Electronic Supplementary Material (ESI) for Soft Matter.

Does polysaccharide glycogen behave as a promoter of amyloid fibril formation at physiologically relevant concentrations?

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Supplementary information:

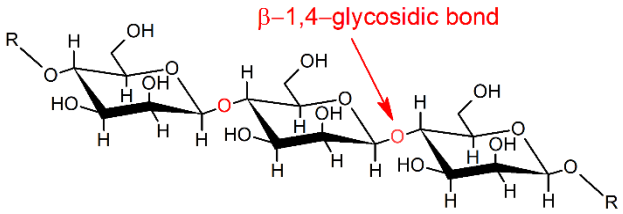


Fig. S1. Structure of mannan (MAN).

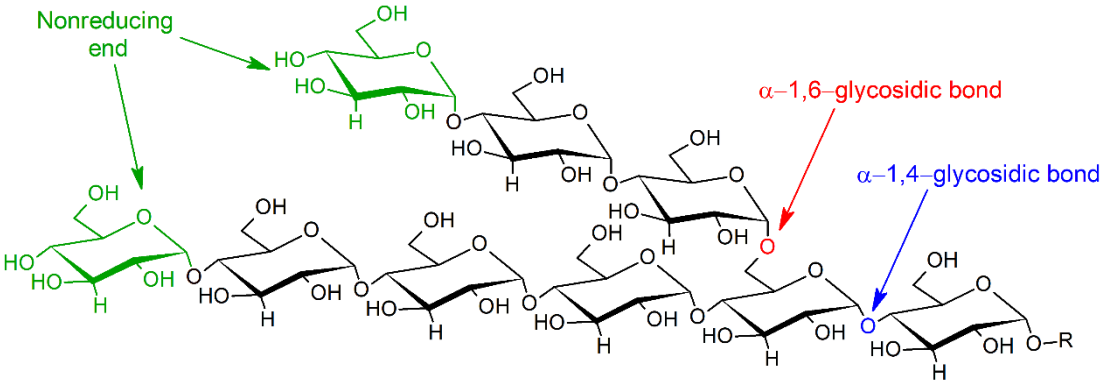


Fig. S2. Structure of glycogen (GG).

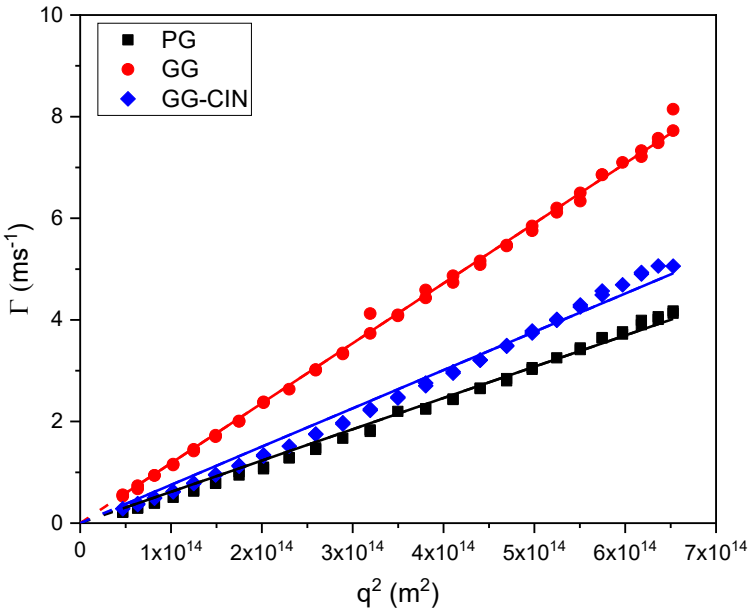


Fig. S3 The graph of Γ dependence on q^2

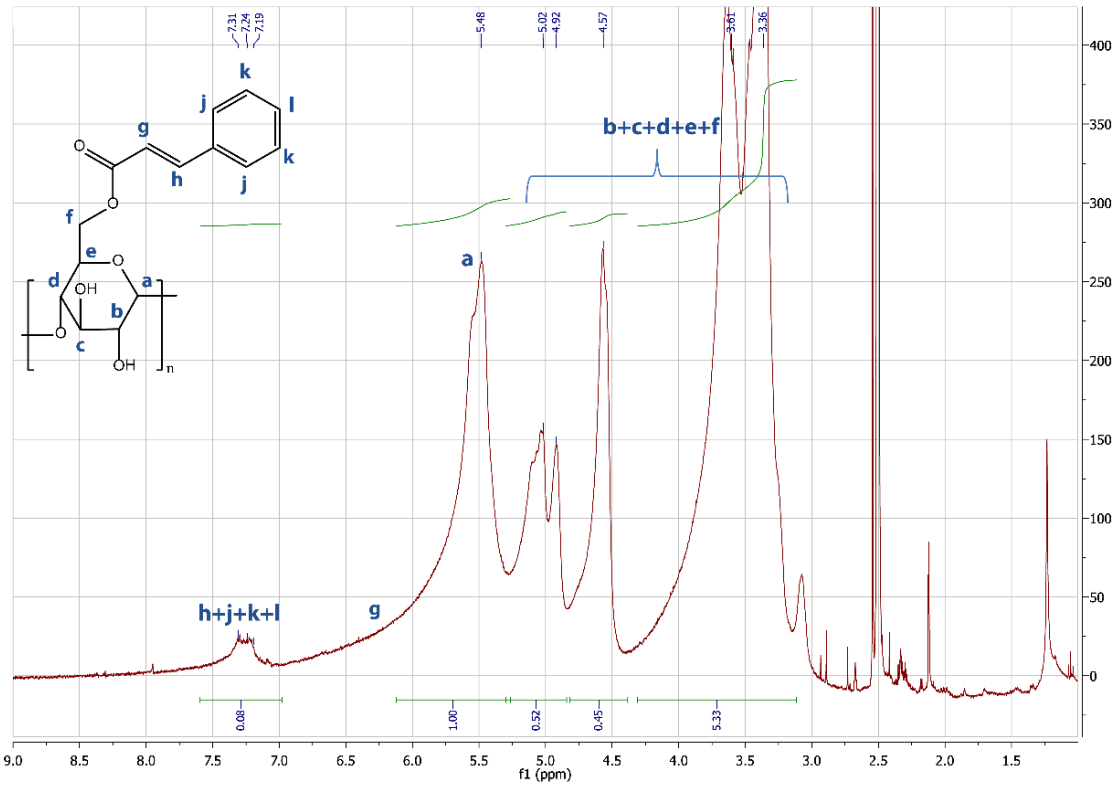


Fig. S4. ^{1}H-NMR of cinnamoylated glycogen (GG-CIN).

Tab. S1. The refractive index increment (d*n*/d*c*), molecular weight (M_W) and intensity-weighted R_h values obtained from DLS of all polysaccharides and GG-CIN.

