

Conference Report

Achievements and Challenges in Biotransformation

30 March 2004 Imperial College, London

RSC Biotechnology Group and RSC Chemical Biology Forum

This one-day Meeting, held at the Sir Alexander Fleming Building, School of Biomedical Sciences, Imperial College of Science, Medicine & Technology, featured seven speakers, two from Academe and five from Industry. By contrast, the delegates from Academe outnumbered those from industry by about two to one.

The total attendance was 54. The delegates clearly enjoyed the Conference, since there were lively Question and Answer Sessions after most of the Lectures and the informal feedback to the Conference Committee on the day was good.

The names of the speakers – including one from the US and one from The Netherlands - and the titles of their lectures are given in the attached Programme. An Abstract of each lecture follows.

We thank Imperial College for their hospitality, *Astrozeneca*, *Pro-Bio Faraday Partnership* and *The Chemical Biology Forum* for generous Sponsorship, and we wish to acknowledge the important contribution to the smooth-running of the administration of the Conference by Elaine Wellingham.

The Conference Committee

Dr Irene Francois (Chair) Consultant and Biotechnology Group

Dr Helen Hailes Chemistry Department, UCL and Biotechnology Group

Dr Paul Dalby Biochemical Engineering Department, UCL and Biotechnology Group

Dr Martin Hayes Astrozeneca and Biotechnology Group

Dr Colin Bedford Chemistry Department, UCL and Biotechnology Group

Dr Simon Edwards, Manager, Life Sciences, Royal Society of Chemistry

14 April 2004

The full Programme and the ABSTRACTS appear below.

Achievements and Challenges in Biotransformation

FINAL PROGRAMME

- 09.30 Coffee and Registration
10.30 Opening remarks and Session I
Chair: Helen Hailes (University College London)
- 10.35 Rapid evolution of genes for improved biocatalysis
Gjalt W Huisman (Codexis Inc. USA)
- 11.15 Directed evolution of an oxidase enzyme for the deracemisation of secondary alcohols
Andrew Carnell (University of Liverpool)
- 11.55 Recent experiences in biocatalysis: Challenges and opportunity
Stephen Taylor (Chirotech Technology)
- 12.35 Lunch
13.35 Session II
Chair: Colin Bedford (University College London)
- 13.40 Expanding the synthetic repertoire of transketolase
Paul Dalby (University College London)
- 14.20 Application of C-C bond forming enzymes in custom manufacturing
Marcel Wubbolts (DSM Pharma Chemicals, The Netherlands)
- 15.00 Tea
- 15.25 Session III
Chair: Martin Hayes (AstraZeneca)
- 15.30 The application of biocatalysis to stereoselective synthesis: From milligrammes to tonnes
Robert Holt (Avecia Pharmaceuticals)
- 16.10 Natural ingredients – naturally
John Sime (Novacta Biosystems)
- 16.50 Closing remarks
16.51

THE BIOTECHNOLOGY GROUP

The Biotechnology Group is the focal point within the RSC for members who have an academic or industrial interest in the chemical aspects of biotechnology. New members of the Committee are currently required to widen the area of its expertise and those with an interest in modern aspects of the chemistry of life are warmly invited to contact either the chairman or the secretary.

Professor Steve Harding (Chairman), or Dr Colin Bedford (Secretary), *c.t.bedford@ucl.ac.uk*

Rapid evolution of genes for improved biocatalysts

Gjalt W. Huisman

Codexis Inc., Redwood City, CA, USA

The utility of enzymes as highly specific catalysts in the manufacture of chemicals was described as long ago as 1894, yet the introduction of biocatalysts in actual chemical processes is still limited due to intrinsic characteristics of these catalysts. The recombinant DNA revolution of the 1970s enabled the production of enzymes at much higher levels, thereby directly making some enzymes available at low price. However, crucial shortcomings of natural enzymes, including insufficient selectivity, productivity, and tolerance to feedstock and/or product, are still impeding the commercialization of biocatalytic processes.

