P 332 Crystal structure of the complex between an insulin-like peptide (DILP5) and an ILP binding protein (IMP-L2) from *Drosophila Melanogaster*

Nikolaj Kulahin[1], Christopher J. Watson[2], Waseem Sajid[1], Gerd Schluckebier[3], Asser Sloth Andersen[3], Andrzej M. Brzozowski[2] and Pierre De Meyts[1].

[1]Novo Nordisk A/S, Gentofte, Denmark; [2]University of York, York, UK; [3]Novo Nordisk A/S, Måløv, Denmark.

The insulin signalling system including the insulin receptor tyrosine kinase (IRTK) is evolutionarily ancient and appears in the first multicellular organisms (Cnidarians). The *Drosophila Melanogaster* genome contains seven genes for insulin-like peptides (ILPs) that are expressed in neurosecretory cells in a highly tissue- and stage-specific pattern, DILP1-7. There is however only one IRTK (dIR). This system is important in the regulation of metabolism, growth, reproduction and lifespan.

We reported in 2011 the crystal structure of Drosophila insulin-like peptide 5 (DILP5) (expressed in *Saccharomyces cerevisiae*) at 1.85Å resolution, as well as its biological and receptor binding properties. DILP5 shares the canonical fold of the insulin peptide family and form dimers that differ from the mammalian and hagfish insulin dimers.

Drosophila Melanogaster also has a circulating ILP binding protein, called imaginal morphogenesis protein-Late 2 (IMP-L2), with no equivalent in mammals. It was cloned, expressed and purified in 2000 and was shown to bind human insulin and insulin-like growth factors. We showed in 2011 that it also binds DILP5. The knockout of IMP-L2 is embryonic lethal. We now report the solution of the crystal structure of IMP-L2 in the free form as well as bound to DILP5. Recombinant IMP-L2 was expressed in a Baculovirus expression system, purified and crystallized with selenium as heavy atom.

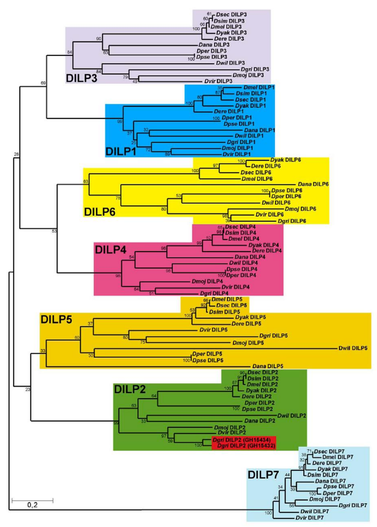
IMP-L2 showed as predicted from the sequence a bilobed structure with two IgG beta sheet modules (3 strands for IgGI and 4 for IgGII), folded together into a "baseball glove". The structure is very different from the known partial structure of IGFBP5 which is mostly alpha-helical.

The complex of IMPL-2 with DILP5 showed a multimeric structure with two DILP5 molecules bound into the grooves between the beta sheets of two distinct IMP-L2s in a symmetrical tetrameric IMPL-2. The structural differences between the free and bound forms will be discussed.

Biological functions of IMP-L2

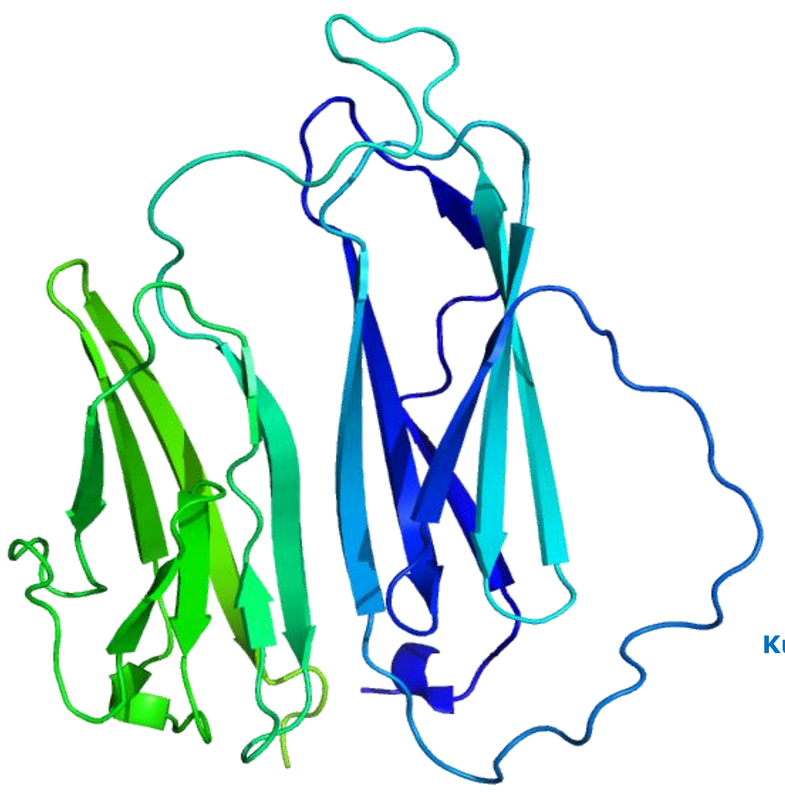
- **Induced by 20-hydroxyecdysone**
- **Secreted immunoglobulin family member**
- **Expressed during imaginal disc and imaginal histoblast morphogenesis**
- **Involved in the formation of the continuous adult epidermis**
- **Gene deletion is embryonic lethal**
- **Overexpression increases lifespan**
- **IMP-L2 is an insulin/IGF binding protein (7)**
- **Homologue of IGF-BP7 tumour suppressor?**
- **Conteracts insulin signalling and essential for starvation resistance**

