Institution: University of Leeds

Unit of Assessment: 9 Physics

Title of case study: New techniques in protein analysis speed up biopharmaceutical R&D

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

Research in protein folding and technological development at the University of Leeds led to the creation of Optim1000, a high throughput microlitre protein stability analyser, through Leeds spin-off company Avacta. Used in the early stages of R&D in the biopharma industry, Optim1000 evaluates the stability and homogeneity of complex biological drugs, using just micrograms of protein sample. This screening reduces the costly development and late-stage failure of unsuitable candidate therapeutics. The platform has been sold to a wide range of global biopharma companies; it is reported to reduce drug stability screening by months. This provides economic impact through saving the industry millions of dollars in R&D costs, along with health impact by speeding up the emergence of new products. Avacta reported revenue of over £3 million in 2012 and employs 70 staff.

2. Underpinning research (indicative maximum 500 words; Leeds researchers in **bold**)

Since 2003 the School of Physics and Astronomy and the Institute of Molecular and Cellular Biology (IMCB) at the University of Leeds have collaborated to study the kinetics and mechanisms of protein folding and unfolding. The primary aim of the research work has been to understand molecular mechanisms of protein stability – and the features and environmental conditions that lead to instability.

Nanosecond protein heating

Early experiments were limited by the rapidity of some proteins' folding kinetics, where conformational changes occur within microseconds of chemical or temperature-induced denaturation. In 2003 Dr Alistair **Smith** (School of Physics and Astronomy) and Professor Sheena **Radford** (Institute of Molecular and Cell Biology) developed techniques to observe protein structural changes during rapid (microsecond) temperature switches [1-3]. In [1] a custom built fluorescence microscope was used to monitor the unfolding of the immunity protein Im9, using diffusion single pair fluorescence resonance energy transfer. In [2] the transition state of the Im7 folding intermediate was characterised using a combination of protein engineering, ultra rapid mixing and stopped flow experiments. All this led to the design and construction of instrumentation capable of heating samples very rapidly. Specifically, the researchers were able to build an instrument which could increase the temperature of a sample by up to 25°C in 8 nanoseconds whilst taking spectroscopic readings over the same timeframe. Using this so-called 'T-jump' apparatus the team reported on the temperature and denaturant dependence of equilibrium and kinetic data for a fluorescent mutant of the staphylococcal protein A in the international peer-reviewed journal PNAS [3].

Small sample rapid spectroscopy

The on-going collaboration between **Smith** and **Radford** led to further advances in spectroscopic instrumentation to examine fluid flow properties. Typically, sample solutions and reagents are mixed rapidly to initiate chemical reactions and trigger dynamic folding processes. However, early events in the folding process, such as the formation of folding intermediates, are difficult to study because they exist transitorily during the 'dead time' while reagent mixing is still taking place.

In 2006 Smith and Radford developed devices so they could monitor unfolding kinetics by fluorescence and Raman spectroscopy on rapid timescales of 20msec upwards, crucially using small protein sample volumes. They developed two novel, different 'T mixers' that use moderate flow rates (0.2-0.4ml/s) to achieve mixing times as low as 20 microseconds. They demonstrated that fluorescence and ultraviolet resonance Raman spectroscopy instrumentation could monitor the early stages of protein folding using only milligrams of a sample – a significant advance where

quantities of available protein are limited.

The microfluidic T mixers were evaluated and shown to enable the spectroscopic monitoring of protein folding reactions of up to several milliseconds [4]. Details of the novel instrumentation developed are also given in [4].

3. References to the research (indicative maximum of six references; Leeds researchers in **bold**)

[1] **Tezuka-Kawakami T**, **Gell C**, **Brockwell DJ**, **Radford SE**, **Smith DA**. Urea-induced unfolding of the immunity protein Im9 monitored by spFRET. *Biophysical Journal* 2006;91(5):L42-L44. http://dx.doi.org/10.1529/biophysj.106.088344 [cited 27 times].

[2] **Friel CT**, **Smith DA**, Vendruscolo M, Gsponer J & **Radford, SE**. The mechanism of folding of Im7 reveals competition between functional and kinetic evolutionary constraints. Nature Structural and Molecular Biology 2009; 16(3): 318-324. http://dx.doi.org/10.1038/nsmb.1562 [cited 28 times].

