

## REVIEW

# Structural insights into ligand-induced activation of the insulin receptor

C. Ward,<sup>1,2</sup> M. Lawrence,<sup>1,2</sup> V. Streltsov,<sup>1</sup> T. Garrett,<sup>2</sup> N. McKern,<sup>1</sup> M.-Z. Lou,<sup>1</sup> G. Lovrecz<sup>1</sup> and T. Adams<sup>1</sup>

<sup>1</sup> CSIRO Molecular & Health Technologies, Parkville, Vic., Australia

<sup>2</sup> Walter and Eliza Hall Institute of Medical Research, Parkville, Vic., Australia

Received 25 March 2007,

accepted 17 August 2007

Correspondence: C. W. Ward,  
903 Rathdowne Street, Carlton  
North, Vic. 3054, Australia.

E-mail: colin.ward@csiro.au

## Abstract

The current model for insulin binding to the insulin receptor proposes that there are two binding sites, referred to as sites 1 and 2, on each monomer in the receptor homodimer and two binding surfaces on insulin, one involving residues predominantly from the dimerization face of insulin (the classical binding surface) and the other residues from the hexamerization face. High-affinity binding involves one insulin molecule using its two surfaces to make bridging contacts with site 1 from one receptor monomer and site 2 from the other. Whilst the receptor dimer has two identical site 1-site 2 pairs, insulin molecules cannot bridge both pairs simultaneously. Our structures of the insulin receptor (IR) ectodomain dimer and the L1-CR-L2 fragments of IR and insulin-like growth factor receptor (IGF-1R) explain many of the features of ligand-receptor binding and allow the two binding sites on the receptor to be described. The IR dimer has an unexpected folded-over conformation which places the C-terminal surface of the first fibronectin-III domain in close juxtaposition to the known L1 domain ligand-binding surface suggesting that the C-terminal surface of FnIII-1 is the second binding site involved in high-affinity binding. This is very different from previous models based on three-dimensional reconstruction from scanning transmission electron micrographs. Our single-molecule images indicate that IGF-1R has a morphology similar to that of IR. In addition, the structures of the first three domains (L1-CR-L2) of the IR and IGF-1R show that there are major differences in the two regions governing ligand specificity. The implications of these findings for ligand-induced receptor activation will be discussed. This review summarizes the key findings regarding the discovery and characterization of the insulin receptor, the identification and arrangement of its structural domains in the sequence and the key features associated with ligand binding. The remainder of the review deals with a description of the receptor structure and how it explains much of the large body of biochemical data in the literature on insulin binding and receptor activation.

**Keywords** binding model, crystal structure, insulin binding sites, insulin receptor ectodomain.

The first evidence for the presence of an insulin receptor (IR) came in 1971 from the pioneering studies of Cuatrecasas using <sup>125</sup>I-insulin to label a protein in the

plasma membrane of insulin-responsive cells (Cuatrecasas 1971). The IR could be solubilized with non-ionic detergents and was subsequently shown by SDS gel

electrophoresis to be a homodimer composed of two  $\alpha$ - and two  $\beta$ -chains held together by disulphide bonds (Hedo *et al.* 1981, Massague *et al.* 1981, Siegel *et al.* 1981). Their existence on the cell surface as disulphide-linked dimers is a major feature that separates the IR subfamily from most other receptor families. Members of the IR subfamily presumably require domain rearrangements rather than receptor oligomerization for cell signalling. The next key discovery was the demonstration that the IR was a tyrosine kinase (Kasuga *et al.* 1982). Similar data have been established for the insulin-like growth factor receptor (IGF-1R) and its activation by IGF-I and IGF-II (Jacobs *et al.* 1983, Rubin *et al.* 1983).

The cDNA for human IR was cloned and sequenced in 1985 (Ebina *et al.* 1985, Ullrich *et al.* 1985). It codes for a 1370 amino acid precursor which is cleaved by furin into an  $\alpha$ - and  $\beta$ -chain. The  $\alpha$ -chain and 194 residues of the  $\beta$ -chain comprise the extracellular portion of the IR, there is a single transmembrane sequence and a 403 residue cytoplasmic domain containing the tyrosine kinase. The cDNAs for the IGF-1R and a third member of the IR subfamily, the insulin receptor-related receptor (IRR), have been cloned and sequenced and are similarly organized (Ullrich *et al.* 1986, Shier & Watt 1989).

