Preface

New Methods for the Discovery and Implementation of Novel Biocatalysts

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The use of biocatalysis as a synthetic tool by organic chemists has been increasing rapidly over the last several years in part because of the rapid advancements in the field. One of the most important breakthroughs has been the advent of new methods for discovering and engineering custom biocatalysts. Another great leap has been the establishment of effective methods for development of new applications that have promise as large-scale manufacturing processes. This issue of *Bioorganic and Medicinal Chemistry* focuses on sampling some of the leading edge solutions which are being developed for the identification of new biocatalysts and the design of high-yield, scaleable bioprocesses.

This issue has been organized into two basic sections. The first section contains contributions on new technologies and methods for the discovery and development of enzymes. The second section documents methods for the implementation of new bioprocesses, often using specific application examples.

Enzyme Discovery

In the past, a number of challenges have impeded the successful development of biocatalytic synthetic processes, preventing biocatalysis from becoming the broadly powerful synthetic tool that had been predicted. One of the most widespread challenges was that isolated enzymes were notoriously unstable. While this perhaps did not hamper the development of biocatalytic syntheses for small-scale applications, it resulted in reproducibility problems that implied an inability to use the enzymes on a larger scale. This, in turn, led to an overall reluctance by many researchers to work with catalysts which could never lead to real-world solutions. In addition, even though the activity of enzymes in organic solvents had been demonstrated, most enzymes lost a significant portion of their activity in organic solvents. Several technologies have now been developed to address these problems, including the use of thermostable enzymes, enzyme immobilization, new whole-cell technologies and directed evolution technologies to create stable enzymes for industrial settings.

Another challenge was the fact that each reaction potentially needs a different custom enzyme. While the same enzyme can often recognize similar substrates, even highly related molecules may need a different catalyst to optimally carry out a reaction. With the availability of larger commercial enzyme libraries and directed evolution technologies to create mutant libraries of enzymes, it is now possible to either discover or engineer enzymes with unique substrate specificities and selectivities that are robust enough for organic synthesis applications.

Several of the papers in this issue address the discovery or engineering of new enzymes. **Schmidt-Dannert** reviews efforts to clone and express recombinant lipases from a number of different organisms so that systems for their eventual engineering and production can be effectively employed. **Brush** and co-workers describe the discovery and characterization of lipases from a fungus that has evolved to help assimilate lipids from wood sources. **Benkovic** et al. describe an approach for the generation of new enzymes by using incremental truncations of proteins, extending the traditional bounds of a directed evolution approach. In addition, **Roberts** and colleagues review the use of shorter polyamino acids as effective catalysts, especially for asymmetric reactions such as epoxidations.

There are a number of challenges to the successful development of a commercial biocatalysis process. Most of these challenges can be addressed at the outset by properly setting up a screen or selection, in order to identify a novel catalyst which has the properties necessary to carry out a reaction that can be scaled-up. Screens are easier to develop, more readily available and can often give quantitative results, but require that

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every single colony be analyzed. Selections are more difficult to develop and are qualitative, but allow much higher throughput since only the colonies with activities of interest grow. However, when they can be developed they allow for the cloning of genes by expression complementation.

Screening of enzymes is an important step in identifying the proper catalyst for a reaction. **Krstenansky and Khmelnitsky** describe a combinatorial biocatalysis approach to screen enzyme libraries for creating compound diversity. As enzyme libraries grow and high throughput screening methods develop, new types of assays that utilize the specific target substrates instead of substrate analogues are needed. A number of papers are directed at developing novel screening techniques to identify clones and enhanced mutant forms of enzymes. **Taylor** et al. developed a novel screening approach using a replica filter to identify clones that expressed a gamma-lactamase from a clone bank. **Bornscheuer** et al. describes novel screening methods for directed evolution of hydrolases such as esterases and lipases. **Chang, Terwilliger**, and colleagues also use a pH-based indicator process to screen for mutants of a haloalkane dehalogenase with improved activity on 1,2-dichloroethane. As described in the paper by **Morís-Varas** et al., the pH monitoring approach is not only potentially useful for enzyme screening but also process optimization applications as well.

Bioprocess Implementation

There are three basic biocatalyst forms that can be used: isolated enzyme, whole cell, or immobilized biocatalyst. In the first strategy, an isolated enzyme can be used in a soluble form to carry out the process. The use of lipases in organic media for the formation of amide and carbamate bonds is reviewed by **Gotor**. **Zaks** et al. describes the use of microsomal glucuronlyl transferases for the synthesis of glucuronidated compounds and preparation of an I^{125} labeled analytical reagent.

The enzyme can also be used as part of a whole-cell biotransformation. This is often the preferred way of implementing biocatalysts which have cofactors that cannot be recycled or membrane bound enzymes, or enzymes which are difficult to produce or use in isolated form for other reasons. New strategies for the metabolic engineering of organisms have also made great advances in the ability to carry out complex multicatalytic processes. Two papers in this issue document the development of novel strains by metabolic engineering of *Escherichia coli*. These methods can be extremely powerful, since they incorporate multiple enzymatic steps into one process step. **Fotheringham** et al. demonstrate the metabolic engineering of *E. coli* with three genes to synthesize L-2-amino butyric acid. **Scott** et al. has demonstrated the production of an isobacteriochlorin by the engineering of *E. coli*.

Finally, an isolated enzyme or whole cell can also be used in immobilized form for recovery and reuse. In addition, immobilization often improves stability and longevity of the enzyme. **Ng** and co-workers describe the development of biocatalytic acylation and deacylation processes using soluble and immobilized penicillin acylase, respectively. **Fischer** and **Peißker** discuss the use of a molecular imprinting method to stabilize proteases in both aqueous and organic media. **DiCosimo** et al. describe the use of immobilized *Pseudomonas chlororaphis* for the effective implementation of a nitrile hydratase process.

The choice of which method to use to implement a biocatalysis process often comes down to chemical engineering considerations, based on the way the process works. In the manuscript by **Hanson** et al., a number of amino acid dehydrogenases were identified which were useful in a reductive deamination process using a cofactor recycling method. **Steckhan** and **Peterson** describe the development of an electrochemical method for the regeneration of galactose oxidase and elimination of hydrogen peroxide build-up.

Despite the many recent advances and the increase in the general acceptance of biotransformation for chemical applications, there is still a perception that biocatalysis has not led to many industrial processes, and is still not practical. In a paper by **Rozzell**, several of these myths are addressed and many large-scale biocatalytic processes that are currently being used in the production of pharmaceutical intermediates have been identified.

Development of a biocatalytic route has historically taken years to complete, impeding the acceptance of biocatalysis in industry. The new methods described in this collection all help speed the biotransformation development process. Recent advances in molecular technology have allowed the rapid and effective discovery and characterization of biocatalysts for chemical synthesis applications. New methods for screening, the creation of enzyme libraries, the development of diverse organism and clone banks, and new methods for assaying enzyme and evolving proteins of interest are helping to solve the challenges of biocatalysis. Effective methods for scaling up and implementing biocatalysts have now been established. At the dawn of the new millennium we can now expect to see biotransformations more generally accepted and integrated into synthetic chemistry processes.

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