Institution: University College London

Unit of Assessment: 12 - Aeronautical, Mechanical, Chemical and Manufacturing Engineering

Title of case study: Biocatalysis integrated with chemistry and engineering (BiCE) to speed development of green pharmaceutical processes

1. Summary of the impact

UCL research has been instrumental in creating critically needed new biocatalysts and bioprocess technologies for industrial biocatalytic process development. These have impact across the UK chemical and pharmaceutical sectors. BiCE enzyme technologies have been exploited through the formation of a spin-out company, Synthace, generating investment of £1.8m and creation of 7 new jobs. Commercial utilisation of BiCE enzymes by company partners has led to environmental benefits through sustainable syntheses and reduced waste generation. BiCE high-throughput bioprocess technologies have also been adopted to speed biocatalytic process development. UCL established a parallel miniature stirred bioreactor system as a new product line for HEL Ltd. [text removed for publication]. Related knowledge transfer activities have also benefited some 157 industrial employees from over 50 companies since 2008.

2. Underpinning research

The multi-disciplinary BiCE (Bioconversion - Chemistry - Engineering Interface) programme at UCL, conducted between 2004 and 2008, established a new integrated approach to development of biocatalysts and biocatalytic processes. This combined aspects of synthetic chemistry, molecular biology and process engineering to generate novel biocatalysts with increased productivity for application in the chemical and pharmaceutical industries. The programme also established a range of technologies to facilitate the rapid design and scale-up of green, more environmentally friendly, industrial biocatalytic processes. This new approach was shared with 13 industrial partners, enabling the early identification of all potential bottlenecks for efficient biocatalytic process development, from biocatalyst discovery through to process design and scale-up.

The underpinning research delivered a range of tools for synthetic biology, enzyme engineering and biocatalyst screening, as well as new enzyme variants and *E. coli* strains, enabling synthesis of important pharmaceutical intermediates (chiral ketodiols and aminodiols). Building on work from 2000 to 2003, which resulted in patents [8], the BiCE programme used this technology to engineer enzyme variants now used by the programme's company partners. Automated, high-throughput methods for biocatalyst process evaluation were also developed, along with novel miniature stirred bioreactor technologies that help facilitate the rapid establishment of scalable biocatalytic and chemo-enzymatic synthetic routes.

Over 70% of pharmaceutical compounds contain amine functionalities and many biologically active natural products contain chiral amino alcohols. While chemical methods exist for their asymmetric synthesis, these are generally step intensive or consume expensive and frequently toxic catalysts or chiral auxilliaries. The underpinning BiCE research addressed key bottlenecks in the development of novel, industrially useful biocatalytic processes for the synthesis of amino alcohols. First, researchers constructed a completely *de novo* metabolic pathway consisting of transketolase (TK) and transaminase (TAm) in *E.coli*, using a novel mix-and-match plasmid technology, to produce the chiral aminodiol 2-amino-1,3,4-butanetriol [1]. To diversify this pathway and access a broader range of substrates and useful aminodiol products, over 100 new transaminases, including the versatile omega-transaminases, were identified from a range of organisms by metagenomics. These were then isolated, cloned into *E.coli*, and screened for broadened substrate selectivity and increased activity [2]. In parallel, novel targeted directed evolution strategies were invented using phylogenetic and structural information to engineer TK mutants capable of processing a range of new substrates including aliphatic and cyclic aldehydes [3], and to have improved product enantioselectivities. Next, automated high-throughput and process modelling approaches were created [4] and researchers designed a miniature and parallel stirred bioreactor system that is predictive of larger-scale bioreactor performance [5]. Underpinning all this work was development of a suite of novel automated high-throughput assays for quantification of TK activity and stability [6]. Finally, complete integration of all the BiCE component elements was demonstrated and published [7]. This utilised all the BiCE technologies to rapidly establish a preparative scale, two-step process suitable for manufacture of 2S-amino-1,3S-pentanediol on an industrial scale.

