Institution: University of Oxford

Unit of Assessment: UOA5

Title of case study:

Glycobiology platforms: enabling technologies for the biopharmaceutical industry

1. Summary of the impact

Research at the University of Oxford's Glycobiology Institute (OGBI) has led to the development of 'state-of-the-art' platform technologies for the analysis of oligosaccharides (sugars) that are linked to proteins and lipids. These enabling technologies have had major impacts worldwide on drug discovery programmes, have enabled robust procedures to be developed for the quality control of biopharmaceutical production, and have been widely adopted by the pharmaceutical industry.

2. Underpinning research

Glycosylation, the complex process that attaches glycans (sugars) to proteins (glycoproteins) and lipids (glycolipids), plays a pivotal role in maintaining normal cell function; and glycoproteins are involved in a wide range of cellular processes including immune responses, cell growth and cell-cell adhesion. Because of this central role in cell function, the potential for glycoproteins to be used as therapeutic agents has long been recognised. Acquiring detailed information about how, and to what extent, proteins are glycosylated is therefore crucial for understanding mechanisms underlying both the normal and the diseased state, and also for developing innovative and effective therapies. Today, one of the fastest growing markets in the biopharmaceutical industry is for therapeutic glycoproteins.

Since 1993, research performed by Professor Raymond Dwek and colleagues in the Oxford Glycobiology Institute (OGBI) at the University of Oxford has substantially advanced methodology for the large-scale analysis of glycosylation. The first breakthrough occurred in 1997 when Dwek and colleagues reported a novel, rapid and sensitive technique for profiling and sequencing of glycoprotein-associated oligosaccharides[1]. Whereas previous sequencing methods required that glycoproteins were totally free of contaminating proteins and small molecules, Dwek developed a method of sequencing oligosaccharides directly from protein gels. This approach precluded the need for time-consuming protein purification procedures prior to sequencing because contaminants were separated during electrophoresis.

The next advance addressed the issue of distinguishing 'glycoforms' (a term coined by Dwek) of individual proteins. Although the same glycosylation machinery is available to all proteins which enter the secretory pathway in a given cell, most glycoproteins emerge with heterogeneous populations of glycans at each site: it is not uncommon for a glycoprotein to be processed with, in excess of, 100 alternative glycans at a single glycosylation site. Resolution of these different forms requires electrophoresis on two-dimensional (2D) protein gels, with the amount of each form necessarily being only a small proportion of the total pool that is resolved on a one-dimensional gel. In collaboration with Professor Pauline Rudd, then at OGBI, Dwek optimised methods of gel extraction and fluorescent labelling to increase the sensitivity of glycan analysis and hence to enable sequencing of different glycoforms from 2D gels[2].

In 2008, research at the University of Oxford further transformed glycan analysis when it led to the development of an automated, sensitive, and robust system for glycan profiling. Based on the previous gel extraction and fluorescent labelling procedures, the new method enabled the quantitative analysis of samples immobilised on 96-well plates[3]. This research facilitated the high-throughput High Performance Liquid Chromatography (HPLC) analysis of serum samples and the detailed analysis of glycans at concentrations required for biomedical applications, including quality control of biologicals used for therapeutic purposes.

The final breakthrough that the OGBI researchers made was to design and develop a novel bioinformatics platform for the interpretation, annotation and assignment of glycan sequencing data. Before the platform was developed, the analysis of HPLC-glycan data was a manual and very time-consuming task that could only be carried out by experts. In collaboration with Matthew Campbell of OGBI, Dwek and Rudd built a relational database (GlycoBase) and an analytical tool (autoGU) that brought glycan analysis within the reach of any well-established laboratory[4]. GlycoBase contained the HPLC elution positions for over 350 labelled glycan structures and AutoGU assigned provisional structures to each integrated HPLC peak. In combination, these databases provided essential bioinformatics resources required for the large-scale analysis of glycoproteins.