By integrating recursive DNA sequence recombination (DNA shuffling) with process-appropriate screening methodology, Codexis has realized significant improvements in the catalytic performance of biocatalysts and fermentation organisms, and some of these have already been commercialized. The fundamental advantage of these technologies is that an enzyme with the desired characteristics is developed for a process, rather than a process being developed around a catalyst. This concept allows the catalyst and the process to improve in concert as increasing pricing pressure becomes relevant during the lifecycle of a pharmaceutical product through clinical trials, launch, eventually facing generic competition.

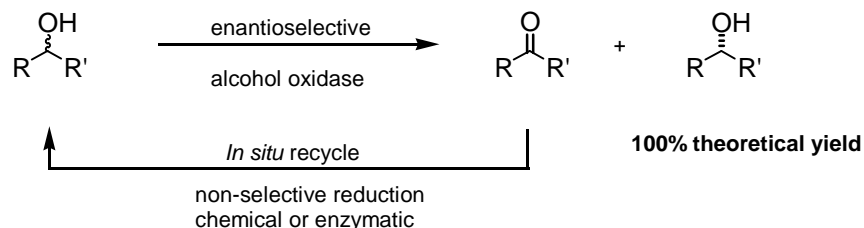
This presentation will give an overview of the DNA shuffling technology and will describe examples of our recent work in the area of biocatalysis and fermentation. It will be illustrated how multiple enzyme traits can be co-evolved to create superior biocatalysts with increased activity, stability and preferred chemo-, regio- and stereospecificity under desirable process conditions.

Directed evolution of an oxidase enzyme for the deracemisation of secondary alcohols

Andrew J Carnell

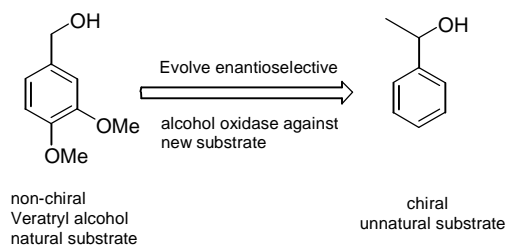
Department of Chemistry, University of Liverpool, Liverpool L69 7ZD

Chiral secondary alcohols are widely used as synthetic intermediates, chiral auxiliaries and analytical reagents and can be obtained by resolution or asymmetric reduction processes. The deracemisation of racemic alcohols is an attractive approach since there is, in principle, no need to discard or recycle an unwanted enantiomer. Biocatalytic deracemisation processes are an efficient method by which chiral secondary alcohols can be obtained.¹ The combination of transition metal-catalysed racemisation with an enantioselective lipase-catalysed esterification has been reported by several groups.^{2,3} A lipase-racemase two-enzyme system has been used to achieve deracemisation of mandelic acid.⁴ We and other groups have reported the deracemisation of alcohols and diols using microbial systems containing redox enzymes.^{5,6} Nakamura has reported the deracemisation of 1-aryl ethanols with *Geotrichum Candidum* IFO 5767.⁷ It is also possible to use two isolated dehydrogenase enzymes with matched selectivity to achieve similar transformations. More attractive is the use of an oxidase, which does not require the addition of nicotinamide cofactors, to catalyse the initial enantioselective oxidation in combination with a chemical reduction leading to overall deracemisation (Scheme 1).⁸



Scheme 1

In this lecture we will see how microorganisms are able to carry out the deracemisation of a range of alcohols, amidoalcohols and diols. The advantages and limitations of using whole cells *versus* isolated enzymes for such a transformation will be discussed. An alcohol oxidase, whose natural substrate is veratryl alcohol has been improved using directed evolution for the enantioselective oxidation of phenyl ethanol (Scheme 2). The design of the screen and future prospects for very high throughput screening to allow higher frequencies of mutation will be presented.



(1) Stecher, H., Faber, K., *Synthesis*, **1997**, 1.

