Supporting information for article:

Changes in active-site geometry on X-ray photoreduction of a lytic polysaccharide monooxygenase active-site copper and saccharide binding

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Supplementary Figures

Figure S1  The main differences between the fungal and bacterially expressed LsAA9_A. The difference density map (F_o-F_c) at contour level ±3.0 σ shown in green and red for positive and negative difference density respectively. The electron density map (2F_o-F_c) is shown as blue mesh and is contoured at 1.0 σ. (a) The N-terminal histidine of LsAA9_A is shown before the methylation was deleted in the refinement process. The red density at the methylation indicates that the model is oversaturated at that location and the methylation should not be there. (b) The glycosylation site at Asn33 exhibit a large negative density, indicating absence of glycosylation in this structure. A positive difference density is seen at the C5 position of the shown N-Acetylglucosamine molecule, where a water molecule should be modelled. PDB code: 7PQR.
Figure S2  Substrate binding of LsAA9_A(Ec) soaked in cellotriose. The substrates are shown as magenta sticks and LsAA9_A(Ec) is depicted as the green cartoon. Cellotriose can be seen bound at the histidine brace (top), while a secondary binding site is found at the opposite side of the enzyme. 2Fo-Fc electron density map shown in blue mesh at 1.0 σ contour level. PDB code: 7PYU.
Figure S3  Substrate binding of *LsAA9_A*(Ec) soaked in cellotetraose. A) An overview of the overall structure with symmetry related molecules. B) A zoomed in view of the active site binding surface. The substrate is shown in yellow sticks with 2Fo-Fc electron density map shown in blue mesh at 1.0 σ contour level. Cellotetraose is bound from subsite -1 to +3, and not from -2 to +2 as has been seen for *LsAA9_A-Cell4* (PDB 6YDG) (Tandrup *et al.*, 2020). This is due to symmetry related molecules (Sym1 and Sym2 in red and green surfaces) occluding the -2 subsite. Symmetry related molecule Sym3 is shown in blue similarly prevents longer substrates from making meaningful interactions at further positive subsites. PDB code: 7PXW.
Figure S4  Active site of *Ls*AA9_A(f) at room temperature. Data were collected at an in-house diffractometer (a) and at a synchrotron (b). The axial position in the structure from in-house data is occupied by a Cl– ion, and by the more commonly observed water in the structure from synchrotron data. Anomalous difference map (purple mesh) is shown at 4.0σ contour level. Active site distances are shown in Å and listed in Table 3 and Supplementary Table 7. 2Fo-Fc map (blue mesh) is shown at 1.0σ contour level. PDB codes: 7PXR (a), 7PXS (b).
Figure S5  Active site of LsAA9_A structure solved from crystal soaked in ascorbic acid. The two structures have a received X-ray dose of a) \(2.08 \times 10^3\) Gy and b) \(1.70 \times 10^7\) Gy. Active site distances in both structures agree with a fully reduced Cu, similar to what is observed for the \(6.65 \times 10^6\) Gy structure of native LsAA9_A(f) presented in Figure 2b. Active site distances are listed in Table 3 and Supplementary Table 7. 2Fo-Fc map (blue mesh) is shown at 0.8 σ contour level in panel a) and 1.0 σ contour level in b). Distances indicated in Å are given from the water molecules to the Cu. PDB codes: 7PXU (a), 7PXV (b).
Figure S6  Active site angles as a function of X-ray dose. The defined angles (see Figure 1) of the AA9 Cu site are recorded for \textit{LsAA9\_A(f)} and \textit{LsAA9\_A(Ec)}, with and without substrate, and \textit{TaAA9\_A}. Values are listed in Table 3 and Supplementary Tables 7 and 8.
Figure S7  Active site of fungally expressed LsAA9_A at increasing X-ray doses. Active site distances are presented in Supplementary Figure 9 and listed in Table 3 and Supplementary Table 7. At increasing X-ray dose both the axial and equatorial water molecules (red spheres) increase their distance to the Cu (orange sphere). In panels b-f) the equatorial water molecule has been modeled in a double conformation. For all panels the 2F₀-Fᵣ map (blue mesh) is shown at 1.0 σ contour level and difference map in green/red mesh at +/-3.0 σ contour level. An animation of the transition between these structures is available as Supplementary Movie 1. PDB codes: 7PXI (a), 7PXJ (b), 7PXK (c), 7PXL (d), 7PXM (e), 7PXN (f).
Figure S8  Active site of LsAA9_A(Ec) at increasing X-ray doses. Active site distances are presented in Supplementary Figure 9 and listed in Table 3 and Supplementary Table 7. The distance between Cu (orange sphere) and the axial water molecule (red sphere) increases with increasing X-ray dose. In panels b,d,e) the axial water molecule has been modeled in a double conformation. On the opposite side of the equatorial water molecule with respect to the Cu a sulfate has been modeled. The distance between the nearest atom (SO₄-O1) and Cu is 4.3 Å (a). As the X-ray dose increase, this distance is reduced to 3.9 Å for the highest dose LsAA9_A(Ec) structure (f). In panel c) it was possible to model a partially occupied sulfate (transparent). In panel d-f) no equatorial water molecule has been modeled, as no supporting electron density was available at this position. For all panels the 2Fₒ-Fc map (blue mesh) is shown at 1.0 σ contour level, and difference map in green/red mesh at +/-3.0 σ contour level. An animation of the transition between these structures is available as Supplementary Movie 2. PDB codes: 7PYL (a), 7PYM (b), 7PYN (c), 7PYO (d), 7PYP (e), 7PYQ (f).
Figure S9  Measured distances as a function of average diffraction weighed radiation dose. Distances are measured from the Cu-atom to the indicated atoms. For $\text{H}_2\text{O}_p$, the distance is measured between the N-terminal nitrogen-atom and the oxygen atom of the nearest water molecule. All distances are listed in Supplementary Table 7.
Figure S10  Secondary coordination sphere of LsAA9_A at 6.65 × 10^6 Gy. The equatorial water (H$_2$O$_{eq}$), modeled in a double conformation, is positioned far from the Cu ion and may instead be H-bonded to either Gln162 or His147. Indicated distances in Å. PDB code: 7PXN.
Figure S11 Active site of TaAA9_A at increasing X-ray doses. Active site distances are presented in Supplementary Figure 9 and listed in Table 3 and Supplementary Table 7. With increasing X-ray dose both the equatorial and axial water molecule increase their distance to Cu. Most notably the axial water molecule, as large distances at the equatorial position is consistently prevented by polyacrylic acid, modeled as acrylic acid. Close to the axial position is also a HEPES molecule with 60% occupancy, though not shown here for clarity. For all panels the 2Fo-FC map (blue mesh) is shown at 1.0σ contour level, and difference map in green/red mesh at +/-3.0σ contour level. An animation of the transition between these structures is available as Supplementary Movie 3. PDB codes: 7PZ3 (a), 7PZ4 (b), 7PZ5 (c), 7PZ6 (d), 7PZ7 (e), 7PZ8 (f).
Figure S12 Active site of LsAA9_A structure solved by serial synchrotron crystallography. The structure was solved from 13 crystals, each estimated to have received an X-ray dose of $7.02 \times 10^4$ Gy. Active site distances are to some extent comparable to the $5.99 \times 10^4$ Gy native LsAA9_A(f) structure presented in Figure 2b. Active site distances listed in Table 3 and Supplementary Table 7. 2Fo-Fc map (blue mesh) is shown at 1.0 $\sigma$ contour level. Distances in Å are measured between the water molecule and the Cu. PDB code: 7PXT.
Figure S13 Active site of fungally expressed LsAA9_A binding Cell₄ at increasing X-ray doses. Active site distances are presented Supplementary Figure 9 and listed in Table 3 and Supplementary Table 7. At increasing X-ray dose, the equatorial Cl-ion (green spheres, present from crystallization conditions) increase its distance to the Cu. Distances in Å are measured between the Cl-ion and the Cu. In panels c) and e) the equatorial water molecule has been modeled in a double conformation. The Tyr164-O to Cu distance is here in all cases shorter than for the substrate free structures presented in Supplementary Figure 7. This distance increases slightly with increasing X-ray dose (see Figure 6). For all panels the 2Fo-Fc map (blue mesh) is shown at 1.0 σ contour level and the difference map in green/red mesh at +/-3.0 σ contour level. An animation of the transition between these structures is available as Supplementary Movie 4. PDB codes: 7PYD (a), 7PYE (b), 7PYF (c), 7PYG (d), 7PYH (e), 7PYI (f).
Figure S14 Active site of LsAA9_A(Ec)-Cell3 at increasing X-ray doses. Active site distances are presented in Supplementary Figure 9 and listed in Supplementary Table 7. Similar to LsAA9_A(f)-Cell4 no axial water molecule is present with cello-oligosaccharide bound. Cell3 binds from subsite -1 to +2, as has been described previously for the fungal expressed LsAA9_A (Frandsen et al., 2016; Tandrup et al., 2020). All panels show a well-defined Cl⁻ ion at the equatorial position. This Cl⁻ ion increase its distance to Cu with increasing X-ray dose. Distances in Å are measured between the Cl⁻ ion and the Cu. For all panels the 2Fo-Fc map (blue mesh) is shown at 1.0 σ contour level, and difference map in green mesh at 3.0 σ contour level. An animation of the transition between these structures is available as Supplementary Movie 5. PDB codes: 7PYU (a), 7PYW (b), 7PYX (c), 7PYY (d), 7PYZ (e), 7PZ0 (f).
Figure S15 Difference maps demonstrating shortening of the Tyr-OH Cu distance on saccharide binding.

