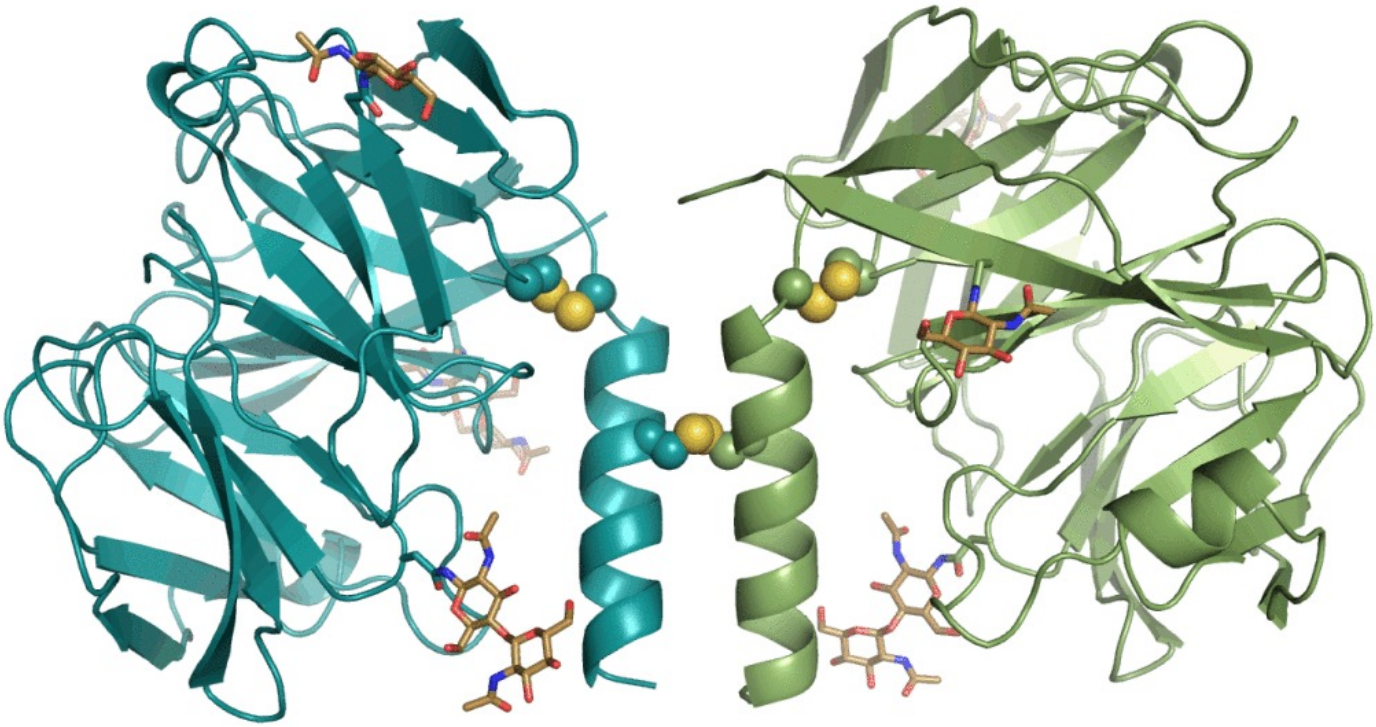
Signaling in the brain studied - a role for receptor clustering?

Olfactomedin-1 is a signaling protein in the brain that plays various roles during early brain development. Brain cells (neurons) secrete the protein to the extracellular matrix, where it is known to be involved in several signaling pathways. How this protein transmits signals to other neurons and how those signals are transferred from the extracellular matrix to their cytoplasm, across their plasma membrane, is not understood.

