as low as ~0.1 photons per an effective pixel as signal for the classification. Theoretically, wave-number of a pixel giving such a limiting photon-count defines the attainable resolution. To estimate attainable resolution even for structure-unknown molecule, we also developed a new concept of standard model protein. As a result, we can estimate attainable resolution by this new technology as a function of incident intensity and sample size.

2P012 ショウジョウバエ由来 GGA の溶液構造解析 Solution structural analysis of *Drosophila* GGA

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GGA is a multi-domain protein comprised of VHS, GAT, and GAE domains, which is an adaptor protein essential for the protein trafficking and targeting. GGA can interact with various proteins. How can GGA recognize its partners and how can GGA bind and release them during the protein trafficking? In order to understand the molecular mechanism of protein trafficking by GGA, it is essential to clarify the structure of GGA in the multi-domain state. The VHS domain recognizes the cytoplasmic acidic-cluster-dileucine (ACLL) sorting signal. Our previous study on mammalian GGA revealed that the domain rearrangement between VHS and GAT occurs when an ACLL peptide is bound to the VHS with the use of the fragment comprised of VHS and GAT. In this study, the effect of the binding of the ACLL peptide to VHS on the domain arrangement of the intact GGA was investigated using *drosophila* GGA (dGGA). We succeeded to purify the intact dGGA. Dynamic light scattering showed that the molecular size of dGGA is 150 Å in the absence of the ACLL peptide. Upon adding the peptide, the size was slightly increased to 155 Å, suggesting a structural change in the intact GGA. To reveal the structural change in detail, we are going to measure X-ray solution scattering profiles in the absence and the presence of the peptide. We will discuss the molecular mechanism of dGGA interaction with its cognate peptides based on the solution structures.

2P013 電子線位相差トモグラフィーによるべん毛基部位と蛋白質輸送装置 FliH/FliI との複合体の構造解析 Structural analysis of the flagellar basal body-FliH/FliI complex by phase contrast electron cryotomography

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The flagellar type III protein export apparatus is composed of six transmembrane proteins that form the export gate and three cytoplasmic proteins, FliH, FliI, FliJ, which promote the export process. The export apparatus translocates flagellar axial proteins into the central channel of the growing flagellum to export them to the distal end for self-assembly. It has been proposed that the export mechanism involves temporary binding of the FliH/FliI/FliJ/substrate complex to the flagellar basal body C ring before the complex is recruited to the export gate in order to facilitate the export process. However, the structure of the basal body with the export apparatus during the translocation process has not been observed yet due to the low stability of their binding and the use of detergents for the purification of the basal body. To visualize the structure of the export apparatus attached to the basal body by electron cryomicroscopy, we purified the FliH/FliI complex and the basal-body separately and cross-linked them by the method called Grafix, which uses a density gradient centrifugation step with weak chemical fixation. We succeeded in visualizing the structure of the basal body that is considered as the initial state of the export process. We are now analyzing the structure of the basal body with the FliH/FliI complex by phase contrast electron cryotomography, which can visualize the structure more clearly than conventional tomography.

2P014 細胞外マトリックス制御因子 RECK タンパク質の単粒子構造解析 Single particle analysis of the membrane-anchored protease inhibitor RECK

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The membrane-anchored protease regulator RECK (reversion-inducing cysteine-rich protein with Kazal motifs) plays important roles in mammalian development and tumor suppression, but its structure has been unclear. Electron microscopy-based single particle reconstruction, a computer-aided structure determination method, reconstructs a three-dimensional (3D) structure

from projections of mono dispersed protein. A large number of particle images are picked up from EM films, aligned and classified to generate two-dimensional (2D) averages, and, using the Euler angle of each 2D average, reconstructed into a 3D structure. From the negatively stained 2,325 particles, the 3D structure of RECK was reconstructed at 25.2 angstrom resolution assuming C2 symmetry. The resulting RECK-His model has a larger dimension than expected from its molecular size, most probably because of an internal large cavity. The RECK dimer seems to anchor to the membrane at its bell top and holds its opening with two protuberances outward. This configuration looks advantageous for docking and interactions with MMPs and other molecules.

2P015 大気圧走査型電子顕微鏡を用いた水中免疫電顕法によるタンパク質局在の高分解能観察 Atmospheric scanning electron microscope with immuno-labeling technique in solution determines protein localization at high resolution

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Determination of protein localization at high resolution is essential for the understanding of the physiological functions of protein. We have developed an immuno-labeling technique for the Atmospheric Scanning Electron Microscope (ASEM), which enables observation of labeled cells in a water solution. In the inverted ASEM system, an electron-permeable window made of pressure-resistant film allows the electron beam to be projected up from underneath the sample. The electrons backscattered from the sample are captured by a detector positioned below. In the immuno-labeling method, target proteins in cells are labeled with an antibody conjugated both with fluorescent dye and gold particles. Expression and rough localization of the proteins are visualized using a fluorescence microscope positioned above the sample, while higher-resolution images in the same area are obtained using the ASEM. In this technique, the gold particle is observed as a strong signal. The antigenicity of proteins and fine subcellular structures are well maintained because the cells are not dehydrated. Using this immuno-labeling method, we obtained high-magnification images of cytoskeletal structures and of ion channel localization in cells.

