3P290 マウス内がん細胞の非侵襲イメージング Noninvasive in vivo imaging of tumor cells in a novel xenograft model

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We developed new noninvasive imaging methods to visualize molecules. We focused on the auricle of mouse for observation of tumor cells because it is very thin and limited hypodermal tissue. We developed a novel xenograft model of the auricle with cancer cells in order to observe them noninvasively. We injected four kinds of human cancer cell lines into the auricle of mice. Tumor was successfully formed using each cell lines. To image molecules, specific antibodies to recognize these cells were labeled with fluorescence quantum dots and then injected to tail vein after the formation of tumor. We could perform noninvasive real time observation of quantum dots within cancer cells and on its membrane.

3P291 バクテリア細胞内 ATP 濃度の一細胞計測 Quantifying the absolute ATP concentration inside single bacteria cells

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Adenosine triphosphate (ATP) is necessary for the progress of many intracellular reactions. Our previous ATP biosensor could report ATP concentration change in single cells, but the absolute value was difficult to measure. Here, we developed a biosensor called BQueen, which can be used to determine the absolute intracellular ATP concentration. Using BQueen, we found that the ATP concentration in *Escherichia coli* cells varies from cell to cell. The ATP concentration was distributed in a positively-skewed shape around 1-8 mM. Interestingly, the cell division rate was the fastest in the moderate ATP concentrations. From the distribution width of ATP concentration in different bacteria growth conditions, we will discuss the change in the metabolic pathways inside the cell.

3P292 細胞内熱伝導率マッピング Mapping of thermal conductivity in single living cells

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Advanced fluorescence thermometry has enabled us to measure the temperature changes in living cells. The details of heat transfer in cells are still not clear due to the lack of information on the thermal properties such as thermal conductivity and heat capacity. In the present study, we mapped two-dimensional distribution of the thermal conductivity in living cells. We applied fluorescent dyes as fluorescent thermometers which uniformly distribute in both nucleus and cytoplasm. The dye is sensitive to temperature changes but resistant to environmental changes such as pH and ionic strength. We locally heated either nucleus or cytoplasm with a focused infra-red laser beam. The temperature distribution indicated heterogeneous thermal conductivity in living cells.

3P293 Structure and fluorescent property of single amino acid insertion mutants of YFP

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Green fluorescent protein based indicators are widely used to detect intracellular environment. We recently found that single amino acid insertion in $\beta 7$ of YFP dramatically affects its fluorescent property in response to environmental change. The fluorescent property highly depends on nature of the inserted amino acid. This finding has opened the door to designing novel fluorescent base biosensors, however, the molecular mechanism of the mutational effect is unknown. To understand the structural basis of the mutational effect, we prepared a series of amino acid insertion mutant YFPs and solved the structures. Here we show the structures of mutant YFPs, (YFP-1E, F, G, L, M, R, Y) and discuss the relationship between the mutant structure and the fluorescent property.

3P294 量子ドットナノプローブを用いたアミロイド β 凝集阻害物質の新規微量ハイスループットスクリーニングシステムの開発 Development of a novel high-throughput screening system of inhibitory substances for amyloid- β aggregation using quantum-dot nanoprobes

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Aggregation of amyloid β (A β) protein causes Alzheimer's disease (AD), suggesting that the screening of inhibitory compounds for A β aggregation can be the first important step to develop preventive and/or therapeutic drugs for AD. In this study, we successfully developed the microliter-scale and high-throughput screening system for A β aggregation inhibitors. First, we evaluated A β aggregation inhibitory activities of ethanolic extracts from 52 spices using this system, and found that mint family showed significantly higher activity. Next, we tried to isolate the main inhibitory compound from summer savory, and revealed it was rosmarinic acid. Inhibitory activity of rosmarinic acid has reported, demonstrating that this system could be applied to the actual screening.

3P295 Simultaneous imaging of intracellular Ca²⁺ and sarcomere length in neonatal cardiomyocytes via expression of cameleon-Nano in Z-discs

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To understand the cardiac E-C coupling at the single sarcomere level, we have developed a novel experimental system for simultaneous measurement of intracellular Ca²⁺ and sarcomere length (SL) via expression of cameleon-Nano, a FRET-based Ca²⁺ indicator, in Z-discs (α -actinin) in primary-cultured rat neonatal cardiomyocytes. Under dual-view microscopy, we measured Ca²⁺ changes by imaging the fluorescence emission ratio (i.e., YFP/CFP) and analyzed SL by measuring the fluorescence profiles. As a result, we found that SL changed in response to a change in the YFP/CFP ratio. The present experimental system is useful for simultaneous imaging of local Ca²⁺ and single sarcomeres at high spatial and temporal resolution in living cardiomyocytes in health and disease.