Trans-Lesion DNA synthesis (TLS) is one strategy of DNA damage tolerance mechanisms to allow continuation of DNA synthesis at damaged template. TLS consists of two major processes, nucleotide insertion at the lesion site and extension. DNA polymerase ξ (Pol ξ) is one of key player in TLS and it acts as both inserter and extender. Pol ξ is composed of catalytic REV3 and regulatory REV7 subunits. Furthermore, REV7 also interacts with REV1, which is also a key DNA polymerase in TLS. In addition, REV7 involves in multiple protein-protein interactions in various cellular functions including transcription and cell-cycle checkpoint. For instance, REV7 interacts with ELK1 and a phosphorylated form of JNK in response to DNA damage and cell stress. Several studies also suggest that REV7 can inhibit the anaphase promoting complex (APC) through interactions with CDH1 in G2/M phase. Recently, it has been reported that a Shigella effector IpaB interferes the inhibition of APC by interacting with REV7, thereby causing cell-cycle arrest. Although REV7 plays a role of an adaptor protein in various cellular functions, the mechanism of those interactions by REV7 still unknown. Here we report the crystal structure of human REV7 in complex with human REV3 fragment. The structure of REV7-REV3 complex revealed that the C-terminal region of REV7 wrapped the REV3 fragment, thereby adopting a knot structure. Furthermore, we performed the pull down assay by using REV7 mutants and REV3 and revealed the mechanism of the interaction of REV7 with REV3.

2P-007 130℃までの熱変性における牛血清アルブミンの構造に対するジェミニ型界面活性剤の保護効果
Protective Effect of Gemini Surfactant on Secondary Structural Change of Bovine Serum Albumin in Thermal Denaturation up to 130 °C

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The effect of gemini surfactant, sodium dilaureamidoglutamate lysine (DLGL), on the helical structure of bovine serum albumin (BSA) was examined at 25 °C and at high temperatures up to 130 °C. The helicity of the protein decreased from 66% to 53% in the DLGL solution at 25 °C, whereas it decreased down to 16% with rise of temperature. Although approximately half of the original helical structures were disrupted upon heating up to 75 °C, most of the structures were maximally protected in the coexistence of 0.30mM DLGL at 75 °C (the protein concentration was 0.010mM). At temperatures below 75 °C, the protected helicity became maximal at such low concentrations of DLGL and then decreased with an increase of the surfactant concentration. In the heat treatments above 80 °C, the protective effect did not appear at low DLGL concentrations, but monotonously enlarged with an increase of the surfactant concentration. On the other hand, upon cooling down to 25 °C after the thermal denaturations below 75 °C, the helicity was maximally recovered to about 60% in the coexistence of DLGL below 0.30mM and then decreased with the surfactant concentration. Upon cooling down to 25 °C from high temperatures above 85 °C, the recovered helicity gradually increased with an increase of the surfactant concentration. The protective effect, which are observed at low DLGL concentrations, might be fulfilled by the monomer ions of the present gemini surfactant ion, since the bound DLGL onto BSA must be smaller than the mixing ratios around 30mol/mol.

2P-008 超好熱始原菌由来 Type III Rubisco の構造を基にした活性最適化
Structure-based optimization for catalytic activity of a Type III Rubisco from a hyperthermophile

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The Calvin-Benson-Bassham (CBB) cycle contributes toward fixing carbon for all plants, algae, and cyanobacteria. Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is an important enzyme in the CBB cycle, and it catalyzes the first major step of carbon fixation, by which ribulose 1,5-bisphosphate reacts with CO₂ and H₂O to form two molecules of 3-phosphoglycerate. Rubisco from a hyperthermophilic archaeon *Thermococcus kodakaraensis* (*Tk*-Rubisco) belongs to a Type III group, and it shows high activity at high temperature. We have previously found that replacement of the entire α -helix 6 of *Tk*-Rubisco with the corresponding region of the spinach enzyme (SP6 mutant enzyme) results in improvement of catalytic performance at mesophilic temperatures both *in vivo* and *in vitro*, but the former and latter half replacements of the α -helix 6 (SP4 and SP5 mutant enzymes) do not show such improvement. We report here crystal structures of the wild-type *Tk*-Rubisco, SP4 and SP6 mutant enzymes, and discuss about relationship between the structure and enzymatic activity. Comparison between these structures shows their movement and increase of temperature factors of the α -helix 6 by the mutations. It is supposed that increase of the flexibility of α -helix 6 and loop 6 regions is important to increase the catalytic activity. The mutation of V330T to the SP5 mutant enzyme (SP5-V330T mutant enzyme) based on structural information significantly increases the flexibility of the above regions, and catalytic activity of the SP5-V330T mutant enzyme is also increased. The thermostability of the SP5-

V330T mutant enzyme is inferior to that of the wild-type *Tk*-Rubisco, which supports this hypothesis focused on the flexibility.

