BDR SEMINAR (Kobe & online hybrid)

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14:00-15:00

1F Auditorium, DB Building C, Kobe / Broadcast online via Zoom

Zoom meeting URL will be announced on the event day by e-mail.

※Non-BDR members: Please register from the following link.

https://krs2.riken.jp/m/bdrseminarregistration (Registration deadline: June 16)

Visualization of endogenous molecular complexes by IRIS, multiplexed super-resolution imaging with antiserum-derived probes

Summary

A variety of proteins form molecular complexes in cytoskeletons, focal adhesions, clathrin-coats and various membranes for diverse cellular functions. However, visualizing the molecular composition of the complex is still difficult, even with several nanometer resolution of the latest super-resolution microscopy. This is because the binding of an antibody (12 nm in size) to a target molecule (e.g., 5 nm in size) interferes with the accessibility of antibodies to another molecule in the complex.

To solve this labeling problem, we have developed fluorescent probes that directly associate with and then rapidly dissociate from the targets. The properties of the probes enable unlimited high-density labeling by capturing numerous binding events and sequential labeling of multiple targets by exchanging the probes. Using the precise localization of these probes, we have developed multiplexed super-resolution microscopy with high-density labeling, called IRIS (Kiuchi et al., Nature Methods, v12: 743-746, 2015).

In this presentation, we show the development of antiserum-derived IRIS probes by modifying the purification and fluorescence conjugation of antibodies, and multiplexed super-resolved visualization of eight endogenous proteins. Their labeling densities reached 3.6 to 6 times the maximum density of antibodies (0.8 labels per 10 nm pixel). In addition, the resolution improved from ±24.2 nm to ±10.7 nm in standard deviation using the mean localization of a long-binding probe. Using the high-density labeling and improved resolution, we also developed an image analysis PC-coloring that evaluates molecular complexes by correlation between two targets at a protein-sized pixel (5 nm). These developments have revealed multi-layered complex formation including EGFR, Grb2 at the rim of clathrin-coats, separation of EGFR and transferrin receptors, and the complex formation of Eps15 with site-specifically different partners.

Clathrin coats are multifunctional devices for membrane receptor recruitment, sorting and endocytosis. The distribution of molecular complexes in clathrin coats indicates the molecular composition for these functions. From antiserum-derived probes produced against arbitrary targets and the faithful distributions of multiple targets, IRIS will powerfully advance the study of molecular complexes in various cellular functions. These results have been published in Structure (Kiuchi et al., Structure, v33: 1-10, 2025).