Chapter 2
Protein Crystallization in Space: Early Successes and Drawbacks in the German Space Life Sciences Program

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Abstract The utilization of microgravity for improving protein crystallization and thereby structure determination started in the early 1980s onboard of TEXUS sounding rockets and of the US Space Shuttle. After the successful pioneering work by Prof. Littke, especially the German space life sciences program put much effort into this topic. In spite of some technical and methodological drawbacks, early successes could be obtained as well. In some cases, microgravity experiments enabled crystallization of certain molecules for the first time; in other examples, improved crystals led to a better structure determination with important application potential for structure-function-analysis or even for drug design. Especially after the development of the APCF (Advanced Protein Crystallization Facility) by German industry on contract by ESA and its utilization in Spacelab missions and on the International Space Station ISS, the potential of microgravity for the improvement of crystallization and structure elucidation became clearly visible.

Keywords Protein Crystallization • Microgravity Conditions • Space Hardware Structure Elucidation • Archaea Surface Proteins • Misletoe Lectin • RNA Molecules

2.1 Introduction: Nobel Prize for Clarification of Ribosome Structure

As pointed out in the introductory chapter, the idea to utilize microgravity for improving protein crystallization was born in Germany with the first space experiments being performed by Littke and co-workers onboard of TEXUS sounding rocket flights in 1981 and during the first Spacelab mission in 1983 (Littke and John 1984). Possibly, within the 1970s and 1980s some crystallization experiments were performed by Russian and Chinese scientists, however only incomplete records of these experiments exist.

Nearly 30 years later, in October 2009 namely, the Nobel Prize Committee awarded the Nobel Prize in Chemistry to two scientists working in the USA, Venkatraman Ramakrishnan and Thomas A. Steitz, and to Israel's Ada E. Yonath for their ground-breaking work on the structure and function of ribosomes.

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From 1979 until 1983 Ada E. Yonath was a guest professor at Berlin's Max Planck Institute for Molecular Genetics; from 1986 until 2004 she headed a Max Planck research group at the German Electron Synchroton (DESY) in Hamburg. During this period she was involved with her experiments in a series of space missions, utilizing conditions of weightlessness to improve ribosome crystallization. By the way, for the sake of simplicity we will use the term protein crystallization consistently to cover other organic macromolecules as well, such as nucleic acids, or protein and nucleic acid complexes such as ribosomes.

It is with the help of ribosomes that organisms produce their necessary protein molecules based on the DNA information. This, in a manner of speaking, makes ribosomes the factories of life. To understand their function one has to know their three-dimensional structure at high resolution. Considering that protein structures were already published and deposited more routinely in the late 1970s, for ribosomes structure it took until the year 2000—although the first few discoveries had appeared also at that time, when Yonath began to produce ribosome crystals and explore their structure with the help of X-rays.

Early attempts had produced extremely thin and fragile ribosome crystals that were unsuitable for X-ray diffraction analysis. At this time the option of research in microgravity conditions became available. The theory in the late seventies had been that, given the absence of sedimentation (particles depositing at the bottom of a fluid) and gravity-driven convection (transfer of thermal energy by means of particle transport) in a weightlessness condition, the crystals that would form were likely to be larger and of greater purity. Although as we know today crystal growth is affected by a number of other factors as well, the assumption, on principle, had been correct and has been followed by many scientists ever since. These considerations that led Yonath in the late eighties to develop an interest in experiments under microgravity.

In the period from 1988 until 1995 Yonath sent her experiments on more than twelve space missions. By doing so, she attempted to improve ribosome crystallization on COSIMA satellite missions (Erdmann et al. 1989) and several American Space Shuttle flights including Germany's Spacelab mission D-2 in 1993 with the German astronauts Ullrich Walter and Hans Schlegel onboard. This was when first signs of progress became apparent. The crystals grown during Shuttle/Spacelab missions D-2 (1993), IML-2 (1994) and USML-2 (1995) were larger, rounder and more evenly shaped, thus pointing and guiding the way to further experiments on Earth, which then ultimately ended up in a successful elucidation of ribosome structures, and finally the Nobel Prize (van Noorden 2009).