	MAN	*GG*	*PG*	*GG-CIN*
dn/dc (mL/g)	0.1355±0.0039	0.1483±0.0017	0.1471±0.0002	0.1505±0.0019
M_W *(g/mol)*	5.5×10^4	5.7×10^6	27.1×10^6	6.1×10^6
Intensity-weighted R_h *(nm)*	5.9±1.5	23.5±8.1	45.1±19.9	30.4±19.0

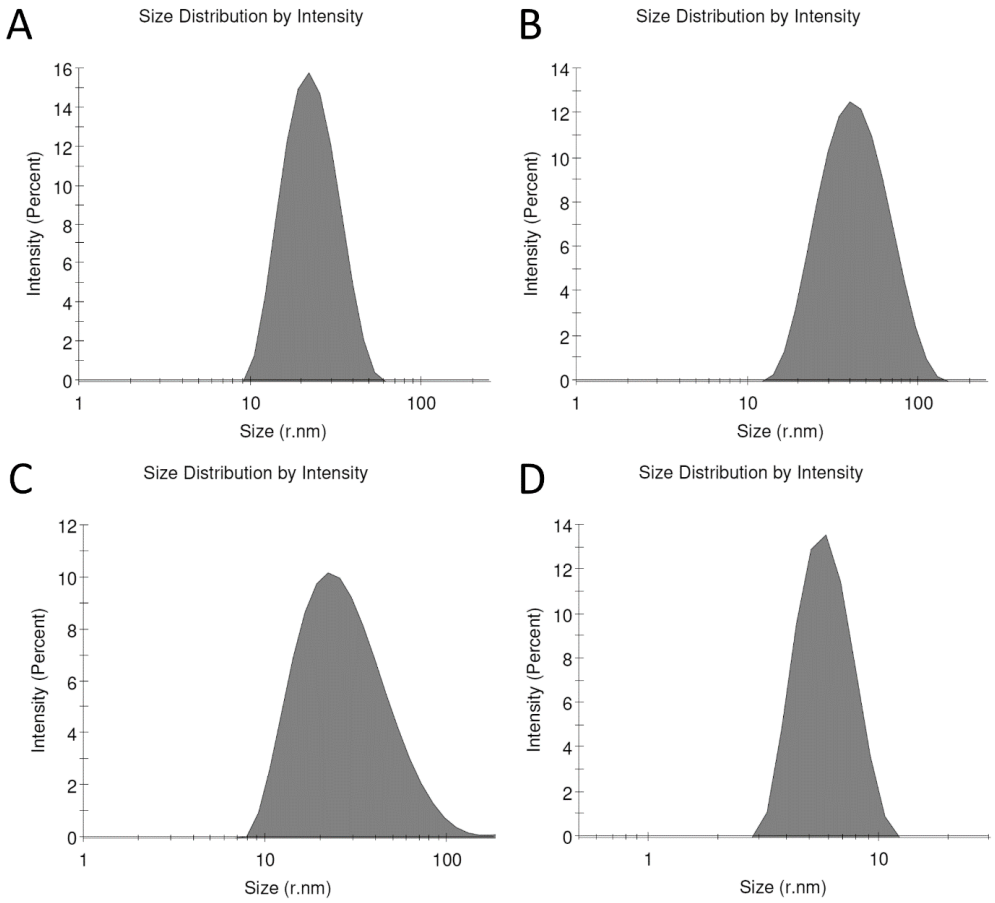


Fig. S5. Size distribution by intensity for (A) GG, (B) PG, (C) GG-CIN and (D) MAN.

Experiments with HEWL, MAN, GG and PG

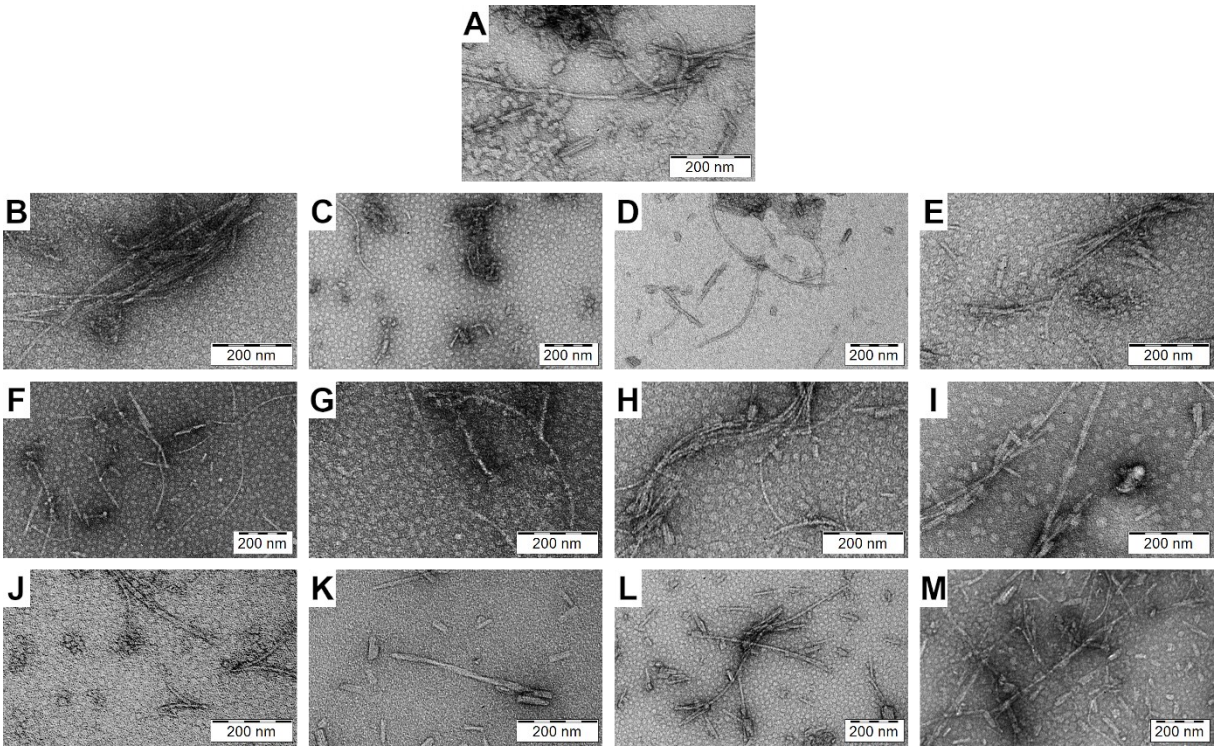


Fig. S6. Experiments with HEWL, MAN, GG and PG: TEM micrographs after 24h: (A) shorter and longer fibrils of HEWL (control) without any polysaccharides; shorter and longer fibrils for samples containing (B) 10 µg/mL, (C) 50 µg/mL, (D) 250 µg/mL and (E) 500 µg/mL MAN; shorter and longer fibrils for samples containing (F) 10 µg/mL, (G) 50 µg/mL, (H) 250 µg/mL and (I) 500 µg/mL GG; shorter and longer fibrils for samples containing (J) 10 µg/mL, (K) 50 µg/mL, (L) 250 PG µg/mL and (M) 500 µg/mL PG. The TEM micrographs were used just to illustrate and check the morphology.

