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Supporting information for article:

Protein crystal structure determination with the crystallophore, a nucleating and phasing agent

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S1. Procedure of production and purification of samples from *Methanothermococcus thermolithotrophicus*

Methanothermococcus thermolithotrophicus (DSM 2095), obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) was grown at 65 °C as previously described (Wagner *et al.*, 2017).

Protein fractions have been enriched and purified using the shotgun approach as described in (Vögeli *et al.*, 2018). The purification of fractions A-D were performed under anoxic atmosphere (95% N₂/5% H₂) at 18 °C and without any freezing step. About 40 g of wet cell pellets were suspended in lysis buffer (50 mM MOPS/NaOH pH 7.0, 10 mM MgCl₂ and 2 mM dithiothreitol) with a ratio of 10 ml lysis buffer per g of pellet. The cells were lysed by osmotic shock at 18°C under gentle stirring with an addition of 2.0 U/ml benzonase to reduce viscosity. The lysate was centrifuged twice at 10,000 ×g for 60 min at 4 °C to remove cell debris. The supernatant was applied on a DEAE Sepharose fast-flow column (65 ml) equilibrated with 50 mM Tricine/NaOH pH 8.0 containing 2 mM dithiothreitol (DTT) (buffer A). The column was washed at a flow rate of 4.5 ml/min for 2 column volumes. The elution step was performed through a gradient of increasing concentration of NaCl from 50 mM to 350 mM in 5 column volumes. The protein fractions eluted between 130 and 240 mM NaCl were pooled and diluted with an equal volume of buffer A and then the sample was loaded on a Q-Sepharose fast-flow column (50 ml) (GE Healthcare, Freiburg) pre-equilibrated in buffer A. The column was washed with 200 mM NaCl, and the proteins were eluted at a flow rate of 3.0 ml/min with a gradient from 200 to 400 mM NaCl in 3 column volumes. Protein fractions eluted between 235 - 370 mM NaCl were pooled and diluted with an equal volume of 25 mM sodium phosphate buffer pH 7.6, containing 2 mM DTT (buffer B) and passed on a hydroxyapatite ceramic type I column (45 ml) (Macrorep; Bio-Rad; München, Germany) equilibrated with buffer B. The column was washed at 3 ml/min with buffer B with 5 column volumes and the flow-through containing the protein from fraction A and B was collected. A gradient of 25 - 500 mM of sodium phosphate was applied to the hydroxyapatite for 4 column volumes and the protein fractions eluted at 240 - 490 mM sodium phosphate pH 7.6 were collected. This latter protein pool containing the fraction C and D was subsequently diluted with three volume of 25 mM Tris/HCl, pH 7.6, containing 2.0 M (NH₄)₂SO₄ and 2 mM DTT (buffer C), and injected on a Source 15 Phenyl column (15 ml) that was pre-equilibrated in the same buffer. After washing the column with 1 column volume of buffer C, the elution was done at a flow rate of 1 ml/min with a gradient from 2.0 to 0 M (NH₄)₂SO₄ in 14 column volumes. Protein of fraction C eluted within a range of 0.90 - 0.77 M (NH₄)₂SO₄ and protein from fraction D eluted within the range of 0.73 - 0.60 M (NH₄)₂SO₄. Each fraction (hydroxyapatite flow-through containing A and B; fraction C and fraction D from Source 15 Phenyl column) was concentrated by passing them through a 50 kDa cut-off filter (Merck Millipore, Darmstadt, Germany). Ultimately, the concentrated sample was injected onto a 10/300 Superose 6 column (GE Healthcare) equilibrated in 25 mM Tris/HCl pH 7.6,

containing 10% glycerol and 2 mM DTT and eluted at a flow rate of 0.4 ml/min. The flow through fraction from the hydroxyapatite containing the protein from fraction A and B were injected on the Superose 6 and both fractions were separated by their elution volume: 10.6 - 13.6 ml for fraction A and 14.1 - 18.1 ml for fraction B. Proteins from fraction C were injected on the Superose 6 and eluted between 11.6 and 14.9 ml. Proteins from fraction D eluted between 13.6 and 16 ml on the Superose 6.

Table S1 Crystallization, soaking and cryo-preserving solutions used for the different proteins studied in the present work.

