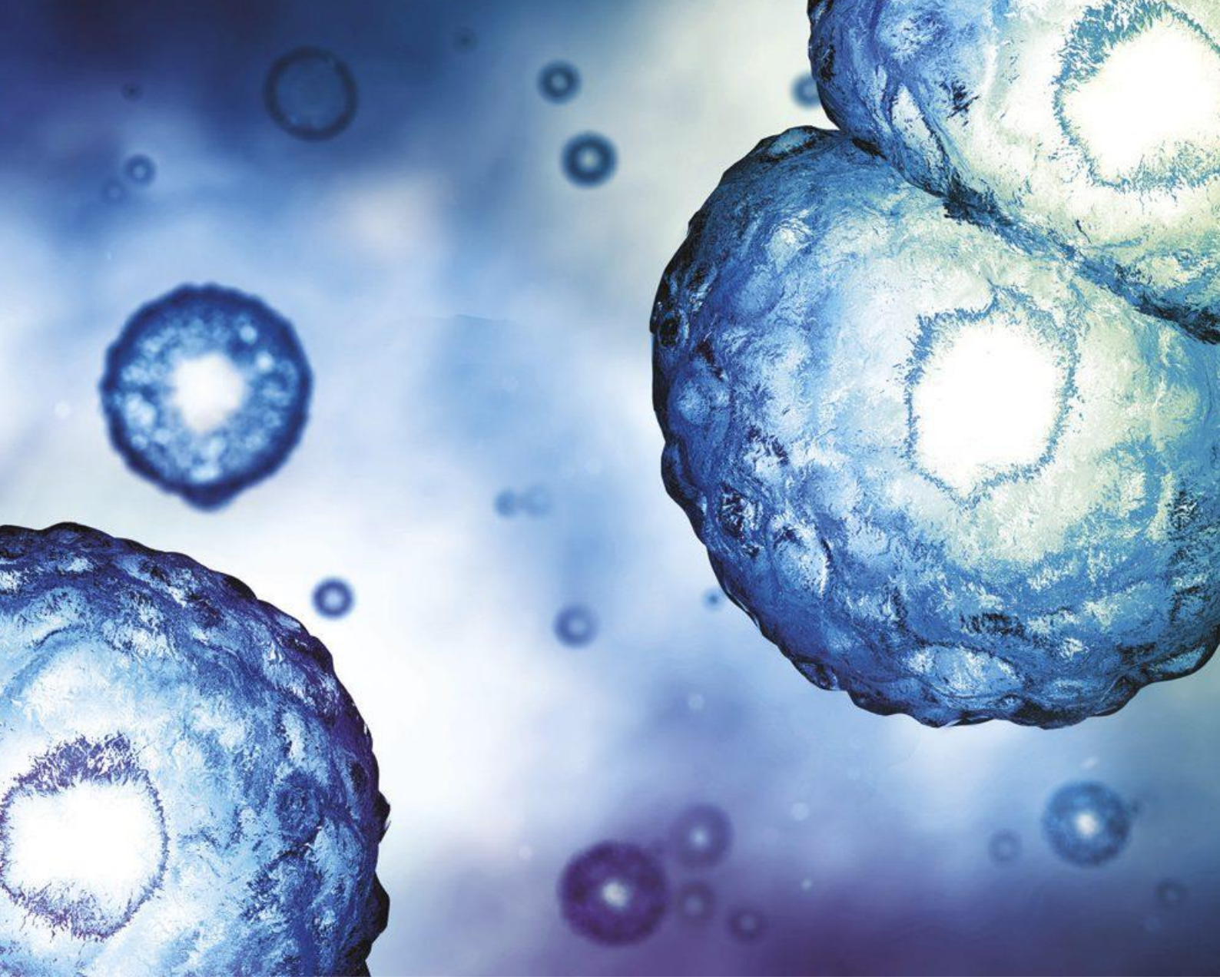

How insulin and IGF-1 bind to their receptors

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The available crystal structure of the insulin receptor extracellular domain (1) and that of the L1-CR-L2 N-terminal domain of the IGF-I receptor (2) unfortunately do not contain the bound ligand.

However, a wealth of information on the mechanism of ligand binding to the insulin and IGF-I receptors has been gathered from a variety of biochemical approaches (for review see 3-6), including studies of the kinetics of radioligand binding (8), photoaffinity crosslinking of ligands to the receptors (9-12), and alanine (or other amino acids) scanning mutagenesis of both the ligands (4, 12-14) and receptors (15-18).

