Conference report

RSC Advances in Biocatalysis

April 21st 2009, University College London
RSC Biotechnology Group and RSC Chemistry Biology Interface Forum

This one-day Meeting, held at the Anatomy Department, University College London, featured nine speakers, eight from Academe and one from the US Industry. The total attendance was 69 and largely from the Biotechnology Industry. The delegates clearly enjoyed the Conference, since there were lively Question and Answer Sessions after most of the Lectures and the informal feed-back to the Conference Committee on the day was good. The poster & lunch buffet session provided an excellent opportunity for lively networking.

The Names and Profiles of the Speakers and the Titles and Abstracts of their presentations are listed in the attached sheet. Also listed are the Names of the Presenters of Posters and their Titles and Abstracts.

We thank University College London for their hospitality and *The Chemistry Biology Interface Forum* for generous Sponsorship, and we wish to thanks the speakers and helpers on the day for ensuring the smooth running of a scientifically exciting conference.

The Conference Committee
Dr Paul Dalby (Chair) Biochemical Engineering Department, UCL and Biotechnology Group
Dr Colin Bedford Chemistry Department, UCL and Biotechnology Group
Dr Brendan Fish, MedImmune, Cambridge.
22 April 2009

Advances in Biocatalysis. 21st April 20009, University College London, UK.

The RSC Biotechnology Group and Chemistry Biology Interface Forum welcome you to this exciting one-day symposium. The purpose of the meeting is to highlight recent achievements and to identify future challenges and drivers in the field of biocatalysis.

09.30 Coffee & Registration

10.25 Opening Remarks and Session I
Chair: Paul Dalby

10.30 David Rozzell: Solidus Biosciences, CA, USA
Biocatalysis: Historical perspective and future directions

11.00 Nick Turner: CoeBio3, Manchester, UK
Biocatalytic routes to enantiomerically pure chiral amines

11.30 Gideon Grogan: York, UK
Intragenomic biocatalytic diversity. The family of Baeyer-Villiger monooxygenases from Rhodococcus jostii RHA1

12.00 Jenny Littlechild: Exeter, UK
Thermophilic enzymes as stable biocatalysts

12.30 Lunch & Poster session

14.00 Session II
Chair: Colin Bedford

14.05 Helen Hailes: UCL, UK
Biocatalytic approaches to ketodiols and aminodiols

14.30 Peter Halling: Strathclyde, UK
Understanding immobilised enzymes and immobilised substrates

15.00 Andy Carnell: Liverpool, UK
Increasing efficiency in the synthesis of pharmaceuticals and chiral ligands using enzymes

15.30 Tea, Posters & Networking

16.10 Session III – short talks chosen from abstracts
Chair: Helen Hailes

16.15 Matt Truppo: University of Manchester (CoEBio3), UK / Merck & Co. Inc., USA
Process development of transaminase catalyzed reactions for large scale industrial use

16.30 Nicolas Szita: UCL, UK
Microfluidic Enzymatic Reactor for Synthesis

16.45 Closing Remarks

Speaker Profiles

David Rozzell, CEO*, Solidus Biosciences, Troy, New York, USA*
Biocatalysis: Historical Perspective and Future Directions"

Biocatalysis has gone through some distinct “Boom” and “Bust” cycles in the past. Previous cycles ended in disappointment when the promise of biocatlaysis did not live up to the hype. Now the field of biocatalysis in another uptrend, riding a wave of new technology that includes rapid DNA sequencing and synthesis, a plethora of methods for creating sequence diversity, and high-throughput screening. Examples of successful biocatalytic processes will be presented, new developments will be highlighted, and future directions for the field will be discussed.

Nick Turner*: CoeBio3, University of Manchester, UK*
Biocatalytic routes to enantiomerically pure chiral amines

This lecture will describe our recent studies on the use of monoamine oxidase enzymes for the deracemisation and desymmetrisation of amines to generate enantiomerically pure building blocks for a wide range of applications. A key aspect of the work is the use of directed evolution technologies to develop enzymes with broad substrate specificity and high enantioselectivity.

Biography
Nick Turner obtained his DPhil in 1985 with Professor Sir Jack Baldwin and from 1985-1987 was a Royal Society Junior Research Fellow, spending time at Harvard University with Professor George Whitesides. He was appointed lecturer in 1987 at Exeter University and moved to Edinburgh in 1995, initially as a Reader and subsequently Professor in 1998. In October 2004 he joined Manchester University as Professor of Chemical Biology where his research group is located in the new Manchester Interdisicplinary Biocentre (MIB: www.mib.ac.uk). He is Director of the Centre of Excellence in Biocatalysis (CoEBio3) (www.coebio3.manchester.ac.uk) and also a co-founder and Scientific Director of Ingenza (www.ingenza.com), a spin-out biocatalysis company based in Edinburgh.

Gideon Grogan*, University of York*
Intragenomic biocatalytic diversity. The family of Baeyer-Villiger monooxygenases from *Rhodococcus jostii* RHA1

The intragenomic complements of enzyme classes can offer small pools of readily accessible enzymes with diverse properties that might each serve as starting points for biocatalyst evolution. Of twenty three open-reading frames encoding putative Baeyer-Villiger monooxygenases (BVMOs) from *Rhodococcus jostii* RHA1, thirteen were expressed in soluble form in *E. coli*. Even within this small group, an impressive diversity with respect to substrate range and enantioselectivity was observed when the targets were challenged with a range of ketone substrates. Activity and selectivity closely correlated with amino acid sequence, with one major exception. This atypical enzyme has been used to target short sections of sequence for mutation, which may assist in conferring some catalytic properties specific to BVMO sub-groups.