3. References to the research

1. Küster B, Wheeler SF, Hunter AP, Dwek RA, Harvey DJ. (1997) Sequencing of N-linked oligosaccharides directly from protein gels: in-gel deglycosylation followed by matrix-assisted laser desorption/ionization mass spectrometry and normal-phase high-performance liquid chromatography. Anal Biochem 250: 82-101. doi: 10.1006/abio.1997.2199 ***Paper outlining a generally applicable, rapid, and sensitive technique for profiling and sequencing of glycoprotein-associated N-linked oligosaccharides from protein gels.***

2. Rudd PM, Colominas C, Royle L, Murphy N, Hart E, Merry AH, Hebestreit HF, Dwek RA. (2001) A high performance liquid chromatography based strategy for rapid, sensitive sequencing of N-linked oligosaccharide modifications to proteins in sodium dodecyl sulphate polyacrylamide electrophoresis gel bands. Proteomics 1: 285-294. doi: 10.1002/1615-9861(200102)1:2<285::AID-PROT285>3.0.CO;2-G ***Paper showing that oligosaccharide sequencing can be carried out on samples extracted from 2D protein gels.***

3. Royle L, Campbell MP, Radcliffe CM, White DM, Harvey DJ, Abrahams JL, Kim Y-G, Henry GW, Shadick NA, Weinblatt ME, Lee DM, Rudd PM, Dwek RA. (2008) HPLC-based analysis of serum N-glycans on a 96 well plate platform with dedicated database software. Anal Biochem 376: 1-12. doi: 10.1016/j.ab.2007.12.012 ***Description of a robust platform enabling the detailed high throughput analysis of low concentrations of glycans released from glycoproteins. An example of its use in rheumatoid arthritis is provided.***

4. Campbell MP, Royle L, Radcliffe CM, Dwek RA, Rudd PM. (2008) GlycoBase and autoGU: Tools for HPLC-based glycan analysis. Bioinformatics 24: 1214-1216. doi: 10.1093/bioinformatics/btn090 ***Database developed to increase the accuracy of analysing structural information on complex mixtures of sugars.***

Funding for research: Between 1993 and 2008, research at OGBI was supported by a £10M endowment from Monsanto, plus grants of ~£3M from Oxford Glycosciences Ltd and £3.6M from United Therapeutics.

4. Details of the impact

The research performed by the University of Oxford's OGBI since 1993 has transformed the field of 'glycomics' in three major respects. First, by making sugar technology available for clinical studies, and for the biotechnology and pharmaceutical industries worldwide it has driven commercial investment and revenue generation. Second, it has led to the design of new drugs that are pioneers in their fields. Third, it has advanced commercial production processes for biopharmaceuticals.

Commercial investment and sustained revenue generation

OGBI's research in the late 1990s was carried out in collaboration with Oxford Glycosciences (UK) Ltd (OGS), a University of Oxford spinout company specialising in integrating proteomics and genomics for drug and biomarker discovery. OGS's first commercial products were based on Professor Dwek's research into methods for sugar detection and analysis. In particular, OGS

'miniaturised' Dwek's gel-based glycoprotein identification and isolation platform[1,2] and sold it worldwide for scientific research. OGS's success was endorsed by its acquisition by CellTech for £103M in 2003[5]. Royalty payments to Oxford University attest to the on-going importance of OGS' commercial activity – **[text removed for publication]**.

Drug discovery

When OGS was sold in 2003, all of the University's share of royalties (approximately £20M) were ploughed back to fund translational research at OGBI, which has since supported further commercial activity. Continued development of the iminosugar drug Zavesca (misglustat) for lysosomal storage diseases[6] is one example of commercial application arising from this research (see impact case UOA5-04 for details). Sales of Zavesca since 2008 have generated CHF 315 million in revenues for Actelion, the company sublicensed to sell it[7]. A second example, as yet without a proprietary name, has been developed by Professor Zitzmann of OGBI, in collaboration with United Therapeutics and Unither Virology. With £12M from United Therapeutics and $45M from the US National Institute of Health, Professor Zitzmann and colleagues have produced a powerful highly innovative antiviral iminosugar, which in December 2013 is due to enter phase II clinical trials of its efficacy against dengue virus[8].

Glycoprofiling biopharmaceuticals

Therapeutic glycoproteins are used to treat various diseases. For example, many different recombinant forms of immunoglobulins are produced to treat life-threatening conditions such as metastatic breast cancer and non-Hodgkin's lymphoma. Control of glycosylation is of major importance during the development and production of these drugs, because glycan chains can have marked effects on stability, activity and antigenicity in intact organisms. However, recombinant glycoproteins are typically produced in cell culture systems and often consist of a mixture of glycoforms. Licensing bodies such as the European Medicines Agency and the US Food and Drug Administration will allow for a certain range of variation in glycoforms, but the manufacturer and the agency must agree on the extent to which such variation is acceptable for a given drug formulation. Once a drug is approved, biopharmaceutical companies need to ensure that every product batch falls within the defined range.

