Review

The crystallization of biological macromolecules under microgravity: a way to more accurate three-dimensional structures?

Bernard Lorber *

*Département 'Mécanismes et Macromolécules de la Synthèse Protéique et Cristallogenèse' UPR 9002,
Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, F-67084 Strasbourg Cedex, France*

Abstract

The crystallization of proteins and other biological particles (including nucleic acids, nucleo-protein complexes and large assemblies such as nucleosomes, ribosomal subunits or viruses) in a microgravity environment can produce crystals having lesser defects than crystals prepared under normal gravity on earth. Such microgravity-grown crystals can diffract X-rays to a higher resolution and have a lower mosaic spread. The inferred electron density maps can be richer in details owing to which more accurate three-dimensional structure models can be built. Major results reported in this field of research are reviewed. Novel ones obtained with the Advanced Protein Crystallization Facility are presented. For structural biology, practical applications and implications associated with crystallization and crystallography onboard the International Space Station are discussed.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Protein; Biological macromolecule; Crystallization; Microgravity; Space

1. Introduction

Well-diffracting crystals are the prerequisite for any crystallographic analysis. This is especially true in biology since X-ray and neutron crystallography are the major methods to accede to the three-dimensional positions of individual atoms in natural or modified macromolecules and supra-molecular assemblies. The body of atomic coordinates of proteins, nucleic acids and sub-cellular or viral particles deposited with the Protein Data Bank http://www.rcsb.org/) inflates each day. It can be foreseen that, in the years ahead, the pool of data for medicine-relevant macromolecules will rapidly be augmented by the increasing flow of proteins coming from post-genomics technologies. To cope with the demand for structural data, new approaches are being implemented to accelerate crystal preparation and analysis. Robots and image processing technologies already handle great numbers of samples for high-throughput crystallization. Progressively, the automation of various steps of the crystallographic analysis, from the freezing of the crystals to the collection of the diffraction data with synchrotron sources, their subsequent computing and the building of three-dimensional structure models shorten the process of structural determination.

The preparation of a pure, homogeneous and monodisperse sample is usually the first rate-limiting step in the crystallization of a biological macromolecule. Afterwards, the search of conditions leading to the formation of crystals is often a bottleneck. Indeed, an aqueous solvent must be found in which the macromolecule is still soluble but tends to aggregate in an ordered way [1,2]. Sparse matrices are an efficient approach to identify a crystallizing agent (either a salt, an alcohol or a polymer such as polyethylene glycol), a buffering substance giving the right pH and any necessary additive. Protein families sharing common surface or solubility properties often crystallize under close solvent conditions. Once a crystal is obtained, it must have satisfactory diffraction properties, and if this is not the case the initial conditions must be refined. Owing to the intense X-ray beams delivered by synchrotrons, crystals measuring no more than a few tens of microns in thickness may be sufficient to collect complete intensity data sets suitable for structure determination.

Crystal quality is generally evaluated using two criteria: (i) resolution (the higher, the better) quantifies the limit of

* Tel.: +33-3-8841-7008; fax: +33-3-8860-2218.
  E-mail address: B.Lorber@ibmc.u-strasbg.fr (B. Lorber).

Table 1
Beneficial effects of microgravity on macromolecular crystallization and crystal quality

(a) Crystallization under microgravity vs. under gravity
More synchronous nucleation and growth
Less heterogeneous nucleation on solid surfaces
Fewer crystals with large volume
Well-shaped crystals with fully developed faces

(b) Crystals prepared under microgravity vs. under gravity
Higher ratio of diffraction intensity vs. background
Higher diffraction limit
Lower mosaicity
Less diffuse scattering (lower thermal motion)

diffraction; (ii) mosaic spread or mosaicity (the lower, the better) measures the misalignment of the microscopic blocks building a crystal or, in other words, the degree of perfection of the lattice. Crystal quality is critical since it defines the amount of details seen in the electron density map. Ultimately, the accuracy of the structure model depends upon the latter.

The aim of the studies dealing with macromolecular crystal formation is to understand how crystal nuclei are formed in a supersaturated solution, how they grow from sub-micron to millimeter size, and how best crystals can be prepared. Most physico-chemical, biophysical or physical methods have been employed to analyze the crystallogenesis of biological particles. All investigations are in agreement with the fact that the laws for small molecules (like table salt) and macromolecules are the same. Hitherto, many variables have been explored to find a way to gain control over crystallization or to produce crystals with minimal defect that diffract X-rays at highest resolution. An important result is that monodisperse solutions have a higher probability to crystallize [3].

Over the last decade, crystallization in a microgravity environment ($\sim 10^{-3}$–$10^{-6} \times g$) has gained strong interest. In quasi-weightlessness, as it occurs in a spacecraft orbiting a few hundred kilometers above earth's surface, phenomena occurring in solution are strongly attenuated. In particular, convection due to density gradients generated by crystal growth and mixing produced by the crystal sedimentation are reduced by several orders of magnitude. Ideally, i.e. under zero gravity, the displacement of all solute molecules should be limited to diffusion (large particles diffusing slower than small ones) and crystals should be immobile from the time of nucleation to that of growth cessation. The hypothesis according to which this unique condition should enhance the properties of the crystals was tested in the mid 1980's. The results were encouraging but since then the burning question whether microgravity-grown crystals can be useful for the determination of three-dimensional structures remained open.

