which contains higher- and lower-order oligomers (e.g., dimers and monomers). The higher-order oligomers of each family include at least one insertion or deletion region in their interface areas. The average length of insertion/deletion region is 15 residual sites, and the loop structures are frequently observed as compared with whole protein. Although the amino acid composition of the insertion regions is similar with that of other interface sites, the delta-ASA of them upon oligomerization is larger than that of other interface sites. We will also estimate the contributions of insertions and amino acid substitutions to interactions and discuss the difference between these two types.

2TP4-03 相互作用プロファイルを用いた 揺らぎをもつタンパク質の相互作用予測解析

Post-docking process for flexible protein docking problems by using interaction fingerprints

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Protein flexibility is important for forming protein complexes. Protein-protein interaction networks include hub proteins, which can interact with various proteins. Many hub proteins are observed as intrinsically disordered proteins. Therefore, it is important to analyze protein-docking problems in terms of protein with conformational changes.

We have been studying post-processing methods in rigid-body docking for searching near-native decoys by using interaction fingerprints (IFP) of molecular complex. IFP is a sequence composed of frequencies of interaction numbers between atoms belonging to amino acid residues. In general, cluster analysis is useful method for discriminating near-native decoys from others. Although RMSD is usually used in cluster analysis, it depends on method of superposing structures of decoys. We compared these 2 methods, IFP and RMSD, in cluster analysis and we could obtain better clusters by using IFP than RMSD.

Various structures of calmodulin (CaM), obtained from structures analyzed by using NMR, were used for docking analysis. Dataset composed of decoys generated from various CaM structures were investigated by cluster analysis by using IFP. We attempted docking simulation with a post-docking analysis for CNG, CaMKK and PMCA as peptide ligands of CaM.

2TP4-04 GPCR 立体構造情報と G タンパク質結合選択性の相関性

Relation between GPCR structure and G protein coupling selectivity.

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The external ligand stimulus to G-protein coupled receptors (GPCRs) induces the coupling with G-proteins ($G_{i/o}$, $G_{q/11}$, G_s , $G_{12/13}$ etc.) followed by different kinds of signal transduction. Though many experimental/theoretical studies have been performed, the mechanism of this coupling selectivity is not yet elucidated.

Recently, new structures of GPCRs were revealed¹⁾ and the GPCR sequences become abundant by various genome projects. In this situation, it is possible to argue about functionally important regions, by connecting comprehensive sequence analysis with new 3D structure information. Here we intend to extract structural regions which participate in selecting G protein type.

We performed a structural classification of GPCRs by drawing dendrograms using inner interaction of the proteins, which are estimated by physico-chemical parameters of amino acid residues on transmembrane regions (TMH). Applying this classification to various combination patterns of TMHs of GPCRs whose coupling selectivity has already known, we narrowed down the domains that participated in G protein selection. Furthermore, we identified the position of the important residues (functional residue sites) by processing statistics such as the Pearson's correlation analysis about G-protein type and structural position relationship. These identified "functional residue sites" really supported the experimental results, suggesting that our results are proper. The results are the quite fruitful information for understanding the G protein coupling mechanism.

Reference

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2TP4-05 NMR による天然変性タンパク質配列の網羅的検証法

A method for systematic assessment of intrinsically unstructured proteins and protein regions by NMR

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[INTRODUCTION] Intrinsically unstructured proteins and protein regions (hereinafter, IUPs), which lack stable 3D structures and have high flexibility in the polypeptide chains, have been widely known, and accurate predictions for

these regions are required. To verify the predictions, an NMR-based method whether the predicted sequences adopt unstructured states has been developed.

[RESULTS and DISCUSSION] For evaluating the physicochemical properties of IUPs by NMR, we designed a novel fusion-protein expression system derived from PH0471, a thermo-stable membrane anchored OB-fold protein. PH0471 is divided into two regions, four membrane helices and an OB-fold domain that are connected by a flexible linker. The NMR signals from the OB-fold region are detectable, only when the linker is totally disordered. Thus, we substituted this linker by candidate sequences of IUPs to be evaluated. 53 candidates were selected by a prediction method, poodle-S, and 40 from them were subjected to PCR-cloning from human or mouse cDNAs. We succeeded in constructing 35 expression vectors. Finally, we obtained ¹⁵N-labelled chimera PH0471 proteins with 25 putative IUPs/IURs as the linkers. From the NMR spectra, we classified them into three categories, very flexible, flexible and less flexible. The less flexible ones involve either membrane-associating or aggregation-prone sequences. The systematic characterization of IUPs will validate the predictions and provide a large amount of data, thereby contributing to improve the prediction method.

2TP4-06 大規模データマイニングから明らかになったタンパク質リン酸化と複合体形成の関係

Macroscopic Relationships between Protein Phosphorylation and Physical Interaction

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Under the recent advances in mass spectrometry-based technology, nearly half of the eukaryotic proteome have been suggested to have potential phosphosites. However, our understanding of protein phosphorylation is still limited. Here, we demonstrated macroscopic relationships between protein phosphorylation and physical interaction by integrating yeast phosphoproteome and protein interactome data. By using liquid chromatography-tandem mass spectrometry, 6,510 phosphosites and 1,993 phosphoproteins were initially identified, and a large-scale dataset of yeast phosphoproteome, including 14,627 phosphosites and 3,433 phosphoproteins, were prepared with a combination of the UniProt data. Then, we mapped the unified phosphoproteome data on the yeast binary protein interaction network. Statistical data mining analyses showed that phosphoproteins have significantly more interacting partners than do non-phosphoproteins. For example, the rate of phosphoproteins having over 10 interacting protein partners was ~2-fold more than that of non-phosphoprotein. Moreover, by comparing with randomly rewired protein interaction networks, we showed that co-phosphorylation of interacting proteins is more common than expected. Indeed, several possible kinases yielding co-phosphorylation of proteins in the same protein complex were illuminated by further *in silico* analyses. These results will provide new insights into the understanding of large-scale intertwining between protein phosphorylation and protein complex formation.

2TP5-01 TEM と SEM によるタンパク質複合体の構造解析

2P-018 Structure analysis of membrane protein complexes using TEM and SEM

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Membrane proteins and their complexes are essential for various kinds of physiological functions in our body. However, they are frequently difficult to make crystal. We determined the 3D structure of a membrane bound protein RECK using Single Particle Analysis (SPA) from EM image. Some Matrix metalloproteinases (MMPs) are up-regulated in cancer cells, and their expression is often associated with poor prognosis. The membrane-anchored protease regulator RECK plays important roles in mammalian development and tumor suppression. RECK was reconstructed using SPA in collaboration with Dr. Makoto Noda (Kyoto Univ). Although small stable protein complexes can be reconstructed using SPA, unstable complexes are hardly visualized after the protein purification step. An Atmospheric Scanning Electron Microscope (ASEM) is developed to observe aqueous samples and cells in solution at atmospheric pressures. Conventional electron microscopes view samples in vacuum and are unable to image wet samples or samples in solution. For this new microscope, a special dish with a window made of thin film (ASEM Dish) was developed to hold samples. The pressure-resistant film of the window allows an electron beam to be projected from underneath the sample. The top of the dish is open, and can contain aqueous sample of several ml.

2TP5-02 Folding simulations of chignolin by self-learning multiscale 2P-002 method

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