Institution: University of York
Unit of Assessment: 8, Chemistry
Title of case study: Short and long-acting insulins for the management of diabetes

1. Summary of the impact

Insulin derivatives that stem directly from structural work carried out within the York Structural Biology Laboratory (YSBL) are now the standard treatment for insulin-dependent diabetes for some 35 million patients worldwide. The successful development of new insulin drugs hinged upon controlling their speed of action following intravenous administration. This speed of action is controlled by insulin's degree of aggregation, which, in turn, is determined by protein-protein interactions. Understanding, modifying and controlling these interactions depended on detailed structural studies of insulin, insulin mutants and insulin derivatives. The most widely used derivatives were developed following structural work carried out within YSBL in the Department of Chemistry. The research has had economic impact through sales of the insulin drugs (over $6 billion in 2012) and major health impacts on diabetics worldwide.

2. Underpinning research)

Background to research. The late GG Dodson FRS played a major role in the initial determination and analysis of the structure of insulin by X-ray crystallography in the Oxford laboratory of Nobel laureate DC Hodgkin. The insulin project moved to York when Hodgkin retired, and over the next twenty years many further structures of native insulin, insulin mutants and insulin derivatives were determined. These structures allowed both academic and industrial scientists to understand how the structure of insulin relates to its biological activity, and how the physico-chemical properties of different preparations of insulin can be engineered to provide therapeutic options.

Naturally occurring insulin is stored in the pancreas in crystals, made up of three insulin dimers coordinated by zinc ions. They dissociate into active insulin monomer upon release into the blood stream. A basal level of insulin (pM) is maintained in the blood stream and increases in response to increased glucose in the blood following a meal. The original therapy for Type I diabetics was injection of insulin crystals extracted from pig or cow pancreas. However, it did not reproduce the physiological blood levels of insulin and caused immune responses to foreign hormones. All these led to the long-term complications associated with insulin therapy.

From 1984 to 2000, YSBL engaged in a major collaboration on recombinant, novel modified insulins with Novo Nordisk A/S (www.novonordisk.com). The structures of these analogues provided a detailed understanding of the nature of insulin aggregation. The focus of the work in 1993 moved towards the rational design of modified insulins in order to obtain clinically applicable monomeric insulins – they are now the basis of the modern "fast-acting" insulins. Furthermore, structures of insulin crystals with various additives and/or modifications identified some general principles on how to increase stability of insulin hexamers, leading to the current "long-lasting" insulin preparations.

Research during period. The key breakthroughs during the assessment period were carried out by J. L Whittingham and R. E. Hubbard with G. G. Dodson:

1. *Design of rapid-acting monomeric insulins:* The crystal structure of insulin hexamers and dimers suggested amino acid mutations, which would lead to a monomeric insulin. The structures of such proteins are reported in references 1 (1993) and 2 (1998). In one of these proteins, B28 proline is mutated to aspartic acid; this is the insulin of the Novo Nordisk product *Insulin aspart*.®

2. *Identifying additives to stabilise insulin preparations:* References 2 (1998) and 3 (1995) describe structures of series of preparations in which hexamer formation is stabilised. The structure of a *m*-cresol-insulin clathrate led to the proposal that mutation to tryptophan at the end of the B chain would disrupt the insulin aggregate. This mutant was found to have improved stability in Novo Nordisk's very complex protein manufacturing processes.

3. *Modifications to generate long-active insulins:* References 4 (1997) and 5 (2004) report the crystal structures of acylated insulins which had been identified by Novo Nordisk as prolonged-

acting insulins. The YSBL research suggested that the prolonged action was more likely to be related to the way that these modified insulins aggregated in solution (and in the crystals). These soluble prolonged-acting insulins are significantly better for diabetics than other long-acting insulins.

Insulin research in York continues. Throughout the 2000s AM Brzozowski of YSBL worked with an international team on the development of super-active analogues of insulin. These have provided the tools that enabled a breakthrough in 2013 (reference 6) in the determination of the structure of the first insulin receptor-insulin complex. Insights from this work will have an impact in the future on the design of further insulin analogues with the prospect of oral delivery of insulin.

Key researchers:

G. Guy Dodson FRS, appointed 01/07/1976 as Lecturer, Professor from 01/08/1993, Emeritus Professor from 01/08/2005. Died 2012.

Roderick E. Hubbard, appointed 01/10/1980 as temporary Lecturer, Professor from 01/10/1995

A. Marek Brzozowski, appointed 06/04/1989 as Research Fellow, Reader 01/10/2002

Jean L. Whittingham, appointed 01/01/95 as Research Fellow

3. References to the research

This research exceeds the quality threshold as is evident from the journal quality and the number of citations. Citations from Scopus (20/09/2013). Refereed publications (authors in publications 1-5 are either from YSBL or YSBL and Novo-Nordisk).