An overview of the progress that was made in this scientific field may be gained by leafing the proceedings of nine international conferences dealing with the crystallization of biological macromolecules held since 1985 [4]. This review presents major achievements along with recent results from experiments performed in the Advanced Protein Crystallization Facility. New perspectives for structural biology opened by the brand-new International Space Station are discussed.
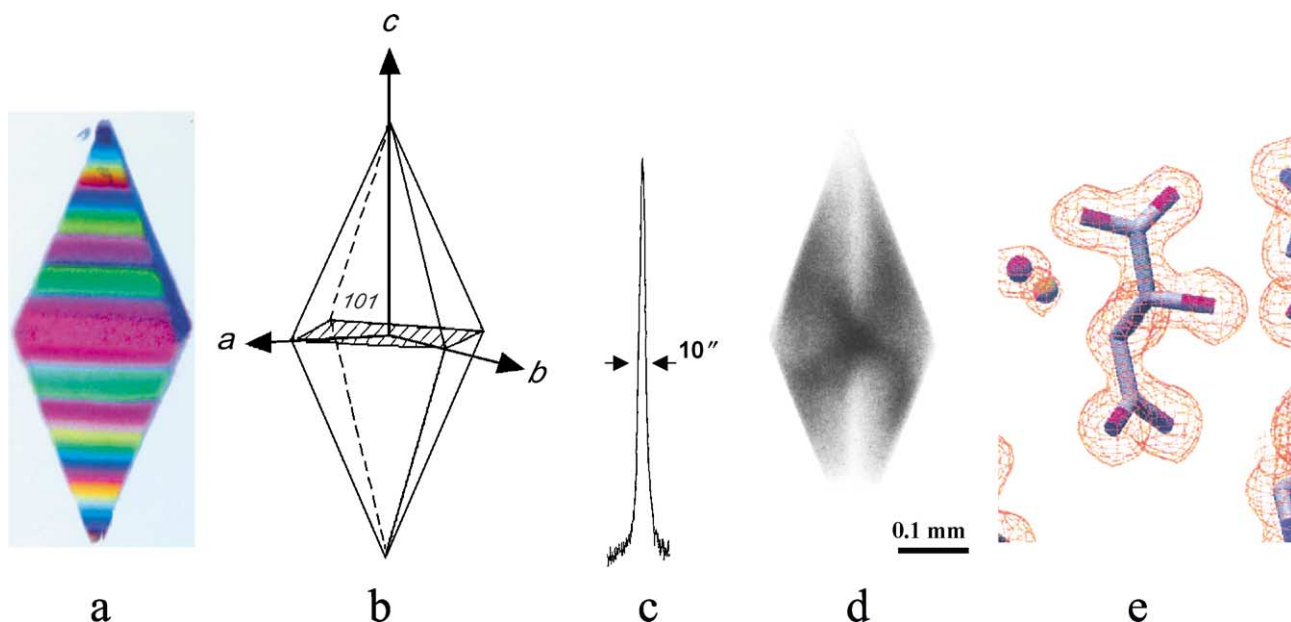

Fig. 1. Thaumatin, an intensely sweet protein crystallized in space. (a) Crystal of the 22-kDa monomer prepared under microgravity. It displays interference patterns in polarized light. (b) Drawing of crystal faces and axes. (c) Profile of a Bragg reflection. (d) Image of the crystal recorded by X-ray topography. (e) Close-up view of the electron density of a tartarate ion located between three protein molecules (adapted from Ref. [47]). The structure at 1.2-Å resolution was derived from crystals grown by dialysis in the APCF.

2. Better crystals from space

To date, more than 15,000 samples of proteins, nucleic acids and other biological particles have been sent to space for crystallization. Many more are planned to go there in the coming years. Amongst the crystallized compounds were proteins serving as models and others that were of great interest for biology or medicine. Most had previously been crystallized under normal gravity in an earth-based laboratory. All experiments were set up under microgravity to attempt to produce crystals diffracting better than those prepared on earth. For this purpose, over a dozen of specialized instruments have been built and patented. Crystallization assays were performed on about half of the first 100 flights of the US space shuttle (for detailed lists see Refs. [5,6]). Others were done on sounding rockets [6,7], on satellites [8–13] and on the *Mir* space station [14–16]. Some have already been conducted on the International Space Station whose construction is in progress. At the same time, laboratories have pursued experimentation on the ground to prepare future microgravity experiments or to simulate reduced gravity [17,18].

Dominant effects of microgravity on protein and virus crystallization are summarized in Table 1. The first investigators [19,20] had observed that crystallization in space often leads to a few voluminous crystals with narrow size distribution. Later, video monitoring revealed that crystals grow more frequently in the bulk of the solution than on solid surfaces, contrary to what happens under normal gravity. New crystal habits were obtained in a few instances and sometimes crystals have grown in space when they did not on earth. Visual inspection through a low-magnification binocular microscope equipped with polarized light has always confirmed that so-called ''space crystals'' are characterized by better morphologies and better optical properties (transparency, birefringence, absence of inclusions or defects) (see, e.g. Refs. [18,20–22]).

