Karlsruher Institut für Technologie

Electro Biotechnology

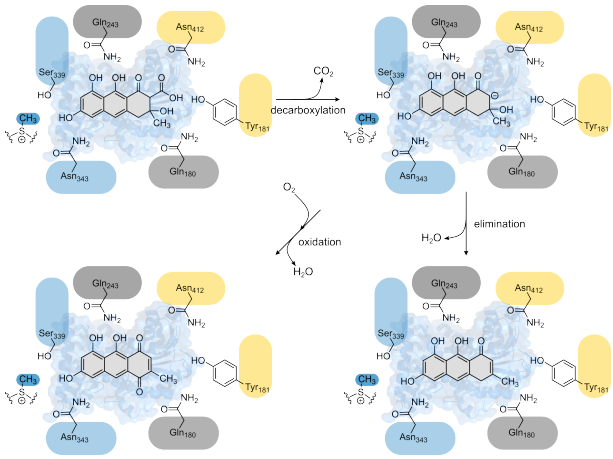
Institute of Process Engineering in Life Sciences (BLT 2)

Electrobiotechnology:
Prof. Dr.-Ing. Dirk Holtmann

Building: 30.43 / 1st floor
Fritz-Haber-Weg 4
76131 Karlsruhe

Call for Applications for a Master's/Bachelor's Thesis on „Multifunctional Methyltransferases: From canonical methyl transfer to moonlighting *extravaganza*"

Background and Research Objective



Methyltransferases (MTs) are widespread enzymes in nature that transfer methyl groups and thereby tune the activity, stability, and fate of small molecules. Beyond this canonical role, several MTs have been shown to catalyze non-canonical *moonlighting* reactions such as decarboxylation, hydroxylation, or eliminations, which expand natural product diversity. Typically, these reactions are orchestrated by single enzymes in large biosynthetic gene clusters. However, in mushroom-forming fungi (Basidiomycetes), BGCs are compact yet yield the same complexity, implying broader enzyme activity, i.e., enzyme promiscuity, than currently assumed. This thesis explores MTs from basidiomycetes to (**i**) establish assays for canonical methyl transfer and (**ii**) test the hypothesis that MTs also perform non-canonical steps that help build polyketide-derived scaffolds. The objective is to map simple sequence–activity rules that explain when and how multifunctionality emerges.

Content of the Thesis

You will build an end-to-end experimental pipeline that takes multifunctional methyltransferases from gene to mechanism. Starting with cloning and heterologous expression, you will produce basidiomycete enzymes in different hosts, e.g., *E. coli*, *P. pastoris*, and *A. niger*. After purification, i.e., IMAC and SEC, you will establish defined *in vitro* assays with *S*-adenosyl-L-methionine (SAM) to quantify canonical methyl transfer on model substrates. You will then challenge the enzymes with native or rationally designed substrates to uncover potential *moonlighting* chemistry such as decarboxylation, hydroxylation, or elimination. Reaction products will be identified and validated by HPLC/LC–MS and, where appropriate, GC/GC–MS and confirmatory NMR for key hits, yielding a tangible link between product profiles and catalytic hypotheses. The outcome is a practical set of rules that explains when and how methyltransferases switch from canonical methyl transfer to true multifunctionality, a direct progress on the central problem of *moonlighting* activity.

Prof. Dr.-Ing. Dirk Holtmann

For applications and further information please contact Dr. Lukas Platz.
E-Mail: lukas.platz@kit.edu