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Review

Lessons from crystals grown in the Advanced Protein Crystallisation Facility for conventional crystallisation applied to structural biology

Alessandro Vergara [a,b], Bernard Lorber [c], Claude Sauter [c], Richard Giegé [c,*], Adriana Zagari [b,d]

[a] *Dipartimento di Chimica, Università di Napoli "Federico II", Monte S. Angelo, 80126, Napoli, Italia*
[b] *Istituto di Biostrutture e Bioimmagini, CNR, Via Mezzocannone 16, 80134, Napoli, Italia*
[c] *Département 'Machineries Traductionnelles' Groupe de Cristallogenèse, UPR 9002, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, F-67084 Strasbourg, France*
[d] *Dipartimento delle Scienze Biologiche, Universitá di Napoli "Federico II", Via Mezzocannone 16, 80134, Napoli, Italia*

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Abstract

The crystallographic quality of protein crystals that were grown in microgravity has been compared to that of crystals that were grown in parallel on earth gravity under otherwise identical conditions. A goal of this comparison was to assess if a more accurate 3D-structure can be derived from crystallographic analysis of the former crystals. Therefore, the properties of crystals prepared with the Advanced Protein Crystallisation Facility (APCF) on earth and in orbit during the last decade were evaluated. A statistical analysis reveals that about half of the crystals produced under microgravity had a superior X-ray diffraction limit with respect of terrestrial controls. Eleven protein structures could be determined at previously unachieved resolutions using crystals obtained in the APCF. Microgravity induced features of the most relevant structures are reported. A second goal of this study was to identify the cause of the crystal quality enhancement useful for structure determination. No correlations between the effect of microgravity and other system-dependent parameters, such as isoelectric point or crystal solvent content, were found except the reduced convection during the crystallisation process. Thus, crystal growth under diffusive regime appears to be the key parameter explaining the beneficial effect of microgravity on crystal quality. The mimicry of these effects on earth in gels or in capillary tubes is discussed and the practical consequences for structural biology highlighted.

Keywords: Protein structure; Crystal quality; Microgravity; Diffusion; Advanced Protein Crystallisation Facility

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Abbreviations: μg, microgravity; ADH, alcohol dehydrogenase; APCF, Advanced Protein Crystallisation Facility; AspRS-1, aspartyl-tRNA synthetase (form 1 of eubacterial type); B, atomic displacement parameter; CcdB, poison of DNA-topoisomerase II complexes; *d*, resolution limit; ESA, European Space Agency; FWHM, Full Width at Half Maximum; g, ground; Hyp, hydroxyproline; I/σ, intensity to standard deviation ratio; ISS, International Space Station; M_r, molecular weight; DCAM, Diffusion Controlled Crystallisation Apparatus for Microgravity; PCDF, Protein Crystallisation Diagnostic Facility; PDB, Protein Data Bank; $(PPG)_{10}$, synthetic polypeptide $(Pro–Pro–Gly)_{10}$; VDA, Vapour Diffusion Apparatus.

* Corresponding author. Tel.: +33 3 88 41 70 58; fax: + 33 3 88 60 22 18.
E-mail address: r.giege@ibmc.u-strasbg.fr (R. Giegé).

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1. Background

Crystal production is frequently a bottleneck in protein crystallography [1–3]. Nowadays, this is especially true for membrane proteins [4] and high-throughput approaches applied to drug discovery [5] and proteomics [6,7]. In the latter context, it is usually observed that only 1 out of 10 target proteins yields crystals that are suitable for structure determination [6,8]. A reason is the plastic architecture of proteins that decreases their propensity to crystallise. Therefore, in all current protein crystallisation methods, homogeneous solutions are driven smoothly towards supersaturation where nucleation and growth occur. On the other hand, the crystallisation process itself is influenced by a diversity of variables including molecular purity, ionic strength, pH, composition, temperature, pressure and gravity, making the whole process complex. Given these difficulties it is understandable that structural biologists eagerly await any effort aimed to develop strategies to increase the success rate of protein crystallisation [9–11].

Early studies on inorganic and small organic molecules had shown that any difference between the crystal and solution densities triggers buoyancy-driven convection and crystal sedimentation or floating. These phenomena are detrimental to crystal quality because they perturb mass transport during crystal nucleation and growth. This gravity-dependent effect is weaker under weightlessness [12]. As a consequence, microgravity was envisioned to have a favourable influence on protein crystal growth. In addition, the small size of many earth-grown crystals and/or the poor diffraction properties of others were as many reasons for undertaking crystallisation under microgravity. In 1984 the feasibility of such experiments was demonstrated by the growth of voluminous lysozyme crystals aboard an orbiter [13]. Despite the potential promises, such studies were immediately criticised (e.g. [14]), among other reasons were the excessive cost of assays and limited flight opportunities. After two decades of experimentation in space, the criticism that stays is the marginal contribution of microgravity research to the development of structural biology [15], compared to that of the advances in X-ray and computing facilities and in high-throughput technologies applied to crystallisation. Despite this apparently negative appraisal, it is generally accepted that microgravity research contributed to the better understanding of the crystallisation process and the parameters governing crystal quality (reviewed in [16]). Further, a number of data obtained recently with other facilities support the view that space-grown crystals can be useful for structural biology [17–23]. However, until now this potential has not been enough exploited because it was not exhaustively analysed and well explained.

