Institution: University of Northumbria at Newcastle

Unit of Assessment: 3 (Allied Health Professions, Dentistry, Nursing and Pharmacy)

Title of case study: Delivering the benefits of enzyme biocatalysis for the pharmaceutical and chemical industries

Period when the underpinning research was undertaken: 2002 – 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Gary Black	Professor	01/09/2000 – present
Justin Perry	Professor	01/09/2000 – present
Darren Smith	Associate Professor	01/09/2011 – present
Nicola Brown	Research Fellow	16/02/2001 – present
Graeme Turnbull	Senior Lecturer	01/09/2012 – present

Period when the claimed impact occurred: August 2013 – December 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact (indicative maximum 100 words)

Chemical synthesis on an industrial scale is usually harmful to the environment as it is often conducted under harsh conditions, typically requires toxic chemicals, and generates hazardous by-products. In pursuit of greener technology, biocatalysis is rapidly changing traditional methods of producing pharmaceuticals and fine chemicals. For more than 15 years, research from Northumbria University, headed by Professor Gary Black and Professor Justin Perry, has explored the potential of biocatalysis to reduce and minimise environmental impacts. Through multiple research projects, this expertise has been used to develop the business of Prozomix Limited, a company specialising in the discovery and production of biocatalysis enzymes. New proprietary enzyme panels have been taken to market, enabling novel environmentally friendly pathways for manufacturing chemicals and opening significant new business opportunities for the company. As a result, the company now has agreements with 10 of the 11 largest pharmaceutical companies. Products sold by the company are now yielding productivity and environmental benefits for users and identifying enzymes for manufacture of drugs targeting COVID-19.

2. Underpinning research (indicative maximum 500 words)

Biocatalysis is the use of natural resources, such as enzymes or cells, to catalyse chemical reactions. The advantages of this process include mild reaction conditions – such as atmospheric pressure, neutral pH levels, and moderate temperatures – as well as the generation of fewer by-products. Northumbria University researchers have made important contributions to the discovery and characterisation of novel enzymes for biocatalysis, exploiting the explosion of microbial genome sequence data to discover naturally occurring enzymes for industrial bioprocesses.

With support from colleagues within the Unit's *Exploring & Exploiting Microbial Diversity* research group, Black and Perry have generated proof-of-concept evidence for the development of a range of nitrile hydratase biocatalysts. Nitriles are chiral molecules, meaning they exhibit a specific chemical 'handedness'. This is critical in pharmaceuticals as the 'left' and 'right-hand' versions of the same molecule (termed enantiomers) can behave differently in a 'handed' environment, such as the human body, and therefore chirality has potential to affect both the effectiveness and the side effects of a drug. Pharmaceutical applications therefore require specific enantiomers of the active molecule.

Nitriles can be difficult to obtain in high yields from chemical synthesis, a process which often produces substantial waste. As an alternative, Northumbria researchers demonstrated that

several novel nitrile hydratase enzymes could be used to efficiently synthesise chiral nitriles with reduced environmental impact [**R1**-**R2**]. This process has delivered products with greatly improved enantiomeric ratios, as enzymes typically present a 'handed' environment that allows the preferred version of the nitrile to form. Examples of pharmaceutical reactions where chirality is important include the important pharmaceutical ingredient naproxen nitrile, an anti-inflammatory [**R1**] and a constituent of one of the world's most widely used chemotherapy drugs, Taxol [**R2**].

Northumbria researchers developed a simple but effective high-throughput screening method to quickly and easily assess panels (collections) of potential biocatalytic enzymes for their ability to perform a nitrilase reaction (Fig. 1). The screen uses a colour indicator for the rapid identification and quantification of nitrilase activity, in cell-free extracts, using a multi-well plate format [**R3**].

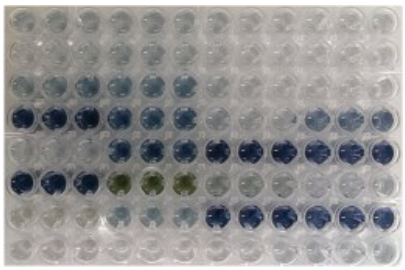

Fig 1: Development of a blue colour indicating level of a nitrilase reaction with 31 nitrile substrates (in triplicate) in a multi-well plate.