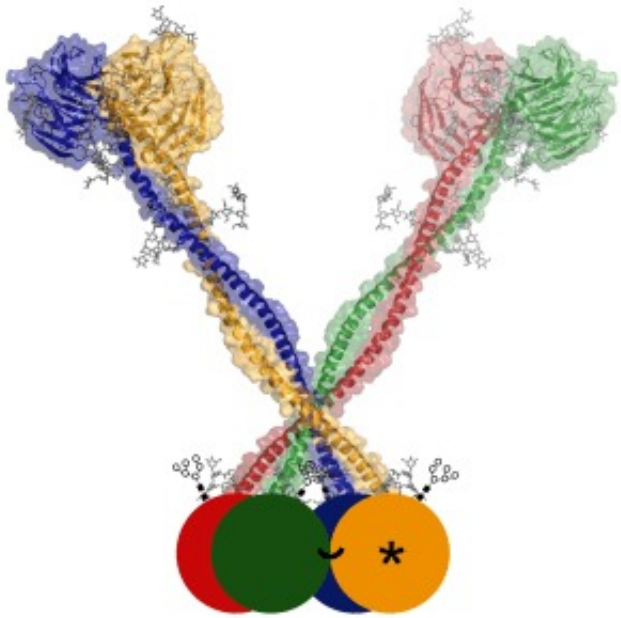


High resolution crystal structure of part of Olfactomedin-1, after limited proteolysis treatment. In the middle, the disulfide bond that links the two copies of the protein (green and teal) together is shown as spheres. The helical region in the middle is known as a coiled coil, that is known to enhance interactions between proteins.

Since the function of proteins is determined by their three-dimensional structure, which is determined by their amino acid sequence (which again is encoded in the DNA of the corresponding gene), we set out to determine this three-dimensional structure.

The typical technique for determining high-resolution protein structures is based on X-ray diffraction from protein crystals. Initial trials did not yield the crystals that are necessary for this technique in case of Olfactomedin-1. Therefore, we used a trick called limited proteolysis, in which we cut up the

protein in smaller chunks, using specific enzymes that cut protein chains (proteases). Since we only use a small amount of these enzymes, the more structured parts should be left intact, whereas floppy and flexible parts that might be hindering crystallization are readily cleaved off. This yielded well-diffracting protein crystals which we could use to determine the structure of the part that crystallized.



Model of the architecture of the full-length Olfactomedin-1 tetramer, based on a combination of techniques, such as X-ray crystallography, electron microscopy and small angle X-ray scattering. The crystallized domains are located at the tips of the "V", whereas tetramerization occurs by domains at the bottom (indicated with an asterix). Each of the four copies is indicated with a different color, and known glycosylations (sugar chains) are shown as sticks.

The structure showed a part of the protein, which appeared as a dimer, i.e. two copies of the same molecule binding to each other. Moreover, the two copies were linked via a strong chemical linkage known as a disulfide bond. This was interesting, as it turned out that the whole molecule (before cutting it up) appeared as a disulfide-linked tetramer, so four copies of the same molecule linked together.

Combining different lower resolution techniques that do not require crystals, we could study how the dimeric part that we observed in the crystals after limited proteolysis, was organized into a tetramer for the full-length molecule. Both electron microscopy and small angle X-ray scattering showed a V-shape with bulky features at the tips of the V. Based on our knowledge of the amino acid sequence, we know that the dimeric part of the crystals is situated at those tips, whereas the base of the V contains domains necessary for the tetramerization.

This unusual architecture, where two pairs of two proteins arrange to form a V-shaped tetramer, suggests that Olfactomedin-1 might signal by simultaneously binding multiple receptors at the same time. This way, receptor clustering would provide a possible mechanism for signal transduction across the membranes of target cells. In follow-up studies, we are indeed trying to prove that this is the case for the signaling pathways that Olfactomedin-1 is known to be involved in.

Publication

Olfactomedin-1 Has a V-shaped Disulfide-linked Tetrameric Structure.
Pronker MF, Bos TG, Sharp TH, Thies-Weesie DM, Janssen BJ
J Biol Chem. 2015 Jun 12