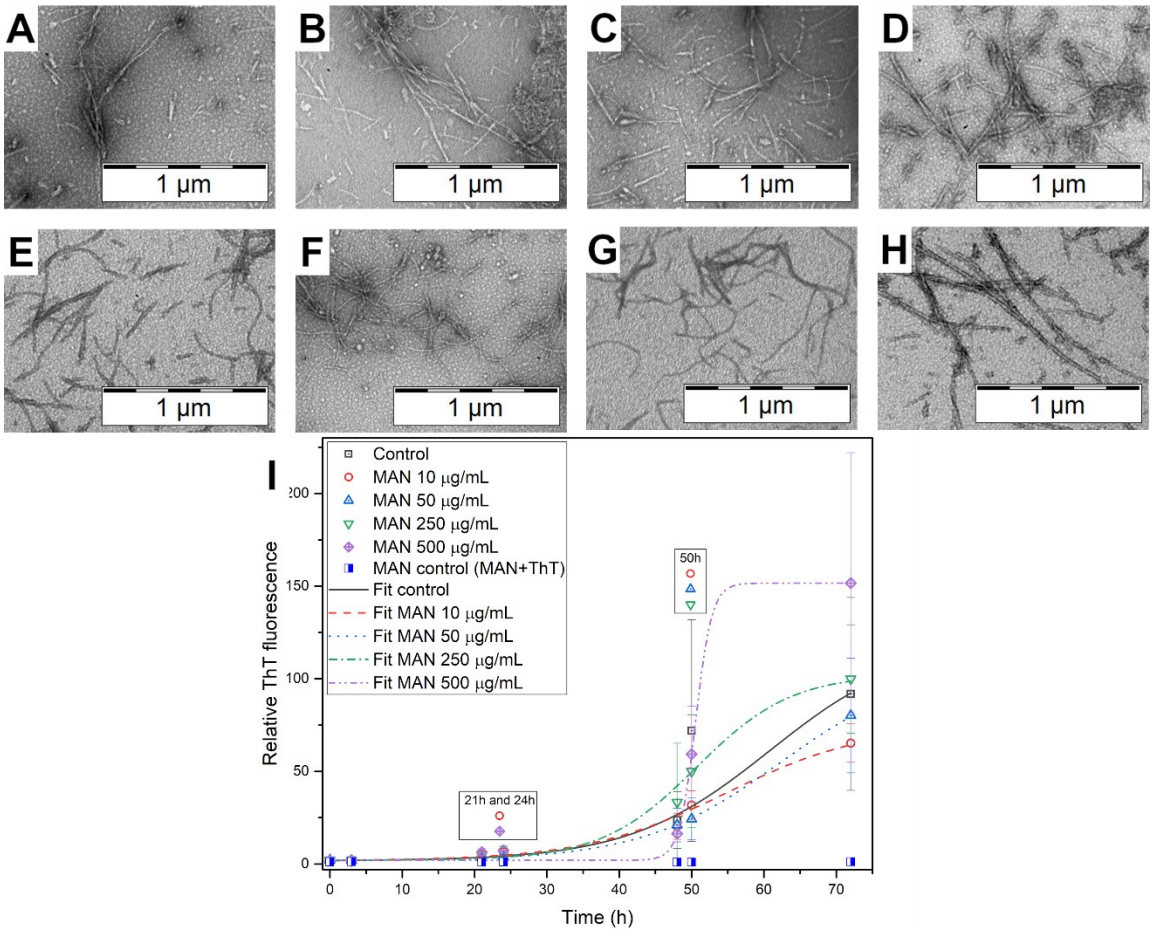


Fig. S7 Experiments with HEWL, MAN, GG and PG: TEM micrographs after 48h: (A-D) shorter and longer fibrils for samples containing (A) 10 µg/mL,(B) 50 µg/mL, (C) 250 µg/mL and (D) 500 µg/mL MAN ; (E) and (F) shorter and longer fibrils for samples containing 10 µg/mL and 50 µg/mL GG; (G) shorter and longer fibrils for the sample containing 10 µg/mL PG; (H) shorter and long fibers for the sample containing 50 µg/mL PG. (I) Graphs of relative ThT fluorescence with the fit of data. The legend above the results from the same time represents a statistically significant difference ($\alpha<0.05$) when compared to control at this time. The TEM micrographs were used just to illustrate and check the morphology.

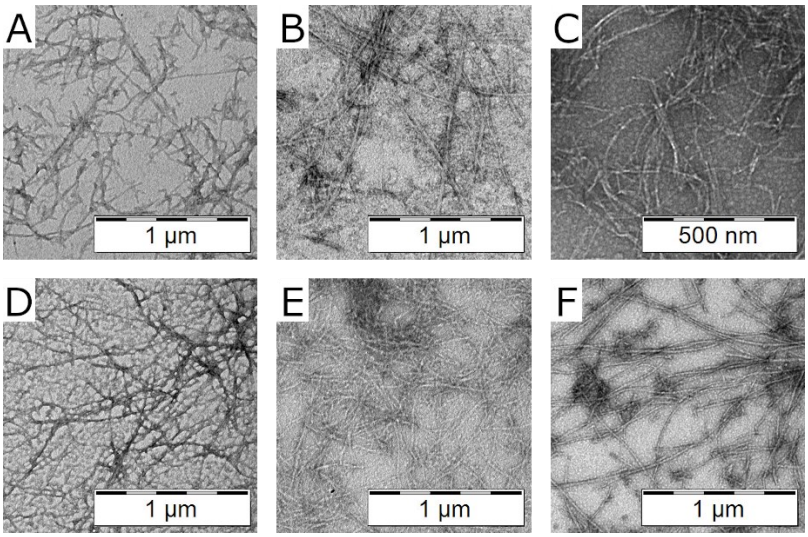


Fig. S8. Experiments with HEWL, MAN, GG and PG: TEM micrographs after 72 h: (A) mature amyloid fibrils of HEWL (control) without any polysaccharides; (B) mature amyloid fibrils of 250 µg/mL MAN; (C) and (D) mature amyloid fibrils for samples containing 10 µg/mL and 50 µg/mL GG; (E) and (F) mature amyloid fibrils for samples containing 10 µg/mL and 50 µg/mL GG. The TEM micrographs were used just to illustrate and check the morphology.

