Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

For

Thermal Unfolding and Refolding of a Lytic Polysaccharide Monooxygenase from *Thermoascus aurantiacus*

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Table S1. Apparent melting temperature (T_m) and half refolding temperature (T_m') of TaLPMO9A at different heating and cooling rates. T_m and T_m' value derived from the unfolding and refolding of TaLPMO9A data recorded using Jasco J-815 CD spectrometer.

Temperature ramp (°C/min)	T_m (°C)	T_m' (°C)
1.0	74.3 ± 0.5	56.7± 0.4
0.5	72.4 ± 0.3	58.6 ± 0.5
0.2	71.8 ± 0.7	63.7 ± 0.3
0.1	71.4 ± 0.5	68.1 ± 0.6

Table S2. Thermodynamic parameters of TaLPMO9A unfolding and refolding. Δ*H* and Δ*S* value derived from the slope and intercept of a Van't Hoff plot (Figure 6B and S6B). Van't Hoff plots for the calculation of thermodynamic parameters of TaLPMO9A unfolding, refolding and average were plotted using a transition curve (shown in Figure 6B and S6B) in the temperature range between 59 to 80 °C.

Transition curve	Δ*H* (kJ mol^{-1})	Δ*S* (kJ mol^{-1})
Unfolding	245	0.71
Refolding	247	0.72
Average	246	0.72

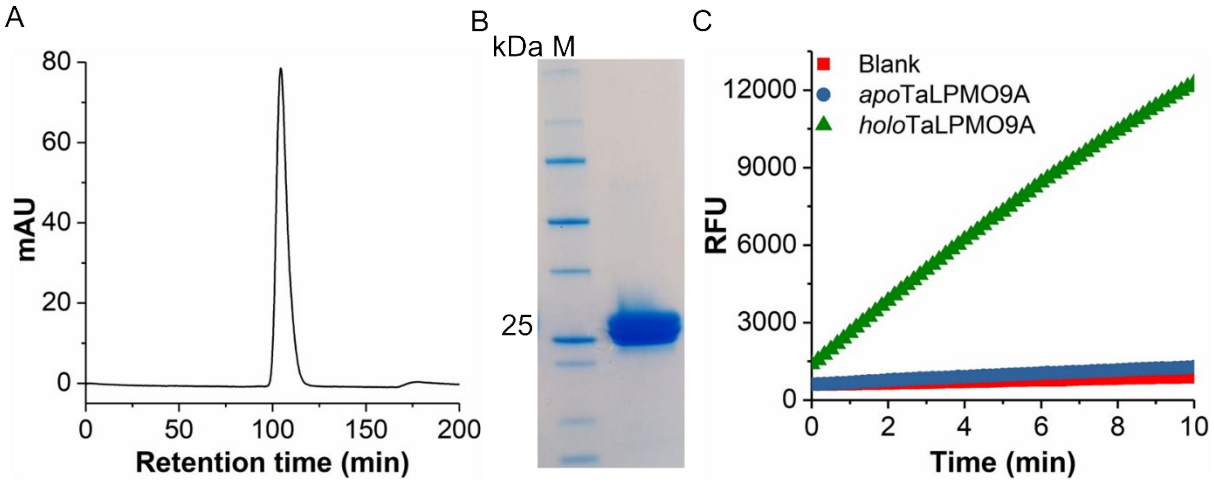

Figure S1. Purification and activity of Cu^{+} loaded TaLPMO9A. (A) Chromatogram showing purification of TaLPMO9A. Cu (I) reconstituted TaLPMO9A was purified using a Hiload 26/60 Superdex 75 prep grade column. Cu/protein molar ratio in *holo*TaLPMO9A and *apo*TaLPMO9A were measured by ICP-MS and determined to be 1.02 and 0.045, respectively. **(B)** SDS-PAGE showing purified TaLPMO9A. M and 1 represent the protein marker lane and purified TaLPMO9A lane, respectively. SDS-PAGE was run on Mini-PROTEAN TGX precast gel with a gradient of 4-15%. **(C)** LPMO activity was measured using an Amplex® Red assay on a SpectraMax M2 multi-detection microplate reader using excitation and emission at 557 and 583 nm. The reaction mixture contained ascorbate (0.08 mM), EDTA (0.1 mM), Amplex red (0.05 mM), HRP (20 U) and TaLPMO9A (2.0 µg mL^{-1}).