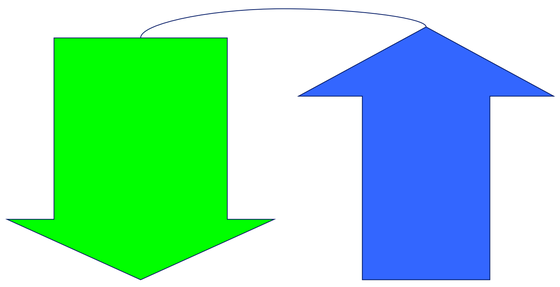
Phylogenetic analysis of 12 drosophila genomes



From ref. 3

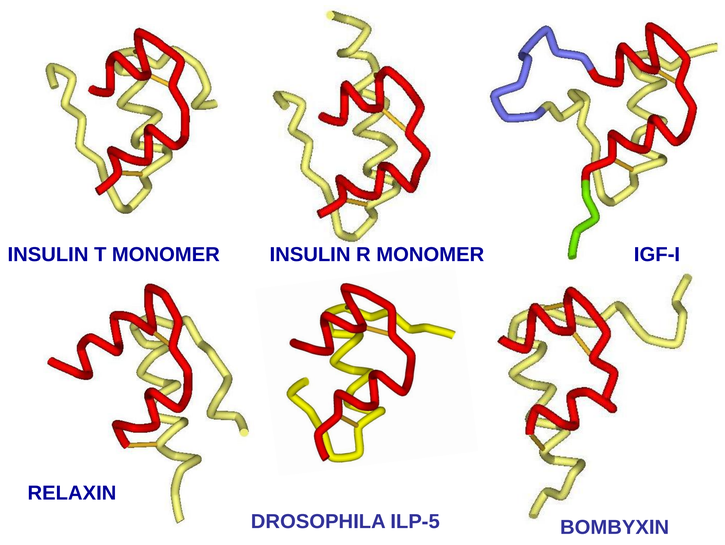
Crystal structure of unbound IMP-L2



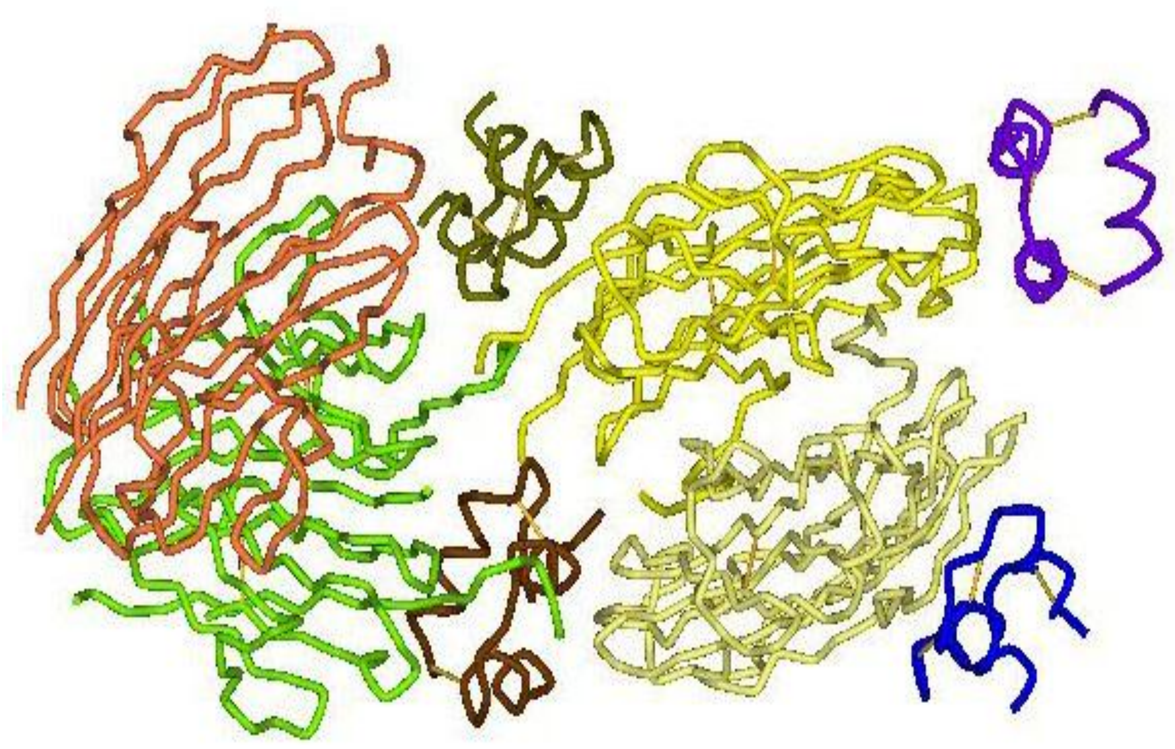


Kulahin N, unpublished

Structure of DILP5 compared to other ILPs

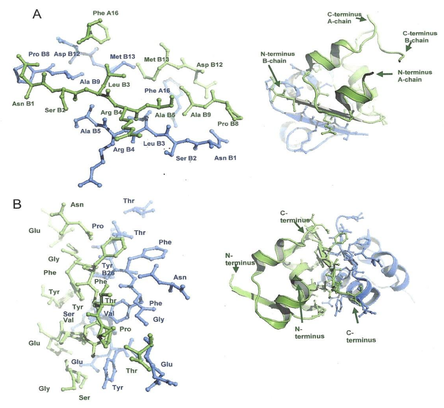


Crystal structure of DILP5-IMP-L2 complex



Structure of ILP dimers

From ref. 5

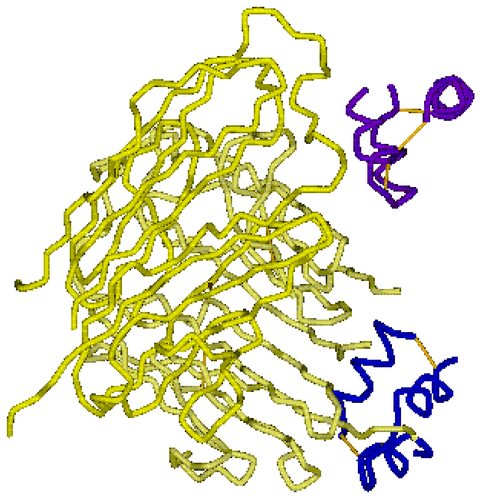


DILP5 dimer

Human insulin dimer

Human relaxin dimer

Two distinct binding modes of DILP5 binding to IMP-L2



Sequence comparison of insect binding proteins