Biography
Gideon Grogan is a senior lecturer in Biochemistry, working in the Structural Biology Laboratory at the University of York. His research interests are in the discovery and application of novel biocatalysts and the elucidation of enzyme mechanism using techniques including X-ray crystallography. He has published over fifty papers in the areas of preparative biocatalysis and structural enzymology and also a book ‘Practical Biotransformations’ to be published by Wiley in Spring 2009.

Jennifer Littlechild*, University of Exeter, Exeter Biocatalysis Centre, UK*

Thermophilic Enzymes as Stable Biocatalysts

The Exeter Biocatalysis Centre specialises in the isolation and characterisation of novel enzymes from extremophilic organisms. Many of these enzymes are used in combination with conventional chemical synthesis for the production of new optically pure drugs of interest to pharmaceutical companies.
The enzymes have been isolated both by screening for the activity of interest directly from the host organism or direct amplification using PCR from already sequenced genomes. The enzymes have been isolated from marine or terrestorial archaea and are cloned and over-expressed in a soluble form in *Escherichia coli.*
Thermophilic enzymes are more robust to organic solvents used in the biocatalytic process or in immobilisation techniques and allow the process to be operated at elevated temperatures where the substrates are more soluble. Enzymes developed at Exeter that are already used commercially are the L-aminoacylase from *Thermococcus litoralis* for the resolution of aminoacids and aminoacid analogues [1], the gamma lactamase from *Sulfolobus solfataricus* for the production of optically pure gamma lactam – the building block for anti-viral carbocyclic nucleotides [2] and alcohol dehydrogenase from *Aeropyrum pernix* for the production of optically pure alcohols [3].
Enzymes in development include a transaminase [4] and a dehalogenase [5] from *Sulfolobus* species. The transaminase can be used for the asymmetric synthesis of homochiral amines of high enantioselective purity. The L-2-haloacid dehalogenase has applications both in biocatalysis and in bioremediation.
Two of these enzymes have been immobilised in micro-reactors to allow rapid substrate screening and biotransformation optimisation [6].
The work in the Biocatalysis Centre also involves molecular structure determination of the enzymes to give an insight into their thermostability, enzymatic mechanistic and substrate specificity.

References

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Biography

Professor of Biological Chemistry and Director of the Exeter Biocatalysis Centre located in the Henry Wellcome Building for Biocatalysis. The Centre was opened in November 2003.
Prof. Littlechild carried out her Ph.D. in the Biophysics Laboratory, Kings College, London University, UK followed by a postdoctoral fellowship at the Biochemistry Department at Princeton University, USA. In 1975 she became a group leader at the Max-Planck Institute for Molecular Genetics in Berlin, Germany. In 1980 she returned to the UK to Bristol and in 1991 to Exeter University. Her current research grants are from UK research councils, BBSRC, EPSRC and the EU and DTI Technology Transfer Initiative. She is the UK representative for the European Section on Applied Biocatalysis and was involved in production of the SusChem documentation for White Biotechnology within the EU Framework 7 programme.

Current research studies involve the structural and mechanistic characterisation of the C-C bond forming enzymes transketolase and aldolase, vanadium haloperoxidases, Baeyer-Villiger monooxygenases, aminoacylases, novel esterases and lipases, gamma lactamases, alcohol dehydrogenases, dehalogenases, transaminases and other enzymes from thermophilic bacteria and archaea. She has published over 110 publications in refereed high impact journals and presented her research work internationally.

Helen C. Hailes, ***Dept of Chemistry, University College London*.**
Biocatalytic Approaches to Ketodiols and Aminodiols
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α,α-Dihydroxyketones are an important motif that can be aminated to the aminodiol functionality (Figure 1), present in many natural and synthetic biologically active molecules including antibiotics[1], alkaloids[2] and amino sugars[3]. Though methodology exists for the asymmetric synthesis of aminodiols, the methods are generally step intensive, have poor regio- or stereoselectivities, and/or consume expensive catalysts or chiral auxilliaries.

We have been working towards the engineering and directed evolution of novel transketolase (TK) (E.C. 2.2.1.1) mutants[4,5] capable of converting a multitude of aliphatic aldehyde substrates (variation of R group) into chiral . -dihydroxyketones and their subsequent transformation into aminodiols using transaminases (TAm) (Figure 1).

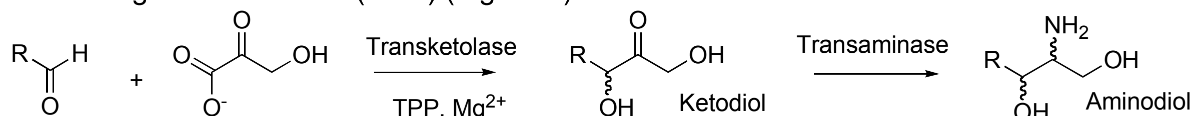

Figure 1. Biocatalytic Synthesis of Aminodiols

We have recently developed a sensitive colorimetric assay to identify active TK mutants from phylogenetic and structural-based libraries.[6] Substrate preferences and enantioselectivities achieved with selected mutants will be presented when using a series of linear aliphatic aldehydes. The use of cyclic aliphatic substrates will also be presented together with a recently developed NMR analytical method.[7] Finally, the use of an -transaminase to convert ketones including 1-phenyl-1,3-dihydroxy-2-propanone to the corresponding aminodiol, 2-amino-1-phenyl-1,3-propanediol will be discussed.[8,9] This work is part of BiCE which in parallel is establishing tools for the rapid characterisation and scale-up of these bioconversions.

