3P302 生物試料中での GFP－CL の観察
Observation of GFP-CL in biological specimens

GFP-CL is a phenomenon that fluorescent proteins like GFP generate fluorescent light by electron irradiation (CL: cathodeluminescence). To develop a new technique of correlative light and electron microscopy (CLEM), we employed GFP-CL and identified GFP-labeled proteins in biological specimens using electron microscope. GFP was expressed in yeast cells, and the CL was observed in the cells using the hybrid light-electron microscope (200kV) developed by Nagayama and coworkers (Iijima et al., submitted). The GFP-CL showed a good matching with the photoluminescence (PL). The spectra of GFP-CL were distributed between 500 to 900 nm. An extreme electron irradiation sometimes enhanced the PL and CL intensities. GFP-CL was also observed in other GFP-transfected cells.

3P303 蛍光蛋白質における光および電子発光の電子線活性化
Electron-beam Activation of Photo- and Cathodo-luminescence in Fluorescent Proteins
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Cathodoluminescence (CL) is a kind of fluorescence (FL) generated by not photon but electron irradiation. Recently we have found that some classes of fluorescent proteins (FPs) act as stable and bright CL substances with an associated enhancement of FL itself, which is quite contradictory to our general comprehension to organic materials prone to be damaged by electron bombardment. To clarify this novel finding, the electron-beam activated luminescence, we have made experiments by focusing on following points; i) generality among FPs, ii) FL and CL spectral properties before and after electron irradiation and iii) efficiency of light emission in CL and FL. Possible causes of the novel luminous event induced with the electron beam is also to be discussed.

3P304 遺伝子制御されたCaイオンリリースカーサイド
Genetically encoded caged Ca²⁺
Noritaka Fukuda1,2, Tomoki Matsuda1, Takeharu Nagai1 (1ISIR, Osaka Univ., 2ebiC, Riken)

In living organism, Ca²⁺ is one of the most important second messenger to mediate between stimuli and biological responses. To decipher biological events, many Ca²⁺ indicators are developed, although development of Ca²⁺ regulators were insufficient.

Here, we report genetically encoded caged Ca²⁺ that termed PhotoActivatable Ca²⁺ Release (PACR). This protein is consist of Ca²⁺ binding protein and light-sensitive domain. Upon photoirradiation, decrease PACR affinity for Ca²⁺ release Ca²⁺. Its direct Ca²⁺ perturbation ability, localizability and inheritance enabled cells and organelle specific Ca²⁺ perturbation and control freely moving organisms. This tool will be useful for studying the role of Ca²⁺ dynamics in complex biological events.

3P305 細胞解析のためのリアルタイム化学刺激システムの構築
Development of the real-time local chemical stimulation system for cell analysis
Masaru Kojima, Takahiro Motoyoshi, Kenichi Ohara, Mitsuhiko Horade, Yasushi Mae, Tatsuou Arai (Grad. Sch. Eng. Sci., Osaka Univ.)

The local environment control technology for single cell analysis is being established. In this study, we try to develop a real-time local chemical stimulation system that can dynamically changing local environment condition and apply this system for analyze and control of bacteria flagella motor. As a first step, we have implemented “fast response measurement” and “solution spouting by micropipette” for this system. And then we confirm the performance of this system by measuring and controlling the rotational speed of bacteria flagellar motor with local ion concentration control.

3P306 DNAナノ構造体を用いたDNA-RNAポリメラーゼ・ハイブリッドナノマシンの構築と活性評価
Construction and functional analysis of DNA origami base DNA-RNA hybrid nanomachine
Takeya Masubuchi1, Hisashi Tadakuma1, Masayuki Endo2, Hiroshi Sugiyama2, Yoshie Harada2, Takuya Ueda1 (1Grad. Sch. Frontier Sci., Univ. Tokyo, 2iCeMS, Univ. Kyoto)

In the cell, gene expression is highly controlled. To create biologically inspired nanoscale device enabling the control of gene expression, we made hybrid nanomachine (T7-tile) using DNA origami tile as the skeletal structure and T7 RNA polymerase (T7-RNAP) as the functional module. T7-tile hybrid allowed us to evaluate the effects of intermolecular distance of enzyme (T7-RNAP) and substrate (target gene containing T7 promoter). We will show our recent achievements.

3P307 人工鞭毛により推進する精子型マイクロマシン
A “sperm-like” micro-machine propelled by an artificial flagellum
Tsuyoshi Yamasaki, Susumu Aoyama, Yuichi Hiratsuka (Japan Advanced Institute of Science and Technology)

Motor proteins form a molecular complex with other proteins by self-assembly, and therefore generate various sophisticated functions, such as contraction of the sarcomere and beating of the flagellum. In a field of mechanical engineering, a device incorporating such functions is expected as a next-generation micro-machine. In this study, we focused on an oscillatory motion of flagellar axoneme among the biological motion driven by motor proteins and we aim to create novel bio-hybrid micro device which is propelled by the oscillatory motion. Currently, we attempt to construct a “sperm-like” micro-machine which is composed of a photolithographically fabricated head part and artificial flagellum synthesized by self-assembly of tubulin and dynein.