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To utilize a microgravity environment effectively, we modelled the benefit of microgravity indexed by a diffusion coefficient and a kinetic coefficient during a protein crystal growth. The concentration of a protein around a surface of a crystal can be estimated by the balance of the protein molecule diffusion toward the crystal and the protein molecule uptake into the crystal. The rate of the diffusion of the protein molecule can be represented by the diffusion coefficient of the protein molecules (D), and the rate of a protein molecule uptake into the crystal can be represented by the kinetic coefficient for a protein trapping into the crystal (beta). The decrease of a supersaturation level on the surface of the growing crystal, which induces the concentration depletion zone formation, can be indexed by D/beta. The smaller D/beta is, the greater the depletion zone around the crystal forms. Therefore, learning D/beta before the crystallization experiment is the efficient way to perform crystallization experiment in microgravity. 'D' can be decreased by using viscous reagent, such as PEG, in the crystallization solution. This was consistent with the results that a high viscous crystallization solution was found to be effective for obtaining high-quality crystals in microgravity in the Japan Aerospace Exploration Agency (JAXA)-GCF project. The purification of the protein sample can increase 'beta'. We will show the effect of microgravity on crystal growth of several proteins using the value D/beta. We thank ESA and Professor Garcia-Ruiz and the members of his laboratory in CSIC-University of Granada for the usage of GCF and their helpful advices, and the Federal Space Agency and RSC Energia for the usage of the Russian Service Module.

Keywords: microgravity crystallization, diffusion, viscosity

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### Protein crystallization under microgravity in JAXA New-GCF project

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Japan Aerospace Exploration Agency-New GCF project (JAXA-NGCF) had three flight opportunities from Jan. 2007 to Apr. 2008, followed by the previous JAXA-GCF project. We produced various know-how of protein crystallization experiment in space in these projects. The protein samples were provided by academic and industrial users. Commercial users took part in the project for the first time. In the JAXA-NGCF, in addition to the main crystallization cell, JAXA crystallization box, we developed a microchip crystallization cell which required only 2 micro-l of a protein solution and could directly be applied for X-ray diffraction experiment. We also developed nucleation technique to help the crystal growth since it was known that a nucleation was sometimes suppressed in microgravity. Applying these techniques, we obtained atomic resolution crystals of prostaglandin synthase-related proteins, nylon-oligomer degrading enzyme, glucose isomerase, alpha-amylase, lysozyme and others. Our unique strategy for the space experiment can offer an opportunity of the usage of microgravity environment for users who want to obtain high-quality crystals on time because of the regular services and technical supports. We will introduce the status of the next protein crystallization experiment in the Japanese Experimental Module 'Kibo' in the International Space Station (ISS). We thank ESA and Professor Garcia-Ruiz and the members of his laboratory in CSIC-University of Granada for the usage of GCF and their helpful advices, and the Federal Space Agency and RSC Energia for the usage of the Russian Service Module.

Keywords: microgravity crystallization, ISS, high-resolution protein structures

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### Investigation of morphology and surface microtopograph of cubic insulin

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We have been investigating crystal growth of cubic insulin from porcine and bovine pancreas using experimental and theoretical approaches. Dodecahedron crystals surrounded by {1 1 0}, cube surrounded by {1 0 0}, and the intermediate form were obtained (Fig. 1). The first one is the stable form, and frequency of appearance is slightly different between porcine and bovine insulin. We had calculated intermolecular interactions of porcine insulin using macrobond [1] and electrostatic energy of transfer (EET) [2] analyses. The surface energy of {1 1 0} was slightly lower than that of {1 0 0}. In this paper, we evaluated the hydration energy and carried out the explicit treatment of the crystal water in EET. The tendency in the interaction energies were not affected by inclution of the hydration energy. On the other hand, the explicit treatment of water molecules in EET improbed the discrepance between EET and macrobond analyses. The growth mechanism and the surface energies of porcine and bovine insulin will be discussed together with the results of surface microtopograph.

[1]Y. Matsuura *et al*. (2003) *Acta. Crystllogr.*, D**59**, 1347. [2]T. Takahashi *et al*. (2003) *J. Mol. Biol.*, **234**, 421.

Figure 1. Morphology of cubic insulin

Keywords: insulin, crystal growth, interacton energy analysis