Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

for

Severe impact of *in vivo*-like microfluidic flow and the influence of gemini surfactants on amyloid aggregation of hen egg white lysozyme

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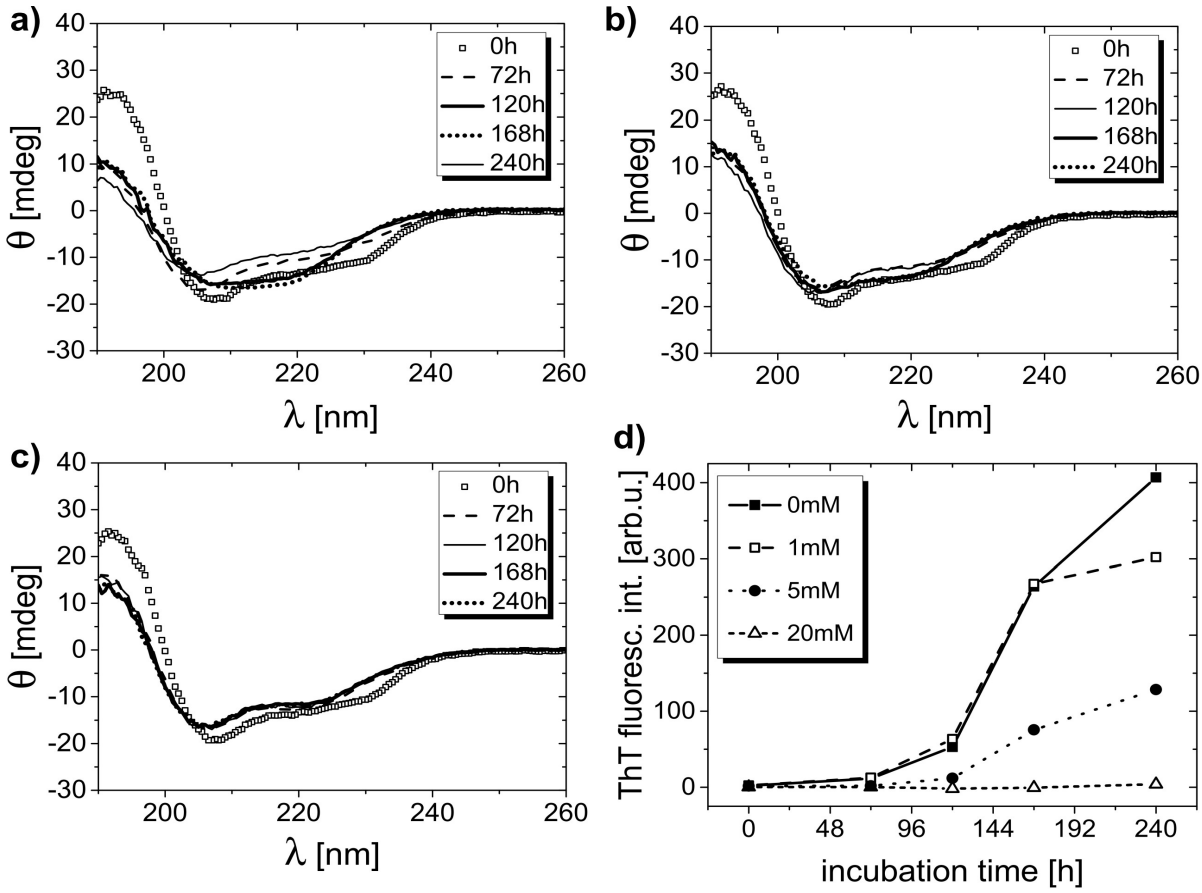

Fig. S1 Circular dichroism curves collected for bulk solution of HEWL with addition of oxyC2 gemini surfactant in concentrations 1mM (a) 5mM (b) 20mM (c) after time of incubation given in the panels insets. Thioflavin T fluorescence signal at λ=490 nm for HEWL/oxyC2 samples as a function of time of incubation (surfactant concentration given in the panel inset) (d).