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Biography

- **1987-1991** PhD in Biological Chemistry, University of Cambridge, with Prof. J. Staunton
- **1991-1992** PDRA, Department of Chemistry, University of Cambridge, with Prof. J. Staunton
- **1992** PDRA, Department of Chemistry, Imperial College, with Prof. S. V. Ley
- **1993-1994** PDRA, Department of Chemistry, Imperial College, with Dr. D. A. Widdowson
- **1994-2002** Lecturer, Department of Chemistry, University College London
- **2002-2005** Senior Lecturer, Department of Chemistry, University College London
- **2005-** Reader in Chemical Biology, University College London

Research activity in our group is focused on the use of synthetic organic chemistry to probe and solve biological problems. Many of our projects involve the development of new synthetic strategies to construct molecules as tools to identify or perturb biological targets, which can lead to the identification of novel compounds with improved biological properties. We are also keenly interested in the use of biocatalytic and chemoenzymatic strategies to biologically active molecules, and this research is carried out as part of the multidisciplinary Bioconversion-Chemistry-Engineering Interface Programme (BiCE) at UCL (http://www.ucl.ac.uk/biochemeng/industry/bice).

Peter Halling, WestCHEM, *Dept P & A Chemistry, Univ Strathclyde, Glasgow G1 1XL*

Understanding immobilised enzymes and immobilised substrates

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Enzymes immobilised in solid particles have been used for many years as practical catalysts. Their catalytic activity is routinely studied, but little is known about the structure of the enzyme molecules within them, because of limitations in available techniques. More recently there has been interest in the inverse system, where dissolved enzymes act on substrates covalently immobilised on a surface, rather than free in solution. Rates in such systems are often much slower than with both enzyme and substrate in solution, but the reasons are not well understood. This talk will describe work aimed at improving understanding of both immobilised enzyme and immobilised substrate systems.

Most spectroscopic techniques used to examine protein structure in solution are difficult or impossible to apply to particle suspensions - light scattering and sedimentation are particular problems. We have shown that circular dichroism (CD) can be adapted to make useful measurements on suspensions of particles at least as large as 100 m [1]. This requires a rotating sample cell, placed close to the detector window to minimise scattering effects, and correction for absorption flattening. The last can best be done using a simulation approach to calculate the magnitude of this effect [2]. CD measurements on subtilisin adsorbed to silica particles are able to demonstrate that the secondary structure of the protein is essentially the same as in aqueous solution, whether the particles were suspended in water or acetonitrile [1]. Near UV data suggests that the tertiary structure becomes more rigid on immobilization, and even more so in acetonitrile. But a sample inactivated after prolonged use in acetonitrile has a substantially changed secondary structure and essentially complete loss of regular tertiary structure [1]. Fluorescence of endogenous tryptophan residues can also be measured on immobilised enzyme suspensions. Lifetime distributions support the view that subtilisin is essentially unchanged, but more rigid, after adsorption on silica, whereas the inactivated biocatalyst has undergone substantial change [3]. A third technique, diffuse reflectance infra-red also supports a shift to much more beta-type structure in the inactivated immobilised subtilisin [3].

When substrates are immobilised on a surface, one important effect is that the chemical equilibrium position of a reaction can be substantially shifted, such that peptide synthesis, for example, is strongly favoured in water [4]. When the substrates are attached to porous beads, it is useful to have a spatially resolved image of the sites of reaction. Images can be produced in real time when enzyme action activates a fluorophore immobilised to the surface, using two-photon microscopy [5]. Reaction rates are enhanced by using a spacer to attach the substrate to the surface, although the length dependence differs between a rigid (glass) and hydrogel (PEGA) surface [6]. The rates are found to be fastest with less than maximal loading of substrate on the surface, and can be increased when permanent charges are also attached to the support [6].

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Biography

B.A. Cambridge, Ph.D. Bristol, both in Biochemistry. Subsequent research work in Biochemical Engineering Section, University College London; and in Unilever Research, Colworth Laboratory. First worked on biocatalysis in low-water media in 1978 at Unilever. Joined Strathclyde 1983, Professor 1990 (currently Robertson Chair of Bioprocess Technology in Department of Chemistry), Fellow of the Royal Society of Edinburgh 1996. Vice-Dean Research 2001-2008, but now pleased to be back with full-time science!