2P-009 張力がアクチンフィラメントの力学特性に及ぼす影響
Effects of tensile force on mechanical properties of actin filament

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Actin cytoskeletons play critical roles in the mechanical processes of a variety of cellular activities, such as shape change, migration and division. The major component of actin cytoskeletons, actin filament, has a double-helical structure consisting of globular actin molecules. Tension changes in the actin filament associated with the above cellular activities will cause alteration of this structure, which may affect the binding of actin-regulatory proteins such as cofilin and profilin, eventually causing global structures of actin cytoskeletons. Therefore, it is crucially important to understand the physicochemical mechanism how tensile forces affect the mechanical property of the actin filament in relation to the binding of actin-regulatory proteins. Towards this goal, we investigated the effect of tension in the actin filament by using steered molecular dynamics simulations which have successfully provided useful insight into biologically relevant questions in a variety of molecular systems. We applied constant tensile forces starting at 200 pN to an actin filament model consisting of fourteen actin molecules in an ionic solvent, and analysed its longitudinal and rotational Brownian motions. We found that the tensile force decreased the torsional angle and torsional fluctuations of the double-helical structure and the interactive energy between actin subunits. Moreover, the tensile force caused changes in the free energy of the whole actin filament. These results suggest the possibility that tensile force alters the binding affinity of actin-regulatory proteins to the actin filament.

2P-010 高電位鉄イオウタンパク質(HiPIP)の0.5 Å 分解能における結晶構造解析
Crystal structure of a high-potential iron sulfur protein (HiPIP) at 0.5 Å resolution

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In electron transfer proteins, outer shell electrons significantly contribute to the electron transfer reaction. Therefore, information of the outer shell electrons is critical for understanding the redox activity of electron transfer proteins. In addition, orientations of bound waters significantly affect redox potentials of the proteins. Therefore, visualization of outer shell electrons as well as hydrogen atoms is important for elucidating the molecular mechanism of electron transfer reaction. However, such information can be derived from crystallographic analyses only at ultra-high resolutions. We have performed crystallographic analyses of the high-potential iron-sulfur protein (HiPIP) from *Thermochromatium tepidum* [1-3]. Recently, diffraction experiments with high energy X-rays were carried out at the beamline BL41XU of SPring-8. The wavelength of incident X-rays was set to 0.4 Å. Consequently, we could observe diffraction spots at 0.48 Å resolution, and data set at 0.50 Å resolution was collected. It is noteworthy that this is the highest resolution data set among protein crystals previously reported. Results from the structure analysis will be discussed at the meeting.

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2P-011 サツマハオリムシ V2 巨大ヘモグロビン (分子量 40 万) の結晶構造
Crystal structure of a 400 kDa hemoglobin (V2Hb) from *Lamellibrachia satsuma*

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Vestimentiferan tube worms, primarily lived in the sulfide rich seabeds, have 400 kDa and 3,600 kDa hemoglobins (Hbs), which are directly dissolved in their blood. In addition, some tube worms have another 400 kDa Hb in the coelomic fluid. To elucidate cooperative and sulfide binding mechanisms of these giant Hb, detailed structural information is primarily important. No crystal structure of vascular Hb of tube worms have been reported. So, we have initiated crystallographic studies of vascular 400 kDa Hb (V2Hb) from a tube worm,

