

Directed Enzyme Evolution

Directed evolution towards functional expression of fungal peroxygenases for selective oxyfunctionalisation of organic molecules

Project work at the autonomous University of Madrid by Christoph K ung, December 2019

During my master's degree, I had the opportunity to conduct one of my practical stays (Projektarbeit) abroad. I did this in Madrid, at the Institute of Catalysis and Petrochemicals of the autonomous university in the lab of Prof. Miguel Alcalde. During this stay of three months, I worked on the directed evolution of fungal peroxygenase of *Marasmius rotula*, aiming to achieve functional expression in the heterologous host *Saccharomyces cerevisiae*.

Fungal peroxygenases represent promising catalysts in synthetic chemistry for selective oxyfunctionalisation of organic molecules, a reaction without such catalyst only feasible under harsh conditions. Therefore, understanding and improvement of fungal peroxygenases have attracted broad interest. The peroxygenase of *Marasmius rotula*, I worked with, hitherto showed to be recalcitrant for heterologous expression which is why it was submitted to a directed evolution experiment. By introducing random mutations in the genome and screening for improved variants, it was tried to achieve expression of active enzymes. Within one round of directed evolution, heterologous expression of active peroxygenases could be accomplished, however underlying details remain to be elucidated.

In the framework of this project, I improved my laboratory skills and learned a lot about workflows in directed evolution experiments from library generation to high throughput screening. The group of Prof. Alcalde consisted of chemists and biologists, therefore suiting the interdisciplinary context of the nanoscience studies. With my background from Nanoscience study program at the University of Basel I felt well prepared joining the group for this stay. The work environment in the lab and during group meetings was very enjoyable which is why I was very happy with my choice. The lab is well equipped and lab members took part in productive discussions about my and other projects leading to constructive solution finding.

The autonomous University is located a bit north of the center of Madrid but is very well connected with the city. Outside of the University routine, I as well enjoyed my stay a lot as Madrid is an amazing, lively and beautiful city. I spent a lot of time wandering around, discovering lovely places, delicious food and interesting people. Because of all these reasons I can only strongly recommend doing a project work or even the master thesis abroad as it for sure comes with an unforgettable experience.

I am very happy to have had this opportunity and would like to thank the SNI for the financial support during my stay in Madrid.



Left: Plaza Mayor in the center of Madrid. Middle: Mutant library after screening. Right: Panorama view from a rooftopbar.