

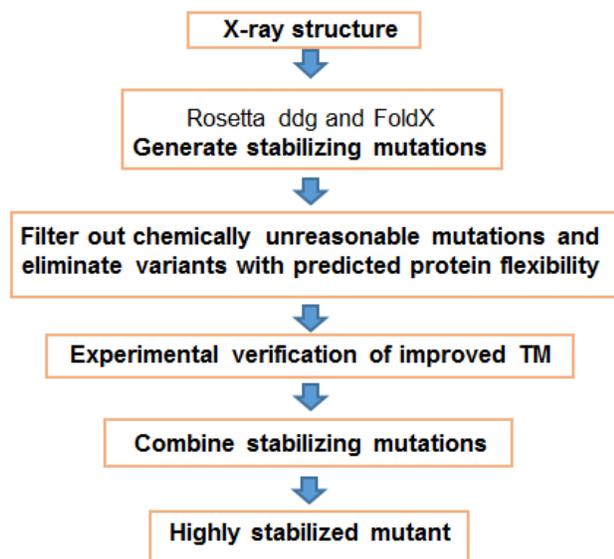
## RAPID ENZYME STABILIZATION BY COMPUTATIONALLY DESIGNED LIBRARIES OF HMF OXIDASE

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HMF oxidase (HMFO) from *Methylovorus sp.* is a recently characterized flavoprotein oxidase [1]. HMFO is able to oxidize 5-(hydroxymethyl)furfural (HMF) into 2,5-furandicarboxylic acid (FDCA). Because HMF can be formed from fructose or other sugars and FDCA is a polymer building block, the oxidase has attracted attention as industrially relevant biocatalyst. The dicarboxylic acid FDCA can be polymerized with ethylene glycol to produce polyethylene furanoate (PEF). This renewable and bio-based polyester can be a valid alternative to the petroleum-based polyethylene terephthalate (PET) thanks to its similar characteristics.

HMFO is a promising biocatalyst for various oxidations and not only for the production of FDCA. The first step to the development of an HMFO with improved catalytic properties is the engineering of the enzyme to enhance its thermostability using the recently developed FRESCO method.



FRESCO (Framework for Rapid Enzyme Stabilization by Computational libraries) is a computational approach to determine thermostabilizing point mutations in a protein structure [2]. FRESCO has the potential to become a more valid alternative to random approaches like direct evolution when the protein structure is known. The first step of this computational method is the application of an algorithm based on FoldX and Rosetta ddG calculations to every possible single amino acid mutant to predict the mutant stability. In the following step MD screening is performed in order to eliminate variants with predicted protein flexibility. After this screening the selected mutants are subject to experimental verification for improved T<sub>M</sub> and preserved catalytic activity. Finally, the combination of stabilizing mutations should lead to highly stabilized variants.

I will present the results obtained by using the FRESCO method: the stability and activity profiles of the generated HMFO mutants.

[1] Dijkman W.P., Fraaije M.W. Discovery and Characterization of a 5-Hydroxymethylfurfural Oxidase from *Methylovorus sp.* Strain MP688. *Applied and Environmental Microbiology*. 2014;80(3):1082-1090. doi:10.1128/AEM.03740-13.

[2] Wijma H.J., Floor R.J., Jekel P.A., Baker D., Marrink S.J., Janssen D.B.. Computationally designed libraries for rapid enzyme stabilization. *Protein Engineering, Design and Selection*. 2014;27(2):49-58. doi:10.1093/protein/gzt061.