Andrew J Carnell and Yunfei Luo, ***Department of Chemistry, University of Liverpool, Liverpool L69 7ZD***

Increasing efficiency in the synthesis of pharmaceuticals and chiral ligands using enzymes

Chiral enol esters are useful synthetic intermediates that can serve as chiral enolate equivalents or undergo oxidative cleavage to produce reactive ester-aldehydes. We have previously shown that enol acetates derived from prochiral cyclic ketones can be deracemised using *P. fluorescens* lipase and then used in the synthesis of NK-2 antagonists.[1-6] Oxabicyclic[3.2.1]ketones have also been desymmetrised using silica-absorbed *Humicola* lipase.[7] In this lecture we present recent results on the extension of this approach to access homochiral [2.2.2] bicyclic diketones *via* enzyme resolution of their mono-enol esters. The chiral diketones can be synthetically elaborated in high yield to give a new range of chiral bicyclic dienes which serve as Hayashi-type ligands in asymmetric catalysis.

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Biography

Andrew Carnell is a graduate from the University of Kent (1988) and obtained his Ph.D. from the University of Exeter (1991) for work on the use of Cycloalkanone Monooxygenase enzymes in Organic Synthesis. As a post-doctoral fellow at the University of Montreal, Canada, he worked with Prof Steve Hanessian on the total synthesis of the indole alkaloid reserpine before returning to Exeter as a Chiroscience Lecturer.
From 1993, Andrew was a Research Chemist in the Chiral Chemistry Group at Associated Octel Ltd., before joining the staff at the University of Liverpool in January 1996, where he is now Senior Lecturer. His research interests are in the discovery, development and application of biotransformations in organic synthesis and medicinal chemistry.

Matthew Truppo*, University of Manchester (CoEBio3), UK / Merck & Co. Inc., USA*

Process development of transaminase catalyzed reactions for large scale industrial use

Chiral amines have proven to be particularly useful building blocks in stereoselective synthesis. The process development of transaminase catalyzed reactions has led to various methods for the production of chiral amines, which overcome the challenges of poor reaction equilibrium and inhibition by both the substrates and products. This has enabled general, scalable processes with high yields (>99% conversion) and high volumetric productivities (>50 g/L). The substrate range of commercially available transaminases has been examined, and excellent enantioselectivities (e.e. > 99%) have been obtained for a variety of amine products. Additionally, the development of rapid screening systems and micro-scale process development techniques for transaminations have been achieved.

Biography

Matt Truppo obtained his BSE in Chemical Engineering and certificate in Engineering Biology from Princeton University from 1998-2002. While at Princeton, he was selected for a joint research internship with Johns Hopkins and the Walter Reed Medical Center, and a Merck Research Laboratory summer internship in the Bioprocess R&D department. In 2002 Matt accepted a Biochemical Engineering position at Merck & Co., working on biocatalyst discovery, biotransformation optimization, and process development and scale-up to deliver pharmaceutical intermediates. Matt was selected for the Merck Doctoral Study Program in 2006, and is currently completing his PhD with Prof. Nick Turner at the Manchester Interdisciplinary Biocentre within Manchester University.

Nicolas Szita*, Department of Biochemical Engineering, University College London*
Microfluidic Enzymatic Reactor for Synthesis

Microfluidic enzymatic reactors have been previously presented for protein digestion and analytical studies [1], yet relatively few studies have explored enzymatic microfluidic reactors for biocatalytic process development and synthesis [2,3]. In this work we report on a microfluidic reactor utilising clarified transketolase lysates for continuous small-scale synthesis. We demonstrate that high conversion is obtained in a microfluidic reactor for two different types of model systems using substrates of two different hydrophobic properties, and yielding L-erythrulose and (3S)-l,3-dihydroxypentan-2-one, respectively.

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Senior Lecturer in the Department of Biochemical Engineering at University College London.

Dr Szita carried out his Ph.D. at the ETH Zurich, Switzerland, in the Department of Mechanical and Process Engineering. At ETH, he created a microfluidic pipetting device from silicon and glass, with integrated capacitive sensors which was patented by the industrial partner. In 2001, he joined the group of Prof K Jensen at MIT's Department of Chemical Engineering as a Post-Doctoral Research Associate, where he fabricated and characterised multiplexed microfluidic bioreactors to demonstrate highly reproducible parallel batch bacterial fermentations which compared favourably with bench-scale reactor data. In 2005, he became a Lecturer at the Technical University of Denmark (DTU), where he worked on perfusion and continuous culture microbioreactors made from polymers. He joined UCL early in 2007 and has established a new Microfluidics Laboratory and researches bioprocess microfluidic systems for regenerative medicine applications and more recently for biocatalysis.

