



**Title of Project : Development of Innovative Methods for
Analyzing Biological Molecules in Living
Cells Based on Protein Chemistry**

Takeaki Ozawa

(The University of Tokyo, Graduate School of Science, Professor)

Research Area : Chemistry, Applied Chemistry, Analytical Chemistry

Keyword : Bio-material analysis

【Purpose and Background of the Research】

Chemical understanding of biological process in living cells is an extremely important for the progress of science. To understand the process, development of a novel technology, which studies on biological phenomena, is strongly requested in all fields of science. We originally found a new phenomenon, named reconstruction of green fluorescent protein (GFP), which enables visualization of various intracellular processes in living cells. This research project aims to develop novel analytical methods for resolving complicate networks and function of biological molecules in living cells. The special targets are

- 1) molecular probes to visualize biological function in living cells,
- 2) methods to identify specific molecules in intracellular signaling,
- 3) molecular probes to control a biological function with light.

Innovative functional molecules and analytical methods are created, using a state-of-the-art technology of molecular science, genetic engineering and protein chemistry.

【Research Methods】

Basic principle of probe molecules is based on the protein reconstitution method (Fig. 1). The probes are first designed reasonably with information on the three-dimensional protein structures and their function. The cDNAs encoding the probes were created and expressed in bacteria and living cells. Evaluation of the probes is performed using optical techniques in

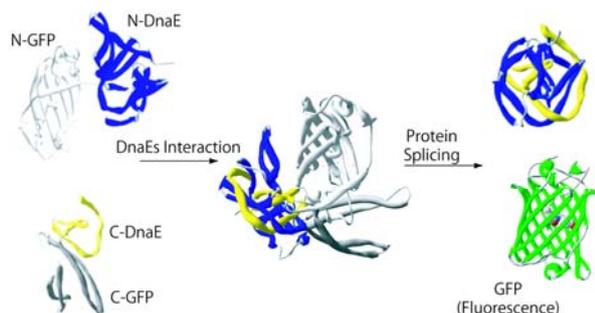


Figure 1. GFP fragments reconstitution. Interaction of DnaEs induces protein splicing and full-length GFP is reconstituted.

vitro and in vivo. In addition, a technique of "molecular evolution methods", which is useful for random mutation, deletion and insertion of amino acids, is used. A desired functional molecule is screened from the protein library.

【Expected Research Achievements and Scientific Significance】

The present 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. It is of great significance to create fluorescent and bioluminescent analytical materials and to guide this analytical field into a worldwide leader like discovery and development of GFP. This study also aims at the development of functional molecules with excellent accuracy, sensitivity, and throughput performance. The present study, therefore, is expected to contribute to the fields like not only the basic biological research but also the drug screening and environmental monitoring.

【Publications Relevant to the Project】

- "Imaging Dynamics of Endogenous Mitochondrial RNA in Single Living Cells", T. Ozawa, Y. Natori, M. Sato and Y. Umezawa, *Nature Methods*, **4**, 413-419 (2007).
- "A genetic Approach to Identifying Mitochondrial Proteins", T. Ozawa, Y. Sako, M. Sato, T. Kitamura, and Y. Umezawa, *Nature Biotechnol.*, **21**, 287-293 (2003).

【Term of Project】 FY2009-2013

【Budget Allocation】 82,100 Thousand Yen

【Homepage Address and Other Contact Information】

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