As will be now shown, Nobel laureate Ada Yonath's crystallization of ribosomes is not the only achievement that deserves attention. Five further particularly successful examples will be presented in more detail. Among them are results of two scientific teams within DLR's microgravity research program that—interesting enough—cooperated closely with Prof. Yonath: Christian Betzel from the University Hamburg at DESY and Volker Erdmann from Berlin Free University, winner of the Leibniz Prize and long-standing spokesman of a collaborative research centre (SFB) at the German Research Foundation (DFG). But before doing that, a few theoretical considerations will provide some further background to the topic in general.

2.2 Some Thoughts on the Theoretical and Methodological Background

Within the last 20 years methods, techniques, instrumentation, software and X-ray radiation sources applied in X-ray crystallography were continuously and substantially further developed and improved, as protein crystallography till now is the most important method and working horse to analyze biomolecules, protein or nucleic acids, at atomic resolution (Su et al. 2015; Lattman and Loll 2008). Only structure function analysis at high and close to atomic resolution provides essential information towards obtaining insights about the various, complex and for each bio-macromolecule unique function. This fundamental knowledge provides for example information required to support the development and design of new pharmaceuticals to treat chronic or infectious diseases, or is providing the required information supporting to engineer enzymes for particular biotechnological applications. However, the use of X-ray crystallography to determine protein structure requires the production of well-ordered protein crystals of sufficient quality and size, named or entitled X-ray suitable crystals. Even today, after installation of third generation and high brilliant synchrotron radiation sources and upcoming X-ray Free Electron lasers (XFELs), which require only nano- or micro-sized crystals (Martin-Garcia et al. 2016), the overall demand to produce well-ordered high quality crystals remains, which means crystals with low internal mosaicity and low incorporation of impurities. The growth of such diffraction quality crystals of biomolecules is often difficult and depends on the molecule itself. For example, high flexibility of a protein or subdomains of the protein, often connected to the function of the biomolecule, is known to restrict crystal growth (Giegé 2013). And till now protein crystal production is the well-known bottleneck of the method and the time limiting factor of a crystal structure analysis (McPherson 2004), beside a wide portfolio of different methods, procedures, hardware, including also robotic procedures, which were developed already and till now are under continuous optimization (Chayen 2003; Chayen and Saridakis 2008). Protein crystal growth is still considered to be an art, as the pathways protein crystal growth remain to some extend unpredictable (Gavira 2016; McPherson 2011).

Only recently latest biophysical methods and advanced diagnostic tools provided insights into early stages of the crystallization processes shed light towards understanding particular the nucleation process in more detail (Vekilov 2004; Schubert et al. 2017). In protein crystallization, a protein solution is typically brought into supersaturation by the presence of a precipitant, which is taking slowly water solvent molecules away from the protein surface and forcing in emergence of intramolecular protein–protein interactions, inducing in consequence the thermodynamically driven crystal nucleation followed by further crystal growth.

Beside purity and homogeneity of the protein solution the solution conditions at which supersaturation is achieved are the most important parameters, as they define a highly specific temporal pathway through the phase diagram (Gavira 2016).