Posters

1. The use of Transketolase and Transaminase in Stereoselective Organic Synthesis.

Armando Cazares & Helen Hailes, Dept of Chemistry, UCL, Gordon Street, London

Transketolase (TK) and transaminase (TAm) are versatile enzymes that produce chiral aminodiols, which makes them useful in organic synthesis. In order to study the tolerance of these enzymes towards non-natural substrates, the aliphatic aldehydes (C3-C8) have been tested in the TK reaction using wild-type and mutant TKs. To explain the behaviour shown by the TKs, docking experiments were carried out and these show that the size of the aliphatic chain seems to play a pivotal role in determining the stereoselectivity of the reaction. Novel, highly-enantioselective, chemoenzymatic synthesis of Baclofen using anaerobic microorganisms containing enoate reductase activity. Anna Fryszkowska, Karl Fisher, John Gardiner, Gill Stephens In recent years, a lot of attention has been devoted to microorganisms possessing enoate reductase activity. These organisms, as well as the enzymes isolated from them, have great potential in industrial stereoselective biocatalysis, as they catalyse a range of synthetically useful reductions of activated C=C double bonds [1]. We have recently demonstrated that Clostridium sporogenes is a versatile biocatalyst for the reduction of α,β-unsaturated nitroalkenes [2]. Here we present a short, chemo-enzymatic, highly-enantioselective total synthesis of the (S)-enantiomer of Baclofen – a selective GABAB receptor agonist used in the treatment of multiple sclerosis. As a starting material, we used 3-aryl-3-cyano-propenoic acid derivatives (p-Cl-Ph-, p-F-Ph, p-MeO-Ph, Ph), which were efficiently reduced by crude extracts of anaerobic bacteria, namely Clostridium sporogenes, Ruminococcus productus and Acetobacterium woodii, to give the respective propanoic acids in high yields and optical purity (≥96% ee). The resulting product was subsequently transformed to (S)-Baclofen in 3 high-yielding steps (>50% overall). This is the first report of the application of this approach for the synthesis of Baclofen, which is a robust and simple methodology to obtain the compounds of this class in a highly enantioselective fashion.

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2. Cancer and glutathione S-transferase enzymes

A. R. Vaz[1], P. G. Winyard[2] & J. A. Littlechild[1] , [1]Henry Wellcome Building for Biocatalysis, School of Biosciences, University of Exeter, UK [2]Peninsula Medical School, Universities of Exeter and Plymouth, UK

One of the major problems with the treatment of cancer in humans is the phenomenon of drug resistance, which can arise in a number of ways, such as the increased expression of phase I and II metabolising enzymes. Glutathione S-transferases (GSTs) are important phase II enzymes, which play a role in cellular detoxification. GST Pi subclass is part of the human cytosolic GST group of enzymes and contains only one protein, GSTP1. Two mutations in the GSTP1 gene result in amino acid substitutions in the GSTP1 protein, and their combination results in four different variants (GSTP1-A, -B, -C and -D) that have different substrate specificities. Their over-expression has been found in a number of different human cancers when compared to the normal tissue, and is believed to be an important mechanism of tumour cell resistance to alkylating agents. Nitrosourea compounds are alkylating agents used as anticancer drugs, and understanding the reactivity between them and the four GSTP1 variants may enable future individual therapy regimens, by prescribing the right drug and dose to the patient in order to ensure the best outcome. The human GSTP1-B DNA sequence has been cloned into the pET-28a expression vector and transformed into Escherichia coli. The other three GSTP1 variant genotypes were obtained by site directed mutagenesis. Expression studies were performed and optimal condition for over-expression set, which were followed by purification on nickel column and gel filtration. Crystallisation of variant D with the drug streptozocin was carried out and small crystals were formed, however the method needs optimization. The four protein isoforms will be assayed for glutathione conjugation activity in the presence of nitrosourea anticancer drugs.

3. Structural Studies on ω-Aminotransferase Enzymes with Applications for Commercial Biocatalysts

C. Sayer, M. Isupov and J. Littlechild
Henry Wellcome Building for Biocatalysis, School of Biosciences, University of Exeter, Exeter, EX4 4QD

ω-aminotransferases (ATs) catalyse the aminotranferase reacion between an omega amine donor and an alpha amine acceptor. The major interest in bacterial ωAT is their potential industrial importance, as they can be used as biocatalysts for the asymmetric synthesis of homochiral amines of high enantioselective purity. The substrate specificity of the *Chromobacterium violaceum* AT and Pseudomonas aeruginosa AT has previously been characterised and they are good candidates in the production of alcoholamines (Kaulmann et al., Ingram et al.,). To date little structural knowledge on the ω-aminotransferases is known. The structure of a ω-AT from *C. violaceum* has been solved in holoenzyme, gabaculine and pyruvate bound forms. The structure reveals significant movements in the regions neighbouring the active site. Interestingly the structures presented differ significantly from the homology based *V. fluvialis* enzyme structure, a related enzyme. The structure of a ω-aminotransferase from *P. aeruginosa* has been solved and compared to the *C. violaceum* structure which helps to explain the different substrate specificities given. These structures offer a basis for site directed mutagenesis for the development of the enzymes in biocatalysis.

4. Characterisation of novel enzymes from the marine virus EhV-86 and evaluation of their use for commercial biocatalysis.

E. L. Reid (1), M. J.Allen (2) and J. A Littlechild (1)
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Viruses are ubiquitous in marine ecosystems representing the most abundant biological entity in the ocean (1). This potentially offers a source of many novel industrial enzymes with a diverse range of catalytic activities. EhV-86 is a large double stranded DNA virus that infects the globally important marine microalga Emiliana huxleyi. EhV-86 is a member of the recently named genus of viruses Coccolithoviridae, belonging to the family Phycodnaviridae. The genome of EhV-86 has recently been sequenced and initial analysis has revealed many novel components. EhV-86 has a 407,339 bp circular genome with 472 predicted genes or protein coding sequences (CDS) with an average gene length of 786 bp. Based on sequence similarity and protein domain matches only 66 or 14% of the 472 CDSs have been annotated with functional protein products (2). In this project we are investigating 2 CDSs from EhV-86. ehv028 and ehv363 have been annotated by the Sanger Institution as a putative lipase and esterase/lipase respectively. The genes have been amplified by polymerase chain reaction and cloned into a variety of Escherichia coli hosts. Over-expression studies are currently being carried out. This will allow the production of sufficient protein for biochemical and structural characterisation. Their commercial applications will also be evaluated regarding optimal activity and substrate specificity.
1. Bidle, K.D. and Falkowski, P.G. (2004). Nature Reviews, 2: 643-654. 2. Wilson, W. et al (2005). Science, 309:1090-1092.

