3N0936 Modification and labeling of complex molecular machines for single molecule manipulation**Morten Bertz**, Kazuhiko Kinoshita (*Waseda University, Dpt. of Science & Engineering*)

In the past decade, single molecule techniques have offered a wealth of insight into the functions of complex biological systems. Molecular machines involved in a great variety of cellular functions ranging from cellular replication over protein homeostasis to active transport and locomotion have successfully been investigated. Using advanced imaging techniques the movements of these fascinating proteins could be elucidated. Taking things a step further, single-molecule manipulation methods have allowed determining the forces involved in molecular transitions and, to a certain extent, to actively reverse a molecular machine's course of action. All these techniques share the need for an attached probe for observation or manipulation. With the ever-increasing complexity of the molecular machines under investigation, however, efficient and specific attachment of these reporters is becoming increasingly difficult, especially if observation in the native context of the protein is desired. Here, we will compare and discuss several methods for labeling protein machines with simple and complex probes for single molecule manipulation, with special emphasis on modifications carried out *in vivo*.

3N0948 分子内複数同時アミノ酸置換による一本鎖抗体の改変**ScFv engineering by simultaneous random mutagenesis of multiple interior residues**Genta Hayashi, Nobuya Itoh, **Yoshihide Makino** (*Dept. Biotechnol., Fac. Eng., Toyama Pref. Univ.*)

Single-chain Fv immunoglobulin fragment (scFv) is expected to be useful in many applications. Expression of scFv in *E. coli* can be advantageous for large-scale production, but often leads to the formation of inclusion bodies. In this study, we engineered scFv for efficient expression without loss of function in *E. coli*. When 4M5.3 scFv (Boder et al. (2000) *PNAS*, 97, 10701) is expressed in *E. coli*, most of the protein become insoluble. 4M5.3 scFv was engineered to achieve higher level of expression while maintaining its affinity to fluorescein. Three amino acid side-chains, which are sterically-proximal in the interior of the protein, were randomly substituted. Each mutant was fused to the green fluorescent protein to monitor soluble expression. The affinity to fluorescein was examined by ELISA. Many clones had higher fluorescence intensity as compared to the original clone. The affinity to fluorescence was also improved in many clones. The integrated clone, which had all of the mutations found in the best clones of the solubility and the affinity, showed more improved affinity to fluorescein. These results suggest the effectiveness of the methodology for scFv engineering.

3N1012 In vitro selection of protease: an approach towards the evolution of an enzyme using in vitro display method**Shingo Ueno**^{1,2}, Naoto Nemoto^{1,2} (¹*Grad. Sch. Sci. and Eng., Saitama Univ.,* ²*CREST*)

The artificial evolution of enzymes is one of the ultimate goals of evolutionary molecular engineering. The display technologies, such as phage display, mRNA display and ribosome display have been widely applied for affinity selection against various targets in the last two decades. On the other hand, although some groups have been challenged to evolve enzymes using phage display, there were only few examples in the case of mRNA display and ribosome display. One of its reasons might be the liability of mRNA moiety in the protein-mRNA conjugate molecules in these technologies. Recently, we have developed cDNA display technique which involves covalent binding of the cDNA and its coded protein via a puromycin linker. Thus, the protein-DNA conjugated library generated by cDNA display could be stable against degradation by contaminated ribonucleases and also employed in sever screening conditions (ex, in blood, serum, cytoplasm for medical enzymes and in high-temperature, alkaline pH, organic solvent for industrial enzymes).

In this study, to evolve a protease "subtilisin" as a model of enzymes, we designed a newly puromycin linker of cDNA display for *in vitro* selection in the same manner of ribozyme selection.

