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The expanding roles of biocatalysis and biotransformation

Editorial overview

Grace DeSantis and Benjamin G Davis

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Grace DeSantis

Principle Scientist, Diversa Corporation, 4955 Directors Place, San Diego, CA 92121, USA
e-mail: grace_desantis@hotmail.com

Grace DeSantis joined Diversa early in 2001 after training in the areas of enzymology and biocatalysis in the labs of Professor JB Jones at the University of Toronto as a doctoral student, and in the labs of Professor C-H Wong at the Scripps Research Institute as a postdoctoral fellow. Her research interests span the application of enzymes as protein therapeutics and biocatalysis for fine pharmaceutical intermediate manufacture and industrial processes. At Diversa, she leads a group of scientists focused on assay development and enzyme characterization aimed at discovery and optimization of enzymes for varied applications. She has contributed to numerous publications and patents in this area. In addition to her responsibilities at Diversa, she remains active in the scientific community through invited presentations and contributions on scientific advisory panels.

Benjamin G Davis

Chemistry Research Laboratory, University of Ocford, Oxford OX1 3TA, UK
e-mail: Ben.Davis@chem.ox.ac.uk

Ben Davis got his B.A. (1993) and D.Phil. (1996) from the University of Oxford. During this time he learned the beauty of carbohydrate chemistry under the supervision of Professor George Fleet. He then spent two years as a postdoctoral fellow in the laboratory of Professor Bryan Jones at the University of Toronto, exploring protein chemistry and biocatalysis. In 1998 he returned to the UK to take up a lectureship at the University of Durham. In the autumn of 2001 he moved to the University of Oxford and received a fellowship at Pembroke College, Oxford. His group's research centers on chemical biology with an emphasis on carbohydrates and proteins. In particular, the group's interests encompass synthesis and methodology, inhibitor design, protein engineering, drug delivery, molecular modeling, molecular biology, and glycoscience. Their work has received the RSC Meldola medal and prize, RSC Carbohydrate Award, the AstraZeneca Strategic Research Award, a DTI Smart Award, a Mitzutani Foundation for Glycoscience Award, the Philip Leverhulme Prize and the Royal Society Mullard Medal. Ben is on the Editorial Board of *Carbohydrate Research* and *Organic and Biomolecular Chemistry*, is co-founder of Glycoform, and in the MIT TR100.

The collection of diverse articles in this year's issue of *Current Opinion in Chemical Biology* is aimed at highlighting the diversity of roles that biocatalysis fulfills. From the very ancient and historic role of enzymes in cheese and wine making to their more fashionable utility in bioremediation, it becomes evident that harnessing the contribution of enzyme transformations in all aspects of science has led to great advances. The scope of influence and effectiveness of enzymes and biocatalysts continues to grow at an exciting pace fueled by increased understanding of enzyme mechanisms, effective metabolic pathway engineering, protein evolution methods and huge advances in the number and methods by which new enzymes may be discovered and characterized.

In an increasingly post-genomic era, the stronger emphasis placed on function rather than simply sequence is bringing more and more techniques into the realm of biocatalysis, a field which by definition has always had function at its core. The articles in this issue therefore highlight the breadth of the field ranging from the large-volume application of enzymes as key catalysts to address the energy shortages via biomass conversion (**Gray *et al.***) to the smaller-volume application of enzymes in diagnostic applications (**Murphy**). Our aim has been to highlight how biocatalysts may be used not only to exquisitely transform but are also vital and increasingly relevant tools for solving and probing biological questions.

While the debate as to what are genuinely sustainable sources of energy rages, it cannot be denied that sugars and, in particular, D-glucose, tied up as polysaccharide, is the most abundant organic molecule on the planet. Accessing its oxidative power allows us to, in part, reap the wonderful productivity of photosynthesis. Enzymes are poised to make accessing biofuel alternatives to petroleum an economically sustainable and attractive prospect. Although fuel ethanol today can be, and currently is, primarily derived from corn grain (starch) and sugar cane (sucrose) substrates, the limits on these raw materials necessitate use of more readily available glucoconjugates (e.g. lignocellulosic biomass) as substrates. Despite the long-standing work on cellulases, viability still rests on effectively reducing capital and operating costs. As **Gray, Zhao and Emptage** highlight, advances in understanding the synergy between carbohydrate recognition and multiple hydrolytic families as well as novel fermentation systems is now bringing the biofuel arena to a critical point. Thus, enzymes and their associated technologies will take center stage as the tools to make these routes economically viable.

The theme of carbohydrate-processing biocatalysts is continued by **Yip and Withers**. Withers' group, amongst others, has for many years generated

illuminating mechanistic insights into the most prevalent mode of glycosylhydrolases that operate through concerted general acid-base and nucleophilic catalysis. However, as nature frequently shows us, there are many ways to skin a cat and in their review here they highlight some elimination-based mechanisms that have been uncovered in recent years. These involve E1 or $E1_{cb}$ pathways, including some with intriguing intervening redox manipulation, to achieve an overall cleavage of the glycosidic linkage. Given the breathtaking diversity of glycoconjugates in nature, such a detailed mechanistic understanding will not only allow future exploitation but shed light on fascinating evolutionary parallels.

Given their ability to accomplish transformations (e.g. stereoselective, C–H bond activation) that traditional synthetic methodologies would be envious of, oxygenases are a powerful class of enzymes that surprisingly have remained largely under-exploited compared with other classes of industrial enzymes. Some challenges that have plagued this class are the need for reduction equivalents and electron transfer partners as well as stability and capricious substrate selectivity issues. **Urlacher and Schmid** review how many of these challenges are being addressed via targeted mutagenesis and directed evolution methods. These methods, targeted primarily at isolated enzymes, together with whole-cell engineering methods, have recently been applied to improve the practicality of cytochrome P_{450} (CYP) Baeyer-Villiger monooxygenases and Rieske dioxygenases. Continued advances in this area are sure to enhance the utility of the oxygenases in both bioremediation and chemical synthesis applications.

Kumar and Clark provide a timely review of the multitude of methods for HTS of biocatalytic activity. Such platforms enable a more facile screen of enzyme targets. The article focuses on the application of various screening platforms such as multiwell, microfluidics, sol-gel entrapment, and strategies such as immunoassay-based detection LC-MS, fluorescent substrates and bioluminescence, to screening of enzymes with the primary aim of small-molecule inhibitor drug discovery. Such approaches not only allow an alternative to more traditional methods of screening against *in vitro* targets but also importantly allow rapid pre-screening of potential metabolic processing (e.g. against multiple CYP isoforms). Moreover, as noted by the authors, these same methods are now starting to be exploited in HTS for activity, specificity and selectivity of enzymes. In some cases they are also allowing the first detailed assessments of more difficult multisubstrate enzyme classes (e.g. ligases, transferases) that have typically been intractable using traditional (e.g. chromophoric) methods.

Wilkinson and Bachmann provide an overview of the recent advances in the application of synthetic biology to prepare and alter small molecules of pharmaceutical importance. As the authors highlight, this emerging discipline is combining a rich tradition of the detailed mechanistic understanding of secondary metabolite biosynthesis with advanced molecular biology to provide an exciting cutting-edge to the realistic development of natural products as therapeutics. Through either stepwise biotransformation or via the application of engineered biosynthetic pathways, a plethora of new and improved natural product structures may be accessed. Often such access provides unrivalled diversity that is based on unrivalled biocatalyst selectivity and breathtaking biosynthetic pathways. Approaches highlighted include: pathway engineering to access variants of the rapamycin scaffold aimed at addressing physiochemical limitations of the natural structures, whole-cell approaches to alter glycosylation patterns (which often modulate the activity of the natural products to which they are attached) and innovative heterologous expression of otherwise inaccessible or cryptic pathways that in essence provide a new window for natural product discovery.

Murphy reviews the use of enzymes in biosensor applications. Biocatalysts in biosensors have a noble history in the development of analytical systems that are now widely used, such as detection of blood glucose levels in diabetes patients. This ability to directly detect exploits both the selectivity and amplification of biocatalysis, often in challenging biological milieu. The drive towards miniaturization through the application of nanotechnology is now starting to bear fruit, and the excitement that methods for direct DNA detection and/or sequencing might offer are truly mouth-watering.

In essence, biocatalysis offers solutions where other methodologies often struggle, and may provide the solution to issues of economic sustainability. Although often seen as a specialism or a curiosity by some, the ability of biocatalysis to get 'undoable' jobs done means that this approach can't be ignored. The scope of influence and effectiveness of enzymes and biocatalysts continues to grow as we, the scientists in this area, drive the technology development and the business needs pull the growth in economically viable and sustainable directions.