CSC-RUB PhD Project Proposal

Title: Enzymatic epoxidation provides access to chiral building blocks for pharma industries

Sector of research: Biology and Biotechnology; Microbial Biotechnology; Biocatalysis

Degree awarded: Dr. rer. nat.

Keywords: protein engineering, biocatalysis, industrial biotechnology, pharma compounds, enzyme reactor, downstream processing

Supervisor of PhD project: Prof. Dr. Dirk Tischler, Microbial Biotechnology, e-mail: dirk.tischler@rub.de

Research focus of supervisor:

We work in the field of functional annotation of novel biocatalysts and their optimization for fine chemical synthesis. We have expertise and equipment to start with microbiology, (meta)genome studies, gene expression and mutagenesis, protein engineering (directed evolution), protein homology modelling as well as biocatalysis. For the latter, we often combine enzymes with chemical synthesis to establish chemo-enzymatic cascades. Here we have enzyme reactors as well as standard fermenters in routine. Focus we put on various oxidoreductases and their biochemical functionality as well as application. We combine knowledge from microbial degradation of xenobiotics and natural feedstocks with the eco-friendly synthesis of industrially relevant compounds such as drugs, pre-cursors, flavor compounds or polymer precursors.

Publications: In the last 5 years we published 53 manuscripts (H-index: 23, 1597 citations)

- Identification of molecular basis that underlie enzymatic specificity of AzoRo from Rhodococcus opacus 1CP: A potential NADH:quinone oxidoreductase; Ngo, A.C.R., Qi, J., Juric, C., Bento, I., Tischler, D. Archives of Biochemistry and Biophysics, 2022, 717, 109123
- Metal binding ability of microbial natural metal chelators and potential applications; Hofmann, M., Retamal-Morales, G., Tischler, D. Natural Product Reports, 2020, 37(9), pp. 1262–1283

Second Supervisor of PhD project

Prof. Eckhard Hofmann or Prof. Thomas Happe
Summary of research plan:

**Background:** Bacterial flavoprotein monooxygenases are able to activate molecular oxygen and to perform numerous oxygenations yielding chiral epoxides or sulfoxides among others. This is achieved by the help of the FAD cofactor and a highly selective active site. To activate molecular oxygen reducing equivalents are needed. FAD can be reduced in solution by means of artificial electron donors to provide a cheap screening procedure. At the end, the enzymes can be applied in whole-cell biotransformation to scale reactions. We are especially working on styrene and indole epoxidases with the aim to uncover the base of enantioselectivity and have access to unpublished structural data. About 30 enzymes of this family have been studied so far, but so far, the limited structural details hamper application.

**Study objective:** A structure-guided investigation should provide access to a set of flavoprotein monooxygenases with reversed enantioselectivity. Further, the enzymes will be investigated to convert non-natural substrates with industrial relevance.

**Expected Results:** The phylogenetic in combination with structural studies will reveal details of the active site of flavoprotein epoxidases which will be engineered to promiscuous but also highly enantioselective monooxygenases (proposed publication). We aim for styrene, indene and hexene as model substrates. Chiral epoxides can be verified, and the screening will guide towards a whole-cell approach. A two-phase system will be used to scale reaction and provide in situ product recovery. This part of the project can lead to 2 publications and international presentations as output.

**Methods:** Molecular biologic methods such as Gibson cloning or error-prone PCR will be used to manipulate genes, proteins will be produced in E. coli or Pichia, protein purification and immobilization will be achieved in flow systems, enzyme reactors as flow cells or rotating bed reactor are used to synthesize products, products will be analysed by HPLC, GC-FID, GC-MS and NMR.

**Candidate Requirements:** A MSc degree in Microbiology, Biotechnology, Biochemistry, or related a discipline is requested. The candidate needs to have experience in molecular biology and microbiology methods as well as some analytical chemistry. We are an international group and request team player attributes! Good English language skills are mandatory.

**Motivation for CSC application:** In our group you have the chance to study interdisciplinary various aspects of biocatalysis: from gene to process. We over a well-equipped laboratory to apply all kinds of molecular biology, fermentation strategies, protein purification at various scales, biochemistry (incl. rapid-mixing), high-throughput screening technologies, sophisticated analytics (including GC, MS, HPLC and combinations thereof) for volatile and non-volatile products, etc. You will also have to chance to interact with students at various stages with different background which allows to gain teaching expertise. Our group is internationally connected to academia and industries which provides an excellent hub for exchange and career development. You will be integrated in our International Graduate School of Biosciences (IGB).