ESRF

ESRF	**Experiment title:** **The Crystal Structure of a Baeyer-Villiger Monooxygenase Reveals a Complex Mechanism of Action**	**Experiment number**: MX129, MX267
Beamline: ID14 1 ID14 4 ID14 4	**Date of experiment**: 04 October 2003 / 06 October 2003 14 October 2003 / 15 October 2003 27 February 2004 / 28 February 2004	**Date of report**: 11 May 2004
Shifts: 5	**Local contact(s)**: **Dominique BOURGEOIS, Joanne MCCARTHY, Andrew MCCARTHY**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Enrico Malito, Andrea Alfieri and Andrea Mattevi

Department of Genetics and Microbiology, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy

Report:

Abstract of relevant article submitted to PNAS on May 4th 2004

Flavin-containing Baeyer-Villiger monooxygenases employ NADPH and molecular oxygen to catalyse 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. Their catalytic activity 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 similar to that of the

disulphide 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 stabilise 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" conformation 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.

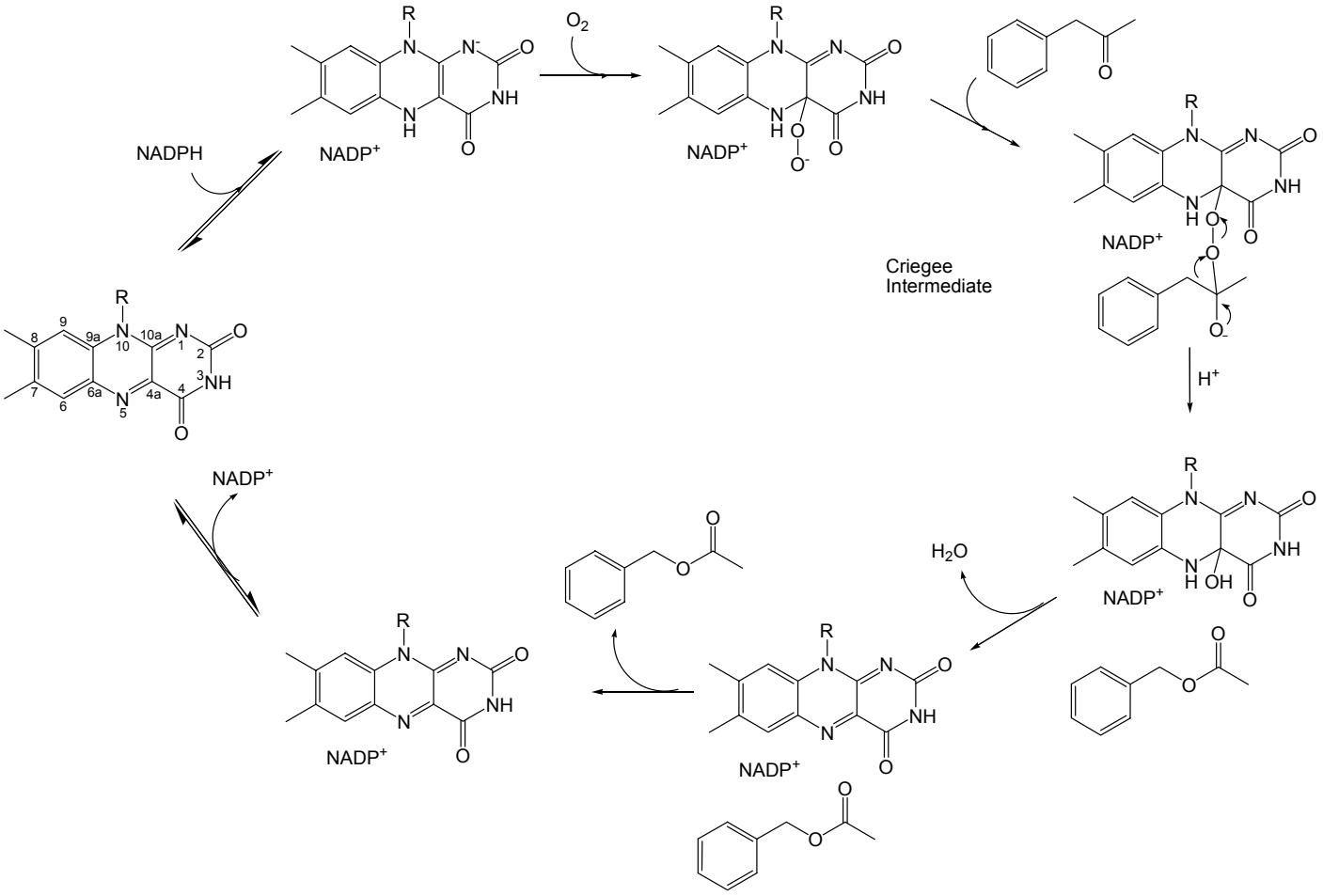

Figure 1. Scheme for the overall catalytic reaction of the Baeyer-Villiger monooxygenases with reference to phenylacetone monooxygenase. The atomic numbering of the flavin ring is shown on the left (in correspondence to the initial step of the reaction).

