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Supporting information for article:

Single-crystal time-of-flight neutron Laue methods: application to manganese catalase from *Thermus thermophilus* HB27

Taro Yamada, Naomine Yano, Takaaki Hosoya and Katsuhiro Kusaka

S1. Enzyme preparation

For the general experimental procedure of enzyme preparation, a TAITEC BR-180LF rotary shaker, a HITACHI CR22G III centrifuge, a Sonics Vibra-Cell Ultrasonic Liquid Processor VCX 500 with a tapered microtip, and a Beckman-Coulter Allega X-15R centrifuge with a bucket rotor SX4750A, were used.

S1.1. Gene expression and purification

Cloning and gene expression of the manganese catalase gene from *Thermus thermophilus* HB27 was done by referring to a previously reported method (Hidalgo *et al.*, 2004). A *T. thermophilus* cloning kit containing a shuttle vector, pMKE1, and *T. thermophilus* HB27::nar strain was obtained from BioTools (Spain, discontinued product) (Moreno *et al.*, 2003). The manganese catalase gene from *T. thermophilus* HB27 was cloned into the shuttle vector pMKE1 using NdeI, HindIII restriction sites by TAKARA Bio Co Ltd. (Japan). The shuttle vector was introduced into the *T. thermophilus* HB27::nar strain. Resulting colonies were picked up from the agar culture plates and glycerol stocks were prepared after checking the gene expression induced by KNO_3.

T. thermophilus HB27 was cultured in 800 mL Thermus broth at 70°C with shaking at 150 rpm for 7 hours after adding 20 mL pre-culture. After shaking was stopped, sterilized solution of 0.5 mL of 1 M $MnCl_2$ and 15 mL of 2 M KNO_3 were added to the culture. The culture was kept still for 14 hours at 70°C. An 800 mL culture yielded ca. 3 g of wet cells after centrifugation at 4000 rpm.

A total of 12 g of wet cells were suspended in 50 mL lysis buffer containing 20 mM Tris•HCl pH 8.0 and 50 mM NaCl in a 125 mL PPCO bottle (Nalgene), and then 55 mg of hen egg white lysozyme (Seikagaku Corporation, Japan) was added. After storing on ice for 1 hour, the lysate was frozen at −85°C overnight. The frozen lysate was thawed with tap water. The lysate was sonicated for 1 min with 1 second on-off pulse cycles, and this procedure was repeated five times, with brief rests in between. The lysate was then centrifuged at 15,000 rpm. Ammonium sulfate fractionations between 35% and 80% saturation afforded crude manganese catalase. The crude pellet was dissolved in 10 mL of 50 mM potassium phosphate buffer (KP buffer) pH 7.2, then loaded into a HiPrep Phenyl HP 16/10 column (GE Healthcare), equilibrated with 1.5 M ammonium sulfate, and 50 mM KP buffer pH 7.2. The column was washed with 200 mL of 1.5 M ammonium sulfate, and 50 mM KP buffer pH 7.2. Manganese catalase was eluted using an ammonium sulfate concentration gradient toward 0 M by running through 200 mL buffer. Active fractions were collected and concentrated using UltraFree 15 (MWCO 10 kD, Millipore). Manganese catalase was then purified with a Superdex 200pg 16/600 (GE Healthcare) column by elution with 20 mM Tris•HCl pH 7.5 buffer containing 100 mM NaCl at a 1

mL/min flow rate. The concentration of active fractions yielded a solution of manganese catalase that was pinkish-brown in color.