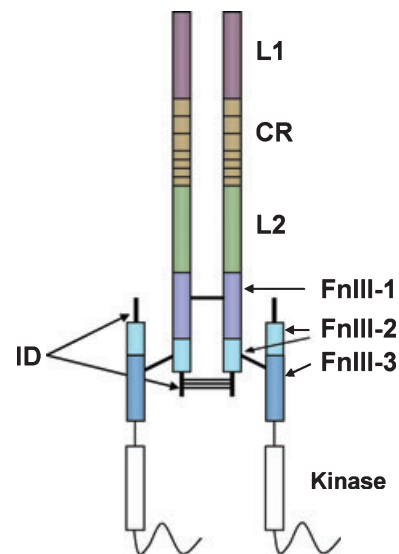
These receptors are heavily glycosylated with the IR carrying both O-linked and N-linked glycans. The O-linked glycans are of the mucin type (Gal.NAc, Gal.NeuAc and Gal.NAc.Gal) and are attached to six sites (Thr744, Thr749, Ser757, Ser758, Thr759 and Thr763) located in a 20 residue segment that begins nine residues downstream of the start of the IR  $\beta$ -chain (Sparrow *et al.* 2007a). There are 19 potential N-linked sites in the IR monomer, 17 of which are used. Most sites are of the complex type with only a few carrying high mannose type glycans (Sparrow *et al.*, 2007b).

The ligands, insulin and the two IGFs, share a common 3D architecture and can bind to IR and IGF-1R in a competitive manner. No ligand has been reported for IRR and its physiological function is unknown. However, the demonstration that a triple *Irf/Igfr/Irr* gene knockout (unlike the single and all possible double knockouts) is incapable of developing the male phenotype indicates that IRR can substitute for the other receptors in mediating the appearance of male gonads and male sexual differentiation (Nef *et al.* 2003).

### Domain organization of IR and IGF-1R

The insulin and the type I IGFR are large, transmembrane glycoproteins. Comparative sequence analyses have shown that the IR family members are composed of a number of different, repeated structural units (Bajaj

*et al.* 1987, Ward *et al.* 1995, Ward & Garrett 2001) as summarized in Figure 1. The N-terminal half of each ectodomain monomer consists of two homologous leucine-rich repeat domains (L1 and L2) of approximately 150 amino acids, separated by a cysteine-rich region (CR) also approximately 150 residues in size and itself predicted to consist of seven smaller repeats (containing either one or two disulphide bonds), similar to those found in laminin and EGF as well as the EGF and TNF receptors (Ward *et al.* 1995). The C-terminal half of each ectodomain monomer (approximately 460 residues) consists of three fibronectin type III domains (FnIII-1, FnIII-2 and FnIII-3). FnIII domains are one of the most common structural modules found in many extracellular proteins (Campbell & Spitzfaden 1994). They are relatively small (~100 amino acids) and consist of a seven-stranded  $\beta$ -sandwich ( $\beta$ -strands A–G) in a three-on-four (EBA : GFCC') topology. FnIII-2 of IR contains a large 120 residue insert (O'Bryan *et al.* 1991) which we termed the insert domain (ID) (Sparrow *et al.* 1997). The ID contains the furin cleavage site that generates the  $\alpha$ - and  $\beta$ -chains of the mature receptor (Adams *et al.* 2000, De Meyts & Whittaker 2002). Intracellularly each IR monomer contains a tyrosine kinase catalytic domain flanked by two regulatory regions (the juxtamembrane region and the C-tail) that contain the phosphotyrosine binding sites for signalling molecules.



**Figure 1** Cartoon of the insulin receptor showing its domain organization. The receptor is synthesized as a single polypeptide and cleaved into an N-terminal  $\alpha$ -chain and a  $\beta$ -chain after dimer formation. Individual domains are coloured as follows: L1, brown; CR, yellow; L2, green; FnIII-1, magenta; FnIII-2, cyan; FnIII-3, blue. The inter- and intra-chain disulphides are indicated by black connecting lines.

The IR splice variant IR-B differs from IR-A by the presence of a 12-amino acid segment (coded for by exon 11) inserted between IR-A residues 716 and 717, three residues prior to the C-terminus of the  $\alpha$ -chain (Adams *et al.* 2000, De Meyts & Whittaker 2002). There are  $\alpha$ - $\alpha$  disulphide bonds at two locations (Cys524 in FnIII-1 and the Cys682, Cys683 and Cys685 triplet in the ID domain), whilst the  $\alpha$ - and  $\beta$ -chains are linked by a single disulphide bond Cys647-Cys860 (IR-A numbering) (Sparrow *et al.* 1997).

