Electronic Supplementary Material (ESI) for Soft Matter

Electronic Supplementary Information for

Hydration effects on the fibrillation process of a globular protein: The case of human serum albumin.

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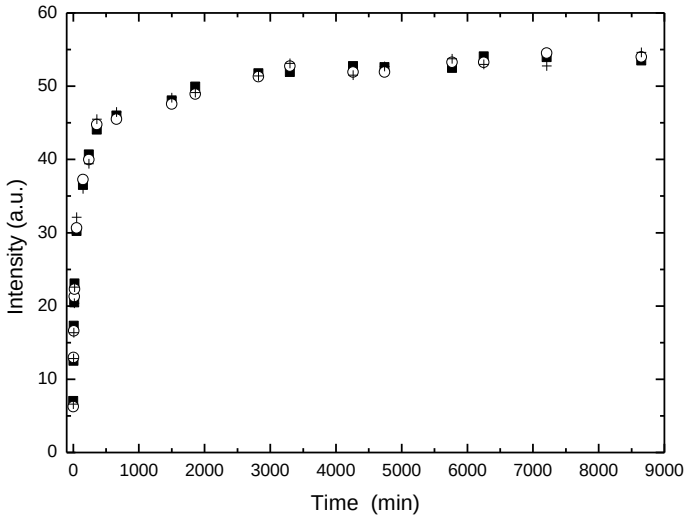

Figure S1: Time evolution of light scattering in HSA solutions incubated at 65 ºC and pH 7.4 at a concentration of 10 mg/mL (■) in the absence and (○) the presence of protein seeds; and (+) at a protein concentration of 1 mg/mL.

Temporal evolution of ThT binding: The temporal evolution of ThT binding to protein molecules/oligomers in selected solution conditions, that is, at 65 ºC and pH 2.0 or 7.4 and alcohol contents of 20, 40 60 and 80% (v/v) are shown as examples. At pH 7.4, it can be observed that the ThT binding kinetics involved the continuous rising of ThT fluorescence during the early periods of the incubation process, and exhibited no discernible lag phase until a quasi-plateau region was attained in the timescale analyzed, in agreement with SLS data. In contrast, at acidic pH and ethanol concentrations below 50% (v/v) the ThT fluorescence intensity is rather lower than at physiological pH and extends over a larger period of time, which points to the formation of oligomeric structures or protofibrils (see below).[40] In particular, at short incubation times a plateau occurs denoting the existence of a lag phase, after which ThT intensity rises. The same behavior was observed by SLS. At larger ethanol contents (> 60% (v/v)), ThT intensity seems to increase steeply and continuously from very short incubation times until a plateau region is attained, in a similar fashion as at physiological pH, with a now discernible lag phase. Nevertheless, the profiles corresponding to these cosolvent concentrations display some differences if compared to those derived from SLS kinetic data (see Figures 1c and S2b). These differences might arise from the different properties we measured: From SLS, we obtained size distributions and, thus, information about aggregate sizes independently from what kind of protein structure composition is, whereas ThT binding fluorescence intensity is related to changes in β-structure content, which are usually connected to protein aggregation processes. In this way, the continuous steep increase in ThT fluorescence intensity at ethanol concentrations above 60% (v/v) might indicate a relatively fast gain of β-structure at the expense of other structural contents; however the aggregate sizes would not have to follow the same trends provided that a sufficient β-structure content and number of

protein clusters/smaller aggregates might be necessary to favor aggregate/fibril fusion and growth, and thus originate important size increases.[S1,S2]

