2.7.4 Methodology Developments in the Directed Evolution of Selective Enzymes as Catalysts in Organic Chemistry and Biotechnology

Biocatalysis by Manfred T. Reetz

ABSTRACT: At the MPI für Kohlenforschung more than 20 years ago, the Reetz group pioneered the concept of directed evolution of stereoselective enzymes, the goals being the generation of useful biocatalysts, and learning lessons concerning the intricacies of enzyme mechanisms. Today the Darwinian methods and strategies that were developed for probing protein sequence space efficiently are used by essentially all academic and industrial groups in the field. During the last three years the focus was on establishing maximal speed and reliability of directed evolution. Highlights include the use of machine learning (artificial intelligence), utility of solid-phase chemical synthesis of designed saturation mutagenesis mutant libraries on Si-chips, evolving high activity of hyperthermally stable enzymes at room temperature, producing enzymes for promiscuous and/or difficult organic transformations, and QM/MM-based mechanistic advances.

Introduction

The Reetz lab in Marburg was closed in November 2017, but the initiated projects were continued in the labs of former Chinese postdocs, now full professors, e.g., Zhoutong Sun at the Tianjin Institute of Industrial Biotechnology (Chinese Academy of Sciences), all efforts leading to >35 publications in the reporting 3-year period with emphasis on methodology development (Figure 1).[1] Recently, Manfred Reetz returned to Mülheim, and is also Adjunct Professor in Tianjin/China. A few highlights published during the research period are listed below:

Artificial intelligence in directed evolution

In collaboration with Frederic Cadet (France), the predictive power of the machine learning algorithm Innov'SAR was successfully tested in directed evolution based on **C**ombinatorial **A**ctive-site **S**aturation **T**est (CAST) and **I**terative **S**aturation **M**utagenesis (ISM).[2] Highly stereoselective epoxide hydrolase mutants were obtained, superior to those previously evolved by first-generation CAST/ISM.

Designed chemical solid phase synthesis of saturation mutagenesis libraries

The inherent limitations of saturation mutagenesis in general, including amino acid bias, were eliminated by exploiting the commercial TWIST technique of chemical gene synthesis on Si-chips. Experimentally, 97% of the designed library members appeared in screening, in contrast to only 50% using traditional molecular biology-based mutagenesis.[1] This advance means an enormous increase in library quality as measured by the frequency of hits, their activity and stereoselectivity, and dramatically less screening effort! If the prices continue to go down, this could well constitute the future of directed evolution.

Figure 1. General concept of directed evolution of stereoselective enzymes;[1] activity and regioselectivity can be handled similarly by this Darwinian approach.
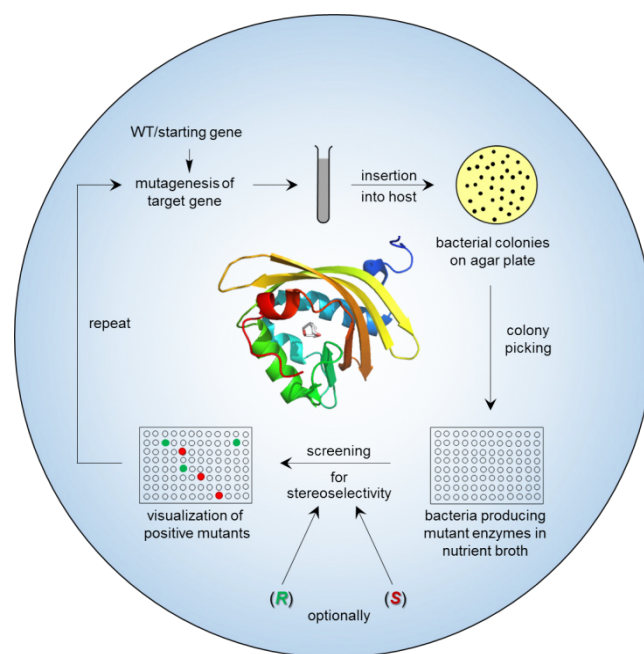
Evolution of high activity of hyperthermally stable enzymes at room temperature

Extremophilic enzymes generally require high operating temperatures. For the first time, extremely high activity of such an enzyme at room temperature was obtained by directed evolution![1]

Promiscuous and/or difficult to achieve chemical transformations

The chemo- and regioselective dihydroxylation of benzene with no overoxidation (!) flanked by the cascade synthesis of arbutin was achieved by directed evolution.[3] In other work, an artificial metalloenzyme[4] was evolved that catalyzes the Kemp elimination, not by the traditional acid/base mechanism, but by single electron transfer.[5] A giant step in solving the challenging problem of P450-based targeted hydroxylation of steroids at any desired position was taken by means of mutability landscaping and mutational scanning,[6] planned oxidation at the C16-position with α- and optionally ß-diastereoselectivity being achieved for half a dozen steroids, while C7- and C11-selectivity was also evolved, likewise for pharmaceutical applications. Overriding electronic effects of Baeyer-Villiger reactions for inverting regioselectivity was also successful by again using second generation CAST/ISM.[7]

QM/MM studies

A 60-year old open mechanistic question was unambiguously settled by directed evolution, enzyme kinetics and QM/MM computations.[8] Another QM/MM study, flanked by X-ray data of mutants, provided mechanistic details of an epoxide hydrolases, which led to surprising insights.[9]

Fusing directed evolution and rational design

Second-generation CAST/ISM[1,10,11] suggested the fusion of directed evolution and rational design as perhaps the ultimate form of protein engineering: First example of a new strategy dubbed Focused Rational Iterative Site-specific Mutagenesis (FRISM) may prove to be a logical step in the right direction.[1,12]

Funding Sources

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