Supporting Information

Material and methods

Sample preparation, peptide synthesis and fibril formation

Tris(hydroxymethyl)aminomethane (Tris), *p*-nitrophenyl acetate (*p*NPA), *p*-nitrophenol (*p*NP) and zinc chloride ($ZnCl_2$) were purchased from Sigma-Aldrich. Trimethylamine-*N*-oxide (TMAO) and urea were purchased from Alfa Aeser, and Ficoll PM70 was obtained from GE Healthcare. All compounds were used without further purification. Tris-HCl buffer containing 25 mM Tris and 1 mM $ZnCl_2$ at pH 8.0 was used to prepare the peptide and substrate solutions. For the cosolvents and crowding studies, the buffer solution was prepared with 0.5 M TMAO, 1 M urea or 25 wt% Ficoll PM70, respectively. The peptide Ac-IHIHIQI-$CONH_2$) were obtained from IZKF Leipzig, Core Unit Peptid-Technologien, at a purity of at least 98% (w/w). The amyloid fibrils were prepared as described in Rufo *et al.*[27] and described by us in ref. 57. Before the measurements, the fibrils were lyophilized and re-dissolved in water to obtain a 0.1 mM stock solution. The substrate *p*NPA was dissolved in acetonitrile to prepare a 0.1 M stock solution. Both the amyloid fibrils and substrate solutions were dissolved with the respective buffer solutions to reach the target concentration right before the experiments.

High-pressure FTIR spectroscopy

The pressure-dependent FTIR data were recorded using a Nicolet Magna 550 spectrometer, equipped with a liquid-nitrogen-cooled MCT detector and a pressure range from 0.1 to 1000 MPa. Each FTIR spectrum was obtained by recording 256 scans at a spectral resolution of 2 cm^{-1}. The infrared light was focused onto the pinhole of a P-series diamond anvil cell (DAC) with type IIa diamonds (High Pressure Diamond Optics Inc., Tucson, AZ, USA).[55] Gaskets of 0.05 mm thickness, made of stainless steel with a 0.45 mm drilling, were placed between the two diamonds, holding a sample volume of about 6 µL. Pressure was determined with co-added $BaSO_4$, which exhibits a characteristic pressure sensitive symmetric stretching mode of the sulfate at around 983 cm^{-1} that increases linearly with pressure (accuracy ~20 MPa).[56] The sample concentration was 60 mg mL^{-1} in 6 µL of pure D_2O and using this volume in the DAC cell yielded a sufficiently high signal-to-noise ratio. An external water thermostat served as a temperature control to maintain a temperature of 25 °C. After each pressure change, the sample was equilibrated for 5 min before collection of the IR spectrum. Spectral evaluation was performed using Thermo GRAMS software as described elsewhere.[56] Two IR regions were analyzed: 1700-1520 cm^{-1} and 3000-2850 cm^{-1}. The first region is related to amide I' (essentially C=O stretch) and amino-acid side-chain vibrations, the second region to the symmetric and asymmetric vibrations of the alkanes present in the amino acid side chains (see Table SI1).[57-59]

Table SI1: Assignment of IR bands.

Vibration	Wavenumber / cm^{-1}
amide I´	1700-1520
υ_s (CH_3)	~2872
υ_{as} (CH_3)	~2962
υ_s (CH_2)	~2853
υ_{as} (CH_2)	~2926

High-pressure stopped-flow (HPSF)

For the high-pressure stopped-flow kinetic studies, the measurements were carried out in Tris-HCl buffer in the absence and in the presence of cosolvents and crowding agents at pH 8. Herein, a final peptide concentration of 15 µg mL^{-1} (~16 µM) was used, while the substrate concentration was varied from 100 to 800 µM. The final concentration of acetonitrile was 2% (v/v) in all reaction mixtures. The enzymatic reaction of 4-nitrophenyl acetate forming the product *p*-nitrophenol (*p*NP) was recorded using a high-pressure stopped-flow instrument (HPSF-56 of Hi-Tech Scientific), which operates up to 200 MPa.[50,60] The molar extinction coefficient of *p*NP at 405 nm was determined experimentally at different temperatures and used to determine the product concentration by UV/Vis absorption spectroscopy. The setup and principle of the HPSF-system was described in detail elsewhere.[50,60] The pressure was controlled by a high-pressure control unit and was increased stepwise from 0.1 MPa to 200 MPa. The temperature was controlled with the help of an external water circuit and kept constant to ± 0.1 °C during all experiments.

Table SI2: Calculated initial velocities of the amyloid fibril reaction for all pressures and solution conditions (at T = 22 °C).

