Institution: University College London/Birkbeck

Unit of Assessment: 5 - Biological Sciences

Title of case study: Combinatorial protein domain hunting to facilitate drug discovery

1. Summary of the impact

Combinatorial Domain Hunting (CDH) technology is a technique for producing fragments of proteins that are soluble and tractable for biophysical analysis. It was developed between 1999 and 2008 at Birkbeck College, in the laboratory of Dr Renos Savva. This technology was patented in 2001 and the biotech company Domainex Ltd was then formed to commercialise it. In 2007, Domainex merged with a UCL spinout company, NCE Discovery Ltd. The company has attracted over £3m in investment and employs about 31 people. In addition to its contract research programme, it has developed an in-house drug discovery programme utilising CDH. Early in 2012 a patent was filed on a series of inhibitors of the protein kinases IKKε and TBK1, which are validated drug targets for cancer and inflammation, and the first of these are expected to begin clinical trials in 2014.

2. Underpinning research (indicative maximum 500 words)

Drug discovery programmes rely on the availability of protein targets for drugs in pure, soluble forms and in relatively large (gram) quantities. There are often difficulties with producing such proteins. Protein truncation can overcome these, but limited proteolysis is not always applicable and bioinformatics-informed truncation may be inaccurate and unsuccessful, or resource-intensive. The technology of combinatorial domain hunting (CDH), developed by Renos Savva and his co-workers at Birkbeck College (Department of Biological Sciences) between 1999 and 2008, reliably produces stable, soluble truncated protein fragments through exhaustively sampling expression of random fragments of the encoding gene **[1, 2]**. These fragments bind ligands and are suitable for structural studies, including X-ray crystallography.

CDH involves subjecting a sequence of DNA that corresponds to a protein-coding gene to an enzymatic process leading to multiple double-strand breaks in the DNA, and thence to a pool of randomly truncated variants. Fragments of the desired approximate size are selected using electrophoresis and cloned to form a library for parallel recombinant protein production in *E. coli*. Detection at various stages is via a short peptide fusion tag on every variant, which also assists purification. Promising samples are then scrutinised using more robust biophysical methods.

In practice, tens of thousands of gene fragments will be expressed within each project, and these will be whittled down within a period of about three months to give up to 20 protein fragments that are soluble and tractable to further analysis, and that may provide suitable targets for downstream drug discovery. These will often cover different parts of the original protein, perhaps sampling different domains with different binding sites and functionalities, increasing the scope of downstream projects to discover small-molecule binders with a range of pharmaceutically interesting properties.

As a proof of principle, CDH was applied to p85a, successfully identifying soluble and highly expressed constructs encapsulating all its known globular domains, and immediately suitable for downstream applications **[3]**. A valuable extension of combinatorial domain hunting, CDH^2, enables empirical discovery of stable protein–protein core complexes. CDH^2 was demonstrated *ab initio* using a previously well-characterized Hsp90/Cdc37 complex **[4]**. Without using *a priori* information, the isolation of stable protein–protein complexes, suitable for further analyses was demonstrated. This resource-efficient process can provide protein complexes for screening of compounds designed to modulate protein–protein interactions, thus facilitating novel drug discovery.

After testing the technology in concept, a patent was filed that also covered the later implementation of the technology, CDH^2 **[5]**. This patented technology led to the spin-out of

Domainex Ltd in 2001, initially as a contract research company for drug discovery.

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

[1] Prodromou C, Savva R, Driscoll PC. DNA fragmentation-based combinatorial approaches to soluble protein expression Part I. Generating DNA fragment libraries. Drug Discov Today. 2007 Nov;12(21-22):931-8. http://dx.doi.org/10.1016/j.drudis.2007.08.012

[2] Savva R, Prodromou C, Driscoll PC. DNA fragmentation based combinatorial approaches to soluble protein expression Part II: library expression, screening and scale-up. Drug Discov Today. 2007 Nov;12(21-22):939-47. http://dx.doi.org/10.1016/j.drudis.2007.08.016