From these studies plausible models have emerged (8, 19). The De Meyts 1994 bivalent crosslinking binding model (8, Fig. 1) was supported by the recent crystal structure of the insulin receptor (1) and by mathematical modelling (20,21), explaining the complex ligand binding kinetics of the insulin and IGF-I receptors which exhibit negative cooperativity, whereby the binding of a second ligand molecule weakens the binding of the first bound molecule by accelerating its dissociation from the receptor.

Figure 1: The insulin receptor symmetrical bivalent crosslinking model. Both a-subunits amino-terminal pairs of binding sites (1 and 2) are represented in asymmetrical anti-parallel arrangement. Insulin has two binding sites, 1 and 2, that each bind to one of the receptor binding sites 1 and 2. The first insulin molecule (shown as a green cone) binds with high affinity by crosslinking receptor sites 1 and 2. On partial dissociation of the first bound molecule, a second insulin molecule can crosslink the remaining sites 1 and 2, causing complete dissociation of the first bound insulin /accelerated dissociation, which is a hallmark of negative cooperativity). At high insulin concentrations, monovalent binding of two extra insulin molecules saturates the leftover sites 1 and 2 and stabilizes the binding of the pre-bound insulin in the first crosslink, explaining the bell-shaped curve for negative cooperativity. From ref. 3, see references 8 and 20 for more complete explanation.