Originally, the enzyme panels screened with these methods (marketed by Prozomix as 'enzyme toolkits') originated from traditionally cultured microorganisms, which limited discovery as cultured microorganisms are a minority of the microbial world. In 2017, Professor Darren Smith introduced the game changing use of metagenomics, a DNA-sequencing based approach that allows the identification of microbial genes within environmental samples without culture [**R4**], thus providing access to a massively expanded pool of enzyme novelty and diversity. The first enzymes to be sourced from metagenomes sequenced at Northumbria were members of the vast ketoreductase (KRED) superfamily [**R5**]. These enzymes provide sources of chiral alcohols with high enantiomeric purity for the production of pharmaceutical intermediates or ingredients. Subsequently Black developed a novel colour indicator assay format (branded kREDy-to-go™) in anticipation of the future availability of additional or expanded KRED panels, as enabled by genomic and metagenomic prospecting. This rapid and highly cost-effective method is suitable for both manual and high-throughput applications [**R6**].

Cumulatively, the Northumbria work has developed a 'green chemistry' approach that has expanded the Prozomix enzyme pipeline and thus allowed industry to produce improved substrates for use in both the pharmaceutical and fine chemical industries.

3. References to the research (indicative maximum of six references)

R1. Van Pelt, S., Zhang, M.*, Otten, L.G., Holt, J., Sorokin, D.Y., van Rantwijk, F., **Gary Black**, **Justin Perry**, and Sheldon, R.A. (**2011**) 'Probing the Enantioselectivity of a Diverse Group of Purified Cobalt-Centred Nitrile Hydratases' *Organic and Biomolecular Chemistry* **9**: 3011-3019 DOI: doi.org/10.1039/C0OB01067G

R2. Wilding, B., Veselá, A.B., **Justin Perry**, **Gary Black**, Zhang, M.*, Martínková, L., and Klempier, N. (**2015**) 'An investigation of nitrile transforming enzymes in the chemo-enzymatic synthesis of the taxol sidechain' *Organic and Biomolecular Chemistry* **13**: 7803-7812 DOI: doi.org/10.1039/C5OB01191D

R3. Gary Black, **Nicola Brown**, **Justin Perry**, Randall, P.D., **Graeme Turnbull**, and Zhang, M.* (**2015**) 'A high-throughput screening method for determining the substrate scope of nitrilases' *Chemical Communications* **51**: 2660-2662 DOI: doi.org/10.1039/C4CC06021K

R4. Tariq, M.**, Everest, F., Cowley, L., de Soyza, A., Giles Holt**, Bridge, S.*, Perry, A., Perry, J., Bourke, S., Cummings, S.*, Lanyon, C.*, Barr, J., and **Darren Smith** (**2015**) 'A metagenomic approach to characterize temperate bacteriophage populations from Cystic Fibrosis and non-Cystic Fibrosis bronchiectasis patients' *Frontiers in Microbiology*, **6**(97) DOI: doi.org/10.3389/fmicb.2015.00097

R5. Thai, Y-C., Szekrenyi, A., Qi, Y.**, **Gary Black**, Charnock, S. J., and Fessner, W.D. (**2018**) 'Fluorogenic kinetic assay for high-throughput discovery of stereoselective ketoreductases relevant to pharmaceutical synthesis' *Bioorganic and Medicinal Chemistry* **26**: 1320–1326 DOI: doi.org/10.1016/j.bmc.2017.05.024

R6. Qi, Y.**, Bawn, M.**, Duncan, R., Lloyd, R., Finnigan, J.**, **Gary Black**, and Charnock, S. (**2016**) 'A Rapid, Inexpensive and Colorimetric High-throughput Assay Format for Screening Commercial Ketoreductase Panels, Providing Indication of Substrate Scope, Co-factor Specificity and Enantioselectivity' In *Practical Methods for Biocatalysis and Biotransformations* 3 (Eds. Whittall, J. Sutton, P.W., Kroutil, W.) pp. 266-273, Willey, Chichester, West Sussex, UK. Available on request

