quality crystals, followed by the development of estimation method of the microgravity effect on crystallization.
In the project, high viscosity of the precipitant solution had positive effects on the quality of the protein crystal grown in microgravity. We have obtained high-quality crystals of alpha-amylase diffracted to 0.89 Å and of lysozyme diffracted to 0.88 Å both at SPring-8 beamline BL12B2 in the project. Both precipitant solutions contained polyethylene glycol either as a precipitant or an additive. It might be because protein and impurity depletion zones are positively formed in high viscous solutions especially in the microgravity environment.
We developed the method for estimating the diffusion coefficient (D) and kinetic coefficient (β) by a simple experiment. The value 'D/β' indicates that protein and impurity depletion zone around the crystal is formed in microgravity if D/β is low enough. Since we can predict the effects of microgravity on the protein crystal growth before performing microgravity experiment, it is possible to select samples and crystallization conditions which have high possibility to improve the crystal quality. Moreover, if we modify the crystallization condition to lower D/β, the improvement of the crystal quality can be expected in microgravity experiment.

We are grateful to the Japan Synchrotron Radiation Research Institute (JASRI) for access to and user support for the synchrotron facilities of BL12B2 at SPring-8, Harima, Japan. We thank ESA and the Belgium government for Odissea mission and the usage of Granada Crystallization Facility (GCF), the Federal Space Agency and RSC Energia for the usage of the Russian Service Module, and NASA for the usage of the incubator in the US module. We would like to thank Professor Garcia-Ruiz and the members of his laboratory in CSIC-University of Granada for their helpful advices.

MS06 P05

JAXA-GCF Project --- The Past, Present and Future

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Keywords: microgravity crystal growth, atomic resolution crystallography, high-resolution X-ray diffraction

Japan Aerospace Exploration Agency (JAXA) finished JAXA-GCF project in 2006. Totally six protein crystallization experimental opportunities in space were provided to crystallographers, more than 250 protein samples were launched, using Russian flight opportunities, twice a year, from 2003. In the project, the success rate of crystallization, that is mostly the improvement of the maximum resolution, has been significantly increased to about 70% of protein that was highly purified and succeeded in the optimization of the crystallization condition. Moreover, the maximum resolution was even improved if the crystal showed already an excellent resolution around 1 Å in the ground-based experiment. We have obtained several know-how to grow high-quality crystals in space.
Based on them, JAXA has started JAXA-New-GCF (JAXA-NGCF) experiment in 2007. Three flight opportunities are scheduled, once in every six months. The first flight has already launched on Jan. 18, 2007 and will be landed in April. The purposes of JAXA-NGCF are to obtain atomic-resolution crystals for precise structural analysis, to cooperate with national project, and to transfer technology to private companies for commercial use.
We thank ESA and the Belgium government for Odissea mission and the usage of Granada Crystallization Facility (GCF), the Federal Space Agency and RSC Energia for the usage of the Russian Service Module, and NASA for the usage of the incubator in the US module. We are grateful to Professor Garcia-Ruiz and the members of his laboratory in CSIC-University of Granada for their helpful advices. We thank Protein 3000 Project (Riken and eight universities), NIAS, PCProt, and other users for providing protein samples.

MS06 P06

Cu co-crystallization and metal-ions cross-influence as a new optimization tools

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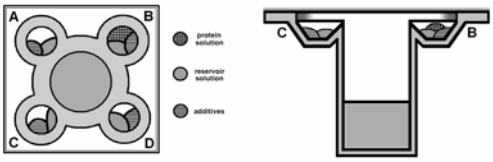
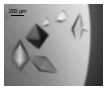
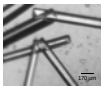
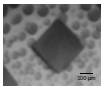
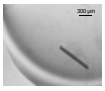
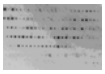
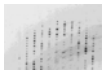
Keywords: Crystal morphology, Cupric compounds, Cross-crystallization

The effect of several metal cations (Cu^{2+}, Cd^{2+}, Co^{2+}, Ba^{2+}) was tested in attempts to improve crystallization and verify a newly discovered cross-crystallization method with two selected proteins; di-heme cytochrome c_4 from anaerobic purple sulphur bacterium *Thiocapsa roseopersicina* and sweet-tasting protein thaumatin from the African berry *Thaumatococcus daniellii*. Cu^{2+} ions promoted the most dramatic improvement in crystal morphology, internal packing and diffraction quality. This investigation qualitatively established the influence of cupric cations on the crystal growth by using the cross-crystallization procedure.



(Fig.: Schematic side and top view of Emerald BioStructures CombiClover Crystallization Plate used for sitting drop cross-crystallization experiments). It was found that influence of Cu^{2+} ions produced evidently different outer morphology and internal packing of thaumatin crystals (hexagonal prism). Usually their shape is presented as a tetragonal bipyramids. In the case of cytochrome, the good diffractable crystals were obtained only by using cross-crystallization method with metal-ion salts. Newly grown crystals (hexagonal prisms) of thaumatin and cytochrome displayed as the same primitive tetragonal system and diffracted up to 1.7 Å. Crystals were suitable for high-resolution structure analysis. (Table: Crystal morphology and internal packing influenced by metal-ion salts).

