2P091 人工酵素に移植した機能エレメントの役割
Roles of functional elements transplanted into the artificial enzyme
Mai Arakawa, Hironari Kamikubo, Yoichi Yamazaki, Mariko Yamaguchi, Mikio Kataoka (Grad. Sch. Mat. Sci., NAIST)
We succeeded to obtain an artificial enzyme, TSN-nuclease, by transplanting a complete set of the function elements extracted from staphylococcal nuclease (SNase) into a TSN domain of human transcription factor, p100. We applied the alanine insertion analysis to TSN-nuclease to clarify the role of the transplanted function elements. We prepared the mutants in which an alanine is inserted into a transplanted function element as well as the mutants in which alanine into an originally TSN region, and examined their activities. The activity was reduced by the insertion into an element, while the activity remained by the insertion into the other regions, indicating that the transplanted function element acts as a function element of TSN-nuclease.

2P092 新規ヘム蛋白質フォールドのデノボデザイン
De novo design of new heme protein folds
Yasuhiro Isogai (Dept. Biotech., Toyama Pref. Univ.)
Designing proteins de novo is a productive challenge to elucidate principles of the protein structure and function. Here we have designed tertiary structures and amino acid sequences of globular heme proteins with an α/β fold that does not occur in nature. The protein structures were modeled to accommodate heme between secondary structure elements by considering basic rules relating local structures to tertiary motifs (Koga et al., Nature 491, 222-229, 2012). The amino acid sequences to fold into a modeled structure were designed with Rosetta. The heme binding site is constructed by positioning two His residues at the sites for coordination of the heme iron and restricting the amino acid composition around the bound heme to leave space within the protein core.

2P093 天然変性タンパクとしての Bach2 ヘム結合領域
Heme binding region of Bach2 as intrinsically disordered protein
Bach2 is a transcriptional repressor, which is involved in B-cell development and plays critical roles in the process of plasma cell differentiation. Heme participates in these functional regulations of Bach2. Bach2 includes the BTB domain at the N-terminal and the b-Zip motif in the C-terminal region. As a heme acceptor, Bach2 has five Cys-Pro motifs. UV-Vis spectroscopic analyses suggested that the central region (331-520) of Bach2 shows similar heme binding manner with the full-length protein. However, no special secondarily structural element was assigned in this region. Here, we focused on this central heme binding region and characterized it based on bioinformatical analyses. The results indicated that the central heme binding region is not fully random structure.

2P094 氧気親和力のヘモグロビンのアロステリック転移
Hemoglobin allosteric transition in a single crystal form
Naoya Shibayama (Div. of Biophysics, Ichi Med. Univ.)
Nine distinct allosteric equilibrium conformers of human hemoglobin in the half-ligated and fully-ligated states have been characterized, within a novel crystal form, by X-ray structural analysis and direct oxygen equilibrium measurements on three isomorphous crystals, each capturing three different conformations. The observed nine conformations cover the complete conformational space of hemoglobin, spanning from T to R (second relaxed state) through R, with various relaxed intermediate forms between R and R2. Moreover, we found a novel intermediate form with an intermediate oxygen affinity between T and R, which may be the missing link between hemoglobin structure and function for over several decades. Details of these results will be presented.

2P095 単一結晶形中でのヘモグロビンのアロステリック転移
Hemoglobin allosteric transition in a single crystal form
Naoya Shibayama (Div. of Biophysics, Ichi Med. Univ.)
Nine distinct allosteric equilibrium conformers of human hemoglobin in the half-ligated and fully-ligated states have been characterized, within a novel crystal form, by X-ray structural analysis and direct oxygen equilibrium measurements on three isomorphous crystals, each capturing three different conformations. The observed nine conformations cover the complete conformational space of hemoglobin, spanning from T to R (second relaxed state) through R, with various relaxed intermediate forms between R and R2. Moreover, we found a novel intermediate form with an intermediate oxygen affinity between T and R, which may be the missing link between hemoglobin structure and function for over several decades. Details of these results will be presented.

2P096 酸化型コバルトミオグロビンへの速度論的配位子結合解析
Kinetic Analysis of Ligand Binding to Co(III) Myoglobin
Saburo Neya, Masaaki Suzuki, Tyuji Hoshino (Chiba University, Graduate School of Pharmaceutical Sciences)
Myoglobin reconstituted with oxidized Co(III) deuteroheme was found to exhibit relatively large affinities to cyanide, azide, pyridine, and imidazole contrary to the early proposal. The relaxation kinetic analysis revealed that the ligand association rates were small and that the dissociation rates were still much smaller. The relatively large ligand affinities in Co(III) myoglobin were found due to the compensation of small association rates with fairly smaller dissociation rates. The rationale for the characteristic ligand-binding behavior of Co(III) myoglobin was provided on the basis of the properties of Co(III) which has an additional negative charge and forms stronger metal-ligand bonds than Fe(III).