Supplementary Material

Methylation of the N-terminal histidine protects a lytic polysaccharide monooxygenase from auto-oxidative inactivation

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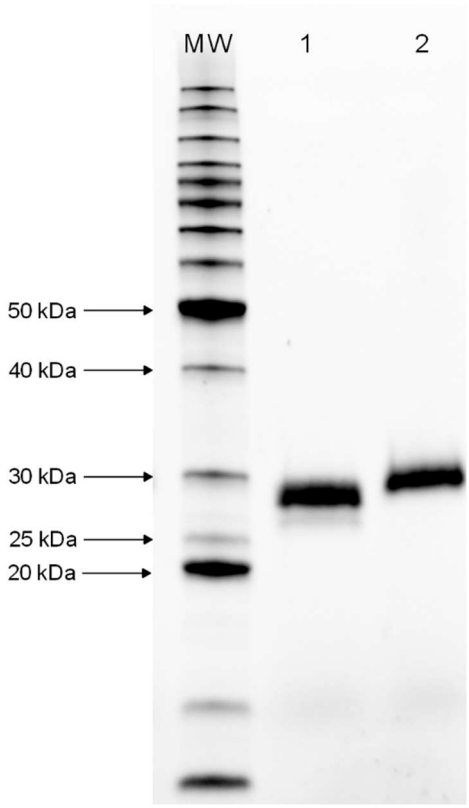

Figure S1. SDS-PAGE of purified *Ta*LPMO9A-Ao and *Ta*LPMO9A-Pp. Lanes: (Mw) Benchmark standard protein molecular weight marker (relevant molecular weight values are indicated); (1) purified *Ta*LPMO9A-Ao after size exclusion chromatography; (2) purified *Ta*LPMO9A-Pp after size exclusion chromatography.