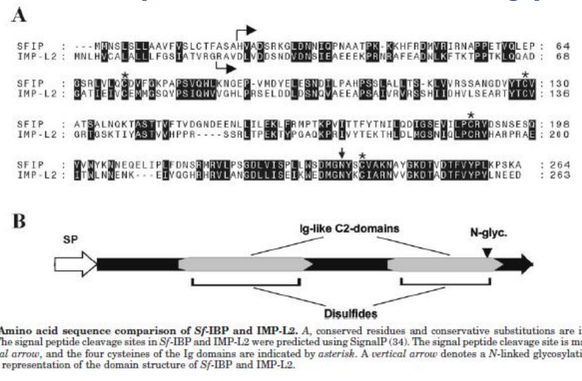


FIG. 4. **Amino acid sequence comparison of *Sf*-IBP and IMP-L2.** *A*, conserved residues and conservative substitutions are in *reversed contrast*. The signal peptide cleavage sites in *Sf*-IBP and IMP-L2 were predicted using SignalP (34). The signal peptide cleavage site is marked with a *horizontal arrow*, and the four cysteines of the Ig domains are indicated by *asterisk*. A *vertical arrow* denotes a *N*-linked glycosylation site. *B*, schematic representation of the domain structure of *Sf*-IBP and IMP-L2.

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