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**Keywords: V-ATPase, asymmetric, rotation mechanism**

## MS08.P06

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**Structural analysis of MamA, a magnetosome associated protein from two different magnetospirillium species**

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Tetra- tricopeptide repeat (TPR) is a structural motif found as such or forming part of a bigger fold in a wide range of proteins. It serves as a template for protein-protein interactions and mediates multiprotein complexes [1]. MamA is a unique, highly abundant, Magnetosome associated protein and predicted to contain 5 TPR motifs as well as predicted putative one. Magnetosome is a subcellular organelle that consists of a linear-chain assembly of inner membrane invaginations each able to biomineralize and enclose a ~50-nm crystal of magnetite or greigite. Magnetosome allows magnetotactic bacteria, a diverse group of aquatic microorganisms, to orientate themselves along geomagnetic fields in search of suitable environments [2]. MamA is one of the most characterized magnetosome-associated proteins *in vivo* and yet, its function is not clear [3-5]. Here, we report on the crystallization and structure analysis of recombinant *M. magneticum* (AMB-1) and *M. gryphiswaldense* (MSR-1) MamA deletion mutants. The structures were determined to a resolution of 2.0 Å and confirmed MamA fold as a five TPR motifs containing protein.

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**Keywords: TPR containing proteins, Magnetosome, Magnetotactic bacteria**

## MS08.P07

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**The mechanisms of self-assembly of the vault, the largest cytoplasmic ribonucleo-protein complex**

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Vaults are the largest cytoplasmic ribonucleo-protein particles found in numerous eukaryotic species. They were first observed in 1986 as contaminants in preparations of clathrin-coated vesicles from rat liver. Rat liver vault comprises three kinds of proteins: the major vault protein (MVP) , the vault poly(ADP-ribose) polymerase (VPARP) , the telomerase- associated protein 1 (TEP1) and a small untransrated RNA consisting of 141 bases (vRNA). The mass of rat liver vault is about 10 MDa, and the particle shell measures about 700 Å in length and about 400 Å in maximum diameter. Most vault particles are present in the cytoplasm, but few of them (about 5% of the total vault fraction) localize to the nucleus. Several studies suggested that vaults might play an important role in the multi-drug resistance (MDR) of cancer cells. Human vRNAs have the ability to bind mitoxantrone, a chemotherapeutic compound, and they may play an important role in the export of toxic compounds (Gopinath et al., Nucleic Acids Res., **33**, 4874-4881 (2005)). The recent study shows that vaults may be involved in innate immunity (M. P. Kowalski et al., Science **317**, 130-132 (2007)). However, their cellular function remains unclear.

We have determined the x-ray structure of rat liver vault at 3.5Å resolution in 2008 [1, 2]. X-ray structure reveals that vault particle has 39-fold dihedral symmetry and shell is made up of 78 identical MVP chains. Each MVP monomer folds into 12 domains: nine structural repeat domains, a shoulder domain, a cap-helix domain, and a cap-ring domain. Side-by-side hydrophobic interactions of the cap-helix domain play a key role for self-assembly of the half-vault. N-terminal residues of MVP domain 1 form intermolecular interactions around two-fold axis including anti-parallel sheet and ionic bond. In this session, we will discuss the mechanisms of self-assembly of the vault.

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**Keywords: vault, ribonucleo-protein complex, self-assembly**

## MS08.P08

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**Modeling the organization of molecules in collagen using the paracrystal concept**

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A characteristic feature of the dense phases formed by fiber-shaped molecules is their organization into parallel rods packed in a hexagonal or pseudo-hexagonal lateral network. This is typically the case for the collagen triple helices inside fibrils, as confirmed by recent X-ray diffraction experiments carried out on highly crystallized fibers obtained by immersing the freshly extracted fibers in a salt-controlled medium.

However such diffraction patterns also generally exhibit additional features in the form of diffuse scattering, which is a clear signature of a low degree of lateral ordering. Only few studies have analyzed and modeled the lateral packing of collagen triple helices when the structure is disordered. Some authors have used the concept of short-range order but this approach does not contain any echo of a hexagonal order. In this study [1], we use an analytical expression derived from the paracrystal model which retains the hexagonal symmetry information and leads to a good agreement with the experimental data in the medium-angle region. This method is quite sensitive to the degree of disorder and to the inter-object distance. *One clear result is that the shift in peak positions, generally attributed to variations in intermolecular distances, can also arise from a change in the degree of ordering without any significant modification of the distances*. This underlines the importance of evaluating the degree of ordering before attributing a shift in peak position to a change in the unit-cell. This

method is generic and can be applied to any system composed of rod-shaped molecules.

