2P085 Rapid monitoring of affinity maturation process for in vitro selection by fluorescence correlation spectroscopy (FCS)

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In vitro selection (one of evolutionary molecular engineering to generate functional biomolecules) has required rapid analysis of each selection process to screen peptides and proteins with a high affinity. This study showed that affinities of the whole selected molecules in the library were measured by fluorescence correlation spectroscopy (FCS) rapidly. We synthesized a fluorescent labeled peptide library by using both a puromycin linker and a cell-free translation system. Then, we could evaluate the affinity maturation process of the whole library by FCS analysis of molecular interaction between the target molecule and each labeled library. As a model selection, in vitro selection against Anti-FLAG antibody with a peptide library was performed by cDNA display.

2P088 cDNA ディスプレイ法を用いた Minimum プロテアーゼの試験管内進化***In vitro* selection of Minimum-Protease by cDNA display**

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Development of more useful enzymes has been expected in various industrial fields. *In vitro* display technologies such as ribosome display, mRNA display and cDNA display enable an efficient artificial protein evolution by using a huge mutant library. However, a few artificial evolution experiments of proteins by *in vitro* display technologies have been succeeded until now.

Recently, we have started the challenge to perform the evolution of a minimum-metalloprotease by cDNA display. The system was designed to select the cDNA coding an active protease by releasing it from the solid-phase when the displayed protease could cleave the peptide substrate in the puromycin-linker. In this presentation, we will show the selection system in detail and discuss about some results.

2P086 DNA 配列相補性を用いた DNA 修飾アクチン繊維の束化制御**Control of bundle formation of DNA-conjugated actin filaments using the complementarity of the DNA**

Masahito Hayashi, Kingo Takiguchi (*Grad. Sch. Sci., Nagoya Univ.*)

We are trying to construct amoeba-like molecular robots based on a giant liposome that encapsulates DNA computers and protein actuators. To make an informational linkage between the computers and actuators, we developed DNA-conjugated actin (DNA-actin) using heterobifunctional cross linkers. The DNA-actin kept its filament structure when the coupling ratio was up to 50%. In the presence of HMM, ATP and methylcellulose, DNA-actin filaments formed aster-like structure, suggesting that DNA-actin filaments keep their basic function as active cytoskeleton. When DNA-actin filaments with sense and antisense ssDNA were mixed, they immediately formed dense bundles, indicating that we can control their bundle formation using the complementarity of the conjugated DNAs.

2P089 ナノ粒子表面セルラーゼモジュールシャッフルリングによる効率的人工セルロームデザイン**Evolutional cellulosome design from module library**

Hikaru Nakazawa, Yuri Ishigaki, Eiko Kobayashi, Do-Myoung Kim, Mitsuo Umetsu (*Grad. Sch. Eng., Tohoku Univ.*)

Recently, we propose a new design of artificial cellulosome to enhance cellulase activity: biotinylated catalytic domain(CD) and carbohydrate binding module(CBM) was clustered on streptavidin-modified inorganic nanomaterials via biotin-avidin interaction, and the design of CBM valency and synergistic effect of CD showed drastic enhancement of catalytic activity. Here, we constructed a biotinylated CD and CBM library with a kind of 65CDs and 21CBMs selected from cellulases reported in PDB, Pfam and Cazy database, and the appropriate cluster format attempted to be identified by analyzing the phosphate acid swollen cellulose degradation. Consequently, we succeeded in conveniently identifying the optimal format of the cellulosome and optimal combination of CD and CBM.

2P087 Regulation of proteasomal degradation through an unstructured initiation site of a substrate

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Efficient proteasome-mediated degradation requires an unstructured initiation site in addition to the ubiquitin modification. We investigated whether the proteolysis by the proteasome could be inhibited by a small molecule that modifies the property of the unstructured initiation site of the substrate using model substrates. As a result, in the presence of the small molecules, the model substrates could escape from the degradation by the purified proteasome and the endogenous proteasome in the HEK293T cells. Our experiments suggest that similar mechanism exist for some of the proteasome substrates *in vivo* and provide a useful strategy to selectively control the cellular concentration of specific proteins.

**2P090 Green Fluorescent Protein からの機能エレメントの抽出
Extraction of Function Elements from Green Fluorescent Protein**

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Function element is defined as a region responsible for function. The chromophore of GFP is reconstituted from own amino acid residues (SYG) involved in the amino acid sequence. We attempted to extract function elements related to the spontaneous reconstruction of the chromophore in GFP. For the purpose, we prepared all possible alanine insertion mutants of GFP ranging from the 50th to the 102nd amino acid residue containing the SYG sequence. The fluorescence of each insertion mutant was examined with the mutant-containing colony on a cultivation plate and supernatant of cell extraction under blue light. From the results, we concluded that the function element responsible for the fluorescence is the region from the 53rd to the 76th residues.