### Features of insulin binding

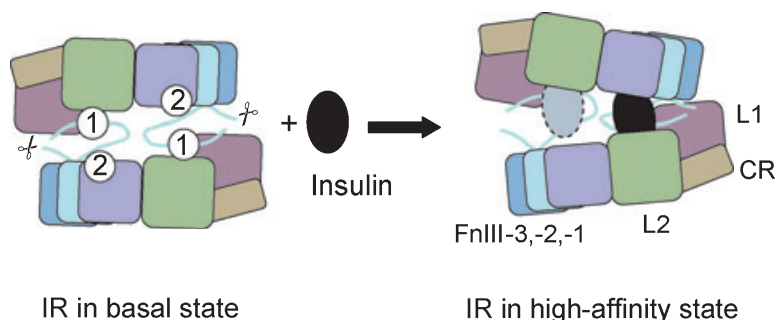
The current model for insulin binding (De Meyts & Whittaker 2002, De Meyts 2004, McKern *et al.* 2006, Ward *et al.* 2007) proposes that each monomer in the IR dimer contains two different binding sites (referred to as sites 1 and 2) located on two different regions of each monomer (see Fig. 2). Binding of insulin to the low-affinity site (site 1) on either of the  $\alpha$ -subunits is followed by a second binding event between the bound insulin and the second site (site 2) of the alternate IR  $\alpha$ -subunit. Negative co-operativity occurs because high-affinity binding can only occur between the pair of sites on one side of the IR dimer (sites 1 and 2'), or the corresponding pair of sites on the other side (sites 1' and 2). Formation of the alternate (site 1'/site 2) cross-link opens up the initial one (site 1/site 2'), promoting dissociation of the ligand bound to the initial site. Two insulin molecules cannot bridge both site 1/site 2' and site 1'/site 2 pairs simultaneously. High-affinity binding is associated with some conformational change within the receptor making it more compact (Lee *et al.* 1997, Florke *et al.* 2001).

The ligand also changes on binding. The B-chain C-terminal peptide (B21–B30) moves away from its

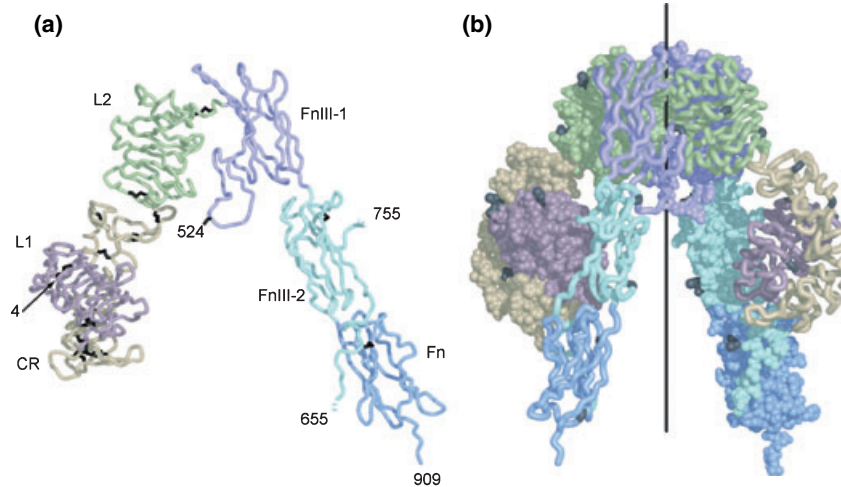
contact with residues A1 and A2 to expose the hydrophobic surface composed of residues A1–A3, A19, A20, B11, B12, B15 and B19 (Derewenda *et al.* 1991, Schäffer 1994, Ludvigsen *et al.* 1998, Xu *et al.* 2004, Wan *et al.* 2005). The B-chain N-terminus is also thought to change from an extended, more stable, but less active, T state (the classical structure) to the less stable, but more active, R-state, where the B-chain helix extends to include residues B1–B8 (Nakagawa *et al.* 2005).

### 3D structures of the IR and IGF-1R

The 3D structures of the L1-CR-L2 fragments of IR (Lou *et al.* 2006) and IGF-1R (Garrett *et al.* 1998) and the structure of the whole IR ectodomain dimer (McKern *et al.* 2006) provide a framework against which the features of insulin binding can be interpreted and allows a tentative description of sites 1 and 2 to be made. The structure of the IR ectodomain monomer and dimer is shown in Figure 3. The L1-CR-L2 fragment structures for IGF-1R (Garrett *et al.* 1998) and IR (Lou *et al.* 2006) reveal that the central  $\beta$ -sheet of the L1 domain is a major contributor to ligand binding. Most of the mutant IRs from patients with defects in insulin binding (Taylor *et al.* 1994, Rouard *et al.* 1999) and all of the L1 domain mutants (generated by alanine scanning mutagenesis) with defects in insulin binding (Williams *et al.* 1995) have mutations on this face (Garrett *et al.* 1998, Lou *et al.* 2006). The central modules of CR, which line one side of the ligand-binding cavity in the L1-CR-L2 fragment (Garrett *et al.* 1998, Lou *et al.* 2006), are also important for IGF-1 binding, based on the properties of IR/IGF-1R chimeric whole receptors and soluble



**Figure 2** Cartoon of insulin binding to membrane-anchored insulin receptor (IR). The symmetrical alignment of the ectodomains of the two monomers in the IR dimer is shown when viewed down the twofold axis. The colours for the domains are the same as in Figure 1. The ID polypeptide is shown as a cyan-coloured line, with the  $\alpha/\beta$  cleavage site highlighted by a 'scissors' symbol. The approximate locations of sites 1 and 2 on each monomer are indicated by the numbered circles, with site 1 being composed of contributions from L1 and CR from one monomer and CT from the other. In the basal state both low affinity sites are equally accessible. In the high-affinity state, one insulin molecule crosslinks sites 1 and 2 on one side of the dimer causing the two monomers to close up on that side and open up on the opposite side. Negative co-operativity is explained by the formation of the alternate cross-link involving the two left over binding sites and the disruption of the first bridging contacts. The monomers in the dimer can be viewed as 'see-sawing' between these two alternatives.



