

## L2-6 Extended substrate scope of a Baeyer-Villiger monooxygenase engineered by multiple-site mutagenesis

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Baeyer–Villiger monooxygenases are interesting enzymes due to their versatile reactivity and often high stereoselectivity. However, like in case of other enzyme classes, their properties often need to be adjusted to meet requirements set by industrial processes. Phenylacetone monooxygenase (PAMO) from *Thermobifida fusca* is a Type I BVMO characterised by thermostability and resistance to organic solvents but relatively narrow substrate scope. [1] As previous studies showed that introducing single mutations usually brings only small changes in the catalytic properties of PAMO, [2] we decided to simultaneously modify multiple positions in the active site of the enzyme. We used the OmniChange mutagenesis [3] to generate a library in which 11 positions were mutated. Screening 1,500 clones of this library using a phosphate-based activity detection method [4] yielded a mutant active on cyclopentanone which contained four substitutions: P253F, G254A, R258M, and L443F. Further characterisation of this mutant revealed that its substrate scope was extended to several aliphatic ketones while activity on aromatic compounds typical for PAMO was preserved. Remarkably, the stability of the quadruple mutant was the same as that of wild-type PAMO. These results demonstrate that screening structure-inspired, focused mutant libraries is a powerful tool for engineering Baeyer–Villiger monooxygenases with new specificities.

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