Institution:
University of Cambridge

Unit of Assessment:
UoA5

Title of case study:
From natural products to medicines by biosynthetic engineering

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

Many clinically-useful natural products fall into the class of polyketides. From 1993, research led by Professors Leadlay (Biochemistry) and Staunton (Chemistry) on polyketide biosynthesis pathways led to the foundation of the spin-out company Biotica Technology Ltd in 1996. Between 2008 and 2013 the company provided continuous employment for on average 15-20 highly-skilled scientists, and attracted additional investments of £4.43M. Its follow-on company Isomerase Therapeutics Ltd, founded by ex-Biotica researchers with Leadlay's support in 2013, has acquired compounds, strains and IP from Biotica. Using the methods developed in the University by Leadlay and Staunton, Biotica developed a HepC antiviral therapy, sold in 2013 to NeuroVive Pharmaceuticals AB and currently entering pre-clinical toxicology tests. Biotica have also licensed their technology to a number of companies globally, including GSK and Amyris.

2. Underpinning research (indicative maximum 500 words)

Microbial polyketides frequently appear as hits in drug screening protocols, but do not usually become drugs in their own right, due to off-target effects and poor pharmacokinetic profile. Solving these issues is beyond the scope of traditional synthetic medicinal chemistry. If variations and analogues of naturally occurring polyketides could easily be manufactured, the portfolio of therapeutic applications for natural products could be greatly increased.

During the period 1993-2000, Peter Leadlay (Herchel Smith Professor of Biochemistry, Dept of Biochemistry, since 2006; previously Lecturer / Reader / Professor of Molecular Enzymology there since 1979) and James Staunton (Professor of Chemical Biology, Department of Chemistry, since 1999; previously Lecturer / Reader there since 1969) carried out fundamental research that showed how this need could be addressed. They introduced a number of innovative ways to reorganise the modular genes encoding the assembly-line enzymes that govern biosynthesis of bacterial polyketide antibiotics, and discovered and characterised new biosynthetic gene clusters. This laid the foundations both for genome mining, and for biosynthetic medicinal chemistry to turn natural product hits into candidate drugs.

Specifying the length of a polyketide by choosing the number of modules in the assembly-line:
In research carried out in the Leadlay and Staunton labs between 1993 and 1995, the 6-deoxyerythronolide B synthase (DEBS) of *Saccharopolyspora erythraea*, which synthesizes the aglycone core of the antibiotic erythromycin A, was modified: a chain-terminating cyclase domain was repositioned to the carboxyl-terminus of DEBS1, the multienzyme that catalyzes the first two rounds of polyketide chain extension. The resulting mutant markedly accelerated formation of the predicted triketide lactone (Ref. 1, Section 3). This proved the concept that repositioning of an enzymatic domain could redirect polyketide synthesis to obtain polyketides of specified chain lengths.

First demonstration of a functional hybrid multienzyme delivering a predicted novel polyketide:
In further research, between 1994 and 1996, the Leadlay and Staunton labs replaced the entire acyltransferase (AT) domain from module 1 of DEBS1 by the AT domain from module 2 of the rapamycin-producing polyketide synthase. As predicted, this lead to the synthesis of two novel triketide lactones (with good yields), and proved that a truly hybrid multienzyme with a rationally altered specificity of chain extension can be generated through rational swapping of core domains (Ref. 2, Section 3). This opened the possibility of generating families of potentially useful analogues that are inaccessible by chemical synthesis.

Demonstration of functional swap of a whole module and engineering of broader specificity in chain initiation: Research carried out between 1995 and 1997 in the Leadlay and Staunton labs grafted the naturally wide-specificity loading module for the avermectin-producing polyketide synthase onto DEBS1 in place of the normal loading module. Expression of this hybrid enzyme in the

erythromycin producer *S. erythraea* produced several novel antibiotic erythromycins derived from endogenous branched-chain acid starter units (Ref. 3, Section 3). This approach opened the way to facile production of novel analogs of antibiotic macrolides, by feeding commercially-available alternative starter acids.

