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RECENT RESULTS AND NEW HARDWARE DEVELOPMENTS FOR PROTEIN CRYSTAL GROWTH IN MICROGRAVITY

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Abstract

Protein crystal growth experiments have been performed on 16 space shuttle missions since April, 1985. The initial experiments utilized vapor diffusion crystallization techniques similar to those used in laboratories for earth-based experiments. More recent experiments have utilized temperature induced crystallization as an alternative method for growing high quality protein crystals in microgravity. Results from both vapor diffusion and temperature induced crystallization experiments indicate that proteins grown in microgravity may be larger, display more uniform morphologies, and yield diffraction data to significantly higher resolutions than the best crystals of these proteins grown on earth.

Introduction:

The study of protein crystal growth in microgravity has generated considerable interest in recent years.[1-7] Through the support of the National Aeronautics and Space Administration (NASA), this laboratory has coordinated a program designed to study protein crystal growth processes in general, and to evaluate the effects of these processes in the microgravity environment existing on U. S. Space Shuttle missions. A large co-investigator group consisting of researchers and/or engineers from universities, NASA, and aerospace or pharmaceutical companies, participated in the hardware development and scientific experiments. Since April, 1985, experiments have been performed on sixteen U. S. Space Shuttle missions using a variety of crystal growth hardware and crystallization techniques. In a number of cases, results from these experiments indicate that proteins grown in microgravity may be larger, display more uniform morphologies, and yield diffraction data to significantly higher resolutions than the best crystals of these proteins grown on earth.[8] This paper will discuss recent hardware developments and results associated with these space shuttle missions.

New Hardware Developments:

Vapor Diffusion Apparatus:

The vapor diffusion apparatus (VDA)[2] was used for protein crystallization experiments on twelve different space shuttle missions. This hardware utilizes a vapor diffusion technique which is closely related to the widely used hanging drop method of protein crystal growth on earth.[9] This method was chosen for several reasons: 1.) most protein crystallography laboratories have extensive experience with this method and a large percentage of the protein crystals described in recent publications have been obtained using this technique; 2.) this technique is particularly amenable to crystallization experiments involving small quantities of protein; 3.) in a microgravity environment, relatively large, stable droplets of protein solution can be formed with minimal surface contacts, thereby decreasing possible nucleation sites and minimizing wall effects that generally accompany

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crystallization experiments on earth. The hardware was developed using a simple piece of equipment that was easily modified and improved throughout the series of shuttle missions.[8]

Although the microgravity experiments with the VDA clearly demonstrate the beneficial effects of this environment for protein crystal growth, only about 20% of the proteins examined were found to exhibit better morphologies or better quality data than their earth-grown counterparts. Approximately 40% of the space experiments failed to produce crystals, and the remaining 40% yielded crystals that were either too small for x-ray analysis or produced data no better than that obtained from the best earth-grown crystals. It should be noted that generally the results from a single space experiment in which each protein is typically allotted five crystallization chambers are being compared with results from the best crystals ever produced for that particular protein by any method on earth. With the VDA hardware, investigators have no ability to optimize the crystal growth conditions in microgravity. It was believed that this often accounted for results in which no crystals were obtained from microgravity, or in which the crystals that were obtained were of poorer quality than the best crystals produced for these proteins on earth.

USML-1 Glovebox Vapor Diffusion Hardware:

This led to the development of new hardware to be flown on the United States Microgravity Laboratory-1 (USML-1) mission in June, 1992. A glovebox was flown in the spacelab module for this mission, thereby providing a partial level of containment so that liquids (i. e. proteins, buffets, etc.) could be worked with using methods similar to those employed in laboratories on Earth. The hardware developed for this mission incorporated several new capabilities including: 1.) optimization of protein crystal growth experiments; 2.) on orbit seeding with small crystals produced in microgravity; 3.) investigation of crystal mounting techniques for future shuttle missions and for Space Station; 4.) utilization of real-time video downlink to facilitate scientific input from the ground regarding new experiments to be prepared on orbit.

