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Commentary and Perspective

Uncovering the design principles of supramolecular assemblies through manipulation of the structures, dynamics, and functions

Makito Miyazaki[1,2,3,4], Takahiro Kosugi[4,5,6,7]

[1] *The Hakubi Center for Advanced Research, Kyoto University, Kyoto 606-8501, Japan*
[2] *Department of Physics, Kyoto University, Kyoto 606-8502, Japan*
[3] *Institut Curie, PSL Research University, UMR144, CNRS, F-75005 Paris, France*
[4] *PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan*
[5] *Institute for Molecular Science (IMS), National Institutes of Natural Sciences (NINS), Okazaki, Aichi 444-8585, Japan*
[6] *Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences (NINS), Okazaki, Aichi 444-8585, Japan*
[7] *School of Physical Sciences, SOKENDAI (The Graduate University for Advanced Studies), Hayama, Kanagawa 240-0193, Japan*

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Cells maintain their life through the assembly and disassembly of various subcellular structures from molecules. These supramolecular assemblies include many types, ranging from nanometer-scale structures such as protein complexes and DNA/RNA-protein complexes to micrometer-scale structures such as the cytoskeleton, chromosomes, classical organelles, and liquid droplets. A growing body of evidence suggests that these ordered and dynamic structures regulate various key functions of the cell, many of which are previously unknown or unnoticed. How do the tiny molecules assemble into ordered structures at appropriate time and space to generate biological functions? From a physical viewpoint, this is a non-trivial question because molecules are able to "communicate" with each other only through their local interactions. Decoding the self-organization mechanisms of ordered structures and biological functions from molecules remains a challenging frontier in life science.

Over several decades, we have accumulated the knowledge of supramolecular assemblies by identifying the molecular components, resolving the molecular structures, and observing these dynamics in living cells. However, in general, the knowledge obtained by "knowing and seeing" is limited. To deepen our understanding on the design principles of the supramolecular assemblies, the authors believe that "creating and manipulating" their structures, dynamics, and functions will be of crucial importance. To summarize recent advances and discuss prospects on the reconstitution and manipulation strategies aiming to understand the supramolecular assemblies, here we organize a symposium at the 60th Annual Meeting of the Biophysical Society of Japan, which will be held at Hakodate, Japan from 28th to 30th of September 2022. In this symposium, we invite talented early-career researchers in various research fields who are developing cutting-edge technologies to reconstitute and/or manipulate the supramolecular assemblies.

Takahiro Kosugi manipulates protein complexes by redesigning with computational protein design methods. Protein complexes, one of the most major supramolecular assemblies, exert the concerted functions by orchestration between subunits. He reports an approach to designing allosteric sites which provide novel orchestration into protein complexes. By the approach, a concerted function, rotation rate, of a molecular motor V_1-ATPase was successfully regulated [1].

Takeshi Yokoyama (Graduate School of Life Sciences, Tohoku University) manipulates ribosomes by an optogenetic

Corresponding authors: Makito Miyazaki, The Hakubi Center for Advanced Research, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto 606-8501, Japan. ORCID iD: https://orcid.org/0000-0002-4603-851X, e-mail: miyazaki.makito.3s@kyoto-u.ac.jp; Takahiro Kosugi, Institute for Molecular Science, National Institutes of Natural Sciences, 38 Nishigo-Naka, Myodaiji, Okazaki, Aichi 444-8585, Japan. ORCID iD: https://orcid.org/0000-0002-6289-5319, e-mail: takahirokosugi@ims.ac.jp

approach. Ribosome, supra-assembly consisting of proteins and RNAs, translates genetic information encoded on mRNA to the corresponding amino acid sequences, the essential machinery in gene expression. He fused an optogenetic tool to the RNA moiety, which comprises the structural core of the ribosome, and has aimed to control ribosomes inside cells by the light. This manipulation of ribosome will contribute to uncover the translation mechanism and control the translation artificially.

Taki Nishimura (Graduate School and Faculty of Medicine, The University of Tokyo) uncovers a control mechanism of a dynamic membrane process driven by ATG proteins, autophagy; by the uncovered mechanism, autophagy is expected to be manipulated in the future. ATG proteins drive autophagy in response to nutrient starvation. ATG3, which is one of the ATG proteins, binds highly curved membranes via its N-terminal amphipathic α-helix (AH_{ATG3}) and executes LC3 lipidation by collaborating with other ATG proteins. He found several key features of AH_{ATG3} essential for the LC3 lipidation *in vivo*. This finding might provide a novel method for manipulating autophagy in the future.

Yuka Iwasaki (School of Medicine, Keio University) manipulates a small RNA-mediated gene silencing to uncovers a control mechanism of gene expression pattern by small RNAs. Heterochromatin is vital to sustaining stable chromosome structure and gene expression patterns, and some classes of small RNAs instruct heterochromatin formation, resulting in regulation of target genes. She has focused on a class of small RNAs, PIWI-interacting RNAs (piRNAs) that form effector complex with PIWI proteins to preserve genomic integrity by repressing transposable elements. By reconstructing piRNA-mediated silencing, she characterized higher-order nuclear architectural change induced upon small RNA-guided heterochromatin formation in a stepwise manner [2,3].

Shunsuke Shimobayashi (The Center for iPS Cell Research and Application (CiRA), Kyoto University) manipulates the liquid-liquid phase separation (LLPS) of biomolecules aiming to understand the nucleation mechanism of biomolecular condensate. In recent years, LLPS has been thought to drive the formation of various intracellular biomolecular condensates having different biological functions. However, we are still largely in the dark about the design principles that decide where and when these condensates form in living cells. Using an optogenetic approach, he demonstrated that despite the complex nature of the intracellular environment, the kinetics of condensate nucleation occurs through a physical process similar to that in inanimate materials, but the efficacy of nucleation sites can be tuned by their biomolecular features [4].

Hideki Nakamura (The Hakubi Center for Advanced Research, Kyoto University) develops synthetic biology tools that manipulate formation and dynamics of intracellular structures. Recent studies have revealed that the intracellular structures undergo constant trafficking, deformation, or diffusion, with incessant turnover of their components. To understand biological roles and biophysical mechanisms of the dynamics, conventional methods often suffer from lack of spatio-temporal resolutions. Tools in synthetic biology field often provide promising solutions to such difficulties. He developed novel tools that can manipulate, design, or analyze various intracellular structures with high resolution [5-7].

To summarize, the six speakers talk about recent researches for various supramolecular assemblies in the symposium. The topics include protein complex, RNA-protein complex, membrane, heterochromatin, LLPS, and organelles. The speakers manipulate the supramolecular assembles by their original approaches and uncover regulatory mechanisms of the molecular and/or biological functions. Their each research for different targets altogether covers the vast range of fields in life science. Moreover, they often discuss with and encourage each other in a community, JST PRESTO research area “Dynamic supra-assembly of biomolecular systems”. Therefore, not only their each research but also future collaborations with each other will open up a new frontier of life science.

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