# Laboratory of Protein Synthesis and Expression

## Institute for Protein Research


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Cells receive external stimuli and process the information inside the cell to determine how to respond to the external environment. In the study of signal transduction, the most important issue is to understand the mechanism by which receptors receive information at the cell surface (i.e., outside the cell) and transmit it to the inside of the cell across the cell membrane. To address this question, the group aims to elucidate the function of the "input terminal" part of signal transduction through structural biology approaches using X-ray crystallography and electron microscopy imaging. In particular, the group will conduct "structure-from-function" research on proteins such as receptors and synaptic components working in the brain and nervous system, molecules involved in neuronal cell death and axonal guidance, and signaling molecules involved in development and morphogenesis of organisms.

### Structure Determination of Receptor-Ligand Complexes

The structure of the complex between the extracellular domain of a receptor and its ligand protein contains important information that will lead to not only the elucidation of the signal transduction mechanism but also the development of drugs such as inhibitors. In order to elucidate the interaction sites and their roles in binding, the structure of such complexes will be determined (1) at high resolution using X-ray crystallography or (2) at low resolution using electron microscopy (EM) imaging, but in multiple conformations simultaneously.

i) Signaling system of neuroguidance factors and their receptors We are searching for candidate inhibitors of semaphorins, which are neuroaxonal guidance factors, and their receptor, plexin, by analyzing the complex structure and clarifying the structural biology of their mechanisms of action (Fig. 1).

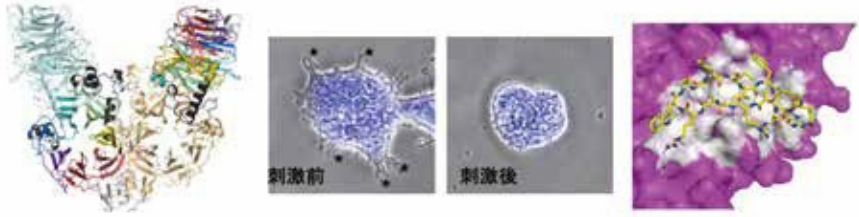


Figure 1: Crystal structure of the semaphorin-plexin complex (left) and Cell morphology before and after semaphorin stimulation (middle), binding sites of pleckin B1 and its inhibitor peptide (right)

ii) Wnt proteins are essential growth factors for stem cell proliferation, and their lipid modifications have made them difficult to purify and analyze. We have determined the three-dimensional structure of the mammalian Wnt protein for the first time in the world, and are elucidating the signaling mechanism based on this structure (Fig. 2).

Figure 2: Predicted structure of the signaling complex (right), combining the crystal structure of Wnt3a (left) and the cryo-EM structure of LRP6 (center).

### Establishment of high-quality recombinant protein production system

Extracellular proteins often require the addition of sugar chains and disulfide bonds to maintain their structure, making it difficult to use simple expression systems in E. coli. The "production" of these difficult recombinant proteins for structural analysis and precise biochemical and physicochemical experiments can be achieved by the following methods.

This will be established through (1) upgrading of animal cell culture systems, (2) development of a new affinity tag system, and (3) improvement and development of expression methods (Fig. 3).

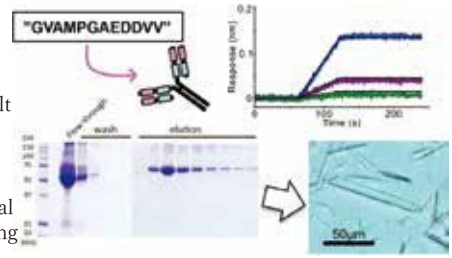


Figure 3: Development of the ultra-high affinity affinity purification system "PA Tag

### Protein engineering based on structural information

Structural information is not only useful for elucidating the mechanism of protein function, but is also powerful for modifying or creating new functions. We are conducting research to give desired functions to proteins and to create useful molecules that do not exist in nature (Fig. 4).

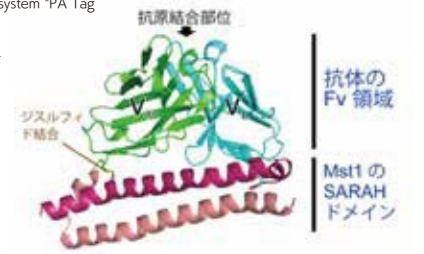


Figure 4: Structure of the new small antibody format "Fv-clasp

Protein research is a traditional craft!


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