Development of micro-channel flow-flash method for time-resolved spectroscopic study of enzymatic reactions

Tetsunari Kimura, Takehiko Toshin, Yoshitsugu Shiro, Minoru Kubo (RIKEN, PRESTO, JST)

Time-resolved spectroscopy is powerful to clarify the molecular mechanism of enzymes. Although the solution-mixing is a general technique to trigger the reaction, high sample consumption and limited time-resolution have prevented its extensive application for enzymatic reactions. To improve the time-resolution, we choose the flow-flash method with caged-compounds, where the flash can quickly release substrates. In addition, based on the approach reported by Nakashima et al. at this Society meeting (31034 in 2012), we are developing a micro-channel flow-cell connected with pulse-synchronized syringe-pump. Only 1 nl sample volume is required to obtain a time-resolved spectrum. The latest developments of the devices will be reported in this presentation.

C-type heme-copper oxygen reductase

Yui Iwamoto, Yuriko Ando, Yoshitsugu Shiro, Kazumasa Muramoto (Grad. Sch. Life Sci., Univ. Hyogo, Harima Inst., RIKEN)

In the aerobic respiratory chain, the heme-copper oxygen reductase (HCOR) families catalyze O$_2$ reduction to H$_2$O coupling to proton pump. To understand energy transduction mechanism, we performed structural and functional analyses of C-type HCOR, one of subfamilies. C-type shows high O$_2$ affinity and transfers both substrate and pumped protons by single pathway. In this study, we analyzed O$_2$ consumption activity of C-type purified from Vibrio cholerae O395-N1 cells. The activity was measured by using ascorbate as electron donor and TMPD as mediator. The maximal activity was observed at pH 7.8 suggesting that proton and/or electron transfer was affected by pH. To examine the activity under physiological condition, we currently construct ascorbate/cytochrome c$_6$ system.

Lateral diffusion of metabotropic glutamate receptor observed in single-molecules on the living cell surface

Masataka Yanagawa, Michio Hiroshimia, Takahiro Yamashita, Yoshinori Shichida, Yasushi Sako (Cellular Informatics Laboratory, RIKEN, Quantitative Biology Center (QBiC), RIKEN, Department of Biophysics, Graduate School of Science, Kyoto University)

G protein-coupled receptors (GPCRs) constitute the largest superfamily of membrane proteins in the human genome. For about two decades, over 140 different GPCR dimers or oligomers have been reported and attracted much attention as potential drug targets. However, the size and dynamics of GPCR oligomers under physiological condition are yet to be cleared. Here we show the dynamics of lateral diffusion of metabotropic glutamate receptor (mGlur) molecules on the living cell surface, which are well known to function as a constitutive homo-dimer, by using single-molecule imaging technique. We will discuss the states of mGlur having different diffusion constants and their relationship with the size of oligomer or with the activation upon agonist stimulation.

Photo-regulation of small G protein K-Ras using photochromic molecules


Ras is one of small G-proteins known as a molecular switch mediating cellular signalling. In this study, we performed basic study to control the function of Ras reversibly using photochromic molecules, 4-phenylazophenyl maleimide (PAM) and monoiodoacetic spiropyran (IASP) upon visible (VIS) light and ultra-violet (UV) light irradiation. We have prepared the three kinds of Ras mutants Y32C, I36C, and Y64C. The mutants were modified with PAM and IASP stoichiometrically. And the GTPase activity of Ras was monitored by the quantitative analysis of GTP and GDP using HPLC with reverse phase column. It was suggested that the GTPase activities of Ras mutants modified with these photochromic molecules were reversibly altered upon VIS and UV light irradiations.

Low molecular weight K-Ras as a potential drug target


The structure of the mitochondrial F-ATP synthase has been characterized by X-ray crystallography of subcomplexes and electron cryo microscopy of the detergent solubilized intact enzyme. Thus far, none of the methods used to study the structure of the F-ATP synthase has the potential to give insights into its conformational state during ATP synthesis under a proton motive force. Here, we present tubes of tightly packed, membrane reconstituted bovine mitochondrial F-ATP synthases, which are suitable for structural studies by electron cryo tomography. The enclosed nature of the tubes make them ideal candidates to elucidate the structure of mitochondrial F-ATP synthase under proton motive force in synthesis mode.

Multi-drug resistance in Gram-negative bacteria


RND-type multi-drug efflux transporters are the major cause of multi-drug resistance of Gram-negative bacteria. They act as a tripartite complex of an exporter, membrane-fusion protein and outer membrane channel, however, the stoichiometry in the active complex has not been known. We constructed the fusion protein gene of an exporter AcrB and a membrane-fusion protein AcrA connected with glycin-serine repeat linker. When this AcrB-AcrA fusion protein was expressed in acrAB deletion strain of Escherichia coli, the resultant strain showed multi-drug resistance, indicating that the AcrB/AcrA stoichiometry is 1:1.