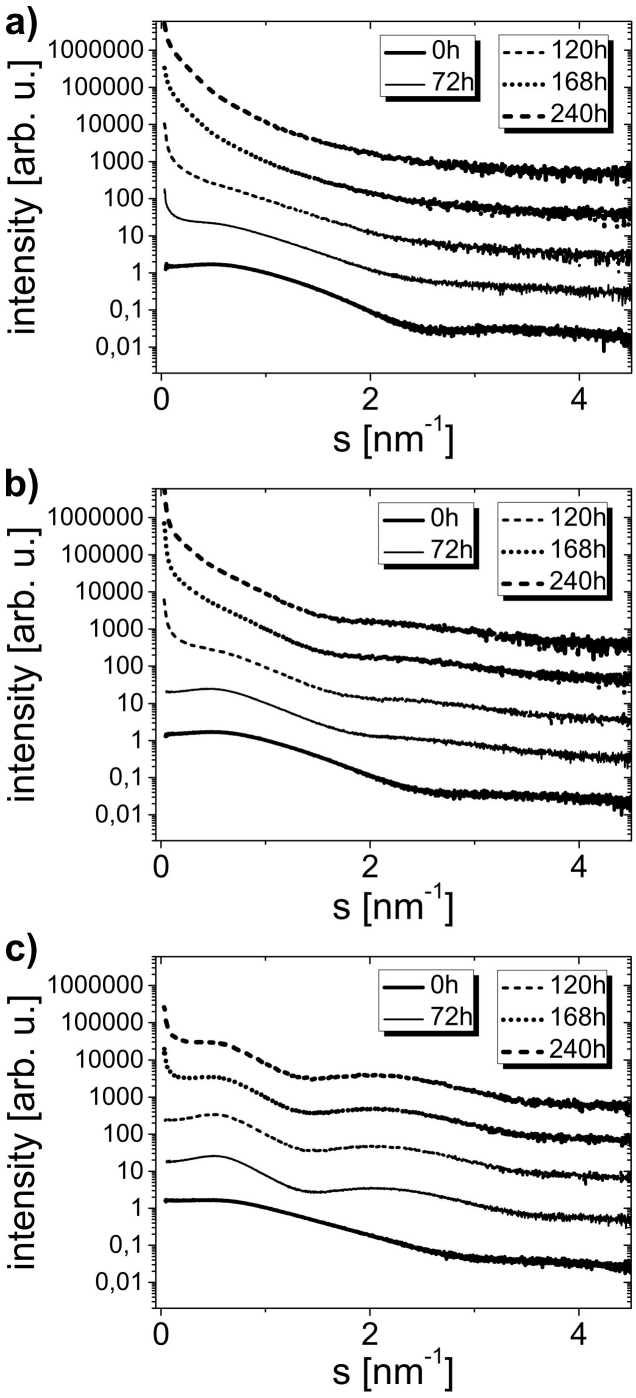

Fig. S2 Small angle X-ray scattering curves of bulk solutions of HEWL with addition of oxyC2 gemini surfactant in concentrations 1mM (a) 5mM (b) 20mM (c) after time of incubation given in the panels insets. Curves were shifted vertically for clarity.