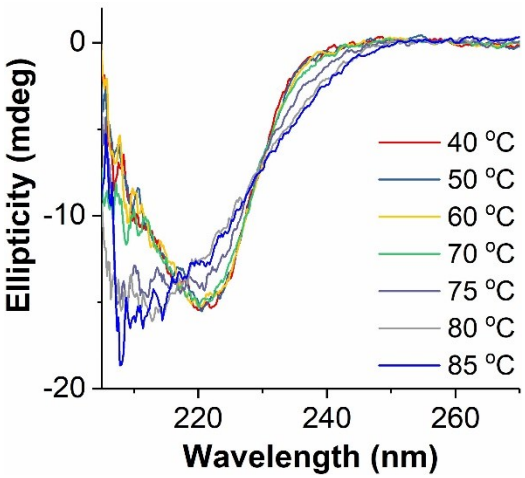

Figure S2. CD spectra in far-UV spectral region for *holo*TaLPMO9A at various temperatures. Purified *holo*TaLPMO9A (0.25 mg mL^{-1}) in 20 mM MOPS buffer (pH 7.0) was subjected to different temperatures ranging from 40 to 85 °C. The CD spectra were recorded using a Jasco J-815 CD spectrometer (1 mm round cuvette) equipped with a temperature-controlled cell holder.

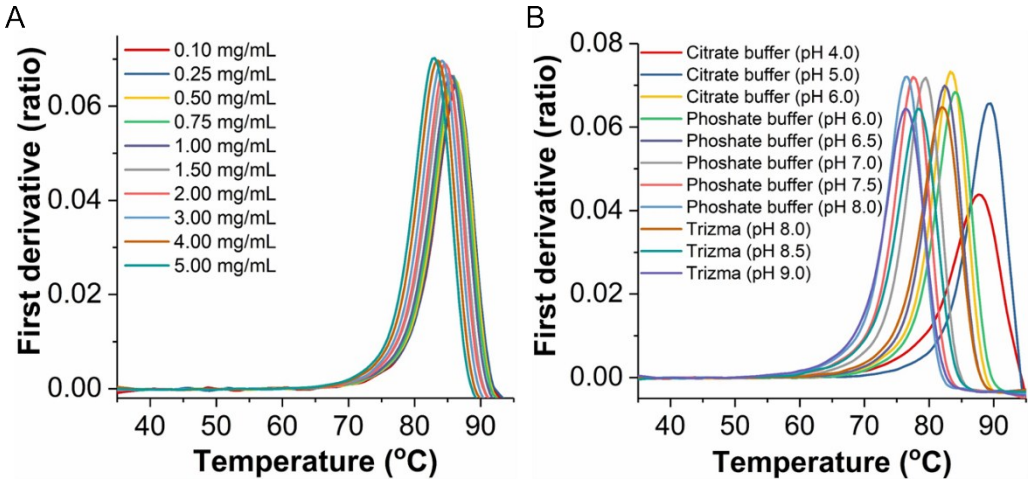

Figure S3. First derivative of the fluorescence traces of TaLPMO9A under different conditions. (A) First derivative of TaLPMO9A at various concentration ranging from 0.1 to 5 mg mL^{-1}. **(B)** First derivative of TaLPMO9A in different buffer solution at various pH (4.0 to 9.0). Stability data was recorded using the NanoTemper Tycho NT.6 temperature-dependent change in tryptophan fluorescence at emission wavelengths of 330 nm and 350 nm**.**

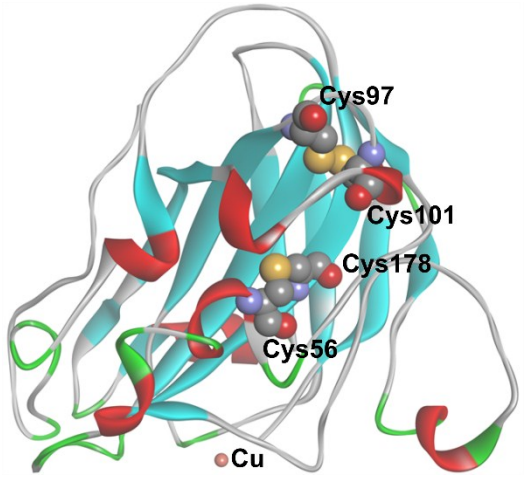

Figure S4. Crystal structure of TaLPMO9A. Structure of TaLPMO9A (PDB: 2YET[1] (A chain)) showing the location of disulphide bonds. TaLPMO9A structure is shown in coloured ribbon (light blue for barrels and red for helices). Residues involved in disulphide bonding are indicated with CPK model. Cu (II) is represented by a sphere.