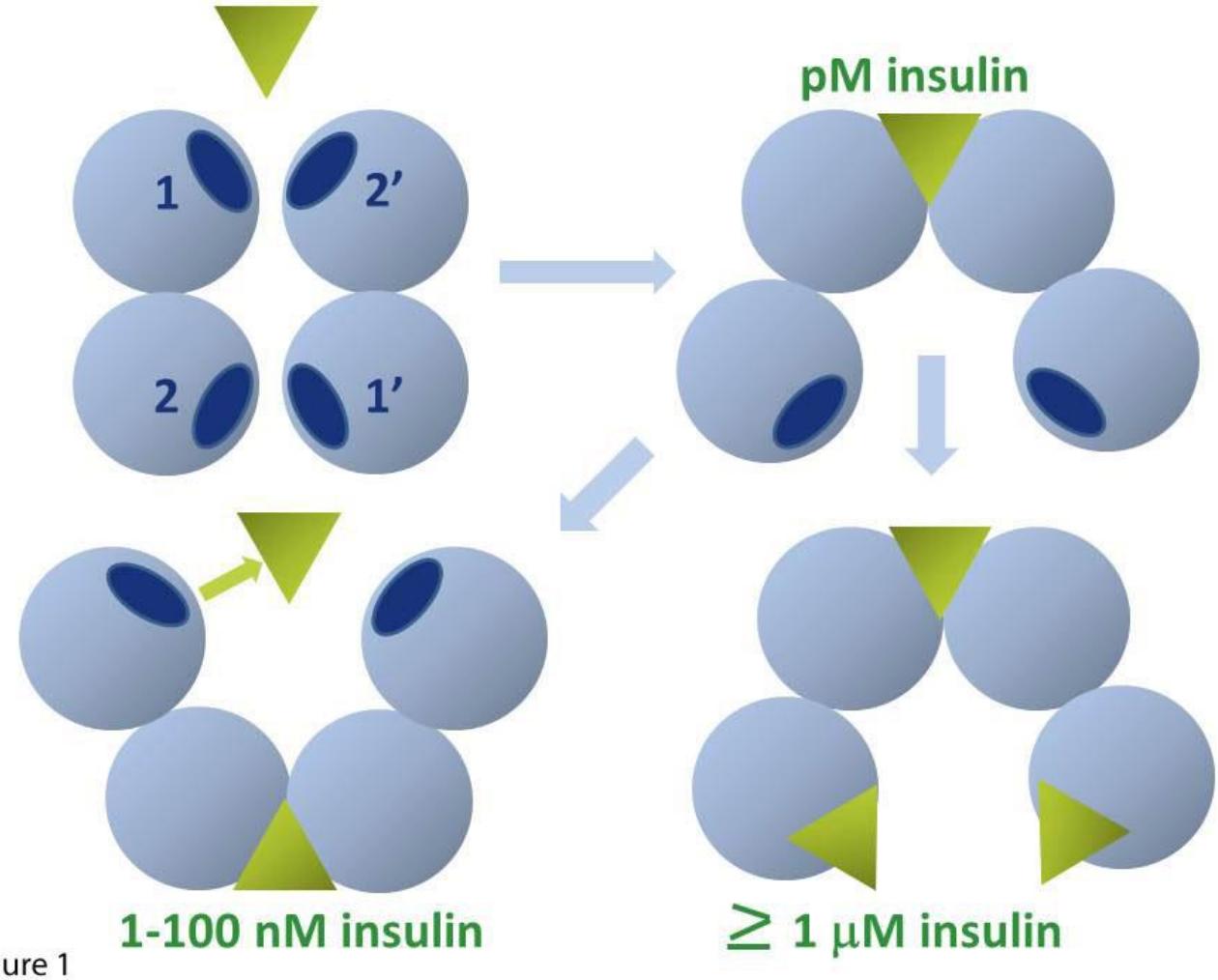

Figure 1

According to the current model for insulin binding to its receptor, insulin has two binding surfaces (site 1 or "classical binding surface") and site 2 (or "novel binding surface") that bind each to two distinct sites (site 1 and site 2', or site 1' and site 2) on the two a-subunits of the insulin receptor (Fig. 2 and 3), and thereby crosslink the two receptor halves into a high affinity, slowly dissociating complex. The alternative crosslinking at sites 1 and 2', and sites 1' and 2, results in the observed negative cooperativity.

Figure 2: Insulin binding site 1 and 2. The two insulin sites that bind to the insulin receptor have been mapped by alanine scanning mutagenesis (refs. 12 and 4). Site 1 (also known as "classical binding surface") is shown in light green and comprises residues Gly A1, Ile A2, Val A3, Gln A5, Tyr A19, Asn A21, Val B12, Tyr B16, Gly B23, Phe B24, Phe B25 and Tyr B26. It overlaps with the insulin surface involved in dimerization (see Fig. 5). Site

2 is shown in blue and comprises Ser A12, Leu A13, Glu A17, His B10, Glu B13 and Leu B17. It overlaps with the insulin surface involved in hexamerization (see Fig. 5). Backbone is shown in dark green. PDB file 9INS.

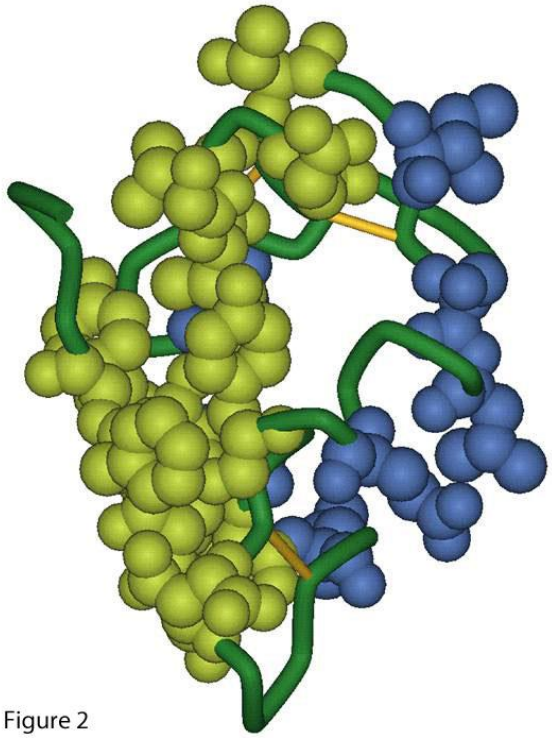

Figure 2

Figure 3: Insulin binding sites 1 and 2. See legend of Figure 2 for description.

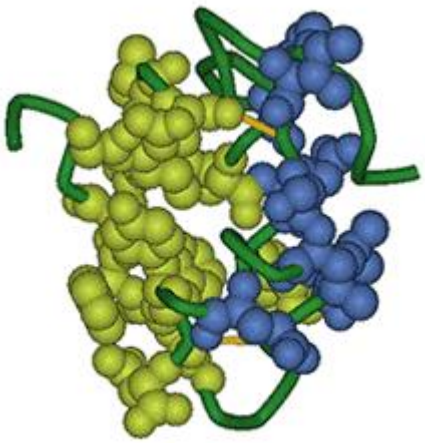

Figure 3

Site 1 of insulin (Fig. 2) was already mapped in the 70's by evaluation of conserved residues in various animal insulins (22,23) and confirmed more recently by alanine scanning mutagenesis (4, 12). It overlaps partially with the insulin surface involved in dimerization (see section on the insulin peptide family). See legend of Fig. 2 for specification of amino acids involved.

