1P001 **心筋収縮制御タンパク質トロポニンの ESR を用いた動的構造解析**
Structural dynamics of cardiac troponin as revealed by spin labeling ESR

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Troponin (Tn) is a molecular switch that undergoes Ca²⁺-dependent regulation of actomyosin in muscle contraction. Ca²⁺ signaling through troponin C (cTnC) differs significantly between cardiac and fast skeletal muscle. The N-terminal lobe of cTnC has a single functional regulatory Ca²⁺ binding site (site II), whereas fast skeletal TnC has two (site I and site II). N-lobe of both TnC undergoes similar but smaller closed-to-open conformational change upon Ca²⁺ binding. PKA-phosphorylation of troponin I, at ser23 and 24, plays a critical role for adrenergic-stimulated regulation of heart beating. In this study, we focused on the interaction between cardiac troponin subunits (cTnC and cTnI) by Ca²⁺ binding using site-directed spin labeling electron spin resonance (SDSL-ESR). We generated cys-mutants of N-lobe of cTnC (84C), and the switch segment (151C, 152C) and the N-terminal residues near phosphorylation sites (ser23, 24) of cTnI which were spin labeled, and then measured the spin-label mobility and interspin distance to examine conformational change and interaction of N-lobe of cTnC and C-terminal region and N-terminal phosphorylation segment of cTnI. Our mobility data suggested that the switch segment of cTnI bound to the N-lobe of cTnC in the Ca²⁺ state. We will present the mobility and distance measurements with cTnC and cTnI in the -Ca²⁺ state and also with spin labeled phosphorylation segment.

1P002 **クロマチンリモデリングタンパク質のリン酸化による構造機能制御機構の解明**
Functional regulation through the phosphorylation to acidic intrinsically disordered region in FACT

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FACT is the chromatin remodeling complex comprising of SPT16 and SSRP1. FACT removes H2A/H2B component from nucleosome to facilitate DNA transcription. SSRP1 contains HMG-box, a type of DNA binding motif, and two intrinsically disordered (ID) regions, acidic ID (AID) and basic ID (BID). AID harbors multiple phosphorylation sites. Our biochemical works have shown that in the absence of DNA, BID-HMG interacts with the AID. In the presence of DNA, AID is peeled to allow the HMG-DNA interaction to facilitate FACT chromatin remodeling. In cell, AID is found to be phosphorylated and tightly binds to BID-HMG in SSRP1, which attenuates FACT binding ability to DNA, thus weakens the chromatin remodeling activity. This phosphorylation-mediated auto-inhibition should have the central role in the functional regulation of FACT. In this study, we have analyzed the molecular mechanism of the auto-inhibition of FACT function by using NMR. We found that the phosphorylation to AID enhanced its binding ability to BID-HMG with increasing the effective number of binding sites. The ID part did not gain specific structure upon binding to the BID-HMG. It is in contrast to the well known phosphorylation-mediated auto-inhibition mechanism where the phosphorylation offers the specific binding site to make a tight inhibitory complex. The AID with negatively charged surface has intrinsic binding ability to BID-HMG as basic part and the phosphorylation to AID gives additional negatively charged parts to facilitate its binding to BID-HMG.

1P003 **HMGB2 タンパク質に含まれる 2 つのドメインの相対配向とリンカーの役割**
Preferential domain orientation of a full length HMGB2 protein and the role of the linker region

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High mobility group B2 (HMGB2) protein is a ubiquitously distributed nuclear protein that binds to DNA without sequence specificity. HMGB2 preferentially binds to distorted or bent double-stranded DNA (dsDNA), thus the protein can recognize the shape of dsDNA rather than sequence. HMGB2 consists of two homologous HMG-boxes which are connected by a ten-residue linker. We found that this short linker specifically interacts with N-terminal HMG-box (A-domain) without any apparent contact to the other HMG-box (B-domain). To analyze the interaction between the linker and the A-domain, we made HMGB2 linker mutants. NMR measurements for the mutants have shown that, among them, only P80G/P81G mutant has shown some chemical shift changes relative to those for the wild-type; the observed signals were

almost identical to those in the isolated A-domain spectrum. This observation suggests the two-residue change in the linker made it detached from the domain. With using NMR, the relative domain orientation in the wild-type HMGB2 was determined to show that it has the DNA binding “wedges” faced to the same side. In considering the interaction between the linker and the domain A, the domain orientation could be speculated to be defined through the linker-domain A contact. To elucidate if the linker can restrict the domain orientation, the domain orientation for the P80G/P81G mutant is now analyzed using NMR. The results will be reported with additional results on the domain dynamics in HMGB2.

1P004 **べん毛フックの長さ制御因子 FliK の NMR による構造解析**
Structural analysis of FliK by NMR

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Many motile bacteria move in liquid using their helical flagellar filaments. The bacterial flagellum consists of three parts: the basal body, the hook, and the filament. In *Salmonella typhimurium*, hook length is controlled at around 55 nm. FliK is known to regulate the hook length, together with the C ring. However, the physical mechanism of the regulation is still unclear. FliK is a soluble protein consisting of 405 amino acid residues, giving the apparent molecular weight of 42kDa. The N-terminal region of FliK is rich in proline and glycine, suggesting that the N-terminal region does not have definitive 3D-structures but has extended structure like a string. Bioinformatics analysis of FliK sequence predicts that FliK N-terminal region could have partially folded structures. We would like to know how these partially folded structures work in length control. Since the FliK N-terminal region is difficult to crystallize due to its unfolded structure, we have tried to solve the detailed structures of FliK by NMR. We purified some types of FliK fragments and assigned most of the residues. We will be discussing the relationship between structure and function of FliK.

1P005 **異方性スピン相互作用を用いた新規 NMR 法による Pin1 の基質依存的な構造変化解析**
Ligand-induced molecular morphological change of Pin1 revealed by a novel NMR approach using anisotropic spin interactions

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Pin1 is a peptidyl-prolyl cis-trans isomerase, which consists of two domains. The N-terminal WW-domain specifically binds to phosphorylated peptide containing Ser/Thr-Pro motif and the C-terminal PPIase domain catalyzes proline isomerization. The domains in Pin1 are linked by a very flexible linker. The crystal structure of the Pin1 in the complex with phosphorylated peptide is available. In this work, we tried to compare the Pin1 structures in the complex of various phosphorylated peptides to see if the domain arrangement is altered according to the bound peptide. One complex structure of Pin1 in the complex with a phosphorylated peptide was determined by our devised NMR method, DIORITE, which uses the orientation dependent TROSY chemical shift changes caused by a weak protein alignment against the magnetic field. The result has shown that the domain orientation in the complex was apparently different from that found in the crystal structure. This seems to be ascribed to the crystal contacts found for the Pin1. In comparing the domain orientation determined in solution with that in the crystal structure, we found that the WW domain shows extended contacts with the ligand peptide in solution. This observed interaction can explain the role of Pin1 on the phosphorylated peptide. In the presentation, the details in the experiments will be discussed with the additional application of the method to the other peptides.

1P006 **TDP-43 の X 線結晶解析**
The crystal structure analysis of TAR DNA-binding protein 43

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Proteins constitute and carry out all kinds of intra- and extra-cellular events and therefore mutations, deletions, misfolding and aggregation of protein molecules, leading to gain or loss of protein functions are related to numerous genetic and sporadic diseases. TAR DNA-binding protein 43 (TDP-43) is a ubiquitously expressed protein whose normal function and abnormal aggregation are directly linked to the common lethal genetic disease, cystic fibrosis and to two neurodegenerative disorders: frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS).