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Review

Microgravity and Macromolecular Crystallography

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ABSTRACT: Macromolecular crystal growth is seen as an ideal experiment to make use of the reduced acceleration environment provided by an orbiting spacecraft. The experiments are small, are simply operated, and have a high potential scientific and economic impact. In this review we examine the theoretical reasons why microgravity is a beneficial environment for crystal growth and survey the history of experiments on the Space Shuttle Orbiter, on unmanned spacecraft, and on the Mir space station. The results of microgravity crystal growth are considerable when one realizes that the comparisons are always between few microgravity-based experiments and a large number of earth-based experiments. Finally, we outline the direction for optimizing the future use of orbiting platforms.

1. Introduction

Macromolecular crystallography is a multidisciplinary science involving the crystallization of a macromolecule or complex of macromolecules, followed by X-ray or neutron diffraction to determine the three-dimensional structure. The structure provides a basis for understanding function and enables the development of new macromolecules (e.g., more efficient industrial enzymes), and structure-guided design of drugs, insecticides, and herbicides. Developments in accelerator technology, detectors, computing and cryogenic techniques have greatly accelerated the determination of new structures, but the production of a crystal of sufficient diffraction quality is often the rate-limiting step.

The crystallization of biological macromolecules is an empirical science of rational trial and error guided by previous results. It requires decreasing the solubility of the macromolecule until it no longer remains in solution and forms an ordered crystal rather than a precipitate. Typically this is carried out at temperatures between 4 and 40 °C using precipitating agents such as salts, polymers, or organic compounds. Three methods are commonly used: vapor diffusion, liquid−liquid diffusion, sometimes across a dialysis membrane, and batch with the precipitant and sample solutions premixed. A summary of macromolecular crystallization methods is presented elsewhere.[1] The term "protein crystal growth" is often historically used to describe these experiments. This is somewhat inaccurate, as the field involves the study of many varied biological molecules, including viruses, proteins, DNA, RNA, and complexes of those molecules. In this review, the terms protein or macromolecule are used to refer to this entire range.

The reduced acceleration environment of an orbiting spacecraft has been posited as an ideal environment for biological crystal growth, since buoyancy-driven convection and sedimentation are greatly reduced. The experiments are also small (an ideal crystal for X-ray diffraction having dimensions on the order of 100 μm) and require simple equipment and minimal intervention—the perfect combination for a microgravity experiment. Macromolecular crystal growth experiments were first flown in 1981. The protein β-galactosidase was grown in microgravity using a sounding rocket. Schlieren optics imaged with a cine camera showed a strictly laminar diffusion process in contrast to turbulent convection seen on the ground.[2] This experiment provided the fundamental basis for subsequent, longer-duration experiments. Gravity is a pervasive force in terrestrial procedures, and much of what has been learned about microgravity experiments has been learned in a trial-and-error fashion that, in hindsight, sometimes seems obvious. Herein, we review the use of microgravity as a crystal growth environment for macromolecules and the practical lessons learned. Much of this material was presented in a recent National Research Council review of NASA's biotechnology program.[3−7] The future use of microgravity for biological crystal growth is also addressed.

2. Microgravity

The term microgravity is used in both colloquial and scientific senses. In the colloquial sense it means an

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acceleration level much less than unit gravity (9.8 m s^{-2}). In the strict scientific sense it means on the order of $10^{-6}g$ (μg). In a looser scientific sense it stands in contrast to milligravity ($10^{-3}g$) and nanogravity ($10^{-9}g$). The colloquial sense is used in this review.

Microgravity is achieved in an orbiting spacecraft because the inertial forces go to zero in free fall. Residual accelerations arise from several sources and can be characterized as quasi-steady, oscillatory, or transient. Quasi-steady accelerations (frequency less than 0.01 Hz) result from atmospheric drag, venting of air or water, and the "gravity gradient" across the spacecraft. They are typically of low magnitude (1 μg or less). The amount of atmospheric drag depends on the attitude of the orbiting vehicle, i.e., a Space Shuttle Orbiter flying nose-first has less drag than an Orbiter flying belly first. Atmospheric drag exerts an acceleration of $\sim 5\mu g$ at an altitude of 250 km for the Orbiter.[8] The orbital drag for the International Space Station is expected to be less than $1\mu g$. The term gravity gradient refers to the forces that arise due to the tendency of different parts of the vehicle to follow different orbital trajectories. To a first approximation, the orbiting vehicle falls as one object and there are no inertial forces between different parts of the vehicle. To a second approximation, only those parts of the vehicle that lie on the orbital trajectory of the vehicle's center of mass are free from inertial forces. Parts of the vehicle not on this trajectory experience a residual inertial force because their orbital trajectory is not the same as the center of mass. A position above the center of mass has a higher orbital radius and slower velocity relative to the center of mass; therefore, an inertial force is required to keep it in the same position relative to the center of mass. The orbital trajectory for a position at the same altitude as the center of mass but not on the orbital trajectory (i.e., "on the side" of the center of mass) follows a different great circle around the Earth than does the center of mass. Restoring forces are provided by the vehicle structure on these points that maintain the vehicle integrity as it orbits. Gravity gradient forces produce accelerations of about $0.1-0.3\mu g$ per meter of displacement from the orbital trajectory of the center of mass. Oscillatory accelerations result from on-board activities such as crew exercise, the operation of experimental and life support equipment, and harmonic structural vibrations of the spacecraft itself. They are characterized by higher frequency ($0.01 < f < 300$ Hz) and larger magnitude ($10-1000\mu g$) and are also referred to as *g*-jitter. Transient accelerations are characterized by the firing of thrusters, docking of spacecraft, closing and opening of hatches and panels, or the startup of equipment. They are random, short in duration, exhibit a broad band of frequencies, and can have peak values as high as $10\,000\mu g$.

3. Why Microgravity for Crystallization?

How can microgravity affect macromolecular crystal growth? Microgravity is not expected to directly affect the molecular interactions required for the formation and growth of crystals. Empirical observations, however, indicate that nucleation times are significantly longer and the overall growth rates slower than on Earth.[9]

The standard model for understanding the effects of microgravity on macromolecular crystal growth is based on the concept of a depletion zone. In zero gravity, a crystal is subject to Brownian motion as on the ground, but unlike the ground case, there is no acceleration inducing it to sediment. A growing crystal in zero gravity will move very little with respect to the surrounding fluid. Moreover, as growth units leave solution and are added to the crystal, a region of solution depleted in protein is formed. Usually this solution has a lower density than the bulk solution and will convect upward in a 1*g* field as seen by Schlerien photography (Figure 1).[10] In zero gravity, the bouyant force is eliminated and no bouyancy-driven convection occurs. Because the positions of the crystal and its depletion zone are stable, the crystal can grow under conditions where its growing surface is in contact with a solution that is only slightly supersaturated. In contrast, the sedimentation and convection that occur under 1*g* place the growing crystal surface in contact with bulk solution that is typically several times supersaturated. Lower supersaturation at the growing crystal surface allows more high-energy misincorporated growth units to disassociate from the crystal before becoming trapped in the crystal by the addition of other growth units. Since microgravity is not zero gravity, the bouyant convection and sedimentation are severely attenuated rather than eliminated. In short, promotion of a stable depletion zone in microgravity is postulated to provide a better ordered crystal lattice and benefit the crystal growth process.

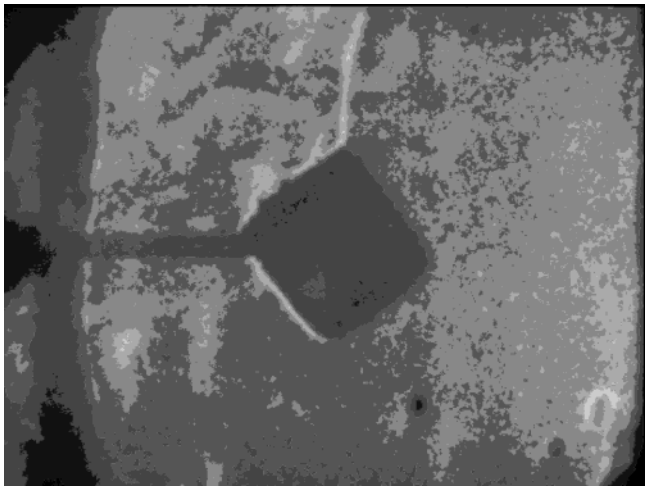

Figure 1. Schlieren photograph of a growth plume arising from a tetragonal lysozyme crystal.[10] The crystal is ca. 1.5 mm in size and was grown on the glass rod used to position it in the observation chamber. Conditions for growth were pH 4.0, 0.1 M sodium acetate, and 5% NaCl at 18 °C.