It is the aim of this review to bring clarification in the microgravity/structural biology dispute and to highlight the positive trends. Conclusions are based on results collected over more than one decade in the Advanced Protein Crystallisation Facility (APCF) with emphasis to those allowing better determination of protein 3D-structures. In order to encourage structural biologists to benefit from the knowledge gained from microgravity research, it describes also how conventional crystallisation assays can be modified to create an environment mimicking the one encountered under microgravity.

2. Experimentation in the APCF

Aboard space shuttles or space stations, the gravity level is 10^3- to 10^6-fold lower than at the earth's surface. Several sophisticated instruments have been built over the last two decades to investigate protein crystallisation in such environments (e.g. [13,24–27]). A research program of the European Space Agency (ESA) that involved a number of research laboratories was directed at developing the APCF [28,29] that was built by Astrium GmbH (Germany). This instrument accommodates 48 reactors (with protein volumes ranging from 4 to 470 μl) and operates according to either of three crystallisation techniques (vapour diffusion, dialysis or free interface diffusion) (Fig. 1). The crystallisation process can be monitored with a video camera and an interferometer [30].

The APCF was aboard 7 space missions from which 6 yielded results (Table 1). From June 1993 to December 2002, 474 individual crystallisation assays in all were conducted on a total of 46 different biological particles, including mutants and various crystal forms. These numbers can be compared with those of ~50 missions that carried into space ~10,000 assays in various

crystallisation facilities between 1988 and 2003 [17,23]. Details about the samples, crystal quality and derived structures are given in Table 2. In the exploration phase, experiments have dealt with proteins that were reluctant to crystallise under routine laboratory conditions and with crystals that were useless for structure resolution. This was the situation of 5S rRNA, the outer surface glycoprotein and octarellin II (Table 1). In the worst case crystals were twinned (e.g. ADH, CcdB), had a high mosaic spread (e.g. CcdB) or poor diffraction properties (e.g. ribosome). On the other hand, a few protein candidates were added to serve as models because their crystals were reproducible and of good quality. Thus, lysozyme, thaumatin, and ferritin were essentially studied to compare their crystallogenesis under microgravity with that on earth. For all samples, the ultimate goal was to prepare crystals that would be suitable for the determination of 3D-structures at atomic resolution. In what follows, the term 'protein' will stand for all types of biological particles including RNA, DNA and nucleoprotein particles such as nucleosomes, ribosomes and viruses. The term 'microgravity' will refer to any level of reduced gravity encountered in orbit. The major results about the physical aspects of crystal growth in the APCF have been reported elsewhere [16].

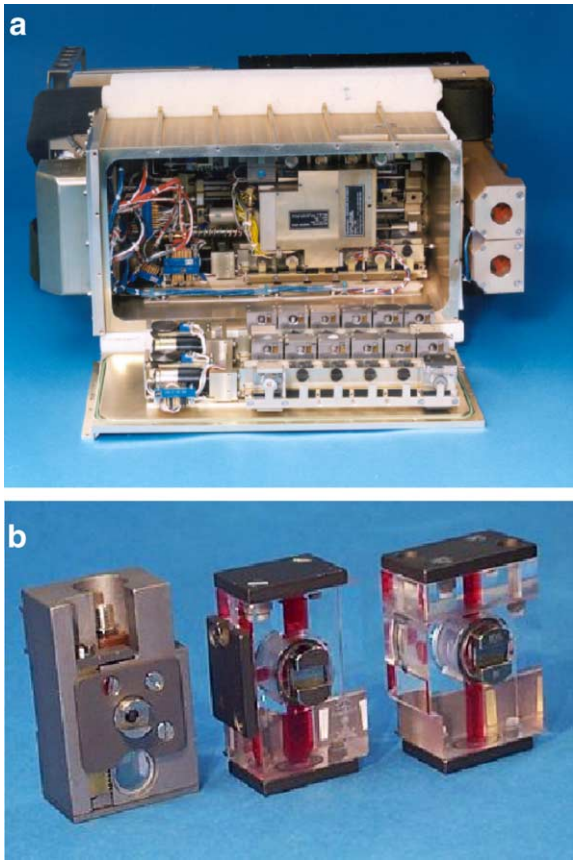

Fig. 1. The APCF. (a) Two racks of six mounted reactors are displayed in front of the open apparatus. (b) Reactors designed for protein crystallisation by vapour diffusion, free interface diffusion and dialysis (from left to right).