Most X-ray diffraction measurements indicated that space-grown crystals consistently exhibit sharp Bragg reflections with strong intensities and low diffuse scattering.
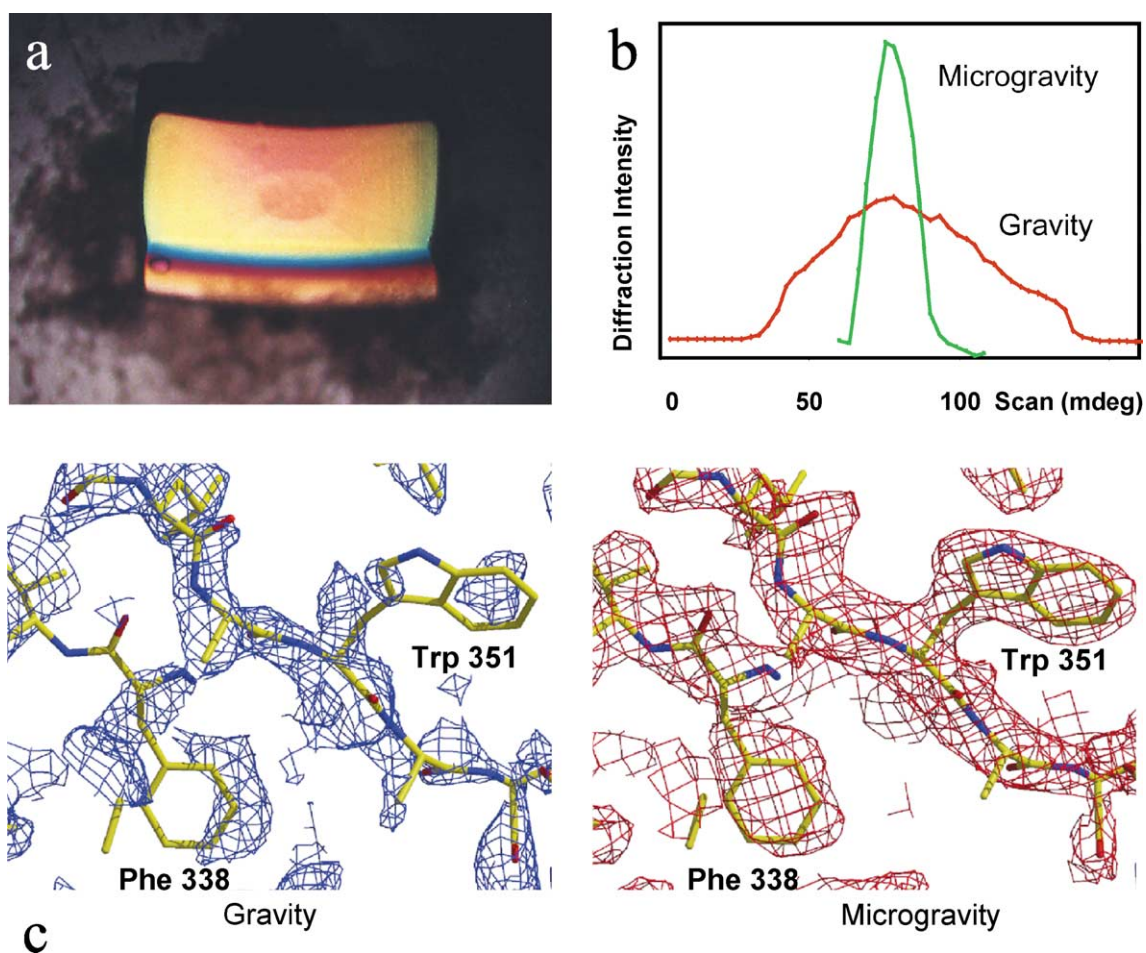

Fig. 2. Dimeric aspartyl-tRNA synthetase from *Thermus thermophilus* crystallized in space within dialysis reactors of the APCF. (a) A 3-mm-long crystal. (b) Comparison of the reflection profiles recorded from crystals that were prepared on earth (in red) or in space (in green). (c) The same region of the electron density map computed from diffraction data collected on crystals prepared either on earth (left) or in space (right) (adapted from Ref. [26]). In both close-up views the structure model is at a resolution of 2 Å.

The diffraction limit of many of them reproducibly exceeds by 0.1 Å to a few angstrom units that of best crystals grown on earth, meaning that the order in the lattice is enhanced [6]. In addition, a 3- to 10-fold reduction of the mosaic spread confirms this enhancement with regard to that of crystals prepared under normal gravity under otherwise identical conditions. The difference in mosaicity was indisputably clear in strictly comparative studies done on model molecules such as lysozyme [23–25], thaumatin [18] (Fig. 1) and aspartyl-tRNA synthetase [26] (Fig. 2) but the same holds for collagenase [27], insulin [28] and phospholipase [16]. Other proteins [5,6], nucleic acids [29], nucleosome core particles [30] and virus crystals [31] also exhibit substantial improvements.

3. More accurate structures from better crystals

A list of biological particles whose three-dimensional structure was determined from crystals prepared under microgravity is given in Table 2. A good quarter of them were solved at a resolution better than 1.5 Å. One out of ten is at a resolution better than 1 Å. The particles are visual pigments, enzymes, components of viruses, ribosomes and

photo- or immune-response systems, oxygen carriers, entire ribosomes or viruses, as well as cellular recognition, plant storage, blood or thermostable proteins. In most cases, the crystals prepared in space were of a previously unachieved quality (at the time they were obtained) and they enabled crystallographers to solve 3D-structures at a superior resolution. Data from several other crystals are currently being processed. Presumably, only a part (roughly estimated to between a third and half) of all the structures determined in this way will ever be publicly available because numerous laboratories from the pharmaceutical industry are actively involved and provide funding. Consequently, the list of compounds that are interesting for human health sciences may be much longer (and the actual diffraction limit for individual molecules may certainly be higher).

