3 1999 Oktober

LOW G JOURNAL

An Intospace Information Service

# ISS – Research Centre and Marketplace

**Commercial Utilization of the ISS becomes now a momentum in Europe with ESA's initiative: »Commercial Agent«**

A major step was taken at the Ministerial Council meeting of ESA, held in Brussels in May 1999, which has a positive impact on Europe's commercialization efforts of the International Space Station. ESA is investigating the option to commercialize the European part of the ISS with respectively two conceptual elements:

- industrial operator responsible for the complete operations of Columbus as well as maintenance and logistics;
- the Commercial Agent, responsible for marketing and sales of the ISS commercial utilization.

A challenging undertaking, for both the respective agency and the space industry in operation, is to serve commercial customers who pay for the ISS utilization instead of having users subsidised by the agencies. Thus the focus of the ongoing definition process for the commercial agent needs to be on the potential customers and the market.

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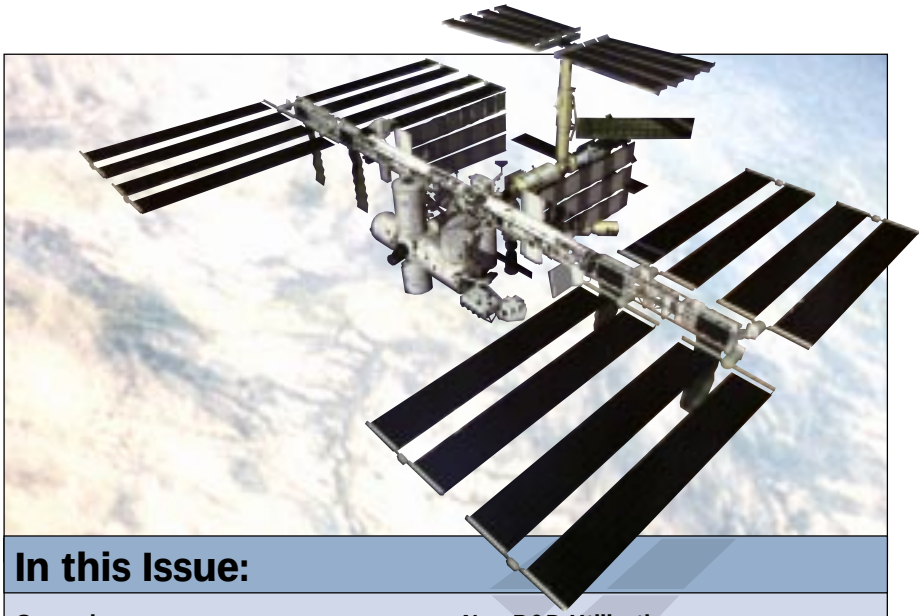

## In this Issue:



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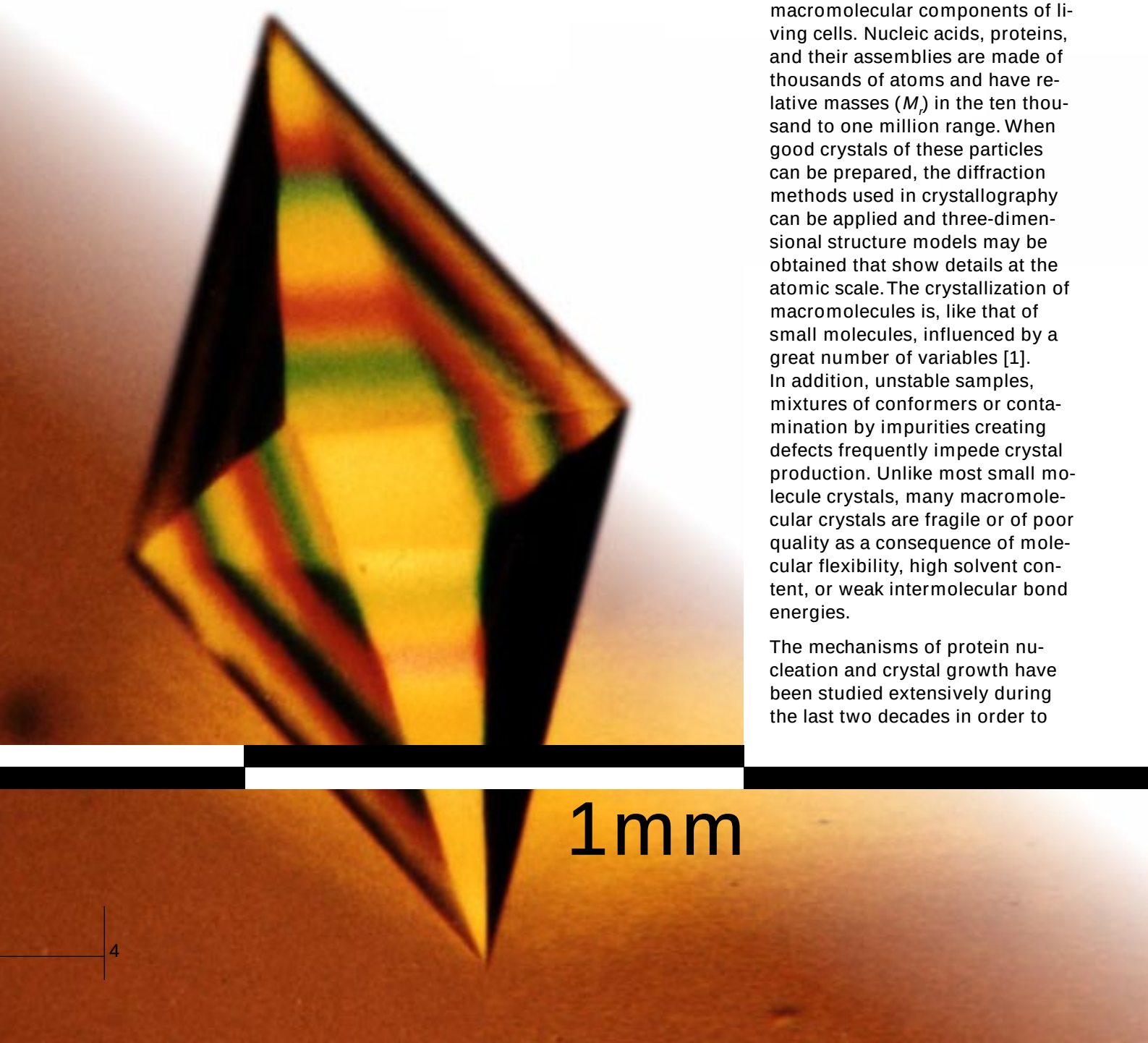
# Results of Advanced (APCF)