The BiCE programme brought together a multi-disciplinary team of staff. The project was managed initially by Prof John Woodley (Biochemical Engineering) and then by Prof Gary Lye (Biochemical Engineering). Key research was led by Prof Paul Dalby (Biochemical Engineering), Prof John Ward (Structural and Molecular Biology, who joined Biochemical Engineering in 2012), Prof Helen Hailes (Chemistry) and Dr Frank Baganz (Biochemical Engineering). The project involved 6 Research Associates (RAs) and 5 associated doctoral students. Of the RAs Dr Martina Micheletti (Biochemical Engineering) joined the UCL staff in 2007 while the other RAs all progressed to industrial careers.

3. References to the research

Since 2004 BiCE staff have **published over 80 multi-authored papers** in leading refereed journals and given over 30 oral presentations at international conferences. The **quality of the research was recognised with a series of awards**: IChemE Innovation and Excellence Award in Bioprocessing (2010); Royal Society of Chemistry Rita and John Cornforth award for Chemical Biology (2010); Evonik European Science-to-Business Award (2010). Key references include:

[1] Ingram, C. U., Bommer, M., Smith, M. E. B., **Dalby, P.A., Ward, J.M.**, **Hailes, H.C.** and **Lye, G.J.** One-pot synthesis of amino-alcohols using a *de-novo* transketolase and β-alanine: pyruvate transaminase pathway in *Escherichia coli.* 2007. *Biotech. Bioeng.* **96**, 559-569. doi.org/dwwvb4

[2] Kaulmann, U., Smithies, K., Smith, M.E.B., **Hailes, H.C.** and **Ward, J.M.** Substrate spectrum of omega-transaminase from *Chromobacterium violaceum* DSM30191 and its potential for biocatalysis. 2007. *Enz. Microb. Tech.* **41,** 628-637. doi.org/dxn5vp

[3] Hibbert, E.G., Senussi, T., Smith, M.E.B., Costelloe, S.J., **Ward, J.M.**, **Hailes, H.C.**, and **Dalby, P.A**. Directed evolution of transketolase substrate specificity towards an aliphatic aldehyde. 2008. *J. Biotechnol.* **134**, 240-245. doi.org/ds8586

[4] Chen, B.H., **Micheletti, M.**, **Baganz, F**., Woodley, J.M. and **Lye, G.J.** (2009) An efficient approach to bioconversion kinetic model generation based on automated microscale experimentation integrated with model driven experimental design. *Chem. Eng. Sci*., **64,** 403-409, doi.org/b8c372

[5] Gill, N.K., Appleton, M., **Baganz, F.** and **Lye, G.J.** (2008) Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up. *Biotechnol. Bioeng.* **100,** 1144-1155. doi.org/bn9jg7

[6] Miller, O.J., Hibbert, E.G., Ingram, C.U., **Lye, G.J.** and **Dalby, P.A.** Optimisation and evaluation of a generic microplate-based HPLC screen for transketolase. 2007. *Biotechnol. Letts.* **29**, 1759-1770. doi.org/c9mh8x

[7] Smith, M.E.B., Chen, B.H., Hibbert, E.G., Kaulmann, U., Smithies, K., Galman, J.L., **Baganz, F., Dalby, P.A.**, **Hailes, H.C.**, **Lye, G.J., Ward, J.M**., Woodley, J.M. and Micheletti, M. A multi-disciplinary approach toward the rapid and preparative scale biocatalytic synthesis of chiral amino alcohols. 2010. *Org. Proc. Res. Dev*. **14**, 99-107. doi.org/b4qqc7

[8] Dalby (2003) “Materials & methods relating to protein and nucleic acid evolution” WO 03004595, Dalby, (2004) “In vitro evolution of enzyme specificity” WO2004024918

References [1], [5] and [7] best demonstrate the quality of the research.

The research outputs were achieved within a number of linked Research Council grants including: the BiCE programme grant “Next generation pharmaceuticals: Linking novel engineering and chemistry to a revolution in biocatalysis” Feb 2004 – Jan 2008, Prof Gary Lye (PI), EPSRC (GR/S62505/01); £1,686,303 plus £300,000 from the industrial consortium; the grant leading to the patent filings: “Substrate walking: Increasing the synthetic repertoire of enzymes using a novel process”. July 2003 – July 2006, Dr Paul Dalby, EPSRC (GR/S02532/01), £98,502. The success of the TAm work led to an EPSRC Follow on Fund grant, 2008-2009 “Creating a user friendly Transaminase toolkit” EP/G005834/1; £155,607. The concept of building synthetic pathways for chiral compounds was extended in the BBSRC grant “Synthetic biology pathways to isoquinoline alkaloids”, BB/G014426/1; £902,058.