Model calculations and some limited data suggest that accelerations greater than $1\mu g$ will perturb macromolecular crystallization. A summary of flow effects on macromolecular crystal growth in microgravity is presented elsewhere.[11] Ramachandran et al.[12] determined that the classical solution to the vertically heated flat plate could be used to describe the velocity and mass transport in the vicinity of a macromolecular crystal. This gave a Sherwood number (the ratio of total mass flux transport to that under diffusive conditions) for biological crystal growth conditions of 1.0 at $10\mu g$; i.e., at this level the mass transport is diffusion-limited. A quasi-steady acceleration environment of $10\mu g$ or less is therefore optimal for biological crystal growth. For

an inorganic crystal simulation, Nadarajah et al.[13] report a complex interaction between the magnitude and frequency of long-term (greater than 100 s duration) vibrations and their effect upon the crystal growth. Low-frequency vibrations lead to intolerable variation in growth rates at $10^3\mu g$ and $100\mu g$. The impact decreases as the magnitude of the acceleration decreases. At $\leq 100\mu g$ low frequencies have no significant effect. If the same pattern applies in biological crystal growth systems, then low-frequency vibrations are to be avoided unless their magnitude can be reduced by vibration isolation. Initial evidence in which CCD video data were used to correlate microgravity crystal growth with measured acceleration data[14] indicates that this may be the case. Accelerations associated with crew exercise ($40\mu g$ at 2.1−2.5 Hz) correlated directly with variations observed in the crystal growth rates, while higher magnitude vibrations at higher frequencies had no apparent effect.

Transient accelerations usually result in a bulk sedimentation or movement of the crystals within the growth cell. During the Spacehab-1 mission lysozyme protein crystals were observed to suddenly move.[15] This motion was related to the retrieval of the EURECA satellite. The measured acceleration was in excess of $1300\mu g$: i.e. milli-g. From Stokes theory,[16] the settling velocity is proportional to the square of the particle diameter; therefore, larger crystals will move further than smaller crystals in response to transient accelerations.

3.1. Micro Effects: Nucleation. The initial processes in macromolecular crystal growth involve solute−solvent/precipitant interactions. For microgravity to have a direct effect implies that it significantly affects the bond energies and assumes that gravitational forces at the molecular scale are comparable in magnitude to the intermolecular forces. If so, then other physical properties such as boiling and freezing points, etc., would be affected as well. This has not been observed to date.[17]

Crystal numbers and sizes can be used to examine the effect of microgravity upon crystal nucleation. The effect of microgravity upon nucleation behavior seems to be macromolecule- and hardware-specific. While some researchers report reduced crystal numbers in vapor diffusion experiments[18,19] and increased crystal numbers in batch experiments[20] in microgravity, Strong et al.[21] find increased or decreased numbers in individual experiments but no general trends overall with either technique. In dialysis and liquid−liquid interfacial experiments both Ng et al.[22] and Strong et al.[21] find consistent and significantly (up to 10 times) fewer crystals in microgravity, while Trakhanov et al.[23] reported nucleation in microgravity where none was reported on the ground. Ries-Kautt et al.[24] also report that although the number of crystals varied according to the method of crystallization, no significant difference was found in the number of crystals in ground-based and microgravity experiments. For catalase[23] and DATase[21] there is also no apparent change in crystal number in microgravity. In terms of nucleation kinetics, De Lucas et al.[9] state that in general macromolecules take longer to nucleate in microgravity than they do under the same conditions on the ground.

Secondary nucleation is the formation of nuclei in solutions that already contain growing crystals. In a $1g$ field and a crystal of size $\sim 10-100\ \mu$m, buoyancy-driven flows develop which not only maintain a high growth rate but may also produce increased secondary nucleation.[25−28] Secondary nucleation is thought to be caused by the removal of partially solvated clusters from near the surface of the crystal (the absorbed layer) by this flow.[29] In microgravity the reduction of buoyancy-driven flows is expected to reduce this effect, as partially solvated clusters are more likely to attach to the growing crystal than be swept out into the bulk to become new nucleation sites.[29] To date, however, there is insufficient experimental evidence to indicate the effect of microgravity on secondary nucleation.

3.2. Macro Effects: Sedimentation and Marangoni Convection. Sedimentation. As nuclei grow, they eventually reach a size where their movement in solution is dominated by sedimentation rather than Brownian motion. For macromolecular crystals, this transition generally occurs around the 1 μm size. The crystal then moves through the solution in the direction of the gravitational force acting on it. The settling velocity as given by Stokes' law is a function of the square of the particle size.[16] After moving through solution, the crystal settles on the bottom of the solution (which may be a vessel wall or an air−solution interface), where it continues to grow. Growth against a vessel wall may prevent one or more of the crystal faces from growing, particularly those adjacent to the wall, and may distort the final crystal shape. Crystals that stay suspended in solution, as is often the case in microgravity, grow free of such boundary restrictions and exhibit a more uniform shape. In microgravity sedimentation is much reduced, but sudden movements can occur due to transient accelerations.

Marangoni Convection. Marangoni convection[30] is the flow of liquid from regions of low to regions of high surface tension. An analysis of the effect of microgravity on Marangoni convection is given by Molenkamp.[31] Marangoni convection can result from concentration and/or temperature gradients along an interface. In macromolecular crystal growth it affects the vapor diffusion and liquid−liquid diffusion crystal growth methods but is generally masked by gravitational effects on the ground.

Marangoni convection can occur in two different forms:[32,33] microconvection and macroconvection. Microconvection is initiated by small random temperature or concentration disturbances that grow in time. Macroconvection originates from concentration or temperature differences due to asymmetry in the system. Macroconvection, through asymmetry, can occur in a vapor diffusion system when the distance between the precipitant reservoir and the drop is not the same everywhere. As solvent leaves the drop, regions of the surface may differ in surface tension due to concentration differences. Surface tension may also vary due to an applied temperature gradient or heat loss due to evaporation, causing thermal fluctuations on the surface. The magnitude of the variation is a function of the dependence of the surface tension on concentration and temperature for the given system. Salts, commonly used as precipitating agents, usually increase surface tension.

Macromolecules decrease surface tension with increasing concentration. Even when no mass transfer occurs between the liquid and gas phases, the formation of a depletion zone around the growing crystal can lead to Marangoni convection. If the depletion zone extends to the interface, then a concentration gradient will exist at the interface. The resulting convection causes higher protein concentration solution (low surface tension) to be drawn toward lower protein concentration solution (high surface tension).

4. Alternatives to Microgravity

Crystallization in gels can suppress fluid and crystal movement following nucleation. This results in growth rates more strictly being a function of the diffusion of solute to the interface, similar to that expected in microgravity. In general, however, there are specific interactions between the gel and the material being crystallized. The number of crystal nucleation sites may increase or decrease.[34]

The few comparisons made between gel-grown and microgravity-grown crystals suggest that gels do not confer the full extent of the benefits of microgravity. Miller et al.[35] report a study of vapor diffusion, gel liquid−liquid diffusion, and microgravity vapor diffusion grown human serum albumin crystals. X-ray analysis showed a marked increase in signal to noise over the entire resolution range for the gel in comparison to the ground grown crystal. The microgravity sample, however, displayed an increase in signal to noise over the gel-grown samples. Dong et al.[36] compared microgravity dialysis grown samples of lysozyme with gel-grown microbatch and dialysis growth on the ground. The resulting structures were very similar, but the most ordered waters were seen in the microgravity crystal and the fewest in the earth-grown.

5. How Have Previous Experiments Performed?

5.1. History. Littke conducted the first microgravity protein crystallization in April 1981 using Germany's Technologische Experimente unter Schwerelosigkeit (TEXUS) sounding rocket. The protein β-galactosidase was crystallized by liquid−liquid diffusion. In microgravity strictly laminar diffusion was observed, in contrast to turbulent convection on the ground. Several single crystals approximately 100 μm in length grew in the 6 min of microgravity. These crystals were of inferior but of comparable visual quality to those grown on the ground over several days.[2] This observation of laminar flow and the quick appearance of crystals spurred interest in the use of microgravity for crystallization experiments.

Table 1 illustrates the history of microgravity biological crystal growth experiments. A brief description of select experiments follows below. Emphasis is placed on NASA Shuttle-based experiments, i.e. flown on the Space Shuttle Orbiter, or experiments transported to the Mir space station by the Orbiter.

5.2. The Space Shuttle Orbiter. The Biological Crystal Growth program has flown samples on 43 flights through 1998 (STS-95). Only five of these flights were dedicated to maintaining a microgravity environment on the Orbiter. The remaining 38 flights had a different primary mission, typically launching a satellite. The NASA Space Shuttle program had its first mission, STS-1, in April 1981 with the first fully operational mission launched in November 1982.

The first Orbiter-based protein crystal growth experiment was STS-9, launched in November of 1983 (STS standing for Space Transportation System). It was a joint NASA−European Space Agency (ESA) science mission carrying Spacelab. The apparatus was based on the TEXUS hardware design but incorporated eight growth cells, four in a freezer and four in an incubator. Crystals of lysozyme and β-galactosidase were grown at 20 °C, and β-galactosidase was grown in a temperature gradient from −4 to 20 °C. This mission carried a three-axis accelerometer that revealed the microgravity environment associated with Orbiter operations. Of note[8] was the effect of the Orbital Maneuvering System (OMS) burns to maintain orbit, which result in accelerations greater than $10^{-2}g$.

