Institution: University of Kent

Unit of Assessment: 5: Biological Sciences

Title of case study: Advancing Technologies for Enhanced Manufacturing of Difficult to Express Proteins: Improving Biotherapeutic Knowledge, Skills, and Capacity Across the National and International Bioprocessing Community

Period when the underpinning research was undertaken: 2010-2020

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:
Professor C. Mark Smales	Professor in Industrial Biotechnology	1997-present

Period when the claimed impact occurred: August 2013-2020

Is this case study continued from a case study submitted in 2014? No

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

This case study captures the impact of research led by Professor Smales into the development of novel technologies and protocols for use in the production of CHO cell-based biotherapeutics. The research led to intellectual property and the advance of industrially relevant skills, know-how, and capacity-building across the national and international bioprocessing community. Specifically, the research enabled the production of higher-quality difficult to express (DTE) proteins in greater yields than previously possible; it provided new strategies that improve manufacturing processes.

As an industrial partner that has applied the technological advancements and know-how developed by Smales' team, Lonza Biologics benefited from new IP, a stronger patent estate, new business opportunities, and new engineered CHO host cell lines and bioprocesses for the commercial manufacturing of DTE proteins. Mologic Ltd applied the research to advance their development and validation processes for COVID-19 diagnostic tests, and in planning and preparing for the increase of their production capacity.

Furthermore, Smales developed and facilitated training across the UK, India, and Thailand. This advanced the industrially relevant skills of early career researchers and helped to establish India's and Thailand's abilities to produce high-value recombinant biotherapeutics for their own needs.

2. Underpinning research (indicative maximum 500 words)

Summary

Since **2010**, Professor Smales and a team in the School of Biosciences at Kent carried out research into the basic biology of Chinese Hamster Ovary (CHO) cells, with the aim of improving the performance of this widely used vehicle for industrial recombinant protein expression applications. The research **[R1-R3, P1-P3]** by Smales' Kent team has advanced knowledge of fundamental biological processes in CHO cells. This enabled the design of novel CHO cell lines and modifications to cell culture procedures that provide an upgrade to outdated CHO cell expression systems and manufacturing processes. These advances are being used to produce difficult to express (DTE) proteins in greater yields than previously possible, thereby facilitating the development of novel therapeutic strategies that might otherwise be lost owing to manufacturing failures.

The Problem

Recombinant protein-based biotherapeutics are frontline drugs (e.g. monoclonal antibodies, growth factors, hormones, blood factors, enzymes) manufactured using biological means that target society's most challenging diseases. They form the basis of a worldwide industry that in 2019 was worth >US$250 billion. Some biotherapeutics, including novel, artificially constructed derivatives of natural antibodies, are classified as difficult to express (DTE) proteins. For instance, this includes bispecific antibodies that enable dual targeting, a desirable feature offering advantages in some applications where single monoclonal antibodies are not suitable. DTE proteins typically fail to achieve the expression levels and/or quality thresholds that are an essential prerequisite for their exploitation as drugs. Thus, the development of many promising new therapies that target otherwise untreatable conditions is frequently impeded by difficulties in expressing the appropriate protein.

Research

Smales and the Kent team outlined a series of scientific hypotheses based around multiple aspects of biological processes in CHO cells that might explain expression difficulties for particular proteins. Most of this work was carried out in close collaboration with industrial partners, funded through mechanisms specifically targeting interactions between industry and academia (e.g. BBSRC CASE studentships S506977, M016013; BBSRC LINK grants R001731, K017640; Innovate UK Knowledge Transfer Partnership KTP010320). Based on cell lines and proteins of interest provided by the industrial partners, novel research investigations were initially conducted at Kent, then validated using the industrial CHO cell-based expression systems housed at the partner companies.