4. Details of the impact

Since 2008, BiCE programme research has had wide-ranging impact, from the establishment of a successful spinout company to commercialisation of a miniature bioreactor technology. A number

of pharmaceutical companies now using BiCE enzymes are benefiting from cost and time savings in their production process, along with reduced waste production. These impacts are summarised below and can be traced back directly to the original research outputs identified above.

Spinout company creation: To capitalise on the knowledge generated in the BiCE programme, Prof John Ward and researchers at UCL set up the synthetic biology spin-out company, Synthace Ltd, in 2011. This aims to make high-value, bio-based chemical and biological products through the application of synthetic biology**.** It is the UK's first dedicated synthetic biology company start-up. Synthace is based in UCL Biochemical Engineering and had completed two rounds of investment funding as of 31 July 2013. These successful funding rounds have brought its total financing to £1.8m with TSB funding of £500,000 and a recent second stage finance of £1.3m from Sofinnova Partners' Green Seed Fund and a syndicate of angel investors [a]. The CEO of Synthace said: "*With its world-leading development and scale-up facilities, UCL Biochemical Engineering is the ideal place for a start-up like Synthace as we seek to industrialise advances in synthetic biology*" [b]. Synthace has licensed enzyme libraries established in the BiCE programme and currently uses these in sizeable commercial contracts from three major companies. In March 2013, the Universities and Science Minister, The Rt Hon David Willetts, recognised the contribution of Synthace to an "*increasingly important*" section of the UK economy, when he said: *"Companies like Synthace can help the UK exploit the massive potential that synthetic biology has both here and abroad. By making investment in technology now, it will ensure that in ten years' time the UK is at the forefront of the global race when it comes to commercialising new technologies"* [b].

Economic benefits in terms of new products and cost and time savings: Since 2008, many of the new bioprocess tools developed as part of the research described above have been adopted by consortium companies for their industrial biocatalytic processes. Examples of the impact of these technologies include:

- **Commercialisation of a miniature stirred bioreactor technology.** The UCL design for a novel miniature stirred bioreactor technology enabling direct scale-up from 30 mL to 10L was commercialised via HEL Ltd and has been on the market since 2008. The miniature bioreactor enables rapid testing of new fermentation applications with data gathering that fully matches the large scale. This saves time and cost in avoiding running large-scale fermentations while testing a fermentation protocol. These miniature bioreactors (30-500 mL) represented a new product line for the company [c] [text removed for publication].
- **Industrial adoption of high throughput bioprocess design methodologies.** A number of BiCE programme industrial partners have now adopted UCL high-throughput and microscale technologies to help speed the design of new biocatalytic processes. These include Lonza, Merck & Co, Evonik and DSM. As an indication of the impact achieved the Executive Director of BioProcess Technology & Expression from Merck & Co has reported a ***3-to-5-fold throughput improvement by the application of microscale-based techniques***. This accelerated the pipeline in multiple ways including: shortening the duration from catalyst screen to delivery of mg quantities of initial material by 70%, thereby enabling a 3-fold increase in the number of projects that can be handled by a single scientist and widening of the catalyst library that can be rapidly screened by 3 to 5 times [d]. This enables them to complete customer projects in a shorter time or with the commitment of fewer FTEs due to the parallel nature of the HEL miniature bioreactor systems. In addition UCL staff are now providing consultancy services to additional companies on how to implement these methodologies.