3N1024 Towards in vitro autonomous evolution of self-referring in vitro virus displaying an RNA replicase, 4**Hideao Arai**¹, Manish Biyani², Yuzuru Husimi³ (¹*Dept. of Functional Materials Sci., Saitama Univ.*, ²*Dept. of Bioeng., Grad. Sch. Eng., Univ. of Tokyo*, ³*Innovative Research Organization, Saitama Univ.*)

We have been tried to construct an autonomous protein evolution system, combining the *in vitro* virus (IVV) and the one-pot isothermal RNA replication reaction. In this system, the norovirus RNA-dependent RNA polymerase (3D^{pol}) is displayed on the IVV. Using this IVV displaying the RNA replicase and two RNA primers, we designed the one-pot isothermal replication reaction system, in which the IVV can amplify itself, and evolve autonomously. Moreover, this reaction system will evolve not only the RNA replicase but also the amplification algorithm itself. 3D^{pol} was synthesized in cell-free translation system. When the enzyme was incubated with RNA template without any primer, 3D^{pol} synthesized a complementary RNA strand in a primer-independent manner. On the other hand, when it was incubated with a template (RNA)-primer (RNA) hybrid, the primer-dependent replication was realized, but this activity of 3D^{pol} was not so high, as was reported [J. Rohayen et al., (2006)]. It was observed also that 3D^{pol} made a long hairpin through the self-priming of an RNA template that has a very small self-priming structure at 3'-terminus. It was shown that 3D^{pol} discerned the 3'-terminal structure and preferred the self-priming structure. Using this replicase, we may achieve the much simpler *in vitro* evolution system than the traditional Q β replicase system, because Q β replicase is a tetramer including three proteins from the *E. coli* translation system, and this fact may lead to complex interference behaviors in the autonomous protein evolution experiments.

3N1036 天然のタンパク質のアミノ酸配列における隠れた反復性の解析**An investigation of pseudo-repetitive amino-acid sequences in native proteins**Koji Inai, Tomomi Nitta, **Masahito Oka** (*Osaka Prefecture University, Faculty of Liberal Arts & Sciences*)

Using currently available protein data base, it is tried to investigate the repetitive amino-acid sequences of periodic proteins for inspecting the hypothesis on the amino-acid sequences of the earliest proteins. A total of 8286132 proteins have the repetitive amino-acid sequences among 12619557 proteins, suggesting that repetitive amino-acid sequences are generally found in the native proteins. It was also shown that they are divided into several groups such as Pro-rich, Gly-rich, Ser-rich, Ala-rich, Gln-rich ones and others. Then, using autocorrelation function method, it is tried to investigate the cryptic periodicity of amino-acid sequence, which cannot be found in the usual method aimed at the strict amino-acid sequence. Zinc finger protein160 has no repetitive amino-acid sequence, however, it is shown that this protein has 28 residue periodicity in its amino-acid sequence by autocorrelation function method. Through the analysis, many quasi-repetitive sequences were found, which suggest that this method is effective for searching the evidences of amino-acid substitutions in molecular evolution process.

3N1048 マイクロ流路を用いたテトラヒメナの一細胞観察**Single cell observation of *Tetrahymena thermophila* using microfluidic devices****Itsuka Kumano**¹, Makoto Sueyoshi¹, Katsuki Hirata¹, Kazufumi Hosoda¹, Hiroaki Suzuki^{1,2}, Tetsuya Yomo^{1,2} (¹*Inf Osaka-u.*, ²*ERATO JST.*)

Endosymbiosis, defined as a symbiosis in which one organism lives inside the other, is ubiquitous in nature. Some of cell organelles are also said to be results of endosymbiosis between two different organisms. The establishment of endosymbiosis is thus important process in evolution; however, the details of how endosymbiosis was established are still unclear because it occurred in the past and cannot be observed directly. To directly observe the establishment of endosymbiosis, we are trying to construct a new endosymbiosis in a laboratory. Specifically, we cocultivated a model predatory protozoan *Tetrahymena thermophila* (Tetra) and a model bacterium *Escherichia coli* (*E. coli*) for the experimental coevolution. In this system, to evaluate the relationship between the two organisms in the evolutionary process, observation of both the growth of the Tetra cell and the growth of *E. coli* inside the Tetra cell is required. However, it is difficult due to their high-motility. In this study, we have constructed a microfluidic device as a new method that enables single-cell observation of living motile cells. In this device, we designed bifurcated channels in which one channel (C1) had a trap with a narrow (~9 μ m) drain for Tetra cell and the other (C2) did not. When the trap in C1 has a Tetra cell and the drain is blocked, other Tetra cells flow into C2. Therefore, living cells can be trapped in isolation. By using this device, we succeeded at trapping Tetra and observing their divisions and dynamics of *E. coli* inside Tetra.