1. E. J. Dodson, G. G. Dodson, R. E. Hubbard, P. C. E. Moody, J. Turkenburg, J. L. Whittingham, B. Xiao, J. Brange, Kaarsholm and H. Thogersen, H. “Insulin assembly: its modification by protein engineering and ligand binding”, *Phil. Trans. R. Soc. Lond. A*, 1993, **345**, 153-164. DOI: 10.1098/rsta.1993.0126. *14 citations*.
2. J. L. Whittingham, D. J. Edwards, A. A. Antson, J. M. Clarkson and G. G. Dodson, “Interactions of Phenol and m-Cresol in the Insulin Hexamer, and Their Effect on the Association Properties of B28 Pro → Asp Insulin Analogues”, *Biochemistry*, 1998, **37**, 11516-11523. DOI: 10.1021/bi980807s. *59 citations.*
3. J. L. Whittingham, S. Chaudhuri, E. J. Dodson, P. C. E Moody and G. G. Dodson. “X-ray Crystallographic Studies on Hexameric Insulins in the Presence of Helix-stabilising Agents, Thiocyanate, Methylparaben, and Phenol”, *Biochemistry*, 1995, **34**, 15553-15563. DOI: 10.1021/bi00047a022. *69 citations.*
4. J. L. Whittingham, S. Havelund and I. Jonassen, “Crystal Structure of a Prolonged-Acting Insulin with Albumin-Binding Properties”, *Biochemistry*, 1997, **36**, 2826-2831. DOI: 10.1021/bi9625105. *77 citations.*
5. J. L. Whittingham, I. Jonassen, S. Havelund, S. M. Roberts, E. J. Dodson, C.S. Verma, A. J. Wilkinson and G. G. Dodson, “Crystallographic and Solution Studies of N-Lithocholyl Insulin: A New Generation of Prolonged-Acting Human Insulins”, *Biochemistry*, 2004, **43,** 5987-5995. DOI: 10.1021/bi036163s. *22 citations*.
6. J. G. Menting, J. Whittaker, M. B. Margetts, L. J. Whittaker, G. K.-W. Kong, B. J. Smith, C. W. Watson, L. Žáková, E. Kletvíková, J. Jiráček, D. F. Steiner, S. J. Chan, G. G. Dodson, A. M. Brzozowski, M. W. Weiss, C. W. Ward and M. C. Lawrence. “How insulin engages its primary binding site on the insulin receptor”, *Nature,* 2013, **493**, 241-245. DOI: 10.1038/nature11781. *11 citations.* Patent PUV 2012-26680.

4. Details of the impact

Worldwide there are some 35 million sufferers of insulin-dependent diabetes. For many years, the adverse symptoms of this condition have been mitigated successfully with regular injections of insulin. Initially these insulins were obtained from bovine and/or porcine sources, but they did not mimic well actions of endogenous insulin, leading to frequent hypo-glycaemia and other complications. In 1980, the first clinical trials were begun of recombinant insulins obtained by

protein engineering methods. From these trials it soon became apparent that the rate of insulin action *in vivo* was a key factor in the therapeutic potential of the enzyme. The best results were obtained when the insulin injectate contained a mixture of fast-acting and slow-acting insulins, where the latter avoids the need to have regular repeated injections and minimise hypoglycemia, and the former is necessary to treat the rapid rise in blood glucose levels that accompanies ingestion of food.

Accordingly recombinant insulin producers concentrated on controlling the rate of action of insulin and its analogues. Amongst these, Novo-Nordisk in collaboration with YSBL, led the way in trying to find a structural rationale for the controlled disaggregation rates of hexameric insulin. YSBL was the world's leading laboratory for insulin structures, and the large majority of the structural work for Novo-Nordisk on insulins from 1993 to 2000 was done in conjunction with YSBL. The protein structures published from York are available as coordinates in the Protein Data Bank.[7] The collaboration between YSBL and Novo-Nordisk was supported by several grants.[8] This development programme was highly successful and consequently today Novo-Nordisk is the leading developer and world's largest producer of recombinant insulin for treatment of diabetes.

The first new product that came from York structural studies was the aspartate mutant described above.[1,2] This fast-acting insulin was launched in 1999, marketed as NovoLog® in the US and Insulin Novorapid® in Europe.[9]

The second development came from the derivatised insulins, which were conceived directly from the structural work described in reference 4 above. It was discovered that the attachment of a fatty acid to insulin led to its prolonged action, resulting in just one daily intravenous administration of the hormone. These derivatised insulins received FDA approval in 2005, and are marketed as the product Levemir® (or Insulin Detemir®) and are now the mainstay of Novo Nordisk's insulin products. Its new, improved generation insulin Degludec® was approved by the EU and Chuikyo (Japan) in 2013.