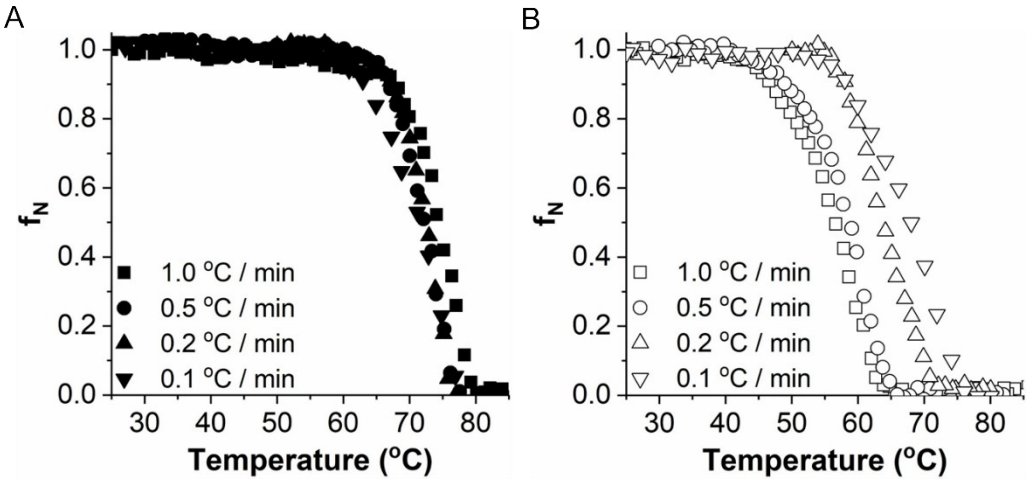

Figure S5. Transition curves for unfolding and refolding of TaLPMO9A. (A) Transition curve showing change in f_N for the ellipticity measured at 222 nm in the temperature range from 25 to 85 °C at different heating rates of 1, 0.5, 0.2, and 0.1 °C . **(B)** Transition curve showing change in f_N for the ellipticity measured at 222 nm in the temperature range from 85 to 25 °C at different cooling rate of 1, 0.5, 0.2, and 0.1 °C.

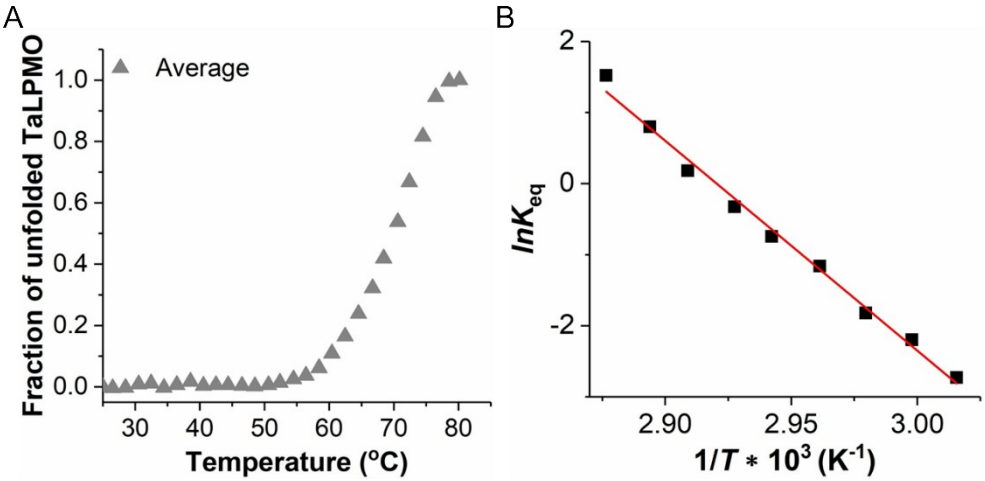

Figure S6. Transition curve and Van't Hoff plot for the reversibility at equilibrium condition. (A) Transition curve for the ellipticity for average of unfolding and refolding. Average of the unfolding and refolding transitions were considered for the reversibility at equilibrium condition. **(B)** Van't Hoff plot for the calculation of thermodynamic parameters of TaLPMO9A at equilibrium condition.