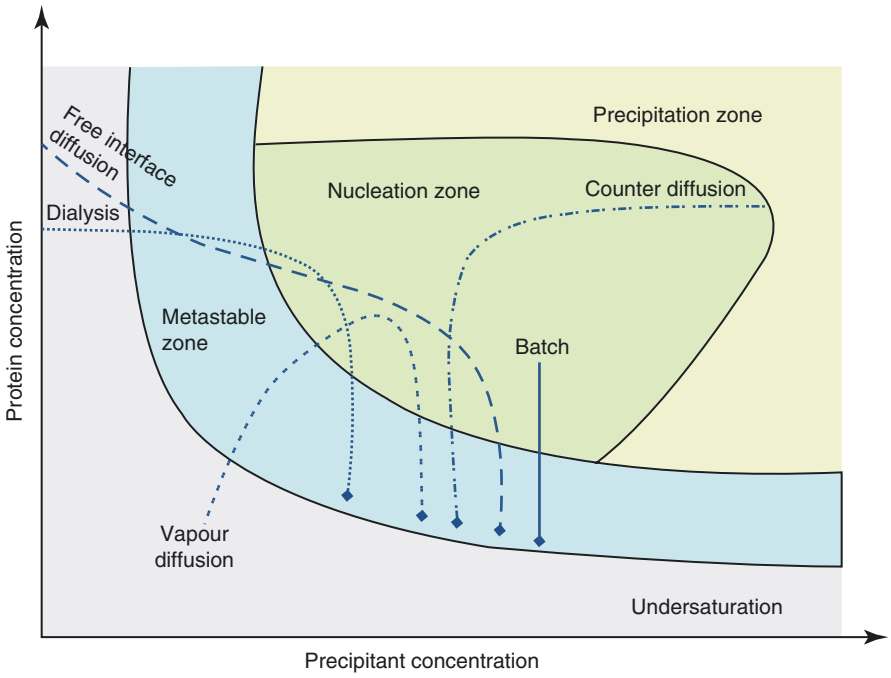

Fig. 2.1 Phase diagram demonstrating effects of protein concentration against precipitant concentration. The solubility curve divides two areas corresponding to the undersaturated and supersaturated state of a protein solution. The supersaturated area harbors the metastable, nucleation and precipitation zones

The phase diagram is commonly used to explain the crystallization process based on the Debye Hückel theory (Debye and Hückel 1923). As shown in Figs. 2.1 and 2.2 a supersaturation state can be achieved either rather straight, using the batch technique, or more smooth applying dialysis, vapor diffusion, or counter diffusion (McPherson 2004; McPherson and DeLucas 2015), shown in Fig. 2.2. The last two methods are the today most commonly used for standard screening experiments. The counter diffusion method, allowing to control and adjust precise and reproducible mass transport in protein crystal growth, was in recent years further developed and optimized for space experiments (Gonzalez-Ramirez et al. 2008).

On Earth density differences near growing crystals, produced by incorporation of protein molecules, solute and ions into the growing crystal, cause convective flows within the vicinity of growing crystals. These convective flows overlap with transport based on diffusion. On Earth the interaction and overlap of both transport phenomena, also shown in Fig. 2.3, determine the kinetics of crystal growth.

In this context, it is well known that the rate and kinetics of the overall mass transport is determining also the incorporation of impurities, such as protein aggregates, partially unfolded proteins etc., which can be present in crystallization solutions to some extend and influence the crystal quality and the final dimensions and form of a crystal. As convection flows are caused by heavy or more light fluids,

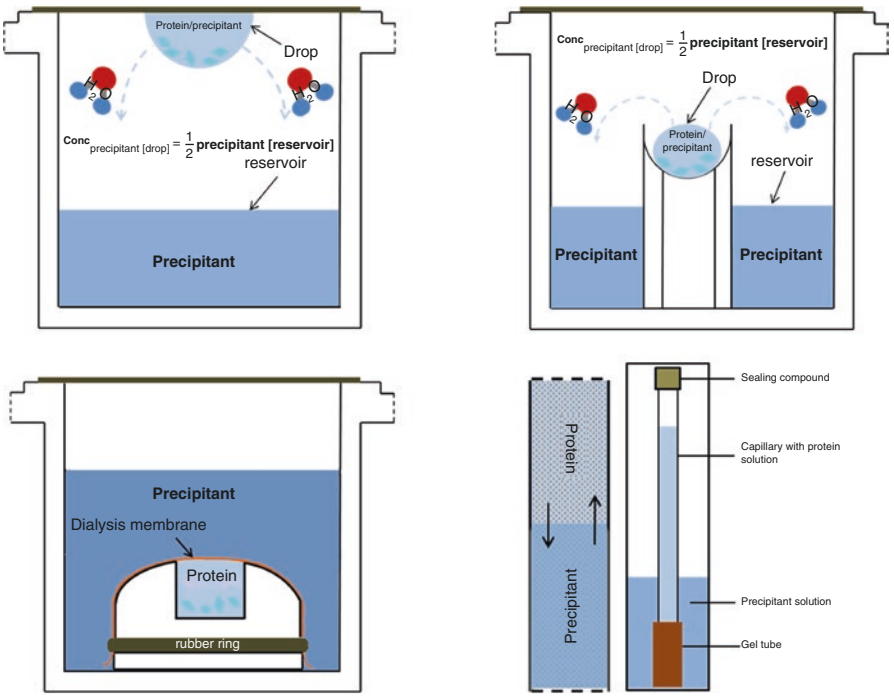

Fig. 2.2 Schemes of the most commonly used crystallization methods. *Top*, vapor diffusion, hanging and sitting drop. Below *left*, dialysis and *right*, counter diffusion

