Technology from Kyoto University

High-specificity and low-affinity antibodies for multiplexed super-resolution imaging

We are looking to out-license the technology for its commercialization.

Imaging probes based on fast dissociation antibodies that maintain antigen specificity

◆Background

Antibody probes used in immunological assays such as immunostaining and immunoassays generally bind strongly to antigens. Once such probes are bound, it is difficult to dissociate the antigen from the antibody probe. The inventors have established a multiplexed super-resolution imaging method called IRIS, which utilizes antibodies with low affinity but high specificity to the antigens. However, the acquisition of such antibody probes was challenging.

◆Description

The inventors have shown that mutations introduced at conserved positions located at the base of the complementary determining region loops in the antibody probe can significantly reduce the half-life of antibody/antigen association while maintaining the binding specificity to the antigen.

➢ **Automated acquisition of multiplexed super-resolution imaging is possible.**

The antibody probe can be easily washed off with saline from the antigen (Fig. 1). Therefore, multiplexed super-resolution imaging is achieved by simply repeating the following three steps on the sample: Step 1: applying the antibody probe to the sample; Step 2: image acquisition; and Step 3: washing of the antibody probe (Fig. 2).

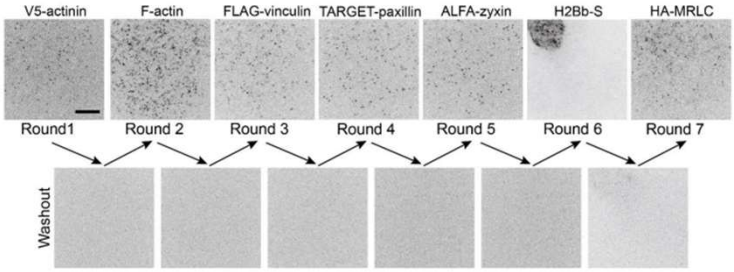

Figure 1. The process of septuple super-resolution image acquisition
Six types of anti-epitope tag antibody probes and lifeact-GFP were sequentially used. Each successive probe was applied after washing the preceding probe with PBS.

➢ **Faithful special distribution of antigens can be captured**

Because the antibody probe of the present invention dissociates from the antigen in a short period, steric hindrance due to the antibody probe bound to the antigen does not occur, unlike conventional antibody probes. This increases the labeling density of the antigen compared to the conventional method (PALM/STORM).

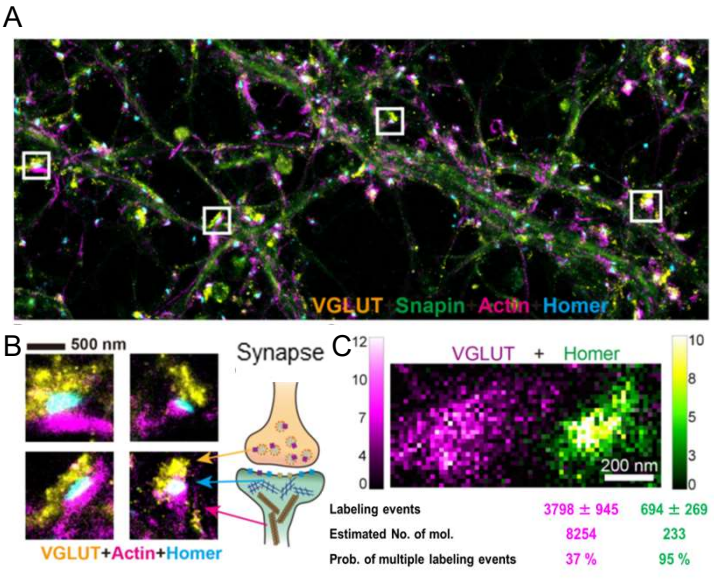

Figure 2. Quadruple super-resolution image of cultured neurons by the IRIS method.
(A) Overall image. (B) Enlarged images of neural synapses. The VGLUT in the presynapse, the adapter molecule Homer in the postsynaptic density, and the actin fibers surrounding the Homer are visualized. (C) The number of times the novel high-specificity low-affinity antibody probes for Homer and VGLUT labeled the target molecules at each synapse (average of 10 synapses) was comparable to the number of each molecule estimated to localize at a synapse. In super-resolution microscopy, if the labeling density is not sufficient, the correct special distribution of the molecules cannot be reconstituted.

◆Development Status

・Antibody probes in Fv-Clasp and vHH formats have been verified to work.

・ Low-affinity and high-specificity antibody probes for 6 types of epitope tags are available.

◆Applications

・Research reagent
(Imaging, immunoblotting, etc)

・In vitro diagnosis
For adjusting the binding affinity of antibodies to antigens

◆Intellectual Property

- Licensing
- Option to license
(for feasibility study)
- MTA（Antibody probes for epitope tags are available）

※Patent pending

◆Publication

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