Summary report of the ISS-Kibo utilization mission, "Growth Mechanism and the Perfection of Protein Crystals under Microgravity by *In Situ* Observation (NanoStep)"

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Many years have passed since 20% of protein crystals grown in space showed better resolution in X-ray structural analysis. However no "convincing" space experiments in the light of crystal growth mechanism and the perfection have been conducted to answer this "Why?". The perfection of crystals are the result of crystal growth history and thus to understand why space grown crystals are "better" in X-ray diffraction, we need carefully to analyze both difference of growth mechanisms in gravity and under microgravity. Such approach has historically been conducted by growing crystals in space followed by transporting them to the ground for statistic investigations. Contrary to such "indirect" approaches, we have adopted a "direct" approach, namely, optical *in situ* observation for crystal growth at molecular level, which has actively been developed and improved by the present investigator.

In order to employ this *in situ* observation system in KIBO at the same resolution level, 10^{-3}nm/s, as in our laboratory on the ground, we developed (1) a modified Mach-Zhender interferometer, by which we measure growth rate *vs* supersaturation and surface morphology *vs* supersaturation, (2) quickly temperature controlled miniature growth apparatus at 5-40 ℃ without disturbing optical measurement owing to the thermal expansion of the cell materials, and (3) a software for *in situ* analysis of the interference data which was counted to be a several Tera-byte data throughout this experiment. In a sense, this experiment is based on not only *in situ* observation but also *in situ* analysis. This was the key issue for this experiment. Thanks to these preparations, all planned experiments were 100% successfully conducted.

Among other experimental results, the growth rate *vs* supersaturation measurement is pronounced. It has been believed that crystals grow slower due to the suppression of convection or flow under microgravity. The present results have denied this speculation. Crystals in space grow faster than in gravity, fig.1. This is attributed to the large reduction of impurity adsorption on the crystal surface under microgravity, the effect of which is often observed in gravity

leading to crystal growth rate retardation. In what follows, the heterogeneous 2D nucleation is suppressed largely due to this reduction of impurity adsorption, fig.2. This suppression also widens the growth regime of spiral growth that favors crystal growth with less micro defects generation in crystals. This widening of spiral growth regime in space might be important for the easier improvement of perfection of protein crystals in space.

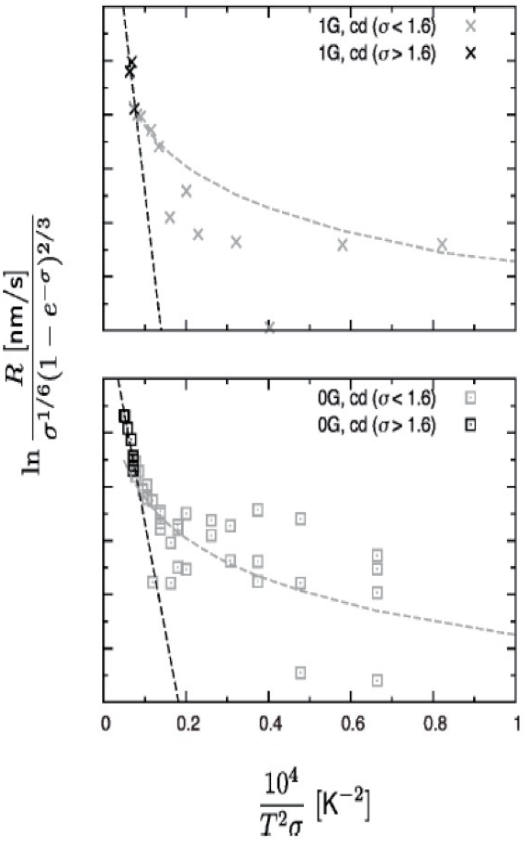

fig. 1 Growth rate vs supersaturation. Crystals grow faster in space.

Solution concentration distribution around growing crystals both in gravy and under microgravity was compared by newly developed 3D interferometric observation using a concept of CT. This observation shows the uniformity of less than 2.6% supersaturation over the growing crystal surface under microgravity. While, in gravity the uniformity got worse down to 26%, which increase morphological instability to form dendrite or hopper crystals, which promote other types of large defects in crystals. This concept of morphological stability would be important for larger crystal size and thus crystals for neutron diffraction would have to be carefully investigated.

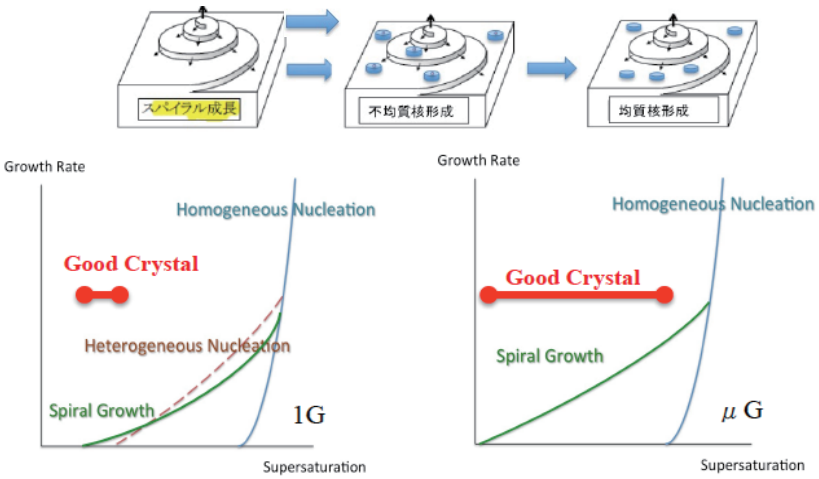

fig. 2 Wider spiral growth regime in space.