- (2) (a) Allen, J.V.; Williams, J.M.J. *Tetrahedron Lett.* **1996**, *37*, 1859. (b) Dinh, P.M.; Howarth, J.A.; Hudnott, A.R.; Williams, J.M.J.; Harris, W. *Tetrahedron Lett.* **1996**, *37*, 7623. (c) Larsson, A.L.E.; Persson, B.A.; Bäckvall, J.E. *Angew.Chem., Int. Ed. Engl.* **1997**, *36*, 1211. (d) Reetz, M.T.; Schimossek, K. *Chimia*, **1996**, *50*, 668.
- (3) Persson, B.A.; Larsson, A.L.E.; Le Ray, M.; Bäckvall, J.E. *J. Am. Chem. Soc.* **1999**, *121*, 1645.
- (4) Strauss, U.T.; Faber, K. *Tetrahedron Asymmetry* **1999**, *10*, 4079.
- (5) Carnell, A.J. *Advances in Biochemical Engineering Biotechnology/ Biotransformations* **1998**, 57.
- (6) Carnell, A.J.; Allan, G. R. , *J. Org. Chem.*, 2001, *66*, 6495.
- (7) Nakamura, K., Inoue, Y., Matsuda, T., Ohno, A. *Tetrahedron Lett.* **1995**, *36*, 6263.
- (8) Kroutil, W.; Faber, K. *Tetrahedron: Asymmetry*, **1998**, *9*, 2901.

Recent Experiences in Biocatalysis: Challenges and Opportunity

Stephen Taylor
Chirotech Technology Ltd, Cambridge

Recent chemistry literature suggests that of the wide range of biocatalysts in use, a high proportion are hydrolases, a situation that has persisted since the early 1990's. In particular, lipases dominate. This reflects the experience of the speaker.

There are two approaches to biocatalysis. In the first, off-the-shelf enzymes may be exploited. This is typically to satisfy customer demands for chiral intermediates in the pharmaceutical fine chemical arena, where speed and cost are prime considerations. In the second approach a longer term view is taken, and investment put into discovering and developing new enzymes for customers and/or products that may not yet exist. Here one seeks a competitive edge and a broadly applicable biocatalyst to secure future revenues. Each approach has their own challenges, but in either the biocatalyst that goes into a process must satisfy the criteria needed for "industrial readiness", in relation to performance, cost, availability and right to use.

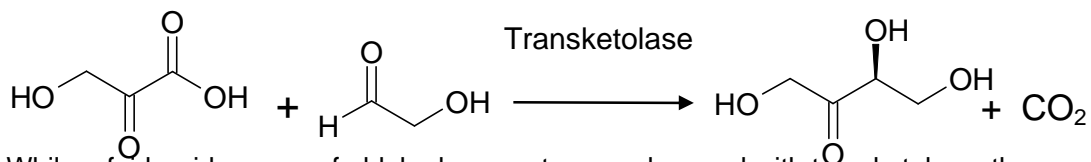
Several approaches to enzymes discovery and development in the latter long-term approach are described. Whilst these may be exploited in traditional fine chemical fashion, they can present new opportunities. For example, their application for the synthesis of some complex cyclic amino acids that have two or three chiral centres is described. Such molecules make ideal building blocks for the drug discovery process. Further value may be demonstrated if one actually takes the effort to elaborate such molecules further and demonstrate biological activity. In this respect, a cyclopentane structure was used to mimic the natural product balanol, which is a potent protein kinase inhibitor. By making and studying the individual isomers it was possible to assess the relative importance of each chiral centre for biological activity, and thereby to identify potent inhibitors. This is one of a number of ways in which biocatalysis can contribute very effectively to the drug discovery process.

Expanding the synthetic repertoire of transketolase

Paul A. Dalby

Dept Biochemical Engineering
University College London
Torrington Place, London WC1E 7JE
Tel: 020 7679 2962
p.dalby@ucl.ac.uk

Transketolase catalyses the transfer of a two-carbon ketol unit from xylulose 5-phosphate to an aldehyde acceptor such as erythrose 4-phosphate, producing a new C-C bond and chiral centre with high enantioselectivity. An alternative ketol donor, beta-hydroxypyruvate, can be used to ensure that the reaction is irreversible, producing CO₂ as a byproduct, and hence cost effective at large scale.



