3P085 X 線 1 分子追跡法を用いたタンパク質過飽和溶液中における核形成前駆体クラスターの動態観察
Dynamical Observations of Prenucleation Clusters in Supersaturated Protein Solution from Diffracted X-ray Tracking

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Recently, comprehension of supersaturated phenomena in protein solution is one of the most important subject. For example, elucidation of amyloid fibril formation process which is known as a main causative agent causing Alzheimer disease. In our study, we utilized Diffracted X-ray Tracking (DXT) as a high time resolution (100us) and high positional accuracy (pm) detection system for the time resolved dynamical interactions between a single gold nanocrystal and localized prenucleation clusters in supersaturated solution (at SPring-8, BL40XU). At this presentation, We explain the DXT method and obtained physical parameters of clusters as a dynamic, size and occupied spaces by DXT analysis. In finally, We present a comprehensive model of protein supersaturated solutions

3P086 細菌由来アルブミン結合ドメインの接触表面の模倣によるアルブミン結合ヒト型タンパク質のデザイン
Design of an Albumin-Binding Humanized Protein by Mimicking the Contact Surface of a Bacterial Albumin-Binding Domain

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Attachment of a bacterial albumin-binding protein module is an attractive strategy to prolong the plasma residence time of protein therapeutics. However, the induction of an unfavorable immune reaction by bacterial albumin binding module-fused protein or peptide is concerned.

To address this issue, we designed an alternative albumin-binding protein by transferring key residues of a bacterial albumin-module, onto human derived 6 helix-bundle proteins. We utilized structural information to avoid the deleterious mutation in the design.

As a result, one of the designed proteins specifically binds to human serum albumin (HSA). Competitive binding assay and thermodynamics analysis showed that the best binder have similar HSA-binding site of bacterial module.

3P087 ファージディスプレイ法で同定した白金結合アミノ酸配列の解析
Analysis of a platinum-binding amino acid sequence identified by phage display

Asumi Kaji, Hiroya Niino, Satoshi Akanuma, Tatsuya Uchida, Akihiko Yamagishi (Tokyo University of Pharmacy and Life Sciences)

Creation of a metal-binding site on a desired position of a protein may contribute to develop a highly sensitive biosensor. Previously, we created a platinum-binding loop on an artificial four-helix bundle protein, LARFH. In that study, we constructed a LARFH library that contained random amino acid sequences within a loop and then selected a LARFH variant interacting with platinum by using the T7 phage display system. The selected LARFH variant contained a 6-residue YKRGYK sequence at the loop. In this study, we analyzed the affinity of the LARFH variant to platinum by means of Quartz Crystal Microbalance analysis. As the result, we found that the YKRGYK sequence really enhances the affinity to platinum.

3P088 エングレイルドホメオドメインを用いた新たな転写因子の設計
Designing a new artificial transcription factor based on engrailed homeodomain

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Recently, zinc finger nucleases which are artificially fused a zinc finger transcription factor and a nuclease have been widely used for genome editing. However, the applications are limited because they only recognize GC-rich sequences. To make such artificial proteins recognize broader sequences other than GC-rich ones, we are rationally designing novel proteins based on an engrailed homeodomain. Engrailed homeodomain proteins recognize six bases. To recognize longer DNA sequences, we connected two homeodomains with a short linker. We will report that the designed molecule show a strong affinity and high specificity for the target sequence. We will also report a DNA sequence profile of the protein obtained by B1H assay.

3P089 Design of a peptide nanotube having the capability of rare metal binding

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There has been compelling need for recovery of precious rare metals from the urban mines these days. We focused on the peptide nanotube (PNT), which does not give a burden on the environment because it is biodegradable and water-soluble. It also leads to energy saving because it can be used at normal temperature and pressure. Therefore, PNT is valuable as a new material for the metal recovery technology. So far we have designed PNT by self-assembly of cyclic peptides. However, it can only bind to base metals. Here we try to transform the PNT to have the ability to bind the rare metals. As a result, it has been confirmed that the newly designed PNT can bind to palladium and silver, indicating that the PNT can be conferred the capability of rare metal binding.

3P090 脂肪酸アシル-ACP 還元酵素の迅速活性評価法の開発
Development of a high-throughput method to evaluate catalytic activity of fatty acyl-ACP reductase

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Alkanes produced in microorganisms are focused on as renewable alternatives of biofuels. Cyanobacterial fatty acyl-ACP reductase (AAR) catalyzes the reduction of fatty acyl-ACP to fatty aldehyde, which is an initial step in alkane biosynthesis. However, due to low catalytic activity of AAR, it is required to improve its activity for industrial use. Directed protein evolution is a powerful method to improve an enzymatic activity, but it requires a rapid and convenient method to evaluate the activity of a library of mutants. Here, we developed a high-throughput method to evaluate the catalytic activity of AAR. This enables high-throughput screening of E. coli colonies expressing active AAR from random mutant libraries generated by error-prone PCR.