Activity, stability and 3-D structure of the Cu(II) form of a chitin-active lytic polysaccharide monooxygenase from *Bacillus amyloliquefaciens*

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Supporting Information

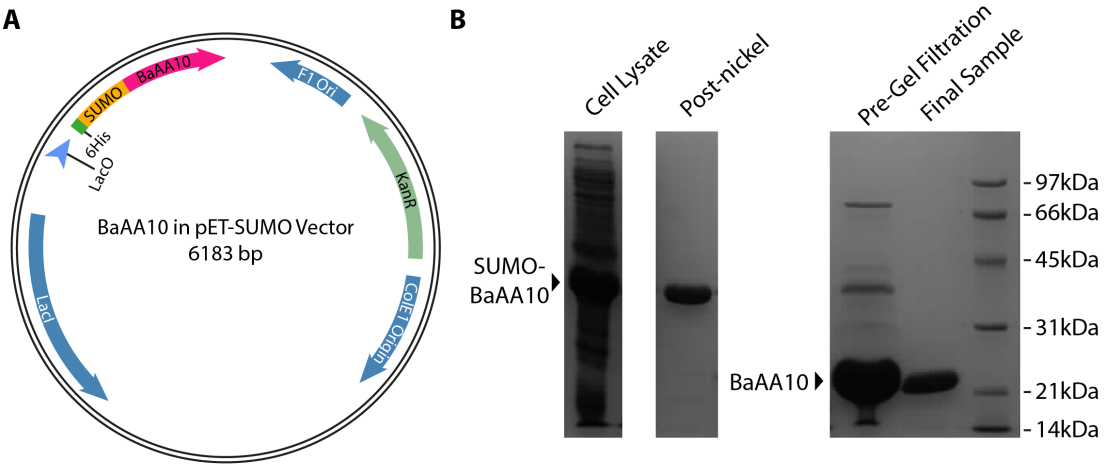

Figure S1. (A) representation of the Champion-pETSUMO(*Ba*AA10) vector. The plasmid encodes the *Ba*AA10 protein (pink arrow) with a SUMO-tag (orange region) attached at its N-terminus. **(B)** SDS-PAGE gel illustrating the purification of *Ba*AA10 from cell lysis to the final pure sample with SUMO tag removed. The expected molecular weight for *Ba*AA10 is 19.2 kDa and it must be noted that LPMO proteins often run at a molecular weight slightly larger than their estimated values on SDS-PAGE gels. (This image derives from a single gel but lanes have been removed for clarity).