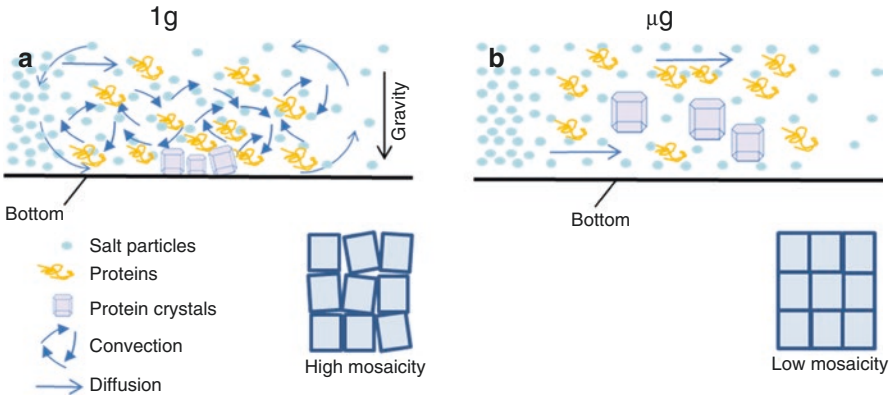

Fig. 2.3 Scheme (**a**) and (**b**) showing comparative mass transport under Earth and microgravity conditions and resulting crystal quality indicated by the mosaicity of the crystal lattice

which move or circulate in counter directions under normal gravity (1 g) they are somehow unfortunate and not really controllable, and they disturb to a certain extent crystal growth and crystal perfection. Therefore, crystal growth at 0 g or in a microgravity environment (10^{-3} to 10^{-7} g) instead, allows to minimize the convective flow

and associated mass transport resulting in an environment with mass transport based only on diffusion, as shown in Fig. 2.3b. As a result, crystals can grow with less incorporated impurities, substantial lower mosaicity and increased volume in comparison to their 1 g controls.

As mentioned before, already the first space experiment, performed by Littke and John in 1984 in the frame of the German TEXUS program (Littke and John 1986), confirmed that microgravity is an attractive environment to produce protein crystals with improved quality. In consequence, since early days of microgravity research growth of protein crystals and other biomolecules in microgravity is a distinct topic in international microgravity research activities. Different hardware and procedures were established to perform crystallization experiments on unmanned satellites, on space shuttle missions and space stations such as MIR and ISS. From early experiments till now considerable and continuous progress was made constructing and adapting crystallization hardware for microgravity experiments, considering the special environment of the spacecraft selected for the experiments. In principle, all crystallization methods mentioned before and shown in Fig. 2.2, applied for lab experiments for protein crystal growth, were adapted to individual space experiments. The following table is showing a summary of hardware most frequently used for protein crystallization experiments (Table 2.1).

The publication by Littke and John (1984) was well recognized by scientists working in the field of X-ray crystallography, followed by emerging new and further concepts to perform protein crystal growth in space. All concepts and following experiments were connected with the expectations that improved protein crystal growth in space will boost X-ray crystallography and structural biology. At the early time of space experiments, it was even considered that protein crystals can be obtained from sample suspensions, which even do not crystallize on Earth, due to the reduction before mentioned unfortunate convection, supporting smooth nucleation and further crystal growth. However already in the middle 1980s all crystallization experiments in space followed a same experimental plan, first the protein depended crystallization procedure was adapted to the flight hardware in the laboratories of the investigators. The final protein crystallization protocol was used to prepare and fill the flight hardware at the lab of the principle investigator, or at the launch site. In parallel the same number and a 1:1 replicate of the crystallization experiment was prepared for ground control experiments. Upon return all experiments and crystals obtained were first visually inspected and analyzed comparative to the ground control experiments. In a next step the samples were transported back to the home lab of the investigator for follow up comparative diffraction data collection and all steps of structure solution and refinement.

