needed to place individual metal atoms precisely in the cluster. Additionally, we have shown in the recent study by X-ray spectroscopy that the Mn_4Ca cluster is highly susceptible to the X-ray radiation damage, particularly under the condition that the diffraction data have been commonly taken (2). In the current study, we have compared the effect of X-ray radiation damage to the two major Mn proteins, PS II and Mn catalase. Unlike PSII, the crystal structures of the Mn catalase have been reported with high resolutions (3,4). We discuss possible differences between these two cases. We discuss the importance of the combination of the spectroscopic techniques and crystallography in order to obtain intact forms of the catalytic complexes (2,5).

References:

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2SC1058 Metallation status and metalloprotein conformations: Tracking subtle changes using time-resolved ESI-mass spectrometry - real title

Martin Stillman, (University of Western Ontario)

Electrospray ionization mass spectrometry (ESI-MS) has provided exquisite detail of the reactions of metalloproteins with metals. Two examples will be discussed: (1) the metallation mechanism of metallothioneins and (2) the heme transport reactions of the Isd proteins in *Staphylococcus aureus*. Metallothioneins (MTs) are ubiquitous metalloproteins. Spectroscopic probes used for equilibrium studies during the step-wise, metal-by-metal metallation have established that metallothionein binds rapidly a wide range of metals, including Zn, Cu, Cd, Hg, As, Bi, and Pb. Time- and temperature-resolved ESI-MS data have provided information on the mechanism of As(III)-metallation. Each of the six As(III) ions bind in a bimolecular, noncooperative manner to human MT1a. The individual rate constants, activation energies and Arrhenius factors were calculated individually for each metal ion bound. *Staphylococcus aureus* is an important human pathogen that causes a range of infections and diseases including life threatening septicemia. There is an absolute requirement for iron by *S. aureus* in human hosts. A series of nine proteins, named Isd, have been identified as a heme acquisition system. Together, these Isd proteins are believed to interact with heme proteins, particularly hemoglobin, extract the heme molecule, and transport it across the cell wall through to the membrane into the cytoplasm where the iron is finally extracted for use. Recent papers: Ngu et al. *Biochemistry*, 2009, 48, 8806 and Muryoi et al. *J. Biol. Chem.* 2008, 283, 28125.

2SC1350 マイクロ-ミリ秒時間領域における蛋白質のフォールディングダイナミクス In vitro protein folding dynamics in the microsecond-to-millisecond timescale

Tetsunari Kimura, (Institute for Molecular Science)

Protein folding is one of the dynamical conformational changes and a complex self-assembly process. Experimental elucidation of the polypeptide conformational dynamics is critical to understanding the molecular mechanism of protein folding reaction. However, protein folding is a very fast reaction, which complete within 10 ms in some cases, and it is difficult to follow the early stage of the reaction by using the conventional methods. The development of the rapid solution mixer, whose mixing dead time is less than 50 μ s, enabled us to directly observe the conformational changes with the time resolution of 100 μ s. By combining this mixer with various spectroscopic methods, we have succeeded in investigating 1) conformational changes including secondary structure formation and the compaction of molecular size, 2) changes in the conformational heterogeneity of the polypeptide chain, and 3) the exclusion of the water molecules from the polypeptide. In this symposium, the molecular mechanism of the *in vitro* protein folding revealed by the experiments will be discussed and compared with the folding energy landscape theory.