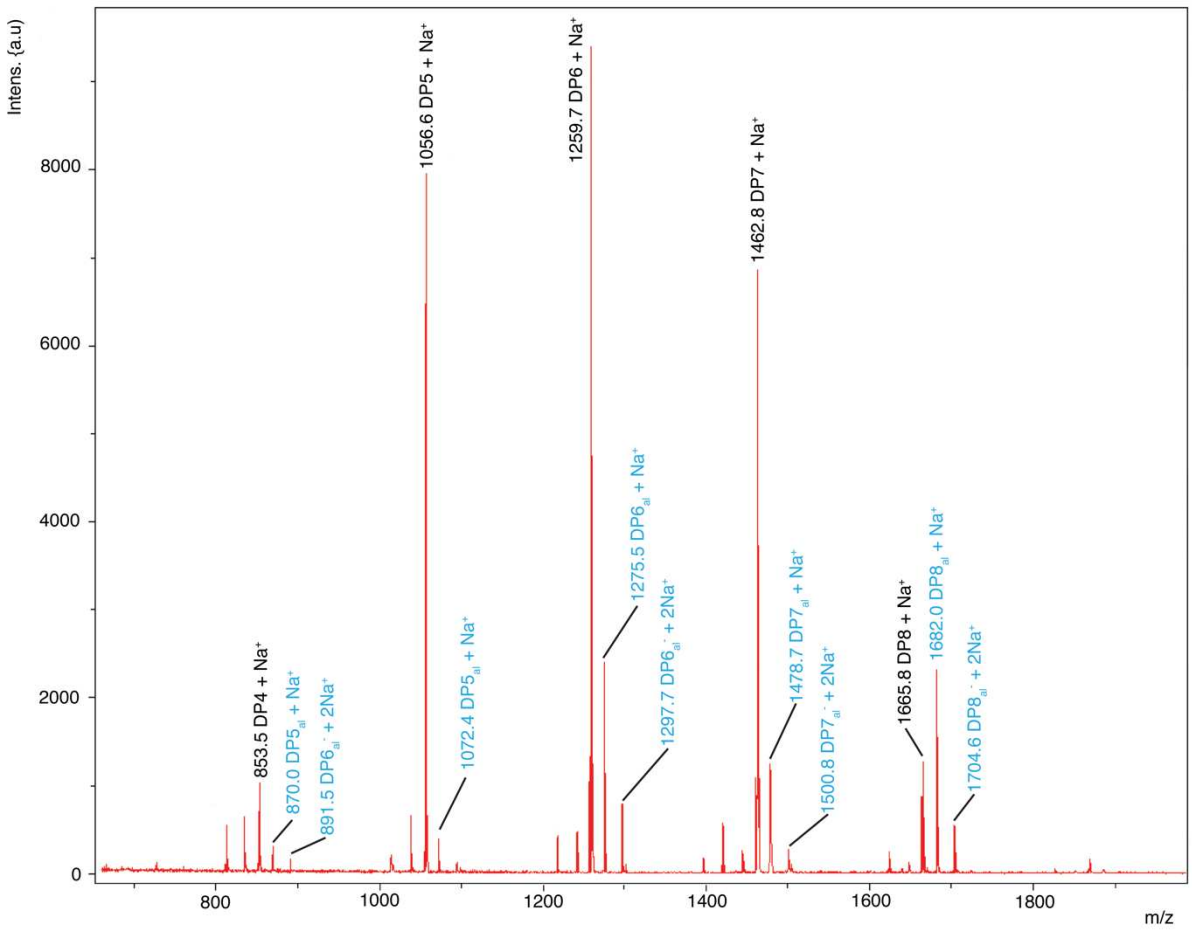

Figure S2. MALDI-TOF Mass Spectrum of the breakdown of alpha chitin from crab shells. The aldonic acid products resulting from oxidative breakdown by *Ba*AA10 can be seen labelled with blue labels (DPn_{al}), however these are overshadowed by the presence of chitooligosaccharides indicated by black labels (DPn), which may already be present in the crab chitin sample.

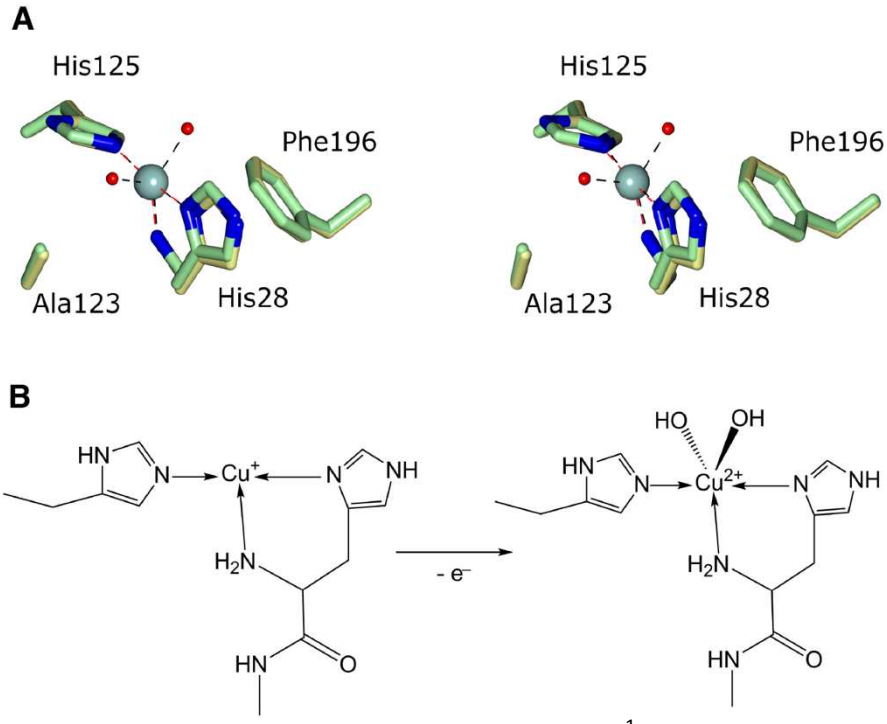

Figure S3. (A) Structure comparison of Cu(I)-*Ba*AA10 (green)[1] with that of the obtained Cu(II)-*Ba*AA10 active site (yellow, with red oxygen atoms). **(B)** Schematic diagram of the difference in geometries between that of the T-shaped Cu^{+} geometry, and the distorted trigonal bipyramidal Cu^{2+} geometry.