Development of the erythromycin-producing strain as a "super-host" for novel polyketide production: In research conducted between 2002 and 2006 in the Leadlay lab and at Biotica, 14-membered macrolides were designed in which the normal 5-*O*-sugar substituent was specifically replaced by a disaccharide from a 16-membered macrolide. The aim of this glycoengineering was to produce a hybrid combining the advantages of the two classes, specifically targeting the additional favourable binding to the ribosomal target involving the disaccharide. The target compounds were successfully produced in *S. erythraea*, illustrating the utility of this actinomycete as a host strain for polyketide antibiotic biosynthesis (Ref. 4, Section 3).

Underpinning work to find and characterise new biosynthetic gene clusters: These efforts were accompanied and reinforced by work on identification of new biosynthetic gene clusters – in total over 50, including (in work carried out between 1993 and 1995 in the Leadlay and Staunton labs) the first example of a hybrid polyketide-peptide synthetase (for rapamycin biosynthesis in *Streptomyces hygroscopicus*). With a total of 70 constituent active sites this was the most complex multienzyme system identified at the time (Ref. 5, Section 3). This work provided the first supply of sequenced genetic material to enable construction of hybrid polyketide synthases.

Foundations of genome mining (accurate prediction of the compound produced from sequencing individual clusters, later extended to prediction of the global biosynthetic potential of an actinomycete strain from the results of whole-genome sequencing): A particular landmark was work carried out in the Leadlay lab between 2004-2007 on the complete genome sequencing of the 8.2 Mbp genome of *S. erythraea*, one of the first commercially-relevant actinomycete strains to be fully characterised in this way (Ref. 6, Section 3). This work revealed a number of hitherto-unsuspected gene clusters encoding apparently novel natural products; and showed that large, high-GC "difficult" genomes could be successfully tackled outside major sequencing centres, giving a significant impulse to the now-widespread interest in genome mining of such bacteria for discovery of novel natural product leads.

The above is but a small sample of the body of underpinning work, which was reported in 133 publications between 1993 and 2013. No fewer than 18 of these publications resulted from joint work between the Leadlay laboratory and Biotica scientists (e.g. Ref. 4, Section 3).

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

1. Cortés, J., Wiesmann, K.E.H., Roberts, G.A., Brown, M.J.B., Staunton, J., and **Leadlay, P.F.** (1995). Repositioning of a domain in a modular polyketide synthase to promote specific chain cleavage. Science, 268, 1487-1489. DOI:10.1126/science.7770773

2. Oliynyk, M., Brown, M.J.B., Cortés, J., Staunton, J. and **Leadlay, P.F.** (1996). A hybrid modular polyketide synthase obtained by domain swapping. *Chem. Biol.* **3**, 833-839. DOI:10.1016/S1074-5521(96)90069-1

3. Marsden, A.F.A., Wilkinson, B., Cortés, J., Dunster, N.J., Staunton, J. and **Leadlay, P.F.** (1998). Engineering broader specificity into an antibiotic-producing polyketide synthase. *Science*, **279**, 199-202. DOI: 10.2307/2893985

4. Schell U, Haydock SF, Kaja AL, Carletti I, Lill RE, Read E, Sheehan LS, Low L, Fernandez MJ, Grolle F, McArthur HA, Sheridan RM, Leadlay PF, Wilkinson B, Gaisser S. (2008) Engineered biosynthesis of hybrid macrolide polyketides containing D-angolosamine and D-mycaminose moieties. *Org. Biomol. Chem.* 6, 315-6327. DOI: 10.1039/b807914e

