CONTROLLING THE FATTY ACID HYDROXYLATION REGIOSELECTIVITY OF CYP152A1 (P450BSβ) BY ACTIVE SITE ENGINEERING

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Regioselective hydroxylation on inactivated C-H bonds is among the dream reactions of organic chemists. Cytochrome P450 enzymes (CYPs) perform this reaction in general with high regio- and stereoselectivity (e.g. for steroids as substrates). Furthermore, enzyme engineering may allow to tune the regioselectivity of the enzyme. Regioselective in-chain hydroxylation of shorter or linear molecules (fatty acids), however, remains challenging even with this enzyme class, due to the high similarity of the substrate's backbone carbons and their conformational flexibility. CYPs are well described for hydroxylating fatty acids selectively in the chemically more distinct α- or ω-position. In contrast, selective in-chain hydroxylation of fatty acids lacks precedence. The peroxygenase CYP152A1 (P450Bsβ) is a family member that displays fatty acid hydroxylation at both, the α- and β-position.

Herein we report the influence of hydrophobic active site residues on the hydroxylation pattern of this enzyme. By site directed mutagenesis and combination of the libraries, double and triple variants were identified, which hydroxylated decanoic acid (C_{10}) with improved regioselectivity in the β-position. Variants were identified with 10-fold increase of β-regioselectivity (expressed as β/α ratio) compared to the wild type. In total 102 variants of CYP152A1 (P450Bsβ) were investigated. Initially all variants were evaluated with the electron transfer system CamAB.

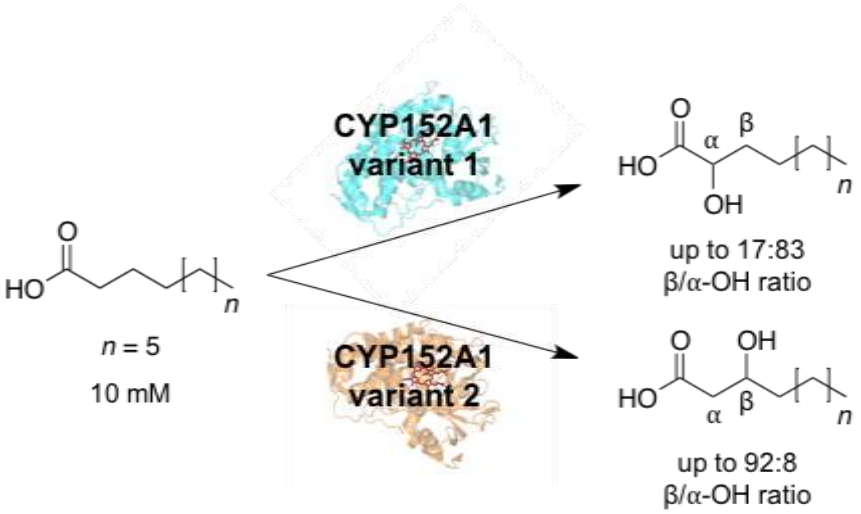

Figure 1 – Hydroxylation selectivity on α- and β-positions of selected CYP152A1 variants