Table S1. *Ba*AA10 Crystallization

Method	Microseeding
Plate type	SWISSCI MRC 96-well Crystallisation Plate
Temperature (K)	19.5 °C
Protein concentration	4.7 mg/ml
Buffer composition of protein solution	20 mM NaOAc pH 5.0; 250 mM NaCl
Composition of reservoir solution	0.1 M NaOAc pH 5.0; 20 % PEG-6000; 0.2 M $CaCl_2$
Volume and ratio of drop	0.25 : 0.30 : 0.05 µl (Reservoir solution : protein : seed bead stock solution)
Volume of reservoir	54 µl

Supporting Information References

1. G. R. Hemsworth, E. J. Taylor, R. Q. Kim, R. C. Gregory, S. J. Lewis, J. P. Turkenburg, A. Parkin, G. J. Davies and P. H. Walton, *Journal of the American Chemical Society*, 2013, **135**, 6069-6077.

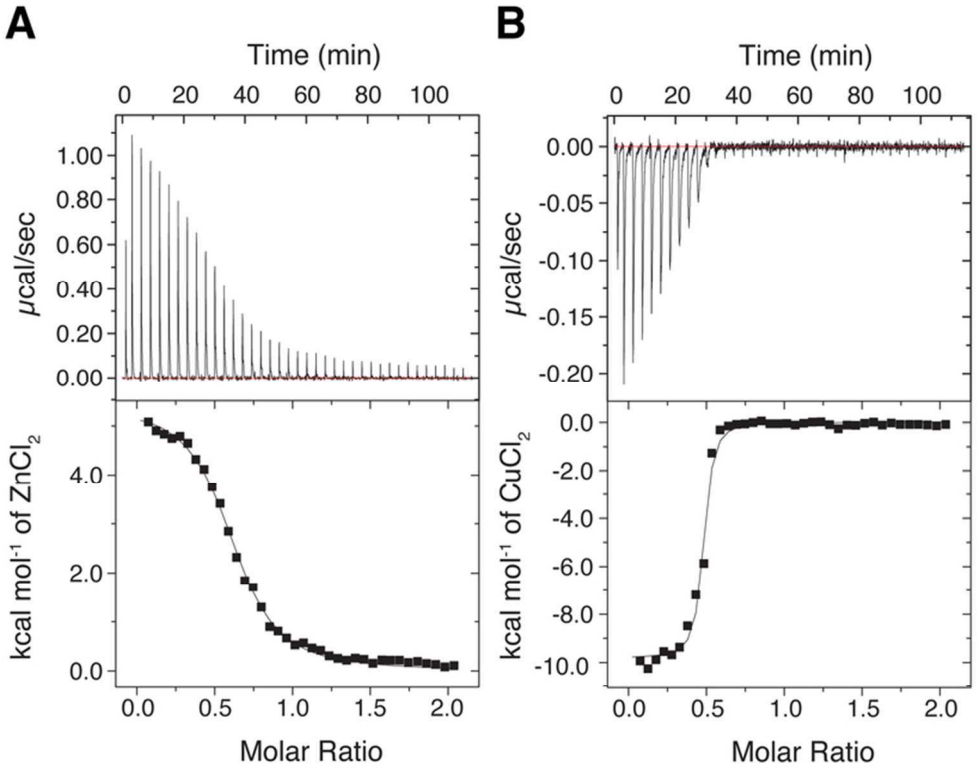

Figure 2. (A) Isothermal titration calorimetry (ITC) plot of Zn2+ binding to *Ba*AA10 at pH 5, to obtain parameters to be used for the Cu^{2+} displacement reaction. The concentration of the protein (*Ba*AA10) inside the cell was 200 µM and the concentration of $ZnCl_2$ was 2 mM. **(B)** The Cu^{2+} displacement result carried out at pH5, whereby the concentration of Cu(II)Cl_2 titrated into the cell was 500 µM.‼ ┼

Figure 2
70x57mm (300 x 300 DPI)