Site 2 of insulin (Fig. 2) was mapped more recently by alanine scanning mutagenesis (4). It overlaps partially with the insulin surface involved in hexamerization (see section on the insulin peptide family). See legend of Fig. 2 for specification of amino acids involved.

Thus, it is not surprising that a small globular protein like insulin uses partially the same domains for receptor binding and for self-aggregation.

IGF-I uses very similar surfaces as those on insulin for binding to the IGF-I receptor, as shown by site directed mutagenesis (for review see ref. 14) (see Fig. 4 and 5 for details).

Figure 4: IGF-I binding sites 1 and 2. The two IGF-I binding sites that bind to the IGF-I receptor have been partially mapped by various mutagenesis approaches (see ref. 14). Site 1 is shown in light green and comprises Ala 8, Phe 23, Tyr 24, Tyr 31, Val 44, Met 59, Tyr 60 and Ala 62. In addition, Arg 36 and Arg 37 which are missing from the crystal structure are depicted by two spheres. Site 2 comprises Glu 9, Asp 12, Phe 16, leu 54 and Glu 58. PDB file 1GZR.

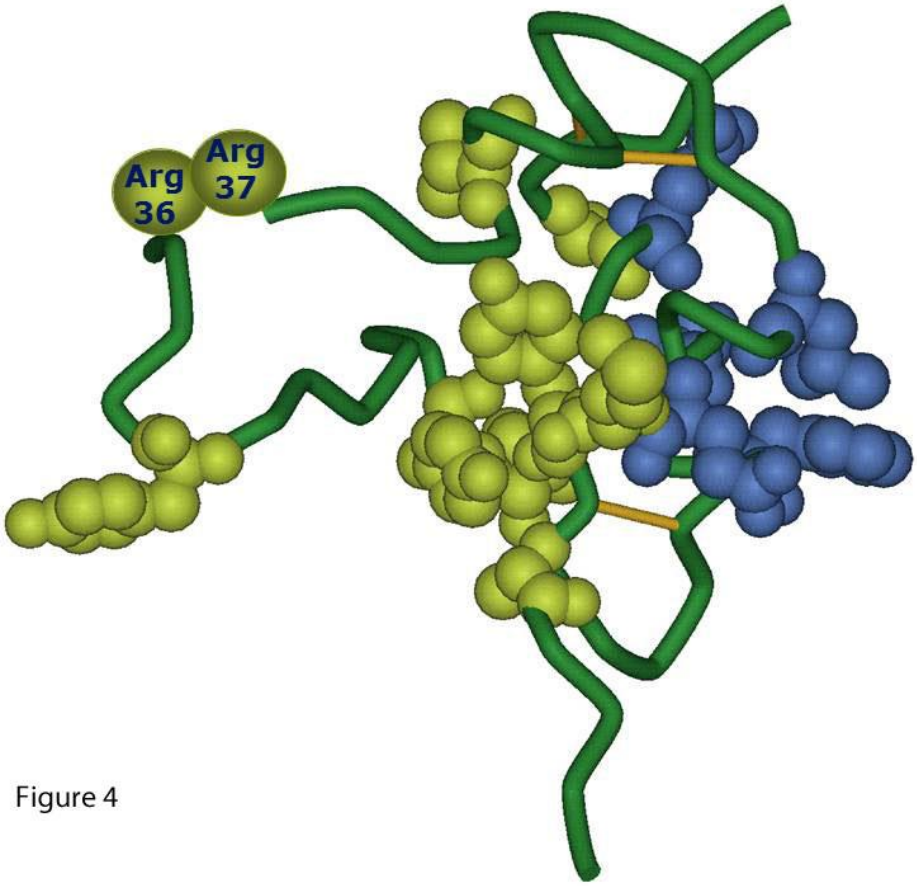

Figure 4

Figure 5: IGF-I binding sites 1 and 2. See legend of Figure 4 for description.

