Institution:	University of Northumbria at Newcastle
Unit of Assessment:	3 - Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Improving green chemistry for the pharmaceutical industry using enzyme biocatalysts

1. Summary of the impact

Biocatalysts provide unique activities that facilitate chemical transformations that are simply not possible using abiotic methods. Northumbria University researchers with expertise in enzymes and biocatalysis have provided biocatalysis services to the pharmaceutical, fine chemical, food and biofuels industries through our business facing innovation unit Nzomics. This has generated significant contract research, collaboration and licence agreements to companies, including the pharmaceutical company GlaxoSmithKline and the services-led company Almac. Biocatalysts produced as a result of Northumbria University research and technology transfer are sold worldwide and benefit business through their use in research and development activities, such as the production of intermediates in drug synthesis.

2. Underpinning research

Based on new opportunities that have arisen from research, process developments and the increased availability of interesting enzymes, biocatalysis as a green technology is revolutionising traditional methods for chemical synthesis. Much research in this area is focused on integrating biocatalysts into a variety of processes ranging from the production of smaller, chiral ('handed') speciality chemicals to the synthesis of more complex pharmaceutical intermediates. Biocatalysis will very soon become the first choice for synthesizing large scale production of industrially relevant chiral products. Northumbria University researchers have made important contributions to the identification and characterisation of novel enzymes for biocatalysis, exploiting the sequence diversity uncovered by the recent explosion of microbial genome sequence data.

Initially, Professor Gary Black (appointed to Northumbria in 2000; Chair of Protein Biochemistry) produced and characterised a series of carbohydrate active enzymes with different substrate specificities for use in carbohydrate research, including hyaluronate lyases (Smith et al., 2005), beta-glucosaminidases (Dennis et al. 2006) and a pectate lyase (Charnock et al. 2005). In 2006, Professor Gary Black and Dr Justin Perry (appointed to Northumbria in 2000; University Enterpise Fellow) formed Nzomics Biocatalysis, a business-facing research unit offering services and products in the field of biocatalysis. In 2007, Dr Meng Zhang joined Nzomics as a Research Associate (University Research Fellow since 2011). Proof of concept funding (£86,350) was provided by Northstar Ventures, a venture capital company, for the development of a range of nitrile hydratase biocatalysts. Specifically, Nzomics screened several novel nitrile hydratases for enantioselectivity (specific 'handedness') against a broad range of chiral nitriles. This research determined properties relevant to the way these enzymes bind chiral nitriles and determined that these are important for selecting the enantioselectivity of all nitrile hydratases tested so far. Impressive enantiomeric ratios of 80 and >100 (i.e. selecting for one product form) were determined for two of the nitrile hydratases that we screened against the important pharmaceutical intermediate naproxennitrile (van Pelt et al. 2011).

Professor Black, Dr Perry and Dr Zhang have also shown that a novel nitrile hydratase can hydrate aliphatic, aromatic and heterocyclic nitriles under very mild conditions and a range of organic solvents, often with excellent product selectivity. The major reaction determinant is the degree of steric hindrance around the nitrile moiety and/or size of the substrates (Black et al. 2010).

Subsequent to these studies, Professor Black, Dr Perry and Dr Zhang, with Dr Lynn Dover (Reader in Biotechnology; appointed to Northumbria in 2007), have recently developed a generic process for the production of UDP-sugar: sterol glycosyltransferase enzymes for the synthesis of sugar–modified sterols and have shown that novel enzymes in this class exhibited exquisite substrate specificity towards steroidal acceptors (Malik et al. 2013).

Much of this research has been funded by national funding bodies including a Leverhulme Trust grant to Black (£55,139) in 2003; two EPSRC Industrial CASE studentships (£83,298 and £85,052) in 2007 with Piramal Healthcare UK Ltd and in 2008 with Prozomix Ltd, respectively; and a Knowledge Transfer Partnership grant (£28,680) in 2010 with Hycagen Ltd. Continuing research is currently being funded via another EPSRC Industrial CASE studentship (£89,117) awarded in 2011 with Prozomix Ltd entitled 'Understanding the biocatalytic processing of renewable platform chemicals'. Cumulatively our work takes a 'green chemistry' approach that has allowed collaboration with industry to produce improved substrates for use in the pharmaceutical, fine chemical, food and biofuels industries.

