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Engineering of an organic solvent tolerant esterase based on computational predictions

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Biocatalytic reactions in synthetic chemistry often require the presence of organic solvents to enhance substrate solubility or steer the reaction equilibrium towards synthesis. Given that enzymes have evolved in aqueous environments, the discovery of rare organic solvent-tolerant enzymes in nature is immensely valuable for elucidating the molecular mechanisms underlying their resistance. The esterase PT35 from Pseudomonas aestusnigri, which was found in crude oil contaminated sand samples, exhibit extraordinary activity and stability (Tm 49℃; t1/2 35 h) in the presence of 50% acetonitrile. Molecular dynamics (MD) simulations comparing PT35 with the organic solvent-sensitive structural homolog ED30 revealed a more pronounced hydration shell around PT35 due to its distinctive negative surface charge. We developed a mutagenesis strategy involving both enzymes, PT35 and ED30, that aimed to decrease or strengthen the hydration shell surrounding the enzyme by modifying its surface charge. Consequently, we generated PT35 variants with reduced negative surface charge and ED30 variants with enhanced negative surface charge. We successfully produced 10 variants for each enzyme, with 7 PT35 variants exhibiting decreased tolerance (e.g. Δt1/2 -37.2 h) and 8 ED30 variants displaying increased tolerance (e.g. Δt1/2 +25.6 h) compared to the wild type. Our findings underscore the potential of engineering an enzyme's surface charge as a means to boost its tolerance to organic solvents. Our next goal is to produce a ED30 variant with 7 mutations evenly distributed across the surface to reinforce the hydration shell around the entire enzyme.

Consent

Yes

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