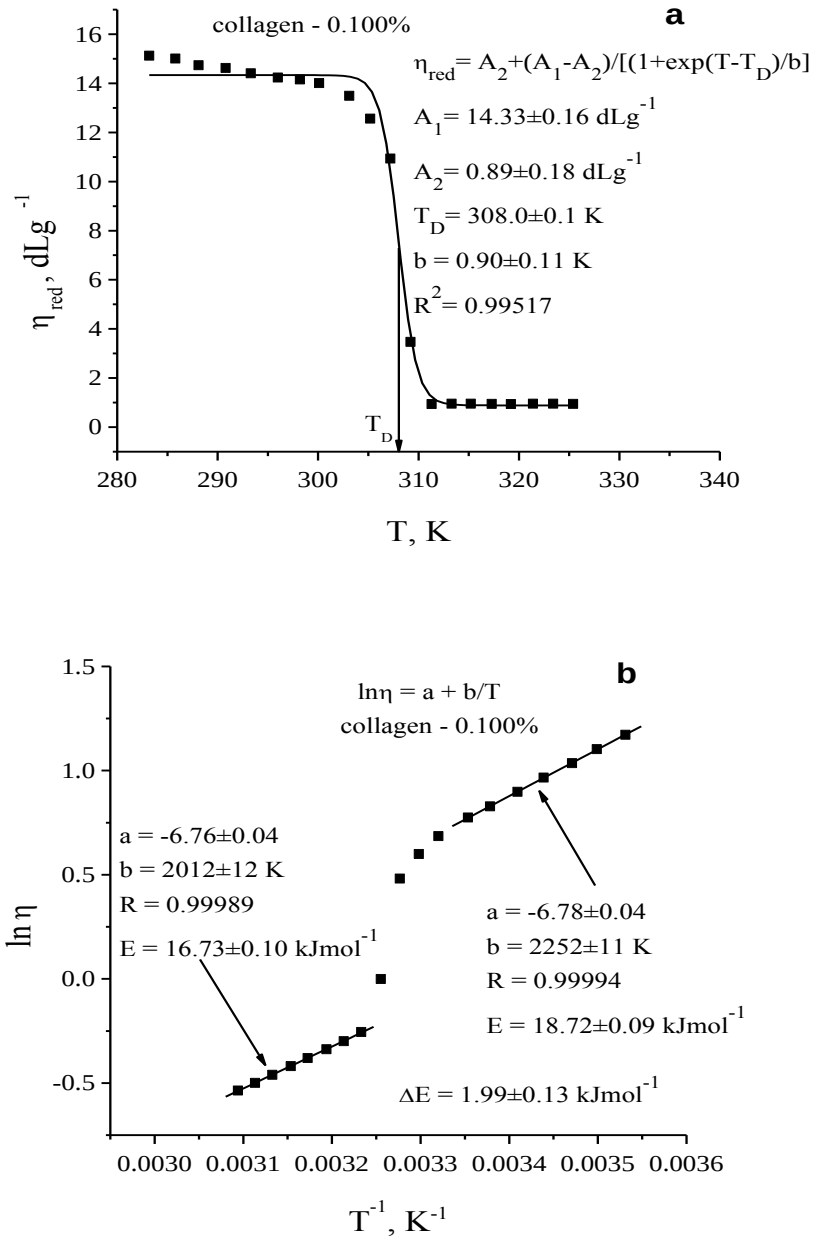

Figure S4. Suitable viscosity-temperature plots for determining (a) denaturation temperature and (b) activation energy of viscous flow for acidic solution of 0.100% CI

