Elucidation of Dynamical Structures of Biomolecules toward Understanding the Mechanisms Underlying Their Functions

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions

KATO, Koichi
Professor

YAMAGUCHI, Takumi
Assistant Professor

YAGI-UTSUMI, Maho
OIIB Research Assistant Professor

YANAGI, Kotaro	IMS Fellow
TANADA, Norio	IMS Fellow
UEKUSA, Yoshinori	Post-Doctoral Fellow
WANG, Ying-Hui	Post-Doctoral Fellow
CHANDAK, Mahesh	Graduate Student
ZHANG, Ying	Graduate Student
ZHU, Tong	Graduate Student
WANG, Jinzheng	Graduate Student
KUNIHARA, Tomoko	Graduate Student
KUMOI, Kentaro	Graduate Student*
OKAWA, Keisuke	Graduate Student*
INAGAKI, Kouya	Graduate Student*
SUZUKI, Kousuke	Graduate Student*
THAMMAPORN, Ratsupa	Graduate Student†
SUZUKI, Mariko	Technical Fellow
ISONO, Yukiko	Technical Fellow
IKEDA, Yukako	Technical Fellow
MIZUKI, Hiroko	Technical Fellow
OKADA, Tomo	Technical Fellow
TANAKA, Kei	Secretary

Our biomolecular studies are based on detailed analyses of dynamical structures of various biological macromolecules and their complexes at atomic level, using NMR spectroscopy in conjunction with other biophysical, biochemical and molecular biology techniques. Here we report our recent structural studies of biomolecular complexes for exploring their functional relevance.

1. NMR Characterization of Specific Glycolipid-Protein/Peptide Interactions

Lipid membranes provide active platforms for dynamic interactions of a variety of biomolecules on cell surfaces, where oligosaccharides covalently modifying lipids are involved in divergent molecular recognition events. Sarcotoxin IA, a ecropin-type peptide from *Sarcophaga peregrine*, exhibits antibacterial activity against Gram-negative bacteria through its interaction with lipid A, a core component of lipopolysaccharides. To acquire detailed structural information on this specific interaction, we performed NMR analysis using bacterially expressed sarcotoxin IA analogs with isotope labeling along with lipid A-embedding micelles composed of dodecylphosphocholine. By inspecting the NMR data, we revealed that the N-terminal segment of sarcotoxin IA formed an amphiphilic α-helix upon its interaction with the aqueous micelles, thereby identifying key lysine residues in the interaction with lipid A and the consequent antibacterial activity.[1)] These results offer unique information for designing chemotherapeutics based on antibacterial peptide structures.

Gangliosides, glycosphingolipids possessing a sialyl oligosaccharide moiety, can be targets for various amyloidogenic proteins that are associated with neurodegenerative disorders. We successfully displayed a series of gangliosides on small bicelles with a uniform confined size, offering nanoscale standardized membrane mimics for spectroscopic characterization of weak encounter complexes formed between ganglioside clusters and amyloidogenic proteins.[2)] This enabled probing of initial membrane-landing processes of α-synuclein as therapeutic target by NMR spectroscopy.

2. NMR Characterization of Interactions of Molecular Chaperones with Model Ligands

Molecular chaperones are involved in various cellular processes in which proteins undergo folding, unfolding, and/or refolding under physiological and stress conditions. It has been proposed that molecular chaperones actively contribute to the suppression of toxic aggregate formation of various amyloidogenic proteins associated with neurodegenerative disorders. Furthermore, molecular chaperones have attracted attention due to their potential applicabilities as intelligent nanodevices in bioenginnering fields.

The chaperonin of *Escherichia coli* GroEL forms a large cylindrical complex that assists in the folding of nascent polypeptides. We characterized GroEL–protein interactions by stable isotope-assisted NMR spectroscopy using chemically

denatured bovine rhodanese and an intrinsically disordered protein, α-synuclein, as model ligands. NMR data indicated that proteins tethered to GroEL remain largely unfolded and highly mobile, enabling identification of the interaction hot spots displayed on intrinsically disordered proteins.[3] Furthermore, we found that GroEL could suppress Aβ amyloid formation by interacting with its two hydrophobic segments involving key residues in fibril formation. The binding site of Aβ was mapped on a pair of α-helices located in the GroEL apical domain.[4] These results provide insights into chaperonin recognition of amyloidogenic proteins of pathological interest.

Moreover, we successfully identified ligand-binding site of Hsp47, a client-specific chaperone for collagen, by NMR spectroscopy in conjunction with mutational analysis.[5] Our findings provide a molecular basis for the design of drugs that target the interaction between Hsp47 and procollagen in therapeutics for fibrotic diseases.

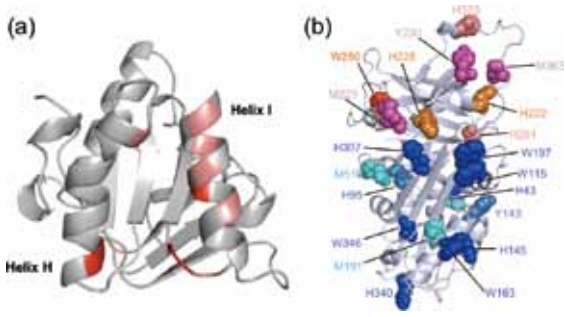

Figure 1. Mapping of (a) the Aβ-binding site on the GroEL apical domain and (b) the collagen-binding site of Hsp47.

3. Elucidation of the Functional Relevance of Quaternary Structures of Proteasome-Associated Proteins

Assembly of the eukaryotic 20S proteasome is an ordered process involving several proteins operating as proteasome assembly factors including PAC1-PAC2 but that of the archaeal 20S proteasome involves spontaneous self-assembly. Recent bioinformatic analysis identified archaeal PAC1-PAC2 homologs PbaA and PbaB. However, it remains unclear whether such assembly factor-like proteins play an indispensable role in orchestration of proteasome subunits in archaea. We revealed that PbaB forms a homotetramer and exerts a dual function as an ATP-independent proteasome activator and a molecular chaperone through its tentacle-like C-terminal segments.[6] Our findings provide insights into molecular evolution relationships between proteasome activators and assembly factors.

In mammals, a major form of proteasome activator PA28 is a heteroheptamer composed of interferon-γ-inducible α and β subunits, which share approximately 50% amino acid identity and possess distinct insert loops. Using deuteration-assisted small-angle neutron scattering, we demonstrated three α and four β subunits are alternately arranged in the heteroheptameric ring.[7] In this arrangement, PA28 loops surround the central pore of the heptameric ring, suggesting that the flexible PA28 loops act as gatekeepers, which function to select the length of peptide substrates to be transported between the proteolytic chamber and the extra-proteasomal medium.

We have also succeeded in encapsulation of ubiquitin, a protein modifier recognized by the proteasome, within synthetic hosts in collaboration with Dr. Makoto Fujita (The University of Tokyo) and his colleagues.[8]

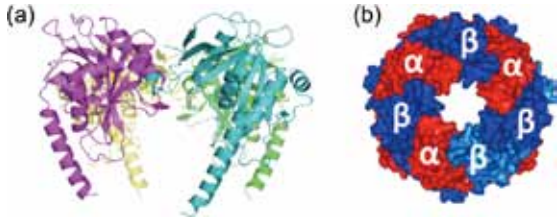

Figure 2. Quaternary structures of (a) the PbaB homotetramer and (b) the PA28 heteroheptamer.

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Award

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* carrying out graduate research on Cooperative Education Program of IMS with Nagoya City University
† carrying out graduate research on Cooperative Education Program of IMS with Kasetsart University