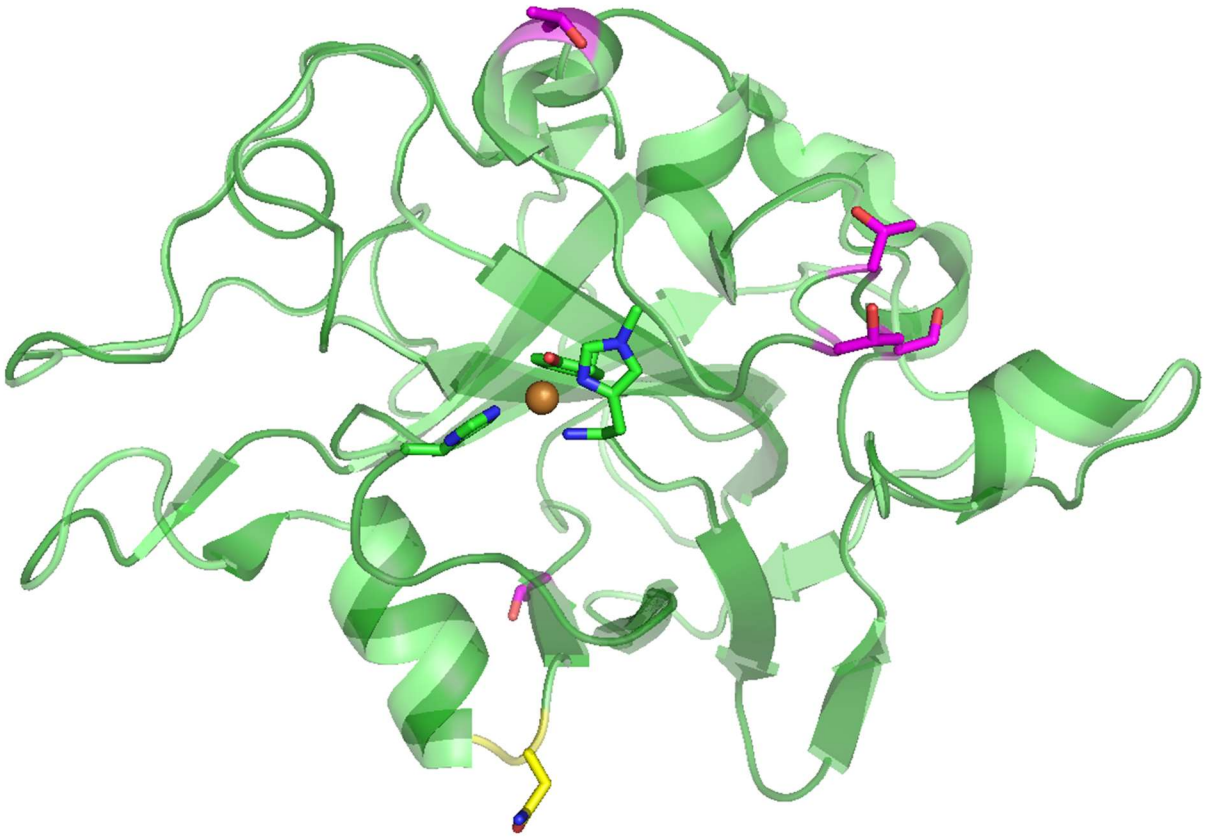

Figure S2. 3D structure of *Ta*LPMO9A (PDB ID: 3ZUD). The side chains of Met-His1, His86 and Tyr175, involved in coordination of the copper (brown sphere) are shown, with green carbons. The side chain of Asn138, which may be *N*-glycosylated, is shown with yellow carbons. The side chains of potentially *O*-glycosylated amino acid residues are shown with purple carbons.

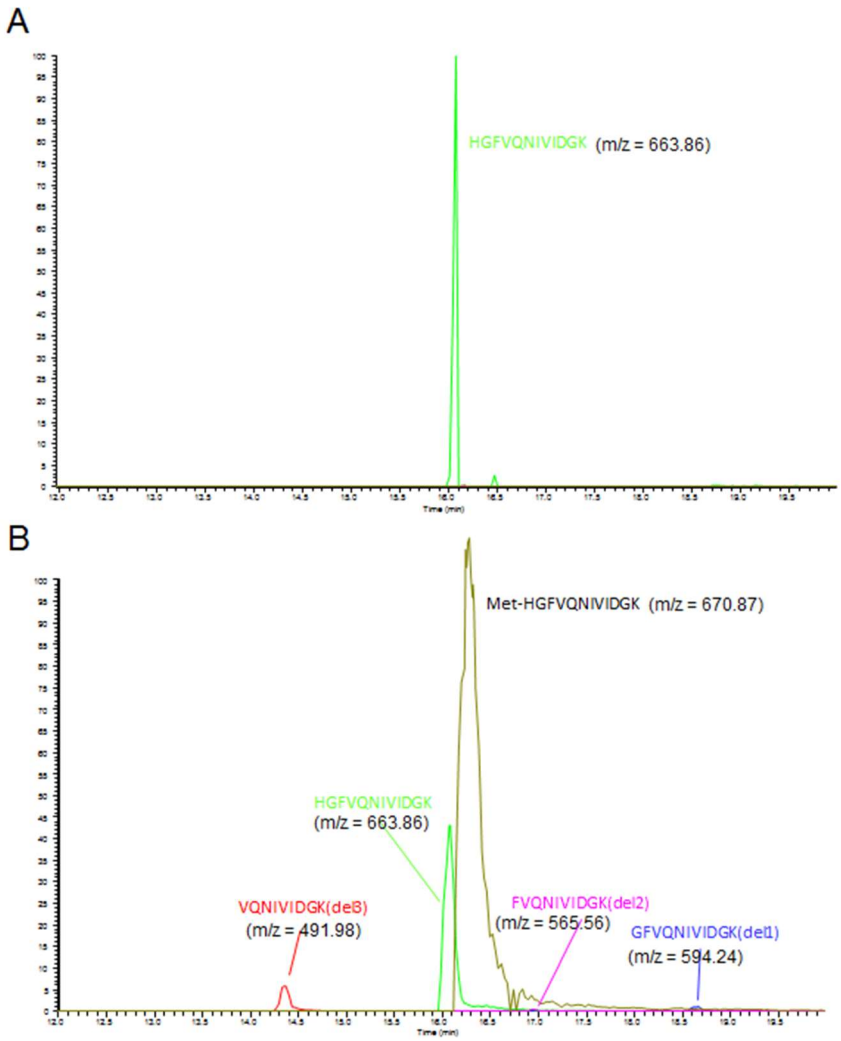

Figure S3. LC-MS extracted ion chromatograms of N-terminal peptides derived from (A) *Ta*LPMO9A-Pp, and (B) *Ta*LPMO9A-Ao. The *Ta*LPMO9A-Pp sample (A) shows a peak with a retention time of 16.10 min and a detected mass of 663.86 Da that can be ascribed to a double charged non-methylated N-terminal peptide (*m/z* for $[HGFVQNIVIDGK]^{2+}$ = 663.26 Da). Other relevant masses, including a mass that corresponds to the methylated N-terminal peptide were not detected. LC-MS analysis of the *Ta*LPMO9A-Ao sample (B) revealed several peaks with retention times between 14.30 and 18.80 min. The most dominant peak appeared at the retention time of 16.28 min and had a mass of 670.87 Da, which corresponds to a double charged methylated N-terminal peptide (*m/z* for $[Met\text{-}HGFVQNIVIDGK]^{2+}$ = 670.29 Da). The second most intense peak

in the sample appeared at 16.08 min and had a mass of 663.86 Da, which corresponds to the double-charged non-methylated N-terminal peptide. Three minor peaks with retention times of 14.36, 16.95 and 18.65 min, and corresponding masses of 491.98, 565.56 and 594.24 Da, respectively, result from enzymes with incorrectly processed signal peptides, as their masses corresponds to double-charged N-terminal peptides that lack one to three N- terminal amino acids. Peak intensity values are presented relative to the intensity of the dominant peak (100 %).