Table 1
Space missions on which the APCF was a payload

Mission/date of flight	Proteins[a]
SpaceHab-01/Jun 1993	5, 7, 14, 20
IML-2/Jul 1994	2, 5, 8, 10, 13–17, 19, 20, 25–27, 29–31, 34, 42, 43, 45, 46
USML-2/Oct 1995	2–6, 10, 12, 13, 16–20, 23, 25–27, 29–32, 36, 37, 41–46
LMS/Jun 1996	2, 3, 5, 10, 12–14, 19, 25–27, 29, 33, 35, 37, 38, 41
STS-95/Oct 1998	1, 5, 9, 11, 13, 14, 16, 17, 19, 21, 24, 28, 29, 33, 37, 38, 41
ISS-3/Aug 2001[b]	1, 2, 5, 9, 13, 16, 17, 20, 22, 29, 38–40
STS-107/Jan 2003[b,c]	–

[a] Each biological particle is represented by the number under which it appears in Table 2.
[b] Experiments carried out on the International Space Station instead of the U.S. Space Shuttle.
[c] Shuttle was lost a few minutes before landing in the morning of 1st February 2003.

3. Contribution of crystal growth experiments in the APCF to structural biology

On several occasions, experiments in the APCF were carried out on model proteins whose 3D-structure was well known; therefore a complete X-ray data collection was not planned. In other few cases, complete diffraction data were not collected due to twinning, instability or radiation damage [31–33]. Considering all the above cases, a comparative analysis between the APCF grown crystals and ground crystals resulted in a percentage of success of 52% [16]. Notice that crystal quality of 2/46 proteins was better for ground-grown crystals, namely triose P isomerase from *Thermotoga maritima* and the photoreactor centre from *Photobacter sphaeroides* [16].

Data reported in Table 2 indicate that in 24% cases (i.e. 11 proteins out of 46 assayed in the APCF), when a complete X-ray data set was collected, microgravity grown crystals led to better 3D-structures than any of the corresponding control crystal previously grown on earth. To find the origin of the positive influence of microgravity, a correlation was searched between the crystal quality enhancement and any system-dependent biophysical variable. Better crystals were obtained for 67% of the smaller proteins, 50% of the medium-size proteins and 31% of the large proteins [16]. There is no correlation with the isoelectric point of the proteins. Opposite to what was suggested [34], there is an anticorrelation between the solvent content and the quality improvement. As for other experiments performed in space [17,23], the proportion of successful crystallisations with the APCF has strongly increased over time. It is worth noting that over the years the APCF has been implemented and that increasing

Table 2
Proteins and other biological particles crystallised in microgravity within the APCF

Code	Biological particles	Source	Mr (kDa)	Solvent content[a] (%)	Resolution (g–μg) (Å)	PDB code [ref]
Small						
1	$(Pro–Pro–Gly)_{10}$	Synthetic	8	40–46[b]	1.8–1.3	1K6F [36]
2	5S rRNA (B, C and E domains)	*Thermus flavus*, synthetic	8–14[c]	53–67[c]		
3	Topoisomerase poison, CcdB	*Escherichia coli*	12	55		
4	Lysozyme	Bacteriophage λ	17	44		
5	Lysozyme, tetragonal form	*Gallus gallus*	14	43	1.33–0.94	194L IBWJ 1IEE [37–39]
6	Lysozyme, monoclinic form	*Gallus gallus*	14	39	na[d]–1.45	1HF4 [40]
7	Ribonuclease A	Beef	16	na		
8	Ribonuclease S	Beef	16	na		
9	Antigen–antibody complex	Camelid	17	na		
10	Apocrustacyanin C1	*Homarus gammarus*	20	48	2.0–1.85	1OBQ [41]
11	Adaptor Grb2	Engineered	(20)[e]	na		
12	Epidermal growth factor receptor		(20)	na		
13	Thaumatin	*Thaumatococcus daniellii*	22	45	1.7–1.2	1KWN [42]
14	Rhodopsin	Bacterioelectrogenic membrane	25	na		
15	Collagenase	*Hypoderma lineatum*	25	50	1.8–1.7	2HLC [43]
Medium						
16	Octarellin II	Synthetic	28	na		
17	Octarellin III	Synthetic	28	na		
18	Concanavalin B		37	na		
19	5S rRNA	*Thermus flavus*, engineered	40[c]	69[c]		
20	Rhodopsin	Beef	40	na		
21	Proteinase K	*Tritirachium album* limber	45	44	1.3–0.98	1IC6 [44]
22	Antithrombin		50	na		
23	Glutathione S-transferase		50	na		
24	Single strand DNA binding protein—ssDNA		60	74		
25	Triose P isomerase	Human	75	na		
26	Triose P isomerase	Human, mutant	75	na		
27	Triose P isomerase	*Thermatoga maritima*	75	72		
28	Outer surface glycoprotein	*Methanothermus fervidus*	76	49		
29	Aspartyl–tRNA synthetase (form 1)	*Thermus thermophilus*	132	62	2.4–2.0	1L0W [45]
30	Canavalin, hexagonal form	*Canavalia ensiformis*	142	51	2.6–2.0	2CAV [46]
31	Canavalin, rhombohedral form	*Canavalia ensiformis*	142	56	2.6–1.7	1DGW [47]
32	Phenylalanyl–tRNA synthetase	*Thermus thermophilus*	(150)	na		
33	Alcohol dehydrogenase, ADH	*Solfolobus solfataricus*	150	59		
Large						
34	Photoreactor centre	*Photobacter sphaeroides*	(200)	na		
35	Nucleosome		206	52		
36	Catalase	Beef liver	240	59		
37	Apoferritin		450	na		
38	Ferritin		474	na		
39	Low density lipoprotein particle	Human	550	na		
40	Lumazine synthase	*Bacillus subtilis*	1000	na		
41	Photosystem I	*Synechococcus elongatus*	1020	75	4.0–3.4	Structure not yet deposited [48,49]
42	Satellite panicum mosaic virus		1200	na		
43	Satellite tobacco mosaic virus, cubic form		1400	na		
44	Tomato aspermy virus		(2000)	na		
45	Ribosome	*Haloarcula marismortui*	2300	na		
46	Turnip yellow mosaic virus		5600	na		

