



**Experiment title:**  
**Crystal structure of a Baeyer–Villiger monooxygenase**

**Experiment number:**  
MX267

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**Report:**

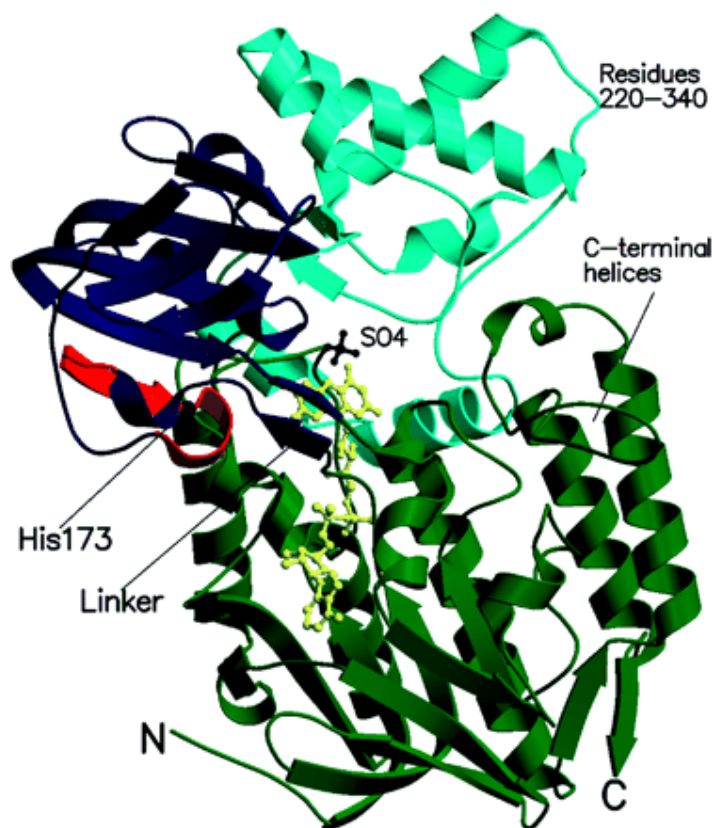
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**Crystal structure of a Baeyer–Villiger monooxygenase**

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Flavin-containing Baeyer–Villiger monooxygenases employ NADPH and molecular oxygen to catalyze the insertion of an oxygen atom into a carbon–carbon bond of a carbonylic substrate. These enzymes can potentially be exploited in a variety of biocatalytic applications given the wide use of Baeyer–Villiger reactions in synthetic organic chemistry. The catalytic activity of these enzymes involves the formation of two crucial intermediates: a flavin peroxide generated by the reaction of the reduced flavin with molecular oxygen and the "Criegee" intermediate resulting from the attack of the flavin peroxide onto the substrate that is being oxygenated. The crystal structure of phenylacetone monooxygenase, a Baeyer–Villiger monooxygenase from the thermophilic bacterium *Thermobifida fusca*, exhibits a two-domain architecture resembling that of the disulfide oxidoreductases. The active site is located in a cleft at the domain interface. An arginine residue lays above the flavin ring in a position suited to stabilize the negatively charged flavin-peroxide and Criegee

intermediates. This amino acid residue is predicted to exist in two positions; the "IN" position found in the crystal structure and an "OUT" position that allows NADPH to approach the flavin to reduce the cofactor. Domain rotations are proposed to bring about the conformational changes involved in catalysis. The structural studies highlight the functional complexity of this class of flavoenzymes, which coordinate the binding of three substrates (molecular oxygen, NADPH, and phenylacetone) in proximity of the flavin cofactor with formation of two distinct catalytic intermediates.



**Figure.** Ribbon diagram of the enzyme monomer. The FAD-binding domain is shown in green, and the NADP-binding domain is shown in blue. The subdomain inserted into the canonical NADP-binding domain topology is depicted in cyan. The fingerprint residues, which characterize the Baeyer–Villiger monooxygenases are outlined in red.