2P085 CS 複合体形成をモデルとした高感度ペプチドタンパク質間相互作用検出系のデザイン

Design of highly sensitive peptide-protein interaction detection system adopting CS complex formation as the model

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Biomolecules such as protein, peptide and nucleic acids interact with each other in the cell controlling its life activities. Therefore, techniques to reproduce biomolecular interactions correctly on the solid substrate would be a good versatile way to clarify the biological phenomena. In this research, we investigated the influence of protein orientation and density on peptide-protein interaction (adopting cysteine synthase (CS) complex formation as the model) detection with employing site specific protein immobilization technique. As the results, consideration of the location of functional site with avoiding the steric hindrance by adjusting the protein density and the degree of freedom of the peptide would be important to design higher interaction detection system.

2P088 α/β フォールドをもつ新規ヘム蛋白質の設計と合成
Design and syntheses of de novo heme proteins with an α/β fold

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De novo protein design is a constructive approach to elucidate principles of the protein structure and function. We have computationally designed tertiary structures and amino acid sequences of globular heme proteins with an α/β fold that does not occur in nature. The protein 3D architectures were modeled to accommodate heme between secondary structure elements. The amino acid sequences to fold into the modeled structures were designed with Rosetta. The heme binding site is constructed by positioning two His residues at the sites for coordination of the heme iron. In the present study, $\beta_2\alpha\beta\alpha_2$ folds with the heme binding site were designed and synthesized. The synthesized proteins exhibited the secondary structure contents and heme-binding activity as designed.

2P086 無機基板表面を標的としたラクダ抗体から着想するスマートなバイオセンサー仕様抗体の設計

Smart interface antibody design for biosensor

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Some biosensors have a sensor chip bearing antibody for detecting a specific target molecule and antibody's capturing action is transduced to physical signals. The density and orientation of immobilized antibody are critical for detection sensitivity of sensors, but several complicated processes should be considered for designing the surface of sensor chip. Recently, we devised a method for creating a camel antibody with high affinity for a specific inorganic material, and the material-binding antibody can spontaneously and high-densely bind to the material surface. In this study, we tried to design the bispecific interface molecules from material-binding antibody and antigen-binding antibody fragments, which can immobilize antigen-binding probes on sensor chip.

2P089 膜たんぱく質の進化学手法、リボソームディスプレイ法による α -ヘモリシンの *in vitro* 分子進化

Directed evolution of membrane protein, alpha hemolysin, by development of liposome display method

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In vitro methods have enabled the rapid and efficient evolution of proteins and successful generation of novel and highly functional proteins. However, the available methods deal only with globular proteins (e.g., antibodies and enzymes), and not membrane proteins. Here, we report the development of a method named liposome display that can evolve the properties of membrane proteins entirely *in vitro*. This method, which involves *in vitro* protein synthesis inside liposomes was applied to the pore-forming activity of alpha-hemolysin (AH), a membrane protein derived from *Staphylococcus aureus*. The obtained AH mutant possessed only two point mutations but exhibited a 30-fold increase in its pore-forming activity compared with the wild-type.

2P087 タンパク質デザインの新たな方法の開発: 蛍光強度の違いを利用してファージを選別する

Development of a new strategy of protein design: the single phage sorting based on fluorescence intensity

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We aim to develop a new strategy of protein design based on the phage display and single-molecule sorting. In the first step of the method, a library of phages expressing a target protein with randomized sequence is prepared. In the second step, the expressed proteins are labeled by fluorophore(s) and are sorted based on the fluorescence signals from single phages. To realize the strategy, we prepared a phage expressing C-terminal fragment (214-230) of GFP. The addition of the N terminal fragment (1-214) of GFP, purified separately, to the phage caused the reconstitution of GFP and the appearance of the fluorescence signal. We are currently conducting the observation and sorting of the phages having the reconstituted GFP on the surface.

2P090 リボソームディスプレイ法によるメリチンの人工進化
In vitro evolution of Melittin using a liposome display

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Recently, a new method was developed, called "liposome display", which can evolve the properties of membrane proteins *in vitro*. So, using this method, we tried to evolve the pore-forming activity of melittin, an antimicrobial peptide derived from bee venom. Melittin consists of 26 amino acids and exhibits antimicrobial activity by lysing cell membrane. Starting from a random mutagenized DNA library of melittin, we obtained a mutant which exhibited higher pore-forming activity and induced higher molecular weight-dependent permeability, compared with the wild type after several rounds of selection using a liposome display. This analogue of melittin which we obtained can be expected to be applied widely, for example drug delivery vesicles.