The X-ray scattering experiments were performed at ESRF microfocus beamline ID13 on collagen tendon fibers extracted from mouse tails.

[1] J. Doucet, F. Briki, A. Gourrier, C. Pichon, L. Gumez, S. Bensamoun, J.-F. Sadoc *J. Structural Biology* **2011**, *173*, 197–201.

**Keywords: collagen, paracrystal, modeling**

## MS08.P09

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**Structural changes of bovine cytochrome *c* oxidase dependent on the redox states**

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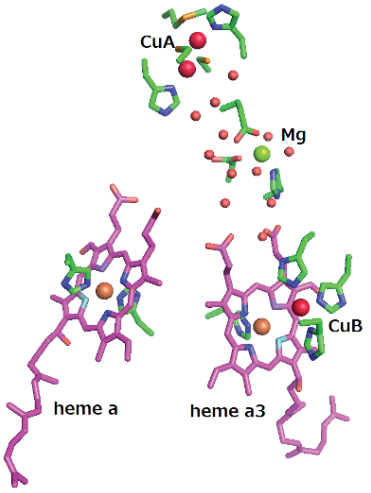
Cytochrome *c* oxidase (CcO) is a terminal oxidase in the respiratory chain. Mitochondrial CcO contains four redox active metal sites, CuA, heme a, heme a3 and CuB. The oxygen molecule is bound to the reduced heme a3-CuB site and reduced to water molecules by electrons from cytochrome c. The oxygen reduction is coupled to the proton translocation across the membrane, generating electrochemical proton gradient. To understand energy transducing mechanism, we have been determined the structural changes of bovine mitochondrial CcO in the resting oxidized and the reduced states. In previous analyses, the conformational changes were found at the peptide segment containing aspartate residue on the CcO surface and the α-helix between hemes a and a3 [1, 2]. It has been suggested that these changes contribute to the proton transfer.

In this study, we prepared bovine CcO crystals in the reduced state which was identified by the measurement of the UV/vis absorption spectrum of the single crystal. We performed X-ray diffraction experiment at BL44XU in SPring-8 and determined the detailed structural changes at 1.4 Å resolution. The FO-FO electron density map calculated by using the data from the resting oxidized and the reduced states indicates that the changes are localized at the inside of CcO, where the redox active metal sites are located. In addition to the previously found conformational changes, it is found that water molecules in the water cluster between subunit I and II are moved dependent on the redox states. These waters are located near the CuA site in the subunit II and the Mg site which is octahedrally coordinated by one aspartate residues in the subunit II, one aspartate and one histidine residues in the subunit I and three water molecules. Furthermore, refined structural changes were found at heme a3 porphyrin plane and the CuB ligand. It is suggests that these changes are involved in the energy transducing reaction.

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**Keywords: Supramolecular_Protein, X-ray_Structure, Redox_Metal**

## MS08.P10

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**High resolution analysis and anomalous dispersion analysis of bovine cytochrome *c* oxidase**

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Cytochrome *c* oxidase (CcO) in the respiratory chain is supramolecular membrane protein. CcO functions as a terminal oxidase catalyzing the molecular oxygen reduction coupled to the proton transfer across the membrane. The X-ray structures of mitochondrial and several bacterial CcOs were determined and its resolutions have been improved step by step [1-2].

To understand proton transfer mechanism, we aim to determine orientations and protonation states of amino acid residues (imidazole and carboxyl group etc.) in the proton pathways. In this study, we performed X-ray diffraction experiment at BL44XU in SPring-8 using bovine mitochondrial CcO crystals in the resting oxidized state and improved the resolution to 1.4 Å. To reduce X-ray irradiation damage, diffraction data were collected from many isomorphous crystals. To improve accuracy of the data, the redundancy of data was increased and low quality images ($R_{merge}$ > 0.3 or mosaicity > 0.3) were removed. Resultant data shows 1.6 of I/ $\sigma$ (I) at 1.42-1.40 Å resolution range. Electron density map shows many multi-conformations of amino acid residues (Met etc.). Structures of phospholipids and ligand at the $O_2$ reduction site are refined.

CcO crystals give anisotropic diffraction intensities dependent on the crystal axis and the direction of X-ray beam. Processing of the diffraction images at anisotropic resolution ranges improved R and $R_{free}$ values without lowering quality of the electron density.

Anomalous scattering analysis was carried out to determine phosphorus atom positions in phospholipids and to identify the atomic species of the ligand at the $O_2$ reduction site. The difference anomalous peaks indicate that 7 phospholipids are located on the CcO surface, so we correct previous models [3]. No difference anomalous peak was observed at the $O_2$ binding site indicating that the ligand is not chloride ion.