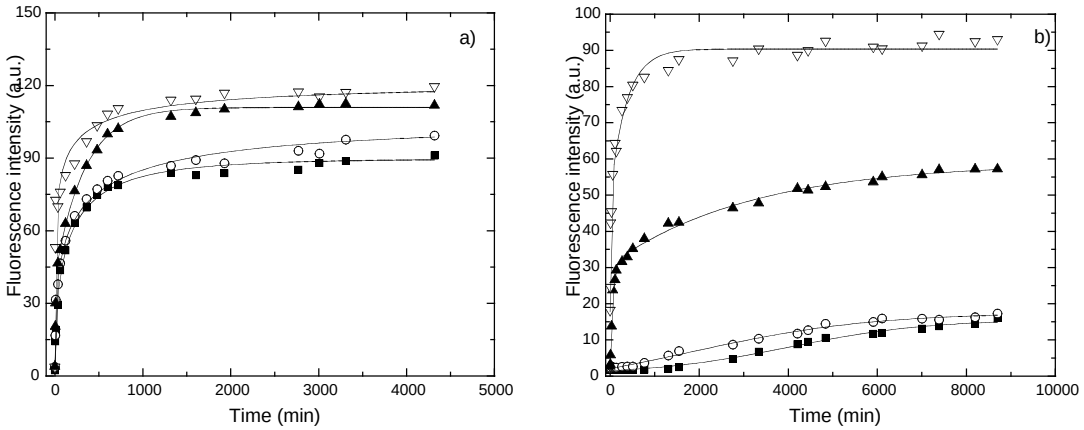

Figure S2: Selected temporal evolutions of ThT fluorescence intensities at ethanol concentrations of (■) 20, (○) 40, (▲) 60, and (▽) 80% (v/v) and 65 ºC at a) pH 7.4, and b) pH 2.0.

Table S1: Values of lag times and apparent aggregation rate constants for HSA solutions under the different solution conditions.

		pH 7.4, 25ºC	pH 7.4, 65ªC	pH 2.0, 25 ºC	pH 2.0, 65ºC
Lag time (min)	20	-	-	-	950
	30	-	-	-	1206
	40	-	-	-	3120
	50	-	-	12490	4865
	60	-	-	16870	-/1170
	70	-	-	15304	-/1549
	80	-	-	11510	-/2890
K_{app} (min^{-1})	20	-	0.011	-	0.0004
	30	-	0.015	-	0.0006
	40	0.002	0.017	-	0.001
	50	0.004	0.027	0.0003	0.003
	60	0.005	0.032	0.0006	0.004/0.001
	70	0.62	0.26	0.001	0.005/0.001
	80	0.92	0.96	0.002	0.007/0.001

Uncertainties ± 10 %. Values of K_{app} at pH 2.0 and 65 ºC in the interval 60-80 % (v/v) correspond to the first and second stages of the growth phases of the aggregation kinetics. Also, lag times in this cosolvent interval under the same solution conditions correspond to those observed before the second growth step. The -/ in the right hand column denotes the absence of a lag time phase before the first growth step occurs.