The vapor diffusion method was used for the first time in microgravity on STS-51D.[37] The Vapor Diffusion Apparatus (VDA) had 17 growth chambers, each containing a single syringe that extruded a drop of protein solution into a closed chamber containing a wicking material saturated with precipitant solution. Two VDA's were flown, and many drops were lost during activation (extruding the drop onto the tip of the syringe) or deactivation (solution withdrawal into the syringe). The mission involved higher levels of accelerations than anticipated,[38] as it involved chasing a satellite that failed to fire its booster. Iterative development and refinement of the VDA hardware took place on subsequent flights. For the STS-61B mission the VDA flew with 48 individual growth cells, and crystals of C-reactive protein, bacterial purine nucleoside phosphorylase, and lysozyme were produced. Diffraction analysis of the samples indicated they were as good as the best ground-grown crystals.[38] These vapor diffusion experiments were all carried out at the ambient temperature of the Orbiter.

An incubator was first used on flight STS-26. During the hiatus following the Challenger accident a temperature control unit, the Refrigeration Incubator Module (RIM), was developed. The RIM was capable of maintaining a set temperature between 2 and 35 ± 0.8 °C. The VDA was further modified for STS-26 to include two syringes, one with protein solution and the other with precipitant. STS-26 carried 48 VDA and 12 dialysis cells with 7 different experiments. The Orbiter cooling system suffered problems, and the ambient temperature was ∼30 °C for most of the 4 days mission, reinforcing the need of active temperature control for experiments.

Large-scale temperature-based protein crystallization was first performed on STS-37. The Protein Crystallization Facility (PCF)[39,40] consisted of four cylinders containing 20−500 mL of solution each, over which a temperature gradient could be established. Activation by temperature ramping occurred on the first day of the mission. This mission flew bovine insulin as the crystallizing protein. Over 7000 crystals from the three missions were measured optically and compared with ground-control samples. Microgravity-grown crystals were up to 10 times longer than the ground-grown crystals. For the microgravity samples a direct relationship between the crystal size and PCF container volume was found. This was not the case for the ground controls.

Table 1. Table of All US Flights with Biological Crystal Growth Experiments Onboard or of Interest to the Crystallographic Community

vehicle	date	apparatus	no. of cells	no. of species	no. of new	comments
TEXUS-3	Jan 1981		2	1	1	sounding rocket
TEXUS-4	Jan 1981		1	1		
TEXUS-5	Jan 1982					
STS-9	Nov 1983		8	2	1	
STS-41B	Feb 1984		3	3	3	failed to activate
STS-51D	Apr 1985	VDA	34	13	12	hand held
STS-51F	Jul 1985	VDA	34	3	1	Spacelab-2
STS-61A	Oct 1985		14	3	1	German spacelab
STS-61B	Nov 1985	VDA	48	5	3	
		dialysis	6			
STS-61C	Jan 1986	VDA	48	6	1	
		dialysis	10			
Photon	Apr 1988		21			USSR satellite
COSIMA-1	Aug 1988		101			China launch
STS-26	Sep 1988	VDA	60	13	7	
STS-29	Mar 1989	VDA	60	16	10	
		dialysis	12			
MASER	Apr 1989		4	2		sounding rocket
COSIMA-2	Sep 1989		101			USSR launch
STS-32	Jul 1990	VDA	140		4	60 @ 40 °C, 60 @ 22 °C, 20 @ ambient
STS-31	Apr 1990	VDA	60	22	5	Hubble deployment
STS-37	Apr 1991	PCF	4	1	1	
STS-43	Sep 1991	PCF	4	1	0	
STS-48	Sep 1991	VDA	60	11	3	22 °C
Photon-3	Oct 1991	KASHTAN	9	1		
STS-42	Jan 1992	VDA	120	14	7	IML-1 mission
		cryostat	4			
STS-49	May 1992	PCF	4	1	0	
STS-50	Jun 1992	VDA	120	40	16	L. DeLucas was
		glovebox				mission specialist
STS-46	Jul 1992	EURECA	12	1	2	long-duration free flyer
STS-47	Sep 1992	VDA	60	5	4	Spacelab-J
		Japanese		6		
STS-52	Oct 1992	PCF	4	1	0	
STS-56	Apr 1993	MDA/ITA		9	0	
STS-55	Apr 1993	cryostat	14	2		Spacelab D2
	Jun 1993	APCF	48	6	3	EURECA retrieval
STS-57		VDA	60			
		PCF	4			
		COS	6			
STS-51	Sep 1993	PCF	4	1	0	
STS-60	Feb 1994	PCF	4	1	0	
		PCFLS				
STS-62	Mar 1994	VDA	80	13	8	PCAM hand held test unit
		PCAM	24			
STS-65	Jul 1994	VDA	60	10	5	
		APCF	96			
STS-68	Sep 1994	VDA	60	1		
STS-66	Nov 1994	VDA	60	9	4	
		COS				
	Feb 1995	VDA	60	25	14	
		PCF	4			
STS-63		PCFLS	4			
		HHDTC	16			
		PCAM	378			
	Mar 1995	MDA		15	3	
STS-67		VDA	80			
		PCAM	378			
STS-71	Jun 1995	Dewar	181	17	8	Orbiter carried expt to Mir
STS-70	Jul 1995	PCF	4	1		
STS-69	Sep 1995	MDA				
		GBA				
	Oct 1995	glovebox		25	14	
		PCAM	756			
		HDTC	16			
STS-73		PCF	4			
		APCF	96			
		DCAM	81			
		VDA	60			
STS-74	Nov 1995	Dewar	166	16	1	Orbiter carried expt to Mir
	Jan 1996	Japan	16	6	3	
STS-72		VDA	80			
		PCF	4			
STS-75	Feb 1996	VDA	128		0	

Table 1. (Continued)

vehicle	date	apparatus	no. of cells	no. of species	no. of new	comments
STS-76	Mar 1996	Dewar	225	31	12	Orbiter carried expts to Mir
		DCAM	162			
	May 1996	HHDTC	32	10		
STS-77		VDA	128			
		PCF	4			
STS-78	June 1996	APCF	96	8	3	
	Sep 1996	Dewar	285	17	5	Orbiter carried expts to Mir
STS-79		VDA	128			
		DCAM	162			
STS-80	Oct 1996	MDA				
STS-81	Jan 1997	DCAM	162	13		Orbiter carried expts to Mir
		Dewar	216			
	Apr 1997	PCAM	630	25	14	mission cut short
STS-83		VDA	80			
		HHDTC	32			
STS-84	May 1997	DCAM	162	25	4	Orbiter carried expts to Mir
		Dewar	106			
		VDA	80			
	Jul 1997	PCAM	630	32	0	repeat of STS-83
STS-94		VDA	80			IPCG deployed on Mir
		HHDTC	32			
STS-85	Aug 1997	PCAM	630	14	4	
STS-86	Sep 1997	IPCG	6			
		CAPE	700			
STS-89	Jan 1998	DCAM	162	25	5	IPCG retrieval
		Dewar	170			
STS-91	Jun 1998	VDA	128			Mir mission
STS-95	Oct 1998	FPA		32	13	
		PCAM	378			
		VDA	80			
		PCF	4			
		CVDA	118			

[a] Also included are a limited number of none US flights where data is available. These figures include only scientific samples. Commercial samples flown are unknown. The numbering system for shuttle flights has changed two times. Initially, shuttle flights were numbered consecutively, beginning with the number 1. Later, a new numbering system was introduced with the first number being the fiscal year, the second the launch site (1 for Kennedy, 2 for Vandenberg) and the letter the order of that launch in the year. The system changed again, resuming almost sequential numbering with STS-26.

STS-40 launched in June 1991 with the Spacelab Life Sciences Mission. Although it did not carry protein crystal growth experiments, it is notable because of the Space Acceleration Measurement System (SAMS) that characterized the acceleration environment on board the Orbiter.[41] During sleep periods acceleration magnitudes of $10^{-6}-10^{-5}g$ were measured with $10^{-4}g$ during crew activity. Vernier thruster firing (used to maintain altitude and attitude) caused acceleration shifts of $10^{-4}g$ and primary thruster firings caused accelerations as great as $10^{-2}g$. It is these primary thruster firings that are used for satellite deployments.

The first flight to have maintenance of a microgravity environmnent as its primary mission was the International Microgravity Laboratory (IML-1) on board STS-42. This mission carried both the German Cryostat hardware and VDA. Cryostat has two thermal enclosures, each with seven growth cells for liquid−liquid diffusion experiments. Satellite Tobacco Mosaic Virus grown in this resulted in a 1.8 Å structure.[42]

The first crystallization experiments conducted by a person mixing solutions in orbit was STS-50. It carried the VDA and a glovebox experiment, operated by mission specialist Dr. Larry DeLucas, enabling iterative techniques in protein crystal growth.[9] The glovebox was used to optimize crystallization conditions, seeding, crystal mounting, and real-time video transfer of data. Crystallizations were set up using 24 different proteins. Approximately 25% of them required 10−14 days of growth in ground-based experiments. In most cases the growth took even longer in microgravity. Of the remaining 75% more than half produced crystals for X-ray diffraction. Four proteins had flown that had not crystallized on previous missions, possibly due to inadequate mixing of the protein and precipitant PEG solution. In the case of malic enzyme[9] an X-ray data set was obtained which was improved from 3.2 Å resolution to 2.6 Å.

