Supporting Information for Publication

Neutron and atomic resolution X-ray structures of a lytic polysaccharide monooxygenase reveal copper-mediated dioxygen binding and evidence for N-terminal deprotonation

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Materials and Methods

Expression, purification, crystallization and data collection were previously described in Bacik *et al*., 2015.

X-ray structure refinement. The X-ray structure of *Jd*LPMO10A was solved by molecular replacement using the 100 K structure of *Jd*LPMO10A, PDB 5aa7 (Mekasha *et al.*, 2016) as a search model, and refined in Phenix using positional, anisotropic B factor (heavy atoms only, including solvent atoms, ions, and the dioxygen species), and occupancy refinement. Waters were automatically added using Phenix. Riding hydrogens and deuteriums were added in the later stages of refinement. No charges were introduced, e.g. the copper ion was modeled as Cu (29 electrons), and not as Cu^{+} (28 electrons) or Cu^{2+} (27 electrons). Likewise, oxygen atoms in the peroxide were treated as neutral species (8 electrons). Data processing and refinement statistics are reported in Table S1.

Neutron structure refinement. Using a partially refined model of *Jd*LPMO10A (~20 % R_{free}), containing isotropic temperature factors, the model was refined against neutron data alone, in Phenix. Deuterium atoms were added to solvent molecules as D_2O, and deuterium was introduced at exchangeable sites on the protein molecule and their relative occupancies were allowed to refine. Nonexchangeable sites remained modeled as H. In the initial maps, solvent molecules that were not visible at 1.0 sigma in the $2F_o$-F_c maps were removed to reflect the slight differences between the crystal used for neutron diffraction and crystal used for X-ray diffraction. The structure was refined using positional, isotropic B factor, and occupancy refinement. The quality of the model was confirmed using a composite omit map in Phenix, which showed the continuous density for the dioxygen species. Data quality and refinement statistics are reported in Table S1.

Table S1. Data collection and refinement statistics

	X-ray (5VG0)	**Neutron (5VG1)**
Data collection		
Diffraction source	Beamline 4.2.2, ALS	MaNDi, ORNL
Wavelength (Å)	1.000	2 - 4
Detector	RDI 8M CMOS	27 SNS Anger Cameras
Temperature (K)	295	295
Crystal-to-detector distance (mm)	100	450
Rotation range per image (deg)	0.2	Fixed
Total rotation range	180	120
Exposure time per image	0.1 s	20 h
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell (Å)	32.0, 75.6, 120.4	32.5, 76.4, 122.1
Mosaicity	0.07	N/A
Resolution range (Å)	32.0 – 1.1 (1.16 – 1.10)	12.0 – 2.1 (2.17 – 2.1)
Reflections	1248110 (170145)	62823 (2757)
Unique reflections	116274 (16352)	13989 (1091)
Completeness (%)	97.3 (95.0)	76.02 (59.4)
Multiplicity	10.7 (10.4)	4.5 (2.5)
$<I/\sigma(I)>$	13.7 (1.5)	9.4 (2.7)
R_{pim}	0.031 (0.436)	0.089 (0.196)
Wilson B ($Å^2$)	9.2	24.7
Refinement		
Resolution	32.0 – 1.10	12.0 – 2.10
Reflections (R_{free})	116060 (2000)	13989 (686)
R_{cryst} / R_{free}	0.114 / 0.128	0.187 / 0.265
Mean B factor ($Å^2$) Overall / chain A / chain B/ solvent	17.2 / 14.5 / 17.5 / 35.3	38.0 / 35.2 / 39.3 / 43.1 (H/D)
Ramachandran	98.3 % most favored 1.7 % allowed 0 % disallowed	97.2 % most favored 2.8 % allowed 0 % disallowed
Rmsd bonds (Å)	0.008	0.002
Rmsd angles (deg)	0.975	0.560
Atoms (non-solvent / solvent / H and D atoms)	2279 / 299 / 2541	2207 / 231 / 2946

Table S2. Cu coordination environment in X-ray structures of *Nc*LPMO9D[a] and *Jd*LPMO10A

	Distance (Å)				
ATOMS	***Nc*LPMO9D**		**ATOMS**	***Jd*LPMO10A**	
	Molecule A	**Molecule B**		**Molecule A**	**Molecule B**
Cu-His1-Nδ	1.93	1.95	Cu-His32-Nδ	2.01	2.01
Cu-His1-Namino	2.19	2.20	Cu-His32-Namino	2.17	2.14
Cu-His84-Nε	1.97	1.90	Cu-His109-Nε	2.00	2.01
Cu-Y168-OH	2.66	2.73			
Cu-H_2O equatorial		2.06			
Cu-H_2O axial	2.36	2.23	Cu-H_2O axial	2.45	2.34
Cu-O1 (proximal)	1.90	3.57[b]	Cu-O2 (proximal)	1.84	1.83
			Cu-O1 (distal)	2.14	2.69
O1-O2	1.44	1.20	O1-O2[c]	1.48	1.46
			B (Cu, $Å^2$)	11.40	11.85
			Occupancy (Cu)	0.84	0.89
			B (O1, $Å^2$)	48.65	33.23
			Occupancy (O1)	0.83	0.95
			B (O2, $Å^2$)	48.62	31.01
			Occupancy (O2)	0.83	0.95