S1.2. Crystallization

Prior to crystallization, the manganese catalase solution was washed with 50 mM AMPSO pD 9.0 buffer using UltraFree 15. A vapor diffusion sitting drop method was adopted. A custom-made small glass vessel was put in the center of an organ culture dish from Falcon BD. Fifty μL of Fluorinert FC-3283 (3M) was added to the glass vessel to provide crystallization support. Two mL of reservoir solution, consisting of 1.95 M ammonium sulfate d-8, 14.5v/v% ethylene glycol, 50 mM AMPSO buffer pD 9.6, and heavy water was added to the outside well of the culture dish. The final pD of the crystallization solution mixture was confirmed as 8.2. 150 μL of 32 mg/mL enzyme and 150 μL of reservoir solution were mixed and centrifuged to remove dust. The mixture (150 μL) was then placed in a small glass vessel. The culture dish was sealed with the lid using high-vacuum grease (Dow Corning Toray Co., Ltd.). The crystallization solution was stored at 20°C for 11 days. A crystal was sealed in a quartz capillary with a custom-made stainless-steel magnet base stored 32 μL of the reservoir solution. The crystal size was 2.4 × 1.6 × 0.7 mm and the estimated volume was 1.3 mm^3. Photos of the type of vessels used for the crystallization and the actual crystal used for this neutron diffraction study are shown in Figures S1 and S2.

S2. Neutron diffraction data

Table S1 lists the statistics calculated for resolution intervals for the neutron diffraction data of *Thermus thermophilus* manganese catalase. Reflections with an overlap ratio less than 0.1 were included.

Figure S3 show a plot of the completeness against the overlap criteria, which were calculated for the neutron diffraction intensity data set over all resolution ranges (15–2.35 Å).

Figure S4 shows examples of TOF profiles of Laue spots with different overlap ratios. The figure shows that the curve fitting libraries provided by the ROOT (Brun & Rademakers, 1997) can determine baselines and resolve targeted Laue spots with small overlap ratios.

References

Brun, R. & Rademakers, F. (1997). *Nuclear Inst. and Methods in Physics Research* **389**, 81–86.
Hidalgo, A., Betancor, L., Moreno, R., Zafra, O., Cava, F., Fernández-Lafuente, R., Guisán, J. M. & Berenguer, J. (2004). *Appl. Environ. Microbiol.* **70**, 3839–3844.
Moreno, R., Zafra, O., Cava, F. & Berenguer, J. (2003). *Plasmid* **49**, 2–8.

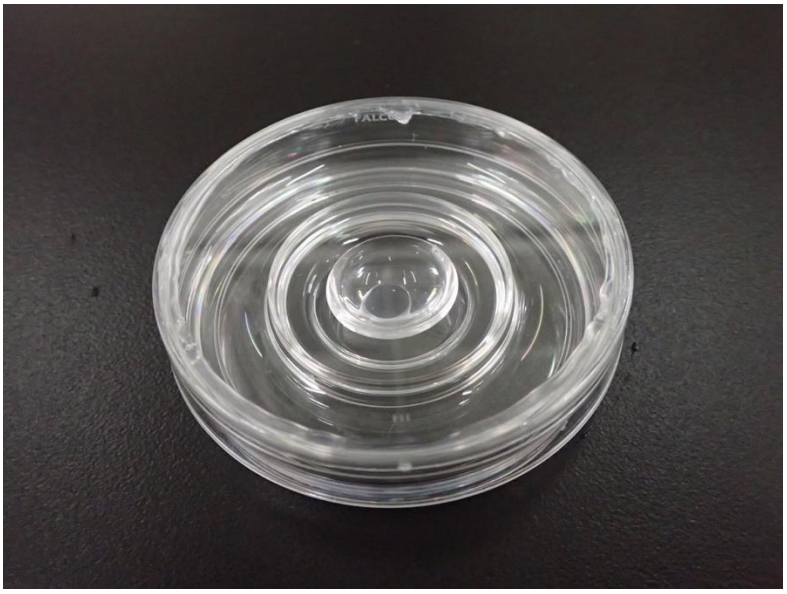

Figure S1 The same type of crystallization set-up as used for this work. A small glass vessel for protein samples is set on the centre well of the BD Falcon Organ Culture Dish. The reservoir can be poured into the outer well. The vessel is sealed with some high-vacuum silicone grease put on the inside edge of the lid.