Protein crystallization experiments under microgravity conditions have resulted till now in more than 100 examples showing clear improvements in crystal quality via X-ray diffraction analysis, however also a number of experiments failed or did not show differences in space and ground control grown crystals. The majority of the crystal improvements resulted in improved resolution of the three-dimensional protein structures determined by X-ray crystallography.

Table 2.1 Protein crystallization hardware applied for microgravity crystallization experiments

Crystallization hardware	Crystallization method	Mission Space Shuttle	Year	References
Protein crystallization facility	Free interface diffusion/ liquid-liquid diffusion	TEXUS rocket	1984	Littke and John (1984)
VDA, Vapor Diffusion Apparatus	Vapor diffusion	STS-51D, STS-51F, STS-61B, STS-61C	1985–1986	Gonzalez-Ramirez et al. (2008), DeLucas et al. (1986)
CRYOSTAT	Liquid-liquid diffusion, free interface diffusion	STS-42	1992	Day and McPherson (1992)
PCF (protein crystallization facility)	Vapor diffusion	STS-37, STS-60	1991–1994	Gonzalez-Ramirez et al. (2008)
HH-DTC (Hand-Held Diffusion Test Cells)	Liquid-Liquid diffusion	STS-94	1997	Gonzalez-Ramirez et al. (2008)
APCF (Advanced protein crystallization facility)	Dialysis, vapor diffusion (hanging drop), liquid-liquid diffusion (free interface diffusion)	STS-95, ISS Mission 7A.1, STS 105, returned on Mission UF-1, STS 108	1993 onwards	Bosch et al. (1992) https://www.nasa.gov/centers/marshall/news/background/facts/apcf.html
PCAM (Protein crystallization apparatus for microgravity)	Vapor diffusion (sitting drop)	STS-62, STS-67	1994–1997	Gonzalez-Ramirez et al. (2008)
GN 2 (Gaseous Nitrogen-dewar)	Liquid-liquid diffusion	ISS Mission STS-110/8A, STS-111/UF-2, STS-71, STS-74, STS-76, STS-79, STS-81, STS-84, STS-89	1995–2002	Gonzalez-Ramirez et al. (2008)
DCAM (Diffusion-controlled crystallization apparatus for microgravity)	Dialysis	STS-76, STS-79, STS-81, STS-84, STS 89, STS-107, ISS, MIR	1996	Carter et al. (1999)

(continued)

Table 2.1 (continued)

Crystallization hardware	Crystallization method	Mission Space Shuttle	Year	References
CAPE (Canadian Protein Crystallization Experiment)	Liquid-liquid diffusion	Mir-24/NASA-6	1997	Schlagheck and Trach (2003)
GCF (Granada crystallization facility)	Counter diffusion	ISS, STS-105, STS-111, STS-113, STS-115	From 2001	Zegers et al. (2006)
HDPCG (High-Density Protein Crystal Growth)	Vapor diffusion	ISS Mission 8A, STS-100, STS-110, STS-111	From 2001	Rahman et al. (2015)
Modul-1	Free interface diffusion, liquid-liquid interface	ISS	From 2005	Smirnova et al. (2009)
SCDF (Solution crystallization diagnostic facility)	Batch, dialysis	ISS	From 2007	Pletser et al. (2006, 2008)
GCF-2 (Granada crystallization facility)	Counter diffusion	ESA-FOTON M-3 capsule	from 2007	Gonzalez-Ramirez et al. (2008)
PCDF (Protein crystallization diagnostics facility)	Batch, dialysis	ISS Mission 1E, STS-122	From 2008	Joannes et al. (2004), Pletser et al. (2009)
SCOF (Solution Crystallization Observation Facility)	Liquid diffusion	JAXA, ISS, STS-123/1J/A	From 2008	Yoshizaki et al. (2013)
DLR, SIMBOX	Counter diffusion	Chinese space mission Shenzhou-8	2011	Drebes et al. (2016)

As these examples demonstrate, the crystallization of organic macromolecules in microgravity is yet another example of how, in research, patience will usually pay off in the end. According to a review published in 2001 (Kundrot et al. 2001), experiments under microgravity have produced better crystals in about 20% of cases worldwide. If we leave out the substances that have been in space only once, the success rate rises to about 35%. If we include only those macromolecules that have been investigated in space more than four times, the success rate jumps to more than 60%. This again demonstrates that research needs patience and some staying power (Fig. 2.4).