Experiment with HEWL, GG and GG-CIN

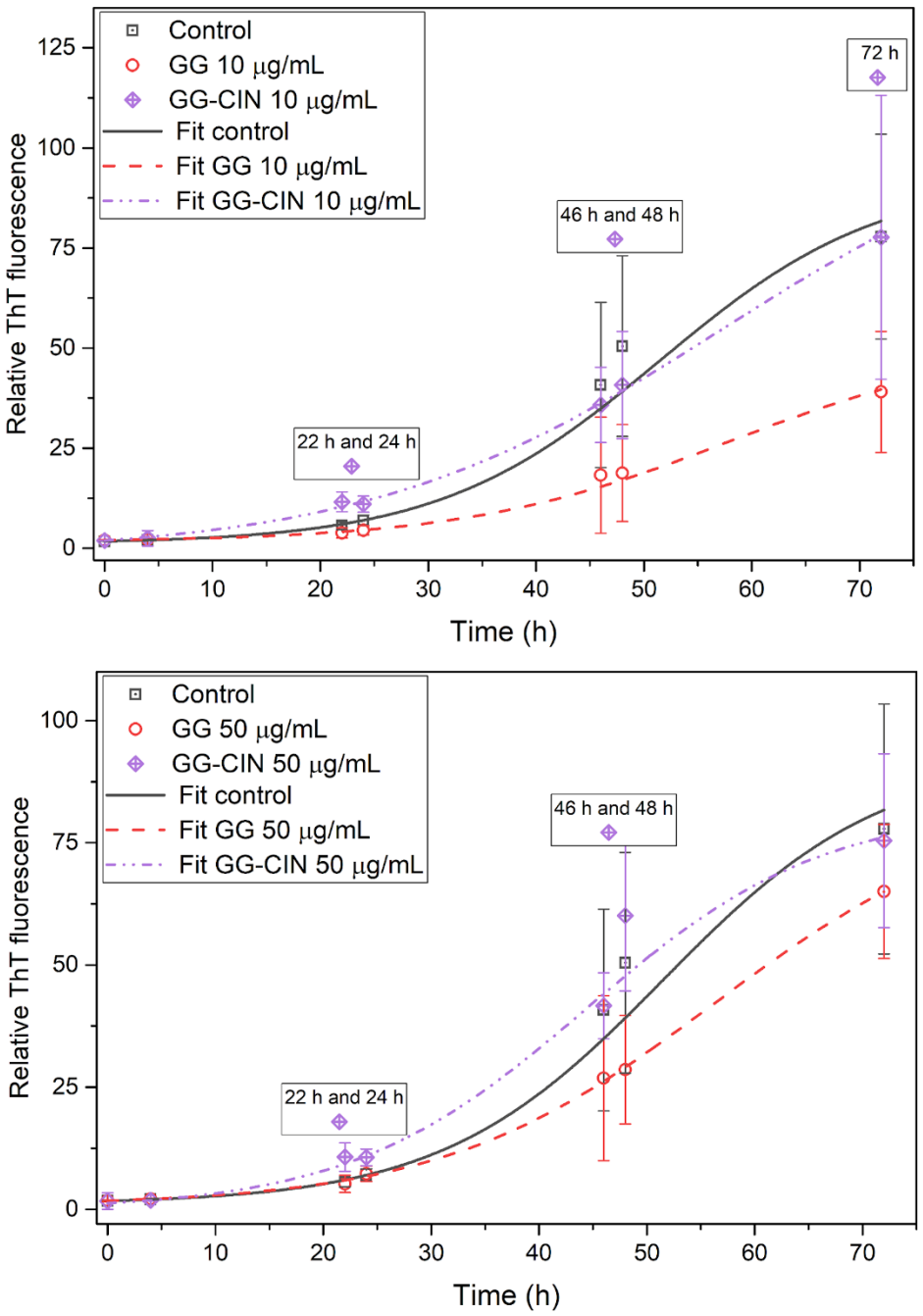


Fig. S9 part A. Experiments with HEWL, GG and GG-CIN: The graphs of comparison same amount unmodified GG and GG-CIN. The legend above the results at the same time represents a statistically significant difference ($\alpha<0.05$) when GG-CIN was compared to GG at this time.

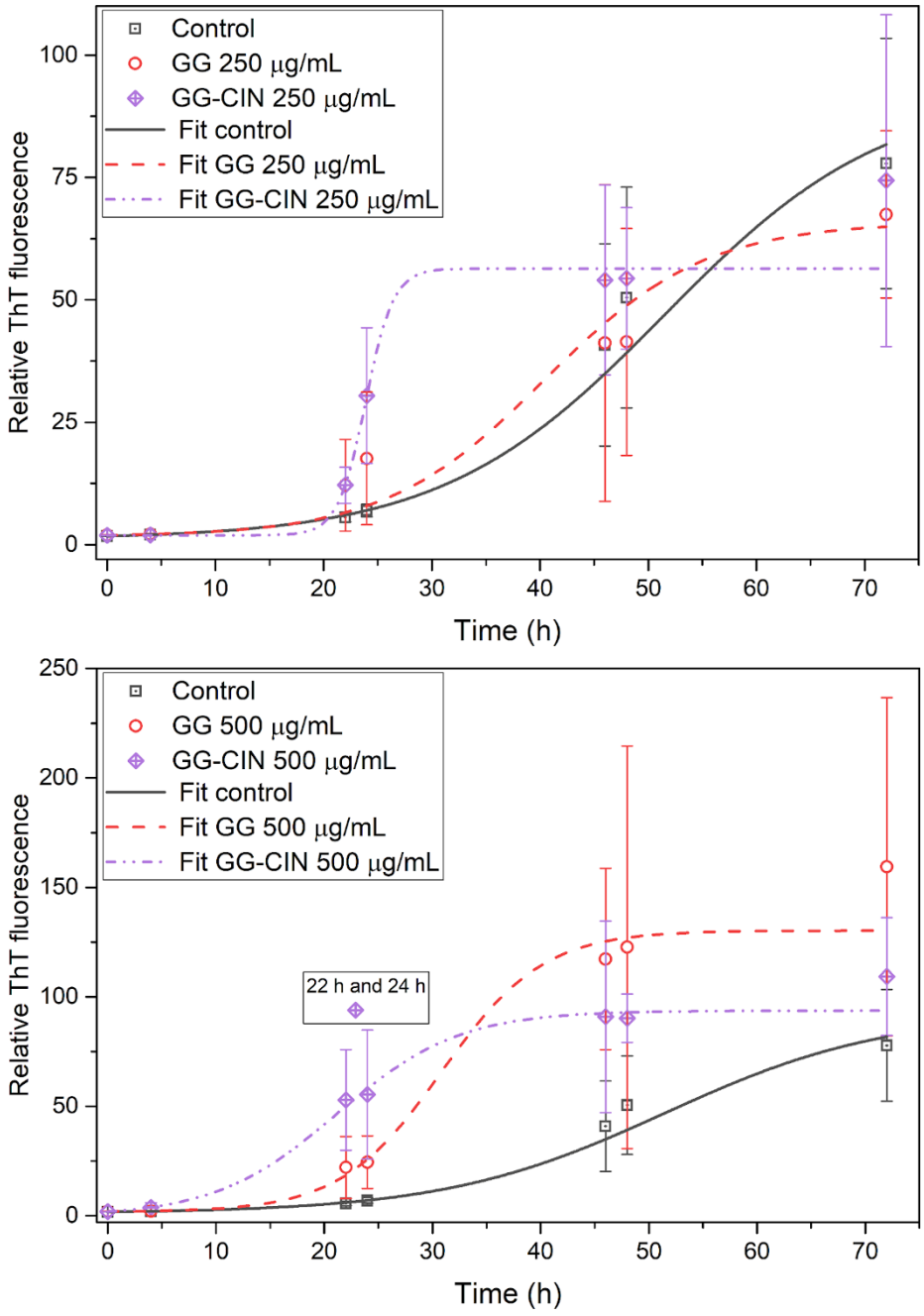


Fig. S9 part B. Experiments with HEWL, GG and GG-CIN: The graphs of comparison same amount unmodified GG and GG-CIN. The legend above the results at the same time represents a statistically significant difference ($\alpha<0.05$) when GG-CIN was compared to GG at this time.

