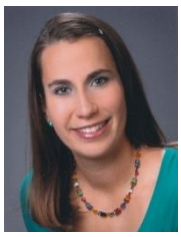
In vitro flow cytometry-based screening platform for cellulase engineering

Körfer, G., Pitzler, C., Vojcic, L., Martinez, R., and Schwaneberg, U., *In vitro* flow cytometry-based screening platform for cellulase engineering*, Scientific Reports,* **2016**, 6: 26128



Development of an ultrahigh throughput flow cytometry-based screening platform with compartmentalized cell-free expression system

Ultrahigh throughput screening (uHTS) plays an essential role in directed evolution for tailoring biocatalysts for industrial applications. Flow cytometry-based uHTS provides an efficient coverage of the generated protein sequence space by analysis of up to 10^7 events per hour. Cell-free enzyme production overcomes the challenge of diversity loss during the transformation of mutant libraries into expression hosts, enables directed evolution of toxic enzymes, and holds the promise to efficiently design enzymes of human or animal origin. The developed uHTS cell-free compartmentalization platform (InVitroFlow) is the first report in which a flow cytometry-based screened system has been combined with compartmentalized cell-free expression for directed cellulase enzyme evolution. InVitroFlow (Fig. 1) was validated by screening of a random cellulase mutant library employing a novel screening system (based on the substrate fluorescein-di-β-D-cellobioside), and yielded significantly improved cellulase variants (*e.g.* CelA2-H288F-M1 (N273D/H288F/N468S) with 13.3-fold increased specific activity (220.60 U/mg) compared to CelA2 wildtype: 16.57 U/mg).

Fig. 1: Principle of InVitroFlow comprising 7 steps. (1.) Mutant library generation using a linear DNA template (approx. 6 h), (2.) entrapment of mutant cellulase library in (w/o) single emulsions within 0.5 h, (3.) cell-free expression of mutant library and generation of (w/o/w) emulsions within 4 h, (4.) sorting of active variants within (w/o/w) emulsions using flow cytometer within 2 h, and (5.) DNA recovery from (w/o/w) emulsions and PCR gene amplification in 3.5 h. A whole round of InVitroFlow (diversity generation, screening by flow cytometry, amplification) can be completed within 16 h. (6.) Cloning and transformation into expression host (2 days), and (7.) screening of up to 2,000 beneficial clones in MTP format and characterization of a few variants (7-12 days).

The InVitroFlow technology platform drastically reduces time requirements for one round of directed evolution from several months to only one day and enables an efficient prescreening of mutant libraries (10^{10}) with high diversity covering a significant portion of the generated sequence space in a time efficient manner. InVitroFlow has an impressive potential to study challenging scientific questions *e.g.* the exploration of combinatorial effects or structure-function relationships within biocatalysts and can –from our point of view - be adapted to other enzyme classes since it uses a widely applicable fluorescence sorting of cell-free expressed active enzyme variants within (w/o/w) emulsions compartments.