COUPLED SECOND SPHERE RESIDUES BOOST H_2O_2 PRODUCTION IN LYTIC POLYSACCHARIDE MONOOXYGENASES: A COMPUTATIONAL STUDY IN AA11 LPMO

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Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that catalyze oxidative degradation of recalcitrant polysaccharides and are central to sustainable biofuel production. LPMOs utilize O_2 or H_2O_2 as co-substrate, with experimental evidence identifying H_2O_2 as the preferred oxidant. Under typical experimental conditions, H_2O_2 is generated *in situ* via LPMO oxidase activity, which may constitute the rate-limiting step of LPMO function. Across LPMO families AA9, AA10, and AA11, elevated H_2O_2 production rates show consistent correlation with the presence of a Glu–Tyr motif in the secondary coordination sphere, suggesting a mechanistic role, though the origin and impact of the interplay between these two residues remains unexplored.

Here, we rationalize the structural basis for high H_2O_2 production by an AA11 LPMO with a unique Glu-Tyr pair in the secondary coordination sphere, using QM/MM calculations. We systematically evaluate multiple pathways for proton and electron transfer, focusing on the roles of key secondary sphere residues (Glu-Tyr) and the water molecule coordinated to the copper center together with the dioxygen species. Our analysis highlights the previously underappreciated relative significance of the Cu(I) oxidation by O_2 versus subsequent proton transfer steps. We further assess the potential dissociation of all reaction intermediates from the Cu center (*e.g.,* superoxo, hydroperoxo) using thermodynamics by fully accounting for the reorganization of the first solvation shell of all species, offering a more accurate picture of the dissociation energetics. Additionally, we apply Local Energy Decomposition (LED) analysis to dissect non-covalent interactions, revealing the specific contributions of individual amino acid residues (both stabilizing and destabilizing) throughout the catalytic cycle. Collectively, our findings provide a deeper mechanistic understanding of LPMO oxidase activity, focusing on the involvement of the secondary sphere residues. These findings offer valuable design principles for engineering improved LPMOs and bio-inspired catalysts, emphasizing the strategic integration of secondary sphere residues and dynamic solvation effects.