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Development of a high-throughput fluorogenic assay for the detection of promiscuous amidase activity

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PURPOSE OF THE ABSTRACT

Carboxyl esterases are powerful catalysts due to their broad substrate specificity and high regio- and stereoselectivity, as well as their ability to catalyze reactions without cofactors.[1]

An esterase from B. subtilis DSM402 (BS2) was found to not only be active towards tertiary alcohols but unlike many carboxyl esterases, shows promiscuous amidase activity.[2] The first steps in unraveling the amidase mechanism of this enzyme have shown that a hydrogen-bond network is essential for stabilizing the transition state.[3] A directed evolution approach resulted in a mutation that improved the amidase activity of BS2 by changing the orientation of the substrate in the active site. This increase was achieved through a π-π stacking network, which enhanced the stability of the tetrahedral intermediate formed during amide bond hydrolysis.[4]

We aim to improve the understanding of the amidase mechanism of BS2 to increase its activity further by applying a computational-aided engineering approach using the 3DM tool.[5] For this purpose, we have established a novel whole-cell high-throughput assay based on fluorescence-activated cell sorting (FACS) to screen large mutant libraries and identify variants with improved amidase activity.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

amidase mechanism | high-throughput screening | flow cytometry

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