

# **Engineering of Industrial Enzymes by Rational Design and Directed Evolution**

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The use of enzymes in industrial processes has become an important factor in modern biotechnology. One large field is biocatalytic production of bulk and fine chemicals. Biocatalytic strategies have various advantages. Most important is the high reactivity, selectivity and specificity of enzymes allowing efficient production of complex molecules including enantiopure compounds. Another important aspect is mild reaction conditions which enables environmental friendly production conditions avoiding toxic solvents or side products.

One prerequisite for efficient biocatalytic processes is the availability of suitable enzymes. Nature with its evolutionary developed biodiversity provides a big palette of enzymes. However, natural evolution has directed the functionality of these enzymes towards the needs of the respective organisms and the needs of the environmental conditions under which these organisms are living. This means that one can easily find an enzyme catalyzing a specific reaction but under the conditions of an industrial process the efficiency of such enzymes is in most cases very limited.

In the past century efficient strategies have been developed which allow adapting enzymes towards specific features (e.g. stability, solvent tolerance, reactivity, substrate specificity, enantioselectivity etc.) by engineering the primary structure, based on creating muteins by mutation or by recombination of genes encoding the respective enzymes. Both, rational design and directed evolution strategies based on random mutagenesis or gene shuffling combined with an efficient screening or selection process, respectively, and combinations of these, have been successfully employed. Examples for successful engineering of enzymes (hydrolases, lyases) towards different features by both strategies will be discussed.