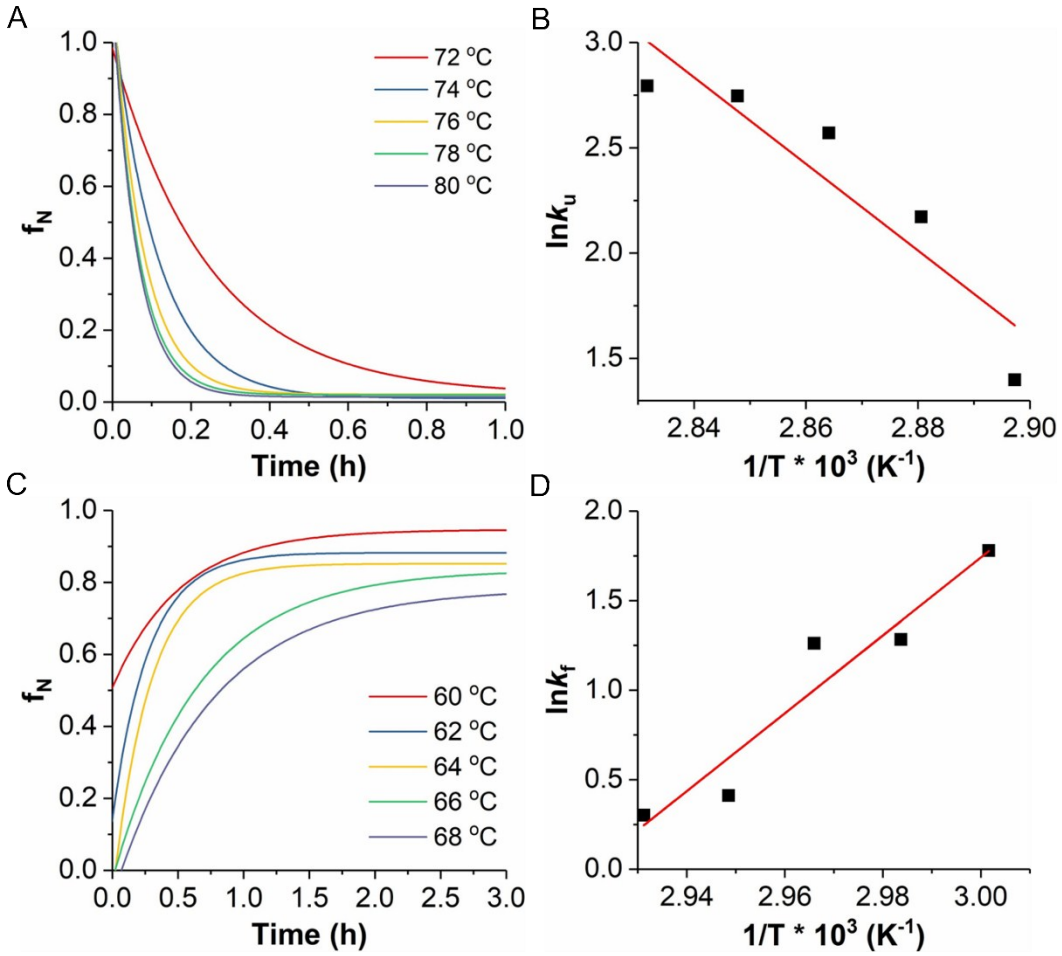

Figure S7. Calculation of unfolding and refolding kinetic parameters. (A) Single exponential decay fitting of f_N with time at different temperatures. **(B)** Arrhenius plot for unfolding of TaLPMO9A showing the linear dependence of $\ln k_u$ over $1/T$. **(C)** Single exponential fitting of f_N with time at different temperatures. (**D**) Arrhenius plot for refolding of TaLPMO9A showing the linear dependence of $\ln k_f$ over $1/T$.

Reference:

1. R. J. Quinlan, M. D. Sweeney, L. Lo Leggio, H. Otten, J. C. Poulsen, K. S. Johansen, K. B. Krogh, C. I. Jorgensen, M. Tovborg, A. Anthonsen, T. Tryfona, C. P. Walter, P. Dupree, F. Xu, G. J. Davies and P. H. Walton, *Proc. Natl. Acad. Sci. USA*, 2011, **108**, 15079-15084.