Institution: WestCHEM
Unit of Assessment: Sub-panel 8 – Chemistry
Title of case study: Therapeutic protein and vaccine stabilisation technology with global reach across the pharmaceutical industry

1. Summary of the impact

A novel self-assembly process, developed at WestCHEM was shown to provide a step-change for stabilising proteins as dry powders. The spin-out company, XstalBio, was created in 2004 and licensed the patented technology with the aim of developing it for delivery and formulation of therapeutic biomolecules and vaccines. Over the period 2008-2012, eight leading international pharmaceutical and animal health companies paid XstalBio over £2.2M for access to its IP portfolio and to undertake evaluation studies with candidate biomedicines and vaccines. XstalBio employed 8 highly skilled research scientists over this period and 4 further patent families were generated. Boehringer Ingelheim licensed the technology for application to its therapeutic biomolecules and in collaboration with XstalBio built a dedicated €5M pilot plant for manufacture of inhalable dry powders.

2. Underpinning research

Context

Medicines based on biomolecules, including vaccines, are currently the major engines of growth in the pharmaceutical industry, with sales predicted to increase from ~$130bn in 2012 to ~$280bn in 2022. The *in vivo* activity of biomolecules such as enzymes, monoclonal antibodies, and vaccines is determined by the tertiary structure, *i.e.*, the three-dimensional conformation. This means that biologic drugs are much less stable and therefore harder to formulate and administer than traditional small molecule drugs.

Research by the research groups of BD Moore (Senior Lecturer and Reader, WestCHEM), PJ Halling (Professor, WestCHEM) and MC Parker (Lecturer, WestCHEM) aimed to facilitate the transfer of active enzymes from aqueous to organic media to harness their catalytic power in organic synthesis. A key observation was that dehydration processes other than lyophilisation resulted in much better retention of enzyme catalytic activity and excellent preservation of protein tertiary structure in the dry state (1). The wider importance of this finding was recognised and the innovative stabilisation technology was translated into commercial formulation of therapeutic biomolecules and vaccines.

Key Findings

The pathway of transferring an enzyme from aqueous solution into non-aqueous media is found to be critical in maintaining catalytic activity and surprisingly, conventional protein drying methods such as lyophilisation give very poor results. Moore and Parker discovered and patented ("Rapid Dehydration of Proteins", *WO0069887)* a much more successful process based on co-precipitation of enzymes with an inorganic salt by addition of an aqueous mixture to an excess of water-miscible solvent (2). This method was designed to rapidly dehydrate the enzyme, as the water dissolved into the solvent, but the additional bonus was that it also resulted in spontaneous formation of microcrystals of the salt coated with protein. Self-assembly of these protein-coated microcrystals (PCMCs) was found to be applicable to all enzyme classes tested and the process coupled with control of protonation state (3) are recognised as benchmark methods for preparing biocatalysts for use in non-aqueous media. The high catalytic activity of enzymes on dry PCMC particles was postulated to be because an unusually high proportion of protein molecules retained a native tertiary conformation in the dry state. This was subsequently proven to be the case by solid-state circular dichroism spectroscopy and active site-titrations in dry solvent.

The excellent retention of protein native structure in PCMCs was recognised to have applications beyond biocatalysis and applied research at WestCHEM explored their potential use as a platform for delivery of therapeutic biomolecules by inhalation (4). This research identified a novel method for forming free-flowing dry powders by supercritical fluid carbon dioxide extraction of suspensions of PCMC in solvent. The formulations and process were patented ("Pharmaceutical Composition",

WO2004062560) in conjunction with an alternate continuous flow process for precipitating PCMC particles ("Process for Preparing Microcrystals*", WO2006010921*). A range of physiologically acceptable water-soluble crystals, including amino-acids and sugars, were investigated with the aim of preparing PCMC particles in the size-range 3-5 µm suitable for delivery to the lung. Dry powders of model proteins with promising aerodynamic properties were identified and found to be unusually stable to high temperature and humidity meaning they could be stored without requiring refrigeration or an inert atmosphere. These properties are highly desirable for formulation and delivery of biologic drugs and vaccines (5).

The spin-out of XstalBio in 2004 provided access to much more aggregation-sensitive proteins such as humanised monoclonal antibodies supplied by commercial partners, and stimulated further improvements to the technology. In research directed by Moore, zwitterionic additives were identified which disrupted aggregation of precipitated protein on exposure to polar solvents and enabled them to be incorporated onto the outer surface of the particles in a native quaternary state (6). These additives were patented ("Precipitation Stabilising Compositions", WO2008132439) and the technology has been successfully applied to the formulation of over ten therapeutic monoclonal antibodies in development by pharmaceutical companies.

