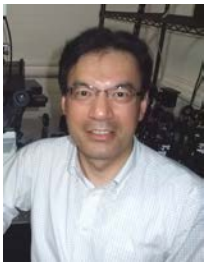
【Grant-in-Aid for Scientific Research (S)】
Science and Engineering (Chemistry)



Title of Project : Methods for the Analysis and Control of Biomolecules in Living Cells Based on Molecular Imaging

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Research Project Number : 26220805 Researcher Number : 40302806
Research Area : Chemistry
Keyword : Bioanalysis, Imaging

【Purpose and Background of the Research】

To deeply understand the intracellular signals as a network of biomolecules, this research project aims to develop novel methods for imaging and controlling biomolecules in living cells. The topics we are interested in are 1) visualization and analysis of small number of biomolecules in living cells, 2) development of methods to control a kinase activity with external light, and 3) development of chemical probes to control GPCR activity. Innovative functional molecules and analytical methods are created, using a state-of-the-art imaging technology with new optogenetic methods (Figure 1).

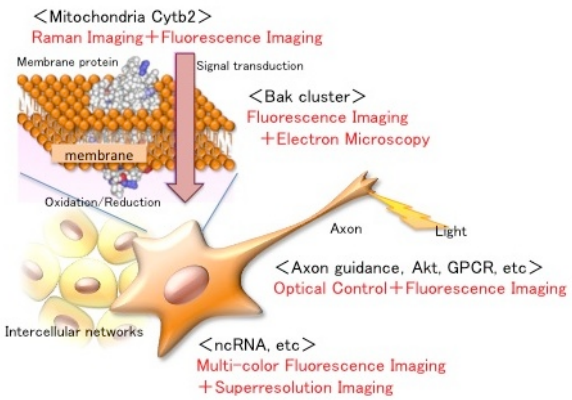

Figure 1. Methods for the analysis of biomolecules and intracellular signaling.

【Research Methods】

1) We develop methods for visualizing and quantitatively analyzing small number of biomolecules in living cells. The target is a telomere RNAs, of which fluorescent probes are designed. Localization of the telomere RNAs and their number in a single cell are analyzed under a fluorescence microscopy. Based on the same approach, we develop a method to analyze Bak cluster formation on the mitochondrial membrane. The number of Bak in a single cluster and their size will be revealed by a technique of superresolution imaging.

2) We develop methods to control a kinase activity with external light. We focused on a serine/threonine kinase of Akt. To control the Akt activity, a photoreceptor derived from a plant cell is used. We will demonstrate that it is possible to control phosphorylation of the substrate and gene expression by external light.

3) We develop chemical probes to control GPCR activity. A new bioluminescent probe for the analysis of GPCR dimerization is developed. We will generate a stable cell line which expresses the probe on the plasma membrane. Using the cells, inhibitors for a target GPCR will be screened from large chemical libraries. New light sensitive chemical probes will be developed.

【Expected Research Achievements and Scientific Significance】

This research is located in the center between the life science and the molecular science. By standing in the cross fields, the study has a possibility to cultivate a new field in the analytical chemistry. The present research will provide new imaging techniques under the control of a biomolecule of interest, which will be expected to contribute to the fields of the basic biological research.

【Publications Relevant to the Project】

・Advances in fluorescence and bioluminescence imaging. T. Ozawa, H. Yoshimura and S.B. Kim, *Anal. Chem.*, **85**, 590-609 (2013).
・Methods of split-reporter reconstitution for the analysis of biomolecules. H. Yoshimura and T. Ozawa, *Chem. Record*, in press.

【Term of Project】 FY2014-2018

【Budget Allocation】 150,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.chem.s.u-tokyo.ac.jp/users/analyt/index.html