

Volume Editors' Preface

The field of biocatalysis, defined as the use of enzymes for the transformation of unnatural compounds, dates back almost a century and in its infancy it was driven by curiosity about biochemical pathways and enzyme mechanisms. It was mainly during the 1980s that the enormous catalytic potential of enzymes was recognized for the asymmetric synthesis of unnatural, high-value targets. Subsequently, the increasing demand for environmentally compatible procedures paved the way for the application of biocatalysts for low-cost bulk chemicals. The ability to develop the next generation of biocatalysts was enabled by major technology advances in the biosciences, which triggered several distinct innovation waves:^[1]

- In the 1980s, only crude commercial enzyme preparations from the food, detergent, and tanning industries were available, and their use for stereoselective synthesis had much of a black-box approach. Aiming to broaden the arsenal of enzymatic reactions, chemists began to screen whole microbial cells in the search for novel activities in the 1990s, but enzyme isolation was still a cumbersome task.
- Rapid advances in molecular biology widened the quantitative understanding of biocatalytic systems by means of genomics, proteomics, and metabolomics. These advances facilitated the sequence-based search and subsequent production of suitably tagged enzymes via cloning and overexpression into a reliable host, which has become simple and affordable enough to be carried out by chemists.
- The exponential growth in the availability of crystal structures of proteins has significantly contributed to the understanding of enzyme mechanisms, which allows biocatalysts to be tuned for improved selectivity and stability under process conditions by site-directed mutagenesis. Exploitation of the “catalytic promiscuity” of proteins has often led to unprecedented catalytic activities.
- New methods for activity testing enable high-throughput screening of large libraries of mutant enzymes generated through selective pressure by directed evolution.
- In the near future, the search for a desired catalytic activity, which is generally guided by sequence analogy today, will include the third dimension of a desired catalytic site derived from crystal structures to accommodate the transition state of almost any organic transformation.^[2]
- The compatibility of enzymes with each other has enabled the design of highly efficient synthetic cascades, thereby avoiding the separation of sensitive intermediates.^[3] It is expected that the ever-increasing complexity of cascade design will merge with the field of metabolic engineering, which allows the use of renewable carbon sources more efficiently as alternatives to petroleum-based platform chemicals.

As a result of these developments, it is now possible to obtain biocatalysts that catalyze a much more diverse range of synthetic transformations, including asymmetric amination of ketones (transaminases), C–C bond formation (aldolases, oxynitrilases), oxidation (amine/alcohol oxidases, P450 monooxygenases, Baeyer–Villiger monooxygenases), and reduction (ene reductases, amino acid dehydrogenases), as well as new enzymes for hydrolysis (nitrilases, nitrile hydratases, epoxide hydrolases). The increased availability of new biocatalysts will become even more prominent in the next five years as new biocatalyst platforms (e.g., imine reductases, alkyltransferases, halogenases) move from academic laboratories into practical application.

One impact of this rapidly changing landscape will be that process and medicinal chemists will have additional options for replacing expensive or toxic chemical reagents with more selective and sustainable biocatalysts. Although replacing a chemical reagent

with a biocatalyst represents a significant step forward for biocatalysis, more transformative opportunities are presented when the use of a biocatalyst enables a new synthetic route to the target molecule to be developed. Such routes can be more efficient and cost effective, since they cut out steps in the synthesis and hence reduce costs and waste. Thus, the synthetic chemists of the future will be able to redesign their routes to target molecules using biocatalysts that can catalyze reaction steps not achievable by alternative chemical approaches. Increasingly, chemo- and biocatalysts will be used in concert to develop efficient and telescoped reaction processes including dynamic kinetic resolution and deracemization reactions.