[3] **Dimitriadis G**, **Drysdale A**, Myers JK, Arora P, **Radford SE**, Oas TG, & **Smith DA**. Microsecond folding dynamics of the F13W G29A mutant of the B domain of staphylococcal protein A by laser-induced temperature jump. *Proceedings of the National Academy of Sciences of the United States of America* 2004; 101(11):3809-3814. http://dx.doi.org/10.1073/pnas.0306433101 [cited 43 times].

[4] **Masca SI**, **Rodriguez-Mendieta IR**, **Friel CT**, **Radford SE**, **Smith DA**. Detailed evaluation of the performance of microfluidic T mixers using fluorescence and ultraviolet resonance Raman spectroscopy. *Review of Scientific Instruments* 2006;77(5): 055105. http://dx.doi.org/10.1063/1.2198800 [cited 6 times].

Relevant Research Grants:

- BBSRC, Testing Models of Helical Protein Folding, reference BBS/B/04803, 15/07/2004-14/07/2007, PI **Radford**: co-investigator Prof David Alastair **Smith**, £198,386, researcher Dr Graham Spence.
- Wellcome Trust, Ultra-Violet Raman Spectroscopy, reference 072763/Z/03/Z, 01/12/2003-30/11/2006, PI Dr A **Smith:** co-investigators **Radford,** Prof Jennifer Kirkham, Prof David Alastair **Smith**, £62,500.

4. Details of the impact (indicative maximum 750 words; Leeds researchers in **bold**)

PATHWAY TO IMPACT

Protein stability studies are essential in the biopharmaceutical industry to identify suitable candidate product formulations and eliminate the costs associated with the development (and later failure) of unstable molecules. **Smith** and **Radford** quickly realised that their T-jump and T-mixer devices for heating and mixing small quantities of proteins with simultaneous spectroscopic monitoring (described in [3]) of denaturation had significant commercial potential as an analytical tool for early stage biopharmaceutical R&D.

Avacta was formed in 2004 as a spin-off company from the research described above [A]. It subsequently raised raised £1.0m through its AIM listing in 2006, with a further £2.7m generated in a placing of ordinary shares in 2007 [B]. Dr Alastair **Smith** was the founding CEO of Avacta and still is, transferring his leadership and expertise from the University of Leeds into the business sector.

IMPACT DURING THE REF PERIOD

Between 2005 and 2009 Avacta further developed the unique rapid temperature switching and T mixer technologies. The Optim1000 platform for high throughput, micro-volume protein stability analysis was launched in April 2009. Towards the end of the REF Impact Period the Optim 1000 was been replaced with an improved product, the Optim 2.

Saving biopharma time and money and reducing risk

Optim1000 has allowed biopharmaceutical researchers to analyse the effects of temperature, time and the chemical environment on small quantities of biomolecule with high throughput. Optim1000

could analyse up to 96 samples in a working day, using only 9µl sample volumes (as little as 0.1µg protein [D]). The Optim 2 can analyse up to 48 temperature controlled samples simultaneously, with an increase to 144 samples per day and has retained the 'best in class' sample volume (9µl) of the Optim 1000 [C].

Studies have shown that even the previous Optim1000 technology "is at least fifty times faster and consumes significantly less protein than alternative technologies to obtain the same information." [D]. The requirement for less protein sample material also allows laboratories to perform stability studies much earlier in the R&D process, reducing waste in reagents and resource and the costs of scaling up production to generate sufficient quantities of sample for analysis. Optim1000 and now Optim 2 marketing asserts that the early analysis facilitated by the system *"eliminates the cost associated with the unnecessary development of candidates or formulations that do not have the potential to proceed and dramatically reduces the chance of late-stage failure."* [C].