Economic benefits from screening and utilisation of BiCE enzyme libraries: Libraries of engineered enzymes have been provided to company partners for use in their internal high-throughput screening programmes. Licensing of these libraries saves each company the time and costs involved in in-house library generation e.g. for a company to construct and validate an enzyme library containing tens to hundreds of mutants, rather than using UCL's libraries, might demand full-time commitment of 2 FTE for 6 months. For example, Almac has used over 100 of these enzymes to identify transaminases that are able to catalyse amination of the substrates Almac uses in its in-house drug discovery programmes and for customer contracts. In 2010 Almac identified one TAm variant that catalyses amine formation on a compound for a pharmaceutical company customer. They are now scaling up production of this TAm ready for commercial-scale utilisation. The head of biocatalysis at Almac said: "*The new enzyme process is* ***one third of the***

cost of the chemical process *and the* ***yield of the process has increased from 10% to over 90%***" [e]. Since then, Almac and UCL have had a royalty-sharing agreement for the commercial exploitation of this and the other transaminases. In a similar vein, Sigma-Aldrich has since 2006 used one of the BiCE TK variants for commercial preparation of D-xylulose-5-phosphate and a TAm variant to prepare pyridoxamine-5-phosphate. The Head of Research Specialities from Sigma-Aldrich (SIAL) notes that *"The direct comparison of selective one-step enzymic reactions to chemical routes of synthesis show the advantage of replacing non-selective chemical routes, which then involve also significant purification efforts after the reaction(s). The beneficial effect on safety, health and environment improvement by the enzymatic route fits well with the current sustainability goals at Sigma-Aldrich."* [f]

Environmental benefits arising from adoption of biocatalysis: It is widely recognised in the chemical industry that biocatalysis generates environmental benefits when used to replace chemical approaches that require multiple steps and the use of protection/deprotection reagents. Industrial adoption of BiCE enzyme variants for commercial production of chemicals and pharmaceutical intermediates, as described above, has major environmental benefits. For example, Almac's head of biocatalysis noted: "*Recent success using a BiCE transaminase enzyme has resulted in the removal of 8 steps of chemistry using a transaminase enzyme from the BiCE project. As you can imagine this has a major input into cost of goods by lowering reagent and energy usage and very importantly, waste production*" [f]. Likewise, SIAL has obtained similar benefits since 2008 through exploitation of a BiCE TK enzyme for the synthesis of xylulose-5-phosphate and a transaminase single step route to pyridoxamine-5'-phosphate, both metabolic intermediates useful for research purposes. These enzyme processes replace multiple chemical steps in the synthesis of these products, with the benefit that no costly and hard-to-dispose-of organic solvents need to be used [f].

New job creation: The establishment of Synthace in 2011 and its subsequent success in raising investment funding has led directly to the creation of 7 new jobs [b]. Likewise, Almac's association with UCL and adoption of our advanced biocatalyst technologies "*has resulted in significant growth of biocatalysis research at Almac including increased staff numbers*" [f]. In a new business plan finalised in July 2013 HEL Ltd committed to the creation of 4 new jobs to support their growing miniature stirred bioreactor product line [d].

Knowledge transfer to the wider industrial community: The wider uptake of BiCE programme outputs has also been achieved through incorporation of BiCE-related material in a number of the industrial modules contributing to the UCL **MBI® Training Programme**. Modules using this material include Rapid Fermentation Process Development, Design of Experiments for Bioprocess Optimisation and Industrial Biocatalysis and Biorefining. These modules have been attended by 157 industrial delegates from over 50 companies since 2008, with several customers having used multiple courses throughout the five-year period covered.

5. Sources to corroborate the impact

[a] A full copy of the statement from the CEO of Synthace, corroborating the impact of company formation, utilisation of underpinning BiCE research and UCL support for the company is available on request.
[b] Quote from David Willetts, 7 March 2013, https://www.gov.uk/government/news/government-invests-5-3-million-in-leading-edge-bioscience.
[c] A full copy of the statement from the Managing Director of HEL Ltd, corroborating sales of the new bioreactor product line and investment in new jobs is available on request.
[d] A statement from Executive Director of BioProcess Technology & Expression, Biologics BioProcess Development, Merck Research Labs, corroborates the time and cost savings to the company. Available on request.
[e] A full copy of the statement from the Head of Biocatalysis at Almac corroborating the economic and environmental benefits arising from utilisation of the BiCE TAm is available on request.
[f] A full copy of the statement from the Head of Research Specialities of Sigma-Aldrich corroborating the use of BiCE TK and TAm enzymes in commercial manufacture and the environmental benefits these bring is available on request.