Rigorous comparisons of structural data from space-grown and earth-grown crystals indicate that microgravity does not alter the conformation of polypeptide chains. The answer to the question raised by crystallographers, whether space-grown crystals provide 3D-structures of superior definition and accuracy, is a strong ''yes'' despite the opinion of the disbelievers. Unequivocal results obtained for hen lysozyme show ions and ordered water molecules that are not visible as clearly in the electron density maps

Table 2
Three-dimensional structures determined using crystals grown under microgravity

Source	Biological particle	Resolution (Å)[a]	PDB code	References
Human	Interferon gamma	2.8[b]	1HIG	[32]
	Serum albumin	2.8[b]	1UOR	[33]
	Factor D	2.0[b]	1DSU	[34]
	Rec. Immunosuppressant binding protein	1.7	1FKJ	[35]
	Interferon alpha		Patent	[36]
	Rec. Insulin	1.4	1BEN	[37]
	Antithrombin III alpha	2.6	2ANT, 1E05	[38,39]
	Purine nucleoside phosphorylase	2.8	1ULA	[40]
Beef	Immunosuppressant binding protein	2.3	1FKK, 1FKL	[35]
Hen	Lysozyme (tetragonal crystal form)	1.8	1BWJ	[41]
		1.33	193L	[25]
		2.1 (neutrons)	n.a.	[42]
		0.94	1IEE	[43]
	Nucleosome core particle	2.5	1EQZ	[30]
Fish	EF-Hand parvalbumin	0.91	2PCB	[44]
Worm	Collagenase	1.7	2HLC	[27]
Plant	Isolectin 1	1.9	1LOE	[45]
	Canavalin	2.0	1DGW	[46]
	Thaumatin	1.2	1KWN	[47]
Fungus	Proteinase K	0.98	1IC6	[48]
Bacterium	Phospho carrier protein Fab complex	2.8	1JEL	[49]
	Aspartyl-tRNA synthetase	2.0	1L0W	[26]
Virus	Neuraminidase	2.5	1NNA	[50]
	Satellite tobacco mosaic virus	1.8	1A34	[31]
	Lysozyme	2.3	1AM7	[51]
Synthetic construct	Collagene-like peptide	1.3	1G9W	[52]

Footnotes: This list of 25 entries results from data mining in Biosis, Chemical Abstracts, Current Contents, Inspec, Medline, Pascal, Science Citation Index, Biological Macromolecule Crystallization Database, NASA Protein Crystal Archive, Protein Data Bank and ESA Microgravity Data Base. The lack of a common terminology was a hindrance in identifying articles containing information about crystal preparation. For this reason a few hundred printed publications had to be combed through to establish this short list. Abbreviation: n.a., not available.
[a] Structures were solved using X-ray diffraction data except otherwise mentioned.
[b] Diffraction data collected from early space-grown crystals were used to refine structures derived from earth-grown crystals.

computed from data collected with earth-grown crystals [41,42]. In the case of plant thaumatin [18] (Fig. 1) and bacterial aspartyl-tRNA synthetase [26] (Fig. 2), crystals having the same volume give diffraction intensity peaks that are significantly less spread. In both cases, the sharpness and the intensity of the diffraction has cut down exposure times and accelerated indexing procedures. More details are visible in some parts of the electron density maps corresponding to the polypeptide backbone and its side chains (Figs. 1 and 2). In the case of insulin, a second *para*-hydroxybenzamide guest molecule could be resolved with certainty [37]. Here, and in several other instances, diffuse scattering due to thermal motion was reduced [5].

Space-grown lysozyme [41], phospholipase A [16] and aspartyl-tRNA synthetase [26] (Fig. 2) crystals share another common feature: more ordered hydrogen-bound water molecules are visible in the hydration layer of the protein. Crystals of other proteins were reported to be less sensitive to radiation damage. It was also found that the quality of the crystals prepared in agarose gel (in order to reduce convection and suppress sedimentation on earth) is intermediate between that of crystals prepared in solution on earth and that of crystals prepared in space [17,18]. In the gel, the number of water molecules per lysozyme monomer was also intermediary [25].

Some guidelines for the preparation of better crystals and for the collection of best diffraction data are summarized in Table 3. In brief, it is advisable to work with purest molecules. Nucleation and growth should take place in an undisturbed medium in which mass transport occurs by diffusion (rather than by convection) so that macromolecules have enough time to position properly on the surface of the growing crystalline lattices. Supersaturation should be moderate to favor growth either by a screw-dislocation or a two-dimensional nucleation mechanism. Only freshly prepared crystals should be used for diffraction analyses.

4. Microgravity influences crystallization and crystal quality

The crystals of most biomacromolecules are soft and fragile. They distinguish from those of small molecules by their big unit cell, their high solvent content, their weak intermolecular forces and exquisitely specific contacts. In most cases, a considerable amount of diffraction data must be collected and atomic resolution cannot be reached because the intensity-to-background ratio is too small. Crystal preparation in space may be a remedy.

By which mechanism(s) is the quality of the crystals enhanced under microgravity? There is strong evidence that the quiescence of the solution plays a central role. Obviously, the zone around a crystal, which becomes depleted in protein as the latter is incorporated, can only be stable in an undisturbed solution. Depletion zones have been visualized with optical methods within capillary tubes and gelled solution on earth and in space (see Refs. [53,54] and references therein). In space, convective mixing becomes negligible and matter is transported in a purely diffusive way. Thereby, the incorporation rate of macromolecular impurities or aggregates declines. This segregation is like a purification process [55,56]. The same phenomenon occurs when crystallization takes place in a gel on earth [57]. Furthermore, under microgravity, an extended network of hydrogen-bound water molecules can hold together weakly assembled macromolecules. It can also stabilize the agitated parts of individual macromolecules or their ligands. Accordingly, crystals with a high solvent content are assumed to be better candidates for microgravity crystallization.