2P016 ヒトプリオンタンパク質における His96 と His111 の Cu²⁺ 結合能力 Cu²⁺ binding ability of His₉₆ and His₁₁₁ in human prion protein

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The N-terminal domain of prion protein (PrP) residues 23-125 includes four octarepeat regions, which has high affinity for binding Cu²⁺ leading to stabilize the structure. In this report, we tried to search the another site to bind Cu²⁺ in the N-terminal domain of PrP. HuPrP₉₅₋₁₁₄ containing His₉₆ and His₁₁₁, outside the octarepeats, was prepared and Cu²⁺ binding ability was assayed using Circular Dichroism (CD) with varying pH. By the addition of 1 mol eq. of Cu²⁺, the growth of the negative band at 200-250 nm and the blue shift of a negative peak at 200 nm were observed. However, further addition of Cu²⁺ did not achieve further change in the CD spectra. CD measurements in the UV region showed that huPrP₉₅₋₁₁₄ bound to Cu²⁺ at both physiological and acidic pH. In addition, to investigate the role of each site of His₉₆ and His₁₁₁ in Cu²⁺ binding, we also made similar experiments for huPrP₁₀₃₋₁₁₄ containing His₁₁₁. The negative peak at 200 nm did not show significant change, but slightly changes in negative band at 215 nm and 230-250 nm were observed by the addition of Cu²⁺ up to 2 mol eq.. At below pH 6.0, even Cu²⁺ was added, CD spectra for huPrP₁₀₃₋₁₁₄ never changed. These results suggest that His₁₁₁ binds to Cu²⁺ at only physiological pH, while His₉₆ even at acidic pH. Hence, we will plan to examine Cu²⁺ binding ability of His₉₆ by using huPrP₉₅₋₁₀₄ containing His₉₆. We will also examine more detailed conformation of Cu²⁺ in PrP with magnetic resonance spectroscopy.

2P017 分子動力学シミュレーションによる高圧における深海魚の α アクチンの構造変化の解析 Structure Change Analysis of Deep-sea Fish α -Actin at High Pressure by Molecular Dynamics Simulations

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α -actin plays important roles in numerous cellular functions in muscle and non-muscle cells. The amino acid sequence of α -actin is highly conserved in

eukaryotic protein. High pressure typically induces depolymerization of the actin filament or loss of the actin ATPase activity in the species living in land or shallow water, whereas deep-sea fish α -actin is high pressure-adaptive. Although it has few amino acid substitutions, they contribute to tolerance to high pressure. ATP binding to monomeric α -actin promotes the polymerization at the end of the filament and ATP is hydrolyzed to ADP during the elongation of the filament. This catalysis needs divalent cation Ca^{2+} or Mg^{2+} that has high-affinity to ATP. The active site is located at the nucleotide cleft in the center of the protein. The divalent cation at active site is coordinated by 6 to 8 oxygens in crystal structures. The oxygen of Q137 side chain is directly coordinated to the cation. However, deep-sea α -actin fish has the substitution Q137K that is expected to contribute to the pressure tolerance. Molecular dynamics simulations of the actin molecules from distinct species at atmospheric pressure and high pressure have been performed in order to analyze the effects of the amino acid substitutions and the mechanism of the tolerance.

2P018 アミノ酸配列の連続性, 順序性を無視した蛋白質立体構造アライメントの新規手法
A novel method for non-sequential protein structure alignment.

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Protein structure comparison is fundamental approach for protein classification, studies of evolutionary relationships, and so on. Today, there are many structure alignment tools, most of which follow a simple sequential rule: two proteins are aligned in sequential order. That is because it is generally assumed that similar protein structures will align to each other in a sequential manner, conserving the direction of the chain. However, recent structural comparison studies have suggested that there are many examples of structural similarity that are non-sequential. The most popular example of these relationships may be circular permutations. Analyzing such non-sequential similarities would give us some new insights into physico-chemical properties and evolution of protein structures.

We have developed a novel method of non-sequential protein structure alignment utilizing geometric hashing technique and vectorial representation of secondary structure elements (SSE). The algorithm can identify the best structural alignment between protein pairs without constraints of sequential connectivity and that of SSE direction. Vectorial representation of SSE used in the method is different from the one which has been often used in the past. One SSE is represented by a number of vectors and the number of vector representing one SSE is depend on the length of SSE. Thus, the method can appropriately treat bent strands or bent helices. We will present the results of benchmark test and comparing to other structural alignment methods.

