strategy to analyze basic biological mechanisms and several diseases through the distribution of fatty acids.

3P327 タンパク質の時間分解蛍光イメージング法の開発 Development of a time-resolved fluorescence imaging method for protein analysis

Taku Yamamoto¹, Shin Mizukami^{1,2}, Kazuya Kikuchi^{1,2}, (¹Graduate school of Engineering, Osaka Univ.; ²Immunology Frontier Research Center, Osaka Univ.)

A lanthanide luminescence labeling probe was developed based on a labeling technology, which was established in our group recently. Mutant β -lactamase was used as the tag protein that can covalently interact with β -lactam compounds. β -lactamase is a 29 kDa enzyme derived from bacteria, and does not exist in mammalian cells, thereby β -lactam compounds could label the mutant β -lactamase(BL)-tag specifically. The synthesized probe with a β -lactam structure and lanthanide complex, tPA-Eu, showed high affinity of binding to BL-tag and long lifetime luminescence.

Long lifetimes of luminescence allow efficient spectral separation of emission signals, and enable time-resolved detection methods to remove scattering and autofluorescence background. Lifetimes of lanthanide luminescence reach micro-milliseconds, although many fluorescent dyes have lifetimes of nanoseconds order. Moreover, lanthanide luminescences have several advantages such as multiple narrow emission bands, large Stoke's shifts and emission wavelength insensitive to external environments.

In this study, time-resolved fluorescence imaging was developed with a synthesized probe tPA-Eu. BL-tag labeled by tPA-Eu could detect with time-resolved method without any background signals. The specific labeling of tPA-Eu with BL-tag expressed on living cell surfaces was visualized using a time-resolved microscope system.

3P328 $\text{I}\kappa\text{B}\alpha$ の NF- κB 活性化制御機構の 1 分子イメージング解析 Single molecule imaging analysis of NF- κB activation mechanism regulated by $\text{I}\kappa\text{B}\alpha$

Akira Okada^{1,2}, Makio Tokunaga^{1,2}, Kumiko Sakata-Sogawa^{1,2}, (¹Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology; ²Research Center for Allergy & Immunology, RIKEN)

NF- κB is an important transcription factor that activates expression of inflammatory genes in response to stimulation. In unstimulated cells, NF- κB is sequestered in the cytoplasm by binding to $\text{I}\kappa\text{B}\alpha$. Stimulation leads to phosphorylation of $\text{I}\kappa\text{B}\alpha$ and their subsequent ubiquitination to be degraded by proteasome. As a result of $\text{I}\kappa\text{B}\alpha$ degradation, liberated NF- κB translocates to nucleus and activates expression of target genes including $\text{I}\kappa\text{B}\alpha$ genes. The nascent $\text{I}\kappa\text{B}\alpha$ binds to nuclear NF- κB and exports to the cytoplasm resulting in negative feedback loop.

In this study, we aim to directly confirm molecular mechanism of multiple functions of $\text{I}\kappa\text{B}\alpha$ by single molecule fluorescence microscopy. For this purpose, we established cell lines expressing both $\text{I}\kappa\text{B}\alpha$ and p65 (NF- κB) fusion proteins of GFP or RFP and confirmed co-localization in cytoplasm. We visualized single molecule movements of $\text{I}\kappa\text{B}\alpha$ both in the cytoplasm and nucleus, suggesting the shuttling of NF- κB / $\text{I}\kappa\text{B}\alpha$ complexes. After stimulation, $\text{I}\kappa\text{B}\alpha$ remained in the cytoplasm, although p65 translocated to the nucleus. We will discuss molecular dynamics of $\text{I}\kappa\text{B}\alpha$ from the results of molecular imaging of $\text{I}\kappa\text{B}\alpha$ and its dominant-negative mutant $\text{I}\kappa\text{B}\alpha\text{M}$.

3P329 蛍光性金ナノクラスターの開発及び生細胞観察への応用 Characterization of Fluorescence Properties of a Blue Emitting Au Nanocluster

Shin-ichi Tanaka¹, Hiroyuki Morimura¹, Dharmendra Tiwari², Jun Miyazaki¹, Takashi Jin², Yasushi Inouye¹, (¹Graduate school of Frontier Biosciences, Osaka University, Japan; ²Immunology Frontier Research Center, Osaka University, Japan)