While a fairly wide range of aldehyde acceptors can be used with transketolase, the range of ketol donors is much more restricted. It is highly desirable to expand the range of both acceptor and donor substrates to obtain a version of the enzyme with broadened substrate specificity. Such an enzyme would be useful as an 'off the shelf' enzyme that can be used more generally in organic synthesis, but also as a valuable starting point for directed evolution in the development of large-scale biocatalytic routes.

We are currently exploring the use of bioinformatics combined with genetic engineering to obtain a broad-range transketolase. The methodology for identifying potential broad-range enzymes will be presented and the results of preliminary kinetic characterisations will be rationalised in terms of structural considerations.

Application of C-C Bond Forming Enzymes in Custom Manufacturing

Marcel Wubbolts

DSM Pharma Chemicals, Center for Advanced Synthesis, Catalysis & Development, P.O.
box 18, 6160 MD Geleen, Netherlands, marcel.wubbolts@dsm.com

Stereoselective carbon-carbon bond forming reactions are a great challenge in organic chemical synthesis. Carbon-carbon bond forming enzymes however, typically exert tight stereo-chemical control during the reaction and can achieve the synthesis of a variety of important chiral intermediates for application in pharma and agro products.

DSM Pharma Chemicals, in collaboration with a number of academic partners, has developed a series of novel enzyme platforms that enable the formation of C-C bonds from a variety of aldehydes and ketones. These platforms include enzymes such as (*R*)- and (*S*)-threonine aldolases, deoxyribose-5-phosphate aldolase and (*R*)- and (*S*)-hydroxynitrile lyases from various sources that have been used for the synthesis of a number of chiral products at excellent e.e's. Examples of syntheses utilizing enzymatic C-C bond formation and the challenges encountered during the implementation of such processes will be presented.

**The application of biocatalysis to stereoselective synthesis:
From milligrammes to tonnes**

Robert A Holt
Avecia Pharmaceuticals
Billingham TS23 1YN

A key target for pharmaceutical companies is to launch new drugs as quickly as possible to make maximum use of the period of exclusivity provided by a patent and hence recover the huge investments expended in the discovery and development of a new drug. A class-leading drug can bring in revenues of well over \$1million per day so every day counts. This pressure to deliver quickly is passed down the supply chain to the pharmaceutical intermediate supplier who is expected to invent, develop and scale-up novel synthetic routes to key intermediates in very short time frames and at reasonable cost. Often the target molecules are very complex containing multiple chiral centres and this makes resolution approaches unattractive due to the high yield losses that inevitably occur. One of the most common functional groups encountered in pharmaceutical synthesis is a chiral secondary alcohol and in this presentation I will describe how we have invented and scaled-up to multi-tonne scale the biological asymmetric reduction of ketones to provide efficient routes to complex molecules containing multiple chiral centres. The identification of the appropriate biocatalyst is often the easy part, developing a process that can be operated at scale is sometimes more of a challenge.

Natural Ingredients – Naturally

Dr John T Sime

Novacta Biosystems Ltd, Norwich, UK

john.sime@novactabio.com

The natural status of biocatalysis, in both perceptual and legal contexts, provides significant commercial opportunities in a number of industrial markets.

The production of natural vanillin, without dependency on the vanilla orchid, has been a target for many research groups for decades. The vision has now become reality and the consequences are being dealt with in the food and flavouring sectors of the world market.

The reasons for the desirability of the process will be discussed as well as the various significant scientific discoveries that led to the creation of the innovative biotransformation reactions required to realise a challenging modification of chemical functional groups in a cost effective, scalable and acceptable manner.

These scientific processes are now leading to further definitions of natural flavours and methods to detect adulteration of high value ingredients.