2P019 分子動力学シミュレーションによるグルタチオンとグルタチオン転移酵素 T2-2 の構造とダイナミクス
Structure and dynamics of glutathione and glutathione-transferase T2-2: a molecular dynamics study

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Glutathione is an antioxidant concerned with the detoxification metabolism in vivo and has a function to defecate toxic compounds from cells. Glutathione-transferase has been known to reduce the reactivity of toxic compounds by catalyzing their conjugation with glutathione. Glutathione-transferase is an enzyme that plays a critical role in the cellular detoxification process and resides in body tissues like kidney, bowel, lung and liver. The function of glutathione and glutathione-transferase has been through to implicate in detoxification. Thus, the understanding of the binding character between the glutathione and glutathione-transferase should be important in the realization of catalytic reaction mechanism and contribute the development of antibiotic drug. In this study, we analyze the structure and dynamics of the glutathione and the glutathione-transferase T2-2 in water solvent by the molecular dynamics simulations to understand the changes of structures due to the binding the glutathione and glutathione-transferase. We carried out the molecular dynamics simulations of the glutathione-transferase T2-2 contains glutathione and the one without glutathione. The dynamics of these model proteins are estimated by principal component analysis.

2P020 残基間平均距離統計に基づくフェレドキシン様フォールドのフォールディング機構の解析
The analyses of the folding mechanism of the Ferredoxin-like folds based on renewed inter-residue average distance statistics

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A protein in the Ferredoxin-like fold forms the same 3D structure even if the two proteins share low sequence identity, i.e., this 3D scaffold constitutes a "superfold". In the present work, we try to identify the important regions in the sequences for the formation of the 3D-scaffold by means of the methods based on inter-residue average distance statistics, i.e., an average distance map and the contact frequency of a pair of residues. In a previous study, we demonstrated that it is possible to identify the folding mechanism of the Ferredoxin-like fold with no 3D information but only sequence information. In this study, on the basis of previous work, we try to identify very important sequence regions or some characteristic structural motifs in the construction of Ferredoxin-like fold by analyzing with high accuracy predicted contact maps that are calculated with renewed inter-residue average distant statistics. The details will be presented at the conference.

2P021 側鎖の効果を取り入れた粗視化 Go モデルと ADM を併用した IgG 結合ドメイン関連タンパクのフォールディング機構の解析
Analyses of the folding mechanisms for the IgG binding domain related proteins based on a coarse grained Go model and ADM

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The Go-model can reproduce protein folding pathway using protein structure information. It accounts interactions which stabilize the native structure of a given protein. In the previous work, we reproduced the differences in the folding pathway of Protein G from that of Protein L using our Ca -Go-model. This Ca -Go-model makes it possible to simulate protein folding pathway with same accuracy to all-atom-Go-model by incorporating the differences in side-chain direction using virtual bond angle around Ca atoms. On the other hand, ADM(Average Distance Map) which is a sequence analysis method based on the statistical average distances between residues can predict the area which folds early. This method was successfully applied to predict folding pathways of globins. In this study, at first, we apply these methodologies to predict folding pathway of G311 and GB95. G311 and GB95 are designed protein based on Protein G. Secondly, we compare the results of predicted folding pathway of G311 and GB95 with the folding pathway of Protein G and Protein L. Protein G, Protein L, G311 and GB95 share same topology but have diverse sequence identity (24% to 64%). These proteins should implicitly contain information to fold same topology. We try to extract the information in the present study.

2P022 Pseudomonas sp. B-0831 由来 3 α -hydroxysteroid dehydrogenase の補因子および基質との複合体構造に関する理論的研究
Theoretical Study of Complex Structure of 3 α -Hydroxysteroid Dehydrogenase from Pseudomonas sp. B-0831, NAD(H), and Substrate

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3 α -Hydroxysteroid dehydrogenase from *Pseudomonas* sp. B-0831 (Ps3 α HSD) catalyzes the reversible oxidoreduction of the steroids between 3 α -hydroxysteroid and 3-ketosteroid. The crystal structure of the binary complex of Ps3 α HSD with NADH exhibited a heterodimer which is formed by *apo*- and *holo*-subunits, and the binding experiments showed the negative cooperativity in the NADH binding to Ps3 α HSD. In this study, we investigate the role of the structural heterodimer on the formation of ternary complex of Ps3 α HSD with NAD(H) and substrate using the computational method. Because the ternary complex structure of Ps3 α HSD with cofactor and substrate has not been determined, we performed docking calculation and molecular dynamics simulation to determine it using NADH and androstandione as cofactor and substrate, respectively, as an oxidized model. When the complex of *holo*-subunit including NADH obtained from the crystal structure was used as a model to reduce computational efforts, no proper conformations were obtained. We next used the heterodimer structure, and the binding conformations were obtained. Since the distance between substrate and NADH is about 3.5 Å, we think that the conformation obtained from the calculation with the heterodimer was proper for the reduction of 3-ketosteroids. We will discuss the structural uniqueness of Ps3 α HSD based on further theoretical analyses and experimental results.

2P023 銅含有アミン酸化酵素触媒反応における時間分割 X 線結晶構造解析
Time-resolved X-ray crystal structure analysis of enzymatic reaction of copper amine oxidase from *Arthrobacter globiformis*