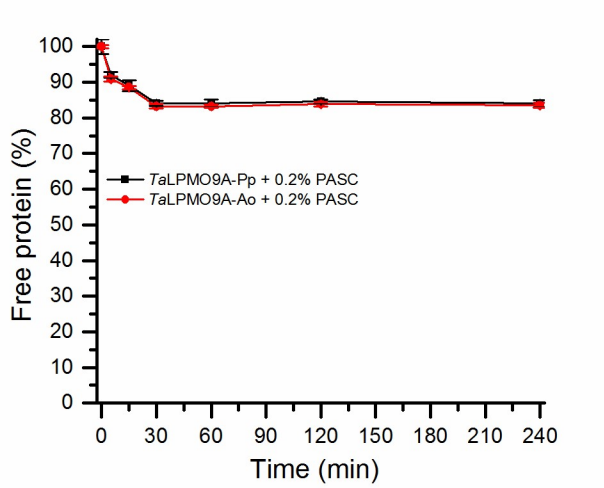

Figure S4. Binding of the *Ta*LPMO9A-Pp and *Ta*LPMO9A-Ao to PASC. The percentage of free LPMO was determined by measuring the reduction in LPMO concentration (A_{280}) over time. The experiments were carried using the same conditions as in LPMO reactions with substrates, but without the addition of a reductant.