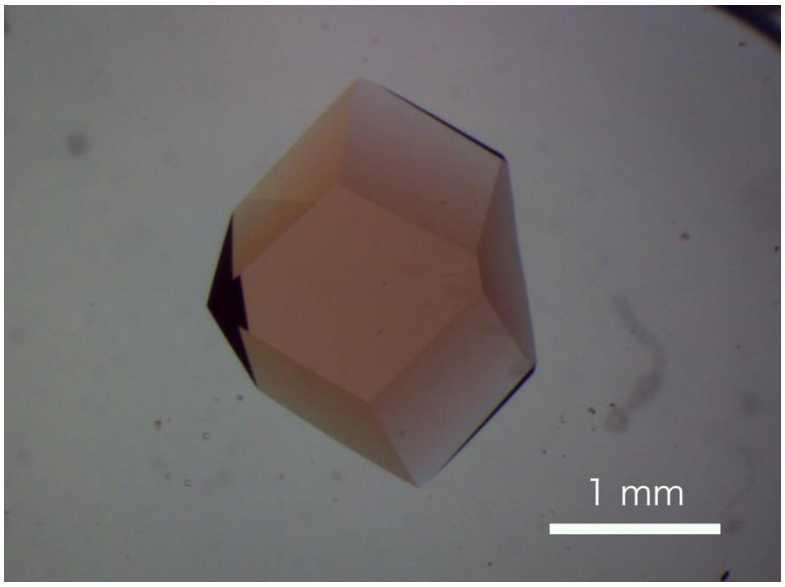

Figure S2 The manganese catalase crystal used for this work. The size was 2.4 mm × 1.6 mm × 0.7 mm.

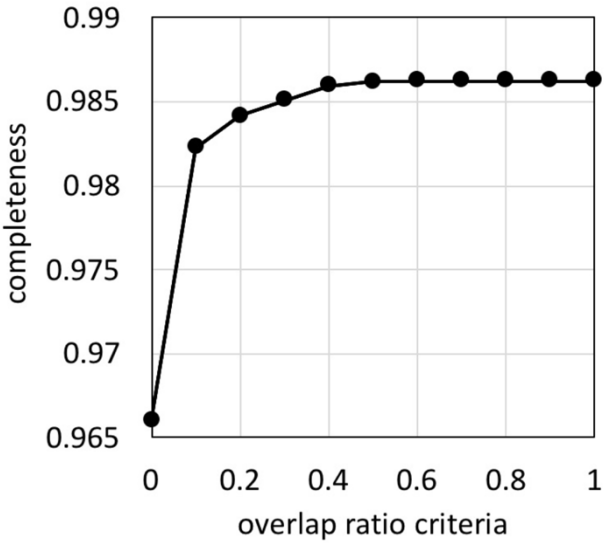

Figure S3 The plot of overall completeness for 2.4 Å resolution data against overlap ratio criteria. Including reflection data with the overlap ratio less than or equal to 0.1 improves the completeness.