3. References to the research

Charnock S.J., Brown I.E., Turkenburg J.P., Black G.W. & Davies G.J. (2002) 'Convergent evolution sheds light on the anti-beta-elimination mechanism common to family 1 and 10 polysaccharide lyases'. *Proceedings of the National Academy of Sciences (USA)* 99, pp12067-12072. DOI: 10.1073/pnas.182431199.

Smith N.L., Taylor E.J., Lindsay A-M., Charnock S.J., Turkenburg J.P., Dodson E.J., Davies G.J. and Black G.W. (2005) 'Structure of a group A streptococcal phage-encoded virulence factor reveals a catalytically-active triple-stranded beta-helix'. *Proceedings of the National Academy of Sciences (USA)* 102, pp17652-17657. DOI: 10.1073/pnas.0504782102.

Dennis R.J., Taylor E.J., Macauley M.S., Stubbs K.A., Turkenburg J.P., Hart S.J., Black G.W., Vocadlo D.J. & Davies G.J. (2006) 'Structure and mechanism of a bacterial beta-glucosaminidase having O-GlcNAcase activity'. *Nature Structural & Molecular Biology* 13, pp365-371. DOI:10.1038/nsmb1079.

Black G.W., Gregson T., McPake C.B., Perry J.J., Zhang M. (2010) 'Biotransformation of nitriles using the solvent-tolerant nitrile hydratase from *Rhodopseudomonas palustris* CGA009'. *Tetrahedron Letters* 51, pp1639-1641. DOI:10.1016/j.tetlet.2009.12.094.

van Pelt S., Zhang M., Otten L.G., Holt J., Sorokin D.Y., van Rantwijk F., Black G.W., Perry J.J., Sheldon R.A. (2011) 'Probing the Enantioselectivity of a Diverse Group of Purified Cobalt-Centred Nitrile Hydratases'. *Organic and Biomolecular Chemistry* 9, pp3011-3019. DOI: 10.1039/C0OB01067G.

Malik V., Zhang M., Dover L.G., Northern J.S., Flinn A., Perry J.J., Black G.W. (2013) 'Sterol 3beta-glucosyltransferase biocatalysts with a range of selectivities, including selectivity for testosterone'. *Molecular BioSystems* 9, pp2816-2822. DOI: 10.1039/c3mb70303g.

4. Details of the impact

As a crucial element of modern biocatalysis, biocatalysis enzyme panels are being increasingly developed for integration into safe, green, more selective and cost effective processes for the synthesis of various compounds, including chiral chemicals. Through Nzomics, Northumbria researchers have now worked with over 20 clients (15 since 2008) providing a range of biocatalysis enzymes for processes in the pharmaceutical, fine chemical, food and biofuels sectors. Since 2008, Nzomics has offered fee-for-service contract research services totalling approximately £200,000 for a range of clients including GlaxoSmithKline and Almac Sciences, in the pharmaceutical sector; Glycoform, Prozomix, Hycagen, Protech Research, Sepagen and Micron Bio-systems, in the biotechnology sector; and Kraft Foods in the food sector. Additionally, Nzomics has entered into research and licence agreements with Almac (2009-present), Prozomix (2009-present) and Megazyme International (2003-present): these companies sell enzymes developed by Nzomics to a worldwide market.