Levemir®, NovoRapid® and NovoLog® (also marketed as Novomix® in a different formulation) are true blockbuster drugs with billion dollar sales (reference 10). Levemir® had US sales of $1.7 billion in 2012 growing from $756 million in 2008. The corresponding figures for Novolog® are $1.6 billion and $1.1 billion, and for Novorapid, $2.7 billion and $1.5 billion. Levemir® and Novolog® are currently number 40 and 42 in the ranking of US drugs by sales. Total worldwide sales of the drugs were $6 billion in 2012 (Figure).[10]

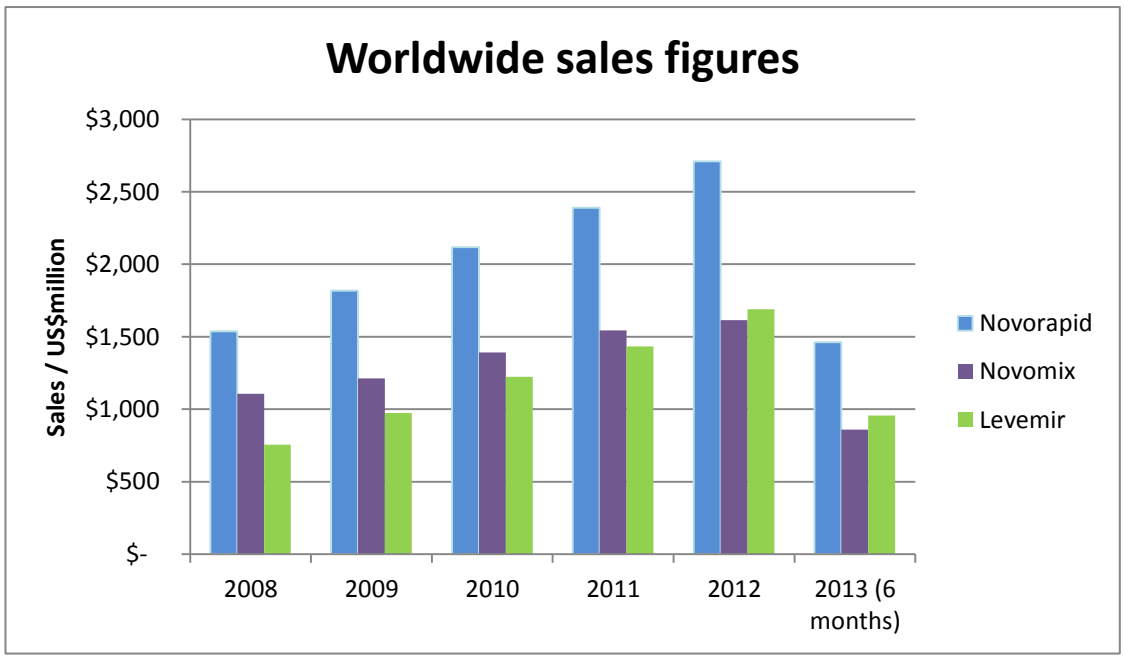

Figure: Worldwide sales of Novorapid, Levemir® and Novomix® (alternative name for Novolog® depending on market), during REF impact period.[10]

Svend Ludvigsen (Vice-President, Diabetes formulation, biophysics and structure) at Novo Nordisk assesses the impact of York's work as follows:[11]

"Throughout the years the collaboration with York has been a continuous source of inspiration for the understanding of insulin structure and insulin as pharmaceutical products. The work of Whittingham et al. 1997 has provided significant insight into some of the protraction principles of the insulin analog, insulin Detemir, developed into a once daily basal insulin product Levemir®. Novorapid® (US: Novolog®) and Levemir® both have blockbuster status and are used by millions of patients all around the globe."

5. Sources to corroborate the impact

7. Deposition of coordinates on Protein Data Bank (PDB). http://www.rcsb.org. 22 insulin structures deposited with Whittingham as co-author (1995 onwards) such as entries - 1UZ9, 1ZEG, 3ZU1, 1MPJ

8. Seven successive, uninterrupted, grant renewals from Novozymes and Novo-Nordisk since 1993– 2013, totalling more than £3.4M.

9. NovoLog® and Novorapid® details: www.ukmi.nhs.uk/NewMaterial/html/docs/insulin.pdf and http://www.globalrph.com/rapid-acting-analogues.htm

10. Sales of insulins from Novo-Nordisk website: www.novonordisk.com e.g. http://www.novonordisk.com/images/investors/investor_presentations/2013/Interim_report/PR130808_H1_results_UK.pdf

11. Vice President, Diabetes formulation, structure and biophysics, Novo-Nordisk A/S