**MS06-1-7 Structure-based modification of omega transaminases from Pseudomonas putida KT2440 for industrial use**
**#MS06-1-7**

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**Abstract**
Transaminases are enzymes that catalyse the exchange of an amino group between an amino acceptor and an amino donor, resulting in the formation of chiral products. A class of these enzymes, known as omega transaminase (ω-TA), can mediate this chiral amination without the requirement of an α-COOH group. These enzymes are also reported to accept unnatural substrates[1], thus increasing their relevance in the production of several pharmaceutical intermediates of commercial interest. However, product inhibition and poor substrate tolerance are some of the several factors that hinder the industrial application of these enzymes despite recent developments in the field of enzyme and process engineering[2]. This study aims at understanding the active site architecture of wild-type transaminases, followed by rational design of the active site to enhance the biotransformation of (*R*)-Phenylacetylcarbinol ((*R*)-PAC) to (*1R*, *2S*)-Norephedrine. Two such ω-transaminases (TA_5182 and TA_2799) were identified in the *P. putida* KT2440 strain and overexpressed in *E. coli* BL21 hosts. Crystals of the purified recombinant enzymes were grown; and the structures of both the enzymes in their apo and co-factor (PLP) bound holo forms were solved. The crystal structure of TA_5182 reveals the position of two flexible loops over the cofactor binding pocket, which are not modelled due to lack of features in the electron density in most of the reported apo structures of the similar enzymes. TA_2799 was solved in the co-factor bound state with a PLP molecule covalently bonded to the catalytic Lys286 as an internal aldimine. Based on their crystal structures, the FoldX stability enhancement algorithm was used to predict probable mutations in sites away from the active site to enhance the stability of the enzymes (Figure 1). Enzyme activity assays show that TA_2799 requires significantly higher concentrations of co-factor than TA_5182 to achieve satisfactory biotransformation of R-PAC. In-silico analyses were performed to generate mutants of TA_2799 to reduce the co-factor dependency of the enzyme. Based on substrate docking studies, several mutants are proposed to modify the substrate-binding pocket of the enzymes to accommodate a wider range of substrate molecules. The results of our studies will generate Pseudomonad ω-TAs with high efficiency for asymmetric synthesis, to be used in host systems for optimal large-scale industrial biotransformation.

**References**
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Overall structure and rational design of ω-TA