Active-Site Modeling and Molecular Dynamics Characterization of CelOCE, a Copper Polysaccharide-Degradating Enzyme

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Abstract: The degradation of complex polysaccharides such as cellulose and hemicelluloses is a key biological and biotechnological process, enabling the release of fermentable sugars and the valorization of biomass into value-added chemicals. Among the enzymes involved, several metalloenzymes (particularly copper-containing ones) play crucial roles by catalyzing oxidative or redox-driven cleavage of recalcitrant glycosidic bonds with high efficiency [1]. In this regard, CelOCE is a newly discovered copper-dependent oxidative enzyme that performs highly specific C1 oxidation of cellulose chain ends [2]. Its active site contains a monocopper center coordinated by H44, H46, H84, and Q50, along with additional water molecules observed in the crystal structure. Together with the rigid PXHXHP loop and the aromatic residue F33, this copper environment shapes CelOCE's distinctive pocket-like architecture and exo selectivity.
To investigate how copper coordination influences the structural pre-organization of the active site, we performed two all-atom Molecular Dynamics (MD) simulations of the apo enzyme, parameterizing the copper center using *Metal Center Parameter Builder* (MCPB.py). One simulation retained the crystallographic water, generating a penta-coordinated Cu(II) environment, while the second model omitted this ligand, yielding a tetra-coordinated state.
Both systems were analyzed using GROMACS tools to assess backbone stability, local flexibility around the metal site, solvation patterns, and the conformational behaviour of functionally relevant residues such as the PXHXHP loop and F33.
These simulations provide a basis for understanding the structural logic of CelOCE and lay the groundwork for future studies, including substrate-bound Molecular Dynamics, mutational analyses, and free-energy evaluations aimed at elucidating the enzyme's mechanism.

[1] R. J. Quinlan, M. D. Sweeney, L. Lo Leggio, H. Otten, J-C. N. Poulsen, K. Salomon Johansen, K. B. R. M. Krogh, C. I. Jørgensen, M. Tovborg, A. Anthonsen, T. Tryfona, C. P. Walter, P. Dupree, F. Xu, G. J. Davies, P. H. Walton, *PNAS*, **2011**, 108 (37) 15079-15084

[2] C.A, Santos, M. A. B. Morais, F. Mandelli, E. A. Lima, R. Y. Miyamoto, P. M. R. Higasi, et al. *Nature*, **2025**, *639*, 1076–1083