*__Internal Northumbria University co-authors__: Dr Meng Zhang, Associate Professor of Microbial Biotechnology (submitted to UoA12); Northumbria UoA3 staff S. Bridge, S. Cummings and C. Lanyon contributed to elements of R4 that are not relevant to this case study

**__Northumbria PhD studentships__: Maria Bawn, awarded 2012 (supervisor: Gary Black), Yuyin Qi, awarded 2015 (supervisor: Gary Black); James Finnigan, awarded 2019 (supervisor: Gary Black); Mohammad Tariq, awarded 2016 (supervisor: Darren Smith); Giles Holt, awarded 2018 (supervisor: Darren Smith)

Research funding
G1. ESPRC Industrial CASE studentship, 2008, GBP85,000
G2. ESPRC Industrial CASE studentship, 2011, GBP89,000
G3. PI Gary Black, BBSRC, June 2015 – June 2016, GBP82,240 (BB/M028496/1)

4. Details of the impact (indicative maximum 750 words)

Impact from Northumbria's biocatalysis research has been primarily delivered through and for Prozomix Limited, a privately-owned, research-intensive biotechnology company based in the UK that is focused on the discovery and production of biocatalysis enzymes. Prozomix is one of only six companies in the world specialising in biocatalysis enzymes and its customer base includes ten of the eleven leading pharmaceutical companies, along with prominent synthetic biology companies. Prozomix has benefitted from the research in three ways: (i) bringing new and improved products to market, (ii) resulting commercial benefits, and (iii) business growth. More broadly, there are efficiency and environmental benefits that have resulted from the new green chemistry processes built on Northumbria's research.

Visiting Professor Simon Charnock, the Managing Director of Prozomix, acknowledges that, without Northumbria research, the company *'would not have been able to gain the market share it has'* [**E1**]. In particular, four areas of product development have been identified as key to the company's success. Northumbria research and collaboration since 2011 has led to *'very large diverse panels of biocatalysts developed that now comprise the popular Prozomix biocatalysis Enzyme Toolkit'* [**E1**, **E2**]. This product has enabled Prozomix to rapidly become a global player in the enzyme screening market [**E1**]. Meantime, the applied metagenomics work enabled Prozomix to *'quickly and cost-effectively develop beyond state-of-the-art "maximum diversity" proprietary enzyme panels*' [**E1**].

Further to this, Northumbria's research led to the development of numerous additional panels of enzymes, key to taking market share, for example of carbonyl reductases from the KRED superfamily. In this case, *'not only did Northumbria devise the metagenomic sequencing and data analysis strategy, but they also aided in the design of a rapid and efficient method of analysing/screening the novel KREDs generated'* [**E1**]. This resulted in the kREDy-to-go™ product, which utilises Northumbria's novel, high-throughput colorimetric screening technology [**R6**; **E1**; **E3**, p3] and has made the screening of enzymes much cheaper, easier, and quicker for Prozomix's customers [**E2**]. Another highly desirable panel of enzymes enabled by the Northumbria collaboration is Prozomix's range of cytochrome P450 enzymes [**E4**, p2]. These

represent a large enzyme superfamily, members of which catalyse the oxidation of a wide range of organic substrates. This has enabled Prozomix to enter the drug metabolism market. These enzymes have generated significant revenues from customers in the flavour/fragrance and chemical industries [**E1**].

The new and improved products that Prozomix has brought to market through their work with Northumbria University have resulted in commercial benefits for the company, including *'lucrative enzyme discovery and supply agreements with 10/11 of the largest pharmaceutical companies in the world, now a source of significant and rapidly rising sales revenue*' [**E5**]. It has also resulted in more than 50% market share of biocatalysis screening within the pharmaceutical industry [**E5**]. As well as growing its market share, the company has adapted its business plan in the light of work with Northumbria [**E6**]. The increased number of enzyme panels gave Prozomix the opportunity to move away from its one-off 'hit fee only' based model to a long-term (20 years per hit) recurring revenue stream from all panels offered by the company [**E6**]. [Text removed for publication] signed-up to the new agreement very quickly, demonstrating that the expanding collection of panels within the Biocatalysis Enzyme Toolkit were compatible with the intended target market. Virtually universal interest from the leading pharmaceutical companies has followed in the current overall Toolkit offering, with Prozomix viewed by this sector as an emerging leader in this highly lucrative industry category [**E6**].