**Figure 3** Schematic diagram of the insulin receptor (IR) ectodomain monomer and dimer showing the relative arrangement of domains. Individual domains are coloured as in Figure 1. (a) The IR monomer is shown in tube representation. Intra-monomer disulphides are shown in black stick representation as are the inter-monomer disulphide at Cys 524 and the  $\alpha$ - $\beta$  disulphide bond between Cys647 at the start of ID and Cys860 in FnIII-3. Convincing electron density was not seen (McKern *et al.* 2006) for  $\alpha$ -chain residues 1–3 and 656–719 or for  $\beta$ -chain residues 724–754 and 910–917. The visible termini (residues 4, 655, 755 and 909) are labelled. (b) The IR homodimer. One IR monomer is shown in tube representation, the other is shown in atomic sphere representation. The location of the potential N-linked glycosylation sites is shown in black on each monomer. Reproduced from McKern *et al.* (2006) with permission.

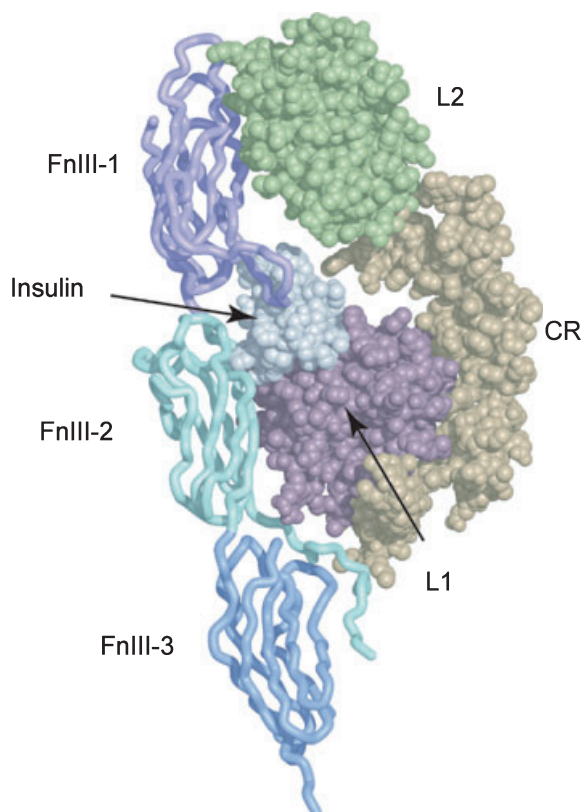
ectodomains (reviewed in Adams *et al.* 2000) and of IGF-1Rs after site-specific mutagenesis (Whittaker *et al.* 2001). Finally, chemical cross-linking (Kurose *et al.* 1994), site-specific mutagenesis (Mynarcik *et al.* 1996, 1997) and the properties of a series of mini and chimeric receptors (Kristensen *et al.* 1999) indicate that the last 16 residues of the  $\alpha$ -chain (referred to as CT) are also important contributors to ligand binding by both IR and IGF-1R.

The IR ectodomain dimer structure (McKern *et al.* 2006) suggests that the CT peptide may be intimately associated with the L1 binding face of the L1-CR-L2 fragment, creating one binding site composed of L1, CR and CT. The binding face of each L1 domain in the dimer had a small segment of electron density lying across its surface which we suggest corresponds to the CT peptide (McKern *et al.* 2006). This CT segment is known to be in close juxtaposition to the L1 domain because the adjacent insulin B-chain residues Phe24 and Phe25 can be cross-linked to the L1 domain and CT region respectively (Xu *et al.* 2004). We have suggested (Ward *et al.* 2007) that there are two possible arrangements for this CT segment in the dimer. One is that it belongs to the same IR monomer as the L1 domain against which it is positioned (as expected in IR half receptors, which can be generated by mild reduction; Treadway *et al.* 1989). The other option is that the CT peptide adjacent to the L1 domain of one monomer is provided by the ID of the second monomer. We hypothesize (Ward *et al.* 2007) that it is this latter arrangement that occurs in the wild-type receptor, given