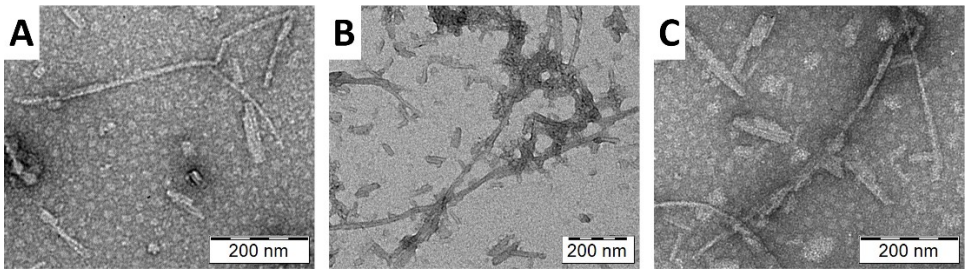


Fig. S10. Experiments with HEWL, GG and GG-CIN: TEM micrographs after 24h incubation: shorter and longer fibrils for samples containing (A) 10 μg/mL, (B) 50 μg/mL and (C) 250 μg/mL GG-CIN. The TEM micrographs were used just to illustrate and check the morphology.

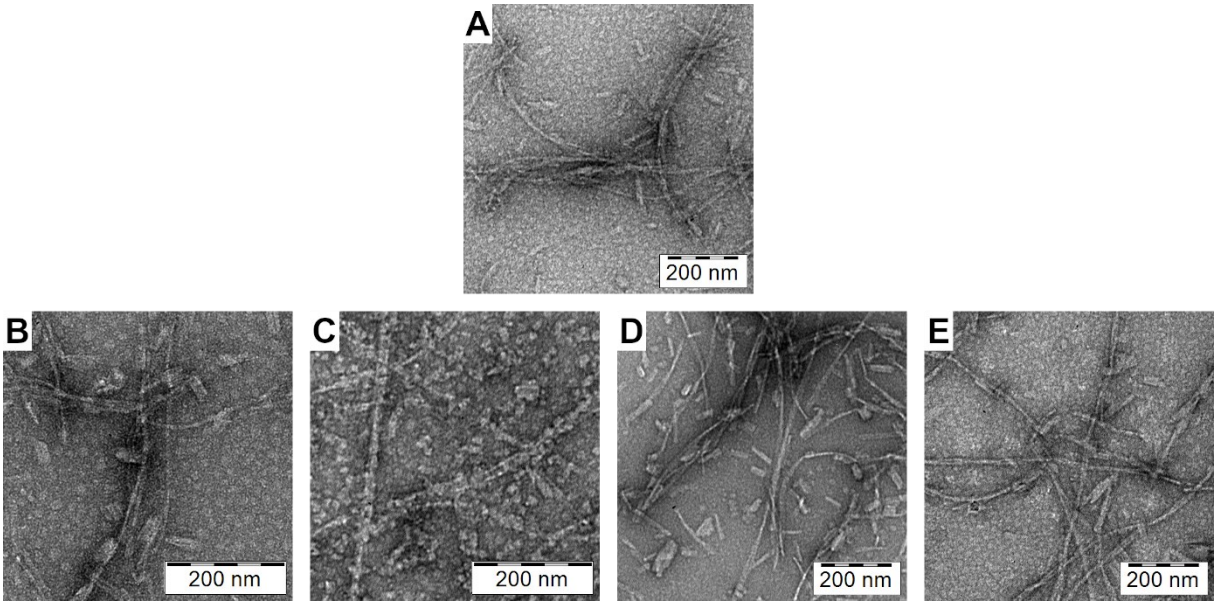


Fig. S11. Experiments with HEWL, GG and GG-CIN: TEM micrographs after 48h incubation: (A) shorter and longer fibrils of HEWL (control); (B) shorter and longer fibrils for the sample containing 10 μg/mL GG-CIN, (C) longer fibrils for the sample containing 50 μg/mL GG-CIN, (D) many shorter fibrils and some longer fibrils for the sample containing 250 μg/mL GG-CIN, (E) long mature fibrils for the sample containing 500 μg/mL GG-CIN. The TEM micrographs were used just to illustrate and check the morphology.

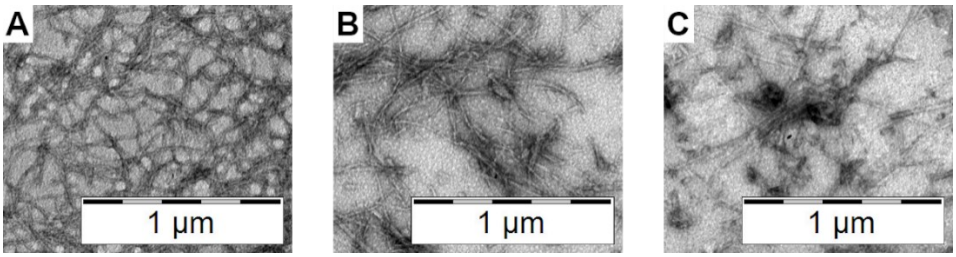


Fig. S12. Experiments with HEWL, GG and GG-CIN: TEM micrographs after 72 h incubation: (A) long mature fibrils of HEWL (control); (B) long mature fibrils and some shorter fibrils of samples containing 10 µg/mL GG-CIN, (C) long mature fibrils and some shorter fibrils of samples containing 250 µg/mL GG-CIN. The TEM micrographs were used just to illustrate and check the morphology.