Lamellibrachia satsuma. We have successfully obtained crystals of V2Hb with typical dimensions of $0.3 \times 0.3 \times 0.5 \text{ mm}^3$. Crystallization experiments were performed using vapor-diffusion method, and the crystals were obtained from a protein solution of 20 mg/ml and a reservoir solution containing PEG 3350 and magnesium acetate (or calcium acetate) as a precipitant. To overcome crystal defects of the non-merohedral twinning, the low-concentration ($< 10 \text{ mM}$) of salts were preferable for the reservoir solution. The crystals belongs to the space group $P6_3$ with unit cell dimensions of $a = 108.9 \text{ \AA}$, $c = 193.9 \text{ \AA}$. Initial phases have been obtained by the molecular replacement method using the structure of the coelomic Hb (C1) of a tube worm (*Riftia pachyptila*) as a search model. Compared to the *Riftia* C1 structure, V2Hb has no potential zinc binding sites in the equivalent position. This fact strongly suggests that V2Hb has completely different mechanism for sulfide binding.

- 2P-012 DFT 計算を用いた *Pseudomonas* sp. B-0831.由来 3α -HSD の補酵素 NAD/NADH の結合性に対する pH 依存性
DFT Studies on the pH dependency of coenzyme NAD/NADH binding to 3α -HSD from *Pseudomonas* sp. B-0831

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3α -Hydroxysteroid dehydrogenase (3α -HSD) from *Pseudomonas* sp. B-0831(Ps. 3α -HSD) belongs to the short-chain dehydrogenase/reductase (SDR) super-family. 3α -HSD catalyzes the reversible inter-conversion of hydroxy and oxo groups at position 3 of the steroid nucleus. Ps. 3α -HSD has the dual nucleotide cofactor specificity for both NAD(H) and NADP(H). The triad of Ser114, Tyr153, and Lys157 in the active site participates in the enzyme catalysis. The role of Lys157 is to lower the pKa of Tyr153 through interaction, and Tyr153 acts as a general base in the extraction of the proton from the hydroxyl group of the steroid. In this work, we focus the roles of Tyr153 and Lys157 in the cofactor binding and analysis of the pH dependency of the Ps. 3α -HSD binding to the NAD⁺ and NADH by using Density Functional Theory (DFT). The previous experimental results showed that the higher pH is more favorable for the binding to NAD⁺, whereas the lower pH is more favorable for the binding to NADH. We investigated the binding energy of the complexes between the neutral and deprotonated tyrosine and Ps. 3α -HSD to determine the role of Tyr153 in the cofactor binding. The results show the same pH dependency of the binding to NAD(H) as the experiment. The role of Lys157 in the cofactor binding also was investigated by the same method as of Tyr153, and the results also were corresponded to that experiment. The amino residues adjacent to Tyr153 and Lys157 were included to the complex models and their roles in the cofactor binding were investigated.

- 2P-013 電子顕微鏡法・単粒子解析法によるアセチルコリン受容体の構造解析
Structure of acetylcholine receptor by electron cryo-microscopy and single particle analysis

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Acetylcholine receptor (AChR) is a protein related to neurotransmitter function. A kind of AChRs work in a nerve-muscle junction. The AChR transits among three types conformational states: the open state, the closed state, and the desensitized state. Previously, the structures of AChR in the open and closed state were solved by electron cryo-microscopy and helical reconstruction, but that of the desensitized state have not been yet unknown because of poor helical symmetry. We also reported the preliminary structure of the desensitized AChR. Here, we examined the refined structure of AChR in the desensitized state by electron cryo-microscopy at 2.3 nm resolutions. Additionally, we compared desensitized state to closed state by single particle analysis. The results gave new understanding to us. The three dimensional structure indicated that one of five subunits considering as δ subunit might become deformed to cover the inside pore of AChR and block the gate so the AChR should be desensitized.

- 2P-014 3つのクラミドモナス外腕ダイニン重鎖の立体配置とその相互作用
Configurational Interactions of *Chlamydomonas* Three Outer Arm Dynein Heavy Chains