that pro-receptor cleavage occurs after receptor dimerization (see Adams *et al.* 2000). The location of residue 755 (at the C-terminal end of the ID) in our crystal structure (Fig. 3a) indicates that dimerization of the uncleaved IR would position the CT region of one monomer against the ligand-binding surface of the L1 domain of the other monomer which is precisely where it is required based on the chemical cross-linking data (Xu *et al.* 2004). This has now been experimentally confirmed by complementation analysis using co-expression of differentially tagged IR midi-receptors (Chan *et al.* 2007). The complementation pairs examined were the Arg14Ala + FLAG/Phe714Gly + Myc pair of mutants or the Phe64Ala + FLAG/Phe714Gly + Myc pair of mutants. Whereas the singly-expressed mutants were all devoid of insulin-binding ability, the hybrid mutants bound insulin with wild-type affinity, showing that the two mutations on the two monomers contributed to the same binding site on one side of the dimer and that their un-mutated counterparts on the alternate side of the IR dimer were still capable of providing a normal (site 1) insulin-binding site composed of L1 and CR from one monomer and CT from the other.

The structure of the IR dimer also indicates that the second binding site of the receptor involves one or more of the AB, CC' and EF loops of the first fibronectin domain FnIII-1. This juxtapositioning of the two binding sites is achieved by the IR monomer adopting an inverted 'V' layout relative to the cell membrane (Fig. 3) where one leg is formed by the L1, CR and L2 fragment and the other by the three fibronectin

domains, FnIII-1, FnIII-2 and FnIII-3. As shown in Figure 4, when the classical binding site of insulin is modelled onto the site involving the L1 binding face of IR, the second binding site on insulin (the hexamer face) is exposed to interact with the C-terminal loops of FnIII-1 (McKern *et al.* 2006). Evidence supporting the requirement of these portions of FnIII-1 for high-affinity binding comes from (1) an analysis of IR/IGF-1R chimeras, (2) chemical cross-linking studies and (3) the differential binding properties of a series of truncated IR ectodomains (reviewed in Ward *et al.* 2007). In addition, insulin molecules with mutations on the hexamer face are more severely compromised in their binding to native receptor compared to their binding to soluble ectodomains and display impaired negative co-operativity (see De Meyts 2004), further implicating this interaction in the generation and stabilization of the high-affinity state.



**Figure 4** Ligand binding to the insulin receptor (IR) ectodomain. Schematic diagram of possible binding location of insulin bridging two monomers in the IR homodimer. The L1, CR and L2 domains are from one monomer, whilst the FnIII domains are from the other monomer. Domains are represented and coloured as in Figure 1, whilst insulin is shown in grey atomic sphere representation. The positioning of insulin is based on the model of insulin bound to L1-CR-L2 (Lou *et al.* 2006). Reproduced from McKern *et al.* (2006) with permission.

## Concluding remarks

We conclude that the low-affinity site which controls ligand-binding specificity seen in wild-type receptors and soluble ectodomain constructs includes contributions from the L1 domain binding face of one monomer (referred to for this discussion as monomer A), the CT peptide and possibly an additional peripheral portion of the ID (Wan *et al.* 2004, 2005) from monomer B, and in the case of IGF binding, the CR region from monomer A. Low-affinity binding involves the large hydrophobic patch on the L1 central  $\beta$ -sheet interacting with the major hydrophobic surface of insulin (the classical site). The nature of the interaction between insulin and the CT peptide is not known apart from the fact that it contacts insulin B25 (Kurose *et al.* 1994). It is tempting to speculate that interaction between the CT peptide (which comes from the second monomer, monomer B), and insulin leads to the insulin B-chain C-terminal region (B21–B30) being displaced from its contact with insulin residues A1 and A2 (Derewenda *et al.* 1991, Schäffer 1994, Ludvigsen *et al.* 1998, Xu *et al.* 2004, Wan *et al.* 2005). In the process the CT peptide from monomer B is in turn displaced from its position over the L1 binding face of monomer A allowing the now exposed hydrophobic core of insulin to interact more effectively with the hydrophobic patch on L1.

The data described in this review also suggest that the second insulin-binding site corresponds to one or more loops at the membrane-proximal end of FnIII-1 (McKern *et al.* 2006). The cross-linking model (see Fig. 2) accounts for the properties of a hybrid IGF-1R dimer comprising a normal monomer and a monomer with a mutation (in site 1) that abolishes binding (Chakravarty *et al.* 2005). This hybrid receptor showed wild-type binding as it could still create one normal, high-affinity binding site with site 1 from the wild-type monomer and the non-mutated site 2 of its partner. However, it could not exhibit negative co-operativity (Chakravarty *et al.* 2005) because the alternate combination of the mutated (defective) site 1 and wild-type site 2 is unable to bind ligand and thus is unable to form the alternate high affinity cross-link. The model also explains why IR-A/IGF-1R and IR-B/IGF-1R hybrids all behave like IGF-1R (Slaaby *et al.* 2006). Analysis of IR/IGF-1R chimeras showed that insulin, but not IGF-1, binding required both sites 1 and 2' to be donated by the IR (Schumacher *et al.* 1993).