To avoid refinement bias, difference maps were calculated using the low dose saccharide-bound data, \( LsAA9\_A(Ec)\)-Cell3 \((1.49 \times 10^4 \text{ Gy})\) after rigid body refinement using the low dose saccharide-free model. A single rigid body including the protein and Cu (at fixed distance) were used for refinement and phasing (a). The difference density clearly indicate that Cu is closer to the Tyr-OH in the saccharide-bound structure.

Similarly, difference maps are shown for low dose \( LsAA9\_A(f)\)-Cell4 \((7.88 \times 10^3 \text{ Gy})\) phased with low dose saccharide-free \( LsAA9\_A(f)\) model \((7.88 \times 10^3 \text{ Gy})\) (b) and previously published low dose \( LsAA9\_A\)-Cell3 data phased with low dose \( LsAA9\_A\) model (Frandsen et al., 2016) (c). The difference map is shown in green mesh for +3.0 \( \sigma \) and red mesh for -3.0 \( \sigma \) contour level.
## Supplementary Tables

| Table S1 | Crystallographic data and refinement statistics for LsAA9_A(f). |
|----------|---------------------------------------------------------------|
|          | LsAA9_A(f) | LsAA9_A(f) | LsAA9_A(f) | LsAA9_A(f) | LsAA9_A(f) | LsAA9_A(f) |
| PDB-ID   | 7PXI       | 7PXJ       | 7PXK       | 7PXL       | 7PXN       |            |
| Crystal size [μm³] | 4 × 10⁶ | 4 × 10⁶ | 4 × 10⁶ | 4 × 10⁶ | 4 × 10⁶ | 4 × 10⁶ |
| Beamline | P11        | P11        | P11        | P11        | P11        | P11        |
| Dose [Gy] | 7.88 × 10⁴ | 5.99 × 10⁴ | 3.39 × 10⁴ | 3.60 × 10⁵ | 1.45 × 10⁸ | 6.65 × 10⁶ |
| Wavelength [Å] | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 |
| Flux [photons/sec] | 1 × 10¹⁰ | 1 × 10¹⁰ | 1 × 10¹¹ | 1 × 10¹¹ | 1 × 10¹² | 1 × 10¹² |
| Degrees exposed per frame | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Degrees exposed | 45 | 360 | 405 | 540 | 765 | 1080 |
| Degrees used in processing | 0-45 | 315-360 | 0-45 | 100-180 | 0-45 | 315-360 |
| Exposure per frame [s] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Space group | P4₁32 | P4₁32 | P4₁32 | P4₁32 | P4₁32 | P4₁32 |
| Cell parameters | (a, b, c) [Å] | 124.98 | 124.98 | 124.99 | 124.65 | 125.08 | 125.51 |
| | (α, β, γ) [°] | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 |
| Resolution [Å] | 50.0-1.63 | 50.0-1.75 | 50.0-1.40 | 50.0-1.30 | 50.0-1.30 | 50.0-1.65 |
| Completeness [%] | (1.67-1.63) | (1.80-1.75) | (1.44-1.40) | (1.33-1.30) | (1.33-1.30) | (1.69-1.65) |
| | 100.0 (100.0) | 100.0 (99.9) | 100.0 (100.0) | 99.9 (99.6) | 99.9 (97.2) | 95.4 (97.2) |
| Rwork [%] | 12.7 (134.4) | 10.3 (77.2) | 6.2 (141.9) | 7.1 (185.2) | 4.3 (108.5) | 4.5 (138.5) |
| I(0)/I | 10.28 (1.12) | 7.64 (1.27) | 17.33 (1.23) | 24.62 (1.46) | 19.10 (1.53) | 16.09 (1.02) |
| CC[100] [%] | 99.8 (47.3) | 99.6 (56.8) | 99.9 (48.2) | 100.0 (61.1) | 99.9 (57.2) | 99.9 (42.1) |
| Unique reflections | 78692 (5864) | 56518 (4340) | 124225 (9227) | 82005 (5955) | 155322 (11417) | 39262 (2902) |
| Observed reflections | 404113 (28206) | 109427 (8249) | 630117 (43707) | 1367411 (85531) | 761407 (47975) | 130811 (9413) |
| Redundancy | 5.14 (4.81) | 1.93 (1.90) | 5.07 (4.74) | 16.67 (14.36) | 4.90 (4.20) | 3.33 (3.24) |
| No. mol./ASU | 1 | 1 | 1 | 1 | 1 | 1 |
| Rwork [%] | 18.30 | 18.31 | 18.68 | 18.73 | 18.46 | 18.02 |
| Rfree [%] | 20.99 | 21.48 | 20.56 | 19.96 | 20.23 | 20.24 |
| Ramachandran [%][§] | Preferred | 93.1 | 92.7 | 92.7 | 92.7 | 94.0 |
| | Allowed | 6.5 | 6.9 | 6.9 | 6.9 | 6.9 |
| | Outlier | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Avr. B-factors [Å²] | Protein | 18.81 | 18.52 | 17.05 | 16.91 | 17.12 | 34.06 |
| | Ligand / ion | - | - | - | 38.37 | - | - |
| | Water | 27.79 | 27.90 | 27.67 | 25.26 | 28.85 | 35.02 |
| Wilson B-factor [Å²] | 27.77 | 27.47 | 24.06 | 23.52 | 22.66 | 30.34 |
| No. of atoms | Protein | 1866 | 1866 | 1866 | 1895 | 1886 | 1901 |
| | Ligand / ion | 0 | 0 | 0 | 3 | 0 | 0 |
| | Water | 311 | 310 | 311 | 299 | 311 | 86 |
| RMSD | - | - | - | - | - | - |
| Bond lengths [Å] | 0.011 | 0.010 | 0.013 | 0.014 | 0.015 | 0.012 |
Table S2  Crystallographic data and refinement statistics for *LsAA9_A* (f)-Cell4.