It was also shown that a double decomposition process could be carried out in a polar organic solvent between (i) phosphate salts that have been co-immobilised with biomolecules on the crystal surface and (ii) calcium chloride dissolved in the solvent. The resultant outer shell of sparingly aqueous-soluble calcium phosphate can be used to tune the rate at which biomolecules are released back into aqueous solution. Patented in 2008 ("Slow Release Compositions", WO2009077732) these findings have led to the development of vaccines in which both antigens and toll-like receptor agonists are co-immobilised on slow-release particles, resulting in enhanced innate and adaptive immune responses. These are being exploited in temperature stable vaccines for treatment of helminths in livestock and *in vivo* trials are on-going in collaboration with the Moredun Research Institute. All of the patents filed have been licensed to XstalBio Ltd.

Key Researchers

Barry D Moore, (employed at WestCHEM from January 1991, Senior Lecturer from April 1997 and Reader from June 2002); Marie Claire Parker, (employed as Lecturer at WestCHEM from 1997-2005, and currently Honorary Research Fellow); Peter Halling, (employed at WestCHEM since August 1983, Professor since August 1990).

3. References to the research

References 1-3 (highly cited primary papers) best exemplify the quality of the body of research.

[1] Practical route to high activity enzyme preparations for synthesis in organic media. Partridge, J.; Halling, P. J.; Moore, B. D., *Chem. Commun.,* **1998**, 841-842; DOI: **10.1039/A800408K**

[2] Enzyme-coated micro-crystals: a 1-step method for high-activity biocatalyst preparation. Kreiner, M.; Moore, B. D.; Parker, M. C., *Chem. Commun.,* **2001**, 1096–1097; DOI: **10.1039/B100722J**

[3] Control of enzyme activity in organic media by solid-state acid-base buffers. Zacharis, E; Moore, B.D.; Halling, P. J., *J. Am. Chem. Soc.,* **1997**, 119, 12396-12397; DOI: **10.1021/ja972635c**

[4] Ex vivo perfusion bioassay: an excellent technique to measure the bioactivity of inhalable insulin coated microcrystals. Ross A. C.; Steve H. N.; Partridge J.; Moore B. D.; Flores M. V.; Parker M. C.; Brown A. J.; Hillier C.; Coleman J., *Abstracts of the AAPS* **2002**; http://abstracts.aaps.org/published/ContentInfo.aspx?conID=32975

[5] Formulation of the adenylate cyclase toxin of Bordetella pertussis as protein-coated microcrystals. Khosravani A.; Parker M. C.; Parton R.; Coote J., Vaccine. **2007**, 25, 4361-4367; DOI: **10.1016/j.vaccine.2007.03.035**

[6] Dry powder therapeutic mAb formulations with enhanced temperature stability. Gebbie, W.; Davidson, K.; Partridge, J.; Vos, J., Moore, B. D.; Abate, J.; Kirchhoff, C., *AAPS National Biotechnology Conference* **2009**; http://abstracts.aaps.org/published/ContentInfo.aspx?conID=19420

4. Details of the impact

From research to impact

XstalBio Ltd was formed as a spin-out company in 2004 to license, commercialise, and extend the intellectual property associated with the protein and vaccine stabilisation technology. XstalBio's technology adds therapeutic value, accelerates the development, and extends the life-cycles of protein-based drugs as well as enabling new product opportunities. The company targets the $50bn currently spent *per annum* by the pharmaceutical industry on biopharmaceutical product development. XstalBio markets its expertise and technology to these pharma companies with the aim of enabling development of improved formulation and delivery methods for new candidate biologic drugs and vaccines.

In an internationally competitive market, XstalBio has succeeded in selling contracts for access to its Intellectual Property Portfolio for over 8 years and has continuously developed the patented technology to meet the new challenges facing the biopharmaceutical industry. These contracts, worth £2.2M over the period 2008-2012, are underpinned by option license agreements taken out on the WestCHEM owned patents. An early client was Boehringer Ingelheim who subsequently licensed the stabilisation technology from XstalBio, primarily for delivery of biologics by inhalation. As part of this license agreement the two companies co-developed and built a dedicated GMP compliant pilot plant for inhalation dry powders, which was commissioned in Biberach, Germany in 2008 at cost of €5M.

Development programmes have also been carried out with, or are on-going with, other major pharmaceutical companies including [text removed for publication] with most contracts being with biologic drug development groups from outside the UK. The specific details of these are subject to stringent commercial confidentiality agreements but in general the role of XstalBio is to accelerate development, enhance therapeutic value, or manage the life-cycle of the biopharmaceutical by providing innovative dry powder formulations which improve the overall product profile.

Type of Impact

An important impact of the original research has been economic with the launch and growth of a sustainable UK based SME with global reach in the pharmaceutical industry. In the period 2008-2012, the company made international sales of its Intellectual Property of £2.2M and employed 8 full-time research and development scientists at BSc and PhD level. The company has also supported and trained 3 PhD CASE studentships based at WestCHEM and the London School of Pharmacy.

