Structural basis for substrate recognition by cytochrome P450 monooxygenases

Supervisors / Institutes:
Main supervisor: Prof. Vlada Urlacher, Institute of Biochemistry,
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Co-supervisor: Prof. Georg Groth, Biochemical Plant Physiology, Heinrich-Heine University Düsseldorf

Project background and description:

The main goal of this project is the elucidation of the structural basis for substrate recognition by homologous cytochrome P450 monooxygenases. Cytochrome P450 monooxygenases are heme-containing enzymes that use molecular oxygen and the electron donor NAD(P)H to catalyse the insertion of one oxygen atom into a vast variety of organic molecules. Selective oxidation of inactivated carbons – one of the most challenging reactions in synthetic chemistry – is conducted under mild conditions by these impressive biocatalysts. All P450 enzymes for which structures have been determined share a common three-dimensional fold. The question is how this conservative architecture is combined with an amazing adjustability of P450s to a vast variety of substrates that are oxidized with high regio- and stereoselectivity?
The central step of the project will be the crystallization of some representative enzymes from two P450 families in order to compare them with existing crystal structures and with homology models, thereby focusing on the active site regions. Further site-directed mutagenesis in the active site regions of selected P450 enzymes will be done to ascertain (i) whether or not, and (ii) if yes, at which extent it is possible to switch the substrate spectrum from one P450 to that of another P450 either within the same subfamily, or the same family, or between two different P450 families. This project will not only shed light on structural features of new P450s and allow generating enzymes with biotechnologically relevant properties, but might also give a hint about evolutionary aspects of P450 functions.
The project will be carried out in close cooperation between two groups: group of V. Urlacher providing expertise in biocatalysis and protein engineering of P450 enzymes; and group of G. Groth providing expertise in the field of X-ray crystallography and structural analysis of enzymes.

Aims of the project:

- Structural analysis and comparison of homologous P450s with respect to their substrate scope
- Evolutionary aspects of P450 functions
- New P450 with biotechnologically relevant features

Requirements:

- Master degree in biochemistry, chemistry or a related discipline
- Experience in cloning, expression and purification of recombinant proteins in *E. coli*

Additional information:

http://www.chemie.uni-duesseldorf.de/Faecher/Biochemie/Mitarbeiter/Urlacher
http://www.biochemplant.hhu.de