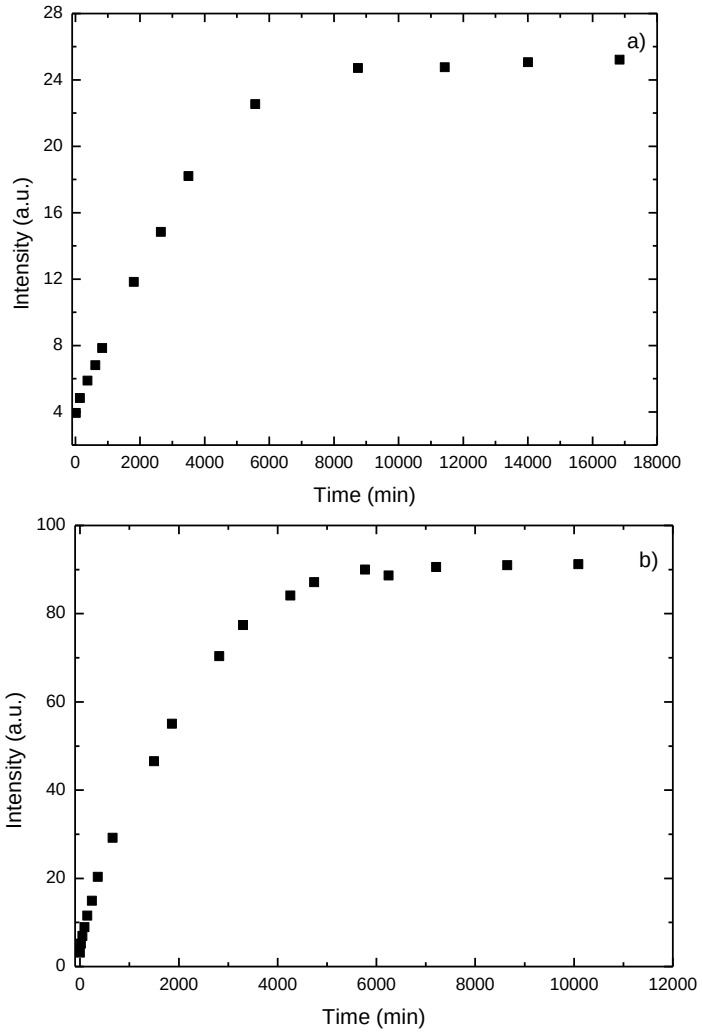

Figure S3: Time evolution of scattered light intensity in a HSA solution incubated at pH 2.0 in the presence of 80 % (v/v) ethanol after seeding addition a) at 25 ºC and b) 65 ºC.

Table S2: Secondary structure compositions of HSA samples upon incubation under the different solution conditions**.**

	% (v/v) ethanol	α-helix	β-strand	Turn	Unordered
pH 7.4, 65 ºC	20	0.12	0.33	0.22	0.33
	30	0.10	0.34	0.22	0.34
	40	0.10	0.34	0.22	0.34
	50	0.10	0.34	0.22	0.34
	60	0.10	0.35	0.22	0.33
	70	0.08	0.36	0.23	0.33
	80	-	-	-	-
	90	-	-	-	-
pH 7.4, 25 ºC	20	0.50	0.03	0.17	0.30
	30	0.48	0.03	0.16	0.33
	40	0.36	0.11	0.16	0.37
	50	0.34	0.09	0.19	0.38
	60	0.14	0.30	0.22	0.34
	70	0.18	0.27	0.21	0.34
	80	0.34	0.14	0.20	0.32
	90	0.45	0.06	0.19	0.30
pH 2.0, 65ºC	20	0.27	0.18	0.22	0.33
	30	0.23	0.22	0.22	0.33
	40	0.21	0.24	0.21	0.34
	50	0.18	0.28	0.21	0.33
	60	0.14	0.31	0.22	0.33
	70	0.12	0.32	0.22	0.34
	80	0.08	0.35	0.23	0.34
	90	-	-	-	-
pH 2.0, 25ºC	20	0.44	0.05	0.18	0.33
	30	0.44	0.05	0.19	0.32
	40	0.46	0.04	0.17	0.33
	50	0.43	0.07	0.17	0.33
	60	0.40	0.11	0.17	0.32
	70	0.33	0.17	0.18	0.32
	80	0.30	0.19	0.18	0.33
	90	0.24	0.25	0.19	0.32

Fourier transform infrared spectroscopy (FT-IR).

FT-IR spectra of HSA solutions were determined by using a FT-IR spectrometer model IFS-66v from Bruker, equipped with a horizontal ZnS ATR accessory. The spectra were obtained at a resolution of 2 cm^{-1} and generally ca. 200 scans were accumulated to get a reasonable signal-to-noise ratio. The sample chamber was continuously purged with CO_2-free dry air. Solvent spectra were also examined in the same accessory and instrument conditions. Each different sample spectrum was obtained by digitally subtracting the solvent spectrum from the corresponding sample spectrum. All the spectra were baseline-corrected and normalized prior to further data processing with the Bruker software provided by the manufacturer (Opus NT). Absorption bands were resolved by Fourier self-deconvolution using Lorentzian parameters of 20 cm^{-1} bandwidth and a noise suppression factor of 0.3. Second derivatives of deconvoluted spectra were calculated using either 9-point (for spectra interpolation with Lorentz curves) or 17-point (in other cases) Savitzky–Golay smoothing. Each sample solution was measured three times to ensure reproducibility and averaged to produce a single spectrum. Protein concentrations were similar to those used in CD experiments.

