Summary and future prospects

The family of Ntn hydrolases includes an assortment of structurally related enzymes that exhibit acylase/amidase activity on a wide range of substrates. Two penicillin V acylases belonging to this family have been studied in this thesis. Biochemical, structural and computational analyses were used to explore the activity and substrate specificity of these enzymes.

In addition to their significant footprint in the pharmaceutical industry, penicillin acylases also possess intriguing catalytic and structural characteristics that make them appealing to study. The PVAs from Gram-negative bacteria (PaPVA and AtPVA) characterized in this work were observed to display considerably enhanced activity and specificity on Pen V over reported PVAs, with unique kinetic behaviour showing cooperativity and substrate inhibition. Expression of these enzymes in E. coli gave significant protein yields, making them very promising systems to develop for industrial applications. Immobilization and biotransformation trials were attempted in this direction with recombinant E. coli – PaPVA. The structure of PVAs from Gram-negative bacteria also displayed unique differences in oligomer interactions and active site residues compared to their counterparts from Gram-positive bacteria. The crucial roles of two tryptophan residues in the active site involved in substrate binding were established in the study. Aromatic stacking interactions between these residues and the phenyl ring of Pen V play a significant part in orienting the Pen V molecule in the active site for maximum activity.

The role of penicillin acylases in the natural environment has remained largely unexplored till recent years. In this study, the ability of PVAs to hydrolyze acylhomoserine lactones (AHLs) involved in bacterial signaling has been established. The addition of PVA to the increasing number of enzymes involved in AHL degradation presents new avenues for use in quorum quenching applications.

The results from this study demonstrate the fascinating nature of PVAs from Gram-negative bacteria and provide new insights into their catalytic behaviour. Further exploration of these enzymes could provide a plethora of opportunities for protein engineering and development of better PVA-based systems for industrial 6-APA production. Moreover, a comprehensive proteomics and metabolomics-based approach would help further explore the place of penicillin acylases within the complex maze of microbial metabolic networks.