The Spacehab-1 mission (STS-57) retrieved the European Retrievable Carrier (EURECA) long-duration satellite launched almost a year earlier on STS-46 and flew ESA's Advanced Protein Crystallization Facility (APCF).[43,44] Each APCF contained 48 individual growth cells that could operate in a dialysis, liquid−liquid, or vapor diffusion geometry. The facility was temperature controlled to ± 0.1 °C and allowed CCD video observation of 12 of the experiments.[11,14,45] Crystal motion was still noticed in the growth cell, due to residual accelerations and a sudden motion during preparation for the EURECA retrieval. Lysozyme crystals grown on this mission were analyzed by polychromatic (Laue) and monochromatic X-rays.[46−49] Interestingly, the lysozyme crystals grown in microgravity displayed a marked reduction in mosaicity (the reflection rocking width with geometric and spectral effects deconvoluted is a measure of the physical crystal perfection independent of the X-ray source or detector used). This mission also carried the PCF facility growing human recombinant insulin complexed with phenol. The resulting crystals were larger than their earth-grown counterparts and dif-

fracted significantly better than the best grown by any method on the ground.[39,40]

Two APCF facilities flew on STS-65, the Second International Microgravity Laboratory (IML-2). Liquid−liquid diffusion was used to grow canavalin, satellite tobacco, satellite panicum, and turnip yellow mosaic viruses.[50] Comparisons to the microgravity samples were made with the best earth-grown ones available. Analysis of rhombohedral conavalin crystals extended the resolution from 2.6 to 2.3 Å, for hexagonal from 2.7 to 2.2 Å. Satellite tobacco mosaic virus data was extended from 6 to 4 Å. Diagnostic experiments were carried out on this mission, looking at lysozyme and apocrustacyanin both during growth and with subsequent X-ray analysis. For lysozyme a series of CCD video images were taken during growth. Motion of the crystals was observed,[11,14] implying a minimum residual acceleration of $\sim 20\mu g$. A single crystal nucleated on the glass wall of the growth cell, and its growth rate showed distinct spurts and lulls. Using measured acceleration data and crew activity logs, the growth spurts were correlated with crew exercise, approaching levels at low frequency of $1000\mu g$ acceleration. The lulls and spurts in the growth rate result from breaking down and reestablishing of the depletion zone by the presence and absence of acceleration. No direct evidence exists as to whether this is detrimental to the crystal quality, but the same experiment performed on STS-57 (Spacehab-1) and STS-65 produced less improvement in crystal mosacity than the STS-57 case.[48,49] The difference was due to the fact that STS-57 was a single-crewed mission with periods of low *g*-jitter during sleep, while the STS-65 mission was double-crewed. The STS-57 mission had a high acceleration event when it retrieved the EURECA satellite, but this was after crystals had grown. In both microgravity cases the crystals had smaller mosaicity than those grown on the ground. Apocrustacyanin-C_1, grown by vapor diffusion, was also observed by CCD video in the APCF. Three needle-shaped crystals appeared at about 46 h and were seen rapidly moving within the drop[11,45] at about 2.1 μm s^{-1}. The movement was in the direction and magnitude predicted by modeling the effects of Marangoni convection on vapor diffusion systems.[51] The improvement seen in these crystals was marginal compared to that of microgravity-grown lysozyme.[52]

Stoddard et al.[53] developed a new vapor diffusion device (VD) reproducing sitting-drop vapor diffusion crystallization techniques rather than the hanging-drop geometry mimicked by VDA. In the deactivated state a plunger depressed a silicon rubber septum, sealing the inner well containing the protein solution from the outer well containing a precipitant solution. For activation the plunger was lifted, allowing a vapor diffusion path between the inner and outer wells. This flew on Mir from December 1989 to February 1990 and is described later. The design was further developed[54] into the Protein Crystallization Apparatus for Microgravity (PCAM). This first flew as a hand held device on STS-62 and evolved into the current design that has flown on 7 Space Shuttle missions to date. PCAM has an experiment density of 378 growth cells in a single temperature-controlled locker or 504 cells without temperature control. Improved structural results from synchrotron data on bacteriophage lambda lysozyme[55,56] (STS-67), synchrotron data on human antihrombin III[57,58] (STS-67), and synchrotron data on pike parvalbumin[59] (11.4 kDa) (STS-94) are some of the systems for which PCAM has produced the highest diffracting crystals.

Ng et al.[22] grew thaumatin crystals in the APCF on STS-78 (Life and Microgravity Science mission-LMS) and STS-73 (United States Microgravity Laboratory-USML-2) by both dialysis and free interface diffusion. Microgravity growth displayed fewer and larger crystals in comparison to the ground controls. A dependence of the average and largest sizes proportional to protein concentration employed was noted. X-ray diffraction data showed the microgravity samples had a resolution of 1.5 Å, with significantly greater *I*/*s* over the entire data set, versus 1.7 Å for the ground. Analysis showed that the microgravity crystals had a mosaic spread of approximately 0.02° fwhm, half that of the ground samples. Otalora et al.[60] grew lysozyme crystals to study the use of long, thin capillary growth cells and high concentration growth. They also used a Mach−Zender interferometer installed in the APCF and CCD video observation for diagnostic work during growth. Maximum growth rates were observed slightly after nucleation with crystal movement seen during the mission. Synchrotron X-ray diffraction analysis gave on average 1.25 Å data with a single example giving 1.15 Å data. Another experiment on the LMS mission compared the quality of crystals of alcohol dehydrogenase grown in microgravity in APCF dialysis chambers with those grown in the APCF using vapor diffusion. The dialysis chambers contained few large (1.0 mm) crystals that diffracted to 1.8 Å, while the vapor diffusion chambers contained many small crystals (0.1 mm) that diffracted very poorly.[61] On the LMS mission sedimentation of lysozyme crystals at 40 Å s^{-1} and sudden jumps of 0.2 mm over 2 h periods (i.e., 0.03 μm/s or 300 Å s^{-1}) were observed.[11]

5.3. Unmanned Experiments. The first unmanned extended-duration, i.e., greater than 6 min, protein crystallization experiments were carried out on the Photon satellite mission, launched in April 1988. Trakhanov et al.[23] flew 5 proteins in a total of 21 liquid−liquid growth cells. A 30 S ribosomal subunit from *Thermous thermophilus* crystallized in microgravity but not on the ground, and catalase produced larger crystals in microgravity. However, experiments under optimal laboratory conditions, rather than ground control hardware, produced larger crystals. The other proteins did not produce crystals in microgravity or on the ground.

In 1988 China launched China 23, carrying CO-SIMA-1 (Crystallization of Organic Substances in Microgravity for Applied research).[62−64] The apparatus consisted of a flexible tube containing protein and salt solution separated by an air gap. The tube was clamped between the two and opened in microgravity, resulting in a vapor diffusion crystallization method. On re-entry the payload experienced a 13*g* force, culminating in a 60*g* jolt when the parachute opened. A total of 101 samples were flown of 7 different proteins. The microgravity crystals generally diffracted to equal or higher (5 out of 7 samples) resolution than the ground controls grown in the same apparatus and had a greater volume

(6 out of 7 samples).[62−64] Interestingly, crystal growth occurred under the same optimum conditions as on the ground, an observation contrary to many other experiments. Crystals grown under optimal conditions on the ground in laboratory apparatus were better than the microgravity or ground controls.

COSIMA-2 was launched from a Soviet Earth observation satellite in September 1989. Thermolysin and a lysozyme from *Streptomyces coelicolor* were crystallized.[65] The microgravity-grown crystals displayed much weaker diffraction than the ground control and laboratory-grown crystals. Asano et al.,[66] however, grew ribonuclease S crystals that diffracted to 2.2 Å, with the best ground-grown crystals (by any method) diffracting to only 3.0 Å.

