Institution: EaStCHEM

Unit of Assessment: 8; Chemistry

Title of case study: Ingenza Ltd; Technologies for new Catalysts and Products across the Industrial Biotechnology Spectrum

1. Summary of the impact

Impact: ***Economic***. Ingenza is a profitable SME based in Roslin, Scotland, with 34 (12 PhD-level) staff, and a turnover of £2.7M in 2012-13.

Significance: Ingenza Ltd is an established industrial biotechnology (IB) and synthetic biology (SB) company which incorporated in September 2002. Its combination of synthetic organic chemistry with efficient methods of genetic screening, fermentation and engineered microbial strains is used to develop competitive and scalable industrial bioprocesses for pharma, chemicals, energy, natural product and other industry sectors.

Beneficiaries are Ingenza's customers (commercial and the public) and its employees.

Research; date; attribution: In 2002 the Turner group (University of Edinburgh, UoE) published in *Angew. Chem.* a new strategy of integrated chemo- and enzymatic catalysed routes to high-value chiral compounds that offered dramatic improvements over existing technologies (high yield and enantiomeric excesses often > 99.9%).

Reach: Ingenza now has moved from a focus on fine chemicals to establish long term technology development and licensing agreements with global leading end-users in the chemicals, polymers, biofuels, food and biologics sectors, for example in the sustainable manufacture of poly-methylmethacrylate with Lucite International.

2. Underpinning research

The Turner group in EaStCHEM developed the simultaneous use of a highly selective oxidase biocatalyst and a chemical reducing agent or catalyst, to prepare a huge variety of enantiopure chiral amines in high yield and optical purity.

In the key paper in 2002 [1] they demonstrated the deracemisation of the amino acid DL-proline using commercially available D-amino acid oxidase (DAAO) and three equivalents of $NaCNBH_4$, a reducing agent of sufficient water stability to be enzyme-compatible, yielding L-proline in 99% yield and 99 % ee (enantiomeric excess), achieving a 94 % conversion of racemate to chiral amino acid after hydrolysis.[1] Previous dynamic kinetic resolutions have been hampered by the harsh conditions required to racemise amines. They extended this to a range of amino acids using commercially available oxidase enzymes.[2]

Another key advance was the use of cloned microbial genes and the development of in-vitro evolution of enzymes. Working in collaboration with GSK, an enzyme from a gene *maoN* that oxidised simple aliphatic amines was identified that showed a clear preference for the oxidation of L-α-methylbenzylamine over D-α-methylbenzylamine. The group optimised this using random mutagenesis and *in vitro* and *in situ* selection, and colorimetric solid phase screening, to generate new biocatalysts and commercially important targets (starting with chiral amines) of interest to pharmaceutical industry customers.[3]

The Turner group recognised three key points of the new method that led them to protect the work [6] and then spin-out Ingenza: (i). The high selectivity of an enzyme allows enantiomeric excesses of >99.9 %. (ii) It converts all the potential substrate into the desired enantiomer product, compared with a standard enzyme-catalysed kinetic resolution of a chiral racemate. (iii). It does not require the harsh conditions required to racemise amines that alternative transition-metal catalyst routes to develop dynamic kinetic resolutions have.

Further UoE research to develop high throughput screening techniques [4] enabled a large number of solid phase screening capabilities to address problems faced by the biopharma industry. For example, novel screen calibration approaches allowed the development of colorimetric oxidase based protein fusion systems to identify the top 50 out of a 500,000-member library of candidates

for a key therapeutic target.[5]

Key researchers:
Prof Nick Turner, EaStCHEM School of Chemistry, University of Edinburgh 10/1998-09/2004. The other co-authors are PDRAs and PhD researchers in the Turner group, and industry collaborators. Enright was a collaborator in Strathclyde at the time of patent filing.
Dr Ian Fotheringham, co-founder of Ingenza in 2002, now President. Glasgow PhD, biocatalysis history with Monsanto.
Dr Robert Speight co-founder. Hired as a PDRA in 2000 to work in Professor Turner's lab, UoE. Awarded an RSE enterprise fellowship in 2002.

3. References to the research

Publications: *Underpinning research has been published in international, high-quality, peer reviewed, academic journals and receives citations from across the research area.*

[1] * Deracemisation and stereoinversion of alpha-amino acids using *D*-amino acid oxidase and hydride reducing agents. T. M. Beard, N.J. Turner, *Chem. Commun.*, **2002**, 246-7. doi:10.1039/b107580m. 46 cits, JIF 6.4.

[2] Stereoinversion of β- and γ-substituted α-amino acids using a chemo-enzymatic oxidation-reduction procedure. A. Enright, F.-R. Alexandre, G. Roff, I.G. Fotheringham, M.J. Dawson, N.J. Turner, *Chem. Commun.* **2003** 2636-7. doi: 10.1039/B309787K. 18 cits, JIF 6.4.