The particles are grouped in three classes according to their molecular mass: the categories are small (M_r<20,000 Da), medium (20,000 Da<M_r<200,000 Da) and large (M_r>200,000 Da). References are given only for structures obtained from μg-grown crystals and deposited in the PDB (last column).

[a] Refers to the global solvent amount and does not distinguish the content of ordered water molecules from the bulk mother liquor inside the crystals.

[b] Variation due to different levels of crystal dehydration.

[c] Refers to domains and full length 5S rRNA.

[d] na, data not available.

[e] Values in parenthesis are tentative molecular weights.

numbers of space-experiments have been conducted. Therefore, the trend also partially reflects concomitant improvements made by molecular biology, X-ray technology and crystallographic methods [35].

4. Value of structures derived from μg-grown crystals

4.1. General considerations

Experiments conducted in microgravity revealed to be extremely useful only if they were accompanied by control experiments carried out strictly in parallel on earth. In what follows, the comparison of the 3D-structures derived from earth-grown vs. μg-grown crystals is based on a variety of data including the ratio of intensity of the reflections over the background noise (mean I/σ), the diffraction limit (resolution), the unambiguous location of all atoms in the electron density map, the number of bound solvent molecules (water) or ions. This comparison covers a subset of 11 different proteins corresponding to 13 structures that have been solved using diffraction data from crystals grown in the APCF while it was on a terrestrial orbit (Table 2) [36–49].

As in many other instances, visual examination showed that crystal dimensions were greater than those of respective controls as in the case of cubic satellite tobacco mosaic virus [50] or photosystem I [48,49]. On the other hand, X-ray diffraction analyses showed that space-grown crystals of the CcdB protein (code # 3 in Table 2) were less twinned [51] and that the diffraction limit of those of an outer surface glycoprotein (code # 28) was extended from 9 to 4.2 Å [32] with respect to earth controls. Similarly, the diffraction limit of tetragonal hen lysozyme crystals was gradually pushed from 1.8 to 0.94 Å in the frame of experiments performed in space [37–39]. Unsuspected structural information was gained from some space-grown crystals. For instance, various monovalent ions (such as Cl^-, Na^+, NO_3^-) were identified in the tetragonal and monoclinic crystal forms of hen lysozyme [37,40] (codes # 5 and 6, respectively) and also in collagenase crystals (code # 15) [43]. Also, the resolution of tetragonal and rhombohedral canavalin crystals (code # 30 and 31) became better than 2 Å [46,47] and that of proteinase K crystals (code # 21) extended beyond 1 Å [44].

More recently, the comparative analysis of electron density maps of proteins crystallised in the APCF has provided evidence that overall better structures may be derived from space-grown crystals. Below, four proteins (arranged by increasing M_r) are described in more detail. The results gained with their studies are corroborated by those coming from crystals of other proteins that were grown under microgravity using different facilities [17,23].

