3SAA-02 Alkyne-Tag Raman Imaging for Visualization of Small Molecules in Live Cells

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Alkyne has a unique Raman band that does not overlap with Raman scattering from any endogenous molecule in live cells. Therefore, alkyne-tag Raman imaging (ATRI) is a promising approach for visualizing small molecules in live cells. An examination of structure-Raman shift/intensity relationships revealed that alkynes conjugated to an aromatic ring and/or to a second alkyne (conjugated diynes) have strong Raman signals in the cellular silent region and can be excellent tags. As a proof of concept, imaging of 5-ethynyl-2’-deoxyuridine (EdU) in living HeLa cells has been demonstrated. Simultaneous imaging of two small molecules, EdU and a CoQ analogue, with distinct Raman tags was also demonstrated.

3SBA-02 シナプス内シグナル分子活性化のイメージングと操作

Imaging and controlling the activity of signaling molecules in dendritic spines of hippocampal neurons

Hideji Murakoshi\textsuperscript{1,2} (National Institute for Physiological Sciences, PRESTO, JST)

Ca\textsuperscript{2+}/Calmodulin-dependent kinase II (CaMKII) is one of the most important signaling molecules for long-term potentiation and associated spine enlargement underlying learning and memory. Here, to understand the function of CaMKII for synaptic plasticity, we developed genetically encoded light-inducible CaMKII inhibitor and photo-activatable CaMKII using LOV2 derived from phototropin. We applied these newly developed optogenetic tools for the study of structural plasticity of single dendritic spines by using 2-photon fluorescence microscope and 2-photon glutamate uncaging, and found that 1) ~60 s of CaMKII activation is sufficient for inducing transient and sustained spine enlargement, 2) CaMKII activation alone is sufficient for triggering structural plasticity.

3SAA-03 ラマン散乱分光顕微鏡を用いた細胞状態を定義する「細胞指紋」の提案

Cellular fingerprints to distinguish and identify the various cellular states with Raman spectroscopy

Tomonobu Watanabe\textsuperscript{1,2,3} (RIKEN, Quantitative Biology Center, Immunology Frontier Research Center, Osaka University, Graduate School of Frontier Bioscience, Osaka University, PRESTO, Japan Science and Technology Agency)

Self renewal and differentiation capability of ES cells are maintained as a result of complex physico-chemical interactions between various elements, such as DNA, protein and lipids, within a nano-scale volume. Microscopy is a powerful tool to observe biological events because of the applicability for observation of living specimens, however, diffusion limit of light restricts the spatial resolution of the microscope to few hundred nanometers. To overcome the problem, we have been challenging to detect the nano-scale events with light that includes the information in nano-scale. Raman scattering is one of the scattered lights, and inheres all the vibration relationships revealed that alkynes conjugated to an aromatic ring and/or to a second alkyne (conjugated diynes) have strong Raman signals in the cellular silent region and can be excellent tags. As a proof of concept, imaging of 5-ethynyl-2’-deoxyuridine (EdU) in living HeLa cells has been demonstrated. Simultaneous imaging of two small molecules, EdU and a CoQ analogue, with distinct Raman tags was also demonstrated.

3SAA-05 スペクトル解析によるバイオ・ラマン研究

Spectral Analysis for Bio-Raman Research

Shin-ichi Morita (Cellular Informatics Laboratory, RIKEN)

Spectral analysis is essential to bio-Raman research to disentangle Raman data since they are complicated and fluctuated multivariable. This talk, we sought to predict cell fates, including cellular differentiation of multicellular system. Two types of cellular systems were used, that is, filamentous cyanobacteria and human breast cancer cell line. For cyanobacteria, Raman technique yielded marker vibrations characteristic to differentiation. The multivariable analysis suggested that the diverse states of undifferentiated cells were converged into a specific state through differentiation.

3SAA-04 ラマン散乱分光顕微鏡を用いた細胞状態を定義する「細胞指紋」の提案

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3SBA-01 抑制性 GABA 作動性シナプス制御におけるカルシウムの驚くべき作用—1分子イメージングで明らかになったこと—

Origo-dependent opposite effect of Ca\textsuperscript{2+} on the regulation of inhibitory GABA\textsubscript{A} receptor diffusion dynamics: a single molecule study

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By analyzing the diffusion properties of type-A GABA receptors (GABA\textsubscript{A,R}) on the cell surface using single molecule imaging technique with quantum dots, we found that Ca\textsuperscript{2+} influx evoked by neuronal excitation increased GABA\textsubscript{A,R} diffusion dynamics and contributed to rapid and plastic reduction in GABAergic synaptic transmission. Conversely, another intracellular Ca\textsuperscript{2+} signaling pathway, i.e. Ca\textsuperscript{2+} release from the intracellular Ca\textsuperscript{2+} stores reduced the surface GABA\textsubscript{A,R} mobility and had an effect to stabilize the GABAergic synapses. These results indicate that Ca\textsuperscript{2+} from different sources could have the opposite effect on the regulation of GABAergic synapses.