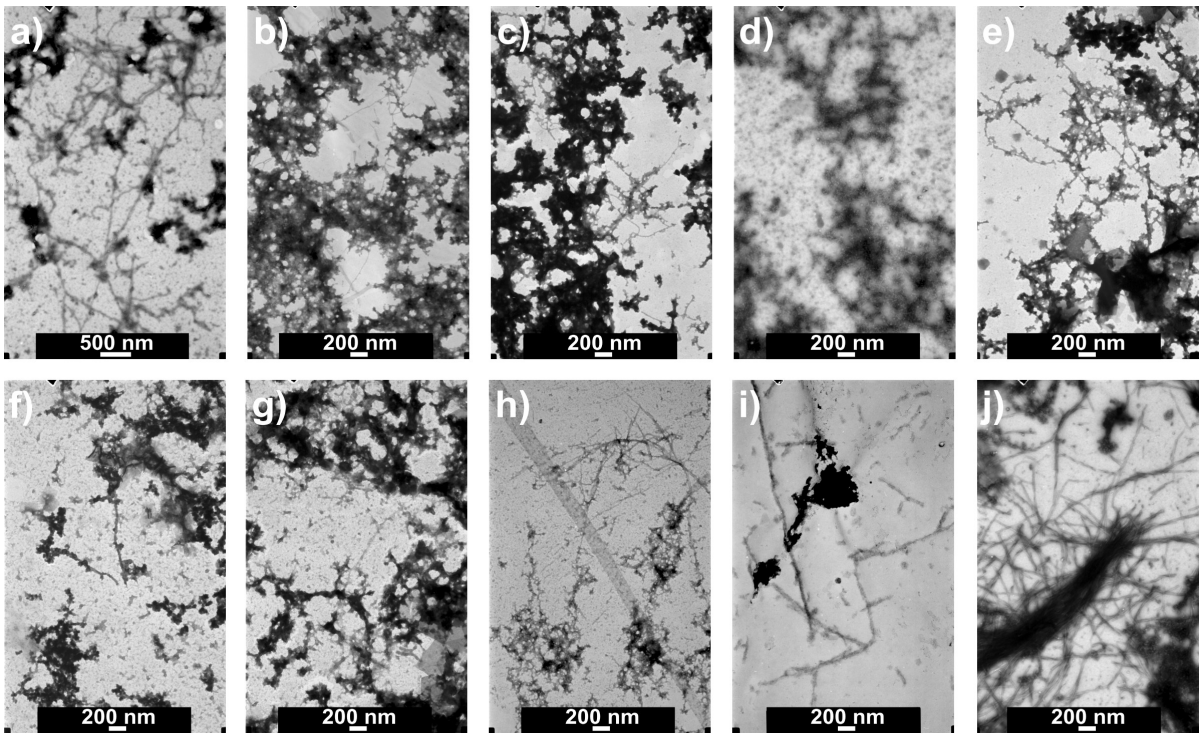

Fig. S3 TEM images of HEWL solutions with addition of different surfactants after 31 days of incubation, for the respective surfactant type and its concentration: no surfactant (a) no surfactant (b) oxyC2, 5mM (c) oxyC2, 20mM (d) oxyC4, 20mM (e) oxyC8, 5mM (f) oxyC8, 20mM (g) oxyC12, 20mM (h) C14S3, 50mM (i) SB3-14, 50mM (j).