5. Schwecke, T., Aparicio, J.F., Molnár, I., König, A., Khaw, L.E., Haydock, S.F., Oliynyk, M., Caffrey, P., Cortés, J., Lester, J.B., Böhm, G.A. Staunton, J. and **Leadlay, P.F.** (1995). The biosynthetic gene-cluster for the polyketide immunosuppressant rapamycin. *Proc. Natl. Acad. Sci. (USA)* **92**, 7839-7843. http://www.pnas.org/content/92/17/7839.long

6. Oliynyk, M., Samborskyy, M., Lester, J. B., Mironenko, T., Scott, N., Dickens, S., Haydock, S. F., **Leadlay, P.F.** (2007) Complete genome sequence of the erythromycin-producing bacterium *Saccharopolyspora* erythraea NRRL23338. *Nature Biotechnol*. 25, 447-453. DOI:10.1038/nbt1297

Funding:
Over the research period (1993-2013), the underpinning research received funding totalling over £13m. For all grants, Leadlay is named principal investigator / co-principal investigator. Where programme grant funding was distributed between HEIs; Cambridge allocation is cited.
BBSRC: 13 grants in period totalling £7,451,371; **Glaxo** Research and Development Ltd: £3,000,000; **EU**: 7 grants in period totalling £739,462; **MRC**: £460,000; **Wellcome Trust**: 2 grants totalling £434,417; **SERC**: 3 grants up to and including 1993 totalling £307,000, 1 grant post 1993: £188,000; **Pfizer**: 2 grants totalling £214,500; **Royal Society**: 3 grants totalling £141,682; **National Kidney Research Fund**: £60,000; **Isaac Newton Trust**: £27,728; **Australian Research Council**: £18,500

For the underpinning work, in the period being considered, Leadlay was awarded the Smets Prize Chair (2010, Universities of Leuven/Louvain-la-Neuve, Belgium); a Humboldt Prize (2011, Alexander von Humboldt Foundation, Germany); and the Inhoffen Medal 2011 of the Helmholtz Foundation, Germany (Ref. 8, Section 5).

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

Research by Leadlay and Staunton from 1993 led them in 1996 to found Biotica Technology Ltd as a means to commercialise and clinically develop novel polyketides as therapeutics. The underpinning research, in particular through the formation of Biotica, has had impact during the eligible period in a number of ways:

Impact on Health
Between 2009 and 2012 Biotica, using the methodologies developed in the Leadlay and Staunton labs, developed in-house a compound (BC556) highly active against hepatitis C virus which reached advanced pre-clinical development. Since March 2013 BC556 (renamed NVP018) is owned by the Swedish company Neurovive, who are continuing its development in a number of high value indications (Ref. 1, Section 5). The CEO of Biotica's follow-on company Isomerase Therapeutics Ltd testifies (Ref. 2. Section 5): "Prof. Leadlay's work has materially advanced the prospects of access to novel natural products-based medicines by sustainable routes".

Impact on Commerce
Industry has invested in R&D (Ref. 2, Section 5)
R&D at Biotica itself was financed in part by Venture Capital and for the rest by research contracts, grants and equity investment (total financing raised 2008-13 £4.43M). Since its formation, Biotica retained scientific links with the Leadlay group. In the period under review, Biotica provided industrial funding to Cambridge in a BBSRC Industrial Partnership Award (2011-14) to study polyketides that selectively kill cancer stem cells; and was a co-applicant with Cambridge and others in a strategic LoLa (total value of award £4.44M; 2013-18) to uncover novel natural product-based agrochemicals. Seven US patents on which Leadlay is a named inventor were granted to Biotica in the period 2008-13 (Ref. 3, Section 5).
In 2009, Biotica signed a three year collaboration and licence agreement with GSK to discover, develop and commercialise novel erythromycin-based macrolides in inflammatory diseases (Ref. 4, Section 5). The deal included an initial cash payment and an equity investment. Under the terms of the deal, Biotica successfully used its technology to produce compounds that are not readily accessible via conventional medicinal chemistry approaches.
A significant research programme was in partnership (2006-11) with Wyeth Laboratories (USA) (later Pfizer Inc. (USA) (total value ~£7.5M) on creation of >100 rapamycin analogues (rapalogs) for pre-clinical testing to combat cancer and multiple sclerosis (Ref. 5, Section 5).
In February 2011, Biotica licensed its technology non-exclusively to the billion-dollar company Amyris Inc. (California) for non-pharmaceutical purposes (Ref. 6, Section 5).

