Lamellibrachia satsuma. We have successfully obtained crystals of V2Hb with typical dimensions of 0.3 x 0.3 x 0.5 mm³. 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 mM) of salts were preferable for the reservoir solution. The crystals belongs to the space group *P*6₃ with unit cell dimensions of *a* = 108.9 Å, *c* = 193.9 Å. 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 3 α -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 3 α -HSD from *Pseudomonas* sp. B-0831

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3 α -Hydroxysteroid dehydrogenase (3 α -HSD) from *Pseudomonas* sp. B-0831 (Ps.3 α -HSD) belongs to the short-chain dehydrogenase/reductase (SDR) super-family. 3 α -HSD catalyzes the reversible inter-conversion of hydroxy and oxo groups at position 3 of the steroid nucleus. Ps.3 α -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 pK_a 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.3 α -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.3 α -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