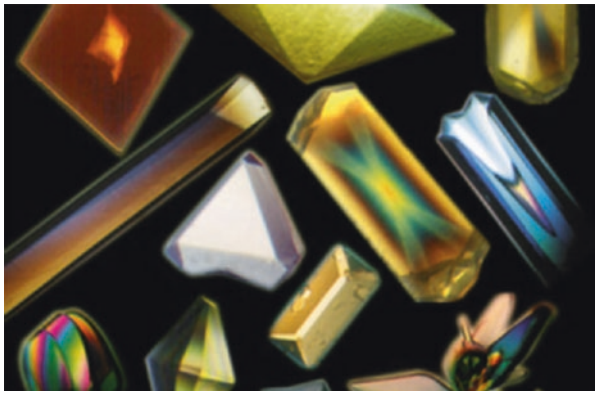

Fig. 2.4 Artist view of a collection of space-grown protein crystals (photo: NASA/Prof. McPherson, modified)

2.3 Early Successes of Structure Elucidation as Obtained in the German Space Life Sciences Program

2.3.1 The Structure and Function of Photo System I

Green plants and algae use photosynthesis to generate their own energy. With the help of sunlight, they convert carbon dioxide and water into sugar and oxygen. The basic chemical equation is very simple while the actual mechanism is extremely complex and has not yet been fully understood. Two major protein cofactor complexes, called photo systems I and II, are of major importance to the process as they ensure that the biological energy conversion process runs extremely effectively—with an energy yield amounting to almost 100%. For comparison: modern photovoltaic cells do not even reach 20%. The multisubunit PSI protein complex with its cofactors and pigments such as chlorophylls and carotenoids uses the energy of sunlight to convert carbon dioxide into oxygen and carbon in the form of carbohydrates, lipids, proteins and nucleic acids as the building blocks of life. PSII—likewise a multisubunit complex with various cofactors—uses the light energy to split water into hydrogen and oxygen for respiration.

It is these two photo systems whose structure, function and dynamics researchers worldwide are eager to work out and to understand in detail. Here again, microgravity is doing its bit. A first breakthrough for photo system I was achieved by scientists from Berlin's Technical University during the USML-2 shuttle mission in 1995. Crystals were grown on this mission whose volume was 20 times that ever reached for crystals grown on Earth. Based on these crystals an improved structural model of photo system I was developed at 4 Ångström (or 10^{-4} µm). This model rendered important functional parts of this large complex visible for the first time (Krauß et al. 1996).

Further experiments conducted during the 1998 STS-95 shuttle mission showed the nucleation rate to be significantly lower in weightlessness, leading to the formation of

larger, almost perfect crystals. Overall, crystals grown under microgravity conditions featured a significantly better resolution and fewer defects than comparable crystals grown on the ground (Klukas et al. 1999a, b).

2.3.2 The Crystallization of Archaea Surface Proteins

So-called S-layers are probably the evolution's first cell wall structures that came into being some three billion years ago. They consist of certain crystalline proteins on the surface of archaebacteria. The name of these organisms is due to the fact that they occur in hostile habitats similar to that of the early Earth. Thanks to their S-layer they have an enormous resistance to heat, extreme pH values and high salt concentrations.

During shuttle flight STS-95 in October 1998, a team of scientists from the universities of Ulm and Mainz together with their Belgian colleagues succeeded for the first time in growing crystals of the S-layer glycoprotein of one of these archaebacteria, *Methanothermus fervidus* (Evrard et al. 1999). Following their return to Earth, the crystals were examined by X-ray crystallography. At a resolution of 3 Ångström it became possible to clearly identify some of the crystals' parameters including their lattice constants and space group (Claus et al. 2002). In a series of experiments conducted on the ISS between June and October 2002, researchers were able to grow S-layer crystals of another organism, *Bacillus sphaericus*, at a resolution of 1.9 Ångström.

These experiments have been a great step forward towards understanding the structure of the S-layer. It is hoped that they will open up new insights into the survival strategies of these organisms and the underlying molecular mechanisms. This knowledge is not only important for science but might also open up options for new applications. Actually, the improved knowledge of S-layer structures has already found its way into the development of ultra-filtration membranes and other molecular nanotech applications.