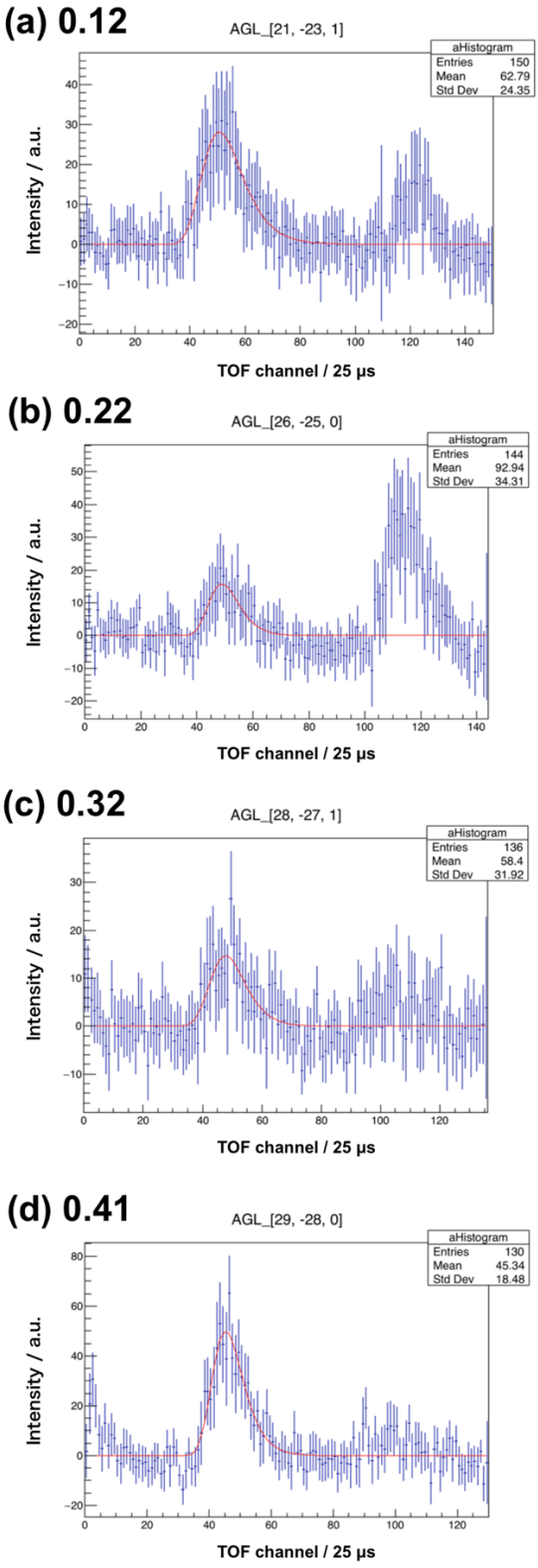

Figure S4 Examples of the TOF profiles for Laue spots with different overlap ratios recorded by the detector #11 (the centre $2\theta = 51.1°$). The curve fittings were done by using asymmetric Gaussian functions described in **Section 3.1**, and the backgrounds estimated were subtracted. Note that the window sizes of the plots are twice of the estimated TOF sizes, (2 × T1 + 2 × T2).

Table S1 Statistics for neutron diffraction data at resolution intervals calculated for the reflections with overlap ratio ≦ 0.1.

d_{max} – d_{min} Å	Ave. I_{obs}	Ave. σ(I)	R_{sym}	R_{rim}	R_{pim}	No. refs.	Redundancy	[a] No. refs. Red. = 1	$CC_{1/2}$	Complete-ness
14.37 – 4.99	7.84	0.58	0.114	0.134	0.068	3,093	3.399	429	0.977	0.905
4.99 – 3.99	7.63	0.80	0.146	0.166	0.076	3,262	4.268	222	0.974	0.972
3.99 – 3.50	6.21	0.77	0.199	0.218	0.087	3,300	5.859	93	0.965	0.993
3.50 – 3.18	4.64	0.85	0.270	0.297	0.121	3,276	5.717	78	0.932	0.993
3.18 – 2.95	3.09	0.81	0.395	0.434	0.175	3,286	5.906	49	0.896	0.998
2.95 – 2,78	2.33	0.79	0.503	0.549	0.214	3,287	6.353	30	0.853	0.999
2.78 – 2.64	2.17	0.88	0.563	0.619	0.252	3,257	5.767	55	0.823	0.998
2.64 – 2.53	1.95	0.96	0.596	0.665	0.288	3,291	5.079	78	[b] 0.758	0.998
2.53 – 2.43	1.73	1.03	0.677	0.760	0.337	3,264	4.726	115	0.683	0.996
2.43 – 2.35	1.59	1.13	0.743	0.839	0.381	3,193	4.417	202	0.606	0.976
All	3.90	0.86	0.322	0.358	0.151	32,509	5.162	1,351	0.949	0.982

a. The number of reflections observed once.

b. The $CC_{1/2}$ value for this shell was unstable and could be as low as 0.36.