Clear evidence of the rapid sample screening advantages of Optim technology is given in the customer testimonies [J]. For example, Royal DSM state *"With the Optim we simply get answers more quickly with far less effort. We test large numbers of formulations, screening many variants to get a new stable end product and can pick the top three to take ahead to more in-depth real-time stability studies. Eliminating the poor candidates very early in the process saves us so much time."* Besides time (and thus cost saving), another very important impact of Optim technology is the reduction of risk in a drug development programme, as highlighted by Syntaxin Ltd [J]: *"The Optim 1000 fluorescence and light-scattering instrument provides data-rich analysis using very small amounts of material. The low sample requirement — <320 mg of material were used for the entire SXN101959 TSI study — enabled Syntaxin to rapidly screen a number of different formulations. Because more formulations could be studied early in development of this drug, and unsuitable formulations could be discarded, risk was reduced for this development program."*

Sales of Optim1000 units have grown steadily since product launch in 2009; 21 units were shipped in the year 2011-12 (compared with 14 in 2010-11) [H], contributing to a 59% increase in revenue. The company has global reach with operations in all in major geographies [A]. Avacta indicates that many of the top global biopharmaceutical R&D companies use Optim technology in their R&D cycle, demonstrating the wide acceptance and reach of the technology.

A conservative estimate of the economic impact that Optim technology enables in the pharmaceutical industry worldwide can be calculated from current annual sales figures of the leading biopharmaceutical product Humira which reported annual sales of $7.9bn in 2011 [I]. Assuming that Optim technology screening had saved Abbott one month in development time (thus increasing the drug's patent exclusivity period by one month), the company could expect an additional ca. $650m in Humira sales before patent expiry. Taking a conservative five-fold scaling factor for the impact of Optim technology across the industry, this technology can be estimated to be worth $3.2 bn in increased sales to the industry. This is simply considering drug sales; clearly additional economic impact is also derived from R&D cost savings, though these are harder to quantify in absolute terms.

Revenue growth and jobs

Since its creation in 2005 Avacta has experienced significant growth through increased sales and acquisitions. In October 2012 the company reported annual revenue over £3M, an increase of 28% from 2011. Sales of Optim1000 increased by 59% from 14 units in 2011 to 21 units in 2012. The company's workforce has grown to its current figure of 70 staff [A].

In September 2012 Avacta signed a deal with the global filtration company Pall Corporation, which will allow Pall Corp to sell Optim1000 into the Indian drug development market, following previous deals to target North America and South East Asia [E, F]. This breakthrough supports the future growth of Avacta and its entry into key new biopharmaceutical markets.

Supporting research

Avacta also runs an analytical contract service which supports biopharmaceutical R&D and fundamental protein stability and kinetic studies serving large pharmaceutical, smaller biotechs and universities [G]. This service contributes to the research of these clients and widens the scope for future benefits and impact based on Avacta technology [A]. This contract service contributes a

steady annual income of around £200,000 to the company [H].

5. Sources to corroborate the impact (indicative maximum of 10 references)
All the web pages used as corroboration of this impact are on record at Leeds, along with all relevant documents as pdfs. All web pages and links last accessed 25/09/2013. A letter from the CEO of Avacta, corroborating all the aspects of the impact, is also on record at Leeds.

[A] Corroborative statement, CEO, Avacta
[B] Avacta Annual Report 2007 http://www.avacta.com/wp-content/uploads/Annual-Report-31-July-2007_1.pdf
[C] Optim1000 webpage, now Optim 2: http://www.avactaanalytical.com/optim-2 . A detailed comparison document is available on the website.
[D] Application note: *Comparing Optim 1000 and DSC* http://www.avactaanalytical.com/wp-content/uploads/Comparing-Optim-with-DSC-121108.pdf
[E] Newspaper report http://www.yorkshirepost.co.uk/business/business-news/avacta-extends-reach-of-flagship-device-to-india-1-4955231
[F] Avacta Annual Report 2011 http://www.avacta.com/wp-content/uploads/AnnualReport2011.pdf
[G] Avacta Analytical Services webpage http://www.avactaanalytical.com/analytical-services
[H] Avacta Annual Report 2012 http://www.avacta.com/wp-content/uploads/AnnualReport2012.pdf
[I] http://www.abbott.com/static/content/microsite/annual_report/2011/18_review1.php
[J] Optim customer testimony and feedback: http://www.avactaanalytical.com/optim-2/optim-customer-testimony