4.2. Collagen-like synthetic polypeptide

Good models for collagen–the most abundant protein in vertebrates–are polypeptides with sequence $(X–Y–Gly)_n$, where X and Y can be any amino acid, but frequently they are Pro and hydroxyproline (Hyp). Crystals of $(Pro–Pro–Gly)_{10}$, $(PPG)_{10}$, contain triple helical molecules $[(PPG)_{10}]_3$ of 90 residues each. Their diffraction patterns are characterized by an uneven distribution of reflections with both very strong and very weak intensities. Initial data collections on earth-grown crystals did not accurately record the weak reflections so that only those originating from a subset could be used for structure determination, leading to an infinite chain model [52]. With better quality $(PPG)_{10}$ crystals grown in the APCF under microgravity (Fig. 2a), the very weak reflections could be detected and thus indexed (Fig. 2b) [53–55]. X-ray diffraction data were collected at room temperature from μg-grown crystals, whose diffraction limit is 1.3 Å compared to 1.8 Å for ground-grown crystals and a new unit cell could be identified (Fig. 2c). In the electron density map a more precise model [36,54–56], including heads and tails of individual molecules, could be visualised as well as a great number of solvent molecules bridging protein side chains in the packing [36]. The final model, refined to a R factor of 0.18, is presently the best 3-D structure for a collagen triple helix [36]. It provides clues regarding the $[(X–Y–Gly)_n]_3$ triple helix folding, its assembly and stability. In particular, the residue Pro in X position–7– adopts a *down* conformation while Pro in Y position is in an *up* conformation (Fig. 2d). The X and Y positions require not only a specific ring puckering but also a specific backbone conformation. According to a previous view, the thermal stability enhancement in the presence of Hyp was either caused by a network of hydrogen bonds [57] or by an inductive effect of the hydroxyl group [58]. A detailed analysis of the high resolution $(PPG)_{10}$ structure, obtained from μg-grown crystals, suggests that a collagen triple helix requires specific and position-dependent conformations for the backbone dihedral angle *phi* [36,59,60]. As a result, the authors put forward a new hypothesis for the high propensity of Hyp occurring in the Y position as being a conformational requirement, thereby explaining the greater stability of the triple helix when hydroxylated in that position [59]. Ultimately, these findings clarify not only the sequence–stability relationship in collagen [59,60] but they also conciliate recent controversial data [61].

4.3. Apocrustacyanin

α-Crustacyanin, a substance responsible for the colouration of some marine crustacean, is composed of 16 protein subunits (M_r ~20,000 Da) each binding one molecule of carotene derivative astaxanthin [41]. Protein variants exist that can be classified into two major types, carotenoproteins C_1, C_2 and A_1 belonging to the first type. The electron density map of apocrustacyanin C_1 dimers at a resolution of 2 Å was derived from μg-grown crystals prepared in vapour diffusion reactors. It shows details at the level of 7 charged surface residues from which two are involved in or located nearby crystal lattice-forming interactions [41]. The electron