| PDB-ID | *LsAA9_A-Cells* | *LsAA9_A-Cells* | *LsAA9_A-Cells* | *LsAA9_A-Cells* | *LsAA9_A-Cells* | *LsAA9_A-Cells* |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|        | 7PYD | 7PYE | 7PYF | 7PYG | 7PYH | 7PYI |
| Crystal size [µm^3] | 4 × 10^6 | 4 × 10^6 | 4 × 10^6 | 4 × 10^6 | 4 × 10^6 | 4 × 10^6 |
| Beamline | P11 | P11 | P11 | P11 | P11 | P11 |
| Dose§ [Gy] | 7.88 × 10^3 | 5.99 × 10^4 | 1.39 × 10^5 | 3.60 × 10^5 | 1.45 × 10^6 | 6.65 × 10^6 |
| Wavelength [Å] | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 |
| Flux [photons/sec] | 1 × 10^10 | 1 × 10^10 | 1 × 10^11 | 1 × 10^11 | 1 × 10^12 | 1 × 10^12 |
| Degrees exposed per frame | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Degrees exposed | 360 | 360 | 720 | 720 | 1080 | 1080 |
| Degrees used in processing | 0-45 | 315-360 | 0-45 | 100-180 | 0-45 | 315-360 |
| Exposure per frame [s] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Space group | P4_32 | P4_32 | P4_32 | P4_32 | P4_32 | P4_32 |
| Cell parameters | | | | | | |
| (a, b, c)[Å] | 125.64 | 125.64 | 125.62 | 125.67 | 125.72 | 126.02 |
| (α, β, γ)[º] | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 |
| Resolution [Å] | 50.0-2.21 | 50.0-2.10 | 50.0-1.90 | 50.0-1.90 | 50.0-1.90 | 50.0-2.05 |
| Completeness [%] | 99.9 (100.0) | 100.0 (100.0) | 99.9 (100.0) | 100.0 (100.0) | 99.8 (99.9) | 95.9 (96.7) |
| R_work [%] | 15.6 (140.0) | 16.2 (251.6) | 7.3 (162.4) | 7.7 (183.0) | 3.8 (57.9) | 5.0 (144.9) |
| I/σ(I) | 9.70 (1.24) | 9.62 (0.68) | 16.43 (1.08) | 20.25 (1.28) | 25.68 (3.17) | 15.87 (1.09) |
| CC1/2 [%] | 99.6 (51.6) | 99.8 (32.8) | 99.9 (45.2) | 100.0 (54.7) | 100.0 (87.3) | 99.9 (48.0) |
| Unique reflections | 32082 (2405) | 37414 (2785) | 50437 (3753) | 50564 (3763) | 50544 (3756) | 21166 (1596) |
| Observed reflections | 170006 (13132) | 263397 (18955) | 262836 (19588) | 486855 (34891) | 261089 (18572) | 69757 (5102) |
| Redundancy | 5.29 (5.46) | 7.04 (6.80) | 5.21 (5.22) | 9.26 (9.27) | 5.17 (4.94) | 3.29 (3.19) |
| No. mol./ASU | 1 | 1 | 1 | 1 | 1 | 1 |
| R_work [%] | 22.47 | 23.94 | 25.40 | 24.29 | 26.15 | 21.80 |
| R_free [%] | 28.71 | 29.42 | 28.68 | 29.29 | 29.00 | 26.00 |
| Ramachandran [%]§§ | Preferred | 91.8 | 90.9 | 91.8 | 91.8 | 92.2 | 93.1 |
| Allowed | 7.8 | 8.2 | 6.5 | 7.8 | 7.3 | 6.5 |
|                          | 0.4   | 0.9   | 1.7   | 0.4   | 0.4   | 0.4   |
|--------------------------|-------|-------|-------|-------|-------|-------|
| **Avr. B-factors [Å²]**  |       |       |       |       |       |       |
| Protein                  | 44.11 | 45.38 | 39.89 | 39.49 | 38.64 | 55.61 |
| Ligand / ion             | 43.54 | 49.14 | 41.94 | 43.29 | 42.89 | 64.04 |
| Water                    | 41.63 | 44.71 | 36.69 | 44.31 | 39.39 | 54.30 |
| Wilson B-factor [Å²]     | 51.77 | 50.84 | 44.09 | 44.29 | 42.64 | 57.06 |
| **No. of atoms**         |       |       |       |       |       |       |
| Protein                  | 1836  | 1836  | 1896  | 1816  | 1845  | 1845  |
| Ligand / ion             | 46    | 46    | 48    | 47    | 46    | 46    |
| Water                    | 139   | 139   | 92    | 201   | 70    | 69    |
| **RMSD**                 |       |       |       |       |       |       |
| Bond lengths [Å]         | 0.017 | 0.077 | 0.0089| 0.0091| 0.0094| 0.019 |
| Bond Angles [°]          | 1.777 | 1.602 | 1.6500| 1.6172| 1.6785| 1.900 |

Crystallization condition: 3.3 M NaCl, 0.1 M citric acid pH 3.5
Drop volume [µl]: 0.5
Ratio: 3:1:1

Soak concentration: 1.0 M
Soak duration [min]: 10

Highest resolution shell shown in parenthesis.
§ Relative dose estimated using RADDOSE-3D (Zeldin et al., 2013).
§§ Ramachandran preferred/allowed/outlier regions calculated by Rampage (Lovell et al., 2003).
§§§ Protein to reservoir to water volume ratio.
| Table S3 | Crystallographic data and refinement statistics. |
|---|---|

| PDB-ID | LsAA9_A(f) (SSX) | LsAA9_A(f)(Ascorbic acid) | LsAA9_A(f)(Acetaldehyde) | LsAA9_A(f)(RT) | LsAA9_A(f)(RT-sync) | LsAA9_A(Ec) -Cell1 | LsAA9_A(Ec) original structure determination |
|---|---|---|---|---|---|---|---|
| 7PXT | 7PXU | 7PXV | 7PXR | 7PXS | 7PXW | 7PQR |
| Crystal size [μm³] | 8 × 10³ | 1.5 × 10³ | 1.5 × 10³ | 4 × 10⁴ | 1.73 × 10⁴ | 1 × 10⁸ | 1 × 10⁹ |
| Beamline | ID29 (ESRF) | P11 | P11 | Diffractometer BioMAX | BioMAX (MAXIV) | BioMAX (MAXIV) | BioMAX (MAXIV) |
| Dose [Gy] | 7.02 × 10³ | 2.08 × 10³ | 1.70 × 10⁷ | - | 1.91 × 10³ | 2.14 × 10⁹ | - |
| Wavelength [Å] | 0.98 | 0.97 | 0.97 | 1.540 | 0.9762 | 0.98 | 1.127130 |
| Flux [photons/sec] | 2.0 × 10¹⁰ | 5.06 × 10⁸ | 4.12 × 10¹² | - | 5.3 × 10¹⁰ | 5.2 × 10¹¹ | - |
| Degrees exposed per frame | 0.1 | 0.1 | 0.1 | 0.25 | 0.1 | 0.1 | 0.1 |
| Degrees exposed | 10 | 360 | 360 | 98 | 50 | 360 | 360 |
| Degrees used in analysis | 0-2.5 | 0-100 | 0-100 | 0-98 | 50 | 0-360 | 0-360 |
| Exposure per frame [s] | 1 | 0.03 | 0.03 | 10 | 0.011 | 0.011 | 0.011 |
| Space group | P4₁32 | P4₁32 | P4₁32 | P4₁32 | P4₁32 | P4₁ | P4₁ |
| Cell parameters | | | | | | | |
| (a, b, c)[Å] | 125.42 | 125.02 | 125.41 | 126.66 | 126.63 | 48.8, 48.9, 48.92, 48.92, 48.92, 48.92, 48.92 |
| (α, β, γ)[°] | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 | 90.0 |
| Resolution [Å] | (4.26-2.4) | (1.85-1.80) | (1.54-1.50) | (1.84-1.80) | (1.95-1.90) | (1.44-1.40) | (1.33-1.30) |
| Completeness [%] | 88.0 (89.7) | 96.9 (99.6) | 100.0 (100.0) | 100.0 (100.0) | 100 (100) | 99.6 (99.7) | 99.4 (93.3) |
| Rwork [%] | 29.3 (91.0) | 22.0 (92.1) | 16.2 (275.2) | 4.7 (50.9) | 18.5 (114.7) | 11.7 (341.7) | 7.7 (125.0) |
| Rcryst [%] | 7.21 (3.37) | 4.84 (1.11) | 16.65 (2.27) | 43.8 (4.8) | 8.67 (1.37) | 12.07 (1.19) | 12.2 (1.09) |
| CC1/2 [%] | 61.9 (66.4) | 98.4 (56.2) | 99.9 (59.7) | 100.0 (92.5) | 99.7 (74.2) | 99.9 (55.0) | 99.9 (48.2) |
| Unique reflections | 21952 (1698) | 30547 (2272) | 54321 (3982) | 32798 (1930) | 27939 (2033) | 49868 (3724) | 63029 (8661) |
| Observed reflections | 77011 (6103) | 130063 (9611) | 1113030 | 562827 | 304633 | 678988 | 763391 |
| Redundancy | 3.51 (3.59) | 4.25 (4.23) | 20.48 (21.19) | 17.2 (10.2) | 9.9 (11.24) | 13.62 (13.57) | 12.1 (5.8) |
| No. mol./ASU | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Rwork [%] | 18.67 | 19.52 | 18.84 | 14.67 | 16.94 | 11.71 | 13.33 |
| Rfree [%] | 24.76 | 22.65 | 20.70 | 16.62 | 19.56 | 15.98 | 15.33 |
| Ramachandran [%] | Preferred | 93.1 | 94.0 | 94.4 | 95.3 | 94.4 | 82.7 | 94.8 |
| Allowed | 6.9 | 6.0 | 5.6 | 4.7 | 5.6 | 12.3 | 5.2 |
| Outlier | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 0.0 |
| Avr. B-factors [Å²] | Protein | 23.15 | 17.92 | 20.13 | 21.75 | 27.94 | 21.5 | 16.85 |
| Ligand/ion | - | 22.95 | 25.84 | 44.83 | - | 35.0 | 36.36 |
| Water | 21.00 | 27.23 | 29.13 | 35.13 | 34.83 | 35.4 | 33.05 |
| Wilson B-factor [Å²] | 36.31 | 26.55 | 25.30 | 27.62 | 36.19 | 26.81 | 22.19 |
| No. of atoms | Protein | 1809 | 1840 | 1833 | 1868 | 1816 | 1812 | 1897 |
| Ligand/ ion | 0 | 4 | 3 | 13 | 0 | 75 | 24 |
| Water RMSD | 245 | 362 | 269 | 182 | 139 | 302 | 466 |
|------------|-----|-----|-----|-----|-----|-----|-----|
| Bond lengths [Å] | 0.0073 | 0.0096 | 0.0124 | 0.0141 | 0.0117 | 0.0145 | 0.0142 |
| Bond Angles [°] | 1.4641 | 1.5930 | 1.7694 | 1.8438 | 1.7356 | 1.8374 | 1.8243 |
| Crystallization conditions | 3.7 M NaCl 0.1 M citric acid pH 4.5 | 3.5 M NaCl 0.1 M citric acid pH 4.0 | 3.5 M NaCl 0.1 M citric acid pH 4.0 | 3.0 M NaCl 0.1 M citric acid pH 3.5 | 3.0 M NaCl 0.1 M citric acid pH 3.5 | 2.3 M ammonium sulfate | 1.8 M ammonium sulfate |
| Drop volume [µl] | 0.5 | 2 | 2 | 20 | 4 | 200 | 0.3 |
| Ratio §§§ | 3:1:1 | 1:1:0 | 1:1:0 | 3:1:0 | 3:1:0 | 3:1:0 | 1:1:0 |
| Soaked with | - | Ascorbic acid pH 5.5 | Ascorbic acid pH 5.5 | - | - | Celloctetraose | - |
| Soak concentration | - | 0.01 M | 0.01 M | - | - | 0.05 M | - |
| Soak duration [min] | - | 20 | 20 | - | - | 30 | - |