2SC1412 Structural and functional plasticity promote smooth evolutionary transition of enzyme

NOBUHIKO TOKURIKI, (Department of Chemistry, University of Cambridge)

Understanding evolutionary dynamics, how protein evolve to new function and structure is crucial to design and engineer proteins for medical and industrial uses. Laboratory evolution can provide such insights as the intermediates of the process can be isolated and characterized. I will describe a complete trajectory created by laboratory evolution, beginning with a weak, promiscuous activity of a highly proficient phosphotriesterase, and evolving the enzyme

to a proficient aryl esterase. The end point of the process exhibits weak phosphotriesterase activity, thus representing a complete functional switch. The transition of the two catalytic activity occurred smoothly passing through bi-functional, generalist intermediates, but neo-specialization as an esterase was accompanied by strong tradeoffs. The functional switch was accompanied by a structural change from a bi- to a mono-metallo active-site. The evolving enzyme variants comprise a series of evolutionary intermediates in which the new function is gradually gained, and the original function is eventually declines, and where the active-site fluctuates between a bi and mono-metal mode. These results demonstrate the capacity of laboratory evolution experiments to provide unique insight into molecular evolutionary dynamics. Moreover, the evolutionary intermediates obtained in these experiments represent excellent model systems that we can use to study the interplay between protein function and structure.

2SC1422 In-cell NMR による細胞内蛋白質の構造とフォールディング安定性 Structure and folding stability of proteins in cells

Masahiro Shirakawa, (Graduate School of Engineering, Kyoto University)

Structure and folding stability of proteins in cells

2SC1444 酵母プリオン Sup35NM のオリゴマー構造はプリオン株の表現型を決定する Structure of yeast prion Sup35NM oligomer determines prion strain phenotype

Motomasa Tanaka, Yumiko Ohhashi, (RIKEN Brain Science Institute, Tanaka Research Unit)

Aggregation-prone proteins often misfold into multiple distinct amyloid conformations dictating their different physiological impacts. Although amyloid formation is triggered by a transient nucleus, the mechanism of how an initial nucleus is formed and allows the protein to form a specific amyloid conformation remains unclear. Here we show that, prior to fiber formation, the prion domain (Sup35NM, consisting of residues 1-254) of yeast prion Sup35, the $[PSI^+]$ protein determinant, forms oligomers in a temperature-dependent, reversible manner. Mutational and biophysical analyses revealed that "nonnative" aromatic interactions outside of the amyloid core drive oligomer formation by bringing different Sup35NM monomers together, which specifically leads to the formation of highly infectious strain conformations with more limited amyloid cores. Thus, transient nonnative interactions in the initial nucleus play pivotal roles in determining the diversity of amyloid conformations and resulting prion strain phenotypes.

2SC1506 Theoretical studies for biomolecular behavior in a liquid state - from the view point of molecular crowding

Yuichi Harano, (Institute for Protein Research, Osaka University)

The life was created and has been evolved in water. When the behavior of biomolecules is considered, the water must play an important role as a surrounding environment for them. Nevertheless, most research of the biomolecular science is tends to be focusing on the nature of biomolecules itself. One of the reasons for that might be due to difficulty in understanding the molecular picture of liquid state from a standpoint of equilibrium or dynamics. Moreover, there still exists the unbridgeable gap between the experiments in biology and the theories in liquid state. The experiments are usually done in the solvent condition that can be experimented regardless of the character of the solvent. If the "third" substance such as salt or sugar for the practical or technical reasons is mixed into the solvent water, the solvent effects to the solute molecules dramatically change. On the other hand, the theoretical studies only can treat the simple system and still cannot catch up with the experimental condition. However, we found the macromolecular behavior in the liquid state can be sketched mainly with the concept of "molecular crowding", and that collective movement of solvent plays a dominant role rather than macromolecule itself. In the talk, the recent studies by liquid state theories are introduced, and the current issues can be shared with experimentalists.

2SC1528 In-cell NMR で生きた細胞の中の蛋白質の立体構造とダイナミクスを見る Seeing proteins inside living cells: structures and dynamics of proteins by in-cell NMR

Yutaka Ito, (Tokyo Metropolitan University)

In-cell NMR has been used to detect various intracellular events such as conformational changes, dynamics and binding events in bacterial cells. In eukaryotic cells, in-cell NMR studies have been performed by injecting proteins into *Xenopus laevis* oocytes or eggs and, recently, cell-penetrating peptides have been used to deliver proteins that can be observed in living human cells.