5. Biopolymerisation and kinetic resolution in fluorous biphasic systems with hydrophobically ion paired enzymes

I. Lapenaite, M. Poliakoff, N.R. Thomas

Screening of lipases of the different origin was performed to select a lipase that could be efficiently ion paired [1] with anionic perfluoropolyether carboxylate surfactant KDP 4606 (DuPont Chemicals) and solubilised in fluorinated solvent, perfluoromethylcyclohexane (PFMC) *Candida cylindracea* (CC), *Candida rugosa* (CR), *Aspergillus niger* (AN), *Mucor miehei* (MM), *Rhizopus arrhizus* (RA) and *Pseudomonas cepacia* (PC) exhibited reasonable complexation efficiency. The catalytic activity of hydrophobically ion paired (HIP) lipases was investigated for synthesis of organic molecules and polymers in fluorous biphasic systems. HIP lipase catalysed kinetic resolution of rac-1- phenylethanol in a PFMC/hexane biphasic system was investigated. Only lipases CC and PC demonstrated reasonable

catalytic activity and enantioselectivity. The ring opening polymerisation of lactones and polycondensation of dicarboxylic acid derivatives with glycols were examined for enzymatic polyester synthesis. ε-caprolactone (ε-CL) was enzymatically oligomerised with different HIP-lipases yielding a polyester. The polymerisation behaviour depended on the reaction conditions and the lipase origin. HIP-lipase gave a much higher conversion of ε-CL than suspensions of lipases but with smaller molecular weights. The ability to solubilise lipases in fluorinated solvents provided an opportunity to perform homogenous polymerisation [2] of highly fluorinated monomers. Several hydroxyl-terminated fluorinated diols and fluorinated diacids were examined in order to generate fluorinated polyesters that may have unique physical and chemical properties.

Hobbs H.R., Kirke H.M. Poliakoff M., Thomas N.R. *Angew. Chem. Int. Ed.* (2007) 46:7860
Hobbs H.R., Thomas N.R. *Chem. Rev.* (2007) 107(6): 2786

6. The role of pyroglutamyl modification of peptides in Alzheimer's disease

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Pyroglutamyl peptidase (Pcp) is an ω-exopeptidase removing an N-terminal pyroglutamyl residue (pGlu). It exists in three distinct forms in mammalian tissues: cytosolic PcpI (EC 3.4.19.3), membrane-anchored PcpII (EC 3.4.19.6) and serum thyroliberinase (EC 3.4.19.-). The enzymes participate in peptide breakdown and the control of the physiological biomolecules. The enzyme is commercially important tool for N-terminal pGlu removal from peptides and proteins prior to Edman degradation. The purpose of this study is to investigate potential ability of Pcps for degradation of Alzheimer's (AD) brain β-amyloid deposits. Amyloid plaques are predominantly composed of various pGlu peptides such as pGlu-Aβ(3-40/42) and pGlu-Aβ(11-40/42). This type of N-terminal modification increases the hydrophobicity and neurotoxicity Aβs and makes them more resistant to proteolysis. Pcp activity was observed to be significantly impaired in pathological AD brain tissue what has an effect in progressive accumulation of pyroglutamyl β-amyloid species. Structure of some archaeal and bacterial pyroglutamyl peptidases are already known. There is no structural information however on human analogues. The human PcpI has been cloned and over-expressed in Escherichia coli BL21-CodonPlus (DE3)-RIPL strain to produce a soluble enzyme. This is being biochemically and structurally characterized. Preliminary studies with human PcpII have resulted in amplification of cDNA fragments encoding for functional enzyme domains and is currently being cloned into expression vectors. Both proteins are promising pharmacological targets for AD treatment.

7. "Industrial Ready Biocatalysts"

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Biocatalysts in the pharmaceutical and fine chemical industry continues to grow as it moves from the domain of specialised technology to mainstream methodology, a move that has been facilitated by the increasing commercial "off-the-shelf" availability of these biocatalysts at large scale. For example, Carbonyl Reductase (CRED) biocatalysts have found applications in enantiomeric resolutions, chemo- and regioselective reductions/oxidations, and synthesis of chiral alcohols from ketones, ketoacids, and ketoesters exhibiting a very broad substrate tolerance. This poster highlights development and application of CRED enzymes from lab-scale through pilot-plant manufacture, with particular focus on the essential criteria for 'industrial readiness', including biocatalyst availability and performance (turnover, selectivity, volume efficiency).