Sweden's MASER (Material Science Experiment Rocket) was used in April 1989[20] to study the growth of bovine ribonuclease (RNase-A) and bovine pancreatic trypsin inhibitor (BPTI). The flight provided about 7 min at 100μg with an experiment consisting of four growth cells, three for RNase-A (one with microseeds) and one for BPTI (with microseeds). The solutions are mixed on activation, giving a batch growth method. Both proteins were chosen because they could be rapidly crystallized in the available time. The test cells were kept at 20 °C with simultaneous ground controls. The BPTI experiment resulted in mass crystallization of 0.2 mm crystals both on the ground and in flight. The flight RNase-A crystals were almost 3.0 mm^3 in volume, compared to 0.5 mm^3 for those grown on the ground. The flight samples yielded diffraction data beyond the instrument capability, 1 Å, unlike the Earth-grown samples that diffracted to only 1.26 Å, and provided new detail in the resulting electron density maps.[20]

The EURECA satellite launched from STS-46 in 1992 carried ESA's Protein Crystallization Facility (E-PCF), consisting of 12 individually temperature controlled liquid−liquid cells monitored by video.[43,67] Observation of α-crustacyanin showed that crystals nucleated and grew without any motion, unlike experiments on the Space Shuttle Orbiter.[11] They were finally disturbed by failure of a temperature control system (on Christmas day), which heated the cells to above 40 °C, destroying the experiment. The acceleration environment[68] never peaked above 62.5μg, and the crystals observed were larger and had a more defined morphology than any previously grown on the Earth.[69] The acceleration environment on board EURECA was probably the best out of all the macromolecular crystallization experiments flown in microgravity. The experiments were close to the satellite center of mass and experienced minimal gravity gradient effects, and there were no extraneous activities like satellite launches or crew activities to create oscillatory or transient accelerations.

5.4. The Mir Space Station. The first macromolecule crystallization experiments on Mir came in 1992, when a progress supply rocket carried up a vapor diffusion device (VD) described earlier.[53] Chicken egg white lysozyme and D-amino transferase were grown. The size and diffraction characteristics of the crystals were superior to those grown using identical hardware on the Earth. Using standard laboratory techniques to grow similar crystals on the Earth, the improvement was small but still measurable.[53] In August of 1994 a Progress vehicle brought the Space Acceleration Measurement System (SAMS)[41] to Mir. During the month of October acceleration data were recorded over 53 h in seven different time periods to survey locations for possible future experiments. During this time, on October 15th, an oxygen generator caught fire and was extinguished. The SAMS data showed that the normal acceleration environment was about 1000μg with quiet periods of 500μg. Larger, transient accelerations were also observed.[70]

An experiment named the Gaseous Nitrogen-dewar (GN2)[71] first flew on STS-71, the first Shuttle Orbiter docking with Mir. Experimentally, the precipitant solution was loaded into Tygon tubing sealed at one end and frozen; then the protein solution was added, the mixture was frozen again, and the tube was sealed. The frozen sample was transferred to a liquid nitrogen Dewar, which was taken to Mir. Over time the liquid nitrogen evaporated, the Dewar was warmed, and the samples thawed, allowing crystallization by free interface diffusion. On this mission GN2 contained 183 samples of 19 proteins (spanning a range of molecular weights, functions, and physical properties), of which 17 were crystallized.[71] GN2 was fixed to an internal partition on Mir, and temperature data were recorded at 1 h intervals. The temperature varied within a range of 17−28 °C over the 4.5 months of the mission. GN2 itself has a large thermal buffering capacity, and Koszelak et al.[71] believe that the samples varied by little more than a few degrees.

The third Shuttle Orbiter mission to Mir introduced the Diffusion-controlled Crystallization Apparatus for Microgravity (DCAM).[72] This experiment was transferred to Mir to be swapped out on the later, STS-79 mission. DCAM consists of two cells containing protein and precipitant solutions, separated by a gel plug that controls the equilibration rate. It requires no activation or deactivation by the crew. A total of 162 experiments were carried in an ambient thermal bag. DCAM experiments were conducted on Mir between the STS-76 and 79 missions, STS-79 and 81, STS-81 and 84, STS-84 and 89, and STS-89 and STS-96. The Jel42 Fab fragment and an HPr complex of the fragment were crystallized in DCAM. Small crystals of the free fragment, which subsequently dissolved, and a few crystals of the complex were produced diffracting to 3.5 Å. In the case of the free Fab the highly viscous precipitant PEG 8000 interfered with the diffusion through the gel plug, impeding crystallization.[73] Carter et al.[72] report that a crystal of a nucleosome core particle, grown on STS-73 as a DCAM proof of concept flight, produced the highest resolution X-ray data yet collected.[74]

STS-86 carried the Canadian Protein Crystallization Experiment (CAPE) to Mir. CAPE had 2 identical sets of over 800 experiments of 32 individual protein samples. One set was grown on the Microgravity Isolation Mount (MIM) and the other on Mir, but without the benefit of active isolation from the Mir *g*-jitter environment. The results are still being analyzed.

5.5. Overview of Results in the NASA Program. Macromolecular crystal growth experiments have been flown by NASA, ESA, RSA, and the Chinese Space Agency on a variety of platforms. In this section, we

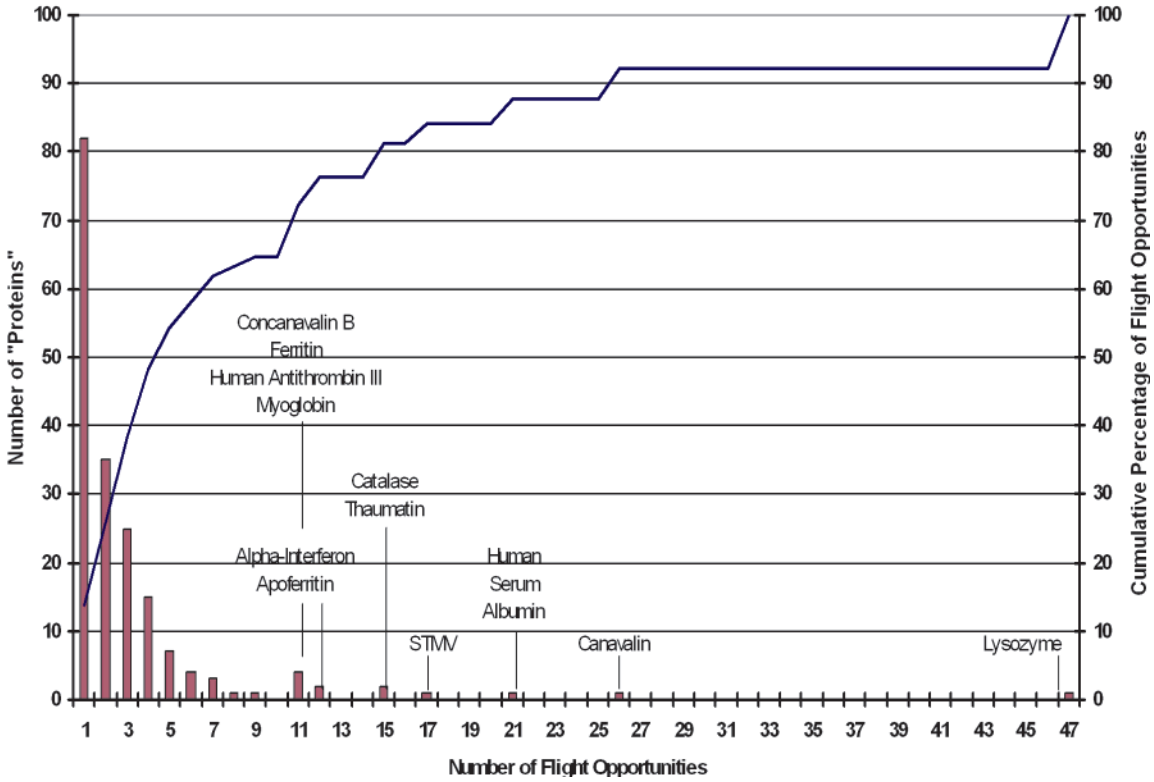

Figure 2. Frequency of flight in the NASA program. The histogram indicates how many "proteins" (meaning proteins, nucleic acids, and viruses), have flown how many times through 1998. Proteins that have flown more than 10 times are identified. The continuous curve indicates the cumulative percentage of flight opportunities versus number of flights.

focus on one program, NASA, and its use of two platforms: the Space Shuttle Orbiter and Mir.

While the NASA program has operated more than 15 years, access to the orbital microgravity environment has been very limited. A total of 185 different macromolecular samples have flown on 43 missions dating from 1983 to 1998. Eighty-one of the 185 macromolecules have flown only once (Figure 2). Less than half that number flew twice. The steep falloff in number of samples versus number of flights is due to several factors: infrequent flight opportunities and discouraging initial results are probably the biggest two factors. Twelve macromolecules, on the other hand, have flown more than 10 times. All 12 of these macromolecules were successful in that they either exhibited higher resolution diffraction than the best earth-grown crystals or they produced crystals of sufficient size for neutron diffraction. Lysozyme is an outlier at 47 flights, because it was used frequently as a positive control and as a model system for several types of crystal growth studies. The continuous curve shows that half of the flight opportunities went to systems that flew four or fewer times. Thus, the number of iterations performed in microgravity for a given macromolecule has generally been rather small. On average, only about 40 crystallization trials (a trial being analogous to a single crystallization setup) were conducted for each sample in microgravity. Assuming that trials were usually conducted in duplicate, only 20 chemical conditions were tested, on average, for each macromolecule.

