agonist and non-agonist mAbs have distinct binding sites on the CD3z/\gamma heterodimer. Agonistic mAbs bind diagonally to the membrane distal CD3z/\gamma lobe adjacent to the lever-like C3 FG loop that facilitates antigen (pMHC)-triggered activation. In contrast a non-agonist mAb targets the cleff between CD3z and CD3\gamma, in a perpendiculur mode. Subsequent biological and biophysical experiments confirmed that the difference in cell triggering is not linked to mAb affinity or CD3z binding stoichiometry per TCR but to the difference in the binding epitope on CD3z/\gamma. More importantly, non-agonistic mAbs is stimulatory only subsequent to the application of an external tangential force (\textasciitilde 20 pN) generated by an optical tweezzer technique. These findings indicate that a dynamic but coordinated receptor quaternary structure change in T cell receptor is important for T cell activation. Common quaternary structure change can better facilitate structural signal initiation, given the vast array of TCRs and their specific pMHC ligands.

The transcriptional co-repressor SHARP contains a transcriptional repressor binding domain, SPOC which is known to bind to the C-terminal region of co-repressor SMRT. SHARP/SMRT complex recruits histone deacetylase to chromatin and regulates transcription negatively. We have determined the three-dimensional structure of SHARP/SMRT complex to establish structural basis for the molecular recognition by using solution NMR. We found that phosphorylated SMRT tightly bound to SPOC domain of SHARP, but the affinity was decreased about 1000 fold when it was not phosphorylated. Interestingly, a comparison of HSQC spectra showed that unphosphorylated SMRT is bound in a similar manner to that of phosphorylated SMRT. We will discuss detailed molecular recognition based on the determined structure, its dynamical aspect derived from 1^H NMR relaxation experiments, and their biological significance.

1SD-03 高圧力NMRから見えた蛋白質の高エネルギー構造の世界
High pressure NMR discloses a rich world of high-energy structures of proteins
Ryo Kitahara (College of Pharmaceutical Sciences, Ritsumeikan University)
A protein in solution is a thermodynamic entity existing in dynamic equilibrium of multiple conformers. The conformational fluctuation could be coupled with protein functions and misfolding events leading to amyloid diseases. NMR is a technique that can, in principle, detect signals from all fluctuating conformers in solution. However, such high-Gibbs energy conformers are not directly observable in conventional NMR techniques, either because of the conformational fluctuation is rapid (less than ms) or the population of high-energy conformers is too low under physiological condition. In order to visualize the actual existence of high-energy conformers of proteins, we use the high-pressure NMR technique. Since the volume is strongly coupled to conformation, pressure shifts the conformation within the range of its fluctuation. Surprisingly, in many proteins, various high-energy conformers with fairly distinct structures have been detected in addition to a fully unfolded one; these include various alternately folded, locally unfolded and molten globule conformers, disclosing a rich world of protein structure beyond the basic folded one. Interestingly, the pressure-stabilized conformers have similar characteristics with those accessing by the thermal fluctuation at physiological condition. Now, we challenge a rational design for high-energy conformers of proteins for further understanding of their structure, dynamics and functional importance.

1SD-04 柔軟なループへの変異で誘導される蛋白質構造ダイナミクスの変動
Protein structural dynamics modality induced by a mutation to flexible loop
Yuji Wada, Eiji Ohmaka, Kunihiko Gekko, Shin-ichi Tate (Hiroshima University, MLS)
Protein structure is maintained in a subtle energy balance, therefore its spatial structure is essentially fluctuating at the physiological temperature. Protein structure dynamics contain a various motions in wide time regimes, from psec to msec. In particular, the motions in the range of psec - msec contain rather large-amplitude motion that correlates the residues in long distances to each other. The mutation to a residue in a correlated motion should be expected to cause motional changes to the other residues in correlated to the mutated residue. This intuitive view prompted us to explore the protein dynamics change caused by mutation to a residue in flexible loop. We analyzed dihydrofolate reductase (DHFR) on its structural dynamics modulation caused by the site-directed mutation to G67 in a flexible loop. The T2 relaxation dispersion experiments at three different temperatures using two magnetic fields were applied. The results showed that the loop mutation actually caused the dynamical modulation to the active loop motion that has the primal role in the enzyme reaction, although the loop is far distant from the mutation site. In the presentation, the caused changes in the motion with its associating thermodynamic parameters by the loop mutation and their functional significances will be discussed.

1SD-05 Molecular recognition of the C-terminal region of SMRT by SHARP and its dynamical aspect in transcriptional regulation
Masaki Mishima (Grad. Sch. of Sci and Tech., Tokyo Metropolitan Univ.)
In eukaryotic cells, each component of the protein complex assembles via weak interactions. These interactions are integrated, and it consequently exerts highly regulated biological functions. I would like to present a NMR study of a regulated protein-protein complex and its dynamical aspect in a transcriptional pathway.

1SD-06 リアルタイムモニタリングによって示された APOBEC3G による DNA 上の核性を有するスライディングとカップルされたタンパク質のアミノ酸基反応
Coupling between cytidine-deamination by APOBEC3G and its sliding along DNA with polarity as revealed with real-time monitoring by NMR
Human APOBEC3G (A3G) exhibits anti-HIV-1 activity by deaminating cytidines of the minus strand DNA of HIV-1. We already demonstrated that the deamination reaction by A3G is successfully monitored using NMR signals in real-time. This method is superior to conventional biochemical methods to examine the enzymatic reaction in terms of its higher spatial and temporal resolution. This time, deamination by A3G of DNA that contains multiple deamination sites has been examined by this method. We have found that the site located closer to the 3' end of DNA is deaminated faster than that located less close to the 3' end. This observation can be rationalized by assuming that A3G slides along DNA with 3' to 5' polarity. Alternative explanation is deamination occurs only if A3G passes the deamination site with 3' to 5' polarity. These two possibilities will be discussed. Further improvement of spatial and temporal resolution of our monitoring method with the aid of 13C labeling will also be presented.

Development of the intelligent enzyme that switches its activity in response to the environmental K⁺ concentration, that was motivated by NMR study of the unique quadruplex structure of RNA, will also be presented.

1SD-07 リン酸化により活性化されるタンパク質の構造変化と生物学的意味
Phosphorylation induced conformation change and its biological implication
Yoshihiro Kobashigawa, Hiroiuki Kumeta, Fuyuhiko Inagaki (Hokkaido University)
Phosphorylation is a major post-translational modification that regulates intracellular signal transduction. Phosphorylation induces conformation change that plays a key role in signaling. Although many X-ray works have been performed, the regulation mechanism by phosphorylation has remained elusive on the structural basis. One reason is that signaling proteins are multi domain proteins and crystallization of signaling proteins is often difficult in the phosphorylated state due to flexibility. NMR is promising for structural study of signaling proteins in the phosphorylated state and gives an insight into the biological implications of phosphorylation. Structural studies require an amount of homogeneous phosphorylated proteins. For this purpose, we devised a novel phosphorylation method that utilizes domain-domain interaction to enhance substrate local concentration. This enhances phosphorylation efficiencies by 10-100 folds and thus, we prepared an amount of homogeneous phosphorylated samples. Using this preparation, we revealed the structure of signaling molecules in the unphosphorylated and phosphorylated states by NMR. We will discuss the biological implication of the phosphorylation on the structural basis.

1SF-01 力学的刺激で引き起こされる細胞内情報伝達について：力依存的な分子の集合および解離
Mechanical force-induced intracellular signaling: assembly and disassembly of adhesion-related proteins in vitro conditions
Hitoshi Tatsumi (Dept Physiol, Nagoya Univ Sch Med)