One reason why crystals prepared on earth are less perfect is that any difference in concentration existing or appearing in solution creates density gradients that trigger convectional currents, and destabilizes depletion zones. Such phenomena are known for a long time; they naturally accompany crystal growth and are independent of any temperature fluctuation. In addition, the destruction of the concentration gradient that is associated with a growing crystal may have deleterious consequences: growth may stop, its mechanism may be altered or the crystal may dissolve. The outcome relies on the balance between diffusive transport and surface kinetics. From this point of view, proteins are a key to advanced crystal growth studies.

Table 3
Guidelines for the preparation of better crystals and the collection of best diffraction data

Requirement	Experimental and comments
1. Pure and homogenous macromolecule	Activity assay, electrophoresis, chromatography, mass spectrometry
2. Monodisperse macromolecular solution	Light, X-ray or neutron scattering analysis
3. Nucleation at lowest supersaturation	Appropriate solution composition and supersaturation to get a few nuclei
4. Growth in a stable environment favoring diffusive transport of matter	Low but sufficient supersaturation to grow a few crystals. Nuclei and crystals immobilized in a gel or in a cubic lipid phase do not settle and can grow in three dimensions. These media also attenuate disturbances in gravity level, residual vibrations, and minor temperature variations. Nucleation in a precipitate and growth by Ostwald ripening may be an alternative means to simulate partially a microgravity environment
5. Crystals harvested just before or upon growth cessation	Monitored with optical and imaging methods (e.g. time-lapse video, image analysis)
6. Immediate diffraction analysis	All parameters kept constant from time of harvest to that of analysis

5. Benefits from microgravity environment

Although protein and virus crystals have been grown in the laboratory for more than a century, the science deciphering the rules of macromolecular crystallogenesis is still in the discovery phase. Microgravity is a new parameter to explore and it opens up wide perspectives for structural biology. For basic and applied research, 3D structures at atomic and even sub-atomic resolution are indispensable to understand the relationship between structure (i.e. conformation, dynamics) and function(s) that is at the heart of macromolecular interactions, catalysis and proton or electron transfer. With flawless crystals prepared in orbit, structures can be solved at previously unachieved resolution using either X-ray or neutron diffraction methods.

A plethora of instruments exist to grow the necessary crystals; they work on different principles (vapor diffusion, dialysis and liquid–liquid diffusion) and accommodate from a dozen to several hundred samples. The space station offers the opportunity for longer crystallization durations (one to a few months) than the shuttle (1 or 2 weeks). Systematic and iterative studies on great numbers of samples covering broad ranges of conditions are now possible. They should rapidly tell if a reduction of the level of gravity influences the crystallogenesis of all macromolecules in the same manner. When the system will reach its cruising speed, periodically fresh samples will go in orbit and crystals will be retrieved for analysis on earth. Monitoring and remote control of individual assays will enable experimenters to act dynamically on the samples. Automation of diffraction analyses and of data collection in orbit will be inevitable to make the most of best crystals [58].

The number of applications of microgravity is expected to increase dramatically, for pharmaceutical industry is a major participant in the frame of the commercialization of space. Inter-disciplinary projects focusing on the study of the structure–function relationship in compounds of proteomes, in difficult-to-crystallize membrane proteins, nucleic acids and large multi-molecular complexes are indispensable. Is not the ultimate goal of structural biology to elucidate life's molecular processes and to comprehend how work machineries performing the sophisticated operations we call transcription, translation, molecular import and export, signal transduction, immune response, photosynthesis or metabolic pathways?

Well-diffracting crystals prepared in space have in fact two purposes: they help us to understand gravity-dependent phenomena (such as nucleation and growth mechanisms) and they can be used for structural determination. Each new high-resolution structure may become the start of a ramifying cascade of investigations to unravel the complexity of the cellular events like growth, division, differentiation, communication, motility, death and their role in the development of multi-cellular organisms. This may accelerate the structure-based design and redesign of drugs targeting pathogens, diseases and degenerative cellular processes as well as of protein and nucleic acid leading to tailor-made enzymes, ribozymes or inhibitors. Monodisperse microcrystalline suspensions produced at a large scale in space might serve as medicinal formulations.

For earth-based laboratories, methodological and instrumental improvements are the payoff of research done in space. Experimentation in space has directly or indirectly promoted our understanding of protein crystal growth. Thanks to studies done under microgravity, media (like gels that change solutions to non-Newtonian liquids and simulate more or less this environment) have been rediscovered and their use has now become widespread. Obviously, crystallographers will continue to solve structures on earth but space may be of priceless help to bypass difficulties linked to crystal preparation. Presently, it may be a gateway to the voluminous crystals required for neutron diffraction experiments [42,59]. Anyway, microgravity should be considered as another variable to test in difficult cases. Gradually, more and more scientists and students [60] are involved in space-based experiments and their rational exploitation. The challenge of the first decade of this millennium is to make an optimal use of the orbiter and of the crystallization facilities. In 2002, each shuttle docking onto the space station will carry hundreds of crystallization assays. Tomorrow, more than today, advances in biology and medicine will rely on the knowledge of three-dimensional structures.

Acknowledgements

References

[1] A. McPherson, Crystallization of Biological Macromolecules, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999.

[2] A. Ducruix, R. Giegé (Eds.), Crystallization of Nucleic Acids and Proteins, A Practical Approach, 2nd ed., IRL Press at Oxford University Press, Oxford, 1999.