Highest resolution shell shown in parenthesis.
§ In-house diffractometer at the Oak Ridge National Laboratory. Rigaku HighFlux HomeLab instrument with MicroMax-007 HF X-ray generator and Eiger R 4M detector.
§ Relative dose estimated using RADDOSE-3D (Zeldin et al., 2013).
 §§ Ramachandran preferred/allowed/outlier regions calculated by Rampage (Lovell et al., 2003).
 §§§ Protein to reservoir to water volume ratio.
| PDB-ID  | LsAA9_A(Ec) | LsAA9_A(Ec) | LsAA9_A(Ec) | LsAA9_A(Ec) | LsAA9_A(Ec) | LsAA9_A(Ec) |
|---------|-------------|-------------|-------------|-------------|-------------|-------------|
| Crystal size [µm³] | 1 × 10⁶ | 1 × 10⁶ | 1 × 10⁶ | 1 × 10⁶ | 1 × 10⁶ | 1 × 10⁶ |
| Beamline | P11 | P11 | P11 | P11 | P11 | P11 |
| Dose [Gy] | 1.49 × 10⁴ | 5.61 × 10⁴ | 2.31 × 10⁵ | 3.33 × 10⁵ | 2.13 × 10⁶ | 6.35 × 10⁷ |
| Wavelength [Å] | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 |
| Flux [photons/sec] | 1.33 × 10⁶ | 1.33 × 10⁶ | 1.31 × 10¹⁰ | 1.31 × 10¹⁰ | 1.36 × 10¹¹ | 1.36 × 10¹¹ |
| Degrees exposed per frame | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Degrees exposed | 360 | 360 | 720 | 720 | 1080 | 1080 |
| Degrees used in processing | 0-80 | 280-360 | 0-80 | 280-360 | 0-80 | 280-360 |
| Exposure per frame [s] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Space group | P4₁ | P4₁ | P4₁ | P4₁ | P4₁ | P4₁ |
| Cell parameters (a, b, c)[Å] | 48.95, 48.86, 48.93, 48.84, 49.01, 49.19, |
| (a, ß, γ)[°] | 90.0, 90.0, 90.0, 90.0, 90.0, 90.0, 90.0, 90.0 |
| Resolution [Å] | 50.0-1.70, 50.0-1.75, 50.0-1.40, 50.0-1.60, 50.0-1.60 |
| Completeness [%] | 91.1 (91.3), 94.0 (91.5), 92.2 (88.7), 93.4 (92.7), 90.1 (90.8), 92.6 (94.6) |
| Rmerge [%] | 14.5 (82.0), 13.0 (64.5), 6.6 (70.6), 5.7 (63.0), 2.7 (10.6), 3.7 (50.4) |
| I/σ(I) | 4.57 (0.87), 4.63 (1.04), 10.0 (1.32), 9.43 (1.25), 22.76 (7.8), 13.37 (1.74) |
| CC1/2 [%] | 99.1 (55.4), 99.1 (63.9), 99.9 (72.9), 99.9 (75.4), 99.9 (98.7), 99.9 (76.8) |
| Unique reflections | 50684 | 47775 (3449), 91816 (6541), 92581 (6814), 60403 (4537), 62736 (4780) |
| Observed reflections | 83579 | 77445 (5462), 185902 (13318), 148360 (10744), 99181 (7562), 100497 (7682) |
| Redundancy | 1.65 (1.63), 1.62 (1.58), 2.02 (2.04), 1.60 (1.58), 1.64 (1.67), 1.60 (1.61) |
| No. mol./ASU | 1 | 1 | 1 | 1 | 1 | 1 |
| Rwork [%] | 14.83 | 16.56 | 14.23 | 13.80 | 12.29 | 14.42 |
| Rfree [%] | 18.83 | 19.39 | 16.08 | 16.32 | 14.66 | 16.78 |
| Ramachandran [%] | Preferred | 94.4 | 94.4 | 94.8 | 94.8 | 94.8 |
| Allowed | 5.6 | 5.4 | 5.2 | 5.2 | 5.2 | 5.6 |
| Outlier | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Avr. B-factors[Å²] | Protein | 17.72 | 17.95 | 13.92 | 14.52 | 13.01 | 22.74 |
| Ligand / ion | 56.11 | 53.29 | 32.64 | 36.27 | 34.35 | 61.02 |
| Water | 36.65 | 26.70 | 26.72 | 31.28 | 27.45 | 34.11 |
| Wilson B-factor[Å²] | 27.65 | 27.70 | 21.72 | 22.40 | 22.92 | 32.41 |
| No. of atoms | Protein | 1807 | 1795 | 1806 | 1832 | 1810 | 1831 |
| Ligand / ion | 20 | 20 | 25 | 20 | 20 | 20 |
| Water | 498 | 260 | 336 | 449 | 350 | 222 |
| RMSD | | | | | | |
| Bond lengths [Å] | 0.0088 | 0.0096 | 0.0125 | 0.0124 | 0.0132 | 0.0132 |
| Bond Angles [''] | 1.4867 | 1.4772 | 1.6995 | 1.6997 | 1.7650 | 1.7369 |

| Crystallization condition | 2.3 M (NH₄)₂SO₄, Sodium acetate pH 4.5 |
| Drop volume [µl] | 4 |
| Ratio§§§ | 1:3:0 |
| Oil ratio§§§§ | 3:2 |

Highest resolution shell shown in parenthesis.