In 2009, Nzomics entered into two six-year collaboration and licence agreements with Almac to provide recombinant DNA clones to the company for the production of two of their selectAZyme™ Enzyme Panels, the Carbonyl Reductase (CRED) Enzyme Screening Panel and the Nitrile Manipulator (NM) Enzyme Screening Panel. The CRED panel is now the largest of the enzyme

panels sold by Almac and was developed solely with Nzomics. CRED and NM sales since 2009 are as follows: 27 CRED panels and the bulk purchase of 17 individual CREDs (corresponding to approx. £250,000 of sales) and 13 NM panels (corresponding to approx. £30,000 of sales). Development and sale of these panels has resulted in the significant expansion of Almac's Biocatalysis team. The sales are mainly to pharmaceutical and fine chemical companies and the enzymes are used in the production of active pharmaceutical ingredients (intermediates in drug synthesis). Almac have confirmed that work with Nzomics *"has resulted in useful commercial opportunities for Almac from medicinal chemistry scaffold supply to actual building block supply at scale across a range of industrial disciplines".*

Nzomics have provided services to GSK since 2009, supplying valued materials, such as enzyme panels and cloned genes. GSK states that this *"has allowed us to attempt some novel enzyme-catalysed chemistry or to explore a scientific hypothesis relating to the synthesis of specific molecules of interest, and all have been incorporated into our general pool of enzymatic tools"*. Indeed, GSK emphasise that *"Nzomics' fee-for-service business model works ideally for our industry"* and *"This has therefore been a highly productive and valued relationship for us.*"

In 2009, Nzomics also entered into a collaboration and licence agreement with Prozomix to provide recombinant DNA clones for the production of several research enzymes. So far this has resulted in approximately £20,000 of sales, to academia, biotechnology, fine chemical and pharmaceutical companies. The collaboration with Prozomix also includes two EPSRC funded CASE studentships, one completed and one just starting. The student associated with the completed studentship became an employee of Prozomix in May 2012. In regard to its Ketoreductase Enzyme Panel flagship product, Prozomix confirms that, "*without the underpinning research from Nzomics, with respect to deciphering which of the many discrete protein families are most relevant to our customers, our product would almost certainly not be recognised as having the highest hit rate of all similar kits available globally"*. Moreover, Prozomics state that *"Nzomic researchers have a unique ability to identify, target and rapidly progress key areas of emerging biocatalysis enzyme technology, and then to disseminate those findings with great overall commercial effect" and that this "has, and we hope will continue to, support new product development within Prozomix".*

These activities demonstrate that the research expertise of Professor Black, Dr Dover, Dr Perry and Dr Zhang in enzyme characterisation and improvement has significantly benefited UK industry (from SME level to 'big Pharma') through the provision of novel reagents and products which have had valuable impacts on their business success.

5. Sources to corroborate the impact

Reports, reviews and web links or other documented sources in the public domain

More information about nitrile hydratase biocatalysts can be found on the Nzomics blog (www.nzomics.blogspot.com).

The link below provides corroborative evidence concerning the relationship between Almac and Nzomics. *"Almac is investing further resources to build on success and to increase its collection of proprietary CREDs. A collaboration has been set up with Nzomics Biocatalysis at the University of Northumbria to accelerate this programme. The collaboration is building upon synergistic skills between the companies to develop a novel panel of CREDs which can be used in organic synthesis."* See: http://www.almacgroup.com/wp-content/uploads/Fuelling-the-Ciral-Line-with-Biocat-power.pdf (reprinted Speciality Chemicals Magazine January/February 2009)

Factual statements by key users/beneficiaries (to be made available if audited) and individual users/beneficiaries who can be contacted to corroborate claims

The Head of Biocatalysis and Isotope Chemistry at Almac has provided a statement and can be contacted to corroborate the claims regarding their relationship with Nzomics and the benefits this has created for Almac

The Synthetic Biochemistry Lead at GlaxoSmithKline Global Manufacturing and Supply has provided a statement and can be contacted to corroborate claims regarding their relationship with Nzomics and the benefits this has created for GlaxoSmithKline.

The Managing Director of Prozomix has provided a statement and can be contacted to corroborate the claims regarding their relationship with Nzomics and the benefits this has created for Prozomix.