[3] A. D'Arcy, Crystallizing proteins—a rational approach? Acta Crystallogr. D50 (1994) 469–471.

[4] Crystallization of biological macromolecules. First International conference, J. Cryst. Growth 76 (1986) 529–718. Second Int. Conf., J. Cryst. Growth 90 (1988) 1–370. Third Int. Conf., J. Cryst. Growth 110 (1991) 1–338. Fourth Int. Conf., J. Cryst. Growth 122 (1992) 1–399 (parts 1/4). Fifth Int. Conf., Acta Cryst. D50 (1994) 337–666. Sixth Int. Conf., J. Cryst. Growth 168 (1996) 1–327. Seventh Int. Conf., J. Cryst. Growth 196 (1999) 185–710. Eighth Int. Conf., J. Cryst. Growth 232 (2001) 1–648. Ninth Int. Conf., Acta Cryst. D58 (2002) in press.

[5] K. Moore, M.M. Long, L.J. DeLucas, Protein crystal growth in microgravity: status and commercial implications, AIP Conf. Proc. 458 (1999) 217–224.

[6] C.E. Kundrot, R.A. Judge, M.L. Pusey, E.H. Snell, Microgravity and macromolecular crystallography, Cryst. Growth Des. 1 (2001) 87–99.

[7] L. Sjölin, A. Wlodawer, G. Bergqvist, P. Holm, K. Loth, H. Malmstrom, J. Zaar, L.A. Svensson, G.L. Gilliland, Protein crystal growth of ribonuclease A and pancreatic trypsin inhibitor aboard the MASER 3 rocket, J. Cryst. Growth 110 (1991) 322–332.

[8] S.D. Trakhanov, A.L. Grebenko, V.A. Shirokov, A.V. Gudkov, A.V. Egorov, I.N. Barmin, B.K. Vainstein, A.S. Spirin, Crystallization of protein and ribosomal particles in microgravity, Dokl. Akad. Nauk SSSR 305 (1989) 1128–1132.

[9] V.E. Erdmann, C. Lippmann, C. Betzel, Z. Dauter, K. Wilson, R. Hilgenfeld, J. Hoven, A. Liesum, W. Saenger, A. Müller-Fahrnow, W. Hinrichs, M. Düvel, G.E. Schulz, C.W. Müller, H.G. Wittmann, A. Yonath, G. Weber, A. Plaas-Link, Crystallization of proteins under microgravity, FEBS Lett. 259 (1989) 194–198.

[10] R. Hilgenfeld, A. Liesum, R. Storm, A. Plaas-Link, Crystallization of two bacrial enzymes on an unmanned space mission, J. Cryst. Growth 122 (1992) 330–336.

[11] M. Hennig, M. Visanji, W. Weber, H. Janczikowski, A. Plaas-Link, C. Betzel, COSIMA-protein crystal growth facility for automatic processing on unmanned satellites, J. Cryst. Growth 135 (1994) 513–522.

[12] P.F. Zagalsky, C.E. Wright, M. Parsons, Crystallization of alpha-crustacyanin, the lobster carapace astaxanthin-protein: results from Eureca, Adv. Space Res. 16 (1995) 891–894.

[13] J. Dong, J. Pan, X. Niu, Y. Zhou, R.C. Bi, Influence of microgravity on protein crystal structures, Chin. Sci. Bull. 45 (2000) 1002–1006.

[14] R.K. Strong, B.L. Stoddard, A. Arrott, G.K. Farber, Long duration growth of protein crystals in microgravity aboard the MIR space station, J. Cryst. Growth 110 (1991) 312–316.

[15] D.W. Zhu, M. Zhou, Y. Mao, F. Labrie, S.X. Lin, Crystallization of human oestrogenic 17β-hydroxysteroid dehydrogenase under microgravity, J. Cryst. Growth 156 (1995) 108–111.

[16] S. Koszelak, C. Leja, A. McPherson, Crystallization of biological macromolecules from flash frozen samples on the Russian Space Station Mir, Biotechnol. Bioeng. 52 (1996) 449–458.

[17] L.J. DeLucas, M.M. Long, K.M. Moore, W.M. Rosenblum, T.L. Bray, C. Smith, M. Carson, S.V.L. Narayana, M.D. Harrington, D. Carter, A.D. Clark Jr., R.G. Nanni, J. Ding, A. Jacobo-Molina, G. Kamer, S.H. Hughes, E. Arnold, H.M. Einspahr, L.L. Clancy, G.S.J. Rao, P.F. Cook, B.G. Harris, S.H. Munson, B.C. Finzel, A. McPherson, P.C. Weber, F.A. Lewandowski, T.L. Nagabhushan, P.P. Trotta, P. Reichert, M.A. Navia, K.P. Wilson, J.A. Thomson, R.N. Richards, K.D. Bowersox, C.J. Meade, E.S. Baker, S.P. Bishop, B.J. Dunbar, E. Trinh, J. Prahl, A. Sacco Jr., C.E. Bugg, Recent results and new hardware developments for protein crystal growth in microgravity, J. Cryst. Growth 135 (1994) 183–195.

[18] B. Lorber, C. Sauter, M.C. Robert, B. Capelle, R. Giegé, Crystallization within agarose gel in microgravity improves the quality of thaumatin crystals, Acta Crystallogr. D55 (1999) 1491–1494.

[19] W. Littke, C. John, Protein single crystal growth under microgravity, Science 225 (1984) 203–204.