Between **2011** and **2018**, Smales' team studied how lipid metabolism in CHO cells underpins functioning of the endoplasmic reticulum (ER), one of the major cellular organelles for protein secretion. This is a multi-step process through which proteins are transported out of the cell. The team's earlier work suggested that culturing CHO cells at reduced temperatures enhanced protein secretion, and this correlated closely with changes in lipid metabolism **[R1]**. The subsequent identification of the underlying cellular mechanisms led to the development of a metabolic genetic engineering strategy based around the manipulation of lipid biosynthesis that enhanced DTE processing in the ER. This development resulted in improved DTE protein yields of the order of 1.9-9x **[R2]**.

Research conducted between **2016** and **2018** on amino acid metabolism in CHO cells focused on poorly soluble amino acids such as proline that need to be supplied in the cell culture growth medium, because CHO cells cannot naturally produce them. Using recombinant DNA technology, key metabolic enzymes involved in the synthesis of these amino acids (e.g. pyrroline-5-carboxylase synthetase) were introduced into CHO cells alongside the biotherapeutic gene of interest. This process enabled the CHO cells to produce these limiting amino acids, thereby removing bioprocessing bottlenecks in the manufacture of the biotherapeutic protein. In addition, the introduction of metabolic enzymes into CHO cells also provided novel and useful selection systems for the manufacturing process that could be used to select for only those cells that had the gene of interest incorporated. As a result, CHO cell lines had higher production capacity in less complex cell culture media **[R3]**.

Since **2013**, Smales' team has studied the proteasome, a major component of the machinery that removes unwanted proteins from cells. Investigations specifically targeted how industry could use inhibition of the proteasome to enable selection of clonal cell lines (those derived from a single cell) with optimal capacity to generate protein-based products. These fundamental investigations resulted in a panel of novel cell lines that produced industrial target proteins, including DTE proteins, with significantly increased yields **[P1]**.

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

The Kent team led by Professor C. Mark Smales comprised of Anne Roobol, Jo Roobol, Martin J. Carden, James D. Budge, Tanja J. Knight, Jane Povey, Ian R. Brown, Gurdeep Singh, and Mark Feary. All University of Kent authors are indicated in bold below.

[R1] Roobol, A., **Roobol, J.**, **Carden, M. J.**, Bastide, A., Willis, A.E., Dunn, W. B., Goodacre, R., and **Smales, C. M.** (2011). 'ATR (ataxia telangiectasia mutated- and Rad3-related kinase) is activated by mild hypothermia in mammalian cells and subsequently activates p53'. *Biochemical Journal* 435: 499–508. doi: 10.1042/BJ20101303

[R2] Budge, J. D., **Knight, T. J.**, **Povey, J.**, **Roobol, J.**, **Brown, I. R.**, **Singh, G.**, Dean, A., Turner, S., Jaques, C. M., Young, R. J., Racher, A. J., and **Smales, C. M.** (2020). 'Engineering of Chinese hamster ovary cell lipid metabolism results in an expanded ER and enhanced recombinant biotherapeutic protein production'. *Metabolic Engineering*, 57: 203-216. doi: 10.1016/j.ymben.2019.11.007

[R3] Feary, M., Racher, A. J., Young, R. J., and **Smales, C. M.** (2017). 'Methionine sulfoximine supplementation enhances productivity in GS-CHOK1SV cell lines through glutathione biosynthesis'. *Biotechnology Progress* 33(1): 17-25. doi: 10.1002/btpr.2372

[P1] Patent Filing 06/01/2017. Inhibition of protein degradation for improved production. WO2017118726A1. Jaques, C. M., **Smales, C. M.**, and **Knight, T. J**. KAR id:85780.

[P2] Patent Filing 03/05/2017. Modulation of lipid metabolism for protein production. WO2017191165A1. **Budge, J.**, **Smales, C. M.**, **Knight, T. J.**, and Young, R. KAR id:85781.

[P3] Patent Filing 01/02/2019. Methods of cell selection and modifying cell metabolism. WO2019152876A2. Young, R., **Smales, C. M.,** Jaques, C. M., Racher, A, **Singh, G.**, **Budge, J.**, and **Roobol, J.** KAR id:85782.

Grants and Awards

Over the period of impact, the research undertaken by Smales and colleagues underpinning this case study has been supported by over £8.5 million in funding, from sources including the BBSRC, EPSRC, EU, Wellcome Trust, and industrial partners.