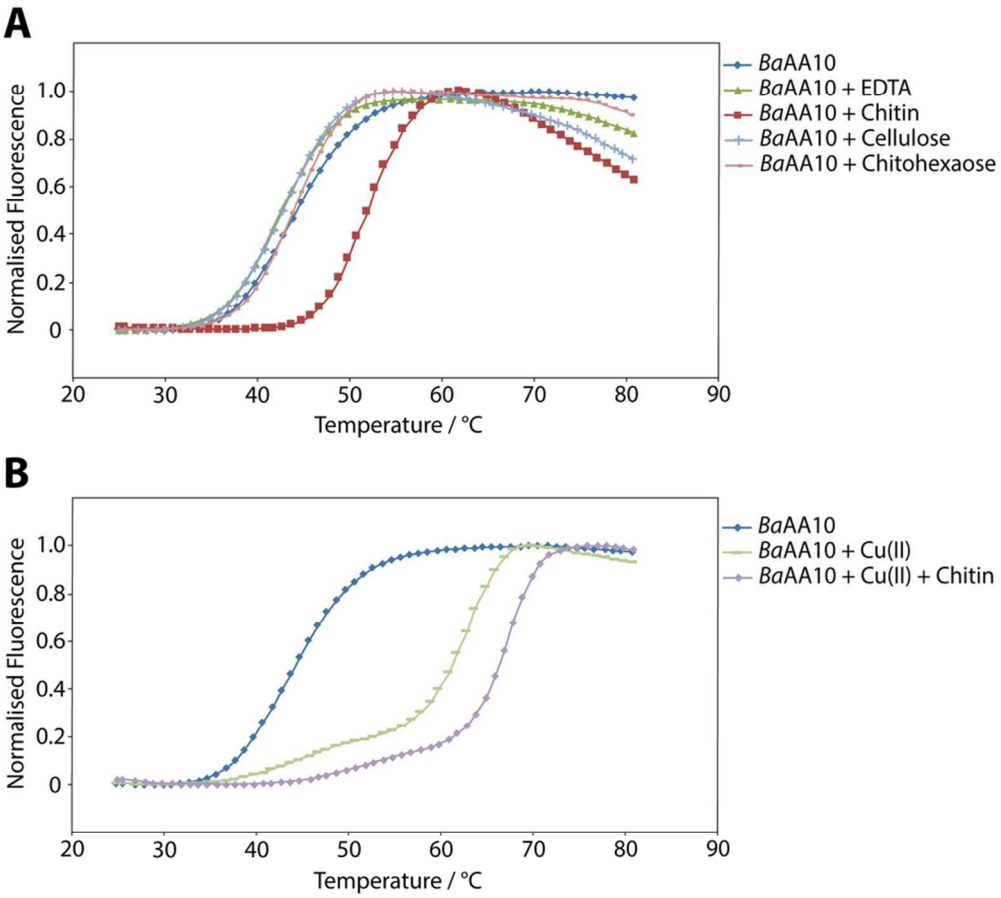

Figure 3. Denaturation curves displaying the normalised fluorescence against temperature for: **(A)** *Ba*AA10 with a variety of ligands including EDTA, chitin, cellulose and chitohexaose, and **(B)** *Ba*AA10 with copper and chitin as the substrate. The corresponding melting temperatures (T_m) determined in analysis were: *Ba*AA10 = 43.6 ºC, *Ba*AA10 + EDTA = 41.8 ºC, *Ba*AA10 + Chitin = 51.9 ºC, *Ba*AA10 + Cellulose = 42.9 ºC, and *Ba*AA10 + Chitohexaose = 44.2 ºC, *Ba*AA10 + Cu^{2+} = 65.2 ºC, *Ba*AA10 + Cu^{2+} + Chitin = 68.7 ºC. Following the initial melting of the protein the fluorescence is observed to decrease again at higher temperatures. This is likely the result of protein aggregation following protein unfolding. As the exposed hydrophobic residues come together during aggregation, the SYPROorange dye is displaced resulting in a loss of fluorescence.

