2P307 細胞内分子混雑感受性蛍光蛋白質の開発
Glycine-inserted mutant Forster resonance energy transfer
(FRET) fluorescent protein to evaluate intracellular crowding

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The Intracellular environment is very crowded, containing various molecules, proteins and nucleotides. This crowded condition is an indispensable factor for cellular functions of proteins. In the past, the diffusion coefficient of a chemical probe has been used as an evaluation index of the intracellular crowded condition. However, crowding depends not only on the mobility, but also the density of the crowding agents. To measure the density of the crowding agents, we developed a sensor for crowding density via hydrophobicity based on fluorescent protein engineering. Combined the sensor with measurement of diffusion coefficient, we revealed the diffusion coefficient alone is insufficient for defining crowding.

2P308 赤外超解像イメージングによる毛髪 α -ケラチンの分子配向観察
Orientation-sensitive IR super-resolution imaging of human hair α -keratins

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Vibrational sum-frequency generation (VSFG) detected IR super-resolution microscopy has a possibility to measure the orientation-sensitive IR image with sub-micrometer scale spatial resolution. In this study, we applied this microscopy to human hair samples and succeeded in the orientation-sensitive IR super-resolution imaging of human hair α -keratins. In the case of the amide III band at 1250 cm⁻¹, the human hair gave strong VSFG signals in the cortex area. This enabled us to observe the distribution of α -keratins. On the other hand, VSFG signals disappeared completely when the amide I band at 1650 cm⁻¹ was monitored. From the polarization dependence of VSFG signals, it is concluded that α -keratins are well oriented along the longitudinal direction of the human hair.

2P309 オルガネラの低温コヒーレント X 線回折イメージング
Cryogenic coherent X-ray diffraction imaging of cellular organelle particles

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Coherent X-ray diffraction imaging (CXDI) bridges a serious gap between optical and electron microscopy regarding the spatial resolution and thickness of sample particles. In CXDI experiment, spatially isolated sample particle is irradiated by coherent X-rays, and the electron density map projected along the direction of X-ray is retrieved from diffraction pattern using the phase-retrieval method. We have developed a suite of X-ray diffraction devices suitable for cryogenic CXDI experiment of organelle particles at a resolution of better than 50 nm using X-ray free electron laser pulses. Here we report our experimental devices, protocols and analysis method for diffraction patterns from chloroplast of schyzon and nucleus of yeast in a period of the cell cycle.

2P310 Real-Time Observation of Single Macromolecular Rotation Using Gold Nanorods

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Gold nanoparticles have higher absorption and scattering cross section. They don't photobleach as seen in fluorescent dyes. These properties make gold nanoparticles suitable for long-time and fast optical imaging. In particular, the scattering light of gold nanorods is highly polarized along the long axis, allowing the anisotropic detection. Using dark-field microscopy and a birefringent prism with a cross-polarization imaging system, we directly visualized the single-molecule anisotropy change of individual gold nanorods indicative of the rotation motion of the attached macromolecule in real-time. We expect that this method can be used to monitor the RecA-family protein assembly process on DNA in high-resolution.

2P311 生細胞における 1 分子内在性 mRNA イメージングのための アンチセンスプローブの開発
Development of potent antisense probes for imaging individual endogenous mRNA in live cells

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Investigating gene-specific and dynamic behaviors of mRNA are important in understanding cell functions. To reveal those mRNA behaviors, we aimed individual endogenous mRNA imaging using antisense probes. Because unbound probes cannot be washed out in live cells, the probes with high binding rate are needed to detect individual mRNA. Here, we investigated relation between probe competence in live cells and the probe sequence. As a result, we suggest a general strategy for designing potent probes toward given mRNA targets. By complying with the strategy, we were able to obtain potent probes effectively, which enabled to observe bright spots derived from individual endogenous mRNA. These results highlighted the advantage of our approach in mRNA study.

2P312 広範囲な細胞内カルシウム濃度に対応する GECO 変異体系列
A series of GECO mutants suitable for calcium imaging in a wide range of calcium concentration

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Ca²⁺ ions have important roles as a second messenger by changing their concentration. To understand mechanisms of the signal transduction systems, it is crucial to image and quantify [Ca²⁺] changes inside living cells. In this research, we tried to capture [Ca²⁺] changes in cells by using GECO, one of the latest cpFP based Ca²⁺ sensors, which gives greater responses (fluorescence change) toward Ca²⁺ compared to previous indicators. However, it has narrow available range of [Ca²⁺] to use in cells where [Ca²⁺] differs drastically depending on compartments. To overcome this problem, we mutated GECO into several variants of different affinities for Ca²⁺ to observe [Ca²⁺] changes in large variety of [Ca²⁺] environments.