8. Oxidoreducing enzymes of Stropharia aeruginosa.

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The organohalogen producing magic mushroom S. aeruginosa secrets an array of potentially interesting oxidoreducing enzymes including oxidases, laccases, peroxidases and haloperoxidases. So far we isolated, purified and characterised biochemically two laccase enzymes which we believe belong to the family of yellow" laccases and show promise in decolorisation of azo dyes and positive preeliminary results in epoxidation of indene. Due to inefficient, expensive and very time consuming process of enzyme isolation we attempted to isolate the genes encoding laccases of S. aeruginosa. We synthesised the cDNA of the organism grown under laccase-expressing conditions and sequenced it using 454 Titanium pyrosequencer. The sequenced data were assembled into contigs and searched for homology to above-mentioned oxidoreductases. BLASTx search identified putative (iso)enzymes belonging to: laccases, aryl alcohol oxidases, manganese peroxidases, cytochromes P450, mono- and dioxygenases, glycoxal oxidases and haloperoxidases, which correspond to enzymatic activities found in crude extracts. Our current work is focused in isolating full-length sequences of laccases and haloperoxidases of S. aeruginosa, their expression in S. ceravisae and will be followed by testing their suitability for enantioselective epoxidations and sulphoxidations.

9. A New, Rapid Screening Method for Ionic Liquid Toxicity

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There is increasing pressure on the chemical manufacturing industry to develop greener protocols. This has created considerable interest in ionic liquids as replacements for conventional organic solvents, especially in the field of whole-cell biotransformations. The flexibility of ionic liquids allows solvents to be specifically designed for a particular application. However, the numbers of ionic liquids available are so vast that it can be difficult to determine which ionic liquids are suitable for a particular application. In whole-cell biotransformations the first criterion to be addressed is the biocompatibility of the ionic liquid with the host. To address this, we have developed a new, high throughput screening method for assessing ionic liquid biocompatibility. A known quantity of ionic liquid is added to a filter paper disc, which is placed on a lawn of microbial cells and incubated. A toxic ionic liquid is identified by the production of an inhibition zone around the filter paper disc. Using the agar screen 100 different ionic liquids have been tested for their toxicity to Escherichia coli MG1655. The screening data have been validated by measurement of growth rates in liquid medium containing ionic liquids. Using the agar screening method we have shown that in combination with imidazolium cations, sulfosuccinate (AOT-) and MeSO4- tend to produce biocompatible ionic liquids, whilst bis(trifluoromethanesulfonyl)imide (NTf2-) anions tend to produce toxic ionic liquids. We have also identified a number of other cation and anion combinations which are also suitable for use with E. coli as a whole cell biocatalyst.

10. Covalent Enzyme Immobilization onto Emulsion – Templated Porous Polymers

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Enzymes are known to catalyse many reactions, and have been used in different areas ranging from chemical synthesis to decontamination of waste streams. Candida Antartica Lipase B (CAL-B) is a particularly useful enzyme for the chemical industry due to its high thermal stability and promiscuity. We have developed polymer scaffolds (polyHIPEs), from the polymerization of the continuous phase of a high internal phase emulsion (HIPE). This support can be used to immobilize covalently the enzyme Candida Antartica Lipase B. Immobilization of enzymes such as CAL-B onto a support has advantages

over the use of the enzyme in solution, such as the separation of the enzyme from the product and a reduction in cost, due to the recycling of the enzyme. Higher enzyme activities on the polyHIPE support were observed compared to commercial supports (Novozyme).

11. **High throughput investigation into excipient factors affecting protein stability**

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The effects of dissolved excipients on protein stability are analysed using high throughput kinetic screening of intrinsic protein fluorescence in a standard microwell plate reader on 96 and 384 well UV transparent plates. We seek to produce unified models of the relationships between excipient and protein properties and their combinatorial effects on protein stability. The methods are able to simultaneously test unfolding kinetics and final stability, which have been shown to correlate well. Refolding studies have also been directly integrated. These techniques may be used to rapidly test stabilising solutions and improve protein refolding yields. The final models may in future be used to tailor or predict optimal additive combinations for improving stability, or to relate protein engineering to improved stability in reactor environments. Work has been performed on two model proteins, studying unfolding and refolding kinetics and equilibria via chemical denaturant (guanidine HCl) concentration jumps. Several stabilizing excipients have been found from a varied range of excipients an the relative impact on stability, folding and unfolding is characterised.

12. High Resolution Structures of E.coli Transketolase Revealing Unusual Mechanistic Angle of Attack of Thiamine Pyrophosphate.

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Stereospecific carbon – carbon bond synthesis is an important reaction in synthetic organic chemistry and may be catalysed by several enzymes including transketolases (E.C 2.2.1.1). The enzyme transketolase in vivo catalyses the reversible synthesis of higher carbon sugars in the pentose phosphate pathway. The transketolase enzyme mediates the transfer of a two-carbon ketol moiety from a ketose to an aldose. The enzyme requires both divalent magnesium ions and thiamine pyrophosphate (TPP) for activity. Hydroxypyruvate can be used synthetically as the ketol donor to release carbon dioxide which renders the reaction irreversible [1]. Various aldehyde acceptors can be used to yield a range of polyhydroxylated compounds that can be cyclised to unusual sugars [2]. In a test case transketolase has been used with glycolaldehyde and hydroxypyruvate resulting in total conversion to the sugar product erythrulose [3]. The TPP cofactor binds at the active sites formed between the two transketolase subunits. The amino group of its aminopyrimidine moiety is close to the dissociable proton, and serves as the proton acceptor. This proton transfer is promoted by a glutamate residue adjacent to the pyrimidine ring. The thiazolium carbanion (ylide) that results from proton dissociation reacts with the carbonyl donor substrate to form the active site intermediate. The positively charged nitrogen in the thiazole ring acts as an electron sink which promotes carbon - carbon bond cleavage in the donor substrate. This releases an aldehyde, leaving a two-carbon fragment attached to the TPP as an enamine intermediate. Condensation of this intermediate with the aldose acceptor to form the ketose product completes the reaction. The high-resolution structure (1.1Ǎ) of the fluoropyruvate complex provides significant insight into the mechanistic details of the C-C transfer, and reveals the angle in which the ketol donor is attacked by the thiazolium carbanion. The complex describes the angle of attack for the SN2 reaction of nucleophiles on carbonyls which leads to the formation of a tetrahedral intermediate. With further computer modelling, the complete mechanistic story of the role of Thiamine pyrophosphate in Transketolase can be told [4].

