Insulin and its receptor: a grand challenge in structural biology

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The discovery of insulin in 1922 by Frederick Banting, Charles Best, and colleagues at the University of Toronto (Banting et al. 1922) through to the determination of the atomic structure of the hormone in 1969 by x-ray crystallography (Adams et al. 1969) forms a fascinating part of the early history of structural biology. A further almost fifty years were then to elapse before structures of insulin bound to the ectodomain component of its receptor finally emerged (Scapin et al. 2018; Weis et al. 2018). These latter structures were obtained through cryo-electron microscopy, though the interpretation of the maps involved was heavily reliant on a set of x-ray crystal structures of fragments of the receptor (both in apo form and in complex with insulin) that had been obtained in the earlier decade or so. Taken together, these achievements in insulin structural biology weave a rich tapestry that highlights not only the persistence of the scientists involved but also the numerous technological advances involved that led progressively to the final picture.

Given my own involvement in a part of this history of insulin and insulin receptor structural biology, I thought that it might be inspirational to present an overview of that history to the students participating in the *Biophysics and Structural Biology at Synchrotrons* workshop held in January 2019 at the University of Cape Town. What follows here is a brief summary of the topics that I touched on in the talk that I gave.

Following insulin's discovery, crystals of the hormone were reported in 1926 (Abel 1926), but almost another ten years were to elapse before diffraction images were obtained from such crystals (Crowfoot 1935). These latter diffraction images allowed Dorothy Crowfoot (later Dorothy Hodgkin) to

deduce that “the shape of the molecule ... appears approximately as an oblate spheroid of diameters 44 Å and 30 Å.” Frederick Sanger in the early 1950s then determined the amino acid sequence of the two chains of insulin (Sanger and Tuppy 1951a; Sanger and Tuppy 1951b; Sanger and Thompson 1953a; Sanger and Thompson 1953b), the first to be determined for any protein. However, the determination of protein structure by x-ray diffraction analysis required a way to determine the phases of the diffracted beams. Max Perutz finally solved this problem by developing the isomorphous replacement method, a technique that involved collecting further diffraction data sets from the same protein crystals derivatized with heavy metals (Perutz 1956). The isomorphous replacement technique was ultimately critical to the successful determination of the crystal structure of the hexameric 2-Zn insulin in 1969 by Dorothy Hodgkin and co-workers (Adams et al. 1969). The structure provided the first insights into how insulin might act at the molecular level, particularly through examination of residues conserved across species.

Understanding the structural biology of the insulin receptor and the manner in which insulin interacted with it then proved to be the next challenge. The amino acid sequence of the insulin receptor was determined in 1985 (Ebina et al. 1985; Ullrich et al. 1985), and in 1994, Pierre De Meyts proposed a two-site anti-parallel model for the receptor's engagement of insulin that could explain the observed negative cooperativity of binding (De Meyts et al. 1973; De Meyts 1994). However, determination of the structure of the insulin receptor itself, either in an insulin-free or an insulin-bound state, was to prove considerably more difficult. In 1990, a laboratory was formed under Colin Ward at CSIRO in Parkville, Australia, with the express goal of obtaining a crystal structure of the insulin-bound receptor. I joined Colin's group in 2001. Progress was incredibly slow, but in 2006, we reported a crystal structure of the apo-insulin receptor ectodomain (McKern et al. 2006). The intervening years had seen the emergence of synchrotron x-ray sources, large-scale mammalian cell culture, and robotic crystallization screening, all of which proved essential to the determination of the ectodomain structure. In addition, the

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structure required the use of a construct designed to display reduced glycosylation, this construct then being complexed with four antibody fragments to provide additional surfaces for crystal contact formation. In 2007, Colin and I moved the quest to determine the receptor's insulin-bound structure to the Walter and Eliza Hall Institute for Medical Research. In 2013, we finally obtained a crystal structure of insulin bound to fragments of the receptor that minimally re-constituted its primary binding site ("site 1") (Menting et al. 2013). The development in the mid-2010s of high-resolution cryo-electron microscopy finally led to the emergence of two papers that revealed the long-sought complex (Scapin et al. 2018; Weis et al. 2018), the latter from our laboratory together with co-workers in Europe. The picture of the insulin-bound receptor was complex, showing extensive re-arrangement of the receptor domains and, in particular, a coming together of the two membrane-proximal domains that is arguably the event that effects signal transduction. Sadly, Colin was not able to see this structure, he passed away in 2017.

Many questions remain unanswered. In particular, the cryoEM structures (Scapin et al. 2018; Weis et al. 2018) show only limited engagement with the receptor of what had been understood to be the secondary receptor-binding surface of insulin. I proposed that the remaining portion of the insulin's secondary binding surface may be involved in only transient engagement with the receptor, perhaps during the process of induced fit to the receptor's primary binding site (Weis et al. 2018). Resolving this question will require an understanding of the conformational pathway that is traversed upon hormone receptor engagement.

At this point, my presentation ended, hopefully leaving the students both inspired and with an appreciation for the time scales and dedication that is involved in answering many of the big challenges in biology. As an exercise, I set the students the task of repeating the molecular replacement that was involved in determining the structure of the apo-insulin receptor ectodomain obtained in complex with antibody Fab fragments (McKern et al. 2006). This task is quite fun and relatively easy: the student can begin with fragments from the earlier structure of the first three domains of the insulin receptor (Lou et al. 2006) and search for and locate these individually. These steps can then be followed by a search for fragments consisting of the variable and the constant modules of antibodies related to those employed in the crystallization. The final step is then to add in models of the three fibronectin domains of the receptor. Not all of the latter domains tackled by molecular replacement: some need to be built into density modified difference maps. The students can take delight in the gradually emerging structure of the ectodomain as the pieces located through modern molecular replacement (McCoy et al. 2007) slot into place.

Compliance with ethical standards

Conflict of interest Michael C Lawrence declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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