2P079 MMV-4SR:シングルセル生物学(SCB)基盤検出系の開発
MMV-4SR : Development of basic detection system in Single Cell Biology (SCB)

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Manipulating a single cell with the conventional system is not easy due to the loss occurring in operations. Since the scale of operation is inevitably minute in SCB, any special handling tools are necessary to deal with such a minute quantity. At the same time, novel detection systems need to be developed besides the conventional microscopic one. Considering these, we have developed the operation system at the micro-scale, that is, MMV (Microarray with Manageable Volume), which does not require pipette operations. In addition, we devised the 4SR (Stacked Slice-gel System for Separation and Reactions) which can detect DNA/RNA derived from a single cell owing to the PCR-amplification and ELISA mechanism equipped with the MMV. Here, we report this novel 4SR system.

2P080 表面増強赤外分光法によるモデル脂質膜上におけるタンパク質フォールディングの動的挙動の解析
Surface Enhanced IR study of Protein folding dynamics at a solid support lipid layer

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Surface Enhance Infrared Absorption (SEIRAS) have unique properties that enhances signals at vicinity of a substrate metal. This property is useful to determine solely the surface chemical process distinguished from that occurred in bulk phase. When a sample of interest is confined to such surface, one can selectively monitor a chemical reaction of the target regardless of the complex ensemble biological process in the bulk phase. We present an application of SEIRAS to in-situ investigation for following folding process of protein on the artificial lipid bilayer, a) aggregation and pore formation of Melittin, b) pH induced pore formation of Anthrax Protective Antigen, c)folding of bacteriorhodopsin during cell-free expression

2P081 Microtubule-kinesin binding assay to differentiate wild and mutant 4R tau proteins

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We have extended the application of kinesin-microtubule molecular set-up for differentiating wild and mutant 4R tau proteins. Microtubule-associated protein tau is a biomarker for neurodegenerative conditions related to tauopathies. We target the excessive need of non-immunological tau detection; by assaying tau-decorated TAMRA-labeled MTs in a kinesin-coated micro device comprising of MT reservoir, channel and collector region. Wild tau intervenes in MT-kinesin binding, eventually reducing the landing rate, density and velocity of MTs, which is reflected by the number of MTs collected at the collectors. By measuring the fluorescent intensity at the collector regions, we were able to distinguish wild and mutant taus.

2P082 高速 AFM による Ascaris 精子由来の MSP 繊維の観察
Direct observation of MSP filaments in cell-free extract from Ascaris sperm by Atomic Force Microscopy

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Crawling movement in eukaryotic cells require dynamic assembly and disassembly of cytoskeleton. In Ascaris sperm, motility is powered by an unique cytoskeletal protein, called Major Sperm Protein (MSP), instead of actin. To understand the property of MSP filament, we have observed them attached to the aminosilane-coated mica surface by AFM. Despite of high protein concentration (~1-2 mg/ml) in cell-free extract from Ascaris sperm, filamentous structures were clearly visible. Analysis of 245 filaments showed that their average length and diameter are 205 nm and 9.2 nm, respectively. Further analysis of filaments attached partially to the surface also revealed that MSP filaments are highly flexible unlike actin filaments.

2P083 固体 NMR を用いた単一細胞あたりの特定タンパク質の分子数計測
Counting of the target recombinant protein molecule in an intact Escherichia coli cell by solid-state NMR

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The structural biology which clarifies the structure and function of proteins is essential to understand lives. A lot amount of target proteins is usually obtained by an expression system of *Escherichia coli*. However the quantification of the protein synthesis ability of *E. coli* was not reported. Here, we report the measurement of the number of recombinant protein molecules in living *E. coli* cells by using quantitative solid-state NMR. First, the relation between the integral value of ^{31}P 1D spectra and the number of cells was obtained by using cell counting. Next, the amount of the protein were evaluated from the signal intensity of the high-resolution ^{13}C NMR. From these results, we calculated the number of the protein molecules in an *E. coli* cell.

2P084 リポソーム内での再構成リボソームの翻訳活性
Translation activity of a reconstructed ribosome in liposomes

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In vitro ribosome reconstruction is a critical step towards the directed evolution of ribosome. Here, as an initial step towards this goal, we report a method to reconstruct E. coli ribosomes in a cell-free translation system (PURE system) and the measurement of the translation activity in liposomes. We reconstituted E.coli ribosome in PURE system in a co-transcriptional manner from purified 16S rRNA, 30S proteins, and 50S subunit. The ribosomes were encapsulated into liposomes, and the translation activities were measured by flow cytometer. This method enables highly sensitive detection of translation activity of the reconstituted ribosome.