Institution: The University of Manchester		
Unit of Assessment: 8 (Chemistry)		
Title of case study: Driving the industrial biotechnology revolution: cheaper and more sustainable chemical manufacturing through enzyme discovery, engineering and scale-up		
Period when the underpinning research was undertaken: 2013 – 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:**
Nicholas Turner	Professor of Chemical Biology	2004 – present
Sabine Flitsch	Professor of Chemical Biology	2004 – present
Nigel Scrutton	Professor of Molecular Enzymology	2005 – present
Jason Micklefield	Professor of Chemical Biology	1998 – present
Michael Greaney	Chair in Organic Chemistry	2011 – present
Nicholas Weise	Lecturer	2009 – present
Christopher Hardacre	Professor of Catalysis and Organic Materials	2016 – present
Sarah Lovelock	Presidential Fellow (2020 – present), Research Fellow (2018 – 2020), PDRA (2014, 2017 – 2018)	2014, 2017 – present
Anthony Green	Lecturer (2016 – present), PDRA (2010 – 2014)	2010 – 2014, 2016 – present
Period when the claimed impact occurred: August 2013 – July 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Researchers in the Centre of Excellence in Biocatalysis, Biotransformations and Biocatalytic Manufacture (CoEBio3) at The University of Manchester (UoM) have discovered and developed a set of >1,000 engineered enzymes across 14 different reaction types. These biocatalysts are commercially available through a licensing agreement with SME Prozomix Ltd, and have been exploited by companies [text removed for publication] in the manufacture of hundreds of kilograms of pharmaceuticals and agrochemicals. [Text removed for publication]

The availability of UoM's biocatalysts and biotechnology methods has led to significant changes of practice in the chemical industry: synthetic chemists now employ biocatalysis alongside traditional synthetic methods. This was enabled by CoEBio3's computational retrosynthetic tool RetroBioCat and Massive Open Online Course (MOOC) in Industrial Biotechnology which has trained chemists globally, with >38,000 learners to date. Students in the 5-18 age group were taught through multi-lingual outreach programmes in Greater Manchester schools. CoEBio3's research has influenced government policy *via* the Industrial Biotechnology Leadership Forum and the "*Growing the UK Industrial Biotechnology Base"* strategy document.

2. Underpinning research

Discovery of new biocatalysts for reductive amination
Reductive amination of carbonyl compounds (aldehydes and ketones) with amines represents the most important method in organic synthesis for preparing amine derivatives. In medicinal chemistry, this synthetic transformation is widely used to prepare libraries of compounds for screening for biological activity. In 2017, the researchers reported the discovery of a new family of enzymes which we termed reductive aminases (RedAms) and demonstrated that these enzymes possess the remarkable ability to couple amines and ketones in water with 1:1 stoichiometry [1]. The enzymes are highly active, with turnover numbers >50,000, and are also highly enantioselective.

Development of biocatalytic hydrogen borrowing methodology
Alongside reductive amination, alkylation of amines is widely practised in both academia and industry for the synthesis of secondary and tertiary amines. However, this seemingly simple reaction suffers from two major drawbacks, namely the requirement for genotoxic alkylating

agents (*e.g.* triflates, mesylates, alkyl halides) and the generation of undesirable by-products through over-alkylation. An elegant solution to these two problems is to use benign alkylating agent equivalents, *e.g.* alcohols and carboxylic acids, with transition metal catalysts (Rh, Ru) under 'hydrogen borrowing' conditions. In 2015 we reported the first procedure for metal-free biocatalytic hydrogen borrowing by combining an alcohol dehydrogenase and amine dehydrogenase to couple an alcohol and amine in the presence of catalytic quantities of nicotinamide cofactor [2]. This biocatalytic approach avoids the use of expensive and potentially harmful metal catalysts, and since this key publication we have extended the method for coupling carboxylic acids and amines [3].

Development of biocatalysts for amide bond synthesis

The amide bond is the most frequently encountered functional group in active pharmaceutical ingredients (APIs). Consequently, new methods for the synthesis of amides are of a high priority, particularly those that involve the use of environmentally benign coupling reagents and that result in minimal generation of waste by-products. In this context biocatalysis presents significant opportunities – we have made major advances in the discovery and engineering of new biocatalysts that are able to couple amines directly with either carboxylic acids or esters, yielding the product amides with only water or a simple alcohol (e.g. ethanol) as the waste product [4].

Cascade biocatalysis

In nature, natural products are produced *via* biosynthetic pathways that involve multiple enzymes acting in sequence. This process results in the conversion of simple starting materials to complex bioactive products (*e.g.* polyketides, terpenes, alkaloids). We have recently shown that such multi-biocatalytic processes can be mimicked in the laboratory, resulting in the construction of synthetic, rather than natural, products [5, 6]. Such biocatalytic cascades represent a new paradigm in enzymatic synthesis of non-natural, biologically active compounds, and this methodology is increasingly being adopted by the pharmaceutical industry.