Overall, of the 179 proteins that flew prior to STS-95, 36 (20%) obtained their highest diffraction resolution to date from a microgravity-grown crystal. If only the proteins that flew more than once are considered, the success rate for better diffraction increases to 35% (34 improvements out of 97 flown). The magnitude of the resolution improvements varies widely (Figure 3). The most improvement seen in the NASA program is pike parvalbumin, which went from a diffraction limit of 1.7 Å for the best earth-grown crystal to 0.9 Å for the best microgravity-grown crystal. The microgravity crystals yielded 6.7 times the data of the earth-grown crystals.

The nucleosome core particle (NCP) is one of several systems that showed greater improvements than expected on the basis of an increase in diffraction resolution alone. The resolution of the best ground-produced crystals diffracted aniosotropically to 2.8−2.7 Å, while the best microgravity crystal diffracted to at least 2.5 Å (the maximum resolution of the detector setting).[74] Reflections were seen beyond that resolution, and no ground-produced crystals matched the diffraction characteristics (diffraction limit and I/σ_I) of the microgravity-grown crystals under comparable situations of data collection. The level of detail visible in the 2.5 Å electron density map of the NCP appears higher than might beexpected due strictly to the improvement in resolution over the published 2.8 Å structure.[74]

The success rate for obtaining improved diffraction quality is higher for the "proteins" that have flown on more occasions (Figure 4). Of 81 "proteins" that flew only once, two showed increased diffraction quality: L-alanine dehdrogenase and neurophysin II/vasopressin complex. In contrast, more than half of the "proteins" that had four flight opportunities produced crystals with increased diffraction quality. Not suprisingly, the ability to perform several iterations of crystal growth in mi-

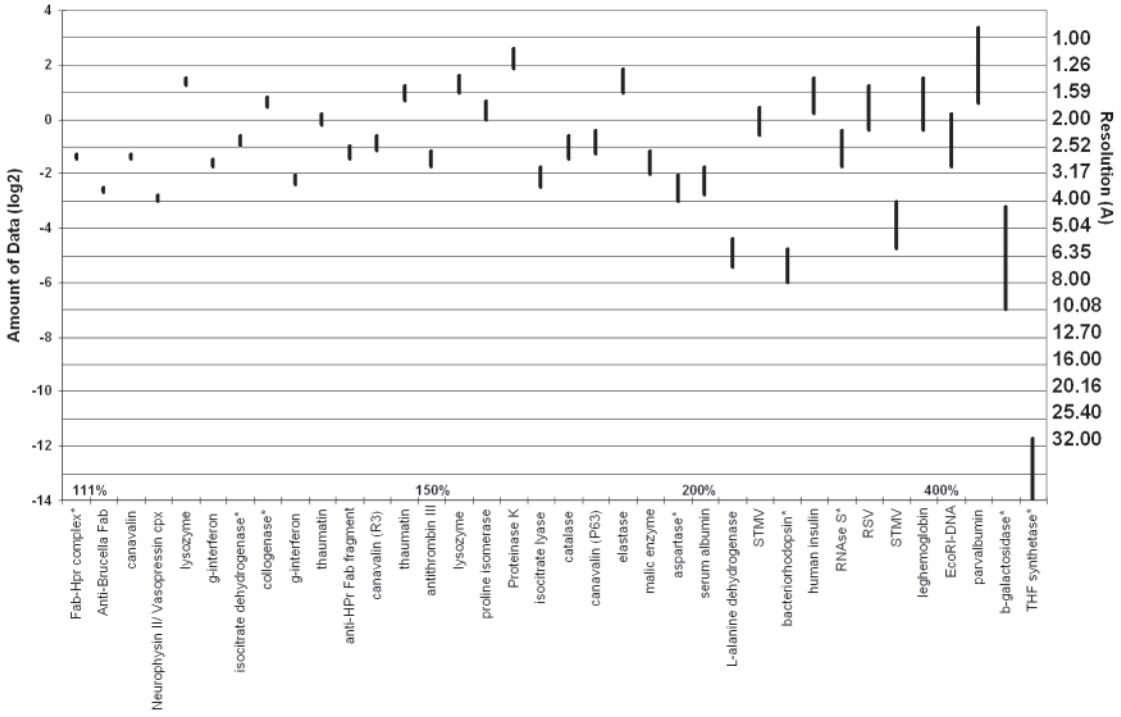

Figure 3. Diffraction resolution improvements from crystals grown in microgravity. For a given system, a bar is drawn between the diffraction limit of the best earth-grown crystal (bottom edge of bar) to the diffraction limit of the best microgravityy-grown crystal (top edge of bar). The space between grid lines represents a doubling of the amount of reflections (the ordinate corresponds to the logarithm, base 2, of the number of reflections obtained from a crystal relative to the number of reflections at 2.0 Å resolution). The corresponding resolutions are shown on the right-hand side of the graph. The systems are ordered from left to right in order of increasing improvement. Systems marked with an asterisk were flown by a non-NASA agency, e.g., the ESA.

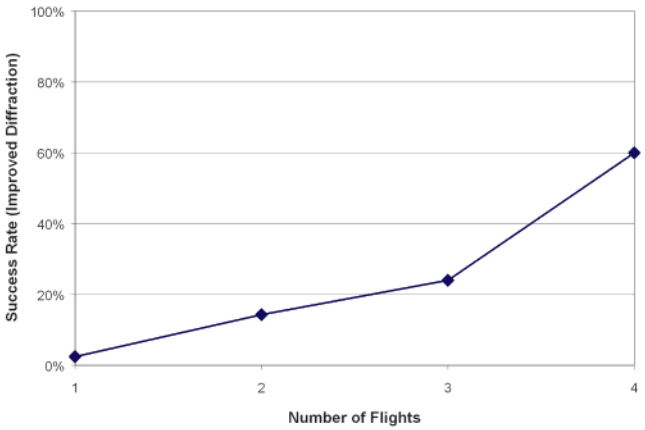

Figure 4. Success rate as a function of flights. The success rate for obtaining improved diffraction quality increases as the number of flight opportunities increases.

crogravity has been important for obtaining superior diffraction.

Both poorly diffracting and well diffracting crystals have benefited from growth in microgravity. If the 2.0−2.5 Å region is used to delimit the poor and well diffracting crystals, one sees that crystals both above and below this region have benefited from microgravity crystallization (Figure 3). In addition to improvements in diffraction limit, there have also been improvements in the signal-to-noise ratio of reflections and improvements in the volume of crystals. This was the case for canavalin[75] prior to STS-65, and marked volume increases were found for STMV[42] and insulin.[40] Thermally ramped canavalin crystals were smaller than those grown on Earth with no significant extension of resolution, although they had an improved signal to noise.[75] Various forms of insulin have been grown in the PCF, a device activated by thermal ramping, and have consistently yielded improved diffraction resolution and larger crystal size.[40]

6. Discussion

The original intent behind conducting biological crystallization experiments in microgravity was to provide a convection-free, quiescent environment for crystal growth. It was anticipated this would produce higher quality crystals. As experimental work progressed, the added benefits of reduced sedimentation were also seen.

The microgravity environment, however, held some surprises. While buoyancy-driven convection was greatly reduced, flows arising from Marangoni convection occurred. These flows, which result from weak surface forces at vapor−liquid interfaces, are usually masked on the ground but can become dominant in a microgravity environment. Marangoni convection may well be a limiting factor in the ultimate perfection of the crystals obtained using vapor diffusion methods. Marangoni convection, however, can be greatly reduced or avoided by using liquid−liquid dialysis or batch crystallization techniques. For example, higher resolution data were obtained from crystals of alcohol dehydrogenase grown in microgravity in APCF dialysis chambers than for those grown in the APCF using vapor diffusion during the same mission.[61]

With the measurement of the acceleration environment aboard an orbiting platform it also became clear that it is not always the quiescent environment it is envisaged to be. Astronaut activity, thruster firings, launching of satellites, the operation of fluid pumps and

other experimental apparatus and the structural harmonics of the spacecraft itself all accelerate the crystallization experiment. Depending on the specific aims of a given Space Shuttle mission, the acceleration environment may be very different from one mission to the next. Low-frequency vibrations in particular appear to stimulate crystal movements and fluid flows. These accelerations can sometimes be avoided by careful scheduling of the crystallization experiment. More generally, vibrations can be damped through the use of active vibration isolation systems. Two such systems, the Glovebox Integrated Microgravity Isolation Technology (g-LIMIT) and the Active Rack Isolation System (ARIS), are currently being developed by NASA for the International Space Station. The Canadian Space Agency has also developed the Microgravity Vibration Isolation Mount (MIM), which performs a similar function.

The empirical nature of optimizing crystallization conditions was not well served by the logistics of the Orbiter-based program. In comparison to the situation for ground-based investigators, who conduct one to two rounds of crystallization experiments per week, the access to microgravity was infrequent. The time between crystallizations in microgravity for a given protein averaged 6 months. Furthermore, the average protein flew slightly over 3 times and explored an average of 40 crystallization conditions per protein with experiment duration time usually limited to ~15 days.