Our 3.8 Å structure of the IR ectodomain (McKern *et al.* 2006) is significantly different to and cannot be reconciled with a previous low-resolution ( $\sim 20$  Å) model (Ottensmeyer *et al.* 2000) of the IR reconstructed from dark-field scanning transmission electron microscopy (STEM) images, which suggested that in the high-affinity state insulin makes contact with the L1 and L2 modules of one IR monomer and the L1 and CR

modules of the other. We note that doubt has been raised about the STEM model by others (De Meyts & Whittaker 2002), whilst our crystal structure of the Fab-complexed ectodomain is in complete agreement with our negative-stain electron microscopy studies of the particle (Tulloch *et al.* 1999, McKern *et al.* 2006).

### Conflicts of interest

The authors have no conflict of interest.

We thank the Australian Synchrotron Research program, the BioCARS sector at the Advanced Photon Source (Chicago, IL) and the Advanced Photon Source and the Photon Factory (Japan) for access to synchrotron radiation. Additional financial support was provided by the Generic Technology component of the Australian Industry Research and Development Act and from Biota Diabetes Research Pty Ltd.

### References

- Adams, T.E., Epa, V.C., Garrett, T.P.J. & Ward, C.W. 2000. Structure and function of the type 1 insulin-like growth factor receptor. *Cell Mol Life Sci* 57, 1050–1093.
- Bajaj, M., Waterfield, M.D., Schlessinger, J., Taylor, W.R. & Blundell, T. 1987. On the tertiary structure of the extracellular domains of the epidermal growth factor and insulin receptors. *Biochim Biophys Acta* 916, 220–226.
- Campbell, I.D. & Spitzfaden, C. 1994. Building proteins with fibronectin type III modules. *Structure* 2, 333–337.
- Chakravarty, A., Hinrichsen, J., Whittaker, L. & Whittaker, J. 2005. Rescue of ligand binding of a mutant IGF-I receptor by complementation. *Biochem Biophys Res Commun* 331, 74–77.
- Chan, S.J., Nakagawa, S. & Steiner, D.F. 2007. Complementation analysis demonstrates that insulin crosslinks both alpha subunits in a truncated insulin receptor dimer. *J Biol Chem* 282, 13754–13758.
- Cuatrecasas, P. 1971. Insulin–receptor interactions in adipose tissue cells: direct measurement and properties. *Proc Natl Acad Sci USA* 68, 1264–1268.
- De Meyts, P. 2004. Insulin and its receptor: structure, function and evolution. *Bioessays* 26, 1351–1362.
- De Meyts, P. & Whittaker, J. 2002. Structural biology of insulin and IGF1 receptors: implications for drug design. *Nat Rev Drug Discov* 1, 769–783.
- Derewenda, U., Derewenda, Z., Dodson, E.J., Dodson, G.G. & Bing, X. 1991. X-ray analysis of the single chain B29-A1 peptide-linked insulin molecule. A completely inactive analogue. *J Mol Biol* 220, 425–433.
- Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J.H., Masiarz, F., Kan, Y.W., Goldfine, I.D., Roth, R.A. & Rutter, W.J. 1985. The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 40, 747–758.
- Flörke, R.R., Schnaith, K., Passlack, W., Wichert, M., Kuehn, L., Fabry, M., Federwisch, M. & Reinauer, H. 2001. Hormone-triggered conformational changes within the insulin-receptor ectodomain: requirement for transmembrane anchors. *Biochem J* 360, 189–198.
- Garrett, T.P.J., McKern, N.M., Lou, M.Z., Frenkel, M.J., Bentley, J.D., Lovrecz, G.O., Elleman, T.C., Cosgrove, L.J. & Ward, C.W. 1998. Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. *Nature* 394, 395–399.
- Hedo, J.A., Kasuga, M., Van Obberghen, E., Roth, J. & Kahn, C.R. 1981. Direct demonstration of glycosylation of insulin receptor subunits by biosynthetic and external labeling: evidence for heterogeneity. *Proc Natl Acad Sci USA* 78, 4791–4795.
- Jacobs, S., Kull, F.C., Jr, Earp, H.S., Svoboda, M.E., Van Wyk, J.J. & Cuatrecasas, P. 1983. Somatomedin-C stimulates the phosphorylation of the beta-subunit of its own receptor. *J Biol Chem* 258, 9581–9584.
- Kasuga, M., Karlsson, F.A. & Kahn, C.R. 1982. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. *Science* 215, 185–187.
- Kristensen, C., Wiberg, F.C. & Andersen, A.S. 1999. Specificity of insulin and insulin-like growth factor receptors investigated using chimeric minireceptors. Role of carboxy terminal of receptor  $\alpha$ -subunit. *J Biol Chem* 274, 37351–37356.
- Kurose, T., Pashmforoush, M., Yoshimasa, Y., Carroll, R., Schwartz, G.P., Burke, G.T., Katsoyannis, P.G. & Steiner, D.F. 1994. Cross-linking of a B25 azidophenylalanine insulin derivative to the carboxy-terminal region of the alpha-subunit of the insulin receptor. Identification of a new insulin-binding domain in the insulin receptor. *J Biol Chem* 269, 29190–29197.
- Lee, J., Pilch, P.F., Shoelson, S.E. & Scarlata, S.F. 1997. Conformational changes of the insulin receptor upon insulin binding and activation as monitored by fluorescence spectroscopy. *Biochemistry* 36, 2701–2708.
- Lou, M., Garrett, T.P., McKern, N.M., Hoyne, P.A., Epa, V.C., Bentley, J.D., Lovrecz, G.O., Cosgrove, L.J., Frenkel, M.J. & Ward, C.W. 2006. Crystal structure of the first three domains of the human insulin receptor: comparison with the insulin-like growth factor receptor. *Proc Natl Acad Sci USA* 103, 12429–12434.
- Ludvigsen, S., Olsen, H.B. & Kaarsholm, N.C. 1998. A structural switch in a mutant insulin exposes key residues for receptor binding. *J Mol Biol* 279, 1–7.
- Massague, J., Pilch, P. & Czech, M.P. 1981. A unique proteolytic cleavage site on the beta subunit of the insulin receptor. *J Biol Chem* 256, 3182–3190.
- McKern, N.M., Lawrence, M.C., Streltsov, V.A., Lou, M.Z., Adams, T.E., Lovrecz, G.O., Elleman, T.C., Richards, K.M., Bentley, J.D., Pilling, P.A., *et al.* 2006. Structure of the insulin receptor ectodomain homodimer reveals an unexpected folded-over arrangement of the N- and C-terminal halves. *Nature* 443, 218–221.
- Mynarcik, D.C., Yu, G.Q. & Whittaker, J. 1996. Alanine-scanning mutagenesis of a C-terminal ligand binding domain of the insulin receptor alpha subunit. *J Biol Chem* 271, 2439–2444.
- Mynarcik, D.C., Williams, P.F., Schaffer, L., Yu, G.Q. & Whittaker, J. 1997. Analog binding properties of insulin receptor mutants – identification of amino acids interacting with the COOH terminus of the B-chain of the insulin molecule. *J Biol Chem* 272, 2077–2081.