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

Advancing Lonza Biologics' Commercial Technology, Practice and Opportunities

Lonza Biologics plc is a multinational company whose UK-based Mammalian-derived Biotherapeutics arm develop CHO cell systems for manufacturing clients' proteins of interest. This arm is part of the company's Pharma, Biotech and Nutrition section, which generated sales of 4.1 billion CHF (£3.4 billion) in 2019 **[a]**. This represented an 11% year-on-year growth in sales compared to 2018, driven predominantly by the Biopharma element **[a]**. The annual report explicitly confirms that their Biopharma activities experience 'growing needs for mammalian expression of more complex, innovative therapeutic proteins' **[a]**.

Lonza has been a longstanding industrial partner of the Kent team (2012-21), in what Dr Andy Racher, Lonza's R&D Director with responsibility for IP Strategy, describes as a 'strong relationship […] between the two partners' **[b]**. Dr Racher affirms that the collaboration 'has exceeded expectations and delivered new IP including knowhow, publications, ways of working, practice and new engineered CHO host cell lines and bioprocesses for the commercial manufacturing of DTE proteins' **[b]**. In **2018,** strategies for improving DTE protein expression **[R2, R4]** were incorporated as upgrades into the Lonza GS (glutamine synthetase) system™ **[a, b, c, d, P3]** in direct response to the growing demand for more complex molecules **[a]**, and validated in the pilot plant at Lonza in **2019**. This resulted in a 'stronger patent estate for the GS system' **[b,**

d, P2, P3], new business opportunities **[b]**, and the creation of three, and retention of another three, FTE jobs **[b]**. In **January 2020**, Lonza invested £609k in continued collaborative work with the Smales lab **[b]**.

The impact of the collaborative work and its ensuing technology developments was confirmed by Michael Carroll, the independent officer appointed by Innovate UK to monitor a collaborative project grant underpinning the adaptation of Kent's research and resulting developments by Lonza. Carroll confirms that the research 'provided impact to Lonza and the wider biotechnology industry by developing an upgrade to the GS system and […] new technologies that allow such protein drug molecules to be expressed at significantly higher yields and quality than previously' **[c]**. Overall, he assesses the project as an 'outstanding exemplar of successful collaboration', with 'underlying science [that is] elegant and underpinning disrupting innovation' **[c]**. In addition to the direct impact on Lonza, Carroll asserts that 'the downstream exploitative outcomes and commercial value-add has the potential to substantially boost the UK knowledge base and the nations' GDP through the realization of better medicinal treatments, improved patient outcomes and enhanced revenue streams' **[c]**. He judges 'the outcomes, deliverables and overall impact of the project as being outstanding', and highlights how the 'project also delivered positive impacts at a societal level', as 'it engaged with the general public and informed on the role of bio-therapeutic drugs' **[c]**.

Informing Mologic's Production and Innovation of Diagnostic Tests and Reagents

Smales has a longstanding relationship with Mologic, a leading UK-based supplier of rapid diagnostic technologies. Since **2015,** Mologic have worked with Smales on their programme of diagnostic product development and taken up Smales' CHO cell-based technology for the production of antigens **[d]**. For instance, Mologic's Chief Scientific Advisor and Co-Founder, Professor Paul Davis, describes how SARS-CoV-2 (the COVID-19 causative virus) antigens produced using Smales' technology played 'an important part in our work on validating, testing and developing the reagents for our commercial COVID-19 diagnostic tests' at low costs and ease, with the antigens usually being of high cost and limited availability **[d]**. Highlighting their 'timely and affordable provisions', and the group's expertise, Davis added that they 'regard the Smales group as a highly valuable asset which will continue to be an important factor in our ongoing work' **[d]**.