It has often been difficult to separate the effects of microgravity from the effects of the flight hardware. Proper evaluation of the effect of microgravity requires comparison of the microgravity experiment to a ground-based control that uses the same flight hardware, sample preparation procedure, operating profile (e.g., temperature), and post-flight analysis. Since the orientation of the ground-based hardware is also a variable, the hardware should be tested in different configurations, e.g., "up", "down" and "sideways". In practice, there has usually not been enough material or hardware to conduct such thorough ground controls. As a result, the relative contributions of the microgravity environment and the hardware for a particular experiment have often been unclear.

Despite these limitations, 35% of proteins flown more than once produced microgravity-grown crystals that diffracted better than any ground-based crystal. The opportunity to do several rounds of crystallization experiments in microgravity is very important for success (Figure 4).

7. Future

To maximize the impact of microgravity crystal growth on structural biology, improvements in both the microgravity environment and the pace of research in microgravity are needed. The International Space Station (ISS) should provide a much better microgravity environment than an Orbiter which is deploying satellites. Dedicated facilities that can invest in vibration isolation hardware will improve the environment further. To increase the pace of research on the ISS, it will be necessary to implement telescience since, after assembly is complete, vehicles may visit the ISS only once every 3 months. As an example of telescience, video images from crystallization trials could be sent from the ISS to the ground for inspection by an investigator. The investigators would then send commands to ISS to set up the next round of crystallization trials. The slower mass transport in microgravity will result in longer equilibration times, but telescience capability would allow the pace of research to more closely mimic the pace of research on the ground.

These improvements should apply to both types of experiments needed: diagnostic and iterative production. Diagnostic experiments are needed to establish the precise relationship between acceleration levels, depletion zones, crystal growth rates, and crystal diffraction quality. Iterative production experiments are needed to produce the crystals for structural biology studies on the ground. Ideally, the capability would include the ability to monitor crystallization trials on the ISS, set up new trials on the ISS, cryostabilize and cryocool crystals on the ISS, and provide video microscopy and X-ray diffraction as measures of crystal quality. Greater use of proper ground controls for both types of experiments will facilitate the identification of hardware-specific effects versus microgravity effects and the development of crystallization strategies based on terrestrial results.

8. Concluding Remarks

In the past the microgravity environment has not been deliberately and systematically optimized. It has provided only limited flight opportunities, yet there have been successes. In fact, the microgravity crystal growth experiment record is considerable when one realizes that the comparisons are always between a few microgravity-based experiments and a large number of earth-based experiments. Past limitations are reminiscent of the difficulties that structural crystallographers experienced at first-generation synchrotrons in the 1970s: infrequent access, few trials, limited experiment time, and variations in beam properties. The development of second- and now third-generation synchrotrons with beamlines dedicated to macromolecular crystallography removed or lessened these limitations. Hopefully, the transition from the Space Shuttle Orbiter based crystal growth program to the ISS-based program will also enjoy such an increase in usefulness to structural biology.

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References

(1) McPherson, A. *Crystallization of Biological Macromolecules*; Cold Spring Harbor Laboratory Press: New York, 1999.
(2) Littke, W.; John, C. *Science* **1984**, *255*, 203−204.
(3) National Research Council. *Future Biotechnology Research on the International Space Station*; National Academy Press: Washington, D.C., 2000.
(4) *Chem., Eng. News* **2000**, *78*(11), 33.
(5) Reichardt, T. *Nature* **2000**, *403*, 114.