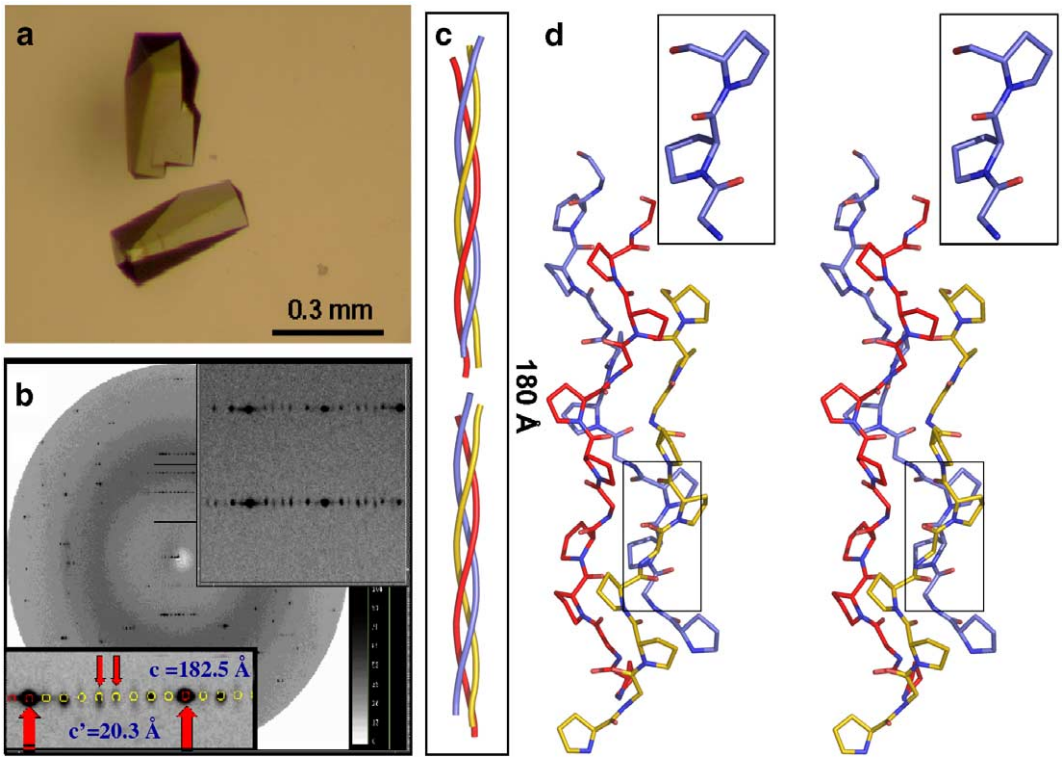

Fig. 2. Crystallographic analysis of $(Pro–Pro–Gly)_{10}$ crystals grown in microgravity. (a) Space-grown crystals with (b) a diffraction pattern. (c) Two independent molecules in the unit cell. (d) Stereoviews of the structure model at 1.3 Å resolution. The insets display a close-up view of the *up* and *down* puckering of adjacent Pro residues. Adapted from Berisio et al. [36,53].

density corresponding to water molecules is also better resolved than in the earth-grown crystals. Afterwards, subtle differences in amidation were found when the 1.3 Å resolution structure of apocrustacyanin C_2 was compared with that of the C_1 form [62].

4.4. Thaumatin

The monomeric protein thaumatin-1 (M_r ~22,000 Da) extracted from the arils of the plant *Thaumatoccocus daniellii* has an intensely sweet taste although it is deprived of any carbohydrate moiety. It was crystallised in 1975 [63] and the first structure determined in 1985 at 3.1 Å resolution revealed three structural domains, the largest one being a flattened barrel formed by 11 antiparallel β-strands [64]. This structure was subsequently refined to 1.65 Å [65]. Later, three other crystal forms were found of which two led to structures at 1.75 Å resolution [66]. When the tetragonal crystals were prepared in the presence of sodium tartrate and of agarose gel (to immobilise the crystallisation nuclei) using dialysis reactors during the STS-95 mission (Fig. 3a), they diffracted X-rays at a resolution better than 1.2 Å [42]. These crystals displayed a characteristic X-ray topography image with a large spiral-like contrast originating at their centre (i.e. at the level of the nucleus) and propagating in the opposite pyramids (Fig. 3b). Their diffraction pattern was essentially composed of sharp and intense Bragg reflections (Fig. 3c). A complete data set at up to 1.2 Å resolution was collected at room temperature (this is noteworthy, since collection of high resolution data using synchrotron radiation generally requires cryo-conditions). The derived electron density map provided a detailed view of a tartrate ion (Fig. 3d). As already known [66], the latter lies at the interface of three symmetry-related protein monomers and plays the role of an additive that contributes to the cohesion of the crystal lattice. In addition, twice as many water molecules as in the starting structure could be positioned unambiguously [42]. The superior quality of the new 3D-structure is a direct consequence of nucleation and growth in a convection-free medium in which crystals with minimal defects grow by a diffusive regime.

4.5. Aspartyl–tRNA synthetase

The high-molecular weight aspartyl–tRNA synthetase (AspRS-1), an enzyme extracted from the hyperthermophilic bacterium *Thermus thermophilus,* was used as a model for crystallogenesis studies in order to identify microgravity-induced effects linked to a large molecular size. Each subunit of this homodimeric protein encompasses 580 residues (M_r ~66,000 Da) [67] and is composed of four well-defined domains that are responsible for specific functions during catalysis. The protein was crystallised in salt solution and a first structure was solved at 2.5 Å resolution [68]. Crystallisation in space has reproducibly yielded a few very large crystals (Fig. 4a) having less