Vaccines and medicines based on biomolecules will be major engines of growth in the pharmaceutical industry over the coming decades. The significant payments by international companies to XstalBio for access to its IP portfolio demonstrate that its biologic formulation and drug delivery technologies lie at the commercial cutting-edge and are impacting on the direction taken by top ten pharmaceutical companies in developing the next generation of biologic medicines.

Unmet product and process needs that XstalBio Technology is being used to address include:

- Delivery by inhalation with a dry powder inhaler requires sensitive biologics to be processed into particles in the size-range of 3-5 microns with no loss of tertiary structure or bioactivity. Techniques such as milling cannot be used and spray-drying produces extremely moisture-sensitive powders which require expensive protective packaging. The impact of XstalBio's PCMC technology in this field was recognised by Boehringer Ingelheim and resulted in a licensing agreement and significant investment in a GMP compliant manufacturing facility (estimated €5M),
- Shipping and storage of biomolecules without the need for continuous refrigeration is a major goal for organisations and companies that intend to supply diagnostics, biological medicines and vaccines in challenging environments such as remote regions in the developing world. XstalBio PCMC dry powders remain stable at high temperatures and in humid conditions and therefore offer considerable cost savings to its partners. Boehringer Ingelheim has noted these more efficient and cost effective manufacturing opportunities compared with conventional

methods:
"Particularly with regard to cost-effectiveness, precipitation methods are attractive. An interesting method is the protein-coated microcrystals (PCMC) technology that stabilises biomolecules on crystalline surfaces by co-precipitation during rapid solvent exchange"

- Administration of therapeutic proteins at very high concentration by subcutaneous delivery would provide an alternative to lengthy infusions, reducing resource use (time in clinic) and improving quality of life for patients with chronic conditions. As was demonstrated with a Pfizer candidate human monoclonal antibody, the patented stabilising additives prevent aggregation and conserve bioactivity. Because the dry mAb powders can also be rapidly reconstituted to very high protein concentration (>200mg/ml) they are providing industry with a radical alternative to concentrating by TFF which becomes very difficult at high viscosities.
- Tuneable drug delivery kinetics: Slowing the release of therapeutic proteins without covalent modification is desirable. Use of a calcium phosphate shell allows release of proteins from PCMCs to be tuned from hours to days.

The collaboration with Boehringer Ingelheim illustrates how the XstalBio technology has had an impact on a client's R&D strategy and investment. Boehringer Ingelheim has stated publicly that *"Boehringer Ingelheim believes that the collaboration with XstalBio will provide improved methods for the formulation and delivery of biomolecules. The technology provides a highly differentiated method for preparing biomolecules as stable, solid formulations, with the particles capable of being engineered for delivery in a range of formulations via various routes of administration. Both partners will jointly develop and scale-up the PCMC technology for GMP manufacturing at Pilot scale."*

The publication of the 6 filed and licensed patent families impacts on the overall knowledge base and direction of the pharmaceutical industry. This is evidenced by the presence of 11 patent filings from other companies, including BASF, Novo Nordisk, Boheringher Ingelheim, Lek, Taisho Pharmaceutical Co. Ltd and Dominó - Indústrias Cerâmicas that reference the technology and/or patents.

5. Sources to corroborate the impact

1. The Non-Executive Chairman of XstalBio will corroborate the company's client base and that XstalBio's technology is being applied to solving a range of biopharmaceutical product challenges for those clients.
2. www.xstalbio.com/technology & bioresearchcentral.pharmaloco.com/company/XstalBio/index.html will corroborate the claims that XstalBio's technology is being applied to solving a range of biopharmaceutical product challenges for its clients.
3. http://formulation.org.uk/index.php?option=com_content&view=article&id=279&Itemid=223 will corroborate the claim that collaboration between XstalBio and Pfizer has improved stability of human monoclonal antibody formulations.
4. http://www.boehringer-ingelheim.ca/en/news/press_releases/2006/16_may_2006.html will corroborate Boehringer Ingelheim's relationship with XstalBio
5. "Development of a pilot-scale manufacturing process for protein-coated microcrystals (PCMC): Mixing and precipitation – Part I", C. König, K. Bechtold-Peters, V. Baum, T. Schultz-Fademrecht,S. Bassarab, K.-J. Steffens, Eur. J. Pharm. Biopharm. 2012, **80**, 490-498 (DOI: 10.1016/j.ejpb.2011.11.012) will corroborate the claim that XstalBio and Boehringer Ingelheim have established a pilot-scale manufacturing process which will provide improved methods for the formulation and delivery of biomolecules.
6. World Intellectual Property Organisation (http://patentscope.wipo.int/), will corroborate filing of the following patents by B.D. Moore *et al.* at Strathclyde:
Rapid Dehydration of Proteins *WO/2000/069887*; Pharmaceutical Composition *WO/2004/062560*; Process for Preparing Microcrystals *WO/2006/010921*; Precipitation Stabilising Compositions *WO/2008/132439*; Slow Release Compositions *WO/2009/077732*; Method for preparing amorphous precipitated protein particles *WO/2013/093524*