§ Relative dose estimated using RADDOSE-3D (Zeldin et al., 2013).
§§ Ramachandran preferred/allowed/outlier regions calculated by Rampage (Lovell et al., 2003).
§§§ Protein to reservoir to water volume ratio.
§§§§ Ratio of Paraffin:Silicon oil used for batch crystallization experiment
Table S5  Crystallographic data and refinement statistics for \textit{LsAA9}_A(Ec)-Cell$_3$.

| PDB-ID | \textit{LsAA9}$_A$(Ec)-Cell$_1$ | \textit{LsAA9}$_A$(Ec)-Cell$_2$ | \textit{LsAA9}$_A$(Ec)-Cell$_3$ | \textit{LsAA9}$_A$(Ec)-Cell$_4$ | \textit{LsAA9}$_A$(Ec)-Cell$_5$ | \textit{LsAA9}$_A$(Ec)-Cell$_6$ |
|--------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Crystal size [µm$^3$] | 1 $\times$ 10$^6$ | 1 $\times$ 10$^6$ | 1 $\times$ 10$^6$ | 1 $\times$ 10$^6$ | 1 $\times$ 10$^6$ | 1 $\times$ 10$^6$ |
| Beamliness [°] | P11 | P11 | P11 | P11 | P11 | P11 |
| Wavelength [Å] | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 | 1.0332 |
| Flux [photons/sec] | 1.33 $\times$ 10$^4$ | 1.33 $\times$ 10$^4$ | 1.94 $\times$ 10$^10$ | 2.2 $\times$ 10$^{12}$ | 2.2 $\times$ 10$^{12}$ |
| Degrees exposed per frame | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Degrees exposed | 360 | 360 | 720 | 720 | 720 | 720 |
| Degrees used in processing | 0-80 | 280-360 | 0-80 | 280-360 | 0-80 | 280-360 |
| Exposure per frame | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| [s] | | | | | | |
| Space group | \textit{P}4$_1$ | \textit{P}4$_1$ | \textit{P}4$_1$ | \textit{P}4$_1$ | \textit{P}4$_1$ | \textit{P}4$_1$ |
| Cell parameters (a, b, c)[Å] | 48.83, 48.83, 108.78 | 48.84, 48.84, 108.79 | 48.84, 48.84, 108.79 | 48.85, 48.85, 108.79 | 48.85, 48.85, 108.79 | 48.90, 48.90, 108.93 |
| (α, β, γ)[°] | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 |
| Resolution [Å] | 50.0 (1.40) | 50.0 (1.40) | 50.0 (1.40) | 50.0 (1.40) | 50.0 (1.40) | 50.0 (1.40) |
| Completeness [%] | 89.3 (87.4) | 96.2 (96.2) | 90.1 (89.6) | 90.2 (86.8) | 90.0 (98.9) | 90.6 (88.6) |
| R$_{int}$ [%] | 10.7 (56.0) | 10.7 (58.8) | 4.3 (8.7) | 5.4 (29.2) | 2.4 (3.8) | 2.4 (12.4) |
| I/σ(I) | 5.08 (0.98) | 5.66 (1.09) | 16.68 (0.93) | 8.78 (2.10) | 33.03 (20.63) | 19.53 (4.45) |
| CC12 [%] | 99.4 (73.2) | 99.4 (72.8) | 99.7 (98.9) | 99.8 (92.4) | 99.9 (99.8) | 99.9 (98.6) |
| Unique reflections | 88368 (6435) | 95196 (7080) | 59815 (4435) | 141907 (10137) | 59808 (4484) | 142855 (10325) |
| Observed reflections | 148178 (10664) | 204486 (14724) | 100546 (7549) | 243974 (16845) | 100249 (7625) | 230046 (15891) |
| Redundancy | 1.68 (1.66) | 2.15 (2.08) | 1.68 (1.70) | 1.72 (1.66) | 1.68 (1.70) | 1.61 (1.54) |
| No. mol./ASU | 1 | 1 | 1 | 1 | 1 | 1 |
| R$_{int}$ [%] | 14.19 | 13.70 | 12.59 | 14.44 | 12.30 | 13.40 |
| R$_{free}$ [%] | 16.22 | 15.34 | 14.62 | 15.78 | 14.38 | 14.75 |
| Ramachandran [%] | Preferred | 94.4 | 94.4 | 95.3 | 94.8 | 94.8 | 94.8 |
| Allowed | 5.6 | 5.6 | 4.7 | 5.2 | 5.2 | 5.2 |
| Outlier | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Avr. B-factors [Å$^2$] | 13.21 | 13.33 | 10.71 | 12.55 | 10.74 | 13.06 |
| Ligand / ion | 29.97 | 30.11 | 25.39 | 25.33 | 25.08 | 31.60 |
| Water | 31.16 | 31.43 | 22.38 | 23.77 | 22.77 | 25.81 |
| Wilson B-factor [Å$^2$] | 21.38 | 21.67 | 20.28 | 17.11 | 20.21 | 17.44 |
| No. of atoms | 1904 | 1904 | 1825 | 1825 | 1825 | 1839 |
| Ligand / ion | 83 | 83 | 50 | 50 | 50 | 88 |
| Water | 452 | 452 | 270 | 270 | 269 | 278 |
| RMSD | 0.0129 | 0.0126 | 0.0134 | 0.0150 | 0.0138 | 0.0178 |
| Crystallization conditions | 2.3 M (NH₄)₂SO₄Sodium acetate pH 4.5 |
|---------------------------|--------------------------------------|
| Drop volume [µl]          | 3                                    |
| Ratio §§§                 | 1:2:0                                |
| Oil ratio §§ §§           | 3:2                                  |
| Soak concentration        | 0.5 M                                |
| Soak duration [min]       | 30                                   |

Highest resolution shell shown in parenthesis.

§ Relative dose estimated using RADDOSE-3D (Zeldin et al., 2013).

 §§ Ramachandran preferred/allowed/outlier regions calculated by Rampage (Lovell et al., 2003).

 §§§ Protein to reservoir to water volume ratio.

 §§§§ Ratio of Paraffin:Silicon oil used for batch crystallization experiment
Table S6  Crystallographic data and refinement statistics for TaAA9_A.

| PDB-ID | TaAA9_A | TaAA9_A | TaAA9_A | TaAA9_A | TaAA9_A | TaAA9_A |
|--------|---------|---------|---------|---------|---------|---------|
|        | TPZ3    | TPZ4    | TPZ5    | TPZ6    | TPZ7    | TPZ8    |
| Crystal size [µm³] | 3.75 × 10⁶ | 3.75 × 10⁶ | 3.75 × 10⁶ | 3.75 × 10⁶ | 3.75 × 10⁶ | 3.75 × 10⁶ |
| Beaml ine | P11     | P11     | P11     | P11     | P11     | P11     |
| Dose [Gy] | 5.37 × 10⁵ | 2.07 × 10⁵ | 9.56 × 10⁵ | 2.22 × 10⁵ | 1.13 × 10⁶ | 3.12 × 10⁶ |
| Wavelength [Å] | 1.0322 | 1.0322 | 1.0322 | 1.0322 | 1.0322 | 1.0322 |
| Flux [photons/sec] | 1.14 × 10⁹ | 1.14 × 10⁹ | 1.11 × 10⁹ | 1.11 × 10⁹ | 1.6 × 10¹⁰ | 1.6 × 10¹⁰ |
| Degrees exposed per frame | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Degrees exposed | 360 | 360 | 720 | 720 | 1080 | 1080 |
| Degrees used in processing | 0-120 | 240-360 | 0-120 | 240-360 | 0-120 | 240-360 |
| Exposure per frame [s] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Space group | P2₁ | P2₁ | P2₁ | P2₁ | P2₁ | P2₁ |
| Cell parameters (a, b, c) [Å] | 34.46, 34.45, 34.41 | 34.38, 34.38, 34.43 | 34.41, 34.41, 34.38 | 34.43, 34.41, 34.38 | 34.41, 34.41, 34.38 | 34.41, 34.41, 34.38 |
| α, β, γ [°] | 87.40, 87.37, 87.29 | 87.21, 87.21, 87.21 | 87.21, 87.21, 87.21 | 87.21, 87.21, 87.21 | 87.21, 87.21, 87.21 | 87.21, 87.21, 87.21 |
| Resolution [Å] | 50.0-1.90 | 50.0-1.85 | 50.0-1.45 | 50.0-1.45 | 50.0-1.80 | 50.0-1.40 |
| Completens [°] | 95.2 (97.4) | 88.2 (92.5) | 89.6 (83.4) | 89.4 (87.2) | 85.1 (83.8) | 91.1 (91.3) |
| Rmax [%] | 23.6 (82.8) | 24.8 (96.5) | 10.4 (76.7) | 10.2 (83.0) | 3.8 (9.3) | 5.8 (50.9) |
| I(0) | 3.08 (0.82) | 2.95 (0.65) | 5.71 (1.0) | 5.22 (0.82) | 16.88 (7.73) | 8.83 (1.49) |
| CC1/2 [%] | 97.0 (50.4) | 97.3 (41.5) | 99.0 (51.1) | 99.4 (57.9) | 99.8 (98.8) | 99.8 (74.0) |
| Unique reflections | 16119 (1194) | 16153 (1273) | 66959 (4641) | 66453 (4836) | 33301 (2405) | 75552 (5685) |
| Observed reflections | 32081 (2349) | 34291 (2456) | 95287 (6365) | 94395 (6335) | 43335 (3028) | 115762 (8276) |
| Redundancy | 1.99 (1.96) | 2.12 (1.93) | 1.42 (1.37) | 1.42 (1.31) | 1.30 (1.26) | 1.53 (1.46) |
| No. mol./ASU | 1 | 1 | 1 | 1 | 1 | 1 |
| Rmax [%] | 20.81 | 23.37 | 16.56 | 16.94 | 13.42 | 15.12 |
| Rmax [%] | 25.63 | 28.58 | 18.58 | 18.85 | 16.98 | 16.89 |
| Ramachandran [%] | 96.4 | 96.4 | 98.2 | 97.3 | 97.8 | 97.8 |
| Allowed | 3.1 | 3.1 | 1.3 | 2.2 | 1.8 | 1.8 |
| Outlier | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Avr. B-factors [Å²] | 21.79 | 21.66 | 13.95 | 14.90 | 11.80 | 14.51 |
| Water | 24.08 | 18.74 | 24.38 | 25.97 | 23.24 | 26.99 |
| Wilson B-factor [Å²] | 29.34 | 28.89 | 21.22 | 21.76 | 20.98 | 21.80 |
| No. of atoms | 1774 | 1761 | 1768 | 1768 | 1748 | 1768 |
| Protein | 160 | 63 | 275 | 275 | 275 | 275 |
| Water | 0.0089 | 0.0071 | 0.0119 | 0.0113 | 0.0123 | 0.0132 |
| Bond lengths [Å] | 1.7822 | 1.6659 | 1.9014 | 1.8482 | 1.9179 | 2.0025 |
Crystallization conditions

