# Department of Biophysical Chemistry

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## Research Projects:

Biomembranes, which play important roles in cell functions, can be considered as supramolecular complexes composed of proteins (such as receptors and ion channels), diverse lipids, and oligosaccharides attached to proteins and lipids. Therefore, to elucidate the structures and functions of biomembranes, understanding of protein-lipid interactions is essential. Our current research projects are listed below.

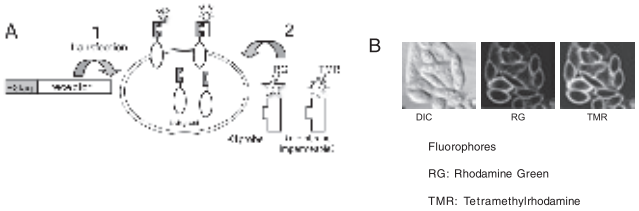
**1) Elucidation of the action mechanisms of antimicrobal peptides:** Antimicrobial peptides, which play an important role in innate immunity, have been isolated from many living species including human for 20 years. Shortly after the discovery of magainin 2, the first antimicrobial peptide from vertebrates, our laboratory started studying the action mechanism of antimicrobial peptides, such as magainin 2 and tachyplesin 1. We revealed for the first time that these peptides bound to bacterial membranes selectively, followed by forming dynamic peptide · lipid supramolecular-complex pores that allow mutually coupled transmembrane transport of ions, lipids, and peptides themselves. We are currently designing hybrid peptides and macromolecule-attached peptides to develop novel therapeutic agents.

**2) Alzheimer's disease:** The conversion of soluble, nontoxic amyloid beta peptide (Aβ) to aggregated, toxic Aβ is considered to be the key step in the pathogenesis of Alzheimer's disease. However, the mechanism of the aggregation remains unknown. It has been shown that, in Alzheimer's disease brain, Aβ is bound to the glycosphingolipid GM1-ganglioside (GM1). We have focused on microdomains in plasma membranes, called 'lipid rafts', which are mainly composed of cholesterol and sphingolipids including GM1, and revealed that Aβ specifically recognizes a ganglioside cluster, the formation of which is facilitated by cholesterol in raft-like liposomes, then undergoes a conformational transition to a β-sheet-rich structure, and the conformationally altered form of Aβ serves as a seed for the aggregation of the protein. Based on these findings, we have proposed "GM1-mediated Aβ accumulation model". In recent studies, we fluorescently visualized time- and concentration-dependent accumulation of Aβ on living cell membranes for the first time.

**3) Elucidation of membrane protein folding:** The folding principles of membrane proteins should be quite different from those of water-soluble proteins. However, experimental examination of the folding of membrane proteins is rather challenging due to their poor solubility and the difficulty in their isolation and purification. Our strategy is to elucidate thermodynamic parameters for forces that generally drive the folding of membrane proteins (e.g., van der Waals, H-bond, and ionic interactions) by using model transmembrane helices (folding units of membrane proteins) in the context of helix-lipid and helix-helix interactions.

**4) Regulation of function of G-protein coupled receptors:** We are developing new methods to control functions of GPCRs, which are important drug targets. We recently developed a labeling method named 'coiled-coil tag-probe labeling system' to quickly label cell-surface receptors in living cells with synthetic fluorescent probes, enabling easy and sensitive detection of receptor internalization after agonist stimulation. We are currently using this technology to elucidate and control complex behaviors of GPCRs in living cell membranes.

**5) Protein structure determination by NMR:** High-resolution NMR spectroscopy is established as a fundamental tool for the determination of detailed three dimensional structures of biomolecules such as proteins and nucleic acids in solution. This technique provides us detailed information about not only static but also dynamic nature of proteins, including protein folding, conformational change upon ligand binding at amino acid residue resolution. We are investigating the folding process of proteins and model peptides by using high-resolution NMR. We are also developing a novel method to analyze highly aggregative proteins to which current NMR is not applicable.




**Coiled-coil labeling method.**
(A) Labeling principle. (B) Confocal images for RG-K4 and TMR-K4 aquired 5 min after incubation with CHO cells expressing E3-β2 adrenoceptors.

## Recent publications


- Okada et al. Toxic amyloid tape: A novel mixed antiparallel/parallel β-sheet structure formed by Aβ on GM1 clusters. ACS Chem. *Neurosci.*, **10**, 563 (2019)
- Nakamura et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* **554,** 249 (2018)
- Yano et al. GXXXG-mediated parallel and antiparallel dimerization of transmembrane helices and its inhibition by cholesterol: Single-pair FRET and 2D IR studies. Angew. Chem. *Int. Ed. Engl.* **56**, 1756 (2017)