high-throughput alternative to FSEC.

3P098 計算機による“理想”タンパク質構造の新規デザイン
3G1150 Computational de novo design of “ideal” protein structures
 Nobuyasu Koga, Rie Koga, David Baker, (University of Washington, Department of Biochemistry)

De novo design of an arbitrary protein structure is a stringent test of our knowledge of protein folding and provides us a challenge to explore the essential factors making amino-acid sequence foldable. Native protein structures belonging to a fold have many varieties of secondary structures (SS) lengths and complicated features such as long loops, bent helices and bulge of strands, due to functional requirements and neutral mutations during evolution. Here, free from functional and evolutionary constraints, we computationally designed “ideal” structures completely from scratch. First, we performed folding simulations of alpha+/beta folds with many different SS lengths using Rosetta, and found the folding probability to a correct topology was dependent on the SS lengths. This finding led to the establishment of the methodology that allows us to design alpha+/beta folds rationally. Choosing the optimal SS lengths that maximize the foldability, we readily created the proteins having smooth funnel-like energy landscape in computation. Finally, the designed structures of Ferredoxin-like and Rossmann2x2 folds based on this method showed actual foldability in experiments. The structures solved by NMR, 2kl8 and 2kpo, were in agreement with the computational models, RMSD <1.3 Å, and were highly stable and did not unfold around 100C.

3P099 Protein engineering approach for point bioconjugation: How
3G1205 a specific lysine residue can be designed on an antibody for
bioconjugation

sanada hideaki, umetsu mitsuo, asano ryutaro, kumagai izumi, (Dept. Biomol. Eng. Grad. Sch. Eng. Tohoku Univ.)

Conjugation of polyethylene glycol (PEGylation) on downsized compact antibodies is an effective method for overcoming rapid elimination of compact antibodies from bodies. In this study, we attempted to design a downsized compact antibody with a diminished number of lysine residues to restrict candidate locations for chemical modification via lysine residues.

A humanized bispecific diabody with epidermal growth factor receptor (EGFR) and CD3 retargeting (hEx3), which is a dimer of two kinds of single-chain Fv (scFv) constructed from humanized anti-EGFR Fv (h528) and anti-CD3 Fv (OKT3), has 20 lysine residues. For the replacement of lysine in complementarity determining regions (CDRs) of 528 domains, we utilized phage display method to select CDRs without lysine residues; consequently, we have selected h528Fv without lysine in CDRs but with native activity. For frameworks (FRs) of h528 and OKT3 domains, we replaced lysine with the other residue with highest frequency of appearance at each location on Kabat database information to avoid inactivation of antibodies.

According to the mentioned replacement, mutant hEx3 diabodies were prepared by *E. coli* expression to measure the binding affinity for EGFR and CD3 by using flow cytometry. Inactivation against CD3 was observed for the mutant hEx3 without lysine residues in all four domains, while the hEx3 mutants without replacement of lysine in OKT3 VL domain retained the binding affinity for CD3. The lysine residues in OKT3 VL domain is important for the binding affinity for CD3.

3P100 共発現による封入体化を利用した効率的な短鎖ペプチド生産技術
Enhancement of the short peptide's expression level by coex-
pression of the aggregation-prone protein

Tomoyasu Aizawa, Satoshi Tomisawa, Masakatsu Kamiya, Takashi Kikukawa, Makoto Demura, Kei-ichi Kawano, (Grad. Sch. of Life Sci., Hokkaido Univ.)

Many protein expression systems have been established to produce recombinant proteins. Moreover, a wide variety of strategies have been developed to improve the yield of recombinant proteins. One such strategy, fusion protein systems have generally been used to improve protein folding and solubility, yielding biologically active products. However, these approaches are not always successful. In particular, it is very difficult to produce target peptides and proteins that are lethal to host cells and/or are easily degraded in soluble form. We present here a novel method of facilitating the expression level in *E. coli* of a recombinant short peptide that is difficult to express in conventional production systems. We demonstrated that coexpression of the aggregation-prone protein remarkably enhanced the target peptide's expression level. It seems that overexpression of the partner protein protects the target peptide from proteolytic degradation by forming insoluble inclusion bodies, thus accounting for the higher observed yields. This method can isolate the target peptide from the partner protein by simple chromatography, and it never requires chemical or enzymatic cleavage of the fusion protein. These advantages make our new

method sufficiently effective and cost-efficient for large-scale production.