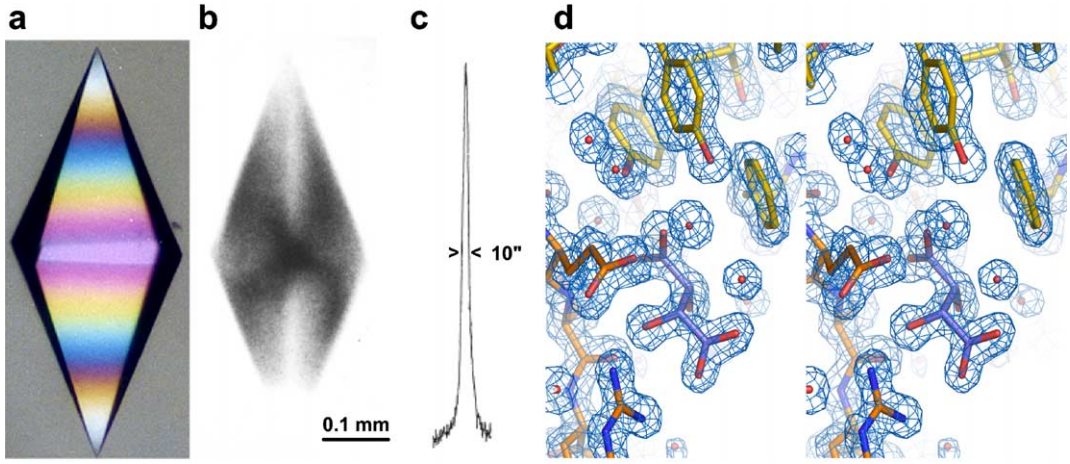

Fig. 3. Crystallographic analysis of thaumatin crystals grown in agarose gel in microgravity. (a) Tetragonal bipyramidal crystal in polarised light. (b) Topograph showing regions with low or high contrast indicative of differences in the density of defects (for details on the method see Robert et al. [88]). (c) Sharp Bragg reflection profile with a full-width at half maximum (FWHM) of 10 arcseconds. (d) Close-up stereoview of the structure model at 1.2 Å resolution fitted in the electron density map. The model shows parts of two of the three protein monomers (in bold and weak orange colours) that interact with a tartrate ion (adapted from Sauter et al. [42]).

surface defects than those grown on earth [45]. The diffraction limit of the crystals grown in dialysis reactors either on orbit or on earth reached 2.0 Å. Space-grown crystals are distinguished from controls by the sharpness of the Bragg reflections (Fig. 4b). A striking difference is visible in the initial *2Fo–Fc* electron density maps (Fig. 4c,d). In the map derived from µg-grown crystals, the atoms located in the backbone or in the side chains of the protein are better resolved in at least three regions distributed along the polypeptide chain [45]. As observed with the three

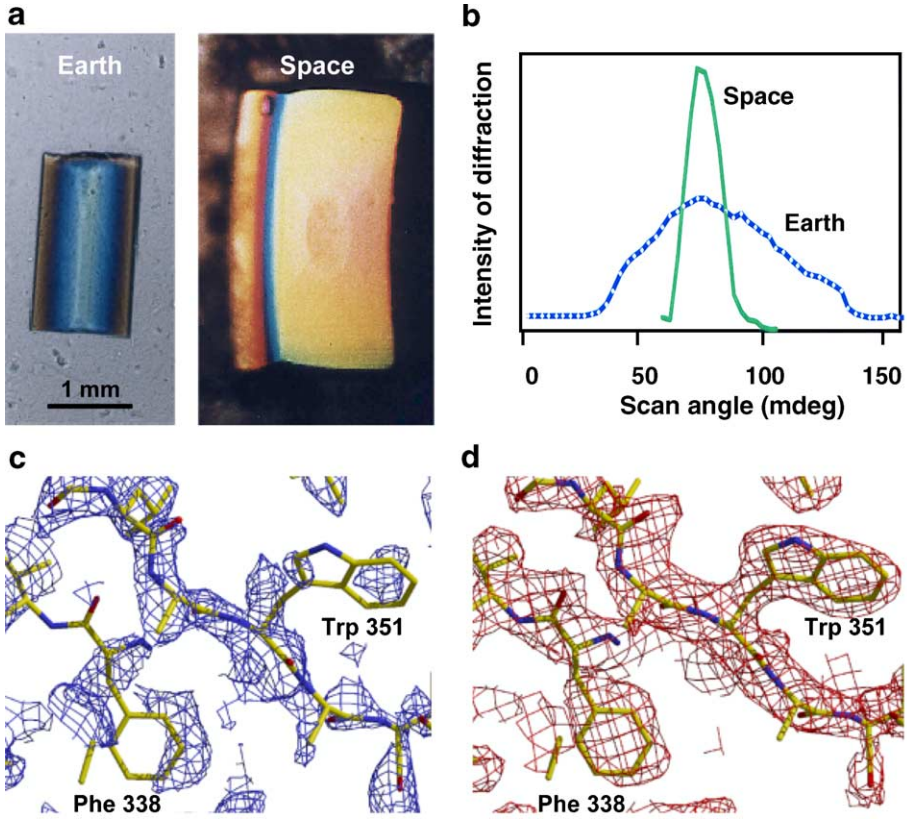

Fig. 4. Comparative analysis of AspRS-1 crystals grown on earth and in microgravity. (a) The displayed space-grown crystal measures $3.0 \times 1.7 \times 1.0$ mm^3 and has a 3-fold greater volume than earth control crystals (average volume 3.4 mm^3 vs 1.1 mm^3). (b) Rocking curves representing the intensity of a same Bragg reflection as a function of the scan angle. (c, d) Close-up views of the same region of the electron density maps derived from ground-grown and microgravity-grown crystals, respectively. The sharper detail on the map in panel d are a useful information for model construction (adapted from Ng et al. [45]).

above proteins, the presence of a greater number of ordered water molecules was correlated with a reduction of the mean B factor in the crystals prepared under microgravity [45].