The conversion of an unnatural substrate in a laboratory or industrial process is often limited by the low performance of commercial “off-the-shelf” biocatalysts, which not long ago required an extensive search from biodiversity for an enzyme variant that is sufficiently effective and stable for an economical operation. In this respect, directed *in vitro* evolution has emerged as a powerful technology enabling us to improve essentially any desired property of an enzyme, including its substrate scope, stereoselectivity, catalytic efficiency, robustness to organic solvents, high substrate concentration, pH extremes, and elevated temperatures, or other external factors frequently dictated by optimum process conditions. Since the proof-of-principle stage two decades ago, significant developments with respect to advanced mutagenesis technologies, smart library design, high-throughput-screening methodology, and the introduction of powerful computer algorithms for the prediction of new enzyme function have revolutionized our abilities to rapidly create tailor-made enzymes with optimized properties. The exponential growth in the field of enzyme engineering by evolutive techniques and semi-rational design, drawing from a rapidly increasing wealth of (genome) sequences, protein X-ray structures, and biochemical data, is currently lifting the traditional limitations of enzymes as practical catalysts for synthetic organic chemistry and for the development of sustainable biocatalytic processes of the future.

As a consequence, it is now routinely possible to adapt enzymes to a specific reaction of interest with predefined process conditions rather than vice versa, as proven by the many success stories including the introduction of various new industrial processes on large scale that are based on specifically designed biocatalysts. Successful reports of enzymes being designed *in silico* (“theozymes”) to catalyze unnatural reactions are already emerging. Although computational enzyme design is in its infancy and its impact on biocatalysis still limited, such methods point the way for the future and promise deeper insights into the origins of efficient enzymatic catalysis.

One way to promote the use of biocatalysis when designing synthetic routes to chemical targets is to embrace the concept of “biocatalytic retrosynthesis”.^[4] The fundamental premise of biocatalytic retrosynthesis is that target molecules are disconnected into smaller fragments based upon the increased availability of engineered biocatalysts to catalyze the forward synthetic reactions. Retrosynthesis is a standard tool used by organic chemists when designing novel synthetic routes, but biocatalysts are rarely considered during this design process; this is not surprising, since only recently has a diverse toolbox of biocatalysts become generally available. The now routine application of protein engineering and directed evolution for the creation of novel, robust biocatalysts has radically changed the landscape. With the current rate of progress, it is clear that during the next few years the number of biocatalysts available for use will greatly increase. One area where biocatalysis is having a major impact is in the synthesis of chiral amines. In the future, the synthesis of enantiomerically pure chiral amines will develop along similar lines to asymmetric ketone reduction, i.e. biocatalysts will become the preferred method of choice rather than a replacement for traditional chemical approaches in second-generation processes.

We believe that this broad contemporary overview on the state-of-the-art in enzymatic methods for asymmetric synthesis will be a useful portal for anyone interested in applying biocatalysis as a highly potent, selective, and sustainable technology complementary to metal catalysis and organocatalysis, and that this three-volume set will be a valuable addition to the acclaimed suite of *Science of Synthesis* resources as part of the *Reference Library*, which has an approach orthogonal to the original concept of focusing on product types rather than methodology. We as editors have benefited enormously from the excellent scientific expertise of the many authors from all over the world, and we are grateful for their outstanding efforts and their precious time dedicated to the successful completion of this unique project. Finally, we also would like to express our sincere appreciation to the entire editorial team at Thieme for their extraordinary efforts made toward a seamless handling of manuscripts throughout the entire publication process, but in particular for the excellent collaboration with volume coordinators Alex Russell, Toby Reeve, Matthew Weston, and Mark Smith, and not least to our colleague Joe Richmond for his initiative.

Volume Editors

October 2014

K. Faber (Graz, Austria)

W.-D. Fessner (Darmstadt, Germany)

N. J. Turner (Manchester, UK)

[1] Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K, *Nature (London)*, (2012) **485**, 185.

[2] Steinkellner, G.; Gruber, C. C.; Pavkov-Keller, T.; Binter, A.; Steiner, K; Winkler, C.; Łyskowski, A.; Schwamberger, O.; Oberer, M.; Schwab, H; Faber, K.; Macheroux, P.; Gruber, K., *Nature Commun.*, (2014) **5**, 4150; DOI: 10.1038/ncomms5150.

[3] *Cascade Biocatalysis: Integrating Stereoselective and Environmentally Friendly Reactions*, Riva, S.; Fessner, W.-D., Eds.; Wiley-VCH: Weinheim, Germany, (2014).

[4] Turner, N. J.; O'Reilly, E., *Nature Chem. Biol.*, (2013) **9**, 285.