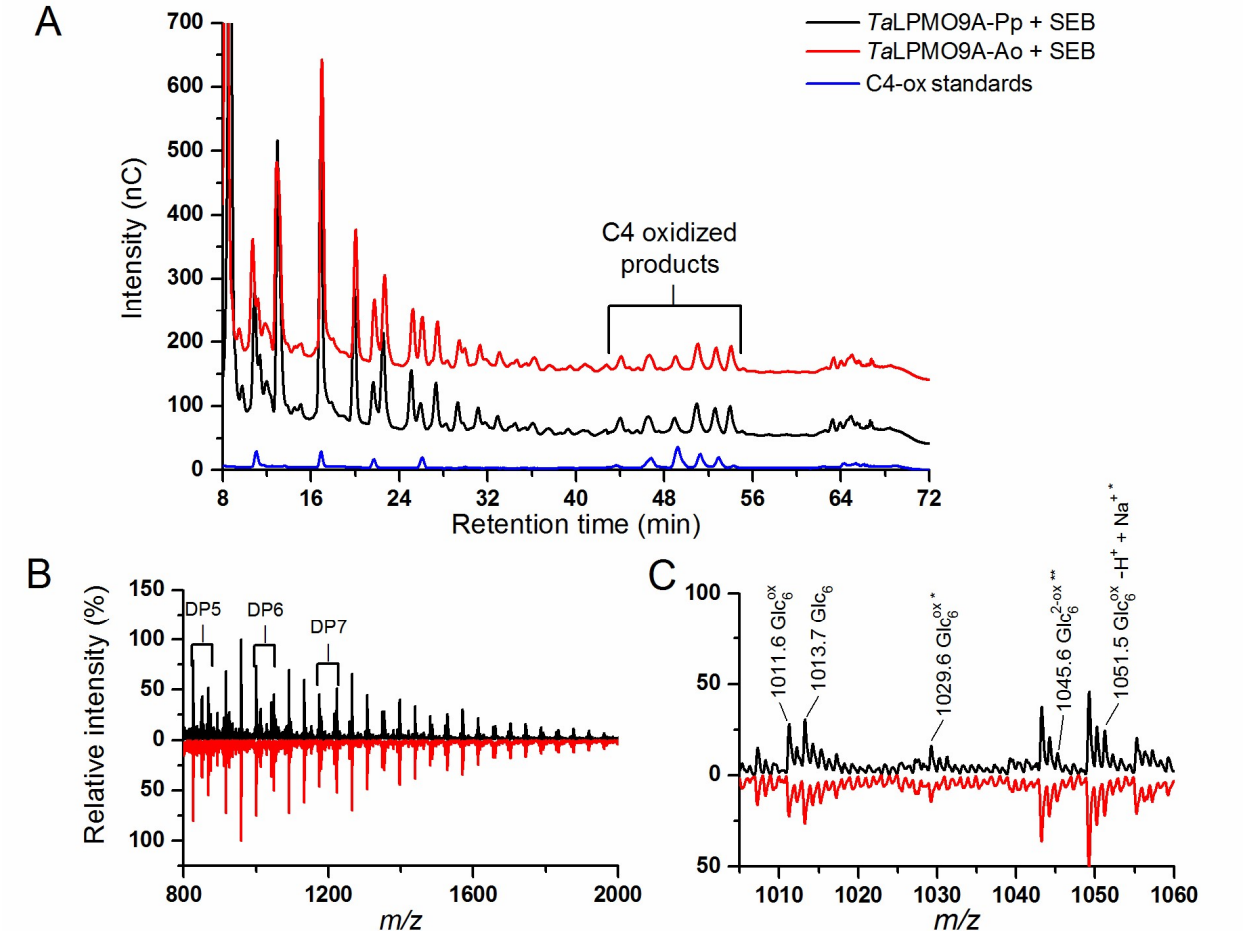

Figure S5. Reaction products generated by *Ta*LPMO9A-Pp and *Ta*LPMO9A-Ao from steam exploded birch (SEB). (A) HPAEC-PAD profiles of various reaction mixtures. Note that ascorbic acid was not added since it is well known that SEB contains sufficient reducing power to drive the LPMO reaction. When using this 75 minute gradient, native cello-oligomers elute between 10 and 26 min, while C4-oxidized products elute between 47 and 56 min as confirmed by analysis of a mixture of C4-oxidized cello-oligomers (solid blue line), obtained by oxidative action of strictly C4-oxidizing *Nc*LPMO9D (NCU01050[1]) on PASC. It was not possible to accurately identify C1-oxidized products due to their lower abundance and the high complexity of the chromatograms in the region where they elute (30 – 50 min). (B) MALDI-ToF MS spectra of products released from SEB by the action of *Ta*LPMO9A-Pp (black line) or *Ta*LPMO9A-Ao (red line), after Na^+ saturation. Characteristic signal clusters belonging to products in the DP5 to DP7 range are

indicated. (C) Close-up of MALDI-ToF MS spectra showing the DP6 product cluster generated by the action of *Ta*LPMO9A-Pp (black line) and *Ta*LPMO9A-Ao (red line). All labeled signals are sodium adducts of native and oxidized products; single oxidation or double oxidation is indicated by "ox" and "2-ox"and single or double hydration is indicated by "*" or "**". The overall signal pattern is characteristic for mixed C1/C4 oxidation.

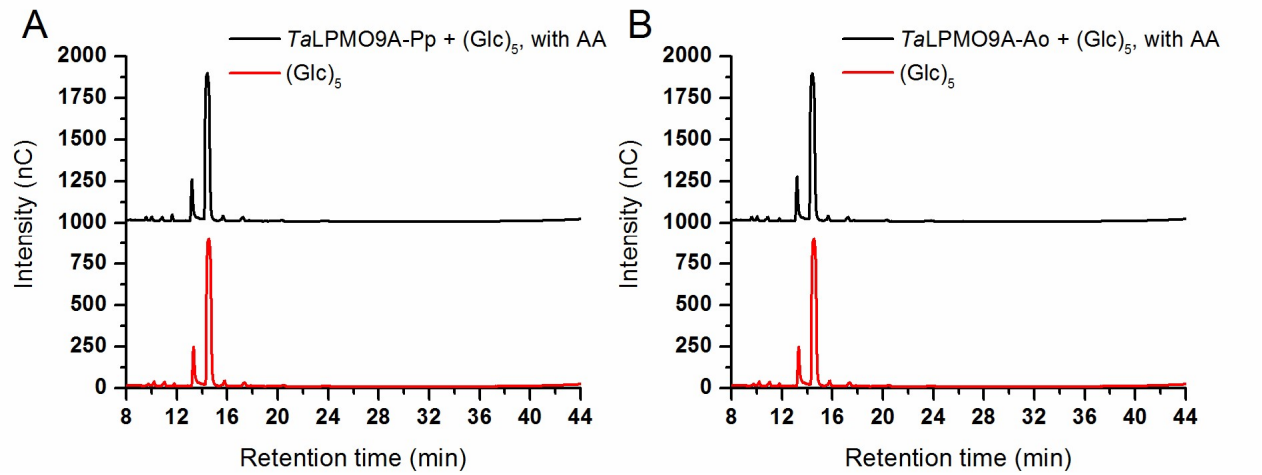

Figure S6. HPAEC-PAD chromatograms showing the outcome of reactions of *Ta*LPMO9A-Pp (A) and *Ta*LPMO9A-Ao (B) with cellopentaose, in the presence of ascorbic acid. Chromatograms for the reaction mixtures are black, while the chromatogram for untreated cellopentaose is shown in red.

