1P301 マルチモーダル超高輝度化学発光タンパク質
Multi-modal super-duper chemi-luminescent protein

Kazushi Suzuki¹, Yoshiyuki Arai^{1,2}, Takeharu Nagai^{1,2} (¹*Grad sch engin, Osaka Univ*, ²*ISIR, Osaka Univ*)

Chemi-luminescence imaging has shed light on bioscience research field in situations when fluorescence cannot be used, for example non-invasive deep tissue imaging of whole organisms and light-induced biological processes. Despite of their potential utility, the universal applications are precluded by low brightness and restricted color hue. To overcome these, we had developed a brighter luminescent protein, Nano-lantern and several functional indicators (Nat. Commn. 3, 1262, 2012). Here we report further development of a super-duper chemi-luminescent protein and its color variant as well as Ca²⁺ indicator obtained by protein engineering, thereby we visualized multiple kind of tumor tissues in freely locomoting mouse, and also multifunctional imaging at single cell level

1P302 生体発光共鳴エネルギー転移 (BRET) を用いたミトコンドリア外膜タンパク質 MAVS の構造基盤解析
A structural analysis of the MAVS-regulatory mechanism using BRET

Osamu Sasaki¹, Takuma Yoshizumi¹, Misa Kuboyama¹, Takeshi Ishihara², Emiko Suzuki³, Shun-ichiro Kawabata², Takumi Koshiba² (¹*Graduate School of Systems Life Sciences, Kyushu University*, ²*Department of Biology, Faculty of Sciences, Kyushu University*, ³*Structural Biology Center, National Institute of Genetics and Department of Genetics*)

In mammals, mitochondria act as a platform for antiviral innate immunity. Mitochondrial-mediated antiviral immunity depends on the participation of mitochondrial antiviral signaling (MAVS), which is localized on the mitochondrial outer membrane. After RNA virus infection, MAVS activates and undergoes a conformational change that is essential for downstream signaling on the mitochondrial outer membrane, although its structural features are poorly understood. Here we examined the MAVS-regulatory mechanism on the mitochondrial outer membrane using bioluminescence resonance energy transfer (BRET) in live cells. Our results reveal structural features underlying the precise regulation of MAVS signaling on the mitochondrial outer membrane.

1P303 ストレス顆粒内一分子 mRNA 立体構造の超解像イメージング
Super-resolution imaging of molecular conformation of single mRNA in stress granules

Yuki Suzuki¹, Kou Sugawara¹, Kohki Okabe^{1,2}, Takashi Funatsu¹ (¹*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, ²*JST, PRESTO*)

During stress, cytoplasmic mRNAs aggregate and form stress granules (SGs), where they are remodeled for repression of translation. It is suggested that circularization of mRNA is important for translation regulation. However, the coordination of translational repression in SGs and circularization of mRNAs has been unknown because a detailed distribution and a conformation of mRNAs in SGs remain unclear. We combined multicolor labeling of single mRNA and super-resolution microscopy to investigate molecular conformation of single mRNA. We observed two spots in close proximity which were most likely two points on single mRNA *in vitro* and in SGs in cells. Analysis of a distance between two spots to confirm whether a circularization occurs in SGs is under investigation.

1P304 転写伸長因子 NELF ダイナミクスの 1 分子イメージング定量解析
Single molecule imaging and quantitative analysis of dynamics of negative elongation factor NELF

Daichi Ikeda¹, Yuma Ito^{1,2}, Makio Tokunaga^{1,2}, Kumiko Sakata-Sogawa^{1,2} (¹*Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech.*, ²*IMS, RIKEN*)

NELF contributes to the regulation of transcription elongation by promoter-proximal pausing, which can be released by phosphorylation of the CTD of RNA polymerase II by a kinase P-TEFb. Despite of the important role in transcription regulation, detailed dynamics of NELF is still unclear.

Aiming to elucidate the dynamic properties of NELF in the living cells, we performed single molecule imaging and quantitative analysis. We constructed SNAP-tag fusion protein of NELF and established a cell-line harboring single copy of SNAP-NELF. By controlling the concentration of fluorescent ligand to SNAP, we visualized the movements of single molecules of NELF in the nucleus. Increase of binding NELF by the inhibitor of P-TEFb supported the single molecule imaging of the molecule.

1P305 AIP1 とコフィリン共存下でのアクチンフィラメント切断の 1 分子リアルタイムイメージング
Real-time imaging of actin filament disassembly in the presence of cofilin and actin interacting protein 1 (AIP1)

Carina Sekiguchi¹, Kimihide Hayakawa², Shoichiro Ono³, Masahiro Sokabe², Hitoshi Tatsumi¹ (¹*Department of Physiology, Nagoya University Graduate School of Medicine*, ²*Mechano-biology Laboratory, Nagoya University Graduate School of Medicine*, ³*Department of Pathology, Emory University School of Medicine*)

Severing of actin filaments by cofilin is thought to be essential for cell survival, growth, and motility, whereas the rate of severing by cofilin is very low *in vitro* (less than $2 \cdot 10^{-3}$ event $\cdot s^{-1} \cdot \mu m^{-1}$). AIP1 enhances the disassembly of actin filaments by cofilin *in vitro* and *in vivo*. However, the molecular process of the enhancement is largely unknown. We directly imaged the binding of Alexa-labeled-AIP1 to actin filaments by total internal reflection fluorescence microscopy, and analyzed the process of the disassembly, which showed that actin filaments were severed within a second at or in the vicinity (<150 nm) of AIP1 binding in most cases. This suggests that AIP enhances the severing activity of cofilin at or near its binding site.

1P306 1 細胞分泌実時間測定による IL-1 β 非古典的分泌機序の解明
Analysis of non-classical secretion of IL-1 β using real-time single-cell secretion imaging

Yoshitaka Shirasaki¹, Ting Liu², Yoshifumi Yamaguchi², Mai Yamaguchi¹, Nobutake Suzuki¹, Kazushi Izawa³, Jun Mizuno⁴, Shuichi Shoji⁴, Yoshie Harada⁵, Ryuta Nishikomori³, Toshio Heike³, Masayuki Miura², Osamu Ohara^{1,6} (¹*IMS, Riken*, ²*Grad. Sch. Pharm., Tokyo Univ.*, ³*Grad. Sch. Med., Kyoto Univ.*, ⁴*Grad. Sch. Sci. & Eng., Waseda Univ.*, ⁵*iCeMS, Kyoto Univ.*, ⁶*Kazusa DNA Inst.*)

Interleukin (IL) 1 β is an important cytokine for inflammatory responses. It does not have a signal peptide for ER/Golgi pathway, and is thought to be secreted via non-classical pathway. Some research suggested that IL-1 β secretion is associated with caspase-1 mediated programmed cell death. We figured out this cell-death associated IL-1 β secretion by our real-time single cell secretion imaging. We found that individual cells digitally secreted massive IL-1 β with inflammasome activating stimuli. We also found that caspase-1 activation, membrane pore formation and IL-1 β bursting occurred sequentially within a few minutes. This result indicates the importance of single cell measurement for research of biological secretion activity.