Figure 3
83x79mm (300 x 300 DPI)

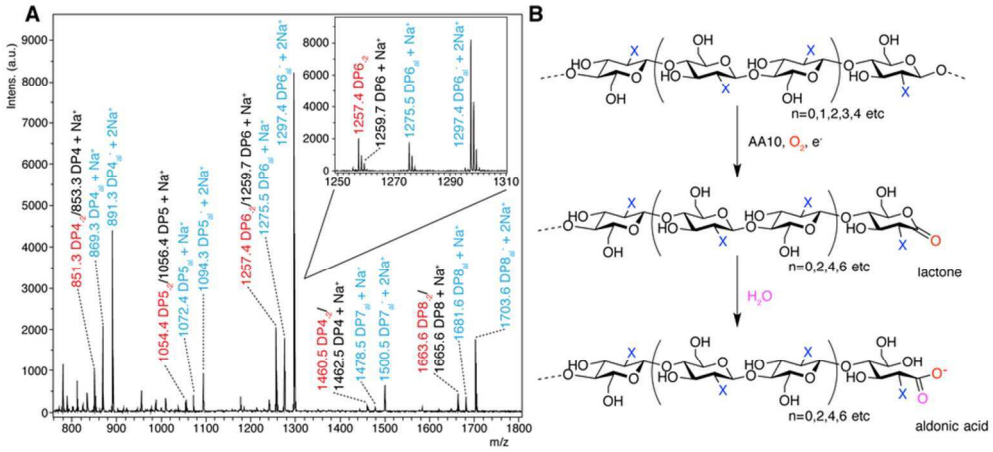

Figure 4. (A) MALDI-TOF mass spectrometry analysis of the action of *Ba*AA10 on β-chitin from squid pen. The spectrum shows varying degrees of polymerisation detected as products, with DPn indicating native oligosaccharides coloured black, DPn_{-2} in red indicating the lactone, and DPn_{al} showing aldonic acids coloured blue. A closer look at the individual ion peaks for the DP6 peaks are shown inlaid. **(B)** Schematic diagram showing the AA10 catalysed C1 oxidation of oligosaccharides (X=NHAc, for chitooligosaccharides) to yield lactones and their ring-opened aldonic acids, following the action of an AA10 enzyme in the presence of oxygen and a source of electrons for the reduction of the AA10. n denotes the most common number of repeating units (bound by parentheses) observed for AA10 LPMO reaction products.

Figure 4
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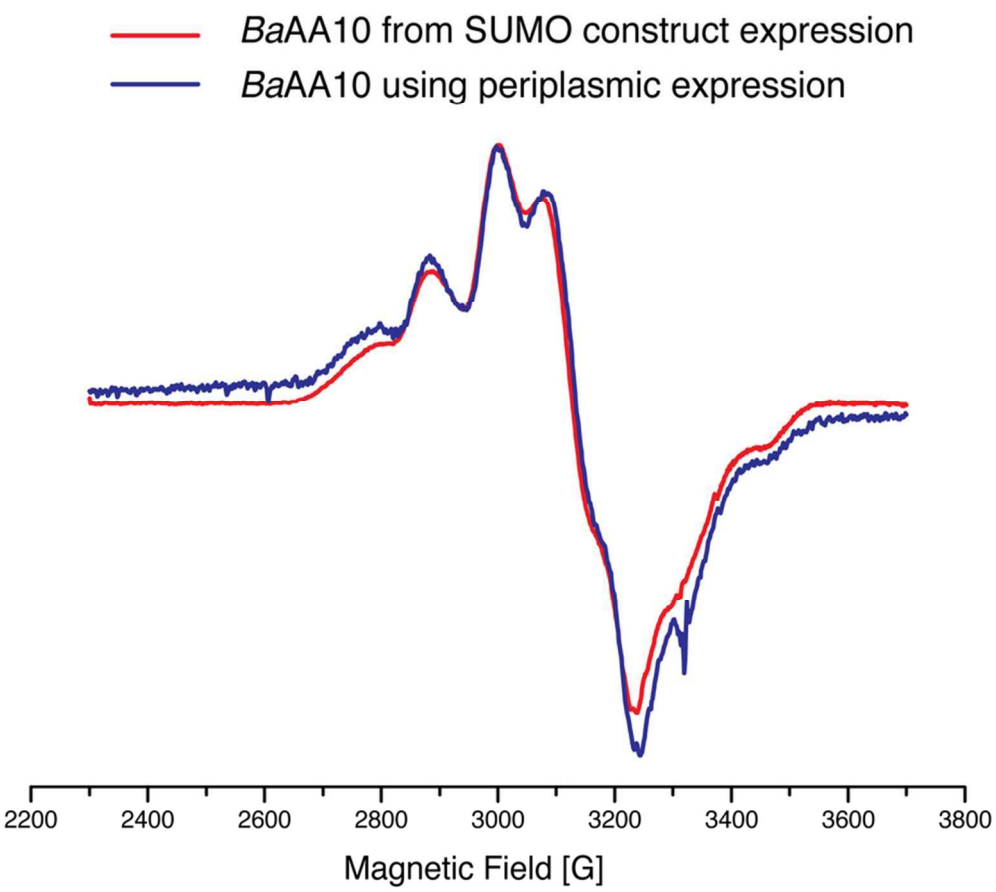