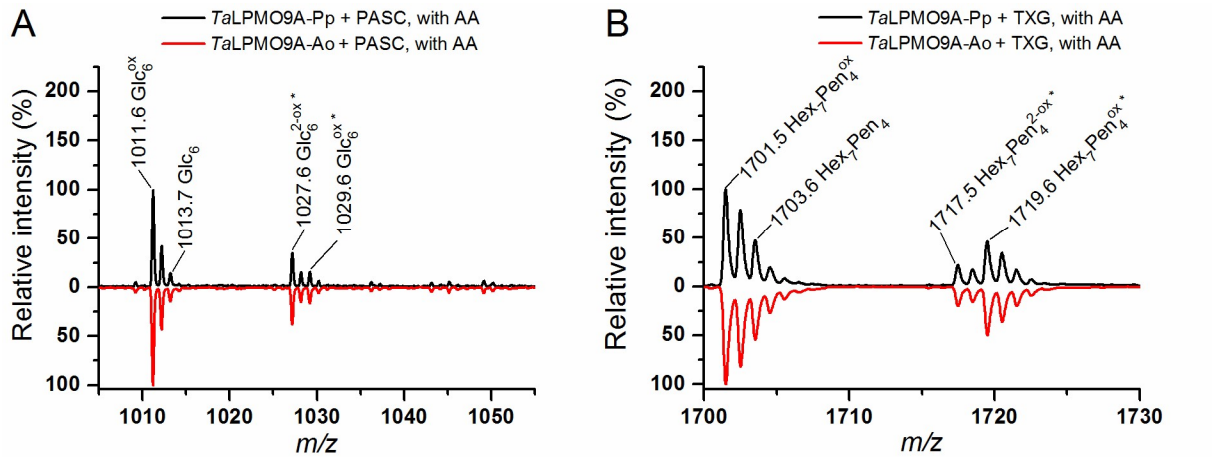

Figure S7. Close-up of MALDI-ToF MS spectra showing products generated by *Ta*LPMO9A-Pp (black line) and *Ta*LPMO9A-Ao (red line). (A) The DP 6 cluster from the reaction with PASC showing products characteristic for mixed C1/C4 oxidation (sodium adducts). The relatively high peak at 1011 (Glc_6^{ox}) indicates C4 oxidation, since the lactone formed by C1 oxidation, which has the same m/z, would be largely in its aldonic acid form (i.e. m/z = 1029 for the single sodium adduct) under these conditions. Furthermore, there is a signal for a hydrated double oxidized species at 1027. (B) The Hex_7Pen_4 cluster from the reaction with TXG, showing oxidized and, possibly, minor amounts of native products. In both spectra, hydration is indicated by "*", single oxidized products are indicated by "ox" and products which *m/z* values corresponding to masses of double-oxidized products are indicated by "2-ox".

