Natural variation in the 'control loop' of BVMO BVMO$_{AFL210}$ and itsinfluence on regioselectivity and sulfoxidation

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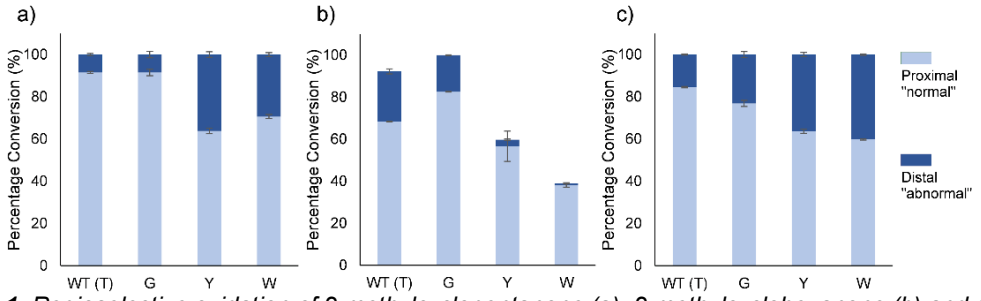

Fig. 1. *Regioselective oxidation of 2-methylcyclopentanone (a), 2-methylcyclohexanone (b) and cis-bicyclo[3.2.0]hept-2-en-6-one (c) by BVMO$_{AFL210}$ wildtype (T) and T513G, Y and W mutants.*

Baeyer-Villiger monooxygenases (BVMOs) are flavoenzymes that use NADPH and molecular oxygen to convert ketones and cyclic ketones to esters and lactones, respectively, as well as catalyse sulfoxidations. The structures of Type I BVMOs have been extensively studied, indicating that these flexible enzymes undergo large domain movements during catalysis [2,3]. Due to this malleability, BVMOs often accept an extensive range of substrates. In the active site, structure transitions of the 'control loop' play a crucial role in the positioning of NADP(H) and substrate during catalysis [4]. The control loop of BVMOs usually contains a conserved tryptophan residue that interacts with NADP(H); however, BVMO$_{AFL210}$ from *Aspergillus flavus* contains a threonine (T513) in this position. Previous studies have indicated that mutating thetryptophan decreases activity and peroxyflavin stability significantly [1,5]. Despite this, BVMO$_{AFL210}$ is highly active and converts a wide range of substrates.

Here we present the crystal structure of BVMO$_{AFL210}$ bound to $NADP^+$ in the 'open' and 'closed' conformations, revealing that T513 does not interact with $NADP^+$. To further probe this, we used site-directed mutagenesis to create T513W, Y and G mutants and performed whole-cell biotransformations with a panel of substrates. The substrate scope was not significantly alteredby any of the mutants; however, the sulfoxidation and regioselectivity of the enzymes were modified in a substrate-specific manner.

As expression levels varied, the mutants were purified, and the turnover frequencies (TOFs) were determined. A general decrease in activity was observed for the mutants in the order of wildtype (T) > G > Y > W, which was more pronounced in the conversion of cyclic and aromatic ketones. In addition, the bulkier substitutions, Y and W, showed a large decrease in peroxyflavin stability. Thus, both the activity and regioselectivity do not only depend on the amino acid at this position but also the substrate evaluated, opening the door to new possibilities of directed-evolution studies for improved activity and/or regioselectivity.

References

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