- Nakagawa, S.H., Zhao, M., Hua, Q.X., Hu, S.Q., Wan, Z.L., Jia, W. & Weiss, M.A. 2005. Chiral mutagenesis of insulin. Foldability and function are inversely regulated by a stereospecific switch in the B chain. *Biochemistry* **44**, 4984–4999.
- Nef, S., Verma-Kurvari, S., Merenmies, J., Vassalli, J.D., Efstratiadis, A., Accili, D. & Parada, L.F. 2003. Testis determination requires insulin receptor family function in mice. *Nature* **426**, 291–295.
- O'Bryan, J.P., Frye, R.A., Cogswell, P.C., Neubauer, A., Kitch, B., Prokop, C., Espinosa III, R., Le Beau, M.M., Earp, H.S. & Liu, E.T. 1991. *axl*, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol Cell Biol* **11**, 5016–5031.
- Ottensmeyer, F.P., Beniac, D.R., Luo, R.Z. & Yip, C.C. 2000. Mechanism of transmembrane signaling: insulin binding and the insulin receptor. *Biochemistry* **39**, 12103–12112.
- Rouard, M., Bass, J., Grigorescu, F., Garrett, T.P., Ward, C.W., Lipkind, G., Jaffole, C., Steiner, D.F. & Bell, G.I. 1999. Congenital insulin resistance associated with a conformational alteration in a conserved  $\beta$ -sheet in the insulin receptor L1 domain. *J Biol Chem* **274**, 18487–18491.
- Rubin, J.B., Shia, M.A. & Pilch, P.F. 1983. Stimulation of tyrosine-specific phosphorylation in vitro by insulin-like growth factor I. *Nature* **305**, 438–440.
- Schäffer, L. 1994. A model for insulin binding to the insulin receptor. *Eur J Biochem* **221**, 1127–1132.
- Schumacher, R., Soos, M.A., Schlessinger, J., Brandenburg, D., Siddle, K. & Ullrich, A. 1993. Signaling-competent receptor chimeras allow mapping of major insulin receptor binding domain determinants. *J Biol Chem* **268**, 1087–1094.
- Shier, P. & Watt, V.M. 1989. Primary structure of a putative receptor for a ligand of the insulin family. *J Biol Chem* **264**, 14605–14608.
- Siegel, T.W., Ganguly, S., Jacobs, S., Rosen, O.M. & Rubin, C.S. 1981. Purification and properties of the human placental insulin receptor. *J Biol Chem* **256**, 9266–9273.
- Slaaby, R., Schäffer, L., Lautrup-Larsen, I., Andersen, A.S., Shaw, A.C., Mathiasen, I.S. & Brandt, J. 2006. Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. *J Biol Chem* **281**, 25869–25874.
- Sparrow, L.G., McKern, N.M., Gorman, J.J., Strike, P.M., Robinson, C.P., Bentley, J.D. & Ward, C.W. 1997. The disulfide bonds in the C-terminal domains of the human insulin receptor ectodomain. *J Biol Chem* **272**, 29460–29467.
- Sparrow, L.G., Gorman, J.J., Strike, P.M., Robinson, C.P., McKern, N.M., Epa, V.C. & Ward, C.W. 2007a. The location and characterisation of the O-linked glycans of the human insulin receptor. *Proteins* **66**, 261–265.
- Sparrow, L.G., Lawrence, M.C., Gorman, J.J., Strike, P.M., Robinson, C.P., McKern, N.M. & Ward, C.W. 2007b. N-linked glycans of the human insulin receptor and their distribution over the crystal structure. *Proteins*. 2007 Epub Oct 23, 2007.
