2P308 セミインタクト細胞リシール技術による細胞内への分子導入と病態モデル細胞構築への応用

Cell resealing technique for introducing molecules into cells and its application for establishment of disease model cells

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We demonstrate the novel method for introducing various molecules into cells by the cell resealing technique. First, the cells were permeabilized with streptococcal pore-forming toxin, streptolysin O (SLO). The permeabilization of plasma membrane allowed various molecules such as proteins, siRNAs, plasmids, and membrane-impermeable chemical compounds to enter into cells by diffusion. Then, the lesion of plasma membrane was repaired by addition of Ca²⁺, making the permeabilized cells become intact again. We call these cells “resealed cells”. In this study, we show the several examples of introducing proteins in resealed cells and the establishment of disease model cells by exchanging the normal cytosol to the pathogenic one as its application.

2P309 アポフェリチン空洞内に合成した Y 化合物を母体とした Eu および Tb ナノ粒子の発光特性

Photoluminescence Property of Eu and Tb Doped Y Based Nano-Phosphor synthesized in an apoferritin cavity

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Eu and Tb doped Y (Y:Eu and Y:Tb) nanoparticles are synthesized in an apoferritin cavity of 7 nm diameter. Electron diffraction pattern of Y nanoparticles corresponds to the Y₂O₃ crystal spacing. Y:Eu nanoparticles exhibit red photoluminescence (emission peaks: 590 and 614 nm), while Y:Tb nanoparticles exhibit green photoluminescence (emission peaks: 488, 544, 582 and 618 nm). The main excitation peak of Y:Eu nanoparticles is detected as 252 nm, which is assigned to be Eu-O charge transfer transition. The intense excitation peak of Y:Tb nanoparticles is observed at 237 nm, which is attributed 4f⁸-4f⁷5d¹ transition of Tb³⁺ ion. The most optimal dopant content for luminescence of Y:Eu and Y:Tb nanoparticles in apoferritin cavity are about 60% and 40%, respectively.

2P310 オズモシス流による FET ナノボア付近の DNA の動き制御
Controlling the fluidic motion of DNA molecules near FET nanopores by electro-osmotic flows

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Recent trends of nanopore related researches are the translocation studies of DNA through nanopores fabricated on conductive membranes called FET nanopores as the membranes for gate electrodes. The gate potentials can be varied relative to the biased potentials applied to trans/cis fluidic solutions. As a result, the polarity and the concentrations of ions near nanopore can be controlled and this induces electro-osmotic flows through the nanopores. We will present the observation of the fluorescent tagged DNA's motions in the flows near nanopores. We have carefully estimated the flow velocities of ionic solutions. Finally, we will discuss the possibility of removing heavily clogged DNA's in pore holes using the electro-osmotic flows.

2P311 ナノ・マイクロファイバゲルマトリックスの弾性設計による三次元細胞運動制御

Mechanical control of 3-D cell movement in elasticity-tunable matrix of nano/micro-fiber gels

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The cell movements are critically affected by the stiffness of extracellular surroundings. To scrutinize the mechanobiology of the cell motility, systematic design of the extracellular mechanical surroundings is quite essential. In this study, we developed elasticity-tunable matrix of nano/micro-fiber gels which consists of nonwoven mesh of swollen fibrous gels, in order to clarify the effect of matrix stiffness on the 3-D cell movement. By regulating the fabrication conditions, elastic modulus of fiber gel sheet was well-tuned. Assessment of movement of 3T3 fibroblasts on the fiber-gel sheets with different stiffness showed that cells tend to get into only harder sheet. This result suggested that 3-D cell mechanotaxis can be driven depending on matrix-stiffness.

2P312 In vitro selection of peptide aptamer binding to reduced ferredoxin

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Photosynthesis is the only known biological process that can convert solar energy into organic substances. The photo-induced electrons produced by the photosystems are transferred to a small redox protein named ferredoxin. If we could use the reduced ferredoxin for our purposes including the productions of solar cell and an artificial substance, we would open up new possibility to harness solar energy. Thus motivates we used ribosome display technique to select the peptide aptamers that binds to the reduced ferredoxin. After the eight rounds of ribosome-display selection, we obtained eleven candidate peptides. We will discuss about those binding assays to the reduced ferredoxin.

2P313 Selection of RNA aptamer binding to a photoredox catalyst

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Aiming for the application of Ru(bpy)₃²⁺ (Ru) as a redox enzyme, we selected its RNA aptamer. We selected 8 RNA sequences after 12 rounds of SELEX. Truncation experiments of one of the RNA sequence identified a minimum functional sequence that possesses high binding affinity, 183nM of K_d. The phosphorescence intensity of Ru increases 3.3 folds upon binding of the RNA; in the same line the phosphorescence lifetime is elongated 4.2 folds. We found that the Ru-bound RNA exhibits a cooperative melting curve, whereas the free RNA does not. Those observations suggest that RNA tightly interlocks the Ru inside and thus the phosphorescence intensity increases due to reduction of the quenching efficiency by dioxygen and energy dissipation to water molecules.