Editorial Commentary

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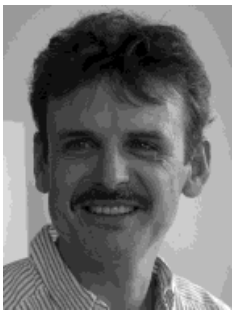

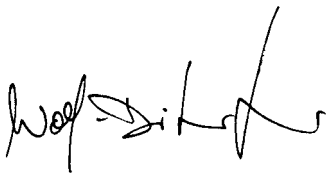

Worldwide, chemists are vigorously taking on the challenge of developing synthetic methodology and "green" processes that meet the criteria of a sustainable, environmentally conscious development. Here enzyme catalysts often have a "natural" advantage that will increasingly be exploited as the demand for enantiomerically pure drugs continues to rise. It is most gratifying to see that biocatalytic transformations today are routinely considered by leading synthetic organic chemists, and by process engineers, as an economically and ecologically competitive technology, and as a matter of course for the development of new production routes to fine chemicals, pharmaceuticals, agrochemicals, and even bulk commodities. The trend is clear, even though a full realization of the potential benefits of biocatalysis in industry has not yet been reached, as pointed out in the accompanying Commentary by Mark Burk. With the growing acceptance of enzymes by the synthetic community, market demands are now pushing for individual tailoring of biocatalysts for specialized applications, driven by the new opportunities that have emerged for the discovery of novel enzymes and the fine-tuning, and even redesign, of their properties.

While the range of enzyme classes to be considered for technical processes is growing remarkably broad, and a steadily increasing number of different bioprocesses are being successfully transplanted from the laboratory to the manufacturing plant, industry continues to demand improved biocatalysts — having customized selectivity, stability, and efficiency. With the advent of the genome age — in which unraveling of entire genome sequences of microorganisms, invertebrates, plants, and mammals is proceeding at an accelerating, almost weekly, rate — an increased emphasis can now be placed on the discovery or evolution of new or improved biocatalysts, including those from previously untapped sources.

In fact, tailoring of enzyme properties to meet the "market demands" of high activity, stability, and enantioselectivity, as well as the appropriate substrate (or cofactor) selectivities, is a long-standing *Holy Grail*-type goal of protein engineering. Designing proteins for a desired function is impeded by the complexity of the problem and by the limitations in our current understanding of the relationships between a protein's sequence, structure, mobility, and function. Traditionally, the rational approach of protein redesign aims at controlled amino acid exchanges by site-directed mutagenesis, which is resource- and labor-intensive and requires a detailed knowledge of the spatial structure of the wild-type enzyme and mechanism-based functional data. Despite the many successful attempts, such re-engineered enzymes are often much less efficient in comparison to their natural counterparts.

On the other hand, recent innovative evolutionary approaches circumvent the problems associated with rational design. Spurred by remarkable advances in genetic mutation techniques, 'directed evolution' takes advantage of the statistical element of Darwinian evolution in a test tube and offers a high potential for speed, flexibility, and efficiency. In this approach, genetic variations are induced by random mutagenesis or by fragment recombination along several generation cycles, and each resulting diversity is subsequently evaluated by rounds of screening of the corresponding protein population to identify enzyme variants having optimized characteristics. Directed evolution of enzymes has yielded biocatalysts with remarkably improved properties. Experimental work has so far demonstrated that directed evolution is particularly suited to "tuning" enzyme function, that is, by improving an activity that already exists at some (if only low) level. In this respect, optimized biocatalysts have been generated showing improvements in substrate or cofactor specificity, enantioselectivity, thermostability, or activity under various environmental conditions (organic solvents, pH, etc.). Surprisingly, it became apparent that mutations or muta-

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tional hot spots are rarely located at the active site but that improved function requires cumulative effects of a number of distant mutations — often more than 20 Å apart and frequently at loops close to the protein surface. Obviously, beneficial mutations can exert their influence on the catalytic function over large distances, in a manner that currently is very difficult, if not impossible, to predict — unexpected observations will thus be the rule rather than the exception.

However, it seems that the philosophically distinct schemes (rational *vs*. "irrational") are no longer mutually exclusive. In fact, the most spectacular examples for the generation of improved or novel biocatalysts are due to a combination of methods: Very recently, even the creation of a new catalytic activity from a given substrate binding site has been successfully realized, by using combined contemporary methods of mutation, recombination, and selection.

Transformations using biocatalysts are now recognized as attractive alternatives for the synthesis of a number of important and commercially significant compounds that are complementary to the traditional chemical approaches. It is obvious that recent landmark advances in molecular biology have started to profoundly alter the character of the field of applied biocatalysis and to accelerate the pace of its future progress. With rapid access to unique enzymes having improved or novel properties from various sources of biodiversity, and from ingenious DNA recombination techniques along an evolutionary approach, it can be anticipated that critically shorter process development cycles will be achievable — in fact, this may be a precondition to overcome a persisting skepticism about biotechnology that so far has limited the full realization of its potential in the chemical industries. This is an exciting time in which fascinating possibilities of fundamental scientific significance are becoming evident that open new playgrounds for creative minds, and that will stimulate the development of effective new biocatalytic tools to tackle the current and future challenges presented by the demands of organic synthesis, in a sustainable manner.

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