

Crystal structure of LGR ligand $\alpha 2/\beta 5$ from *Caenorhabditis elegans* with implications for the evolution of glycoprotein hormones

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A family of leucine-rich-repeat-containing G-protein-coupled receptors (LGRs) mediate diverse physiological responses when complexed with their cognate ligands. LGRs are present in all metazoan animals. In humans, the LGR ligands include glycoprotein hormones (GPHs) chorionic gonadotropin (hCG), luteinizing hormone (hLH), follicle stimulating hormone (hFSH) and thyroid stimulating hormone (hTSH). These hormones are $\alpha\beta$ heterodimers of cystine-knot protein chains. LGRs and their ligand chains have co-evolved. Ancestral hormone homologs, present in both bilaterian animals and chordates, are identified as $\alpha 2\beta 5$. We have used single-wavelength anomalous diffraction (SAD) and molecular replacement to determine structures of the $\alpha 2\beta 5$ hormone from *Caenorhabditis elegans* (*Cea2\beta 5*). *Cea2\beta 5* is unglycosylated, as are many other $\alpha 2\beta 5$ hormones. Both *Hsa2\beta 5*, the human homolog of *Cea2\beta 5*, and hTSH activate the same receptor (hTSHR). Despite having little sequence similarity to vertebrate glycoprotein hormones, apart from the cysteine patterns from core disulfide bridges, *Cea2\beta 5* is generally similar in structure to these counterparts; however, its $\alpha 2$ and $\beta 5$ subunits are more symmetric as compared with α and β of hCG and hFSH. This quasi-symmetry suggests a hypothetical homodimeric antecedent of the $\alpha 2\beta 5$ and $\alpha\beta$ heterodimers. Known structures together with AlphaFold models from the sequences for other LGR ligands provide representatives for the molecular evolution of LGR ligands from early metazoans through the present-day GPHs. The experimental *Cea2\beta 5* structure validates its AlphaFold model, and thus also that for *Hsa2\beta 5*; and interfacial characteristics in a model for the *Hsa2\beta 5*:hTSHR complex are similar to those found in an experimental hTSH:hTSHR structure.

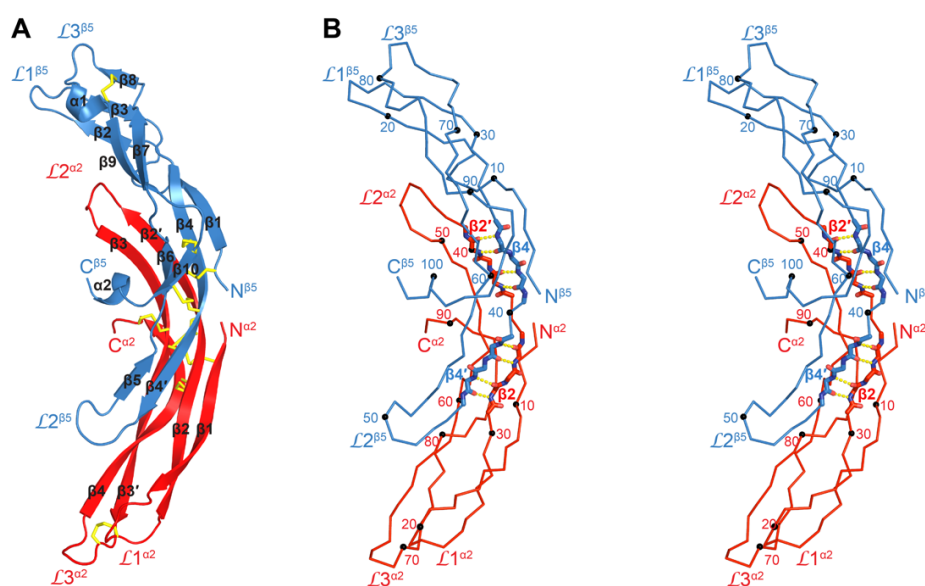


Figure 1. Overall structure of *Cea2\beta 5*. (A) Ribbon diagram of *Cea2\beta 5*. (B) Stereo C α trace of *Cea2\beta 5* in the same orientation as (A), emphasizing the heterodimer interface.

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