[a] Data taken from the ascorbate-treated X-ray structure (5TKH) (O'Dell *et al.*, 2017)
[b] In molecule B, the oxygen is in a proposed "pre-bound" state, interacting primarily with His157. The oxygen does not directly interact with the copper and does not displace the water molecule occupying the equatorial copper coordination position.
[c] Cu-bound O_2-species are modeled as Cu-bound peroxide (Cu-O-O^-)

Figure S1. Omit maps for the dioxygen species bound to the copper in the RT X-ray structure of *Jd*LPMO10A. A-C. F_o-F_c omit map for molecule A, omitting O1 (A), O2 (B), axial water (C). D-E. F_o-F_c omit map for molecule B, omitting O1 (D), O2 (E). Maps are contoured at +5.0 sigma (green) and -5.0 sigma (red).

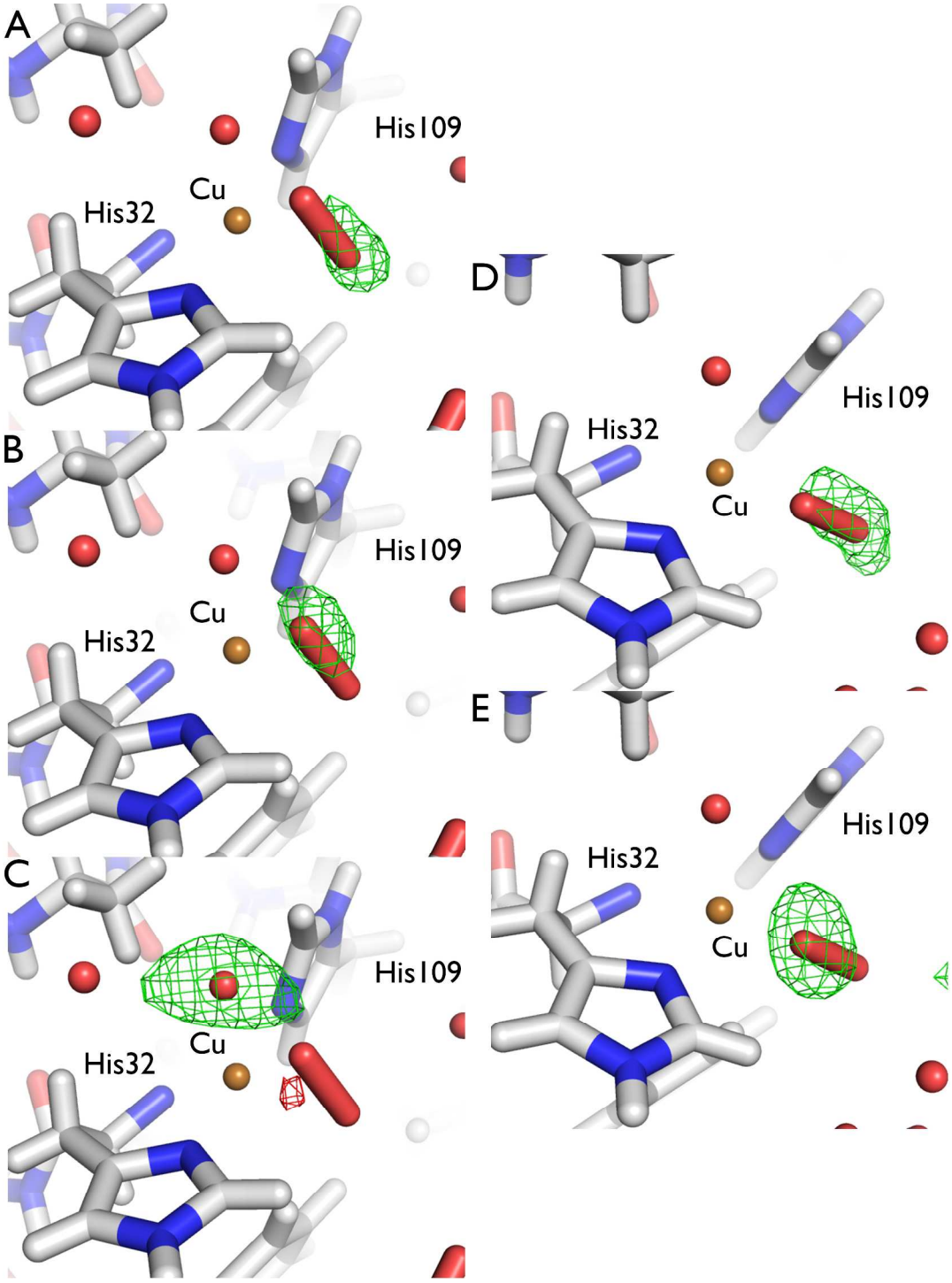


Figure S2. Nuclear density omit map of molecule B N-terminal region, contoured at +3.0 sigma (green), and -3.0 sigma (red). The deuterium on the right, which