|                          |                     |
|--------------------------|---------------------|
| Drop volume [µl]         | 4                   |
| Ratio§§§                 | 3:1:0               |

Highest resolution shell shown in parenthesis.

§ Relative dose estimated using RADDOSE-3D (Zeldin et al., 2013).
§§ Ramachandran preferred/allowed/outlier regions calculated by Rampage (Lovell et al., 2003).
§§§ Protein to reservoir to water volume ratio.
**Table S7** Structural parameters of the LPMO Cu-site.

| PDB ID | Res. [Å] | Rwork/Rfree [%] | Dose [Gy] | Solvent ligands (Eq, Ax) | Cu-N61 [Å] | Cu-N40 [Å] | Cu-N2 [Å] | Cu- Oxy [Å] | Cu-Eq [Å] | θ₃, θ₁, θ₅ (°) | θ₉ (°) | RMSD T (Å) | RMSD H1 (Å) | RMSD HN (Å) | RMSD HN (°) |
|--------|----------|------------------|-----------|--------------------------|-------------|------------|----------|------------|----------|----------------|---------|-------------|-------------|--------------|--------------|
| 7PXi   | 1.57     | 18.30/20.99      | 7.88 × 10⁻⁵ water, water | 1.9 | 2.2 | 2.0 | 2.7 | 2.2 | 2.7 | 92.9, 89.6, 177.1 | 1.5 | 0.12 | 66.9 | 0.03 | 5.8 | 0.02 | 4.3 |
| 7PJK   | 1.75     | 18.31/21.48      | 5.99 × 10⁻⁵ water, water | 1.8 | 2.3 | 2.0 | 2.7 | 3.1 | 3.0 | 93.1, 90.9, 175.2 | 2.7 | 0.07 | 64.7 | 0.03 | 4.7 | 0.02 | 3.8 |
| 7PKX   | 1.40     | 18.68/20.56      | 1.39 × 10⁻⁵ water, water | 1.9 | 2.3 | 2.0 | 2.8 | 3.1 | 3.1 | 92.0, 95.1, 172.5 | 2.2 | 0.04 | 64.5 | 0.03 | 0.9 | 0.01 | 8.6 |
| 7PLX   | 1.30     | 18.73/19.96      | 3.59 × 10⁻⁵ water, water | 1.9 | 2.2 | 2.0 | 2.8 | 3.0 | 3.1 | 92.1, 96.7, 170.3 | 4.2 | 0.04 | 63.7 | 0.03 | -0.8 | 0.02 | 7.4 |
| 7PMX   | 1.30     | 18.46/20.23      | 1.45 × 10⁻⁵ water, water | 1.9 | 2.2 | 2.0 | 2.8 | 3.1 | 3.2 | 93.3, 96.6, 169.6 | 3.1 | 0.03 | 63.0 | 0.03 | -3.2 | 0.01 | 8.6 |
| 7PXN   | 1.65     | 20.35/22.18      | 6.65 × 10⁻⁵ water, water | 1.9 | 2.3 | 2.0 | 2.7 | 3.3 | 3.3 | 91.7, 99.7, 167.5 | 5.1 | 0.04 | 58.6 | 0.03 | -3.2 | 0.02 | 7.4 |
| 7PLY   | 1.70     | 14.83/18.83      | 1.49 × 10⁻⁵ water, water | 2.0 | 2.1 | 2.0 | 2.7 | 1.9 | 2.6 | 91.8, 94.9, 172.6 | 3.3 | 0.12 | 66.2 | 0.03 | 0.5 | 0.02 | 6.7 |
| 7PYM   | 1.75     | 15.66/19.39      | 5.61 × 10⁻⁵ water, water | 2.1 | 2.2 | 2.0 | 2.7 | 3.0 | 3.1 | 92.9, 96.3, 168.6 | 6.7 | 0.10 | 64.3 | 0.03 | -6.1 | 0.03 | 6.8 |
| 7PYN   | 1.40     | 14.23/16.08      | 2.31 × 10⁻⁵ water, water | 2.0 | 2.2 | 2.0 | 2.8 | 1.8 | 3.1 | 92.6, 97.9, 168.8 | 4.0 | 0.05 | 65.8 | 0.03 | -6.8 | 0.02 | 10.1 |
| 7PYO   | 1.40     | 13.80/16.32      | 3.33 × 10⁻⁵ sulfate, water | 1.9 | 2.3 | 2.0 | 2.8 | - | 3.7 | 92.3, 99.8, 167.2 | 4.0 | 0.02 | 64.2 | 0.03 | -9.9 | 0.03 | 11.1 |
| 7PPY   | 1.60     | 12.29/14.66      | 2.13 × 10⁻⁵ sulfate, water | 2.0 | 2.3 | 2.0 | 2.8 | - | 3.6 | 92.7, 99.6, 170.0 | 4.3 | 0.03 | 64.3 | 0.03 | -8.4 | 0.02 | 11.5 |
| 7PYQ   | 1.60     | 14.42/16.78      | 6.35 × 10⁻⁵ sulfate, water | 2.0 | 2.2 | 2.0 | 2.8 | - | 3.8 | 93.3, 102.9, 162.0 | 7.8 | 0.05 | 62.8 | 0.03 | -13.3 | 0.03 | 11.3 |
| 7PXU   | 1.80     | 19.52/22.65      | 2.08 × 10⁻⁵ water, water | 1.9 | 2.3 | 2.0 | 2.9 | 4.0 | 3.7 | 94.8, 96.3, 168.4 | 3.4 | 0.02 | 65.1 | 0.03 | -3.7 | 0.03 | 9.7 |
| 7PVX   | 1.50     | 18.74/20.70      | 1.70 × 10⁻⁵ Water, water | 1.8 | 2.3 | 2.0 | 2.8 | 4.0 | 3.5 | 93.2, 97.4, 168.6 | 4.4 | 0.03 | 59.3 | 0.03 | -4.9 | 0.02 | 9.8 |
| 7PXR   | 2.40     | 18.67/24.76      | 7.02 × 10⁻⁵ water, water | 1.9 | 2.3 | 1.9 | 2.7 | 3.9 | 2.9 | 84.9, 99.6, 171.5 | 7.1 | 0.10 | 69.9 | 0.03 | -4.1 | 0.02 | 11.1 |
| 7PX5   | 1.45     | 16.56/18.58      | 9.56 × 10⁻⁵ water, water | 1.9 | 2.2 | 2.0 | 3.0 | 2.1 | 2.6 | 93.7, 93.8, 172.5 | -0.2 | 0.08 | 75.4 | 0.03 | -1.8 | 0.02 | 10.1 |
| 7PZ6   | 1.45     | 16.94/18.85      | 2.22 × 10⁻⁵ water, water | 1.9 | 2.2 | 2.0 | 3.0 | 2.3 | 2.7 | 94.8, 92.9, 172.3 | 0.5 | 0.06 | 76.9 | 0.03 | -0.6 | 0.02 | 10.8 |
| 7PZ7   | 1.80     | 13.42/16.98      | 1.13 × 10⁻⁵ water, water | 1.9 | 2.3 | 2.0 | 3.0 | 2.6 | 2.8 | 94.6, 95.2, 170.3 | 0.5 | 0.03 | 75.3 | 0.04 | -3.7 | 0.02 | 14.1 |
| 7PZ8   | 1.40     | 15.12/16.89      | 3.12 × 10⁻⁵ water, water | 1.9 | 2.2 | 2.0 | 2.9 | 2.5 | 3.0 | 94.6, 96.1, 169.3 | 1.0 | 0.02 | 74.4 | 0.03 | -4.1 | 0.02 | 14.1 |
| 7PYD   | 2.21     | 22.47/28.71      | 7.88 × 10⁻⁵ chloride, NA | 2.0 | 2.3 | 2.0 | 2.5 | 2.3 | - | 96.4, 94.1, 161.1 | 15.6 | 0.08 | 71.0 | 0.04 | -12.6 | 0.02 | 16.1 |