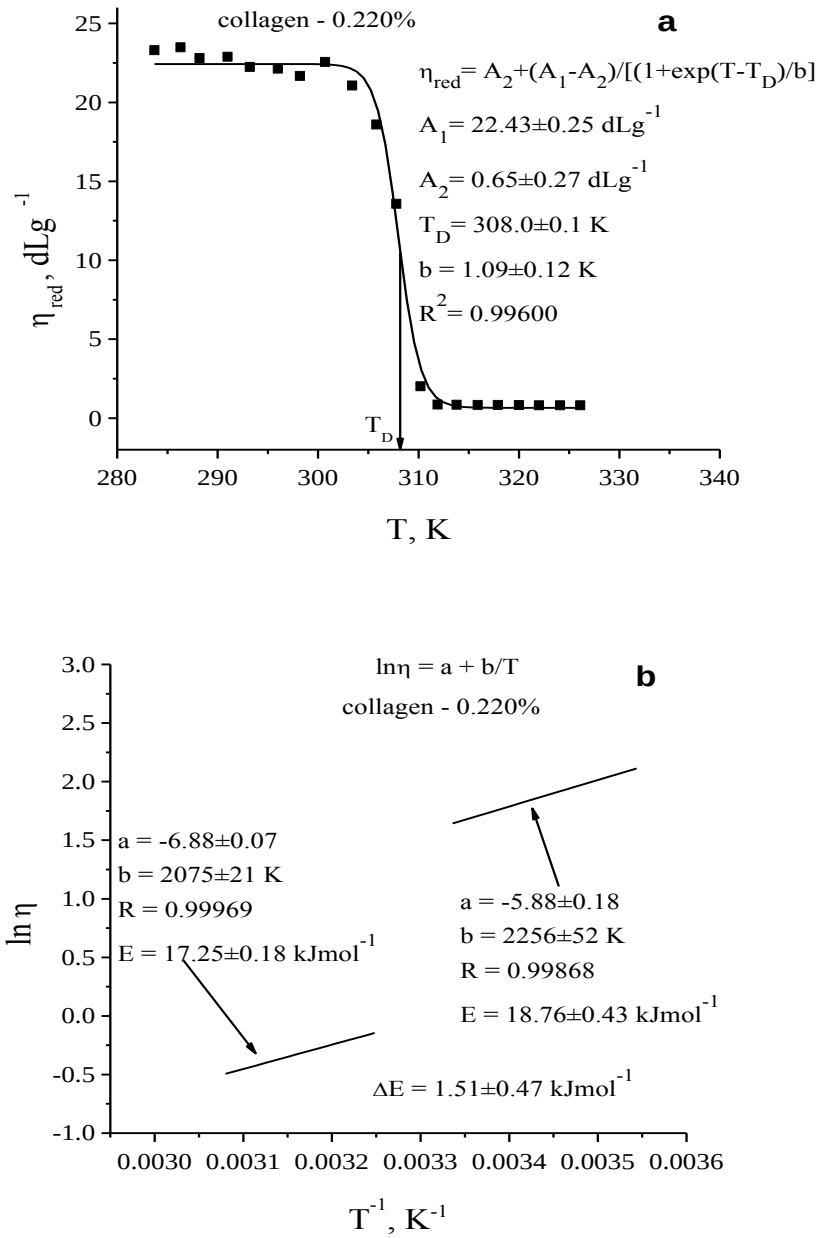

Figure S5. Suitable viscosity-temperature plots for determining (a) denaturation temperature and (b) activation energy of viscous flow for acidic solution of 0.220% CI