Experiment with biologically relevant concentrations of GG

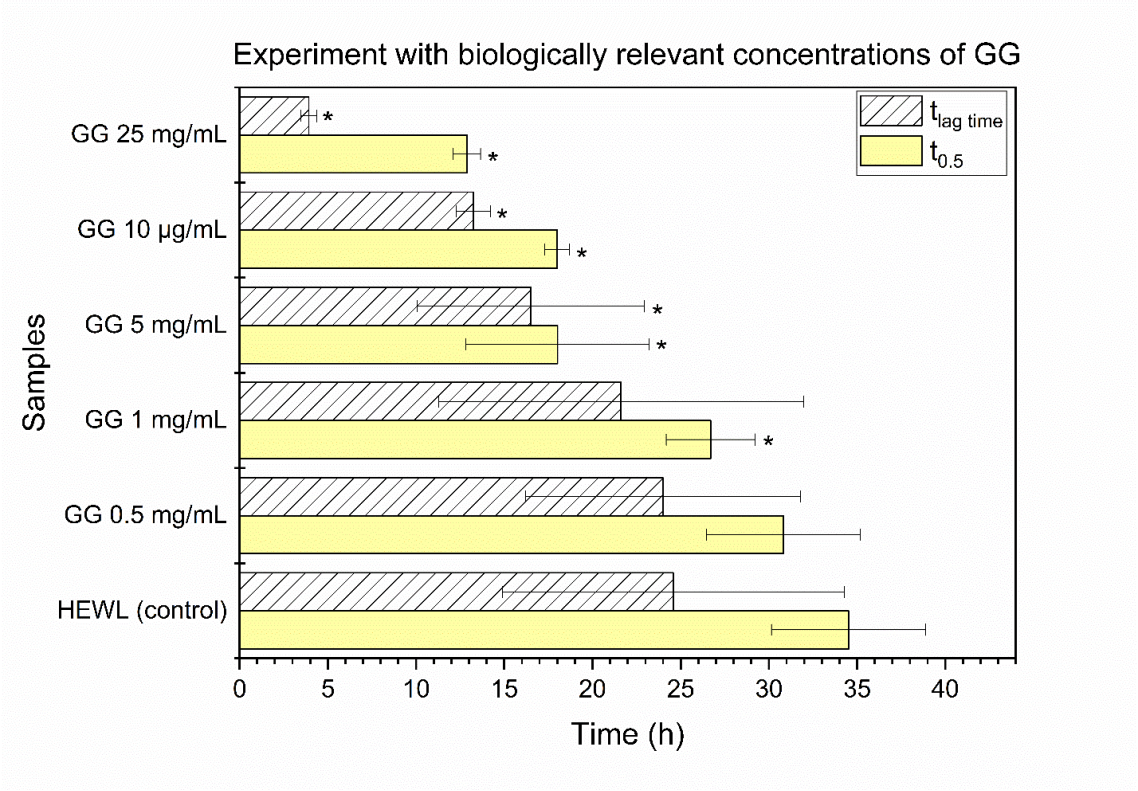


Fig. S13 The graph of the lag times ($t_{lag\ time}$) and times ($t_{0.5}$) at 50% maximal fluorescence for experiments with high concentrations of GG. The star (*) means a statistically significant difference ($\alpha<0.05$) when compared to the control.

Experiment for Aβ1-42, MAN, PG, GG-CIN and GG.

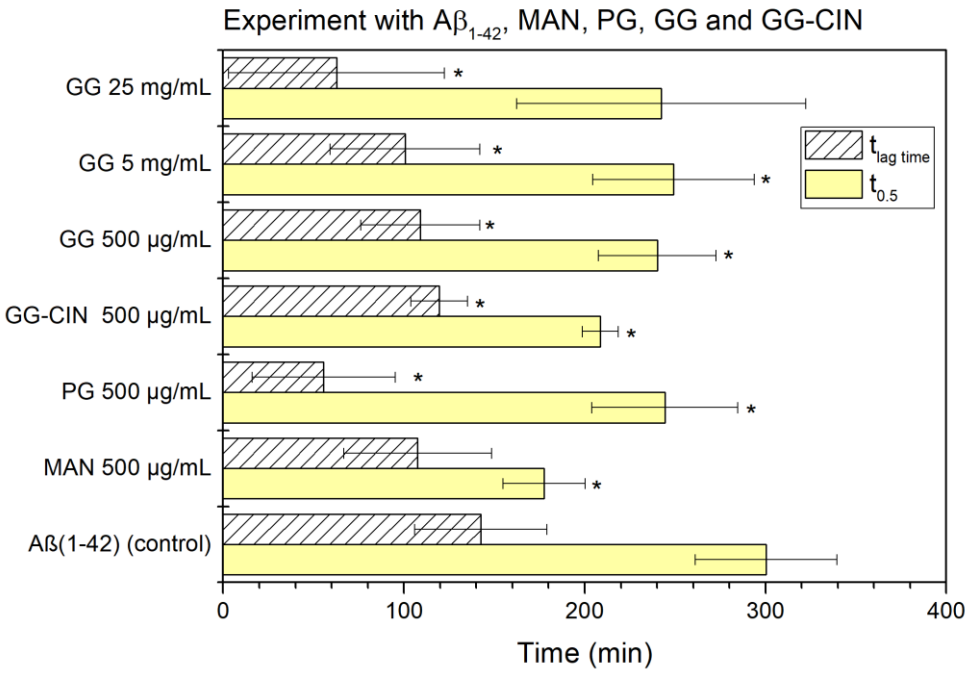


Fig. S14 A graph of the lag times ($t_{lag\ time}$) and times ($t_{0.5}$) at 50% maximal fluorescence for experiments of Aβ1-42 fibril formation without and with polysaccharides. The star (*) means a statistically significant difference ($\alpha<0.05$) when compared to the control.