[3] Reich S, Puckey LH, Cheetham CL, Harris R, Ali AA, Bhattacharyya U, Maclagan K, Powell KA, Prodromou C, Pearl LH, Driscoll PC, Savva R. Combinatorial Domain Hunting: An effective approach for the identification of soluble protein domains adaptable to high-throughput applications. Protein Sci. 2006 Oct;15(10):2356-65. http://dx.doi.org/10.1110/ps.062082606

[4] Maclagan K, Tommasi R, Laurine E, Prodromou C, Driscoll PC, Pearl LH, Reich S, Savva R. A combinatorial method to enable detailed investigation of protein-protein interactions. Future Med Chem. 2011 Mar;3(3):271-82. http://dx.doi.org/10.4155/fmc.10.289

[5] Patent for CDH and CDH2: WO 03/040391 Method for producing and identifying soluble protein domains. Driscoll P., Savva R., McAlister M., Pearl L., Prodromou C. Filed 8 November 2002; granted in Australia (2002341204), the EU (02 774 994.4), Canada (2465377), and Japan (2003-542637), and pending in the USA (10/494,895). http://www.lens.org/images/patent/WO/2003040391/A2/WO_2003_040391_A2.pdf

Major Grants:

BBSRC Exploiting Genomics Initiative Postdoctoral Project Grant (02/2003 – 07/2008).

- Microarrays to soluble proteins: exploitation of expression profiling through high throughput protein production at the BCSB.
- Awarded value: £1,337,456.
- Dr R. Savva (Co-applicant) acting as lead scientist/ supervisor.

In collaboration with Professor. P.C. Driscoll (Principal Applicant) UCL, and Professor L. H. Pearl (Co-applicant) ICR Chester Beatty Labs, Fulham Road, London.

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

Domainex Ltd was incorporated as a private company in December 2001 to exploit the innovative technology of combinatorial domain hunting developed in the Savva laboratory for drug discovery purposes. The patent protecting this technology has now been granted in Australia, the EU, Canada, and Japan, and is pending in the USA. Domainex has received over £5m in private investment, contracts and grant funding in the 11 years since it was established. The most recent investment round closed in June 2010 with the raising of around £1.3m from Longbow Capital, The Capital Fund (managed by YFM Venture Finance), Bury-Fitzwilliam and from Takeda Research Investments **[a]**.

Domainex was originally set up as a fee-for-service drug discovery company and, following an initial investment of £250,000 from the Bloomsbury Bioseed Fund, the first company employees were hired in 2002 to develop the CDH technology into a process that could be offered commercially via this model. Since then a number of medium and large biotechnology, agrochemical, and pharmaceutical companies have placed contracts of ~£30,000 to >£100,000 for CDH studies; this still forms part of Domainex's work and helps fund the in-house drug discovery. By 2007, contractual milestones with the founder institutions were met, and Domainex personnel

moved to an incubator lab at the London Bioscience Innovation Centre (LBIC) in Camden. A £1m trigger point in the company's revenues resulted in the transfer of IP to Domainex, and an accrued overheads payment of £78,000 to the host department at Birkbeck **[b]**.

In 2007, a merger with the chemistry contract research organisation, NCE Discovery, greatly expanded the remit of in-house research at Domainex by allying the core CDH technology with state-of-the-art medicinal chemistry expertise. This enabled the company to initiate internal drug discovery programmes with the aim of reaching the lead optimisation stage before seeking to partner, or outsource, for toxicology and clinical trials of lead molecules. Following the merger, Domainex relocated to the Cambridge Science Park. It moved to larger premises on the same site in mid-2011, doubling its space, increasing its capacity for in-house medicinal chemistry and drug discovery, and enabling it to expand its staff to the present size. Late in 2009, Takeda Ventures, Inc., the corporate venture arm of Takeda Pharmaceutical Company Limited, took an interest on the Domainex board through acquiring an 8% stake during an investment round. The company's primary investors are now Longbow Capital and YFM Equity Partners; previous funders include The Capital Fund, and founder scientists and institutions also remain stakeholders.