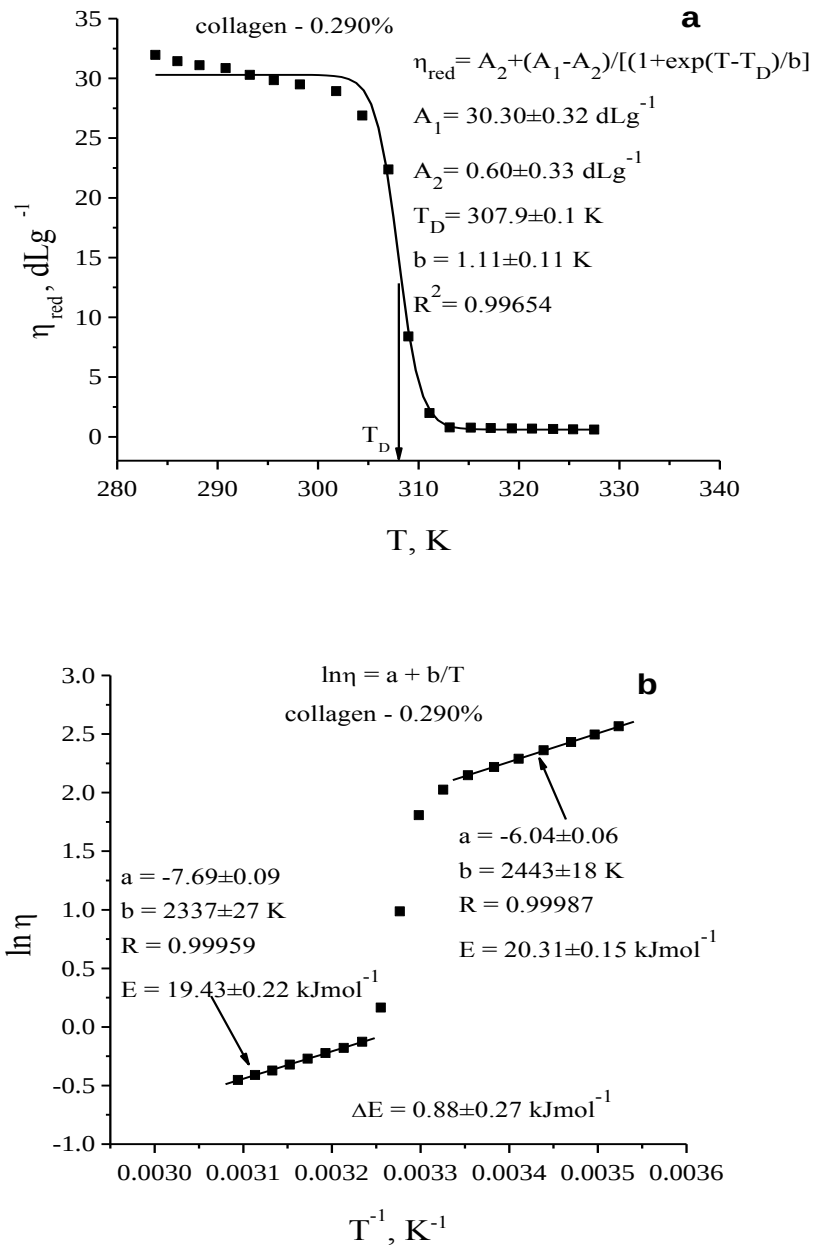

Figure S6. Suitable viscosity-temperature plots for determining (a) denaturation temperature and (b) activation energy of viscous flow for acidic solution of 0.290% CI

Normal distribution of polydispersity profile with respect to the CI aggregates masses in solution

Let the polydispersity profile be a normal distribution (Gaussian distribution) given as:

$$f(M) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(M-M_0)^2}{2\sigma^2}} \quad (1)$$

where M is molecular mass of CI aggregates, M_0 – population's central and σ^2 – population's variance. This means that the number of individual CI molecules that assemble to lead to a certain molecular mass M obeys a normal distribution. Practically, for such a distribution centered in M_0 and depending on the system's variance, there is a finite domain delimited by a minimum (M_{min}) and a maximum mass (M_{max}), respectively, over which the distribution should be normalized to unity. In the studied cases of CI solutions, M_{min} should be the molecular mass of CI in monomeric form (3×10^5 Da) and M_{max} – the molecular mass of CI aggregates with a value symmetrically placed about M_0. At the same time, the quantity f(M)dM defines the weight of CI molecules that are assembled in aggregates with molecular masses ranging from M to M+dM, all with respect to the total weight of the collagen in the sample. In fact, this quantity is numerically equal to the weight fraction of the CI aggregates with the range of molecular masses just mentioned. Particularly, considering a central of distribution of 360 kDa with a total range of 295-425 kDa and a variance of 300 (Fig. S1), a discrete profile of CI polydispersity after an appropriate splitting of the distribution range into even intervals and choosing their midpoints as relevant molecular masses is shown in Table S2. Based on the significance of the weight average molecular mass ($\overline{M}_w$) [1], it is obvious that the distribution's central of 360 kDa is exactly $\overline{M}_w$ of the system. At the same time, according to the expression of the viscosity average molecular mass [1]

$$\overline{M}_v = \left(\frac{\sum_i N_i M_i^{a+1}}{\sum_i N_i M_i} \right)^{1/a} = \left(\sum_i w_i M_i^a \right)^{1/a} \quad (2)$$

a value practically as high as $\overline{M}_w$ (360 kDa) was obtained considering the weight fractions (w_i) and related molecular masses shown in Fig. S2 and listed in Table S2.

1. Tanford C, Physical chemistry of macromolecules. New York: John Wiley & Sons, Inc; 1961. pp. 145-50.