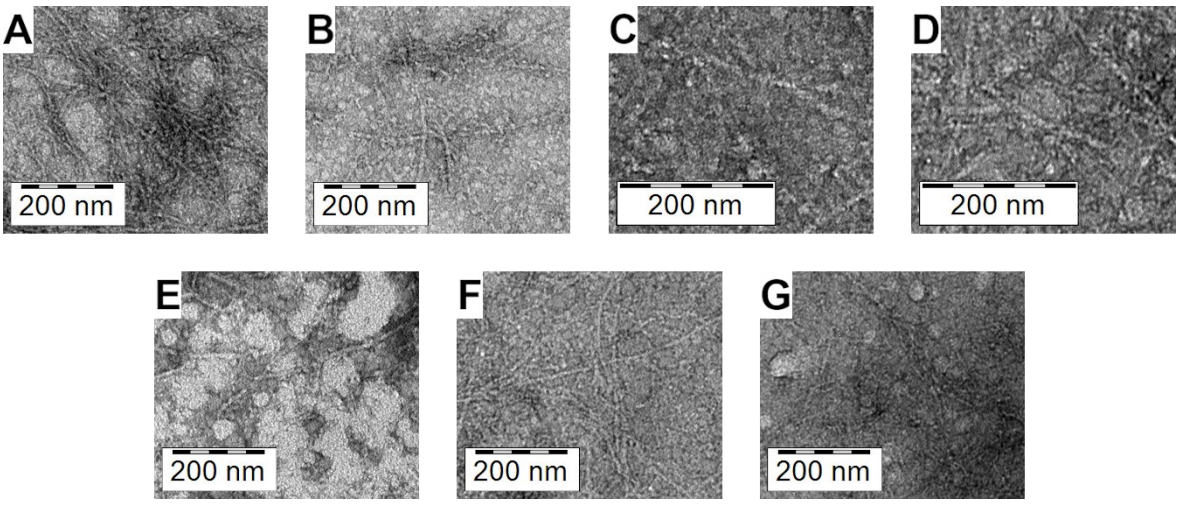
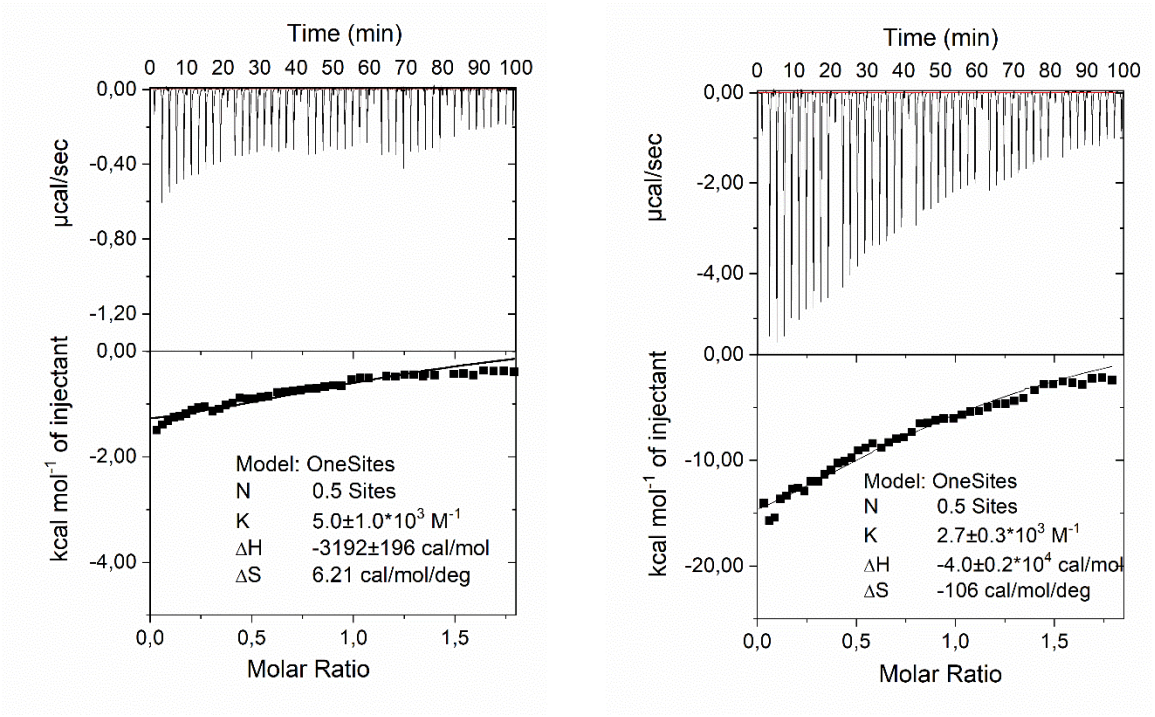


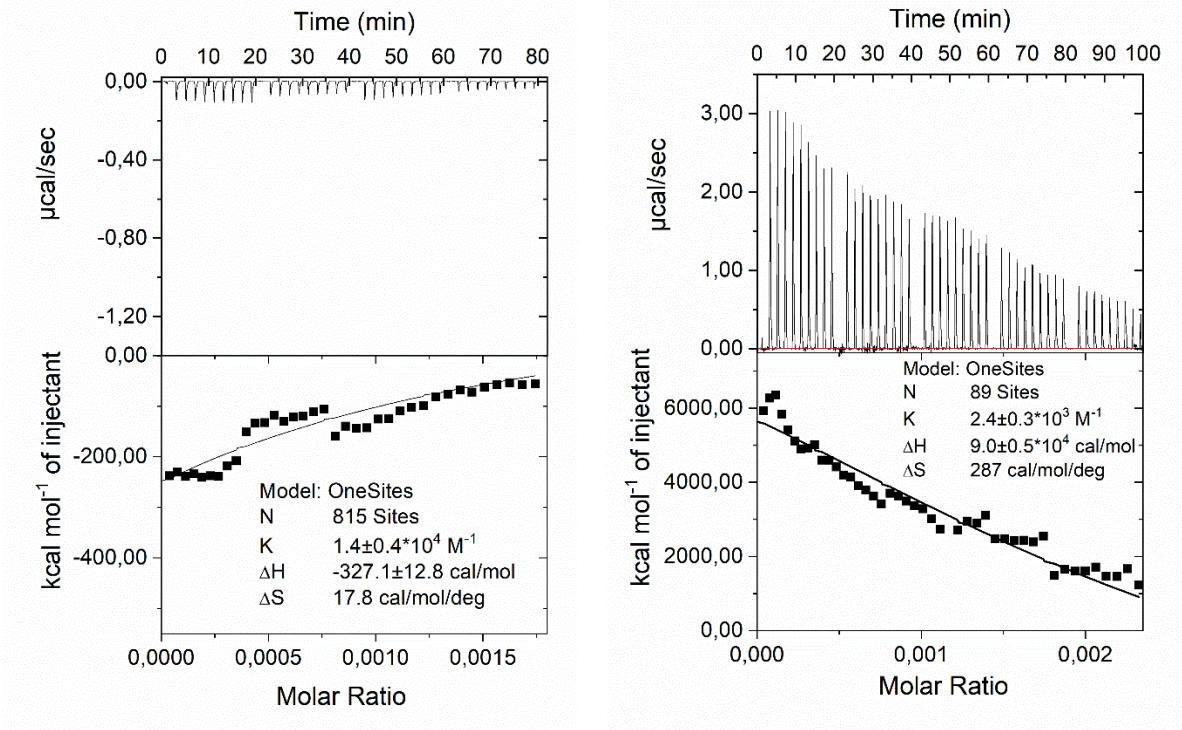
Fig. S15. TEM micrographs after 26 h incubation: (A) fibrils of Aβ1-42 (control); (B) hint of fibrils of the sample containing 500 µg/mL PG, (C) hint of fibrils of the sample containing 500 µg/mL MAN, (D) hint of fibrils of the sample containing 500 µg/mL GG-CIN; a hint of fibrils the sample containing (E) 500 µg/mL, (F) 5 mg/mL and (G) 25 mg/mL GG. The TEM micrographs were used just to illustrate and check morphology.

