1SBP-02 微小重力下の結晶化
Protein crystallization under microgravity conditions

Microgravity conditions can improve the quality of protein crystals mainly due to the suppression of the natural convection. The space and the high magnetic fields are able to attain such microgravity environments. We have developed a protein crystallization system with strong magnetic force that enables to cancel out the gravity of a water droplet and with the in-situ observation device for crystal growth. Crystallization has been carried out and a series of photographs of crystal growth have been taken. The time-lapse movie showed that protein crystals exhibited magnetic orientation while growing. X-ray diffraction experiments indicated that the quality of crystals generated in the system was more homogeneous and better than that of the control crystals.

1SBP-03 結晶スポンジ法による非結晶性・極少量化合物のX線結晶構造解析
Crystalline Sponge Method: X-ray Analysis without Crystallization on the Microgram Scale
Makoto Fujita (The University of Tokyo)

X-ray single crystal diffraction (SCD) analysis has the intrinsic limitation that the target molecules must be obtained as single crystals. Recently, we report a new protocol for SCD analysis that does not require the crystallization of the sample (Nature 2013, 495, 461-466; Nat. Protoc. 2014, 9, 246-252). In our method, tiny crystals of porous complexes are soaked in the solution of a target, where the complexes can absorb the target molecules. The crystallographic analysis clearly determines the absorbed guest structures along with the host frameworks. As the SCD analysis is carried out with only one tiny crystal, the required sample amount is of the nano-to-microgram order. We demonstrate that even ~50 ng of a sample is enough to be analyzed.

1SBP-04 膜タンパク質の結晶化法
Crystallization methods of membrane proteins
Takeshi Murata1,2 (1Science/Chiba-U, 2PRESTO/JST)

Membrane proteins play crucial roles in many biological functions and are of key importance for medicine. Over 50% of commercially available drugs target membrane proteins. We need to understand membrane protein structures to provide a basic understanding of life at the molecular level and for computer aided rational design of new drugs. However, structural studies of membrane proteins have not been progressed very fast because of difficulty of the crystallization. In my talk, I would like to introduce several strategies for crystallization of membrane proteins and our recent work, and like to discuss about the future of X-ray crystallography of membrane proteins.

1SBP-05 抗体を用いた膜蛋白質の結晶化
Crystallization of membrane proteins using antibody fragments
So Iwata1,2 (Kyoto Univ. Grad. Sch. Med., RIKEN SPring8 Center)

Antibody fragments, including Fab and Fv fragments, are known to be effective to stabilise and crystallise membrane proteins. However, it has been difficult to raise monoclonal antibodies to recognise conformational epitopes of native membrane proteins using the conventional mouse hybridoma system. We have recently succeeded to raise antibodies against native membrane proteins using the system combined with improved immunization and screening methods focusing on mammalian membrane proteins. In my talk, I will present several successful examples of crystallisation of membrane proteins including human A2A adenosine receptor (A2AAR), human anion exchanger 1 and RCE1 membrane proteinase.

1SBP-06 対称性を持つタグを利用したタンパク質結晶化確率の向上
Use of symmetric tag to increase the probability of protein crystallization
Min Yao (Fac. of Adv. Life Sci., Hokkaido Univ.)

Protein crystallography becomes more powerful and useful method for life science, due to astonishing progress in its techniques in the past decade. However, protein crystallization still remains as a major bottleneck. It is dependent on the accidental methods searching for special crystallization reagents and the crystal growth conditions. Therefore further development of more advanced crystallization methods is required to increase the probability of successful crystallization.

In order to increase the probability of protein crystallization, we developed a novel method by fusing target protein with crystallization tags named 2/3RS-tag. These 2/3RS-tags polymerize target proteins with 2 or 3-fold axial symmetry, and consequently accelerate formation of crystal.