Furthermore, in **2020,** Mologic established a Bill and Melinda Gates Foundation-supported project aimed at increasing the company's production capacity to 16 million tests per day **[d]**. Manufacturing at such high volumes is a prerequisite for making critical diagnostic tests available cheaply to low- and middle-income countries (LMICs), but requires access to unprecedented amounts of recombinant antigen which Smales' research is enabling the company to meet. Professor Davis confirms that the Smales lab technology is 'critically important […] to make billions of affordable, rapid diagnostic tests available for the first time on a truly global scale'. In **December 2020**, Smales took up one of the first Innovation Scholar Secondments awarded by Innovate UK, which will enable him to spend 50% of his time with the company. It will also further support the new business activity arising from the high-volume manufacturing initiative **[d, e]**.

Contributing to the Development and Knowledge of Bioprocessing in Thailand

In recognition of the particular needs of LMICs where patients frequently cannot afford expensive biopharmaceutical proteins produced in industrialised countries, Smales has used his research expertise in recombinant protein production to actively develop training opportunities that would benefit such countries. In **2017**, Smales received funding from the Global Challenges Research Fund (GCRF) to lead a collaboration with Thailand's first state-owned contract manufacturing facility for the production of clinical research grade biopharmaceuticals, the National Biologics Facility (NBF) at King Mongkut's University of Technology Thonburi (KMUTT). In this project, Smales' expression technologies were employed to produce biotherapeutics particularly relevant to this country. These included monoclonal antibodies against Dengue antigens, which serve as treatments and prophylactic vaccines against dengue fever **[g]**. Dr Lalintip Hocharoen, process development specialist at NBF, confirms that this provided Thailand 'with the ability to produce

recombinant monoclonal antibodies ourselves from cultured CHO cells' **[f]**. He also confirmed that it has facilitated a substantial increase in the biotechnological capability of this country, thereby helping to 'establish Thailand's ability to produce high value recombinant biotherapeutics for its own need' **[f]**.

Building UK Capacity and Industry-Relevant Skills

An integral part of Smales' research has been the training of early career researchers in both basic research and industrially relevant skills. This resulted in the collaborative training and completion of 18 (CASE-, EU/ITN-, and directly industry-funded) PhD students in the lab during the REF2021 period. These students and other early career researchers received training that underpinned their employment at Lonza **[b]**, as well as other UK-based commercial and academic organisations **[c]**. As Carroll highlights: 'the critical area of human resource development and up-skilling benefitted substantially' from the Lonza–Smales partnership. 'Several personnel were trained in leading edge techniques and skills and […] have subsequently progressed to be employed either at Lonza or at other UK based organisations to lead new work in cell line developments. A further person employed on the work has now been employed at Kent in a Lonza Funded Centre of Excellence Centre to develop further new technologies for commercialization by Lonza' **[c]**.

Supporting the Indian Government's National Biopharma Mission

Since **2017**, Smales has co-chaired the Scientific Advisory Group of the Indian Government's National Biopharma Mission (NBM) **[h]**. The NBM is a $250 million initiative, 50% co-funded by the World Bank, for growing the country's biopharma sector and enabling it to meet its own needs in terms of vaccine and biopharmaceutical development. The NBM's mission director, Dr Kavita Singh, confirms that Smales' involvement is directly based on his 'work in the development of CHO cell expression platforms for the production of biopharmaceuticals'. She continues that Smales has had essential 'impact and input into delivering the Mission' **[h]**. In turn, this has enabled the Indian Government to 'deliver the National Biologics Mission which is, and will continue to, impact every-day life of the population in India' **[h]**.

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

[a] Report: Lonza 2019 Full Year report.

[b] Letter of Support: Director of Research and Development and IP Strategy, Lonza Biologics plc.

[c] Letter of Support: Innovate UK Independent Project Monitoring Officer, from Carroll Pharma Consulting.

[d] Letter of Support: Chief Scientific Officer and Co-Founder, Mologic Ltd.

[e] Award: Innovate UK Innovation Scholars award letter.

[f] Letter of Support: Process Development Specialist, King Mongkut's University of Technology Thonburi (KMUTT), Thailand.

[g] Letter of Support: Director of the Center of Excellence for Antibody Research and Head of the Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand.

[h] Letter of Support: Mission Director, National Biopharma Mission, Biotechnology Industry Research Assistance Council, India.