2.3.3 Bacteriorhodopsin: A Promising Compound for Biotechnological Applications

Bacteriorhodopsin is the major photosynthetic protein of archaea such as *Halobacterium salinarium*. It converts the energy of green light—wavelengths between 500 and 650 nm—into an electrochemical protein gradient, which in turn is used for ATP production by the enzyme ATP synthase. It functions as a light-driven proton pump, transporting protons out of the cell. Bacteriorhodopsin has come into focus of much interest roughly 30 years ago, since its reversible light-triggered color change has allowed to develop biotechnological applications e.g. in

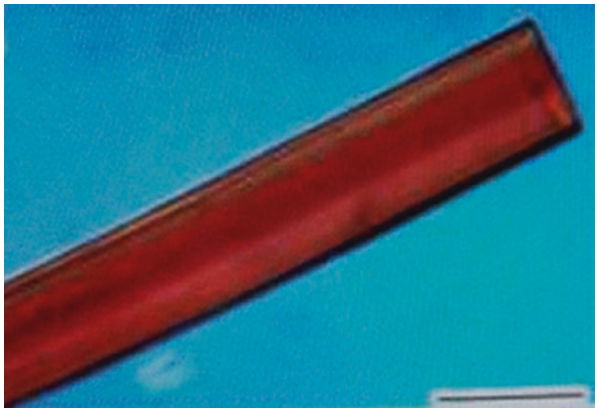

Fig. 2.5 Bacteriorhodopsin crystal grown during shuttle mission STS-95 (Zörb et al. 2002)

optical information recording (for an early review see Oesterhelt et al. 1991; Hampp 2000). Therefore, it is not surprising that elucidation of the structure and function of bacteriorhodopsin has got much attention.

Also, attempts were made to increase the size and perfectness of bacteriorhodopsin crystals by experiments under microgravity conditions. Indeed, experiments during the space shuttle flight STS-95 and on the Russian space station MIR in the late nineties demonstrated that large needles of the molecule could be grown (Fig. 2.5) that were not only larger in size, but also more homogenous and thus superior to those of the parallel ground controls (Zörb et al. 2002). Unfortunately, no further experiments were performed due to the retirement of the scientists, so that the final goal to achieve crystals with dimensions of 1 cm by 1 cm by 1 cm—desirable for successful application on ground—could not be achieved.

2.3.4 Mistletoe Lectin as an Agent in Immune Stimulation and Cancer Treatment

Bare trees bearing strange globular objects are a common sight along the railway lines as you travel from Cologne to Paris on a winter's day. The globular shapes are mistletoe (*Viscum album*), an evergreen plant living in symbiosis on trees (Fig. 2.6). For many centuries people have used it as a medicinal plant. We now know that the main component in mistletoe extracts, frequently used to strengthen the human immune system and in cancer treatment, is a compound called mistletoe lectin-I. Its precise mode of action is still to a large extent unexplained. A closer investigation of its three-dimensional structure is hoped to provide clarification. For some years now, this question has been Professor Betzel's area of research at Hamburg University (see Chaps. 3 and 4). Experiments carried out on the ISS in 2001, 2002 and 2006 provided crystals that were suitable for an improved structural analysis (Krauspenhaar et al. 2002; Meyer et al. 2008). Thus, it became possible for the first

Fig. 2.6 Mistletoe in poplar trees

time to explain what goes on in their active centers. The ribosome blocking protein mistletoe lectin-I (ML-I) is built from two separate protein chains, called A and B. Scientists today assume that subunit B is able to recognize certain sugar molecules on the membrane of the cell to be attacked, and thus helps subunit A to penetrate into that cell. This process then inhibits the cell's ribosome activity and ultimately leads to the death of what could be a cancer cell.

ISS-borne experiments also gave a clear indication that a wide variety of galactose and lactose sugar chains can adhere to the high-molecular protein complex (Mikeska et al. 2005). This also explains why crystals grown under regular laboratory conditions are of only moderate quality, limiting the possibility of an exact structural/functional analysis. Thanks to the space-borne experiments and accompanying investigations the necessary groundwork has now been done to improve the pharmaceutical application of this protein.