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13. Immobilisation and Use of Thermophilic Biocatalysts in Miniaturised Flow Reactors

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The exploitation of enzymes for biocatalysis and biotransformation and their potential application in drug intermediate synthesis have been the focus of much academic research. While a number of enzymes are commercially available, their use in an industrial setting is often limited to functions that have been proven to be cost-effective and they are rarely investigated further. However, the development of miniaturised flow reactor technology has meant that the cost of such research, once considered cost- and time-inefficient, would be much less prohibitive. The use of miniaturised flow reactors for enzyme screening offers a number of advantages over batch enzyme assay systems. Since the assay is performed on a miniaturised scale, enzyme, substrate and co-factor quantities required are significantly reduced, thus reducing the cost in the case of expensive substrates and co-factors for laboratory-scale investigations. Since they use microfluidic systems, where the substrate and products flow out of the system, the problems of negative feedback encountered upon build-up of products by certain enzymes are avoided. Quite often enzymes fulfill a single use function in biotransformation, however, enzyme immobilisation allows enzyme re-use and, in some cases, helps to increase enzyme stability. Two enzymes with potential for biotransformation reactions have been successfully immobilised in miniaturised flow reactors. Both are from thermophilic archaea, an L-aminoacylase from *Thermococcus litoralis* and an amidase from *Sulfolobus solfataricus*. Two approaches to enzyme immobilisation were examined, both involving enzyme cross-linking. The first reactor type used monoliths, to which the enzymes were attached, the second contained previously cross-linked enzymes trapped using frits, in the microfluidic channels. Two different microreactor designs were used in the investigation, microreactor chips for the monoliths and capillary tubes for the cross-linked enzymes. These systems allowed passage of the substrate(s) and product(s) through the system while retaining the enzyme performing the catalytic conversion.

14. Chris Grant. Dept Biochemical Enigneering, University College London

The alkane hydroxylase (Alk) system native to P.oleovorans is of considerable interest for the oxidation of medium-chain aliphatic alkanes to primary alcohols and carboxylic acids. This reaction remains difficult to perform by conventional chemistry and biocatalysis offers an attractive near-ambient temperature alternative with reduced energy demand. Product limitations have consistently been observed in two-liquid phase studies of n-octane to 1-octanol oxidation in both P. putida and E. coli hosts containing the necessary Alk genes (Bosetti et al 1992). Our own studies of n-dodecane and n-octane hydroxylation have also shown that this phenomenon occurs to differing degrees with the two substrates; at a 1-dodecanol concentration of 2g/Lapolar in the E.coli GEC137 host; and 3.8g/Lapolar 1-octanol in the same host. The complexity of the two-liquid phase whole cell bio-oxidation can make the cause of this limitation difficult to isolate. With the n-octane substrate, the scientific literature reports inactivation of Alk gene expression (Chen et al 1996) and mass transfer limitations due to bioemulsifier accumulation (Schmid et al 1998) to be possible causes. In the case of the n-dodecane conversion, we have now shown that the product concentration itself is critical to the cessation of enzyme activity. By spiking (at inoculation) the limiting concentration of 1-dodecanol in a two-liquid phase fed-batch fermentation, we observed that no further 1-dodecanol is accumulated, yet there is no significant observed effect on cell viability or specific growth rate. SDS-PAGE indicates that the cessation of activity is likely to be related to 1-dodecanol related repression of Alk expression.

Organising committee Dr. Paul A. Dalby (Dept of Biochemical Engineering, UCL), Dr. Colin T. Bedford (Dept. of Chemistry, UCL), Dr Brendan Fish (MedImmune), and Dr Martin Hayes (AstraZeneca), under the auspices of the Industrial Technology Forum's Biotechnology Group of the Royal Society of Chemistry. The organizers are grateful to the Chemistry Biology Interface Forum of the Royal Society of Chemistry for their sponsorship and support of student bursaries. 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 from both industry and academia are currently required to widen the area of its expertise. Those with an interest in helping to organize Conferences or Courses in any aspect of biotechnology that is underpinned by chemistry are warmly invited to contact either the Chairman, Paul Dalby (p.dalby@ucl.ac.uk), or the Secretary, Colin Bedford (c.t.bedford@ucl.ac.uk). The Group has annually run conferences or symposia on a diverse range of topics, including Biotransformations, Plant and Microbial Metabolites as Drug Leads, Proteomics, and Glycomics. It also runs Courses with titles such as Biotechnology for Chemists, Freeze Drying of Biologicals and Pharmaceuticals, Protein Biotechnology in the 21st Century, and Understanding Polysaccharide Biotechnology.