POCKEMO – THE STRUCTURE OF A ROBUST POLYCYCLIC KETONE MONOOXYGENASE AS A SCAFFOLD FOR ENGINEERING BIOCATALYSTS ACTIVE ON BULKY SUBSTRATES

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The Baeyer-Villiger oxidation yields esters or lactones from ketones, and this valuable reaction for the introduction of oxygen functionalities is well established in both classic chemistry as well as biocatalysis. It can be particularly beneficial to use flavin-containing enzymes called Baeyer-Villiger monooxygenases (BVMOs), when regio- and stereoselectivity is desired. Achieving this is all the more difficult for bulky, functionalized substrates and while considerable progress has been accomplished by protein engineering, most of the well-studied BVMOs are still limited to relatively small molecules. Another shortcoming of these enzymes is their notorious susceptibility towards heat and solvents.

We present here the biochemical and structural characterization of a novel BVMO from the thermophilic fungus Thermothelomyces thermophila. Phylogenetic analysis shows that this protein is a representative of a subgroup of BVMOs natively active on bulky substrates. We probed the enzyme’s stability and found that it is more stable than most BVMOs described to date. By performing small scale bioconversions with a cofactor recycling fusion variant, we found a very promiscuous substrate acceptance. Besides small linear, aromatic and cyclic ketones, this monooxygenase is particularly active on bulky, poly cyclic ketones, hence we gave it the name PockeMO. We analyzed the conversion products of several steroids, which PockeMO can oxidize on the A and D ring, as well as the C17 sidechain. Determination of the crystal structure allowed us to link this remarkable activity to unique structural features, characteristic for this BVMO subgroup. This characterization lays the foundation for future engineering efforts for this type of enzymes towards larger substrates.