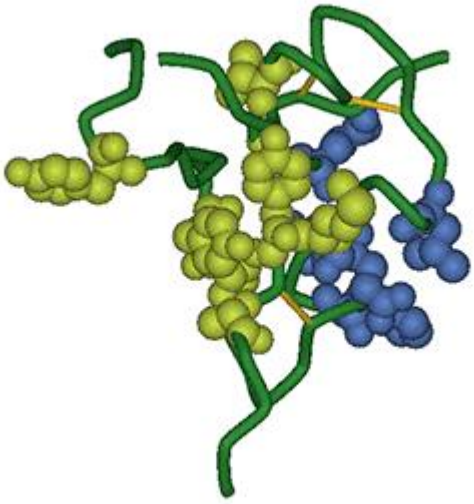

Figure 5

In addition, the permanent C-peptide of IGF-I, absent on insulin, is involved in binding to the IGF-I receptor, binding to residues in the cysteine-rich region of the receptor (3,5). The low affinity of insulin for the IGF-I receptor is due to the lack of this C-peptide domain, while the low affinity of IGF-I for the insulin receptor is due to 4 substitutions in site 1 (24).

The ligand binding sites on the insulin receptor have been mapped by a combination of site-directed mutagenesis, construction of chimeric receptors with the IGF-I receptor, and alanine-scanning mutagenesis (for review see ref. 3 and other refs quoted above).

Site 1 of the insulin receptor is made of a combination of the central b-sheet of the L1 domain and the C-terminal peptide from the insert domain, binding in trans to complete the binding site (25, for review see ref. 6) (Fig. 6).

Figure 6: Insulin receptor with binding sites 1 and 2. The 3-D structure of the insulin receptor extracellular domain (PDB file 2DTG) is shown. One ab half is shown in light blue, the other one in green. Binding sites 1 and 2 have been mapped by alanine scanning (refs. 15, 19, 26). The insulin receptor backbone of one ab half is shown in light blue, the other in dark green. Sites 1 and 1' are shown as CPK spheres in light green, sites 2 and 2´ in blue.

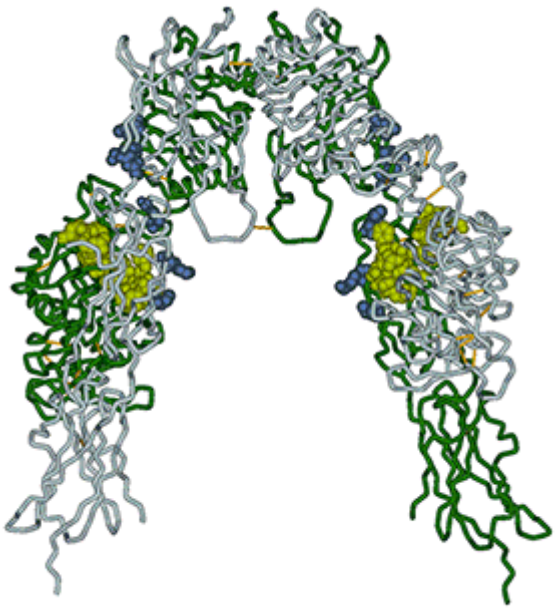

Figure 6

In site 1 of the IGF-IR, the homologous domain is extended to a part of the cysteine-rich domain that binds the C-domain of IGF-I (18).

Site 2 of the insulin receptor is less well defined but comprises the loops at the junction of FnIII-1 and-2 (for review see ref. 6) (Fig. 6).

The see-saw mechanism whereby insulin alternatively crosslinks sites 1 and 2' and sites 1' and 2 ("harmonic oscillator") is shown in detail and explained in Fig. 6.

Figure 7: How insulin binds to its receptor. The domains of the insulin receptor that contain binding sites 1 (1') and 2 (2') have been "extracted" from the 3D structure. The backbones of the L1 domain of one ab half (top right) and of the FnIII-1 and N-terminal part of the FnIII-2 domains (bottom left) are shown in dark green. The backbone of the corresponding parts of the second ab half are shown in light blue. The binding sites 1 and 1´are shown as CPK spheres in light green, sites 2 and 2' in blue. Site 1 is made of residues Asp 12, Arg 14, Asn 15, Gln 34, Leu 36, Leu 37, Phe 64, Leu 87, Phe 89, Asn 90, Tyr 91, Glu 97, Glu 120 and Lys 121. Site 2 is made of residues Lys 484, Leu 552, Asp 591, Ile 602, Lys 616, Asp 620 and Pro 621.