Domainex is now a thriving and successful small biotech company employing about 30 people, mainly highly skilled scientists engaged in molecular biology, biochemistry, computational and medicinal chemistry. It thus makes a significant contribution to the local and national economy. It had a turnover of £1.95m for the year ended 30 April 2012, up from £82,000 in the year to 30 April 2008 **[c]**.

Client companies and partners from the period 2008-13 include Ark Therapeutics, Syngenta, ICR, and Sigma Aldrich **[d]**. Domainex was also a partner on the EU Framework 6 Spine II Complexes consortium 2006-2009 **[e]** and has received grants from public bodies including the Technology Strategy Board **[f]**. One major scientific achievement of such collaboration was the structure of a CDH-optimised protein kinase in complex with lead compounds from the pharmaceutical company UCB **[g]**.

The in-house drug discovery programme has initially centred on proteins that are considered to be useful targets for oncology and anti-inflammatory drugs and is now producing candidate compounds. The most advanced programme in the pipeline focuses on two closely related kinases, TANK-binding kinase 1 (TBK1) and IkappaB kinase epsilon (IKKε). This has yielded patented inhibitors **[h]** that are now in the lead optimisation stage. Domainex is seeking partners within the pharmaceutical industry for the clinical development of these compounds, having raised £4m by end August 2013 **[i]**. The first are expected to be nominated as candidate drugs in 2014 and to enter Phase I in 2015. Another patent has been filed on the synthetic lethality of TBK1/IKKε inhibitors and other oncogenes, which includes the use of these oncogenes as biomarkers and in combination therapies. The company also has an active programme targeting a number of lysine methyltransferases, enzymes that are involved in the epigenetic regulation of gene expression and that are known to play a part in cancer development. Inhibitors of these enzymes are currently at the lead optimisation stage.

Domainex has received several accolades both for its innovative technology and for its proactive work on promoting collaborations between academia and industry. The most recent of these was the 2010 Genesis Life Science Innovation and Enterprise Programme Of The Year Award **[j]**.

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

[a] http://www.domainex.co.uk/investors.asp

[b] All company details can be verified by the CEO, Domainex. Contact details provided.

[c] Financial Statements for year ended 30th April 2008 and year ended 30th April 2012. Copies available upon request.

[d] http://www.domainex.co.uk/news.asp e.g. Sigma-Aldrich collaboration: http://www.domainex.co.uk/TSBEpigenetics.asp

[e] http://www.spine2.eu/SPINE2/index.jsp

[f] http://www.domainex.co.uk/IKKeTBK1TSBFundingDomainexOncologyDrugDiscoveryKinaseResearch.asp

[g] http://www.cambridgesciencepark.co.uk/sectors/bio-medical/ucb-domainex-collaboration-provides-valuable-information-on-cancer-drug-target/
Meier C, Brookings DC, Ceska TA, Doyle C, Gong H, McMillan D, Saville GP, Mushtaq A, Knight D, Reich S, Pearl LH, Powell KA, Savva R, Allen RA. Engineering human MEK-1 for structural studies: A case study of combinatorial domain hunting. J Struct Biol. 2012 Feb;177(2):329-34. http://dx.doi.org/10.1016/j.jsb.2012.01.002.

[h] Patent covering IKKε inhibiting compounds: WO 2012/010826 Pyrimidine compounds as inhibitors of protein kinases IKK epsilon and/or TBK-1, processes for their preparation, and pharmaceutical compositions containing them. Perrior T.R., Newton, G.K., Stewart M.R., Aqil, R. Filed 26 January 2012. http://w.pat.tc/WO2012010826A1

[i] http://www.domainex.co.uk/DomainexAnnouncesInvestmentRound.asp

[j] http://www.pharmafile.com/news/piramed-and-domainex-named-among-best-british-biotech
http://www.bbk.ac.uk/news/archive/20070712b/
http://www.domainex.co.uk/documents/Domainex-Genesisaward.pdf