		$v_0\,[E]^{-1}$ /s^{-1}				
	p / bar	$c_{Substrate}$ / 0.1 mM	$c_{Substrate}$ / 0.2 mM	$c_{Substrate}$ / 0.4 mM	$c_{Substrate}$ / 0.6 mM	$c_{Substrate}$ / 0.8 mM
TRIS buffer	1	0.0098 ± 0.0006	0.0125 ± 0.0024	0.0161 ± 0.0019	0.0159 ± 0.0025	0.0151 ± 0.0011
	500	0.0127 ± 0.0010	0.0193 ± 0.0017	0.0243 ± 0.0018	0.0232 ± 0.0024	0.0240 ± 0.0013
	1000	0.0172 ± 0.0014	0.0279 ± 0.0016	0.0332 ± 0.0025	0.0363 ± 0.0019	0.0363 ± 0.0025
	1500	0.0208 ± 0.0014	0.0386 ± 0.0019	0.0465 ± 0.0017	0.0520 ± 0.0012	0.0530 ± 0.0020
	2000	0.0266 ± 0.0016	0.0470 ± 0.0022	0.0632 ± 0.0029	0.0666 ± 0.0027	0.0722 ± 0.0033
TRIS buffer + 0.5 M TMAO	1	0.0047 ± 0.0011	0.0070 ± 0.0005	0.0113 ± 0.0002	0.0110 ± 0.0004	0.0112 ± 0.0012
	500	0.0079 ± 0.0013	0.0125 ± 0.0005	0.0201 ± 0.0003	0.0177 ± 0.0004	0.0177 ± 0.0010
	1000	0.0139 ± 0.0020	0.0199 ± 0.0002	0.0339 ± 0.0013	0.0336 ± 0.0009	0.0338 ± 0.0025
	1500	0.0210 ± 0.0023	0.0317 ± 0.0005	0.0528 ± 0.0009	0.0628 ± 0.0019	0.0616 ± 0.0052
	2000	0.0304 ± 0.0033	0.0486 ± 0.0006	0.0837 ± 0.0017	0.1017 ± 0.0084	0.1047 ± 0.0007
TRIS buffer + 1 M urea	1	0.0061 ± 0.0011	0.0111 ± 0.0003	0.0152 ± 0.0016	0.0147 ± 0.0015	0.0130 ± 0.0006
	500	0.0073 ± 0.0013	0.0140 ± 0.0018	0.0169 ± 0.0016	0.0196 ± 0.0013	0.0206 ± 0.0008
	1000	0.0116 ± 0.0024	0.0230 ± 0.0008	0.0279 ± 0.0024	0.0311 ± 0.0016	0.0345 ± 0.0011
	1500	0.0167 ± 0.0023	0.0354 ± 0.0013	0.0462 ± 0.0016	0.0419 ± 0.0050	0.0541 ± 0.0011
	2000	0.0293 ± 0.0024	0.0488 ± 0.0024	0.0697 ± 0.0046	0.0690 ± 0.0034	0.0739 ± 0.0028
TRIS buffer + 25 wt% Ficoll PM70	1	0.0090 ± 0.0004	0.0139 ± 0.0010	0.0163 ± 0.0013	0.0192 ± 0.0034	0.0196 ± 0.0025
	500	0.0127 ± 0.0003	0.0218 ± 0.0008	0.0296 ± 0.0010	0.0342 ± 0.0024	0.0322 ± 0.0010
	1000	0.0184 ± 0.0001	0.0326 ± 0.0022	0.0449 ± 0.0018	0.0528 ± 0.0059	0.0471 ± 0.0053
	1500	0.0270 ± 0.0004	0.0410 ± 0.0008	0.0580 ± 0.0045	0.0636 ± 0.0099	0.0807 ± 0.0120
	2000	0.0331 ± 0.0012	0.0519 ± 0.0056	0.0878 ± 0.0137	0.0956 ± 0.0077	0.1185 ± 0.0064

For statistical verification/analysis, a hypothesis testing two-sample *t*-test was used to obtain *p*-values; values below 0.05 indicate that comparisons are significant (Table SI3). Here, the corresponding values of the comparison of the initial rate of the reaction with osmolyte or crowding agent vs. the negative control (TRIS buffer) are given for the lowest and highest measured substrate concentrations.

Table SI3: *p*-Values calculated using the hypothesis testing two-sample *t*-test, with significance level fixed at 0.05 for the comparison of selected kinetic data.