- Taylor, S.I., Wertheimer, E., Accili, D., Cama, A., Hone, J., Roach, P., Quon, M. J., Suzuki, Y., Levy-Toledo, R., Taouis, M., de la Luz Sierra, M., Barbetti, F. & Gorden, P. 1994. Mutations in the insulin receptor gene: update. *Endocr Rev* **2**, 58–65.
- Treadway, J.L., Morrison, B.D., Goldfine, I.D. & Pessin, J.E. 1989. Assembly of insulin/insulin-like growth factor-1 hybrid receptors *in vitro*. *J Biol Chem* **264**, 21450–21453.
- Tulloch, P.A., Lawrence, L.J., McKern, N.M., Robinson, C.P., Bentley, J.D., Cosgrove, L., Ivancic, N., Lovrecz, G.O., Siddle, K. & Ward, C.W. 1999. Single-molecule imaging of human insulin receptor ectodomain and its Fab complexes. *J Struct Biol* **125**, 11–18.
- Ullrich, A., Bell, J.R., Chen, E.Y., Herrera, R., Petruzzelli, L.M., Dull, T.J., Gray, A., Coussens, L., Liao, Y.C., Tsubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M. & Ramachandran, J. 1985. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* **313**, 756–761.
- Ullrich, A., Gray, A., Tam, A.W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J. & Fujita-Yamaguchi, Y. 1986. Insulin-like growth factor 1 receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J* **5**, 2503–2512.
- Wan, Z., Xu, B., Huang, K., Chu, Y.C., Li, B., Nakagawa, S.H., Qu, Y., Hu, S.Q., Katsoyannis, P.G. & Weiss, M.A. 2004. Enhancing the activity of insulin at the receptor interface: crystal structure and photo-cross-linking of A8 analogues. *Biochemistry* **43**, 16119–16133.
- Wan, Z.L., Huang, K., Xu, B., Hu, S.Q., Wang, S., Chu, Y.C., Katsoyannis, P.G., & Weiss, M.A. 2005. Diabetes-associated mutations in human insulin: crystal structure and photo-cross-linking studies of A-chain variant insulin Wakayama. *Biochemistry* **44**, 5000–5016.
- Ward, C.W. & Garrett, T.P.J. 2001. The relationship between the L1 and L2 domains of the insulin and epidermal growth factor receptors to leucine-rich repeat modules. *BMC Bioinformatics* **2**, 4.
- Ward, C.W., Hoyne, P.A. & Flegg, R.H. 1995. Insulin and epidermal growth factor receptors contain the cysteine repeat motif found in the tumor necrosis factor receptor. *Proteins* **22**, 141–153.
- Ward, C.W., Lawrence, M.C., Streltsov, V.A., Adams, T.E. & McKern, N.M. 2007. The insulin and EGF receptor structures: new insights into ligand-induced receptor activation. *Trends Biochem Sci* **32**, 129–137.
- Whittaker, J., Groth, A.V., Mynarcik, D.C., Pluzek, L., Gadsboll, V.L. & Whittaker, L.J. 2001. Alanine scanning mutagenesis of a type 1 insulin-like growth factor receptor ligand binding site. *J Biol Chem* **276**, 43980–43986.
- Williams, P.F., Mynarcik, D.C., Yu, G.Q. & Whittaker, J. 1995. Mapping of an NH<sub>2</sub>-terminal ligand binding site of the insulin receptor by alanine scanning mutagenesis. *J Biol Chem* **270**, 3012–3016.
- Xu, B., Hu, S.Q., Chu, Y.C., Huang, K., Nakagawa, S.H., Whittaker, J., Katsoyannis, P.G. & Weiss, M.A. 2004. Diabetes-associated mutations in insulin identify invariant receptor contacts. *Biochemistry* **43**, 8356–8372.