5. Beneficial effects of microgravity on protein crystal growth

Partial data coming from analysis of other proteins strongly support the conclusions drawn from the rigourous comparison of the quality of space- vs. earth-grown crystals of the four above proteins based on standardised protocols. For instance, the structure of proteinase K derived from μg-grown crystals clearly identifies hydrogen bonds in the serine protease catalytic triad (Ser–His–Asp) and an unusual short H-bond between Asp and His that is part of an extended network [44]. In the frame of a long-term project, the diffraction limit of space-grown crystals of the membrane protein photosystem I, was progressively enhanced. The improved electron density map obtained in the frame of the microgravity experiments of this photosynthetic reaction centre has revealed a second phylloquinone molecule that completes the set of cofactors involved in the electron transfer [48,49].

For both proteins, the enhancement of crystal quality has given access to information essential to understand their molecular mechanism.

The results obtained with the APCF have also been compared to those obtained with other facilities (e.g. [24–27]) such as the VDA [69], the DCAM [25] and the Granada Crystallisation Box [27]. Independently of the hardware used in space, crystal growers come to the conclusion that the quiescence of the fluid medium (at a 10^3- to 10^6-fold lower gravity) is favourable to more order in the macromolecular arrangement and to the visualisation of an extended network of H-bonded water molecules. Protein side chains appear to be less mobile as witnessed by a low B factor, indicating a lesser thermal agitation and a lower static disorder. This was well documented with a second phenolic compound specifically bound to insulin only in μg-grown crystals. Its stabilisation is accompanied by a transition from an insulin trimer to a hexamer [70]. Here also the reduced mean B factor allowed description of more solvent sites. The same was observed for isocitrate lyase, γ-interferon, malic enzyme and proline isomerase [71].

6. Practical considerations for protein crystallisation on earth

Experimentation in orbit has highlighted the crucial role of convection during crystal growth. This was first verified on small molecules and is now known to be very detrimental to the preparation of crystals of proteins and of other large biological particles. There are at least two ways to minimise or even suppress convection in solution on earth and to create diffusive environments. The first consists in performing the crystallisation inside a very thin tube where capillary forces counterbalance gravity forces. In such a device assays can easily be set up using techniques like batch, liquid–liquid (or free-interface) diffusion or counter-diffusion [72,73]. In practice, X-ray capillaries–available on the market in various diameters–are very convenient for the visualisation of crystal growth and for in situ diffraction analyses [74–77].

The alternative is to grow the crystals inside the network of a hydrogel as first assayed in the fifties within gelatin gels [78] and rediscovered 30 years later [79,80]. So far, the favourite gels are agarose and silica at very low concentrations [81,82]. Besides decreasing solutal convection, the gel immobilises the crystals and promotes their isotropic growth. In the peculiar case of thaumatin, it was demonstrated that agarose gel has suppressed crystal motion under microgravity, prevented crystals from settling upon shuttle landing, and helped to save their optical and diffraction properties [83]. As a general application, gels are a good means to protect crystals during transportation, for instance on trips to synchrotrons. Moreover, they may stabilise the crystalline network during cryo-cooling [84]. Finally, other methods mimicking some aspects of the microgravity environment, like crystal growth in containerless volumes [85] are at the disposal of the crystal growers.

7. Perspectives for future experimentation in space and on earth

A novel Protein Crystallisation Diagnostics Facility (PCDF) supported by ESA has been built based on the reliable and efficient APCF [86]. It will be essentially dedicated to the study of the physics of crystal nucleation and growth under reduced gravity by digital imaging, light scattering and interferometry techniques. In addition, uncoupling nucleation and growth in batch and dialysis reactors will be possible by modifying the degree of supersaturation upon temperature or solution composition variation. The PCDF will be installed aboard the European module Columbus of the ISS for long-duration low gravity sessions. Independently of this, an automated diffractometer has been designed under contract of NASA to do on board crystal handling and analyses [69,87]. In order to optimise the use of these high-tech instruments, much effort will be needed to quantify the influence of each of the countless variables of protein crystal growth in ground-based laboratories.

The investigations conducted so far with the APCF and other crystallisation facilities have demonstrated that protein crystals grown under quasi-weightlessness tend to have lesser defects with respect to crystals grown on earth's surface. As a consequence of their better internal order, space-grown crystals can produce diffraction patterns with sharper reflections and extending to higher resolutions.

Their augmented volume is another advantage for neutron diffraction experiments. Altogether, these enhancements result in better data collection statistics and ultimately lead to more accurate 3D-structure models. As illustrated above, any supplementary structural information acquired owing to such crystals gives a more precise insight into the relationship between the molecular structure and its function. In the coming years, fundamental crystal growth studies should run side by side with high-throughput crystal production. The spin-offs of the latter [5–9] will complement crystallogenesis studies towards the understanding of protein crystal growth.

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