Some of the underlying technology was licensed in March 2013 non-exclusively to Warp Drive Bio Inc., a Boston-based company conducting combinatorial biosynthesis. (Ref. 2, Section 5)

A new company has been created

At the beginning of 2013, Biotica's VC funders (BVF) declined further investment and the company was broken up. Three key ex-Biotica staff co-founded a successor company, Isomerase Therapeutics Ltd (www.isomerase.co.uk), with Leadlay as chair of the scientific advisory board, that has acquired compounds, strains and intellectual property from Biotica. It is developing Biotica's rapalogs as anti-infectives, and also offers services to collaborators for microbial natural product-based drug discovery programs, by applying a combination of biosynthetic engineering and semi-synthetic chemistry, based on the methodologies discovered in the Leadlay and Staunton labs (Ref. 2, Section 5).

Employment has been created

Between 2008 and 2013 Biotica continued to conduct research near Cambridge employing on average 15-20 highly-skilled scientists. Its follow-on company Isomerase Therapeutics Ltd, founded in 2013 by ex-Biotica scientists, with Leadlay as chairman of the Scientific Advisory Board, now employs 3 staff (Ref. 2, Section 5).

Strategy of business has been influenced

The CEO of Isomerase Therapeutics testifies: "Prof. Leadlay's guidance and support have been essential in setting the strategic direction for Isomerase Therapeutics Ltd." (Ref. 2, Section 5)

The domain swapping technology for production of novel polyketides was also adopted by others, particularly by the California-based venture-backed start-up KOSAN. In 2007, KOSAN sought a European patent for broad technology to make novel polyketides but were defeated by Biotica in an interference hearing at the European Patent Office (Ref. 7, Section 5). KOSAN successfully turned to in-licensing and development, and were later sold (2008) to Bristol Myers Squibb for $190M.

Specialist advisory roles

Leadlay has served (2011-present) on the Seeding Drug Discovery Committee of the Wellcome Trust (Ref. 8, Section 5), helping to identify promising translational projects for unmet medical needs in which the Trust might invest.

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

1. http://www.neurovive.com/Research--Development/Project-overview/; http://www.genengnews.com/gen-news-highlights/neurovive-isomerase-team-up-to-study-cyclophilin-inhibitor/81248479/
2. Letter from CEO of Isomerase Therapeutics Ltd
3. US 6,271,255 granted 09/214,454; US 6,437,151 granted 09/896,357; US 7,381,546 granted 10/307,595; US 7148045 granted 09/720,840; US 7,198,922,257 granted 010/782; US 7,595,175 granted 10/344,738; US 7,560,252 granted 10/534,210.
4. Biotica Technology Limited Strikes Significant Collaboration Deal With GlaxoSmithKline (GSK) (Dec 2009) http://www.biospace.com/news_story.aspx?NewsEntityId=122617
5. Biotica inks $195M licensing deal with Wyeth (Oct 2006) http://www.fiercebiotech.com/story/biotica-inks-195m-licensing-deal-with-wyeth/2006-10-04
6. Biotica Technology Limited Extends License to Amyris for Polyketide Engineering Technology (Feb 2011) http://www.biospace.com/news_story.aspx?NewsEntityId=210978
7. http://www.biospace.com/news_story.aspx?NewsEntityId=63437 m
8. http://www.wellcome.ac.uk/Funding/Technology-transfer/Awards/Seeding-Drug-Discovery/WTD027712.htm