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Supporting information for article:

High-Pressure Single-Crystal Diffraction at the Australian Synchrotron

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S1. Crystallization conditions, data processing and refinement of Lyophilised chicken egg white Lysozyme (THEWL)

THEWL (Sigma-Aldrich) was dissolved to 40 mg/mL in 20mM sodium acetate pH 4.7. Tetragonal crystals were grown by vapour diffusion, as described previously [REF: 10.1107/s1399004715000292] (**Table S1**). Diffraction data were processed in space group $P4_32_12$ (**Table S2**). Refinement of atomic coordinates was performed using *phenix.refine (Afonine et al., 2012)* with PDB 2yvb as the starting model, and manual rebuilding using Coot (Emsley *et al.*, 2010) (**Table S3**). Refinement of anisotropic *B*-factors was restricted to seven translation-libration-screw groups (defined automatically by *phenix.refine*). Structure factors and final model coordinates are deposited at the Protein Data Bank (dataset ID: D_1000268609 and PDB ID: 8F2G). To calculate RMSD across all α-carbon pairs, superposition of the current structure with 4wld was performed using UCSF ChimeraX (v1.3) (Pettersen *et al.*, 2021) *Matchmaker* tool, with iterative pruning of long atom pairs switch off. Superposition with the crystal structure of lysozyme previously determined at atmospheric pressure (0.1 MPa) (Yamada *et al.*, 2015) reveals no significant structural changes; RMSD between 129 atom pairs is 0.154 Å.

Table S1 Crystallization conditions

Method	Vapour diffusion, hanging drop
Plate type	Hampton Research VDX™ Plate (24-well) with siliconized glass cover-slips
Temperature (K)	293
Protein concentration	40 mg/mL
Buffer composition of protein solution	20 mM Na acetate pH 4.7
Composition of reservoir solution	1 M NaCl, 100 mM Na acetate pH 4.7
Volume and ratio of drop	6 μL at 1:1 ratio of reservoir:protein solution
Volume of reservoir	1 mL

Table S2 Data collection and processing. Values for the outer shell are given in parentheses. Data were collected from a single crystal.

Wavelength (Å)	0.71092
Space group	$P4_32_12$
a, *c* (Å)	79.045, 37.865
α, β, γ (°)	90, 90, 90
Mosaicity (°)	0.06
Resolution range (Å)	55.9–1.84 (1.88–1.84)
Total No. of reflections	31390 (1609)
No. of unique reflections	6894 (383)
Completeness (%)	63.2 (57.3)
Redundancy	4.6 (4.2)
$\langle I/\sigma(I)\rangle$	7.0 (0.7) #
R_{meas}	0.124 (1.557)
$R_{p.i.m.}$	0.055 (0.708)
$CC_{1/2}$	0.994 (0.440)
Overall *B* factor from Wilson plot (Å^2)	19.6

Mean $I/\sigma(I)$ falls below 2.0 at a resolution of 2.10 Å. High resolution cut-off for data processing was selected primarily by reference to $CC_{1/2}$ as recommended by (Karplus & Diederichs, 2015).

Table S3 Structure refinement of THEWL. Values for the outer shell are given in parentheses.

Resolution range (Å)	35.35 - 1.842 (1.908 - 1.842)
Completeness (%)	63.10 (57.74)
No. of reflections, working set	6547 (593)
No. of reflections, test set	303 (26)
Final R_{work}	0.217 (0.334)
Final R_{free}	0.263 (0.337)
No. of non-H atoms	
Protein	993
Water	44
Total	1037
R.m.s. deviations	
Bonds (Å)	0.0023
Angles (°)	0.57
Average *B* factors (Å^2)	
Protein	28.04
Water	29.18
Ramachandran plot	
Most favoured (%)	96.85
Allowed (%)	3.15

Table S4 L-threonine Crystallographic Experimental details. For all structures: $C_4H_9NO_3$, M_r = 119.12, orthorhombic, $P2_12_12_1$, Z = 4. Experiments were carried out at 293 K. H-atom parameters were constrained. CCDC 2227429-2227431 contain the supplementary crystallographic data. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Data set	L-threonine under ambient conditions	L-threonine at 0.15 GPa (laboratory data)	L-threonine at 0.15 GPa (synchrotron data)
Crystal data			
a, *b*, *c* (Å)	5.1456 (2), 7.7415 (4), 13.6223 (6)	5.1419 (2), 7.7217 (4), 13.613 (5)	5.1489 (5), 7.7304 (5), 13.602 (6)
V (Å^3)	542.64 (4)	540.49 (19)	541.4 (3)
Radiation type	Mo *K*a	Mo *K*a	Synchrotron, l = 0.71073 Å
m (mm^{-1})	0.12	0.13	0.13
Crystal size (mm)	0.2 × 0.1 × 0.1	0.2 × 0.2 × 0.1	0.2 × 0.2 × 0.1
Data collection			
Diffractometer	XtaLAB Synergy, Single source at home/near, HyPix	XtaLAB Synergy, Single source at home/near, HyPix	MX1
Absorption correction	Multi-scan *CrysAlis PRO* 1.171.41.115a (Rigaku Oxford Diffraction, 2021) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.	Multi-scan *CrysAlis PRO* 1.171.41.123a (Rigaku Oxford Diffraction, 2022) Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.	Multi-scan *SADABS2012*/1 (Bruker,2012) was used for absorption correction.
T_{min}, T_{max}	0.706, 1.000	0.203, 1.000	0.579, 0.745
No. of measured, independent and observed [I > 2s(I)] reflections	5981, 1304, 1110	7737, 567, 482	1209, 468, 428
R_{int}	0.044	0.059	0.013
(sin q/l)$_{max}$ (Å^{-1})	0.682	0.625	0.654
Refinement			
R[F^2 > 2s(F^2)], wR(F^2), S	0.040, 0.104, 1.08	0.040, 0.125, 1.13	0.040, 0.142, 1.22

No. of reflections	1304	567	468
No. of parameters	76	76	70
No. of restraints	0	48	48
$D\rho_{max}$, $D\rho_{min}$ (e Å^{-3})	0.25, -0.21	0.17, -0.17	0.22, -0.22
Absolute structure	Flack x determined using 383 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 170 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).	Flack x determined using 157 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	-0.2 (10)	-0.6 (10)	-0.2 (10)

References

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