3P101 Aβ42 結合ペプチドの生物活性を測定するセル/ウェルベース
ストバイオアッセイの開発
Development of a cell/well-based bioassay for the biological
activity of Aβ42-binding peptides

Ran Gu¹, Mizuho Sawada², Sachika Ueno-Tsuji¹, Masayuki Komatsu¹, Koichiro Kitamura¹, Yuko Hotta³, Kenji Sakimura³, Miho Suzuki¹, Naoto Nemoto¹, Koichi Nishigaki¹, (¹Saitama University; ²Janusys Corporation; ³Niigata University)

Cell-based bioassay, royal road for finding biological functions of molecules, is now advancing to a new stage where exhaustive searches of functional molecules are possible. Our novel approach that exploits the well-type microarray (MMV) has several advantages in this trend: it can accept specifically different conditions/molecules for each micro-well owing to its open aperture arrayed in a regular mode, thus enabling thousands or more different reactions monitored at the same time. In addition, hundreds fold reduction of reagents required per sample makes otherwise impossible experiments due to its high cost performable. In this study, we tried to construct a bioassay system for which one can find Aβ-induced apoptosis-inhibiting peptides. That is, the esterase activity of a neuron cell (Neuro-2a), which is an indicator of the viability of cell and can be monitored using a fluorogenic substrate, was successfully measured in micro-wells in an MMV, denoting that the MMV can work as culture vessels as well as reaction chambers. Now, the MMV turned out to be a thousand of micro-dish usable for culturing cells and can be combined with a specific peptide for each wells leading to the measurement of 1000-ple different reaction.

3P102 シュードゲノム解析：セルタイプ/セルフーズの迅速モニター
Pseudo-Genome Analysis: Rapid monitoring of cell-types
and cell-phases

Takahiro Watanabe, Ran Gu, Yuta Nomura, Shun Komazaki, Shamim Ahmed, Miho Suzuki, Naoto Nemoto, Koichi Nishigaki, (Saitama university)

In cell researches, we often come to the necessity to identify cell types and cell phases: multi-typed cell system analysis or cell phase difference analysis. For this purpose, antibody-based and microarray technologies have been developed; e.g., fluorescence immunostaining and cDNA microarray analysis. These are powerful and effective for a wide range of studies. However, they need to be fabricated in advance and applicable to limited samples, which is very inconvenient for Cell Biology at large that needs to deal with cells of various phases and diversities of cells.

In this study, we have developed a compact and general method to identify cell types and cell phases: *Pseudo-genome analysis* composed of 1) reverse transcription of the whole mRNA, 2) random PCR, and 3) microTGGE (temperature gradient gel electrophoresis) processes. This method is equivalent to random sampling of the whole mRNA population (i.e., only a small portion of mRNAs, yet sufficient to characterize the whole population just as random sampling used for the poll prediction) and sequence-analyzing by μTGGE without sequencing. This is relatively rapid and convenient and, what's more, does not require sophisticated apparatuses. Therefore, this method is very plausible to be used for various purposes. We have just begun to use this technology to determine the effect of cell-stimulating reagents such as hormones and apoptosis-inducing peptides. We will be able to report on these study also.

3P103 Characterization of artificial SH3 domains with primitive al-
phabets

Junko Tanaka, Hiroshi Yanagawa, Nobuhide Doi, (Keio Univ., Grad. Sch. Sci. and Tech.)

Although the modern proteins consist of 20 kinds of amino acids, it has been proposed that primordial proteins consisted of a limited set of primitive amino acids, such as Gly, Ala, Val, Asp and Glu, which could have been formed abiotically. To examine whether a set of primitive amino acids can generate folded and functional proteins, we constructed three kinds of random-sequence libraries of a SH3 gene whose half positions were replaced by random codon YNN (Y = T or C; N = T, C, A or G), RNN (R = A or G) or NNN. The RNN library mainly consists of the primitive amino acids, whereas the YNN library is mostly constituted of the amino acids that are assumed to have lately recruited to the genetic code. We searched for sequences of SH3 domains capable of binding to a specific ligand peptide from these libraries using mRNA display technique. The active SH3 domains were enriched in RNN and NNN libraries, while they were not enriched in YNN library. This result supports that the primitive alphabets are important for acquiring protein function.

In this study, we characterized affinities and thermal stabilities of the selected proteins from RNN and NNN libraries by Trp fluorescence and CD spectra measurements. The active RNN and NNN proteins showed similar biophys-