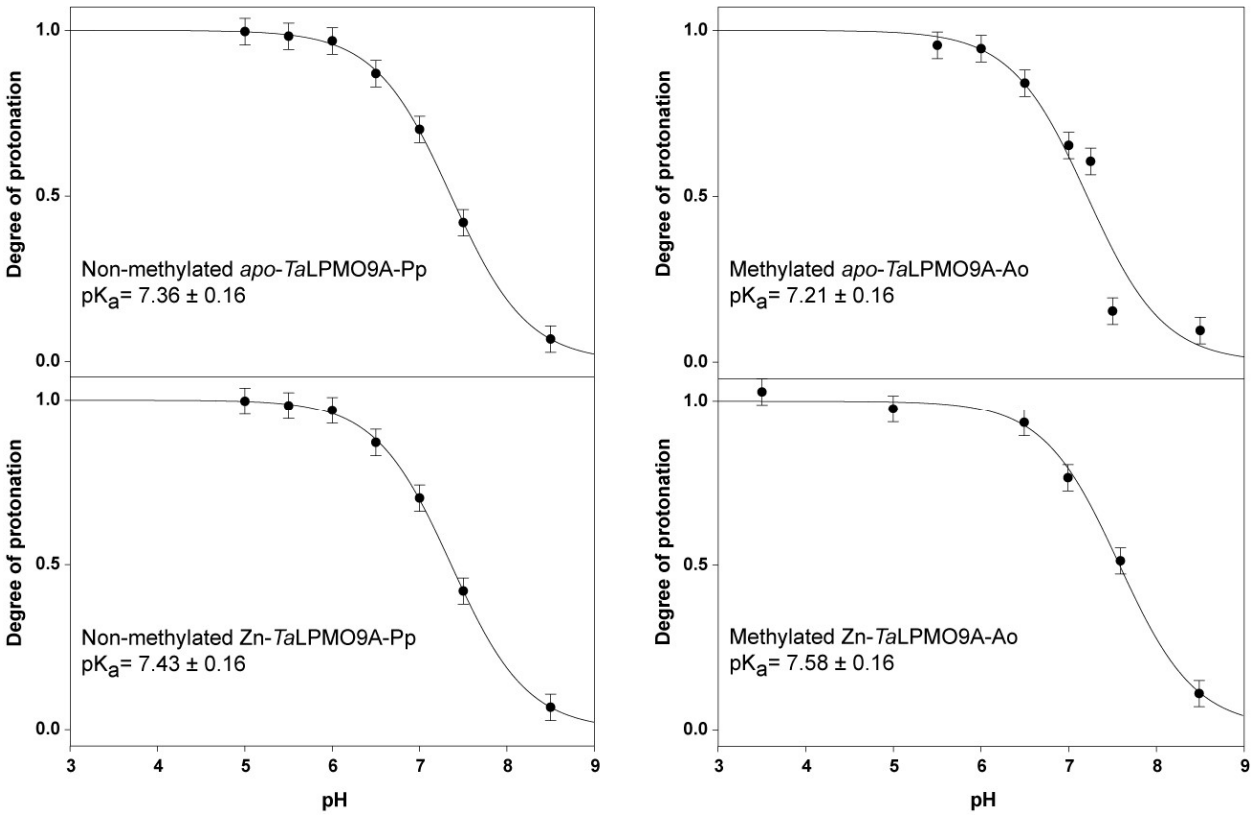

Figure S8. Determination of pK_a values of His1. The graphs show titration curves derived from ^{1}H chemical shift values at each pH point for LPMOs with a methylated (*Ta*LPMO9A-Pp) or a non-methylated (*Ta*LPMO9A-Ao) His1 either in the *apo* form or with Zn^{2+} present. The curves are the result of fitting Equation 1 to the data, and all pK_a values are indicated with their SD.