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Dynein is a minus end-directed microtubule motor that utilizes ATP binding and hydrolysis to convert chemical energy into mechanical energy. Dynein heavy chain that has motor activity consists of three structurally distinct domains, head, stem and stalk. The head has a ring-like structure and binds ATP to be hydrolyzed, while the stem and stalk protrude from each of the head. The stalk can associate with microtubules, while the stem allows heavy chains to multimerize and/or fix on A-microtubule. Recent reports have proposed that the dynein should generate

power by changing the stalk and stem positions against the head relatively. Dynein participates in important cell processes, for example cytoplasmic dynein is engaged in intracellular transport in cytoplasm, while axonemal dynein supplies the motility of flagella and cilia in axoneme. Many kinds of axonemal dyneins are along the axoneme and provide movement of flagella and cilia cooperatively. Axonemal dyneins are separated inner arm dyneins (IADs) and outer arm dynein (OAD). There has been an increase of research into motility and structural studies of OAD using *Chlamydomonas* that have triple head OAD. Thus, we purified OAD from *Chlamydomonas* flagella and observed that. In these observations, two ring-like heads of OAD are parallel while the other head is in different direction from the two. These results are different from previous studies. In deep-etch replica method, three heads were parallel in the same plane but one of them was in different side from the other heads. In cryo-tomography method, three heads stacked on microtubules. We are attempting to reveal that which ring is each head by observing OADs lacking one head. Hereby, this may help to know how three heads of OAD are configured in flagella and interact with one another.

- 2P-015 β 2-Microglobulin フラグメントが形成するアミロイド線維の真空紫外 CD スペクトルの理論的解析
Theoretical Analysis of Vacuum-Ultraviolet CD spectrum of Amyloid Fibril Formed by β 2-Microglobulin Fragment

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The N21-H31 fragment of β 2-microglobulin forms amyloid fibrils at pH 7.5, but its tertiary structure in solution remains unclear because it is a challenging target for X-ray and NMR spectroscopy. Circular dichroism (CD) spectroscopy has a great advantage for the structural analysis of these complex targets and recent CD theory allows us to calculate the CD spectrum from the three-dimensional coordinates. In this study, we theoretically analyzed the CD spectrum of N21-H31 in amyloid fibrils by the combined analysis of vacuum-ultraviolet (VUV) CD, molecular dynamics (GROMACS), and CD theory. The VUVCD spectrum of N21-H31 in amyloid fibrils exhibits small negative peaks at 225 and 185 nm, and a large positive peak at 200 nm. The initial structures of N21-H31 fragments were extracted from the K3 peptide (S20-L41) in amyloid fibrils obtained by solid-state NMR. The average CD spectrum of these structures was calculated by CD theory with the matrix method, but the spectrum exhibited large differences from experimental one. The MD simulation for one-sheet model of N21-H31 fragments showed disassembly within 10 ns, but the two-sheet model with an SS bond between the sheets were successfully simulated for 20 ns. The average spectrum of these simulated structures showed very similar peak positions to experimental data, and a further improved spectrum was obtained from the two-sheet model simulated in NaCl solution. These results indicate the usefulness of this combined method for the structural analysis of amyloid fibrils in solution.

- 2P-016 HMGB2 タンパク質に含まれる2つのドメインの溶液中における相対配向の決定
Preferential domain orientation of a full length HMGB2 protein determined by a novel NMR technique

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High mobility group B2 (HMGB2) protein is a ubiquitously distributed nuclear protein that binds to DNA without significant sequence specificity. HMGB2 preferentially binds to distorted or bent double-stranded DNA (dsDNA), thus the protein can recognize the shape of the DNA rather than sequence. HMGB2 also enhances the DNA bending through its binding to dsDNA, which helps the dsDNA wrapping around the histone octamers. HMGB2, thus, has a role as nucleosome chaperon. HMGB2 contains two HMG-boxes and the HMGB2 function requires the synergistic engagement of both domains. These two HMG-boxes are linked by a short, 10 residue-long, and natively unstructured element. We have found that this short linker interact specifically N-terminal HMG-box (A-domain) without any significant contact to the other HMG-box at C-terminus (B-domain), which was found through the NMR chemical shift differences of each HMG-box between the isolated domain and the intact forms where two domains are linked by the short linker. Their relative domain orientation was determined by our newly devised NMR method, DIORITE. From the analysis, two domains may have preferential orientation that makes the DNA binding “wedges” face to the same side. The DIORITE analysis provides the accurate relative orientation of the two HMG-boxes in the intact form of HMGB2, but cannot give any distance information between them To add the distance restrains for the domains, we further introduced the lanthanide labeling that induces the chemical shift change according to the distance between each 1H and the labeled lanthanide ion (pseudo contact shift; PCS). The structure of the intact form of