**Notes:**
- HN (Å) and RMSD values are provided for chloride, water, and other ligands as indicated.
- Cu-site parameters for LsAA9_A(f) are given for different solvents and ligands.
- Residuals and R-values are reported for each structure.
- Significant data points are marked with asterisks (*) for clarity.
- Specific crystallographic data for each PDB entry are detailed in the table.
| Code  | a  | b   | c  | d   | e  | f  | g  | h  | i  | j  | k  | l  | m  |
|-------|----|-----|----|-----|----|----|----|----|----|----|----|----|----|
| 7PYE  | 2.10 | 23.94/29.42 | 5.99 × 10^4 chloride, NA | 1.9 | 2.2 | 2.0 | 2.5 | 2.9 | - | 95.6, 100.2, 154.1 | 20.4 | 0.05 | 64.3 | 0.03 | -18.1 | 0.02 | 17.5 |
| 7PYF  | 1.90 | 25.40/28.68 | 1.39 × 10^4 chloride, NA | 2.0 | 2.1 | 2.0 | 2.5 | 3.7 | - | 90.8, 102.0, 152.9 | 23.5 | 0.07 | 55.3 | 0.03 | -20.8 | 0.02 | 7.1  |
| 7PYG  | 1.90 | 24.29/29.29 | 3.60 × 10^4 chloride, NA | 2.0 | 2.1 | 2.0 | 2.7 | 3.8 | - | 92.9, 103.7, 153.7 | 20.0 | 0.05 | 60.1 | 0.03 | -20.8 | 0.02 | 13.6 |
| 7PYH  | 1.90 | 26.15/29.00 | 1.45 × 10^4 chloride, NA | 1.9 | 2.3 | 2.0 | 2.6 | 4.0 | - | 94.8, 107.2, 150.9 | 18.4 | 0.16 | 57.7 | 0.03 | -20.7 | 0.02 | 17.9 |
| 7PYI  | 2.05 | 21.80/26.00 | 6.65 × 10^4 chloride, NA | 1.9 | 2.5 | 2.0 | 2.7 | 3.8 | - | 98.1, 110.1, 147.9 | 14.5 | 0.27 | 55.4 | 0.04 | -21.7 | 0.02 | 24.1 |
| LsAA9_A (Ec)-Cell3 | 7PYJ  | 1.90 | 14.19/16.22 | 1.49 × 10^4 chloride, NA | 2.0 | 2.1 | 2.0 | 2.6 | 2.3 | - | 91.3, 91.7, 169.2 | 10.4 | 0.14 | 67.7 | 0.02 | -6.2  | 0.03 | 4.9  |
| 7PYW  | 1.40 | 13.70/15.34 | 5.62 × 10^4 chloride, NA | 2.0 | 2.2 | 2.0 | 2.6 | 2.4 | - | 92.3, 93.6, 169.2 | 9.0  | 0.09 | 66.4 | 0.03 | -8.3  | 0.03 | 8.8  |
| 7PYX  | 1.60 | 12.59/14.62 | 2.74 × 10^4 chloride, NA | 1.9 | 2.2 | 2.0 | 2.7 | 2.7 | - | 92.6, 96.8, 167.4 | 8.3  | 0.03 | 65.3 | 0.02 | -8.9  | 0.03 | 10.6 |
| 7PYY  | 1.20 | 14.44/15.78 | 5.05 × 10^4 chloride, NA | 1.9 | 2.3 | 2.0 | 2.7 | 2.8 | - | 92.5, 97.2, 167.2 | 8.3  | 0.01 | 64.7 | 0.03 | -10.0 | 0.03 | 11.2 |
| 7PYZ  | 1.60 | 12.30/14.38 | 2.97 × 10^4 chloride, NA | 1.9 | 2.3 | 2.0 | 2.7 | 3.6 | - | 92.8, 98.8, 165.8 | 8.1  | 0.02 | 64.2 | 0.03 | -9.1  | 0.02 | 11.0 |
| 7PZ0  | 1.20 | 13.40/14.75 | 9.81 × 10^4 chloride, NA | 1.9 | 2.3 | 1.9 | 2.7 | 3.8 | - | 93.0, 98.8, 165.7 | 8.2  | 0.03 | 63.4 | 0.03 | -11.5 | 0.03 | 10.0 |
| LsAA9_A (Ec)-Cell4 | 7PXW  | 1.40 | 11.71/15.98 | 2.14 × 10^4 chloride, NA | 2.0 | 2.3 | 2.0 | 2.7 | 3.9 | - | 92.4, 99.5, 165.3 | 8.5  | 0.04 | 62.3 | 0.02 | -10.4 | 0.02 | 8.0  |
### Table S8  Structural parameters of the LPMO Cu-site.