Isothermal titration calorimetry (ITC) – the upper graph – heat flow per injection corrected to the heat of dilution, lower graph – integrated heat vs molar ratio (the line is a fit to the experimental data)



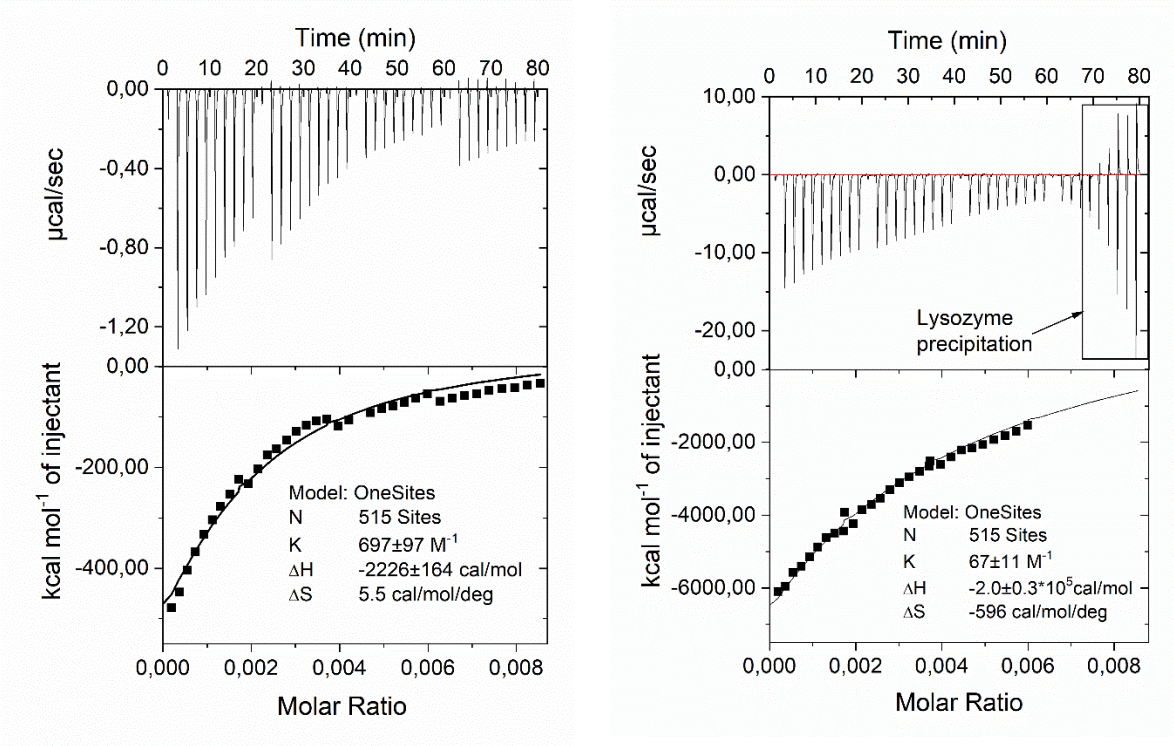
A B

Fig.S16 Titration of 1mM HEWL with 1.35mM MAN at 25°C (A) and 57°C (B).



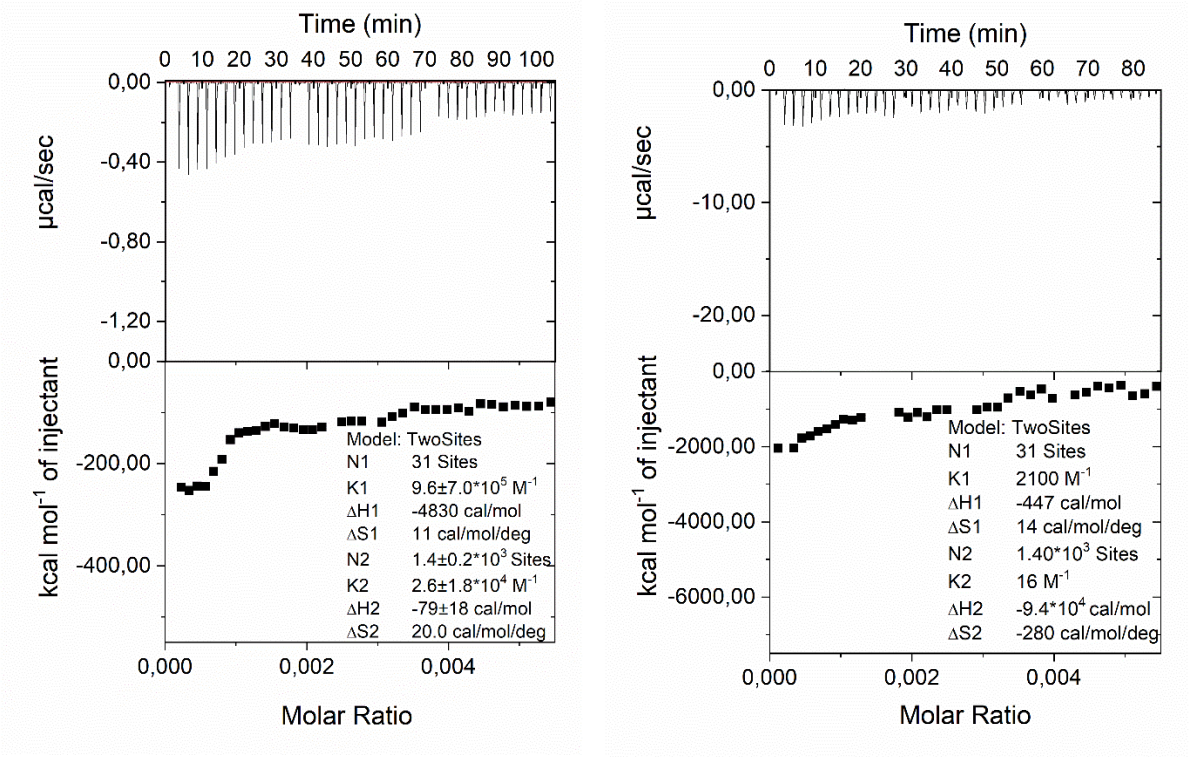
A B

Fig.S17 Titration of 1mM HEWL with 2.76 µM PG at 25°C (A) and 57°C (B).



A B

Fig.S18 Titration of 1mM HEWL with 8.83 µM GG at 25°C (A) and 57°C (B).



A B

Fig.S19 Titration of 1mM HEWL with 7.26 µM GG-CIN at 25°C (A) and 57°C (B).

Tab. S2 Intensity R_h of polymers at temperatures of 25 °C and 57 °C and their corresponding relative excluded volume and density.

		25 °C			57 °C		
	concentration (mg/mL)	R_h *(nm)*	*Density (g/mL)*	*Relative excluded volume (%)*	R_h *(nm)*	*Density (g/mL)*	*Relative excluded volume (%)*
GG	0.5	23.5	0.174	0.29	20.6	0.259	0.19
	1			0.58			0.39
	5			2.87			1.93
	10			5.74			3.87
	25			14.36			9.67
MAN	0.5	5.9	0.106	0.47	6.2	0.091	0.55
PG	0.5	45.1	0.118	0.43	38.2	0.193	0.26
GG-CIN	0.5	30.4	0.086	0.58	27.7	0.113	0.44