3. References to the research

All of the papers appear in high-quality peer-reviewed journals including *Science*, *Nature Chemistry*, *Nature Communications*, and ACS journals. CoEBio3 was a major contributor to the 2019 Queen's Anniversary Prize for Higher and Further Education, which was awarded to the Manchester Institute of Biotechnology.

[1] Aleku *et al*., *Nat. Chem.*, **2017**, *9*, 961-969 DOI: 10.1038/nchem.2782
[2] Mutti *et al*., *Science*, **2015**, *349*, 1525-1529 DOI: 10.1126/science.aac9283
[3] Ramsden *et al*., *J. Am. Chem. Soc.*, **2019**, *141*, 1201-1206 DOI: 10.1021/jacs.8b11561
[4] Wood *et al*., *Angew. Chem. Int. Ed.*, **2017**, *56*, 14498-14501 DOI: 10.1002/ange.201707918
[5] France *et al*., *ACS Catal*., **2016**, *6*, 3753-3759 DOI: 10.1021/acscatal.6b00855
[6] Latham *et al*., *Nat. Commun.*, **2016**, *7*, 11873 DOI: 10.1038/ncomms11873

Underpinning grants

This work has been supported by a grant portfolio in excess GBP58,000,000 with GBP16,500,000 of direct funding to UoM. Major grants (UoM funding) include:

- "Chemical Manufacturing Methods for the 21st Century Pharmaceutical Industries", Innovative Medicines Initiative (IMI) 2012-2018, EUR1,580,000;
- "Developing the Next Generation of Biocatalysts for Industrial Chemical Synthesis", EU FP7, 2011-2014, EUR1,180,000
- BIO-H-BORROW, ERC, 2017-2022, EUR2,340,000 (Turner);
- "Programmable Enzymatic Synthesis of Bioactive Compounds (ProgrES)", ERC, 2018-2023, GBP2,030,000 (Flitsch);
- "EPSRC/BBSRC Centre for Doctoral Training in Integrated Catalysis", EP/S023755/1, 2019-2027, GBP7,000,000 (Greaney);
- "Rapid evolution of enzymes and synthetic micro-organisms for the development of industrial biocatalysts", BB/K00199X/1, BBSRC, 2012-2017, GBP4,490,000 with an industrial contribution from GSK of GBP500,000 (Turner, Flitsch, Micklefield, Scrutton);

- "EPSRC/BBSRC Future Biomanufacturing Research Hub", EPSRC, EP/S01778X/1, 2019-2026, GBP10,000,000 plus GBP4,500,000 pledged by industry (Scrutton).

CoEBio3 Industrial Affiliates provided direct funding of precompetitive research projects: >GBP1,600,000 in direct industrial funding (2013-2019, Turner, Flitsch, Micklefield, Scrutton).

4. Details of the impact

Development of chemical manufacturing processes using engineered biocatalysts
Pathway to impact

[Text removed for publication]

Reach and significance

[Text removed for publication]

Training the next generation of industrial biotechnologists – changing attitudes, behaviours and understanding

i) Changing practice in the chemical industries

The CoEBio3 researchers have created training courses in Industrial Biotechnology, based partly on their research. This includes a Continuing Professional Development (CPD) course on 'Biocatalytic Retrosynthesis', delivered to industrial chemists at AstraZeneca, GSK, Gilead, Novartis and Lonza. To accompany this, they produced a book, "*Biocatalysis in Organic Synthesis: The Retrosynthesis Approach*." (N.J. Turner and L. Humphreys, 2018), which has sold 500 units to date, as well as a computational tool (RetroBioCat: www.retrobiocat.com) which has received 557 downloads and has been read >1,600 times on chemrxiv.org (as of 23/10/20).

The CPD course developed and delivered to industrial chemists has proven remarkably successful. It has been delivered 10 times between 2013 and 2020 and over 500 participants have attended the course. The course has built the capacity of these participants to apply biotechnology, including CoEBio3's engineered enzymes, in their industrial workplaces. Furthermore, RetroBioCat is now being used by many pharma companies for synthetic route design. Examples of this include (emphasis added):

- Gilead, who stated: "*This training has resulted in an expansion of additional opportunities in the way our scientists work day-to-day with biocatalysis as part of their tool kit. It is* ***now commonplace for our scientists to consider using biocatalytic transformations****...*" [E]
- Bayer, who stated: "*as a direct consequence of working with the University of Manchester and the MIB,* ***we have altered our working practices investing significantly in a new screening laboratory****."* [A]
- AstraZeneca, who stated: "*We are now also evaluating the computational tool RetroBioCat developed by the Manchester group and believe that this online tool has* ***changed the way our synthetic organic chemists view biocatalysis***". [F]
- GSK, who stated: "*These courses have helped fundamentally changed* (sic) *the way both lab-scale and process-scale synthetic chemists at GSK Stevenage work. These* ***chemists now routinely apply the concepts of biosynthetic retrosynthesis to their work****. This has led to the more common use of engineered enzymes as biocatalysts in both lab-scale and process-scale synthesis*." and also: "*MIB represents by far the most fruitful source of talent in biocatalysis for GSK*". [G]

