Structural Insights into Catalytic Versatility of the Flavin-dependent Hydroxylase (HpaB) from *Escherichia coli*

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The enzyme 4-Hydroxyphenylacetate 3-hydroxylase (EcHpaB) from *Escherichia coli* is capable of efficient *ortho*-hydroxylation of a wide range of phenolic compounds and demonstrates great potential for broad chemoenzymatic applications. To understand the structural and mechanistic basis of its catalytic versatility, we elucidated the crystal structure of EcHpaB by X-ray crystallography [1], which revealed a unique loop structure covering the active site. We further carried out mutagenesis studies of this loop to probe its role in substrate specificity and catalytic activity. Our results not only showed the loop has great plasticity and strong tolerance towards extensive mutagenesis, but also suggested this flexible loop that enables the entrance and stable binding of substrates into the active site is the key factor to the enzyme catalytic versatility. These findings laid the groundwork for editing the loop sequence to effect loop structure that led to the generation of EcHpaB loop mutants with improved enzymatic performance against larger non-natural substrates. Details of the analysis will be presented.

References

Shen, *et al.* "Structural Insights into Catalytic Versatility of the Flavin-dependent Hydroxylase (HpaB) from Escherichia coli." *Scientific reports* 9.1 (2019): 7087.