[20] L.J. DeLucas, F.L. Suddath, R. Snyder, R. Naumann, M.B. Broom, M. Pusey, V. Yost, B. Herren, D. Carter, B. Nelson, E.J. Meehan, A. McPherson, C.E. Bugg, Preliminary investigations of protein crystal growth using the Space shuttle, J. Cryst. Growth 76 (1986) 681–693.

[21] J. Day, A. McPherson, Macromolecular crystal growth experiments on International Microgravity Laboratory-1, Protein Sci. 1 (1992) 1254–1268.

[22] S. Koszelak, J. Day, C. Leja, R. Cudney, A. McPherson, Protein and virus crystal growth on International Microgravity Laboratory-2 (IML-2, July 1994), Biophys. J. 69 (1995) 13–19.

[23] E.H. Snell, S. Weisgerber, J.R. Helliwell, E. Weckert, K. Hölzer, K. Schroer, Improvements in lysozyme protein crystal perfection through microgravity growth, Acta Crystallogr. D51 (1995) 1099–1102.

[24] J.L. Ferrer, J. Hirschler, M. Roth, J.C. Fontecilla-Camps, Mosaic spread measurement of space-grown and earth-grown protein crystals, ESRF Newsl. (1996) 27–29.

[25] J. Dong, T.J. Boggon, N.E. Chayen, J. Raftery, R.C. Bi, J.R. Helliwell, Bound-solvent structures for microgravity-, ground control-, gel- and microbatch-grown hen egg-white lysozyme crystals at 1.8 Å resolution, Acta Crystallogr. D55 (1999) 745–752.

[26] J.D. Ng, C. Sauter, B. Lorber, N. Kirkland, J. Arnez, R. Giegé, Comparative analysis of space- and earth-grown crystals of an aminoacyl-tRNA synthetase: space-grown crystals are more useful for structural determination, Acta Crystallogr., D Biol. Crystallogr. 58 (2002) 645–652.

[27] I. Broutin-L'Hermitte, M. Riès-Kautt, A. Ducruix, 1.7 Å X-ray structure of space-grown collagenase crystals, Acta Crystallogr., D Biol. Crystallogr. 56 (2000) 376–378.

[28] G.E.O. Borgstahl, A. Vahedi-Faridi, J. Lovelace, H.D. Bellamy, E.H. Snell, A test of macromolecular crystallization in microgravity: large well ordered insulin crystals, Acta Crystallogr. D57 (2001) 1204–1207.

[29] S. Lorenz, M. Perbandt, C. Lippmann, K. Moore, L.J. DeLucas, C. Betzel, V.A. Erdmann, Crystallization of engineered Thermus flavus 5S rRNA under earth and microgravity conditions, Acta Crystallogr. D56 (2000) 498–500.

[30] J.M. Harp, B.L. Hanson, D.E. Timm, G.J. Bunick, Asymmetries in the nucleosome core particle at 2.5 Å resolution, Acta Crystallogr. D56 (2000) 1513–1534.

[31] S.B. Larson, J. Day, A. Greenwood, A. McPherson, Refined structure of satellite tobacco mosaic virus at 1.8 Å resolution, J. Mol. Biol. 277 (1998) 37–59.

[32] S.E. Ealick, W.J. Cook, S. Vijay-Kumar, M. Carson, T.L. Nagabhushan, P.P. Trotta, C.E. Bugg, Three-dimensional structure of recombinant human interferon-gamma, Science 252 (1991) 698–702.

[33] X.M. He, D.C. Carter, Atomic structure and chemistry of human serum albumin, Nature 358 (1992) 209–215.

[34] S.V.L. Narayana, M. Carson, O. El-Kabbani, J.M. Kilpatrick, D. Moore, X. Chen, C.E. Bugg, J.E. Volanakis, L.J. DeLucas, Structure of human factor D. A complement system protein at 2.0 Å resolution, J. Mol. Biol. 235 (1994) 695–708.

[35] K.S. Wilson, M.M. Yamashita, M.D. Sintchak, S.H. Rotstein, M.A. Murcko, J. Boger, J.A. Thomson, M.J. Fitzgibbon, J.R. Black, M.A. Navia, Comparative X-ray structure of the major binding protein for the immuno-suppressant FK506 (Tacrolimus) in unliganded form and in complex with FK506 and rapamycin, Acta Crystallogr. D51 (1995) 511–521.

[36] P. Reichert, C. McNemar, N. Nagabhushan, T.L. Nagabhushan, S. Tindal, A. Hruza, Metal-interferon-alpha crystals (1995) US Patent No. 5,441,734.

[37] G.D. Smith, E. Ciszak, W. Pangborn, A novel complex of a phenolic derivative with insulin: structural features related to the T→R transition, Protein Sci. 5 (1996) 1502–1511.

[38] M.R. Wardell, R. Skinner, D.C. Carter, P.D. Twigg, J.P. Abrahams, Improved diffraction of antithrombin crystals grown in microgravity, Acta Crystallogr. D53 (1997) 622–625.

[39] R. Skinner, J.P. Abrahams, J.C. Whisstock, A.M. Lesk, R.W. Carrell, M.R. Wardell, The 2.6 Å structure of antithrombin indicates a conformational change at the heparin binding site, J. Mol. Biol. 266 (1997) 601–609.

[40] S.E. Ealick, S. Babu, C.E. Bugg, M.D. Erion, W.C. Guida, J.A. Montgomery, J.A. Secrist, Application of crystallographic and modeling methods in the design of purine nucleoside phosphorylase inhibitors, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 11540–11544.

