(Form 1)

Kyoto University	Doctor of Philosophy In Life Sciences	Name	Qianli Zhang
Thesis Title	Development of fast-dissociating recombinant antibodies for high-density multiplexed IRIS super-resolution microscopy		

(Thesis Summary)

Single-molecule localization super-resolution microscopy (SMLM) has greatly surpassed the diffraction limit of conventional optical microscopy. The imaging fidelity, however, is limited by the labeling density of antibodies. Image reconstruction by integrating exchangeable single-molecule localization (IRIS), which uses exchangeable probes that transiently bind to endogenous targets, overcomes the problem. Generation of fast-dissociating IRIS probes has been challenging in the previous studies. Because the number of available antibodies is expanding rapidly, generating recombinant probes from existing antibody sequences may greatly expand the usability of IRIS, but such an approach would require an efficient strategy to accelerate the dissociation of the antibody-target interaction without compromising the binding specificity.

In this study, the applicant found that mutagenesis at the base of complementarity determining region (CDR) loops may effectively accelerate dissociation of antibodies without loss of binding specificity. The amino acids at these sites are conserved and often occupied by tyrosine residues, which play dominant roles in mediating molecular contacts. By combining multiple site-directed mutagenesis at these conserved sites, a versatile strategy that can rapidly convert the existing antibody cDNAs to fast-dissociating fluorescent probes for IRIS multiplexed super-resolution imaging was developed.

The applicant successfully generated dozens of fast-dissociating antibody-derived IRIS probes and demonstrate multiplexed localization of endogenous proteins in primary neurons that visualizes synaptic connections as small as ~0.1 μm. The average binding events of IRIS probes against Homer and VGLUT in individual synapse were 694 and 3798, respectively. Because the number of Homer and VGLUT molecules in a synapse are reported to be 233 and 8254, approximately 95% of Homer and 37% of VGLUT molecules were estimated to be labeled at least once.

In addition, the applicant shows evidence of the spatial interference between multiple antibodies in small synapse areas, which could give rise to sparse labeling in conventional SMLM such as stochastic optical reconstruction microscopy (STORM) and DNA-based point accumulation for imaging in nanoscale topography (DNA-PAINT). Fast-dissociating IRIS probes do not suffer from this problem. Furthermore, multiple localization points may arise from repeated detection of a single label in STORM and DNA-PAINT. IRIS thus achieves higher label density than conventional super-resolution approaches, and visualizes features of synaptic components with higher fidelity.

(Form 2)

(Thesis Evaluation Summary)

The imaging fidelity of super resolution microscopy is restricted by the labeling density of antibodies. Integrating exchangeable single molecule localization (IRIS) achieves high labeling density using fast exchangeable probes (IRIS probes) that quickly bind to and dissociate from the target proteins. However, it has been challenging to develop IRIS probes for individual targets.

The applicant Qianli Zhang developed a new strategy to convert existing antibodies and nanobodies into fast-dissociating and exchangeable IRIS probes. A recombinant antibody format Fv-clasp was used to express the antibody variable region. EGFP was conjugated to Fv-clasp or nanobodies. To accelerate dissociation rate of Fv-clasp or nanobodies, the applicant focused on several conserved amino acid residues at the boundary of the framework regions (FRs) and the complementary determining regions (CDRs). Combination of alanine and glycine substitution at these sites increased the dissociation rate by 2~100-fold. The specificity of probes was well preserved. Multiplexed IRIS imaging of six epitope-tagged proteins expressed in XTC cells and four neuronal proteins was demonstrated. In the IRIS image of primary neuron, 95% of Homer and 37% of VGLUT molecules are estimated to be labeled at least once. In addition, the data indicates that spatial interference between antibodies may deteriorate the labeling density in other super-resolution methods but not in IRIS.

The strategy of generating fast-dissociating antibody fragments developed in this study expands the application of IRIS super-resolution imaging to various targets. The methods will extend the application of existing monoclonal antibodies developed in other biological and medical researches. This study thus contributes to elucidating fine architectures of super-structures and distribution of molecules in the body.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of life science, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On October 12th, 2022, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

(Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) : mm dd yyyy