The National Institute for Bioprocessing Research and Training (NIBRT) in Dublin is an organisation set up by the Irish government to support the biopharmaceutical industry by providing operator-training courses. Another key area of focus for NIBRT is to assist the industry in maintaining the exacting quality standards needed for biopharmaceutical production. To this end, the NIBRT GlycoScience Laboratory (headed by Professor Pauline Rudd, formerly of OGBI), in collaboration with the Waters Corporation and Beckmann, developed a state-of-the art version of the Glycobase database that was first developed at Oxford[4]. Glycobase 3+ provides data for 650 *N*-linked and *O*-linked glycan structures including both published and proprietary glycans[9]. The database is being used to bring greater control and predictability to products produced by pharmaceutical companies. The database is open access, enabling it to be freely used by both academic and commercial users. However, a specialised subset of the database relevant to pharmaceutical companies, as well as the NIBRT bioinformatics platform for interpreting the data, has been incorporated into the Waters UNIFI 1.7 platform[10]. Combined with an automated platform for glycan release and labelling, thousands of samples can be processed each week, making it invaluable for monitoring cell cultures and for evaluating product batches. On average the Glycobase 3+ database is accessed from over 150 independent IP addresses each day[11], with the largest concentrations of users in the US, Ireland, Germany, Spain, France and Australia.

In addition to providing support through Glycobase 3+, the NIBRT GlycoScience Laboratory provides a high-end contract glycoanalytical service for pharmaceutical companies[12]. The high-throughput analytical and bioinformatic platform at NIBRT is based on the prototype that was first developed in the OGBI at the University of Oxford[3]. A large number of pharmaceutical companies use NIBRT's service to analyse their products, and to carry out basic research into effector functions of potentially novel therapeutic agents such as monoclonal antibodies. These companies include Shire, Agilent Technologies, GeneMediX, GE Healthcare, Lilly, Janessen, Waters, BD, Merck Serono, Astellas, Reliance Life Sciences, Unither, Pfizer, Merck, L'Oreal and Roche[13]. **[text**

removed for publication] [14].

5. Sources to corroborate the impact

5. Isis Innovation Ltd Spinout Companies. Oxford Glycosciences plc. http://www.isis-innovation.com/spinout/oxglycosciences.html ***Website stating the flotation of Oxford Glycosciences plc on the London Stock Exchange and its acquisition by Celltech in 2003.***

6. Actelion Pharmaceuticals Ltd Zavesca®. http://www.actelion.com/sites/en/healthcare-professionals/products/zavesca/index.page ***Actelion webpage describing Zavesca.***

7. Actelion Pharmaceuticals Ltd Annual report archive. Switzerland: Actelion Ltd 2013 http://www1.actelion.com/en/our-company/annual-report/annual-report-archive.page ***Archive of Actelion's annual reports detailing Zavesca sales from 2008 to present.***

8. Sayce AC, Miller JL, Zitzmann N. (2013) Glucocorticoids as dengue therapeutics: Resolving clinical observations with a primary human macrophage model. Clin Infect Dis 56: 901-903. doi: 10.1093/cid/cis1048 ***Paper outlining the use of iminosugars in the development of anti-viral therapies for dengue virus.***

9. Waters Corporation. Improving characterization of glycans: Waters' HILIC-UPLC chromatography combines with NIBRT's robust online glycan database. http://www.waters.com/waters/promotionDetail.htm?id=134654015&locale=en_US ***Description of Glycobase 3+.***

10. http://www.waters.com/webassets/cms/events/docs/WatersUNIFIGlycanAnalysis2013biopharm_2.pdf ***Documentation describing Waters' UNIFI platform that is due to be released December 2013.***

11. Record of database hits obtained from NIBRT (held on file).

12. http://www.nibrt.ie/index.jsp?p=158&n=185 ***Details of NIBRT's contract glycan profiling service.***

13. http://www.nibrt.ie/ ***Evidence of the extent and range of NIBRT's commercial clients.***

14. NIBRT's financial information held on file.