Directed Evolution of Epoxide Hydrolase: Exploring the Fitness in Desymmetrization of *meso*-Epoxides

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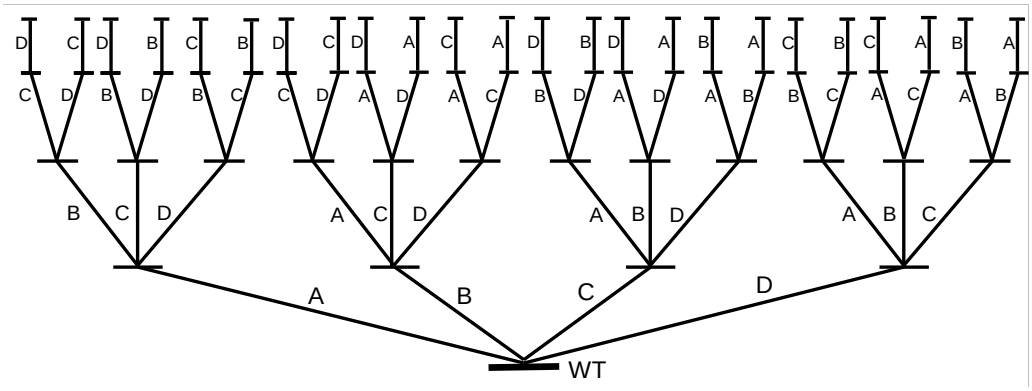
Directed evolution has emerged as a powerful means to engineer the properties of enzymes as catalysts in synthetic organic chemistry and biotechnology. It is based on repeated rounds of mutagenesis, expression and screening (or selection). Following proof-of-concept in the field of directed evolution, many enzymes have been engineered for enhanced enantioselectivity and broadened substrate scope using a variety of different gene mutagenesis methods, including epPCR, saturation mutagenesis and DNA shuffling. We recently developed iterative saturation mutagenesis (ISM) as an expedient method for rapid laboratory evolution, which can be used to control enantioselectivity, substrate scope as well as thermostability[i].

In nature, Limonene-1,2-epoxide Hydrolase (LEH) participates in limonene degradation in *Rhodococcus. erythropolis* DCL14[ii]. It is composed of 149 amino acid residues with a molecular mass of 16.5 kDa and no cofactors are required for its activity. Here, we reported the directed evolution of LEH on desymmetrization of *meso*-epoxide. Wild type shows the 15% of ee (R, R). After several cycles of saturation mutagenesis, we could improve and invert the ee of diols up to 80% (R, R) and 92% (S, S), respectively.



Scheme 1. Simplified Iterative Saturation Mutagenesis (ISM) employing four sites A, B, C and D, each site in a given upward pathway being visited only once.

[i] Reetz, M. T., L. W. Wang, et al. (2006). Angewandte Chemie-International Edition 45(8): 1236-1241.
[ii] van der Werf, M. J., R. V. A. Orru, et al. (1999). Applied Microbiology and Biotechnology 52(3): 380-385.