Gold nanoclusters (Au NCs) as a new molecular probe for bio-applications have been attracting interest in recent years. Also, Au NC is non-toxic, which makes it well suited for biomolecular imaging. Au NCs are often synthesized by utilizing polyamidoamine (PAMAM) dendrimers as molecular template. However, the fluorescence properties of Au NCs synthesized through this route have not been characterized well. This is partly due to the oxidized PAMAM dendrimer itself which exhibits an emission band at 450 nm with excitation band at 380 nm, strongly overlapping with that of the Au NC. Here, we demonstrate for the first time that the Au NCs have fluorescent properties different from PAMAM dendrimers. By mixing PAMAM dendrimer and HAuCl₄ in ultrapure water, Au ions were encapsulated and reduced to form Au NCs. After ligand-exchanged with mercapto acetic acids, the fluorescent Au NCs and free PAMAM dendrimers were isolated and purified by using a size-exclusive high performance liquid chromatography method. The resulting Au NCs solution showed an emission band at 470 nm with the excitation

band at 380 nm, different from that of the PAMAM dendrimer. Furthermore, the fluorescence lifetime (8.7 nsec.) of Au NC was significantly different with that (7.0 nsec.) of PAMAM dendrimer. For the bio-application of Au NCs, fluorescence imaging of HeLa cells was performed. This work demonstrates that Au NCs have fluorescent properties different from PAMAM dendrimer and show great potential as a fluorescent probe for live cell imaging.

3P330 Development of Photoactive Yellow Protein-based Protein Labeling System with Designed Fluorogenic Probe

Yuichiro Hori¹, Kyohei Nakaki¹, Tomoya Norinobu¹, Kazuya Kikuchi^{1,2}, (¹Graduate School of Engineering, Osaka University; ²Immunology Frontier Research Center, Osaka University)

Fluorescence labeling of proteins is a powerful approach to study the localization, dynamics and function of proteins in living cells. So far, various fluorescent proteins (FPs) have been created and applied to cellular imaging, and the development of new FPs is still regarded as important. On the other hand, protein labeling techniques utilizing protein tags have been recently developed as an alternative of FP-based methods. In this technique, a protein of interest (POI) is genetically fused with a protein tag, which is labeled with its specific ligand connected to a fluorescent molecule. The advantage of this system is that a variety of fluorescent molecules are potentially available as labeling reagents, and that a protein tag is conditionally labeled with its fluorescent ligand. In this research, we developed a protein labeling technique by using photoactive yellow protein (PYP) as a novel protein tag. PYP is a small protein (14 kDa) derived from purple bacteria and binds to 7-hydroxycoumarin-3-carboxylic acid as well as to a natural ligand, 4-hydroxycinnamic acid. Based on the structure and fluorescence property of this coumarin derivative, we designed a fluorogenic probe that binds to PYP. The live-cell imaging and specific labeling of PYP was achieved by using the probe. In this conference, we report the detail of the probe design and cell-imaging experiments.

3P331 Investigation of starch granule inner structures by atomic force microscopy

Shigeru Sugiyama, Kazumi Tsukamoto, (Nanobiotechnology Lab., National Food Research Institute, NARO)

Starches have been used by humans for at least a few thousand years and provides major part of the world's kilocalorie supply. Starches are the most interesting bio-polymers not only in food industry but also in non alimentary fields, such as in fiber industries, papermaking industries, etc. In the native form, the starch is organized by two types of polymer amylopectine and amylose with alternating semi-crystalline and amorphous layer. However, detailed inner structures of starch granules have not been clarified yet.

Recently, a blocklet model that the starch granule consists of two kinds of blocklets with different size (~500 nm and ~50 nm) and crystallinity (hard and soft) was proposed and the both sizes of blocklets have been observed by scanning electron microscopy (SEM). Atomic force microscopy (AFM) images of both the granular surfaces also supported the blocklet models but only the smaller size (~50 nm) of blocklet has been detected.

Thus, we developed a resin-embedded section method specific to the starch and performed the structural investigations of the blocklets and growth ring by AFM. As the results, both the big and small blocklets were clearly observed within the growth rings in the section surface of corn starch granule. Furthermore, we found a new structure with fibrous form (10~12 nm in height) at a center of a granule, which might be a cluster structure of amylopectin molecule with a diameter of 10~15 nm.

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3P332 脂質二重膜刺激系の構築による T 細胞シグナル伝達活性化の 1 分子イメージング定量解析 Single molecule imaging and quantitative analysis of activation of T cell signaling with stimulatory lipid bilayers

Yuma Ito^{1,2}, Kumiko Sakata-Sogawa^{1,2}, Makio Tokunaga^{1,2}, (¹Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology; ²Research Center for Allergy & Immunology, RIKEN)

T cell activation is triggered by contact between T cells and antigen-presenting cells (APCs). Full T cell activation requires an adequate quantity of intracellular signaling by ligand-engaged T cell receptor (TCR) complexes. Although analyses of T cell activation have been conducted by using stimulatory antibody, dynamic mechanism of the signal transduction through the strength of the stimulation remains elusive.

Aiming at quantitative analysis of T cell activation, we developed a model system on supported planar lipid bilayers containing the mobile stimulatory anti-CD3 ϵ (signaling subunit of the TCR) antibodies. For monitoring move-