[3] * Deracemization of alpha-methylbenzylamine using an enzyme obtained by *in vitro* evolution. M. Alexeeva, A. Enright, M. J. Dawson, M. Mahmoudian, N. J. Turner, *Angew. Chem., Int. Ed.*, **2002**, *41*, 3177-80. doi:10.1002/1521-3773(20020902)41:17<3177::AID-ANIE3177>3.0.CO;2-P/abstract. 74 cits, JIF 13.7.

[4] Identification of broad specificity P450(CAM) variants by primary screening against indole as substrate. A. Celik, R.E. Speight, N.J. Turner, *Chem. Commun.* **2005**, 3652-54. doi:10.1039/B506156C. 18 cits, JIF 6.4.

[5] * Chemo-Enzymatic Synthesis of Unnatural Amino Acids *in* Asymmetric Synthesis and Application of α-Amino Acids. I. V. Archer, S. A. Arnold, R. Carr, I. V. Fotheringham, R. E. Speight, P. P. Taylor. *ACS Symposium series.* 2009, Vol. 1009, ch 20, p. 322-336.**ISBN13:** 9780841269743 **eISBN:** 9780841224841 Eds. V. A. Soloshonok and K. Izawa. [peer-reviewed book chapter] http://pubs.acs.org/doi/abs/10.1021/bk-2009-1009.ch020.

First patent:

[6] Patent with GSK (industrial collaborators at the time) M. V. Alexeeva, A. Enright, N. J. Turner, M. Mahmoudian, R. M. Thornley (WO.2003.080855.A2, GB0206415.2, filed 19 March 2002); 'enzymatic deracemisation of amines'.

Key Grants:
Dec 2001: Ligand discovery at Edinburgh University funded by Cyclacel (UK industry) (£702,360).
Jan 2002: Development of enantioselective amine oxidases for application in the deracemisation of racemic chiral amines, BBSRC (£124,027).
July 2009: SMART R&D: A new scalable biocatalytic technology to produce enantiomerically pure Unnatural Amino Acids (£475,108).
July 2010: To develop an improved bedside diagnostic (£69,747).
Nov 2010: Two Biosciences KTN SPARK Awards: Improved fed-batch fermentation protocols and Genome sequencing of improved yeast for biofuel applications (£5k each).
March 2011: TSB 100970 (with Edinburgh and Aberdeen Universities): Mining new enzymes in the rumen for biomass processing and chiral synthesis (£360,336).
August 2011: TSB 100962 (with Edinburgh University). Adapted yeast for superior carbon conversion (£578,890).

4. Details of the impact

Ingenza is an industrial biotech company spun out from EaStCHEM research, which employs 34 people and had a turnover last year of £2.7M. [F1] It is based in Roslin BioCentre, a science park in Midlothian for research-intensive and commercial life-science related SMEs. Ingenza was set up to optimise and exploit the new robust, general, and scalable biocatalysis platform technology described above. The new screening methods for strain-engineering developed in the research has helped the company expand its portfolio of biocatalysed transformations and engineered

production microbes into other industry sectors, with wide-ranging catalysed transformations for polymers, biofuels, feedstocks, nutrition and other applications. These enabling technologies have helped establish Ingenza as the leading UK industrial biotechnology and synthetic biology company. As a direct result, Ingenza's staff has grown 2-fold in the past 4 years and revenues have grown 5-fold. The President of Ingenza, in a corroborating letter, states *" Turner's group conducted important research at the School of Chemistry between 2001 and 2003…This work was an important foundation on which we originally built Ingenza's business base…Since that time Ingenza has evolved and diversified into the leading industrial biotechnology and synthetic biology company in the UK with global reach in its customer base and technology implementation…I can attest to direct linkage between research …and the significant societal and economic impacts that followed the spinning out of Ingenza and its ongoing expansion"* [F1]. The Life Sciences Director at Scottish Enterprise said "*The company ... is a great example of Scotland's thriving life sciences sector"* [S2].

Economic:
In the financial year 2012-2013 Ingenza turned over £2.7M and expects to continue strong and profitable growth as its capabilities and interface with multiple market sectors expand. Ingenza's revenue growth reflects the unique capabilities of the company's technology in a challenging economic climate and its adaptability to other industries' awareness and uptake of sustainable manufacturing practices. Total turnover for 2008-July 2013 = £7M. [F1]

a. Development of bioprocess routes to pharmaceuticals/chemical building blocks
Industrial biotechnology as a means to produce platform chemicals, polymer or important drug intermediates is of high and increasing value. Enzyme expertise (engineering and catalysis optimisation by directed evolution) has been applied to the large-scale manufacture of pharmaceutical intermediates. The value of sales for 2008-July 2013 is £3M. [F1]