Protein acronym	Crystallization condition	Cryo-preserving solution	Soaking
pb9	10 % PEG8000	10 % PEG8000	In cryo solution
	8 % Ethylene glycol	25 % Ethylene glycol	Soaking time 2 min
	100 mM HEPES pH 7.5	100 mM HEPES pH 7.5	
	10 mM Tb-Xo4	100 mM Tb-Xo4	
PhP1	2.8 - 3.2 M Malonate pH 5.5	3.4 M Malonate pH 5.5	In cryo solution
	10 mM Tb-Xo4	100 mM Tb-Xo4	Soaking time 2 min
FprA form 1	20 % PEG 8000	20 % PEG 8000	None
	200 mM Ammonium sulphate	200 mM Ammonium sulphate	
	10 % 2-propanol	10 % 2-propanol	
	100 mM HEPES pH 7.5	100 mM HEPES pH 7.5	
	10 mM Tb-Xo4	25 % Glycerol	
FprA form 2	28 % PEG 400	28 % PEG 400	None
	200 mM Calcium chloride	200 mM Calcium chloride	
	100 mM HEPES pH 7.5	100 mM HEPES pH 7.5	
	10 mM Tb-Xo4	10 mM Tb-Xo4	
ThiS form 1	1.6 M Magnesium sulphate	1.6 M Magnesium sulphate	None
	100 mM MES pH 6.5	100 mM MES pH 6.5	
	10 mM Tb-Xo4	25 % Glycerol	
ThiS form 1 soaked with Tb-Xo4	44 % PEE 797	44 % PEE 797	In cryo solution
	100 mM HEPES pH 8.0	100 mM HEPES pH 8.0	Soaking time 9 min
		100 mM Tb-Xo4	
ThiS form 2	28 % PEG 400	28 % PEG 400	None
	200 mM Calcium chloride	200 mM Calcium chloride	
	100 mM HEPES pH 7.5	100 mM HEPES pH 7.5	
	10 mM Tb-Xo4	10 mM Tb-Xo4	
ThiS form 3	45 % PEP 629	45 % PEP 629	None
	300 mM Potassium chloride	300 mM Potassium chloride	

	100 mM TRIS pH 8.5	100 mM TRIS pH 8.5	
	10 mM Tb-Xo4	10 mM Tb-Xo4	
AdkA	25 % PEP 629	25 % PEP 629	In cryo solution
	50 mM Magnesium chloride	50 mM Magnesium chloride	Soaking time 6 min
	100 mM HEPES pH 7.5	100 mM HEPES pH 7.5	
	10 mM Tb-Xo4	50 mM Tb-Xo4	
GlnA	35 % PEE 797	35 % PEE 797	None
	200 mM Ammonium sulphate	200 mM Ammonium sulphate	
	100 mM HEPES pH 7.5	100 mM HEPES pH 7.5	
	10 mM Tb-Xo4	10 mM Tb-Xo4	

PEP 629 stands for Pentaerythritol propoxylate (17/8 PO/OH); PEE 797 stands for Pentaerythritol ethoxylate (15/4 EO/OH)

Table S2 X-ray refinement statistics.

	Protease 1	AdkA	ThiS form1
PDB code	6HF6	6HF7	6HK1
Resolution (Å) ^a	19.99 - 2.00 (2.08 - 2.00)	48.92 - 1.96 (2.03 - 1.96)	49.09 - 2.55 (2.64 - 2.55)
No. of reflections	69161 (6821)	55799 (5378)	79189 (7534)
Reflections used for R _{free} ^b	3384 (338)	2824 (264)	3957 (362)
R _{work}	0.162	0.171	0.189
R _{free} ^b	0.181	0.190	0.213
No. of atoms			
Proteins	3931	4494	12405
Ligand/ion	115	76	315
Water	553	550	438
B factors (Å) ²			
Protein	37.67	50.24	56.23
Ligand/ion	53.41	42.56	66.14
Water	56.10	55.50	61.36
R.m.s deviations ^c			
Bond lengths (Å)	0.013	0.015	0.009
Bond angles (°)	1.57	1.58	1.44
Clashscore	0.62	0.87	1.51
Ramachandran favored (%)	99.39	98.24	97.80
Ramachandran allowed (%)	0.61	1.59	2.20
Ramachandran outliers (%)	0.00	0.18	0.009

^a Values relative to the highest resolution shell are within parentheses. ^b R_{free} was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. ^c R.m.s, root mean square.

Table S3 Raw data availability.

Data set	Corresponding DOI
Protease1 <i>MeshAndCollect</i>	https://doi.org/10.5281/zenodo.2640319
pb9 <i>MeshAndCollect</i>	https://doi.org/10.5281/zenodo.2640356
FprA crystal form1	https://doi.org/10.5281/zenodo.2641965
FprA crystal form2	https://doi.org/10.5281/zenodo.2641991
ThiS crystal form3	https://doi.org/10.5281/zenodo.2641868
AdkA	https://doi.org/10.5281/zenodo.2641812