# Characterization and Engineering of Fatty Acid Hydratases: From Regioselectivity Modification to Cascade Reactions

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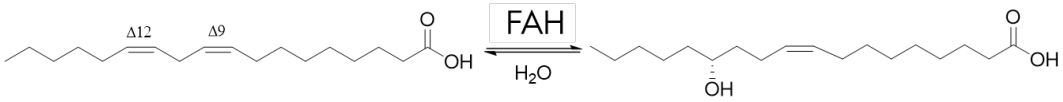
Fatty acid hydratases (FAHs) catalyze regio- and stereo-selective addition of water to isolated carbon-carbon double bonds of unsaturated fatty acids. The resulting "hydroxy fatty acid" (HFA) products are high value oleochemicals with potential uses in materials industry and cosmetics as well as in the medical field.




Linoleic Acid (*cis*-9, *cis*-12-octadecadienoic acid) (*R*)-13-hydroxy-*cis*-9-octadecenoic acid

Over the years, we generated (semi)rational mutant libraries of different FAH orthologs based on comparative active-site structure and sequence analysis, especially for modulating regioselectivity and substrate specificity, in order to expand product scope for HFA regioisomers [1,2]. Significant regioselectivity shifts towards various polyunsaturated fatty acids were observed with engineered FAH mutants. For example, screening of the site-saturation library of a FAH from *L. acidophilus* towards EPA (eicosapentaenoic acid) resulted in mutants that produce varying ratios of 12-hydroxy and 15-hydroxy EPA isomers. Notably, some of these mutants can generate a single isomer with 100 % selectivity, unlike the mixture obtained from wild-type enzyme [2]. We interpreted these changes in selectivity based on structural, kinetic, and statistical analysis. Our engineering studies also provided valuable information on the mechanism of hydration. We also demonstrated semi-prep scale enzymatic synthesis of selected HFAs. Furthermore, we set-up bi-enzymatic cascade reactions for biocatalytic synthesis of various isomers of fatty acid esters of HFAs (FAHFAs) directly from fatty acids. More recently we have identified a new thermostable FAH ortholog, which showed interesting properties such as a unique regioselectivity profile and absolute NaCl requirement for its purified form. We provide in silico structural explanation on the observed regioselectivity patterns. Moreover, although this enzyme showed high thermostability, its highest activity was observed at a much lower assay temperature of 20 °C, which might be related to physiological function of the enzyme.

[1] B. E. Eser, M. Poborsky, R. Dai, S. Kishino, A. Ljubic, M. Takeuchi, ... & Z. Guo, *ChemBioChem* **2020**, *21.4*, 550-563.

[2] Y. Zhang, N. M. D. Breum, S. Schubert, N. Hashemi, R. Kyhnau, M.S. Knauf, ... & Eser, B. E. **2022**, *ChemBioChem*, *23(4)*, e202100606.