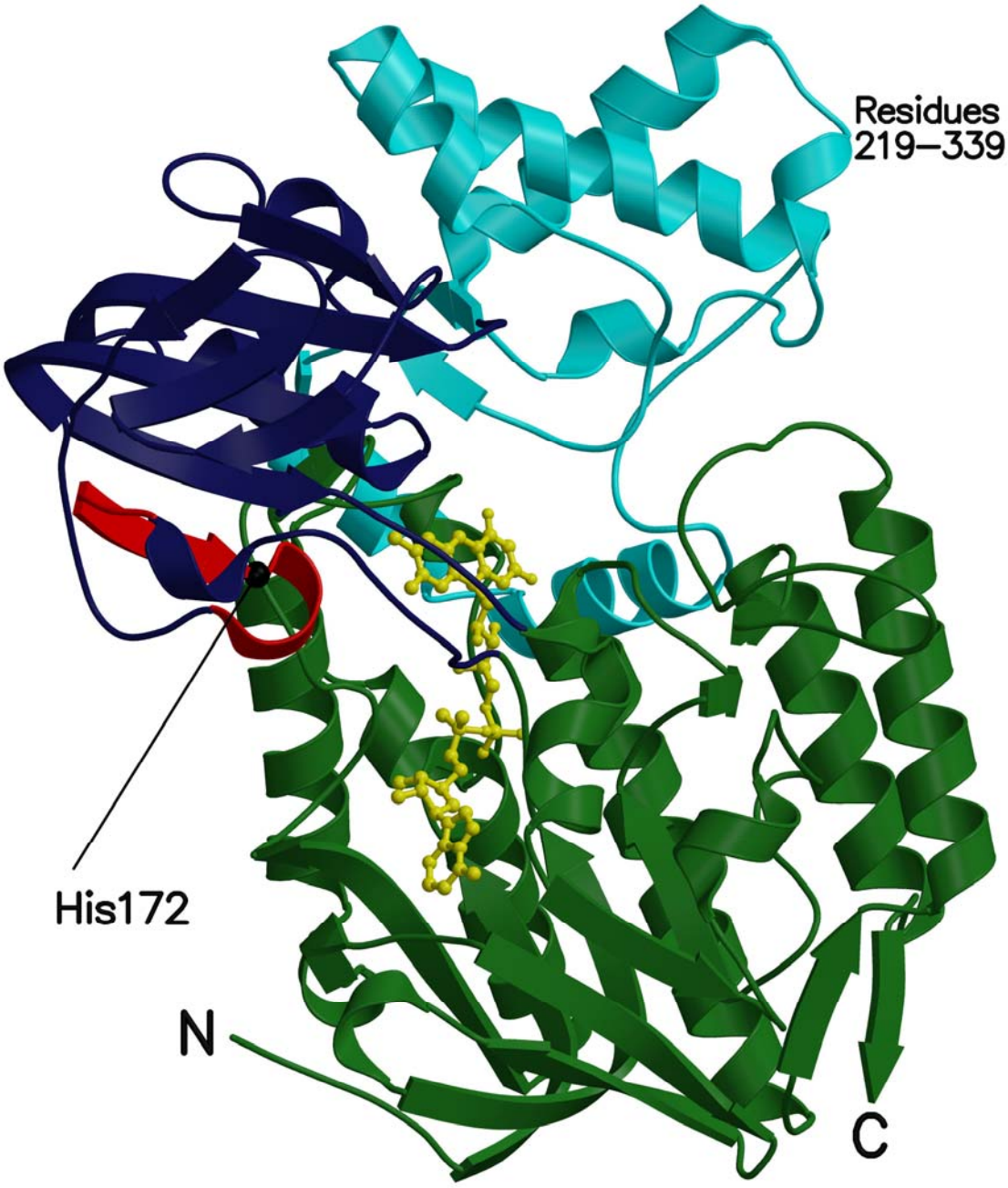

Figure 2. Ribbon diagram of the phenylacetone monooxygenase monomer. The FAD-binding domain is in green and the NADP-binding domain is in blue. Residues 219-339 which form a sub-domain inserted into the canonical NADP-binding domain topology are depicted in cyan. The fingerprint residues which

characterise the Baeyer-Villiger monooxygenases are outlined in red. His172 is a strictly conserved residue of the fingerprint motif that has been shown to be crucial for catalysis.