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Structural and spectroscopic observation of an enzyme at work

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Baeyer-Villiger monooxygenases (BVMOs) are promising targets for biocatalytic applications in synthetic and pharmaceutical chemistry. These flavoenzymes mainly convert ketones into their corresponding esters using NAD(P)H as electron donor by catalyzing the insertion of a single oxygen atom nearby the carbonyl group. Fundamental property of BVMOs is their ability to stabilize the flavin-peroxide intermediate. Understanding its formation is crucial to understand the catalytic mechanism in flavin dependent monooxygenases, gaining insight into the reactivity of flavoproteins with oxygen. Along this way, phenylacetone monooxygenase (PAMO) from Thermobifida fusca has been adopted as a model: its flavin cofactor has well-defined spectroscopic properties which makes it ideal for absorption spectroscopy investigations. Each flavin redox state, including the flavin-peroxide intermediate, could be characterized by UV/Vis absorbance. X-ray crystallography and single crystal microspectrophotometry experiments were performed on PAMO crystals. Microspectrophotometric studies were most useful to correlate diffraction X-ray data with UV/Vis solution studies. They also showed the formation of hydrated electrons and chlorine radicals during data collection. In parallel, we are currently investigating the reactivity of the flavin-peroxide intermediate performing chemical engineering experiments on the catalytic core, by replacing the original flavin cofactor with other FAD analogues.

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