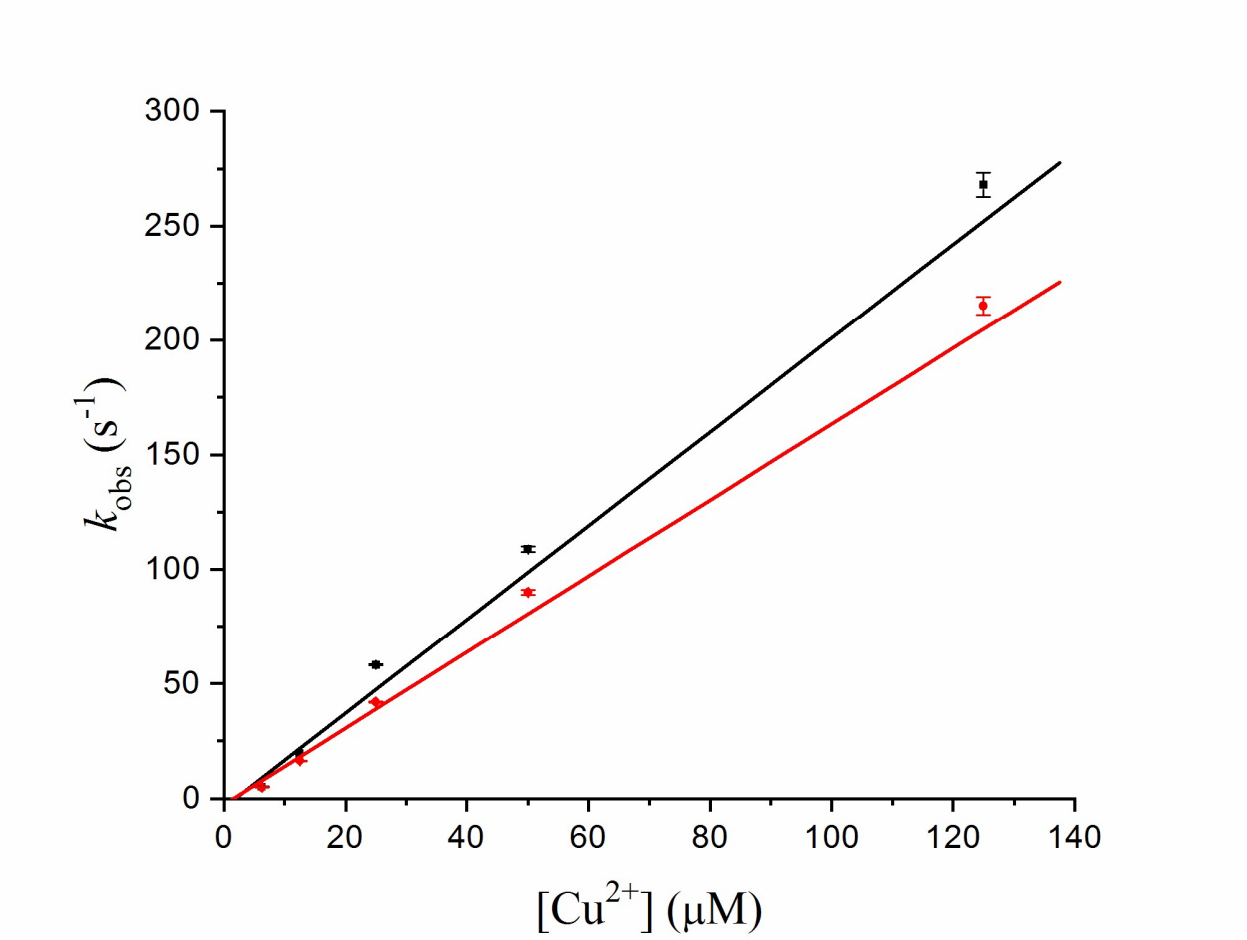

Figure S9. First order rate constants (k_{obs}) for binding of Cu^{2+} as a function of the copper concentration for *Ta*LPMO9A-Pp (●) and *Ta*LPMO9A-Ao (■). The points represent average values derived from four measurements ± the standard deviation.

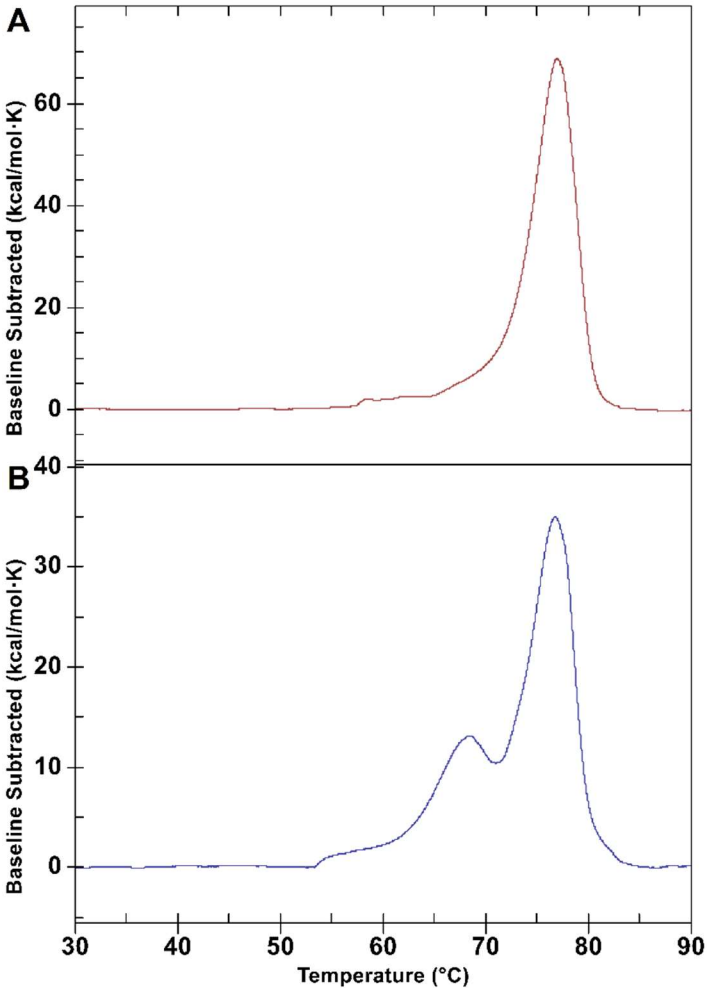

Figure S10. Differential scanning calorimetry (DSC) analysis. The panels show protein unfolding curves for (A) *Ta*LPMO9A-Ao (red), and (B) *Ta*LPMO9A-Pp (blue).