| PDB ID | Dose [Gy] | Eq-Cu-Ax(°) | Nax-Cu-Eq(°) | Nat-Cu-Ax(°) | Nal-Cu-Eq(°) | Nal-Cu-Ax(°) | Nax-Cu-Eq(°) | Oxy-Cu-Eq(°) | Oxy-Cu-Ax(°) | Nam-Cu-Oxy(°) | Nat-Cu-Oxy(°) | Nax-Cu-Oxy(°) |
|--------|-----------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|---------------|---------------|
| **LsAA9_A (f)** | | | | | | | | | | | | | |
| 7PXL  | 7.88 × 10³ | 94.26 | 169.77 | 95.08 | 91.49 | 88.62 | 86.27 | 89.64 | 89.23 | 174.91 | 81.73 | 87.58 | 94.29 |
| 7PXJ  | 5.99 × 10³ | 98.06 | 168.28 | 93.64 | 86.62 | 85.28 | 88.01 | 91.74 | 87.74 | 170.37 | 80.77 | 87.19 | 96.16 |
| 7PXK  | 1.39 × 10³ | 96.19 | 169.26 | 94.46 | 90.22 | 85.45 | 83.2 | 91.74 | 90.52 | 170.02 | 79.1 | 87.14 | 96.4 |
| 7PXL  | 3.59 × 10³ | 95.19 | 170.35 | 94.45 | 89.44 | 83.56 | 82.57 | 91.58 | 91.69 | 168.48 | 78.86 | 87.3 | 98.47 |
| 7PXN  | 1.45 × 10³ | 97.41 | 169.6 | 92.98 | 87.56 | 83.7 | 83.24 | 92.69 | 90.74 | 167.16 | 78.97 | 86.76 | 98.13 |
| **LsAA9_A (Ec)** | | | | | | | | | | | | | |
| 7PYL  | 1.49 × 10³ | 93.84 | 170.53 | 95.55 | 87.7 | 85.83 | 86.28 | 90.22 | 85.92 | 174.69 | 84.62 | 88.86 | 95.06 |
| 7PYM  | 5.61 × 10³ | 96.92 | 163.54 | 99.47 | 88.08 | 81.07 | 85.04 | 90.75 | 79.75 | 171.55 | 83.8 | 91.02 | 96.66 |
| 7PYN  | 2.31 × 10³ | 107.18 | 166.18 | 86.5 | 86.74 | 85.52 | 84.05 | 91.13 | 84.77 | 166.17 | 81.41 | 88.33 | 97.13 |
| 7PYO  | 3.33 × 10³ | NA | NA | 96.72 | NA | 81.74 | NA | 92.72 | NA | 168.84 | 78.44 | 88.37 | 92.72 |
| 7YPY  | 2.13 × 10³ | NA | NA | 96.39 | NA | 81.28 | NA | 92.94 | NA | 169.69 | 78.33 | 87.82 | 98.88 |
| 7PYQ  | 6.35 × 10³ | NA | NA | 89.43 | NA | 78.47 | NA | 93.66 | NA | 162.71 | 79.47 | 88.9 | 101.67 |
| **LsAA9_A (Ascorbic acid)** | | | | | | | | | | | | | |
| 7PXU  | 2.08 × 10³ | NA | NA | 89.0 | NA | 81.3 | NA | 95.4 | NA | 161.0 | 78.6 | 85.3 | 100.2 |
| 7PXV  | 1.70 × 10³ | NA | NA | 89.0 | NA | 82.9 | NA | 92.9 | NA | 163.9 | 79.5 | 86.6 | 99.6 |
| **LsAA9_A (SSX)** | | | | | | | | | | | | | |
| 7PXU  | 2.08 × 10³ | NA | NA | 89.0 | NA | 82.9 | NA | 92.9 | NA | 163.9 | 79.5 | 86.6 | 99.6 |
| **LsAA9_A (RT)** | | | | | | | | | | | | | |
| 7PXZ  | 1.91 × 10³ | 94.91 | 170.19 | 94.85 | 89.00 | 85.67 | 84.66 | 87.82 | 88.86 | 172.98 | 81.54 | 88.48 | 98.44 |
| **TaAA9_A** | | | | | | | | | | | | | |
| 7PZ3  | 5.37 × 10³ | 97.91 | 165.0 | 96.53 | 89.36 | 93 | 85.2 | 87.4 | 82.53 | 179 | 83.1 | 86.1 | 93.54 |
| 7PZ4  | 2.07 × 10³ | 102.12 | 166.92 | 89.35 | 91.86 | 90.24 | 84.2 | 91.29 | 81.66 | 173.17 | 87.59 | 83.92 | 94.77 |
| 7PZ5  | 9.56 × 10³ | 108.42 | 159.05 | 92.2 | 84.37 | 85.77 | 88.56 | 94.16 | 77.22 | 171.13 | 81.87 | 88.03 | 92.8 |
| 7PZ6  | 2.22 × 10³ | 103.4 | 165.93 | 90.17 | 89.01 | 90.19 | 83.67 | 89.32 | 84.36 | 171.01 | 82.44 | 85.36 | 96.12 |
| 7PZ7  | 1.13 × 10³ | 104.62 | 168.2 | 87.18 | 86.71 | 86.7 | 83.9 | 93.23 | 86.08 | 167.63 | 82.25 | 87.76 | 94.09 |
| 7PZ8  | 3.12 × 10³ | 105.57 | 168.13 | 85.93 | 89.05 | 86.97 | 80.67 | 92.78 | 86.03 | 167.08 | 82.84 | 87.57 | 94.75 |
| **LsAA9-A-Cells** | | | | | | | | | | | | | |
| 7PYD  | 7.88 × 10³ | NA | 169.61 | NA | 88.7 | NA | 78.68 | NA | 99.51 | NA | 89.17 | 94.44 | 101.47 |
| 7PYE  | 5.99 × 10³ | NA | 175.32 | NA | 86.77 | NA | 76.23 | NA | 98 | NA | 85.95 | 93.05 | 108.44 |
| 7PYF  | 1.39 × 10³ | NA | 171.78 | NA | 97.1 | NA | 69.85 | NA | 96.04 | NA | 85.35 | 94.72 | 109.92 |
| 7PYG  | 3.60 × 10³ | NA | 170.28 | NA | 96.45 | NA | 66.6 | NA | 97.84 | NA | 84.02 | 94.63 | 107.18 |
| Compound | 7PYH | 7PYI | 7PYU | 7PYW | 7PYX | 7PYY | 7PYZ | 7PXW | 7PXW |
|----------|------|------|------|------|------|------|------|------|------|
|          | 1.45 × 10^6 | 6.65 × 10^6 | 1.49 × 10^4 | 5.62 × 10^4 | 2.74 × 10^4 | 5.05 × 10^4 | 2.97 × 10^4 | 9.81 × 10^6 | 2.14 × 10^6 |
| LsAA9_A (Ec)-Cell3 | NA | NA | 177.99 | 178.81 | 175.13 | 176.29 | 174.62 | 175.24 | 171.42 |
| LsAA9_A (Ec)-Cell4 | NA | NA | NA | 86.44 | 86.02 | 81.84 | 78.5 | 89.21 | 88.07 |
|          | NA | NA | NA | 90.63 | 88.26 | 88.99 | 90.61 | NA | NA |
|          | NA | NA | NA | 96.55 | NA | NA | NA | NA | NA |
|          | NA | NA | NA | 82.9 | NA | NA | NA | NA | NA |
|          | NA | NA | NA | 93.66 | NA | NA | NA | NA | NA |
| NA:      | Not applicable, as one atom is not considered coordinating to Cu. |
Table S9  Structural parameters of the LPMO Cu-site.

| PDB   | Tyr164-OH (σ) | Cu (σ) | O-Cu (Å) | Bond length error | θT (°) |
|-------|---------------|--------|----------|-------------------|--------|
| LsAA9_A(f) low dose | 5ACG | 0.092 | 0.109 | 2.72 | 0.143 | 2.3 |
| LsAA9_A(f)-Cell: low dose | 5ACF | 0.092 | 0.105 | 2.47 | 0.140 | 10.10 |
| LsAA9_A(f) low dose | 7PXL | 0.065 | 0.068 | 2.74 | 0.094 | 1.5 |
| LsAA9_A(f)-Cell: low dose | 7PYD | 0.166 | 0.221 | 2.48 | 0.276 | 15.6 |
| LsAA9_A(Ec) low dose | 7PYL | 0.072 | 0.082 | 2.68 | 0.109 | 3.3 |
| LsAA9_A(Ec)-Cell: low dose | 7PYUL | 0.038 | 0.037 | 2.55 | 0.053 | 10.4 |
| Average (low dose, no substrate) | | | | 2.71 ± 0.03 | 2.37 ± 0.9 |
| Average (low dose, bound substrate) | | | | 2.50 ± 0.04 | 12.03 ± 3.1 |

§: Published previously (Frandsen et al., 2016)
σ: Single atom diffraction precision index (Kumar et al., 2015).
Bond length error (according to eq 4 in Gurusaran et al., 2014).
References

Frandsen, K. E. H., Simmons, T. J., Dupree, P., Poulsen, J. N., Hemsworth, G. R., Ciano, L., Johnston, E. M., Tovborg, M., Johansen, K. S., von Freiesleben, P., Marmuse, L., Fort, S., Cottaz, S., Driguez, H., Henrissat, B., Lenfant, N., Tuna, F., Baldansuren, A., Davies, G. J., Lo Leggio, L. & Walton, P. H. (2016). Nat. Chem. Biol. 12, 298–303.

Gurusaran, M., Shankar, M., Nagarajan, R., Helliwell, J. R. & Sekar, K. (2014). IUCrJ 1, 74–81.

Kumar, K. S. D., Gurusaran, M., Satheesh, S. N., Radha, P., Pavithra, S., Thulaa Tharshan, K. P. S., Helliwell, J. R. & Sekar, K. (2015). J. Appl. Crystallogr. 48, 939–942.

Lovell, S. C., Davis, I. W., Arendall, W. B., de Bakker, P. I. W., Word, J. M., Prisant, M. G., Richardson, J. S. & Richardson, D. C. (2003). Proteins Struct. Funct. Bioinforma. 50, 437–450.

Tandrup, T., Tryfona, T., Frandsen, K. E. H., Johansen, K. S., Dupree, P. & Lo Leggio, L. (2020). Biochemistry 59, 3347–3358.

Zeldin, O. B., Gerstel, M. & Garman, E. F. (2013). J. Appl. Crystallogr. 46, 1225–1230.