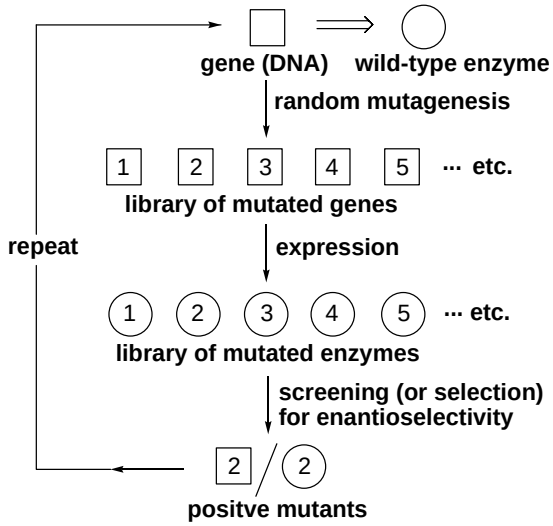
Evolution in the Test Tube as a Means to Create Functional Enzymes

Manfred T. Reetz

Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1
45470 Mülheim/Ruhr, Germany
Email: reetz@mpi-muelheim.mpg.de

Some time ago we developed a fundamentally new approach to asymmetric catalysis, specifically the directed evolution of enantioselective enzymes.[1] It is based on the appropriate combination of gene mutagenesis/expression and high-throughput *ee*-screening.[2] The organocatalytic enzymes that we have studied and are continuing to investigate are lipases, Baeyer-Villigerases and epoxide hydrolases. Highly enantioselective mutants have been evolved by the Darwinistic approach. QM/MM studies help to illuminate the source of enhanced enantioselectivity. In doing so we have used strategies for scanning protein sequence space which are based on error-prone PCR, saturation mutagenesis at the hot spots identified by epPCR and DNA shuffling. Although successful, these strategies are time-consuming. More recently, we have developed the Combinatorial Active-Site Saturation Test (CAST) and iterative CASTing, which we find particularly effective for expanding the substrate scope and enantioselectivity of enzymes.[3] Iterative CASTing is an example of a more general concept, namely "iterative saturation mutagenesis". Another example pertains to the drastic enhancement of protein stability as a result of applying this new form of directed evolution.[4]



(1) First example: M. T. Reetz, A. Zonta, K. Schimossek, K. Liebeton, K.-E. Jaeger, *Angew. Chem*. **1997**, *109*, 2961-2963.; *Angew. Chem., Int. Ed. Engl*. **1997**, *36*, 2830-2832.
(2) Comprehensive review: M. T. Reetz, Directed Evolution of Enantioselective Enzymes as Catalysts for Organic Synthesis. In: *Advances in Catalysis, Vol. 49* (Eds.: B. C. Gates, K. Knözinger), Elsevier: San Diego, **2006**, pp. 1-69.
(3) M. T. Reetz, M. Bocola, J. D. Carballeira, D. Zha, A. Vogel, *Angew. Chem.* **2005**, *117*, 4264-4268; *Angew. Chem. Int. Ed.* **2005**, *44*, 4192-4196. M. T. Reetz, L.-W. Wang, in part M. Bocola, *Angew. Chem.* **2006**, *118*, 1258-1263; *Erratum*, 2556; *Angew. Chem. Int. Ed.* **2006**, *45*, 1236-1241; *Erratum*, 2494. D. Belder, M. Ludwig, L.-W. Wang, M. T. Reetz, *Angew. Chem.* **2006**, *118*, 2523-2526; *Angew. Chem. Int. Ed.* **2006**, *45*, 2463-2466.
(4) M. T. Reetz, J. D. Carballeira, A. Vogel, *Angew. Chem.* **2006**, *118*, 7909-7915; *Angew. Chem. Int. Ed.* **2006**, *45*, 7745-7751.