## Improved Protein Crystal Growth in Space

One goal of biology is to decipher the structures and functions of the macromolecular components of living cells. Nucleic acids, proteins, and their assemblies are made of thousands of atoms and have relative masses ($M_r$) in the ten thousand to one million range. When good crystals of these particles can be prepared, the diffraction methods used in crystallography can be applied and three-dimensional structure models may be obtained that show details at the atomic scale. The crystallization of macromolecules is, like that of small molecules, influenced by a great number of variables [1]. In addition, unstable samples, mixtures of conformers or contamination by impurities creating defects frequently impede crystal production. Unlike most small molecule crystals, many macromolecular crystals are fragile or of poor quality as a consequence of molecular flexibility, high solvent content, or weak intermolecular bond energies.

The mechanisms of protein nucleation and crystal growth have been studied extensively during the last two decades in order to

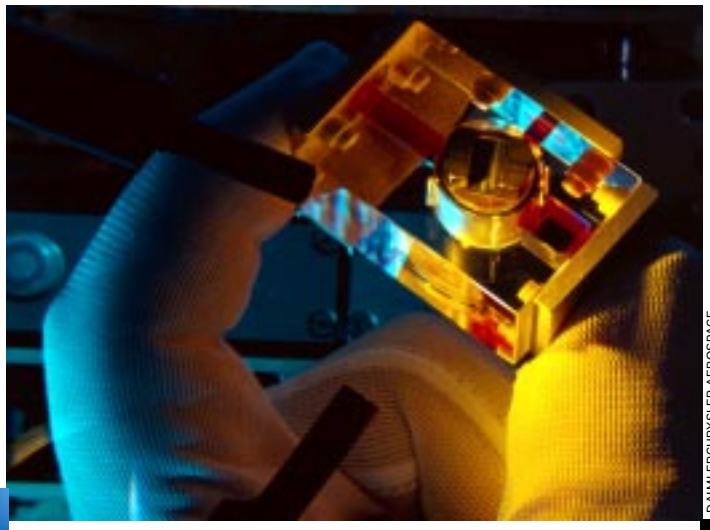


# five experiments in the Protein Crystallization Facility

find a way to improve crystal quality. From the beginning, weightlessness was anticipated to be a means to reach this end because it reduces convection in solution. The preliminary positive results obtained by Littke and John in 1984 [2] have triggered a strong enthusiasm for crystallization in microgravity. Afterwards, space was gradually dismissed because rigorous proves of crystal quality enhancement were lacking. However, investigations destined to rationally evaluate and to understand the effects of low gravity levels were pursued. As an alternative, investigators have attempted to reduce the effects of gravity in the terrestrial environment.

