1P085 Attempt of expression of the glycoprotein from Richadella dulcifica

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A plant Richadella dulcifica has a glycoprotein; miraculin. This protein makes homodimer structure by forming a disulfide bond at the position of Cys138. In addition, this protein has three disulfide bonds in one subunit. Generally, when it carried out overexpression of protein using E.coli, the various problems, such as the formation of inclusion bodies, can occur. In this study, we constructed an overexpression system using E.coli BL21 with plasmid pET-16b in order to obtain the active form of the protein. As a result of expression, the protein was confirmed but it was isolated insoluble fraction for the inclusion body. We examined the various plasmid, species, strain and culture conditions. Further, the purification procedure was also examined.

1P086 アルカンを合成するラン藻由来アルデヒド脱カルボニル化酵素のアラニンスキャン変異解析

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Cyanobacteria synthesize a small amount of alkanes from fatty acyl-ACP using aldehyde decarboxylase (AD). However, little is known about its function. To clarify which residues are responsible for its catalytic activity, we have been carrying out alanine-scanning mutagenesis of AD from Nostoc punctiforme. So far, 184 sites among 231 residues of AD (79%) are substituted into Ala one at a time. The amount of hydrocarbons produced in E. coli, in which mutant AD is overexpressed, is measured by GC-MS. We find that some residues are essential for the activity of AD, because their substitutions into Ala greatly reduced the activity. On the other hand, changes at some other sites enhance the activity of AD. These mutations are useful in improving the alkan production of AD.

1P087 An Information Theoretical Approach to Local Equilibrium State Analysis for Single-Molecule Time-Series

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Extraction of sub-ensemble molecular properties and behavior is now a well-known capability of single-molecule experiments. Recently, local equilibrium state (LES) analysis was developed as a means of extracting 'local equilibrium states' from a scalar time series. LES analysis is comprised of three components: (1) constructing short-time distributions, (2) computing distortion among these distributions, and (3) clustering the distributions into local equilibrium states. Here we apply the methods of rate-distortion theory to LES analysis and demonstrate its ability to identify conformational states within both simulated and experimental single-molecule FRET data without a priori knowledge of the number of states that underlie the empirical data.

1P088 理想タンパク質構造のデザイン原理

Principles for designing ideal protein structures

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We describe an approach for designing ideal protein structures stabilized by completely consistent local and non-local interactions. The approach is based on a set of rules relating local structures to non-local structures, which was identified using folding simulations and analyses of naturally occurring proteins. Building backbone structures according to the rules, and placing sidechains stabilizing the backbones, we can readily design the proteins which have funnel-shaped energy landscapes. Using the approach, we designed ideal protein structures consisting of α-helices, β-strands and minimal loops with the Rosetta program. Designs were found to be monomeric and very stable and to adopt structures in solution nearly identical to the computational models.

1P089 理想的な構造を持つ機能タンパク質の理論設計

Theoretical design of functionalized proteins with ideal scaffold

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Many artificial functionalized proteins, for example enzymes and small molecule binding proteins, have been reported, because they are interesting and important for both academic research and industry. Some proteins of them have higher affinity than that of the native proteins. However, these designed proteins have almost the same structure with a native protein because they are not designed from scratch and the functions are improved based on a native structure. Recently, a theoretical approach to make an arbitrary ideal protein scaffold from scratch has been developed and some stable proteins were designed successfully. In this study, we improved this approach and made some functionalized proteins from scratch without using the structure of native protein.

1P090 リポソーム内遺伝子発現を利用した進化工学によるβ-グルクロンダーゼの機能変改

Directed Evolution of β-glucuronidase Using Liposome-based IVC

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Liposome-based in vitro compartmentalization (IVC) is one of the selection methods for the directed evolution of proteins. This method is experimentally performed using cell-sized liposomes for in vitro protein synthesis and fluorescence activated cell sorter (FACS) for high-throughput screening of liposomes encapsulating the gene of our interest. In this report, we focus on the role of liposome size for screening β-glucuronidase (GUS). Liposomes exhibiting catalytic activity were sorted by following the criteria for fluorescence intensity of reaction product and liposome sizes. Iterative rounds of gene screen experiment using 80FL-sized liposomes enriched active variants of GUS and finally identified GUS variants with capability of faster assembly of tetramer.