Initial rate of the reaction for the lowest substrate concentration (0.1 mM)		Initial rate of the reaction for the highest substrate concentration (0.8 mM)	
compared data sets	*p*-value	compared data sets	*p*-value
1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M urea	0.00560	1 bar, TRIS buffer x 1 bar, TRIS buffer + 1 M urea	0.05263
1 bar, TRIS buffer x 1 bar, TRIS buffer + 0.5 M TMAO	0.00407	1 bar, TRIS buffer x 1 bar, TRIS buffer + 0.5 M TMAO	0.00023
1 bar, TRIS buffer x 1 bar, TRIS buffer + 25 wt% Ficoll PM70	0.08747	1 bar, TRIS buffer x 1 bar, TRIS buffer + 25 wt% Ficoll PM70	0.06399
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M urea	0.07527	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 1 M urea	0.00997
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 0.5 M TMAO	0.00011	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 0.5 M TMAO	$4.5\cdot10^{-6}$
2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 25 wt% Ficoll PM70	0.00084	2000 bar, TRIS buffer x 2000 bar, TRIS buffer + 25 wt% Ficoll PM70	0.00006

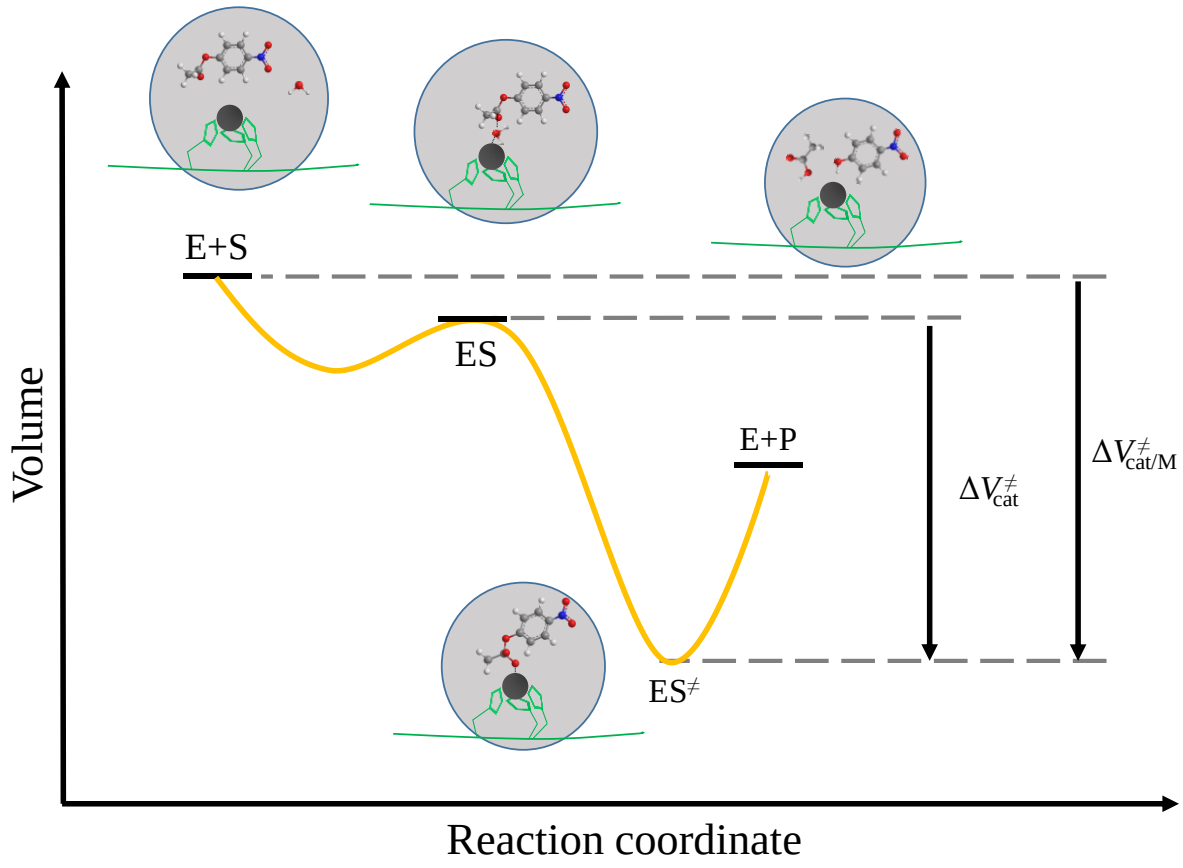

Figure SI1: Schematic representation of the volume profile of the enzymatic reaction (E = enzyme, S = substrate, ES = enzyme-substrate complex, $ES^{\neq}$ = transition state, P = product), where pressure favors product formation.

Additional references

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