In the quest for a comparative quantification of the effects of gravity reduction, three proteins have been studied thoroughly. Within the Advanced Protein Crystallization Facility APCF [3], lysozyme from hen egg-white [4], aspartyl-tRNA synthetase from bacterium *Thermus thermophilus* [5], and thaumatin extracted from fruit of *Thaumatococcus daniellii* [6] were crystallized in microgravity on five Shuttle flights. All experiments were duplicated simultaneously in similar setups on Earth. The two first proteins are enzymes with $M_r$ 14500 and 132000: one hydrolyses bacterial cell walls and the other activates aspartic acid and esterifies a specific transfer ribonucleic acid during the initial step of protein synthesis. Although thaumatin ($M_r$ 22500) is deprived of any attached carbohydrate, it has a sweetness potency exceeding 2000 times that of table sugar and several times that of synthetic compounds on a weight basis. This natural and non-toxic sweetener has industrial applications such as the addition in food and drinks, the prevention of dental caries, and the treatment of obesity linked to diabetes. The following essentially deals with the latter protein.

Thaumatin can be crystallized as tetragonal bipyramids using dialysis and free-interface diffusion techniques. On USML-2 in 1995 and LMS in 1996, the crystals grown in solution had well-developed faces and were of superior crystallographic quality with regard to Earth controls [5]. Video images recorded in space have shown that half of the crystals had nucleated and grown in solution (the others being attached to the reactor walls) and that the proportion of unattached ones had strongly increased after the transportation on Earth. In microgravity, free-floating crystals had grown 2.5 times faster and reached 15-fold larger volumes with regard to crystals attached to walls in Earth reactors [7]. At the beginning of their quasi-synchronous growth, the former were located at regular interval in the solution. The average distance to closest neighbors was constant (the same was observed for lysozyme crystals grown in SPACEHAB-01 on STS-57 in 1993)[7]. This observation has reinforced the idea that every crystal might be surrounded by a depletion zone in which the protein concentration is low enough to prevent the nucleation of other crystals. Another observation was in agreement with this hypothesis: when AspR grow from a precipitate they are in very small number



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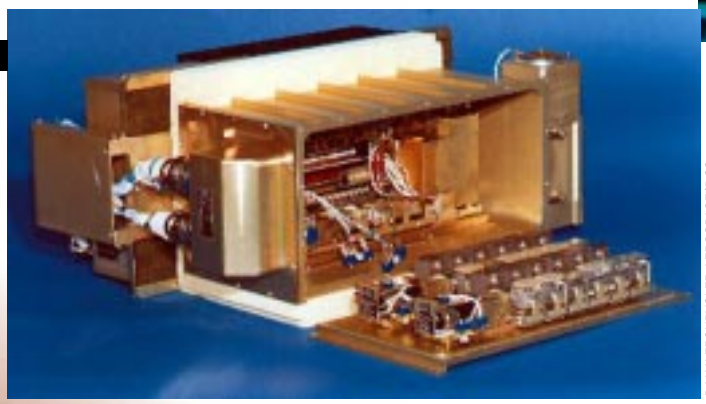

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*The Advanced Protein Crystallization Facility (left) and one of its single experiment chambers (top)*

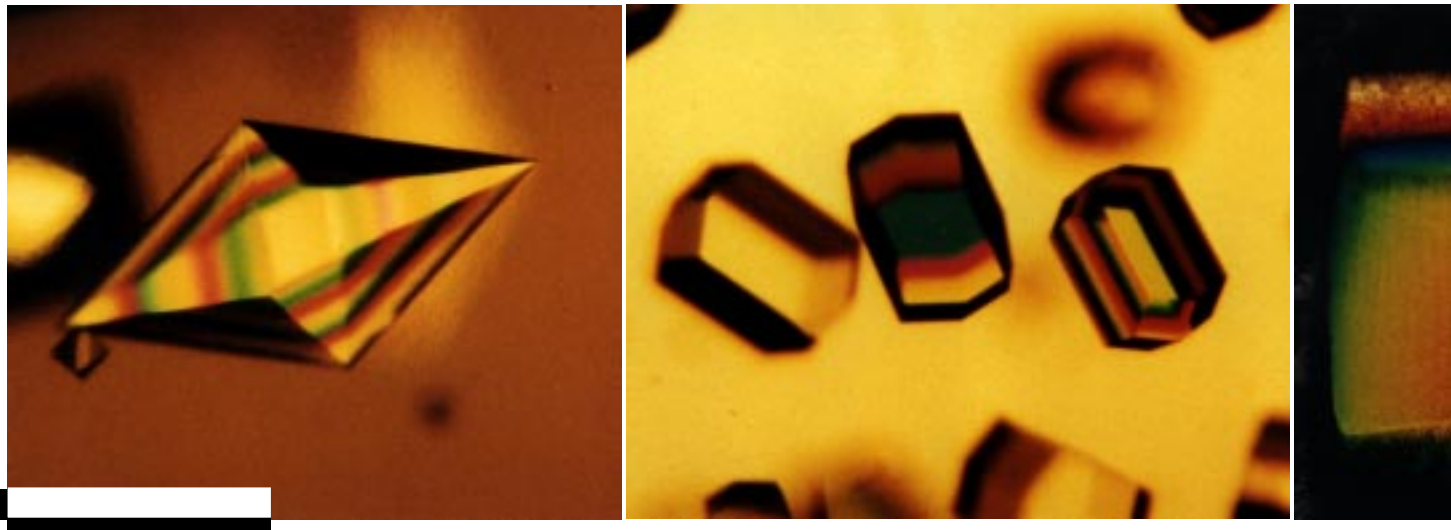
and reach unusually large volumes on Earth as well as in space (this was observed on IML-2 and LMS). Taken together, these results are in agreement with a correlation between the better crystallization parameters existing in microgravity and the improved crystal quality.

