Our findings suggest that C-terminal region of β chain is crucial for normal degranulation and cytokine secretion in mast cell, however, secondary structure and conformation change in deletion mutant do not occur at room temperature.

see 2TP2-02

- 2P-082 酢酸菌のセルロース合成酵素複合体における GxCesA と GxCesB との直接相互作用
Direct interaction between GxCesA and GxCesB in cellulose synthase complex of *Gluconacetobacter xylinus*

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Cellulose is natural polysaccharide that exists as a nanofiber in nature. In a bacterium *Gluconacetobacter xylinus*, it is hypothesized that cellulose synthase complex are formed by four proteins encoded by *GxCesABCD* operon. It is shown that two of these (GxCesA and GxCesB protein) are required for cellulose synthesis, because both of them are purified together with cellulose synthetic activity of *G. xylinus*.

GxCesA protein is a transmembrane glycosyltransferase that belongs to GT-2 superfamily and transfer glucose from UDP-glucose to the tail of growing cellulose. On the other hand, GxCesB is a regulatory subunit that stimulates the activity of GxCesA. Although it is supposed that these two proteins directly interact with each other, there is no clue at all.

In this study, the co-expression system of GxCesAB is established, which allows the putative GxCesAB protein complex to be analyzed. GxCesB was successfully expressed only when expressed together with GxCesA as far as tried. Pull-down assay using His-tagged protein indicated the interaction between GxCesA and GxCesB, supporting the direct protein-protein interaction between GxCesA and GxCesB.

- 2P-083 SMR 型多剤排出輸送担体と基質の相互作用に伴う熱量変化
Thermodynamic parameters of interaction between SMR-type multidrug transporter and its substrates

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EmrE is a small multidrug resistance (SMR) transporter from *Escherichia coli* that extrudes a wide range of toxic compounds from the cell. Glu14 residue plays the essential role for substrate binding and proton coupled transport. It has been thought that it is greatly influenced whether this residue is protonated on the substrate binding. In this study, we had investigated pH dependency of thermodynamic parameters of interaction between EmrE and its substrate by isothermal titration calorimetry (ITC). We could obtain EmrE in high amount using cell-free expression system supplemented with cholate and egg PC. Large heats transfer by non-specific binding hampered an accurate determination of the binding of ethidium to EmrE integrated into lipid bilayer without reconstitution step used cell-free expression system by ITC. Thus, we analyzed thermodynamic parameters of interaction between purified EmrE into detergent micelles and ethidium. At alkaline condition (pH 8), the value of dissociation constant (K_D) was 8.8 μ M. ΔG was observed to be -6.9 kcal/mol with ΔH and ΔS at -15.2 kcal/mol and -27.8 kcal/mol per K, respectively. At acidic condition (pH 6), heat of binding of ethidium to EmrE was not observed. We will analyze the binding at various pHs between EmrE and substrates. We will discuss the mechanism of proton coupled substrate transport.

- 2P-084 ハロロドプシン NpHR の三量体構造は F150 残基の対称配置によって安定化される
Stabilization of trimeric assembly of halorhodopsin, NpHR by C_3 -symmetric geometry among F150 aromatic rings

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see 2TP2-01

- 2P-085 DMPC、DPPC 二重層に再構成したバクテリオロドプシンの可視領域における円二色性測定
Circular dichroism measurement of bacteriorhodopsin reconstituted in DMPC and DPPC bilayer in visible region

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- 2P-086 転写因子のゲノムスケールでのターゲット予測：相互作用情報を用いた方法
Genome-wide target prediction of transcriptional factor: a method based on interaction data

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The regulation of gene expression is one of the most vital functions in organisms achieved by a network of transcription factors and their target genes. In recent years, a large amount of sequence and structural information for transcription factors and the target genes have emerged from various ongoing genome projects. However, the mechanisms by which the transcription factors regulate their target genes are not well understood yet. To achieve better understanding of the gene regulation mechanism, it is vital to elucidate how transcription factors identify their binding sites. One of the methods to predict binding sites of transcription factors is to use experimental protein-DNA interaction data. In order to apply this method to a genome-wide prediction, we conducted a pilot study for the Maf protein, for which a systematic interaction data are available. We have predicted potential binding sites of Maf on the genome scale. Together with the information of known binding sites of other transcription factors, we analyzed the context of binding sites in the regulatory regions of Maf target genes, and tried to decipher the relationship between the context and the functional annotations of the target genes.

- 2P-087 水と核酸塩基間の相互作用自由エネルギーランドスケープ
Interaction free energy landscape between nucleobase and a water

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We calculate the interaction free energy (ΔA) landscape between DNA and a water. ΔA landscape represents the 3D-distribution of water and at the same time the stability of the system when a water locates at a specific position around DNA. Thus obtained ΔA landscape shows sequence specific water distribution along DNA groove and the difference in water distribution between the major and the minor grooves. We also calculate ΔA landscape between nucleobase and a water based on the ab initio MO energy calculation. The inability of TIP3P potential to produce the angle dependency in hydrogen-bonding is clearly visualized as the difference in ΔA landscape. Interaction configurations are sampled based on Monte Carlo method. Since DNA sequence is quite diverse, we calculate ΔA landscape for the 32 kinds of 3bp B-DNA (5'-XAY-3' type and 5'-XGY-3', here X and Y represent A, T, G or C). The obtained ΔA landscape is visualized as isosurface. We find that in the major groove, XGY type has larger ΔA isosurface than that of XAY type and minimum ΔA value of XGY type is almost always lower than XAY type. In the minor groove, on the other hand, XAY type has larger ΔA isosurface than that of XGY type and the minimum ΔA value of XAY type is lower than XGY type. Thus, it is expected that a water molecule favors the major groove side of GC-rich sequence and the minor groove side of AT-rich sequence of B-DNA. By combining ΔA isosurface for 3bp DNA, we construct ΔA isosurfaces of some DNA sequences for which crystallographic structures are available. We find that there is a correspondence between the calculated ΔA isosurface and a water distribution in PDB; namely, a water molecule locates in the minor groove side of AT-rich region and the major groove side of GC rich region. By using ΔA landscape, it is possible to predict recognition sites of water along DNA groove.

- 2P-088 ベイズ統計学的解釈によるタンパク質-DNA 認識における indirect readout の評価
Role of indirect readout in protein-DNA recognition assessed by a Bayesian approach

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Sequence-specific recognition of DNA by proteins plays a critical role in regulating gene expression. Accurate recognition is achieved by a combination of two different mechanisms. The first is direct readout, in which recognition is mediated by direct interactions between the protein and the DNA bases. The second is indirect readout, which is caused by the sequence-dependence of the conformation and deformability of the DNA structure.

In our previous study, the contributions of indirect readout to binding affinity were evaluated for every tetrameric step in given protein-DNA complex structures. Using sequence-structure threading, potential energy differences between native and non-native tetrameric sequences for given tetrameric step conformation were