Figure S4 plots the second derivative of FT-IR spectra of HSA samples incubated at selected different experimental conditions. As observed from this Figure, incubation at 25 ºC and physiological pH at ethanol concentrations of 20-60% (v/v) involved a progressive decrease of the amide I band at ca. 1655 cm^{-1}, and the development of a new one at ca. 1625 cm^{-1}. This finding is representative of the progressive formation of β-sheet strands, as can be also observed upon incubation at 65 ºC. A red-shift of the latter band suggests progressively stronger inter-strand hydrogen bonds between protein

molecules as the ethanol concentration increases. This effect and a larger hydrophobic surface exposed to the solvent favor mutual interactions between side chains,[S1] driving the fibrillation process. In addition, the presence of a strong peak at ca. 1688 cm^{-1} is a clear halfmark of the antiparallel arrangement of β-sheet strands.[S2] Other all α-proteins which undergo a transition to β-sheet rich structure upon incubation in mixed solvents are, for example, *Borrelia burgdorferi* VlsE,[S3] cytochrome *c*,[S4] and myoglobin,[S5] insulin[S6] and lysozyme.[S7] Regarding incubation at acidic pH, FT-IR spectra reveal that α-helix and unordered conformations predominate at low and medium alcohol concentrations (up to 60% v/v). At larger alcohol contents, there exists a development of the band at ca. 1628 cm^{-1} denoting the formation of additional β-structure (not shown). At high temperature the behavior is similar to that previously noted by CD, that is, a little increase of the amide I band, at alcohol concentrations between 20-50% (v/v) occurs, and a subsequent decrease of the former band is observed demonstrating additional β-sheet structuring, as featured by the further development of the peak centered at ca. 1626 cm^{-1}.

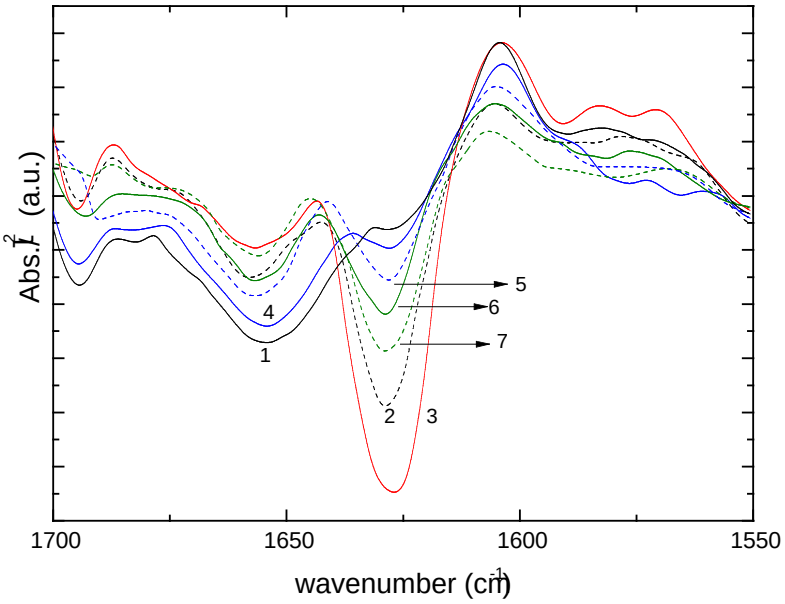

Figure S4: Selected second-derivative FT-IR spectra of HSA samples incubated at pH 7.4 and 25 ºC at 1) 20, and 2) 60% (v/v) ethanol; 3) at pH 7.4 and 65 ºC at 60% (v/v) ethanol; at pH 2.0 and 25 ºC at 4) 20 and 5) 60% (v/v) ethanol; and at pH 2.0 and 65 ºC at 6) 20, and 7) 60% (v/v) ethanol, respectively.

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