

Supplementary Information

The Multifaceted Effects of DMSO and High Hydrostatic Pressure on the Kinetic Constants of Hydrolysis Reactions Catalyzed by α -Chymotrypsin

Lena Ostermeier, Rosario Oliva, and Roland Winter*

Physical Chemistry I - Biophysical Chemistry, Faculty of Chemistry and Chemical Biology,
TU Dortmund University, Otto-Hahn Strasse 4a, D-44227 Dortmund, Germany

*E-mail: roland.winter@tu-dortmund.de

Additional Figures and Tables

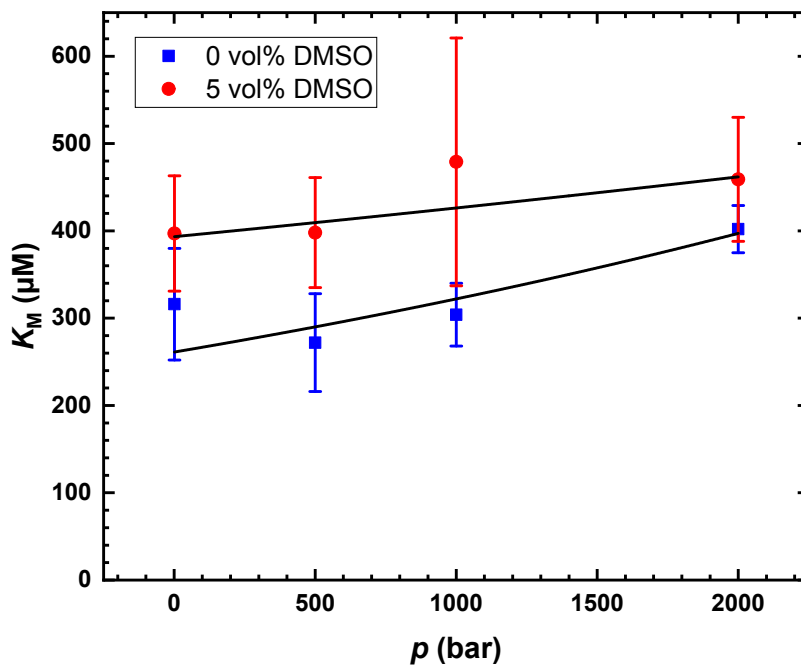


Fig. S1. Pressure dependence of the Michaelis constant, K_M , for the hydrolysis of the substrate AAF-AMC catalyzed by α -CT in neat buffer (blue squares) and in buffer containing 5 vol% of DMSO (red circles). The binding volume was calculated by fitting the experimental points to Eq. 6.

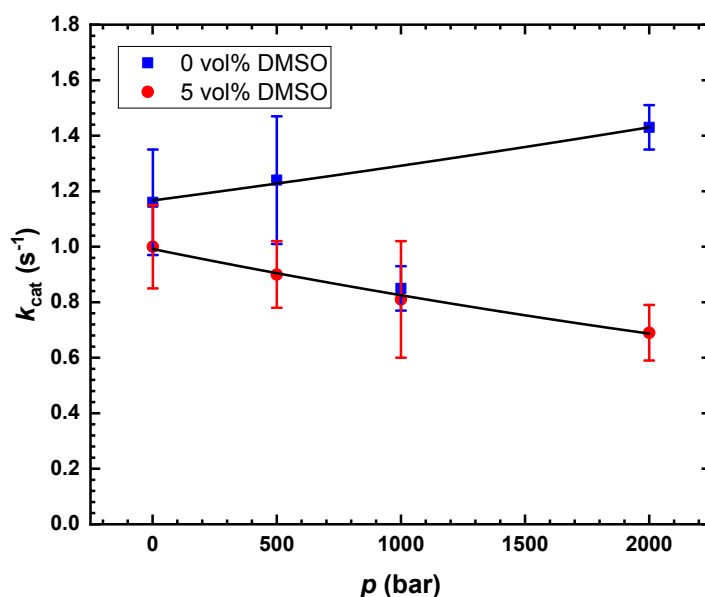


Fig. S2. Pressure dependence of the turnover number, k_{cat} , for the hydrolysis of the substrate AAF-AMC catalyzed by α -CT in neat buffer (blue squares) and in buffer containing 5 vol% of DMSO (red circles). The activation volume was calculated by fitting the experimental points to Eq. 6. Since the turnover number value in neat buffer at 1000 bar is outside the linear range, it was disregarded for the determination of the activation volume.

Table S1. Kinetic parameters for the hydrolysis of AAF-AMC catalyzed by α -chymotrypsin at ambient conditions ($T = 20$ °C and $p = 1$ bar) at different vol% of DMSO.

DMSO (vol%)	k_{cat} (s ⁻¹)	K_M (μ M)	k_{cat}/K_M (10^{-3} s ⁻¹ μ M ⁻¹)
0	1.16 ± 0.19	316 ± 64	3.67 ± 1.34
5	1.00 ± 0.15	397 ± 66	2.52 ± 0.80
10	0.88 ± 0.15	481 ± 88	1.83 ± 0.65
20	0.25 ± 0.03	328 ± 42	0.76 ± 0.19

Table S2. Kinetic parameters for the hydrolysis of AAF-AMC catalyzed by α -chymotrypsin in neat buffer and in buffer containing 5 vol% of DMSO at different pressures and $T = 20$ °C.

DMSO (vol%)	pressure (bar)	k_{cat} (s ⁻¹)	K_M (μ M)	k_{cat}/K_M (10^{-3} s ⁻¹ μ M ⁻¹)
0	1	1.16 ± 0.19	316 ± 64	3.67 ± 1.34
5	1	1.00 ± 0.15	397 ± 66	2.52 ± 0.80
0	500	1.24 ± 0.23	272 ± 56	4.56 ± 1.78
5	500	0.90 ± 0.12	398 ± 63	2.26 ± 0.66
0	1000	0.85 ± 0.08	304 ± 36	2.80 ± 0.59
5	1000	0.81 ± 0.21	479 ± 142	1.69 ± 0.94
0	2000	1.43 ± 0.08	402 ± 27	3.56 ± 0.44
5	2000	0.69 ± 0.10	459 ± 71	1.50 ± 0.45