Video images recorded in space have also revealed that thaumatin crystals (like lysozyme but unlike AspRS crystals nucleating essentially on solid surfaces) had been subjected to motion. Their individual or collective displacements had been provoked by forces that were either external (acceleration changes during Shuttle maneuvers, vibrations caused by instruments or crew) and/or internal to the reactors (Marangoni convection at air-solution interfaces, flow accompanying the diffusion of the precipitant) [7].

On the fifth flight of the APCF on STS-95 in November 1998, thaumatin was crystallized in four dialysis reactors, each containing 188 microliters of protein solution gelified with 0.15% (m/v) agarose. This algal polysaccharide forms a stable, transparent, and neutral entanglement of rigid chains having a porosity compatible with the diffusion of particles up to hundred times larger than thaumatin. The purpose of the soft hydrogel was to immobilize the free-floating thaumatin crystals and to prevent their sedimentation upon landing. Crystals prepared in space and simultaneously in the laboratory under otherwise identical conditions have been compared on the basis of qualitative and quantitative criteria, including morphology as well as optical and crystallographic properties. On one hand, the limit of X-ray diffraction (resolution) was used to characterize the short-range order in the crystalline network. It determines the dimension of the smallest detail that will be discernible in the final structure model computed from diffraction data collected with a synchrotron radiation. On the other, the misalignment of the microcrystalline blocks forming any crystal (mosaicity) was used to evaluate the long-range order. It reflects the degree of perfection of the network. These analyses have been conducted in collaboration with M.C. Robert and B. Capelle at the Laboratoire de Minéralogie et Cristallographie of the Université de Paris.

According to the above criteria, the crystals grown in microgravity were of superior quality in any respect [8]. They had optimal shapes and excellent optical properties. Their diffraction was more isotropic with sharper and more intense reflections. Control crystals prepared in gel on Earth were of better overall quality than the reference crystals grown in solution which had less perfect shapes [8].

In addition, the images recorded in space and after landing have confirmed that the unattached crystals had not moved during their growth. The gel has had further advantages: it has attenuated mechanical shocks as well as thermal fluctuations and was easily removable. The high resolution (1.2 Å) of the diffraction data collected for the space-grown crystals has significantly shortened the time-consuming steps such as data processing and model building. The derived




*Crystals of lysozyme, aspartyl-tRNA synthetase, and thaumatin grown in the APCF*

electron density map is of exceptional quality and yields a more accurate structure than that obtained from crystals prepared on Earth [9].

To summarize, the crystallization of AspRS and of thaumatin in microgravity has repeatedly produced crystals with less defects. These crystals have greatly facilitated and improved the structure determination. The experience gained with these model proteins is transposable to other macromolecular compounds of biological interest.

**Acknowledgements**

This work was supported by CNES, CNRS, and ESA. We wish to thank our colleagues J.D. Ng, M.C. Robert, and B. Capelle as well as DaimlerChrysler Aerospace, the National Synchrotron Facility LURE (Orsay), and the European Molecular Biology Laboratory at DESY (Hamburg).

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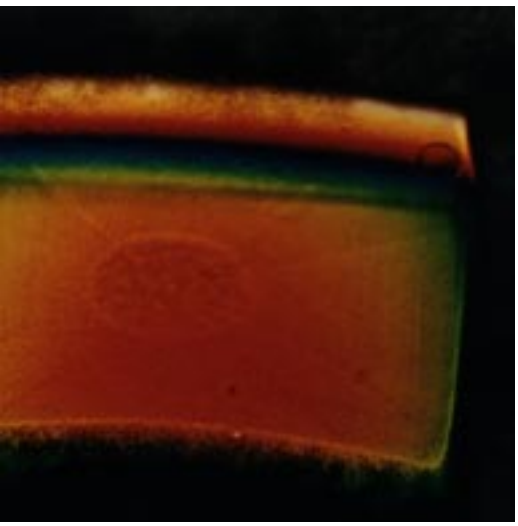

C.N.R.S. & I.B.M.C. STRASBOURG

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