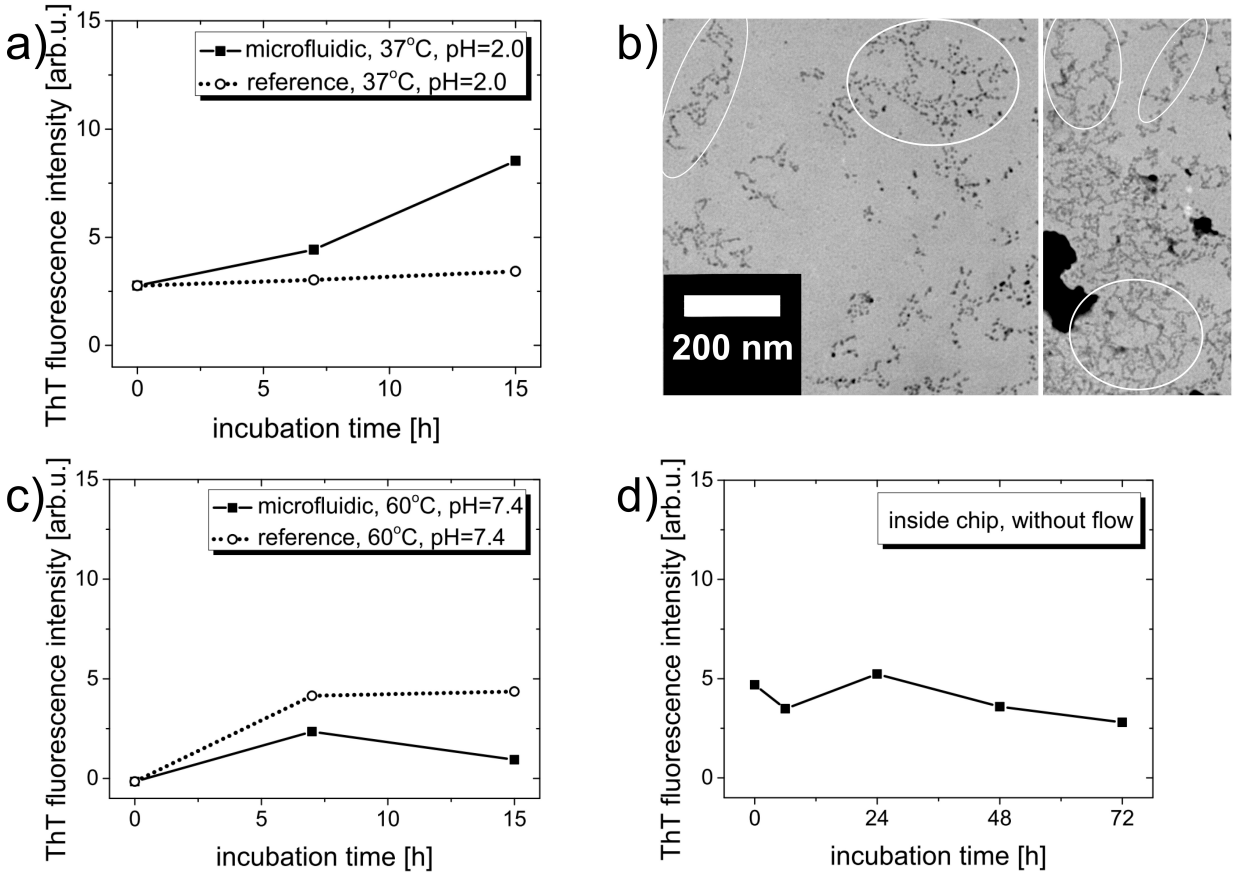

Fig. S4 Thioflavin T fluorescence signal at λ=490 nm in dependence on time of circulation in the microfluidic chip with average flow rate of 50 µL/min for solution of lysozyme without surfactant, together with results for a reference sample (staying outside the chip for the same time in the same conditions) (a). The temperature of measurement was lowered to 37 °C. TEM image of the microfluidic sample from (a) after 15 h of circulation (b). The white oval marks highlight exemplary areas of the image, where smaller aggregates presumably tend to combine and align along lines, forming fibrillar aggregates. Thioflavin T fluorescence signal at λ=490 nm in dependence on time of circulation with average flow rate of 50 µL/min for solution of lysozyme without surfactant staying at temperature of 60 °C, put in pH increased to 7.4, together with results for a reference sample (staying outside the chip for the same time in the same conditions) (c). Thioflavin T fluorescence signal at λ=490 nm in dependence on time of experiment for solution of lysozyme without surfactant, at temperature of 60 °C, pH 2.0 (d). Sample was staying in the microfluidic chip, but with no circulation, under applied pressure of 2500 mbar.

Table S1 Thioflavin T fluorescence signal at λ=490 nm of lysozyme samples (without surfactant) for given time of sample incubation, for identically prepared samples from different series. Data show the distribution of aggregation time and strength. Series were numbered for the purpose of this table, in a random manner. Sample ID is given only as an indication that each record in the table represents a different sample.

		number of days of incubation							
series no.	sample ID	0	3	5	7	10	14	21	31
		ThT fluorescence intensity [arb. u.]							
I	*M*	2	4	59	156	256			
	R	2	12	53	264	407			
II	*0*	2	1	5	13	191			
	4	1	4	13	37	60			
III	*A*			32		84			
	E			9		22			
IV	*a*	2					5	15	55
	b	2					15	22	53

Table S2 Radii of gyration of HEWL samples subject to microfluidic circulation, obtained from SAXS curves measured for these samples.

sample flow rate [µl/min]					
50		100		20	
time of circulation [h]	R_g [nm]	time of circulation [h]	R_g [nm]	time of circulation [h]	R_g [nm]
0	1.6	0	1.6	0	1.5
8	non-determin-able	4	non-determin-able	9	1.6