Protein	Thaumatin		Cytochrome	
Crystallization method	Standard crystallization	Cross-crystallization	Standard crystallization	Cross-crystallization
Crystallization conditions in protein solution	30-40% PEG 3350 15% PEG 6K 0.1 M TRIS pH 6.5	30-40% PEG 3350 15% PEG 6K 0.1 M TRIS pH 6.5 5 mM cupric chloride	3.2 M ammonium sulfate 0.1 M citric acid pH 5.0	3.2 M ammonium sulfate 0.1 M citric acid pH 5.0 5 mM cupric chloride
Crystal outer shape	 tetragonal bipyramids 	 hexagonal prisms 	 quasi crystals – plates	 hexagonal prisms
Crystal system	orthorhombic	tetragonal	no diffraction	tetragonal
Space group	$P2_12_12_1$	$P4_12_12$	no diffraction	$P4_12_12$
Mosaicity	 0.89	 0.44	no diffraction	 0.580
Resolution	1.7 Å	1.5 Å	no diffraction	1.72 Å

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MS06 P07

High-Temperature Crystallization of Thermostable T1

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Keywords: thermostable lipase, high temperature, protein crystallization

The gene encoding thermostable T1 lipase secreted by *Geobacillus* sp. strain T1 was overexpressed in a prokaryotic system. Preliminary crystallization was conducted with crystal screen and crystal screen II through a sitting drop vapor diffusion method with 0.5 mg/mL purified T1 lipase at 16°C. Crystallization at 16°C using formulation 21 of crystal screen II at 2.5 mg/mL yielded bigger and more defined crystals. Good crystals could easily be obtained as the temperature was increased further while retaining other conditions. In fact, crystallization of T1 lipase is still possible at 60°C and this is new in lipase crystallization.

MS06 P08

Structural investigation of human thrombomodulin domains. Lou KL, Chen KT and Wu HL. Graduate Institute of Oral Biology, College of Medicine, National Taiwan University, Taipei, Taiwan.
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Thrombomodulin (TM) is a multifunction glycoprotein expressed on the endothelial cell surface. This glycoprotein is structurally organized into 5 distinct domains. From the C- to N-terminus, TM has a short cytoplasmic tail on the intracellular side of plasma membrane, a transmembrane helical segment, and the extracellular part containing a serine/threonine-rich region, EGF-like repeats, as well as an N-terminal C-type lectin domain. Each of the distinct domains has different biological functions that impact on coagulation, fibrinolysis, inflammation, cell Adhesion, and cell proliferation. To understand how this single molecule may play different important roles through distinct domains, we commenced the crystal structural analysis of TM with domain variants combination. Various domains of TM were constructed and expressed in *Pichia Pastoris*: (i) the TMD-1 construction contains the C-type lectin domain, (ii) the TMD-23 construction contains the EGF-like repeats and the serine/threonine-rich region, and (iii) the TMD-123 construction contains all the extracellular domains. Crystallization screening indicated successful conditions for TMD-23 and potential ones for TMD-1 requiring optimization.

MS06 P09

Pulsed, high-voltage, inhomogeneous electric fields improve nucleation and crystal growth

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In order to avoid multiple nucleations, leading to showers of micro crystals, we investigate the effect of an high-voltage, inhomogeneous electrical fields on the protein concentration in crystallization drops.
Our results indicate that the high voltage, inhomogeneous E-field increases the probability, for nucleation from lower protein concentration. Temporally controlled exposure of proteins in sitting or hanging drops to strong inhomogeneous E-fields of 3000 -5000 V leads to nucleation even in dilute protein solutions. Switching the E-field off allows for the formation of large single crystals.

[1] Taleb M., Didierjean C., Jelsch C., Mangeot J.P., Capelle B. and Aubry A.. J. Cryst. Growth, 1999, 200, 575.
[2] Taleb M., Didierjean C., Jelsch C., Mangeot J.P., and Aubry A.. J. Cryst. Growth, 2001, 232, 250.

MS06 P10

Dynamic screening experiments to maximize hits for protein crystallization Naomi E. Chayen, Sahir Khurshid and Lata Govada *Department of Bio Molecular Medicine Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Faculty of Medicine, Imperial College Londo,n Sir Alexander Fleming Building, London SW7 2AZ, UK*
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Keywords: Screening, Vapour diffusion, Nucleation

In the first step of crystallization screening, the protein is exposed to a wide variety of reagents at different concentrations. Once a "hit" deemed to be conducive to crystallization is identified, parameters such as precipitant concentration, pH and temperature are used to produce crystals suitable for analysis by X-ray diffraction. Crystals, crystalline precipitate and phase separation are usually considered leads that are worth pursuing. Clear drops are mostly disregarded. This poster presents a screening technique that makes use of clear drops. Clear drops are subjected to evaporation with the aim of driving them to supersaturation. The findings reported bring a new dimension to screening and open up the scope for utilizing a potential wealth of crystallization conditions that are currently being ignored. Furthermore, this technique enables the utilization of far less protein sample as well as obtaining the 'hits' in shorter times.