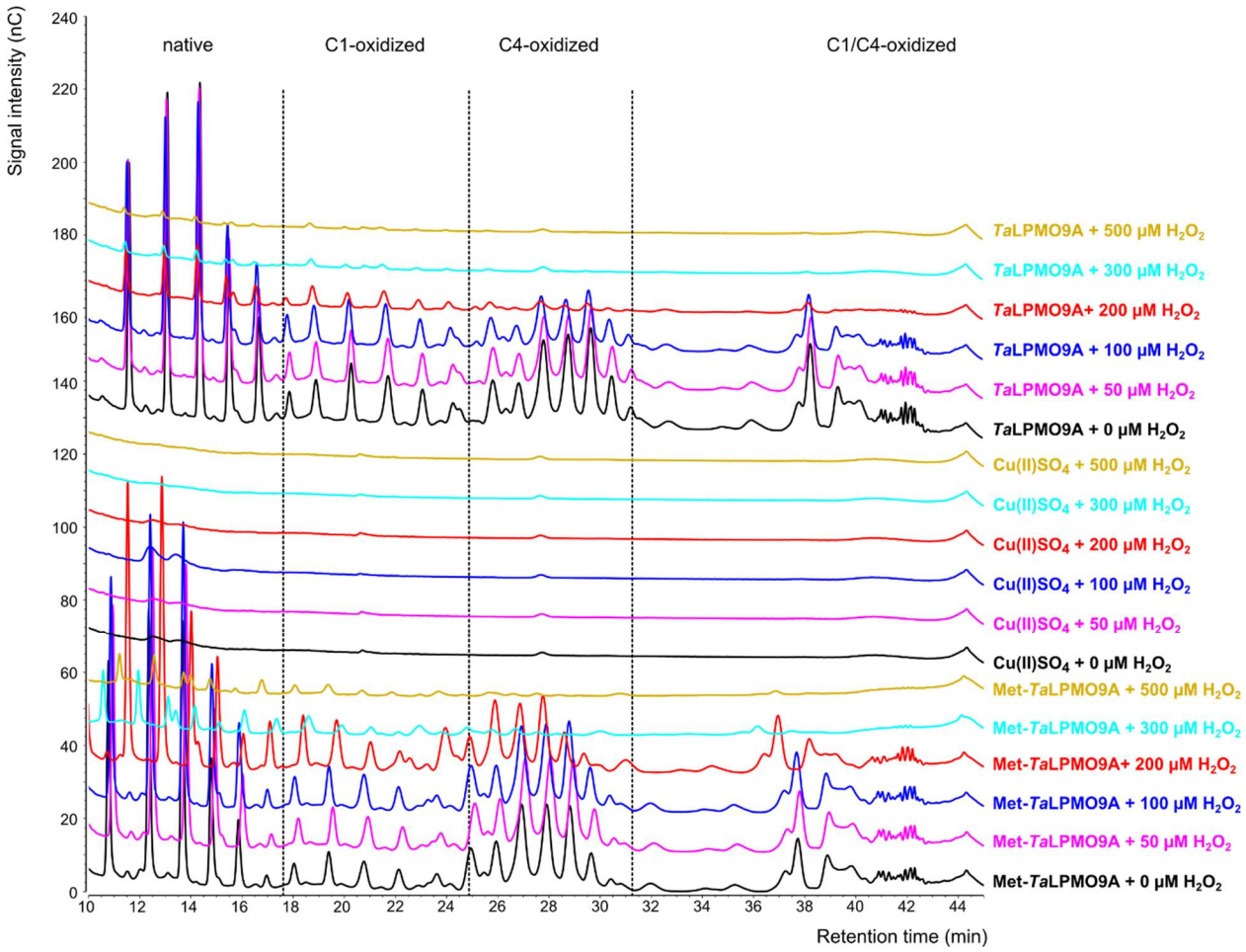

Figure S11. Control reactions to check for occurrence of copper-catalyzed Fenton-type chemistry. The Figure shows HPAEC-PAD product profiles obtained after incubation of 1 µM *Ta*LPMO9A-Ao ("Met-*Ta*LPMO9A"), 1 µM *Ta*LPMO9A-Pp ("*Ta*LPMO9A"), or 1 µM Cu(II)SO_4 with PASC in the presence of different initial concentrations of exogenous H_2O_2 (0 – 500 µM). All reactions were carried out in 40 mM BisTris buffer, pH 6.5, with 2.5 g/L PASC and 1 mM ascorbic acid, at 45 °C and 1000 rpm and the reaction time was 60 min.

[1] Vu, V. V., Beeson, W. T., Phillips, C. M., Cate, J. H., and Marletta, M. A. (2014) Determinants of regioselective hydroxylation in the fungal polysaccharide monooxygenases, *J. Am. Chem. Soc. 136*, 562-565.