[41] M.C. Vaney, S. Maignan, M. Riès-Kautt, A. Ducruix, High-resolution structure (1.33 Å) of a HEW lysozyme tetragonal crystal grown in the APCF apparatus. Data and structural comparison with a crystal grown under microgravity from SpaceHab-01 Mission, Acta Crystallogr. D52 (1996) 505–517.

[42] J.X. Ho, J.P. Declercq, D.A.A. Myles, B.S. Wright, J.R. Ruble, D.C. Carter, Neutron structure of monoclinic lysozyme crystals produced in microgravity, J. Cryst. Growth 232 (2001) 317–325.

[43] C. Sauter, F. Otalora, J.A. Gavira, O. Vidal, R. Giegé, J.M. Garcia-Ruiz, Structure of tetragonal hen egg-white lysozyme at 0.94 Å resolution from crystals grown by the counter-diffusion method, Acta Crystallogr. D57 (2001) 1119–1126.

[44] J.P. Declercq, C. Evrard, V. Lamzin, J. Parello, Crystal structure of the EF-hand parvalbumin at atomic resolution (0.91 Å) and at low temperature (100 K). Evidence for conformation a multistates within the hydrophobic core, Protein Sci. 8 (1999) 2194–2204.

[45] Y. Bourne, C. Abergel, C. Cambillau, M. Frey, P. Rouge, J.C. Fontecilla-Camps, X-ray crystal structure determination and refinement at 1.9 Å of isolectin I from the seeds of Lathyrus ochrus, J. Mol. Biol. 214 (1990) 571–584.

[46] T.P. Ko, J. Day, A. McPherson, The refined structure of canavalin from jack bean in two crystal forms at 2.1 and 2.0 Å resolution, Acta Crystallogr. D56 (2000) 411–420.

[47] C. Sauter, B. Lorber, R. Giegé, Towards atomic resolution with crystals grown in gel: the case of thaumatin crystals seen at room temperature, Proteins 48 (2002) 146–150.

[48] C. Betzel, S. Gourinath, P. Kumar, P. Kaur, M. Perbandt, S. Eschenburg, T.P. Singh, Structure of s serine protease proteinase K from Tritirachium album Limber at 0.98 Å resolution, Biochemistry 40 (2001) 3080–3088.

[49] L. Prasad, S. Sharma, M. Vandonselaar, J.W. Quail, J.S. Lee, E.B. Waygood, K.S. Wilson, Z. Dauter, L.T.J. Delbaere, Evaluation of mutagenesis for epitote mapping. Structure of an antibody–protein antigen complex, J. Biol. Chem. 268 (1993) 10705–10708.

[50] P. Bossart-Whitaker, M. Carson, Y.S. Babu, C.D. Smith, W.G. Laver, G.M. Air, Three-dimensional structure of influenza A N9 neuraminidase and its complex with the inhibitor 2-deozy 2,3-dehydro-N-acetyl neuraminic acid, J. Mol. Biol. 232 (1993) 1069–1083.

[51] C. Evrard, J. Fastrez, J.P. Declercq, Crystal structure of the lysozyme from bacteriophage lambda and its relationship with V and C-type lysozymes, J. Mol. Biol. 276 (1998) 151–164.

[52] L. Vitagliano, R. Berision, L. Mazzarella, A. Zagari, Structural bases of collagen stabilization induced by proline hydroxylation, Biopolymers 36 (2001) 459–464.

[53] A. McPherson, A. Malkin, Y.G. Kuznetsov, S. Koszelak, M. Wells, G. Jenkins, J. Howard, G. Lawson, The effects of microgravity on protein crystallization: evidence for concentration gradients around growing crystals, J. Cryst. Growth 196 (1999) 572–586.

[54] F. Otalora, M.L. Novella, J.A. Gavira, B.R. Thomas, J.M. Garcia Ruiz, Experimental evidence for the stability of the depletion zone around a growing protein crystal under microgravity, Acta Crystallogr. D57 (2001) 147–412.

[55] D.C. Carter, K. Lin, J.X. Ho, B.S. Wright, P.D. Twigg, T.Y. Miller, J. Chapman, K. Keeling, J. Ruble, P.G. Vekilov, B.R. Thomas, F. Rosenberger, A.A. Chernov, Lower dimer impurity incorporation may result in higher perfection of HEWL crystals grown in microgravity. A case study, J. Cryst. Growth 196 (1999) 623–637.

[56] B.R. Thomas, A.A. Chernov, P.G. Vekilov, D.C. Carter, Distribution coefficients of impurities in ferritin and lysozyme crystals. Self-purification in microgravity, J. Cryst. Growth 211 (2000) 149–156.

[57] A.A. Chernov, J.M. Garcia-Ruiz, B.R. Thomas, Visualization of the impurity depletion zone surrounding apoferritin crystals growing in gel with holoferritin dimmer impurity, J. Cryst. Growth 232 (2001) 184–187.

[58] W.B. Crysel, L.J. DeLucas, L.D. Weise, C.D. Smith, W.T. McDonald, The international space station X-ray crystallography facility, J. Cryst. Growth 232 (2001) 458–467.

[59] E.H. Snell, N.E. Chayen, J.R. Helliwell, Crystallization of biological macromolecules in microgravity, The Biochemist (Dec. 1999) 19–24.

[60] A. McPherson, S. Koszelak, G. Jenkins, M. Myers, J. Perkey, A. Holmes, J.D. Ng, ISS: a science classroom for America, AIP Conf. Proc. 504 (2000) 479–487.