b. Provision of process development and bioprocess optimisation for other industries
The enabling technologies developed in Section 2 are being used much more broadly by Ingenza for bioprocess optimisation and supply of improved production microbes, to provide large industries with new, sustainable manufacturing processes from renewable rather than petrochemical feedstocks. For example, multi-year partnerships have been established with leading global companies, such as Lucite International, the world's leading manufacturer of poly-methylmethacrylate [S3]. Ingenza piloted one of its improved biofuel strains with another end-user in the US at full production scale of 2.4 million litres; the Ingenza President can provide information on the number and range of other companies that have benefitted. A major two-year development programme employing five people at Ingenza to work with Invista, one of the world's largest integrated producers of polymers and fibres (10,000 employees in 20 countries), has also just completed successfully, with ten people starting on this project in the near future as a result of the expansion of the project. The Ingenza President can provide corroboration on this [F1]. The President of Invista is quoted in the most recent press announcement "*We see Ingenza's capabilities as very complementary to our own and are pleased to announce this extended collaboration."* [S4]
In a corroborating letter, the co-founder of Ingenza, who recently moved to Australia to be involved in new biotechnology start-up initiatives writes *"The deracemisation research and the interest that was generated by the high impact publications in Nick Turner's group was really the foundation of Ingenza … and the interest that was generated by the high impact publications. As well as the research, the School of Chemistry really supported Ingenza in those first few years in an operational sense and that support was key to starting the company on a strong footing…The ongoing relationship with the School of Chemistry has also been important for Ingenza… The international impact of the published research from Prof Turner's lab meant that Ingenza had a standing on the world stage from day one to the present day, with the majority of company's business coming from abroad."* [F2]

Human Capital:
Ingenza's scientific team spans the disciplines of molecular biology, biochemistry, enzymology, fermentation science and synthetic organic chemistry, and has recruited and trained numerous scientists, including 12 scientists trained to PhD or post-doctoral level at Edinburgh University. Currently 34 staff are employed, 12 of whom have PhDs.[F1]
There is also strong R+D collaboration with EaStCHEM staff, with joint awards from TSB, SPARK, ERA-NET, and collaborative PhD students. Ingenza has engaged with the Government's Modern apprentice scheme since 2009, winning 'Small Employer of the Year 2011' at the Scottish Modern Apprenticeship Awards.[S4] In 2012 Scottish Minister for Youth Employment, said "*I commend Ingenza for their forward thinking approach in fostering a new generation of young scientists, and offering up some excellent opportunities for training in this exciting sector*".[S5]

Impact Development Timeline:
2002 First paper and patent; Ingenza launched and incubated within School of Chemistry.[F1]
2003 The Edinburgh (University) technology fund invested £25,000 in Ingenza for a 5% stake; Ingenza won £120,000 funding from the Scottish Executive, Scottish Enterprise and the Royal Society of Edinburgh.
2005 Scottish Enterprise helped Ingenza with direct funding (£20,000) to offset costs of new business development. This helped Ingenza to establish its initial base of customers and strategic partners.
2007 Commercialisation partnership established with Richmond Chemical Corporation.

2008-2010 Awarded 2 new SMART awards and 3 Technology Strategy Board grant awards, two in partnership with the University of Edinburgh, totalling in excess of £1M. Receives two Biosciences KTN SPARK Awards.
2011 1) A multi-year bioprocess development agreement is signed with Lucite International for bio-manufactured monomers.[S3] 2) Awarded over £500k from TSB for three new biotechnology projects on Industrial Biotechnology and the commercial application of high-throughput genome sequencing, demonstrating Ingenza's diversity and leading position in industrial biotechnology.
3) Ingenza named 'Small Employer of the Year 2011'[S4] for its technical apprenticeship scheme.
4) 5 people at Ingenza are employed to work on a large bioprocess development project for Invista.
2012 1) Opening of a new GMP compatible clean room with additional local council investment of £50,000.[S6] 2) Ingenza is featured case study in Scottish Parliament Life Sciences event.[S7]
2013 Currently 34 staff employed [F1], Current turnover in excess of £2.7 M.

5. Sources to corroborate the impact
[F1] Letter corroborating the link between EaStCHEM research and Ingenza; President of Ingenza. Can be contacted to corroborate staff numbers, turnover and sales

[S1] Scottish Enterprise support for Ingenza, http://news.ingenza.com/?p=354, includes a quote from their Life Sciences Director

[S2] 2011 Ingenza and Lucite International in multi-year bioprocess development agreement. http://news.ingenza.com/?p=331.

[F2] Corroborating support letter from the co-founder of Ingenza [now Business Manager, Australian Institute for Bioengineering and Nanotechnology].

[S3] Press release from Invista Intermediates - extension of collaboration set up before July 2013 (includes quote from company President)

[S4] 2011 Small Employer of the Year (Skills Development Scotland) http://news.ingenza.com/?p=340

[S5] Visit to Ingenza from Scottish Minister for Youth Employment http://news.ingenza.com/?p=377

[S6] 2012 Ingenza new clean room with council investment: http://www.midlothian.gov.uk/press/article/402/50_000_council_loan_sets_midlothian_biotechnology-business_ingenza_ltd_on_expansion_drive).

[S7] Scottish Parliament –Life Sciences event http://www.lsscommunity.com/profiles/blogs/life-sciences-scotland-exhibition-at-the-scottish-parliament.