(6) Lawler, A. *Science* **2000**, *287*, 1728−1729.
(7) Ember, L. *Chem., Eng. News* **2000**, *78*(11), 33.
(8) Hamacher, H.; Merbold, U. *J. Spacecraft* **1997**, *24*, 264−269.
(9) DeLucas, L. J.; Long, M. M.; Moore, K. M.; Rosenblum, W. M.; Bray, T. L.; Smith, C.; Carson, M.; Narayana, S. V. L.; Harrington, M. D.; Carter, D.; Clark, A. D., Jr.; Nanni, R. G.; Ding, J.; Jacobo-Molina, A.; Kamer, G.; Hughes, S. H.; Arnold, E.; Einspahr, H. M.; Clancy, L. L.; Rao, G. S. J.; Cook, P. F.; Harris, B. G.; Munson, S. H.; Finzel, B. C.; McPherson, A.; Weber, P. C.; Lewandowski, F. A.; Nagabhushan, T. L.; Trotta, P. P.; Reichert, P.; Navia, K. P.; Wilson, K. P.; Thomson, J. A.; Richards, R. N.; Bowersox, K. D.; Meade, C. J.; Baker, E. S.; Bishop, S. P.; Dunbar, B. J.; Trinh, E.; Prahl, J.; Sacco, A., Jr.; Bugg, C. E.. *J. Cryst. Growth* **1994**, *135*, 183−195.
(10) Pusey, M. L. *J. Cryst. Growth* **1992**, *122*, 1−7.
(11) Boggon, T. J.; Chayen, N. E.; Snell, E. H.; Dong, J.; Lautenschlager, P.; Potthast, L.; Siddons, D. P.; Stojanoff, V.; Gordon, E.; Thompson, A. W.; Zagalsky, P. F.; Bi, R.-C.; Helliwell, J. R. *Philos. Trans. R. Soc. London* **1998**, *A356*, 1−17.
(12) Ramachandran, N.; Baugher, Ch. R.; Naumann, R. J. *Microgravity Sci. Technol*. **1995**, *VIII/3*, 170−179.
(13) Nadarajah, A.; Rosenberger, F.; Alexander, J. I. D. *J. Cryst. Growth* **1990**, *104*, 218−232.
(14) Snell, E. H.; Boggon, T. J.; Helliwell, J. R.; Moskowitz, M. E.; Nadarajah. *Acta Crystallogr.* **1997**, *D53*, 747−755.
(15) Garcia-Ruiz, J. M.; Otalora, F. *J. Cryst. Growth* **1997**, *182*, 155−167.
(16) McCabe, W. L.; Smith, J. C.; Harriot, P. *Unit Operations in Chemical Engineering*, 4th ed.; McGraw-Hill: Singapore, 1985; p 143.
(17) Giachetti, E.; Ranaldi, F.; Fiusco, A.; Tacconi, M.; Veratti, R.; Falciani, P.; Vanni, P. *Microgravity Sci. Technol.* **1999**, *12*, 36−40.
(18) Bi, R. C.; Gui, L. L.; Han, Q.; Shen, F. L.; Shi, K.; Wang, Y. P.; Chen, S. Z.; Hu, Y. L.; Niu, X. T.; Dong, J.; Zhou, Y. C.; Lin, N. Q. *Microgravity Sci. Technol.* **1994**, *7*, 203−206.
(19) Borisova, S. N.; Birnbaum, G. I.; Rose, D. R.; Evans, S. V. *Acta Crystallogr.* **1996**, 67−271.
(20) Sjolin, L.; Wlodawer, A.; Bergqvist, G.; Holm, P.; Loth, K.; Malmstrom, H.; Zaar, J.; Anders Svensson, L.; Gilliland, G. L. *J. Cryst. Growth* **1991**, *110*, 322−332.
(21) Strong, R. K.; Stoddard, B. L.; Arrott, A.; Farber, G. K. *J. Cryst. Growth* **1992**, *119*, 200−204.
(22) Ng, J. D.; Lorber, B.; Giege, R.; Koszelak, S.; Day, J.; Greenwood, A.; McPherson, A. *Acta Crystallogr.* **1997**, *D53*, 724−733.
(23) Trakhanov, S. D.; Grebenko, A. I.; Shirokov, V. A.; Gudkov, A. V.; Egorov, A. V.; Barmin, I. N.; Vainstein, B. K.; Spirin, A. S. *J. Cryst. Growth* **1991**, *110*, 317−321.
(24) Ries-Kautt, M.; Broutin, I.; Ducruix, A.; Shepard, W.; Kahm, R.; Chayen, N.; Blow, D.; Paal, K.; Littke, W.; Lorber, B.; Theobald-Dietrich, T.; Giege, R. *J. Cryst. Growth* **1997**, *181*, 79−96.
(25) Pusey, M. L.; Naumann, R. *J. Cryst. Growth* **1986**, *76*, 593−599.
(26) Pusey, M. L.; Snyder, R. S.; Naumann, R. *J. Biol. Chem.* **1986**, *261*, 6524−6529.
(27) Pusey, M. L.; Witherow, W.; Naumann, R. *J. Cryst. Growth* **1998**, *90*, 105−111.
(28) Grant, M. L.; Saville, D. A. *J. Cryst. Growth* **1991**, *108*, 8−18.
(29) Larson, M. A. In *Advances in Industrial Crystallization*; Garside, J., Davey, R. J., Jones, A. G, Eds.; Butterworth-Heinemann, Oxford, 1991; pp 20−30.
(30) Marangoni, C. G. M. *Ann. Phys. Chem (Poggendorf)* **1871**, *143*, 7, 337−354.
(31) Molenkamp, T. Marangoni convection, mass transfer and microgravity. Ph.D. Thesis, Rijksuniversiteit Groningen, 1998.
(32) Bakker, C. A. P.; van Buytenen, P. M.; Beek, W. J. *Chem. Eng. Sci.* **1966**, *21*, 1039−1046.
(33) Hoefsloot, H. C. J.; Janssen, L. P. B. M.; Sibbald, R. T.; Hoogstraten, H. W. *Microgravity Sci. Technol.* **1991**, *IV/1*, 55−59.
(34) Provost, K.; Robert, M.-C. *J. Cryst. Growth* **1991**, *110*, 258−264.
(35) Miller, T. Y.; Xiao-min, H.; Carter, D. C. *J. Cryst. Growth* **1992**, *122*, 306−309.
(36) Dong, J.; Boggon, T. J.; Chayen, N. E.; Raftery, J.; Bi, R.-C.; Helliwell, J. R. *Acta Crystallogr.* **1999**, *D55*, 745−752.
(37) DeLucas, L. J.; Suddath, F. L.; Snyder, R. S.; Naumann, R.; Broom, M. B.; Pusey, M.; Yost, V.; Herren, B.; Carter, D.; Nelson, B.; Meehan, E.; McPherson, A.; Bugg, C. E. *J. Cryst. Growth* **1986**, *76*, 681−693.
(38) Naumann, R. J.; Snyder, R. S.; Bugg, C. E.; DeLucas, L. J.; Suddath, F. L. *Science* **1985**, *230*, 375−376.
(39) Long, M. M.; DeLucas, L. J.; Smith, C.; Carson, M.; Moore, K.; Harrington, M. D.; Pillion, D. J.; Bishop, S. P.; Rosenblum, W. M.; Naumann, R. J.; Chait, A.; Prahl, J.; Bugg, C. E. *Microgravity Sci. Technol*. **1994**, *VII/2*, 196−202.
(40) Long, M. M.; Bishop, J. B.; Nagabhushan, T. L.; Reichert, P.; Smith, G. D.; DeLucas, L. J. *J. Cryst. Growth* **1996**, *168*, 233−243.
(41) DeLombard, R.; Finley, B. D.; Baugher, C. R. NASA Technical Memorandum-105652, 1992.
(42) Larson, S. B.; Day, J.; Greenwood, A.; McPherson, A. *J. Mol. Biol*. **1998**, *277*, 37−59.
(43) Snyder, R. S.; Fuhrmann, K.; Walter, H. U. *J. Cryst. Growth* **1991**, *110*, 333−338.
(44) Bosch, R.; Lautenschlager, P.; Potthast, J.; Stapelmann, J. *J. Cryst. Growth* **1992**, *122*, 310−316.
(45) Chayen, N. E.; Snell, E. H.; Helliwell, J. R.; Zagalsky, P. F. *J. Cryst. Growth* **1997**, *171*, 219−225.
(46) Weisgerber, S.; Helliwell, J. R. *Collaborative Computing Project 4, European Science Foundation, European Association for the Crystallization of Biological Macromolecules Newsletter on Protein Crystallography* **1993**, *29*, 10−13.
(47) Weisgerber, S. Developments in synchrotron radiation data collection techniques for macromolecular crystallography (Laue and LOT) and application to concanavalin A. Ph.D. Thesis, University of Manchester, Manchester, U.K., 1993.
(48) Snell, E. H.; Weisgerber, S.; Helliwell, J. R.; Weckert, E.; Hölzer, K.; Schroer, K. *Acta Crystallogr.* **1995**, *D51*, 1099−1102.
(49) Helliwell, J. R.; Snell, E. H.; Weisgerber, S. In *Proceedings of the 1995 Berlin Microgravity Conference*; Ratke, L., Walter, H., Feuerbache, B., Eds.; Springer-Verlag: Berlin, 1996; pp 155−170.
(50) Koszelak, S.; Day, J.; Leja, C.; Cudney, R.; McPherson, A. *Biophys. J*. **1995**, *69*, 13−19.
(51) Savino, R.; Monti, R. *J. Cryst. Growth* **1996**, *165*, 308−318.
(52) Snell, E. H.; Cassetta, A.; Helliwell, J. R.; Boggon, T. J.; Chayen, N. E.; Weckert, E.; Hölzer, K.; Schroer, K.; Gordon, E. J.; Zagalsky, P. F. *Acta Crystallogr.* **1997**, *D53*, 231−239.
(53) Stoddard, B. L.; Strong, R. K.; Farber, G. K.; Arrott, A.,; Petsko, G. A. *J. Cryst. Growth* **1991**, *110*, 312−316.
(54) Carter, D. C.; Wright, B.; Miller, T.; Chapman, J.; Twigg, P.; Keeling, K.; Moody, K.; White, M.; Click, J.; Ruble, J. R.; Ho, J. X.; Adcock-Downey, L.; Dowling, T.; Chang, C.-H.; Ala, P.; Rose, J.; Wang, B. C.; Declercq, J.-P.; Everard, C.; Rosenberg, J.; Wery, J.-P.; Clawson, D.; Wardell, M.; Stallings, W.; Stevens, A. *J. Cryst. Growth* **1999**, *196*, 610−622.
(55) Evrard, C.; Declercq, J.-P.; Fastrez, J. *Acta Crystallogr.* **1997**, *D53*, 217−219.
(56) Evrard, C.; Fastrez, J.; Declercq, J.-P. *J. Mol. Biol.* **1998**, *276*, 151−164.
(57) Wardell, M. R.; Skinner, R.; Carter, D. C.; Twigg, P. D.; Abrahams, J.-P. *Acta Crystallogr.* **1997**, *D53*, 622−625.
(58) Skinner, R.; Abrahams, J.-P.; Whisstock, J. C.; Lesk, A. M.; Carrell, R. W.; Wardell, M. R. *J. Mol. Biol*. **1997**, *266*, 601−609.
(59) Declercq, J.-P.; Evrard, C.; Carter, D. C.; Wright, B. S.; Etienne, G.; Parello, J. *J. Cryst. Growth* **1999**, *196*, 595−601.
(60) Otalora, F.; Novella, M. L.; Rondon, D.; Garcia-Ruiz, J. M. *J. Cryst. Growth* **1999**, *196*, 649−664.
(61) Eposito, L.; Sica, F.; Sorrentino, G.; Berisio, R.; Carotenuto, L.; Giordano, A.; Raia, C. A.; Rossi, M.; Lamzin, V. S.; Wilson, K. S.; Zagari, A. *Acta Crystallogr.* **1998**, *D54*, 386−390.
(62) Plaas-Link, A.; Cornier, *J. Appl. Microgravity Technol.* **1988**, *I(3)*, 123−132.

(63) Erdmann, V. A.; Lippmann, C.; Betzel, C.; Dauter, Z.; Wilson, K.; Hilgenfeld, R.; Hoven, J.; Liesum, A.; Snager, W.; Muller-Fahrnov, A.; Hinrichs, W.; Duvel, M.; Schulz, G. E.; Muller, C. W.; Wittmann, H. G.; Yonath, A.; Weber, G.; Stegen, K.; Plass-Link, A. *FEBS Lett.* **1989**, *1*, 194−198.
(64) Plaas-Link, A. in *Proceedings of the 7th European Symposium on Materials and Fluid Sciences in Microgravity*; ESA-SP-295, European Space Agency: Noordwijk, The Netherlands, 1990; pp 41−44.
(65) Hilgenfeld, R.; Liesum, A.; Storm, R. *J. Cryst. Growth* **1992**, *122*, 330−336.
(66) Asano, K.; Fujita, S.; Senda, T.; Mitsui, Y. *J. Cryst. Growth* **1992**, *122*, 323−329.
(67) Schmidt, H. P.; Koerver, W.; Patz, B. *J. Cryst. Growth* **1992**, *122*, 317−322.
(68) Eilers, D.; Stark, H. R. *NASA Conf. Publ.* **1993**, *No. 3272* (*Vol. 2*), 869−891.
(69) Zagalsky, P. F.; Wright, C. E.; Parsons, M. *Adv. Space Res.* **1995**, *16*, 91−94.
(70) DeLombard, R.; Rodgers, M. J. B. *NASA Tech. Memo.* **1995**, *No. 106835*.
(71) Koszelak, S.; Leja, C.; McPherson, A. *Biotechnol. Bioeng.* **1996**, *52*, 449−458.
(72) Carter, D. C.; Wright, B.; Miller, T.; Chapman, J.; Twigg, P.; Keelink, K.; Moody, K.; White, M.; Click, J.; Ruble, J. R.; Ho, J. X.; Adcock-Downey, L.; Bunick, G.; Harp, J. *J. Cryst. Growth* **1999**, *196*, 602−609.
(73) Quail, J. W.; Delbaere, L. T. J.; Prasad, L.; Pugazhenthi, U. In *Spacebound 1997 Conference Proceedings*, Canadian Space Agency, 1998; pp 295−297.
(74) Harp, J. M.; Hanson, B. L.; Timm, D. E.; Bunick, G. J. *Acta Crystallogr.*, in press.
(75) Day, J.; McPherson, A. *Protein Sci.* **1992**, *1*, 1254−1268.

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