As a result of the success underpinned by Northumbria research, in 2015 Prozomix's business model expanded by entering the contract bulk enzyme production market. This expansion involved increasing manufacturing space from 3,000 ft^2 to 11,000 ft^2 by purchasing a factory in Northumberland, and further significant investment in pilot and production equipment. Despite such significant investment, take-up by clients was such that Prozomix benefitted from a return on investment after only two years [**E5**]. Prozomix is currently expanding its fermentation capacity further still, from 1,000 L to 7,000 L total working volume, sufficient to supply the global annual demand for most enzymes requested. Prozomix will thus shortly become the only company in the sector offering initial enzyme discovery through to annual production capacity, all in-house [**E5**].

Without the specialist expertise of Northumbria researchers [**R1**-**R4**], Prozomix would not have been able to secure participation in its first EU FP7 grant, KYROBIO (2011-2015), success in which led the company to participate in five further Horizon 2020 projects, with grant income totalling [text removed for publication] [**E1**]. Additional evidence of the importance of the relationship between Prozomix and Northumbria University is the rapid creation of a highly experienced company workforce, through employment of PhD level scientists trained at Northumbria University [**E1**].

Beyond Prozomix operations, Northumbria's green chemistry research has benefitted users of the products and wider society. A Prozomix customer, [text removed for publication], was able to identify a transaminase enzyme from a Northumbria sequenced metagenome, which is now used to manufacture weed suppressant [**E5**]. In 2020, [text removed for publication] acquired this enzyme from [text removed for publication] for a process that will produce 25,000 tons of material [**E7**]. The new transaminase enzyme makes the product significantly more efficient, reducing the amount of material that farmers need to apply to control weeds by up to 50%, which will substantially reduce operating costs and environmental impact and thus improve the sustainability of the weed control process [**E7**]. Finally, numerous recent enzyme hits have been identified by Prozomix pharmaceutical clients for use in the manufacture of drugs against COVID-19. These KRED hits were mined from metagenomic data sequenced by Northumbria, identified through the clients' use of kREDy-to-go™ screening [**E5**].

Overall, Prozomix confirms [**E1**, **E5**] that, without Black and colleagues, the company would not have been able to secure such an excellent reputation, market position and turnover [text removed for publication] in such a short period of time. Prozomix acknowledges that the relationship with Northumbria University has been invaluable in driving their strategy, in developing their network of contacts, supplying the company with new talent, helping to develop

new in-house techniques, and supporting their knowledge and understanding of the enzyme discovery field from research. As Dr Ruth Lloyd, the Operations Director, noted: *'I can absolutely affirm that our commanding strategic technical position and well-respected global brand would not have been achieved without the support from the Black Group and others at Northumbria'* [**E5**].

5. Sources to corroborate the impact (indicative maximum of 10 references)

Ref.	Source of corroboration	Link to claimed impact
E1	Testimonial - Simon Charnock, Managing Director, Prozomix	Corroborates Northumbria contribution to Prozomix business model, new products, and economic success
E2	Biocatalysis Enzyme Toolkit Concept (source: www.prozomix.com)	Details the enzyme tool kit developed by Prozomix, with the help of Northumbria University
E3	Prozomix Biocatalysis Enzyme Kits catalogue (source: www.prozomix.com)	Evidences the existence of the KRED diversity panel and the kREDy-to-go kit and demonstrates they are for sale from Prozomix
E4	Prozomix – Technology Snapshot (source: www.prozomix.com)	Confirms development of P450s
E5	Testimonial - Dr Ruth Lloyd, Operations Director, Prozomix	Corroborates Northumbria contribution to Prozomix business development and product success
E6	Innovate UK, Project Completion Report, 2017	Confirms Northumbria contribution to new fee model and success of this with clients
E7	[Text removed for publication] announcement re. acquiring biotechnology from [text removed for publication]	Confirms acquisition, and benefits of the new transaminase enzyme