In 2018, in collaboration with AstraZeneca (GBP1,000,000 investment) and Prozomix, CoEBio3 established the Centre for Biocatalytic Manufacturing of New Modalities (CBNM) to develop new scalable biocatalytic technologies for the cost effective and efficient synthesis of new pharmaceutical modalities [F]. In 2020, CoEBio3 was selected by the Bill & Melinda Gates Foundation to develop new biocatalytic routes to global health drugs for treatment of HIV, COVID-19 and tuberculosis (GBP600,000). This programme of work has the specific aim of applying industrial biotechnology to the manufacture of medicines at greatly reduced cost, making them affordable to healthcare systems with severely limited financial resources.

ii) Shaping UK Government policy through an industrial biotechnology strategy

In 2008, by virtue of their research achievements and expertise, researchers from CoEBio3 were invited to join the UK Government-backed Industrial Biotechnology Leadership Forum (IBLF) [H]. The IBLF is formed of influential members drawn from industry, academia, finance, NGOs, funding agencies (BBSRC, EPSRC, InnovateUK) and government [H]. Through their participation with the IBLF, CoEBio3 researchers directly co-authored parts of our report *"Growing the UK Industrial Biotechnology Base",* which was issued as a UK road map and strategy for Industrial Biotechnology to 2030. This report has directly shaped the UK Government's policies in life science investment, and biotechnology and is foundational in the UK's Bioeconomy Strategy that was formally launched by Government six months later in December 2018 [H]. This strategy is now the guiding document for UK bioeconomy policy – with a goal of creating a 2030 bioeconomy that has a GVA of GBP440,000,000,000 – double its size in 2018 [H].

iii) Inspiring the next generation of scientists to study industrial biotechnology

Alongside the specialist industrial course, CoEBio3 researchers designed and delivered a MOOC in Industrial Biotechnology (IB MOOC), available through the learning platform Coursera (https://www.coursera.org/learn/industrial-biotech). This IB MOOC has made teaching that was traditionally available to students studying at a biotechnology specialist institution freely accessible to anyone with an internet connection. The course has had 47,465 total learners and [5,700] course completers, ~75% of whom are aged 18-34, from Africa, Asia, Europe and the Americas (1,795 ratings; average score = 4.7/5) [I]. 12% started a new career after completing the course and 17% got a tangible career benefit from this course [I].

The researchers also created and co-ordinate a programme of public engagement with research/researchers at the Manchester Institute of Biotechnology, which is delivered to younger, non-specialist audiences from underprivileged backgrounds. This CoEBio3 Public Engagement Programme has enabled over 100 researchers from our institute to engage locally and nationally with non-specialist audiences. This unique approach which has now been adopted by others, has included partnering bilingual researchers with ~400 young people from immigrant backgrounds. As of June 2020, 12 such community visits have been organised with engagement in some of the most widely spoken non-English languages in Manchester (Bengali, Arabic, Polish and Mandarin) as well as other minority languages (Greek, Spanish, Italian, Hindi and Tamil). This initiative has inspired participants into further study and influenced career ambitions. For example, one participant said, *"I think this lesson has inspired me to pursue science as a future career"*, whilst another said, *"It was really nice to learn about the different aspects of chemistry and it has got me to want to know more about chemistry. I am now thinking to do Chemistry at university*" [J]. Delivery of the lessons in non-English languages was also viewed very positively by participants, with one saying "*…I really liked how the session was in Polish because it meant we had more knowledge about science but in Polish*" [J]. Teachers at these multilingual schools also greatly valued the classes, with one saying, "*The activity such as this gives children an opportunity to talk to the young scientists on interesting subjects in the language they are learning, which is a great encouragement to the children and a big help to the schools involved. This was clearly reflected in the highly positive feedback given by the participating children*" [J].

5. Sources to corroborate the impact

[A] Letter from [text removed for publication]
[B] Letter from [text removed for publication]
[C] Letter from [text removed for publication]
[D] Letter from [text removed for publication]
[E] Letter from [text removed for publication]
[F] Letter from [text removed for publication]
[G] Letter from [text removed for publication]
[H] Letter from [text removed for publication]
[I] Coursera Industrial Biotechnology course reviews, available at: https://www.coursera.org/learn/industrial-biotech/reviews

[J] PDF compilation of feedback from schools outreach sessions, available on request