is bonded to the carbonyl group of Ala107, was set to an occupancy of 1.0, as full exchange for D is expected. The occupancy of the left D was set to 0 (A), 0.25 (B), 0.50 (C), 0.75 (D), and 1.0 (E). As can be seen in (E), full occupancy of the site results in the presence of a negative peak (red), indicating a mix of ND^- and ND_2 species.

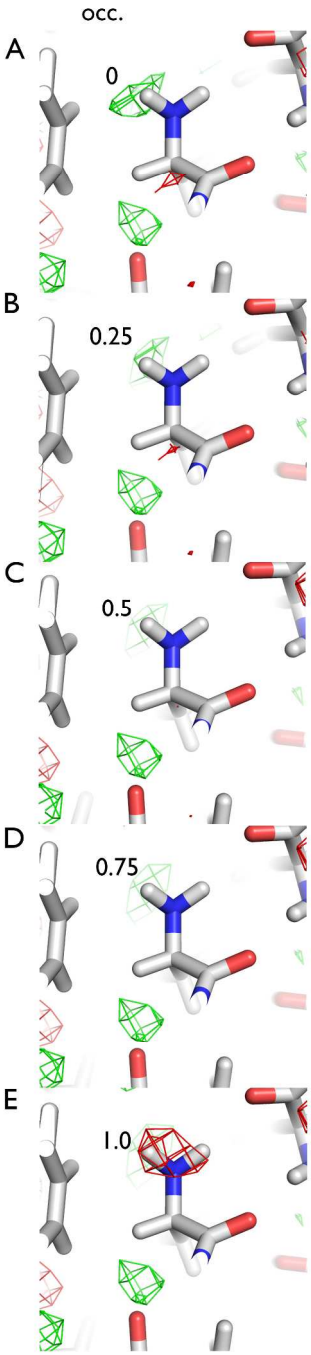
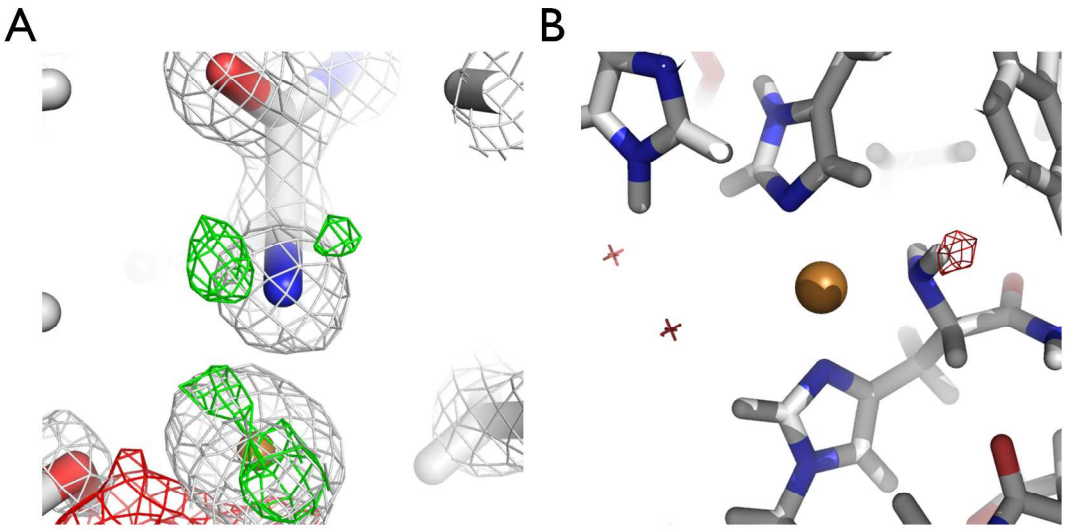


Figure S3. A. F_o-F_c difference density for hydrogen atoms at the N-terminal region of molecule B, showing a mix of ND^- and ND_2 forms. The map is contoured at +3.0 sigma (green) and -3.0 sigma (red). B. F_o-F_c difference density for the N-terminal region of *Nc*LPMO9M (also known as NCU07898; PDB 4EIS) (Li *et al.*, 2012), showing a negative (red) peak at the position of one of the hydrogen atoms. The map is contoured at -3.0 sigma.



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