The backbone of insulin is shown in dark green, site 1 as CPK spheres in light green, site 2 in blue.

The Cys 524 disulphide bond that links the two receptor halves is shown in yellow. Lys 460 on each receptor half (which plays a role in negative cooperativity, see ref. 6) is shown as CPK spheres in dark blue.

See insulin move into its binding sites.

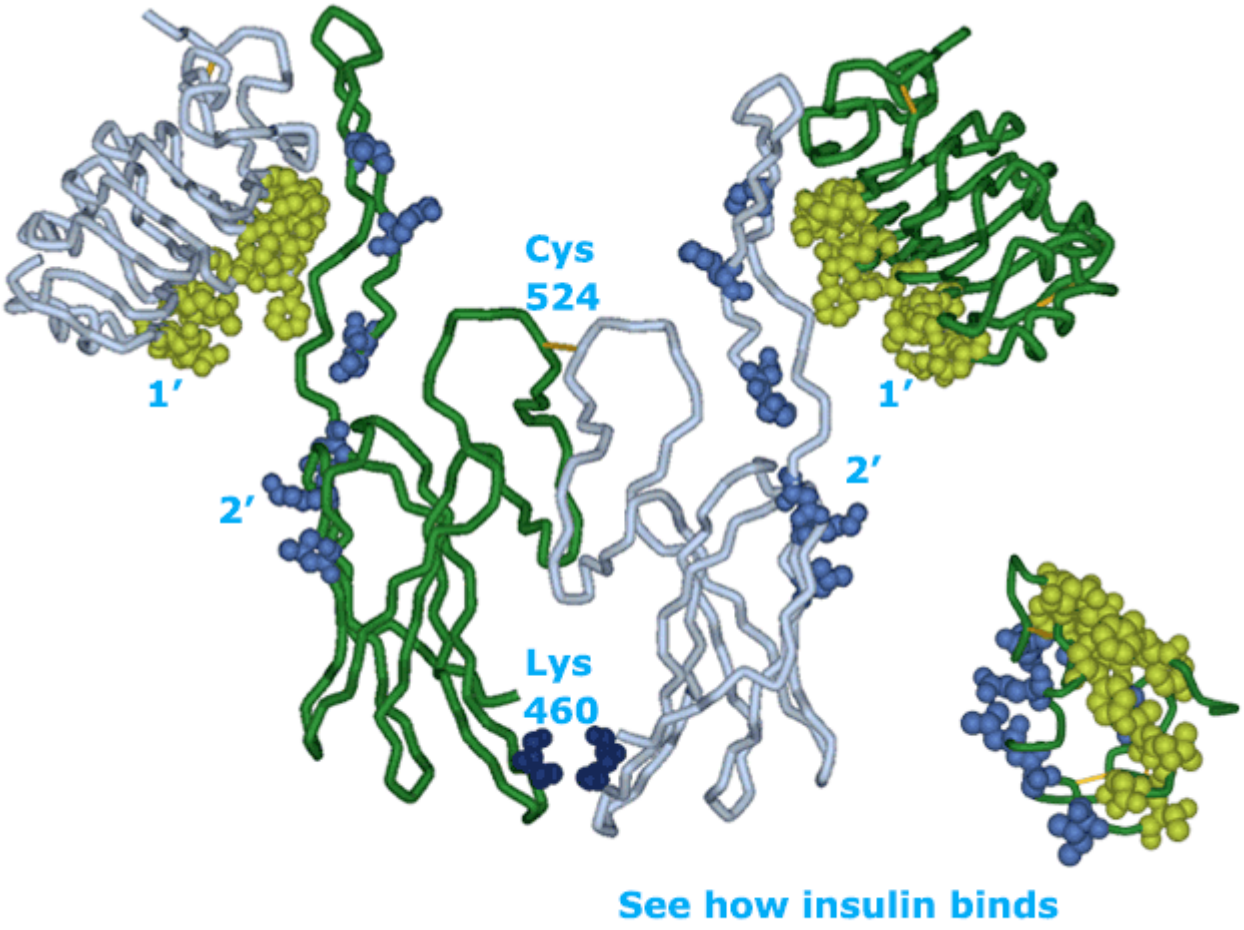

Figure 7

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