Figure 5. X-band EPR spectra of the original Cu-BaAA10 protein prepared via periplasmic secretion (9 GHz, 155 K, coloured blue)[32], and Cu-*Ba*AA10 produced using a SUMO tag (9 GHz, 150 K, coloured red). The spectra clearly overlay showing the distorted axial coordination geometry is maintained in the SUMO protein purification method described here.

Figure 5
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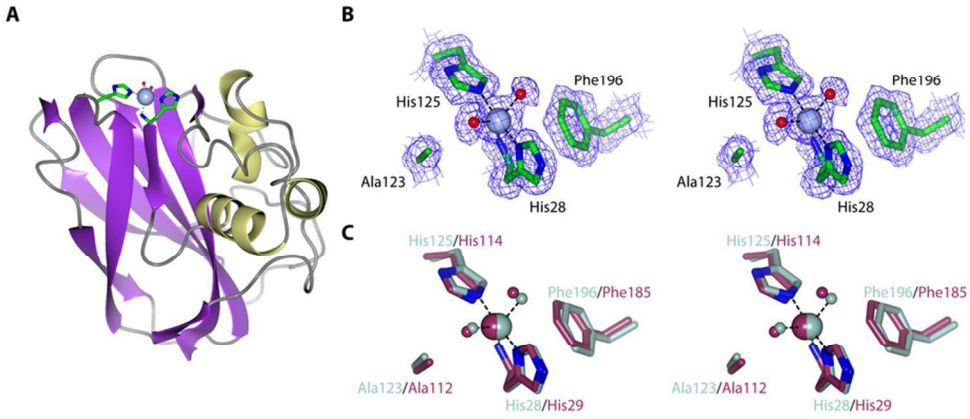

Figure 6. (A) Structural representation of *Ba*AA10, clearly portraying the Cu(II) active site with the familiar 'histidine-brace' and two water molecules. **(B)** Stereo structure of Cu-*Ba*AA10 displaying the $2F_{obs}$-F_{calc} density map at 0.48 e/A^3 (1 σ) contour level, showing the two water molecules coordinated to the copper atom. (C) Stereo structure comparison of the AA10 site from *Bacillus amyloliquefaciens* (blue/grey) with that from *Enterococcus faecalis* (purple) [26].

Figure 6
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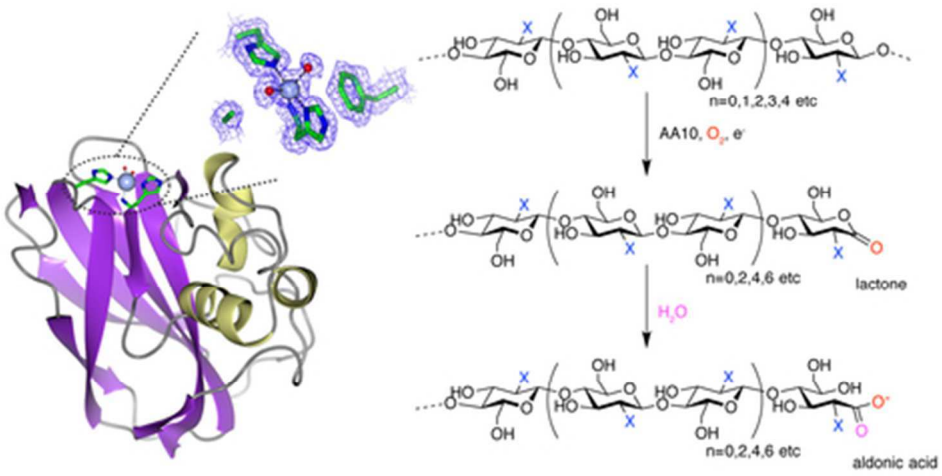

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