These results are a good example of how basic medical research can benefit from a detailed investigation of the structure of organic macromolecules and from understanding important molecular phenomena. The knowledge gained can be put to subsequent use in the development of specific inhibitors or in optimizing molecules which can then be fabricated in large quantities using methods of modern molecular biology.

2.3.5 Mirror-Image RNA Molecules

Ribonucleic acid, or RNA for short, provides the link between the DNA's genetic information and the proteins assembled by ribosomes as a result. Professor Erdmann and his team at Berlin Free University (FU Berlin) have focused research activities for many years on investigating the structure and crystallization of RNAs as well as their protein complexes. The starting point of their investigation was the ribosomal 5S rRNA, an important component of the ribosomes at which protein synthesis takes place. Crystals produced on shuttle flight STS-95 and on a 4 months' worth of experiments on board the ISS in 2001 have resulted in a detailed structure of domain B of the 5S rRNA (Vallaza et al. 2002; see Fig. 2.7). For the first time a diffraction pattern of the 5S rRNA/L18 protein complex was obtained.

Further experiments conducted in cooperation with the pharmaceutical company NOXXON AG on the ISS in 2002 led to the first successful crystallization of mirror-image RNA (Vallaza et al. 2004). The advantage of mirror-image nucleic acid compared to 'natural' RNA molecules lies in their long life in the human blood. This makes them particularly suitable for an effective treatment of tumors or viral infections such as AIDS. In addition, thanks to their great stability they can be chemically synthesized in large quantities and high levels of purity.

However, in order to understand the function of these molecules it is mandatory to understand their structure. One main focus of the ISS experiments was analyzing the interaction between nucleic acids and water molecules. The exact array of water molecules within the helix and its surroundings is important for the maintenance of the spatial structure of RNA. The model of 5S rRNA obtained as a result of these experiments opens up new insights into the interaction between antibiotics and ribosomal RNAs, thus permitting the development of more effective drugs.

Further experiments were conducted on the ISS in late 2008 and in October 2009. Researchers are hoping for further structural details of these important molecules. The ultimate expectation of the structural analysis of RNA samples was to provide a better understanding of ribosomal functions, hoping that the results will find their way into molecular medicine. This is where we reach a full circle: more than 20 years ago Professor Erdmann together with Professor Ada Yonath and Professor Heinz-Günter Wittmann laid the groundwork for the investigation of ribosomal structures.

2.4 Perspectives for Protein Crystallization in Space

What does the future hold in store? It is obvious that, as things have been going so well, the crystallization of organic macromolecules under microgravity conditions will continue to be part of Germany's space program, and those of space agencies worldwide, too. It would be wrong, though, to count on easy answers and to place excessive expectations in the benefit of weightlessness, as people did in the

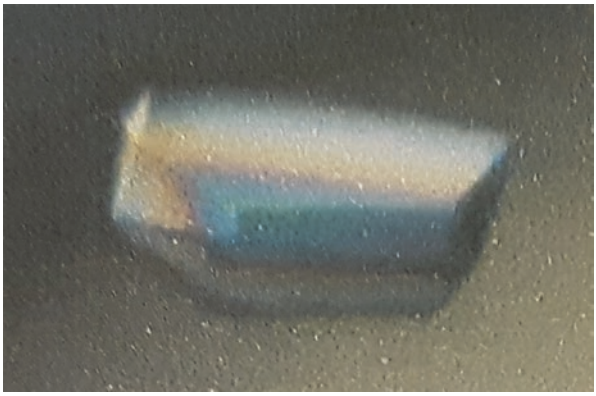

Fig. 2.7 5S RNA crystal grown during IML-2 shuttle mission STS-65 applying the APCF hardware (Förster et al. 2011)

program's early days. After all, gravity is only one of about 20 factors influencing the crystallization process. Yet, as part of a combined effort with Earth-based research, experiments in space will continue to deliver the desired progress. Also, as NOXXON GmbH and RiNA GmbH—both founded in Berlin in the context of the before mentioned biotechnology and structural biology investigations—have demonstrated, we can expect further start-up companies to be successfully placed in the market in the area of drug discovery and design.

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