Activated dioxygen intermediates at the copper-active site of a lytic polysaccharide monooxygenase

Flora Meilleur[1], Cathleen Castello[2]

[1]NA [2]Simons Electron Microscopy Center

meilleurf@ornl.gov

Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that oxidize the glycosidic bond of polysaccharides. Since their discovery in 2010 LPMOs have been intensely studied by structural, spectroscopic, and biochemical methods however their catalytic mechanism remains to be established. Experimental data to unambiguously identify the activated copper-oxygen intermediates and their mechanism of formation are especially required to settle the early steps. We will present how we used neutron crystallography to investigate the initial steps of oxygen activation directly following active site copper reduction in Neurospora crassa LPMO9D. Cryo neutron crystallography allowed us to capture an activated dioxygen intermediate, identify its chemical nature, and reveal a conserved second coordination shell residue as a proton donor essential in intermediate formation. The chemical insights from the neutron crystal structure were supported by density functional theory (DFT) calculations and by mining minima free energy calculations.