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Copper binding and reactivity in bacterial lytic polysaccharide monooxygenases: effects of first and second coordination sphere residues

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Fungal and bacterial lytic polysaccharide monooxygenases (LPMOs) are a recently discovered class of mononuclear copper monooxygenases. LPMOs participate in the degradation of recalcitrant polysaccharides such as cellulose or chitin in synergy with hydrolytic carbohydrate-active enzymes. From a molecular point of view, LPMOs catalyze the oxidative cleavage of glycosidic bonds using dioxygen or hydrogen peroxide as co-substrate and an electron donor (Figure 1b) (lactone and ketoaldose products are in equilibrium with the corresponding aldonic acid and *gem*-diol, respectively (not shown)).[1,2] The topology of their solvent-exposed active site is unique among copper-containing oxygenases. Their mononuclear copper(II) ion is coordinated by both the side chain nitrogen and the main-chain amine of the *N*-terminal histidine, and by another histidine side chain (Figure 1a).[1] This coordination motif is known as "histidine brace" and is relatively rare in biology. In bacterial LPMOs, a conserved second coordination sphere alanine residue was proposed to control the access of the co-substrate (dioxygen of hydrogen peroxide) to the axial coordination on the copper ion.[3] Nevertheless, it was found to be replaced by bulkier amino acid in some bacterial LPMO sequences.[4] Both first- and second coordination sphere residues appear to control and modulate the reactivity of the copper ion in bacterial LPMOs. We are currently investigating first- and second-coordination sphere mutants of the well-studied chitin-active LPMO *Sm*AA10 (from *Serratia marcescens*) and other bacterial LPMOs without the conserved alanine residue using a combination of biochemical, biophysical, and structural techniques. Efforts to decipher the structure-reactivity relationships of the copper-"histidine brace" motif will be discussed.

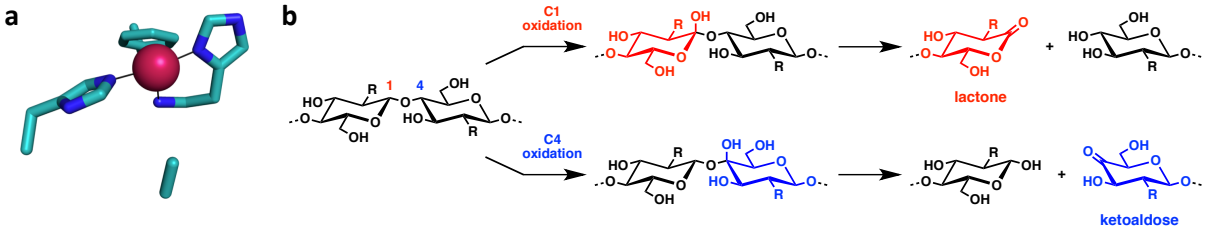

Figure 1. a) Copper-"histidine brace" coordination motif at the active site of a chitin-active bacterial LPMO and **b)** reaction catalyzed by LPMOs (cellulose: R = OH, chitin: R = NHAc)

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