Prof. Dr. Ulrich Schwaneberg
RWTH Aachen
Institute for Biotechnology
Biologiesammelbau 2
Worringerweg 3
52074 Aachen

Aachen, 01.02.2019

Bachelor / Master Thesis / Internship

Development of a FACS-based high-throughput screening system for directed evolution

Background

For succesful directed evolution campaigns it is necessary to generate, cultivate and screen several thousands of enzyme mutants, in order to obtain an improved desired enzyme property. Hereby, 60 – 80 % of the amino acid substitutions, which improve the enzyme properties, cannot be identified, because screening capacity is a limiting factor. In this project a flow cytometer-based method for screening of mutant enzyme libraries is developed, which enables ultra-high-throughput flow cytometer-based screening of millions of mutants in relatively short time.

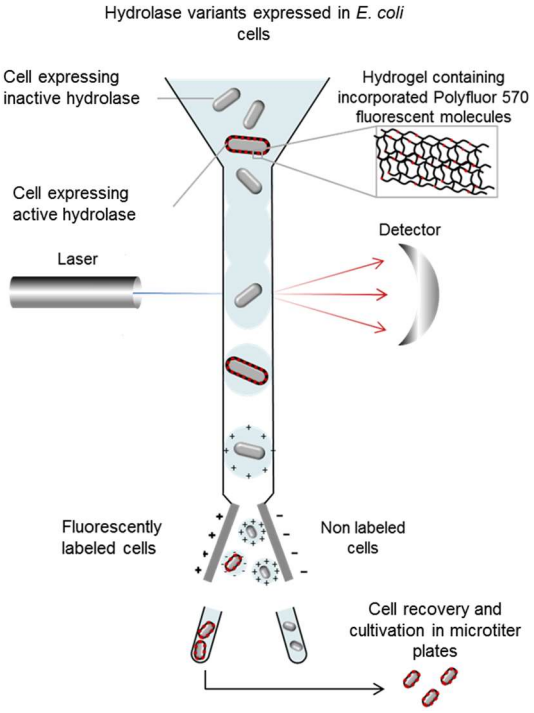

Figure 1: **Flow Cytometer-based sorting principle of the Fur-Shell screening technology.** Enzymes expressed in cells mediate the formation of a fluorescent hydrogel shell. Fluorescently labeled cells are sorted out and can be further characterized in detail.

Aim of the study: The Fur-Shell screening platform[1] was already established for different hydrolases. Now the system will be expanded to different enzyme classes.

Methods

- Plasmid preparation
- Primer design, random mutagenesis (i.e. epPCR)
- Cloning
- Expression in microtiter plates & flasks
- DNA- and protein analytics
- Microtiter plate-based screening assays
- FACS

Language: German & English

Contact: **Volkan Besirlioglu**, room 4.132 (new building)
Email: v.besirlioglu@biotec.rwth-aachen.de
Phone: +49241 / 80 23602

[1]Pitzler *et al* (2014). A fluorescent hydrogel-based flow cytometry high-throughput screening platform for hydrolytic enzymes. *Chemistry & Biology*, *21*(12), 1733–1742.