Figures 1a and 1b show the design utilized for the vapor diffusion experiments. Each experiment chamber (constructed from clear polysulfone material) consists of a rectangular upper and lower half sealed by a neoprene septum. The upper half contains the protein solutions to be used for three separate vapor diffusion experiments (a, b, and c), while the opposing half contains depressions which hold their respective reservoir solutions. The protein and reservoir solutions are prepared on the shuttle using Hamilton syringes in conjunction with a dispensing device that allows microliter quantities to be extruded accurately and rapidly (figure 2a). The solutions used to prepare these experiments are contained in vials/bottles with rubber septums so that they can be accessed using the Hamilton syringes (figure 2b). Solutions can be mixed by withdrawing and re-extruding them from the syringes and/or by gentle stirring using the end of the syringe needle. After all three crystallization experiments have been prepared, the crystallization chambers are placed together and sealed by tightening a thumbscrew. The experiments can be deactivated (the vapor diffusion process terminated) by a 180° rotation of the upper half of the experiment chamber. Eight experiment chambers (24 vapor diffusion experiments) are stored in a holder which fits into a lexan containment tube thereby providing a second level of containment for the fluids. Thirty two of these containment tubes (768 experiments) can be placed into a tray which slides into the refrigerator/incubator module (R/IM) so that a precise temperature can be maintained throughout the crystallization process (figure 3). In addition, a commercially purchased x-y-z translation stage was adapted to hold a manostat syringe onto which capillaries could be attached for crystal mounting procedures (figure 4). The translation stage is attached to a magnetic base so that it can be used on the metal floor of the USML-1 glovebox. Each vapor diffusion experiment could be observed by placing the experiment chamber on a x-y-z translation stage with fiber optic backlighting (figure 4). This stage was subsequently used to prepare experiments and observe experiments via a Zeiss binocular microscope with a special adapter that allowed 35 mm photography and/or real time video downlink (via a glovebox video camera).

Temperature Induced Crystallization:

In a microgravity environment, temperature induced convective flows are suppressed. Temperature provides a subtle, precise way to affect protein solubility and crystal growth rates. Since other crystallization parameters such as protein concentration, precipitant concentration, and solution volume remain constant throughout the crystallization experiment, temperature induced crystallization, as an alternative way to grow protein crystals, should be particularly suited for microgravity studies. Therefore, this laboratory designed, constructed and tested a new crystallization apparatus, the protein crystallization facility (PCF), which utilizes temperature as the sole means of affecting protein saturation and solubility for crystallization. The facility consists of four cylindrical containers which hold milliliter quantities of protein solution. The containers were designed so that one end (made of stainless steel) interfaces directly with the cold plate within the R/IM. The remainder of each cylinder (made of polysulfone material) is surrounded by an insulating pocket of air and another large diameter container which provides a second level of containment (figure 5). Crystals are grown in each container by varying the temperature of the R/IM thereby creating a temperature gradient down the long axis of each cylinder. The four bottles varied in size (500 ml bottle, 250 ml bottle, 100 ml bottle, and 50 ml bottle) and were contained in one R/IM. The R/IM temperature can be varied between 42.0° C and 2.0° C (an improved version of the R/IM, the C-R/IM, is programmable so that a variety of temperature gradients can be created.) This simple hardware design allowed temperature induced crystallization to be investigated as an alternative method for microgravity crystallization.

Results:

Vapor Diffusion Apparatus:

The present flight VDA has been instrumental in serving the needs of a large group of co-investigators who require crystals of higher quality for x-ray diffraction studies. It has also demonstrated that microgravity can be beneficial to protein crystal growth for a variety of proteins when crystallization conditions are optimized. Beginning with flight STS-26 (September, 1988) protein crystal growth experiments were conducted for the first time within the R/IM. Details of the procedure used for the analysis and the results from this mission and the three succeeding missions have been presented.[3,8,10] For these four space shuttle missions, the microgravity experiments yielded crystals that were larger (as much as 2 times the volume), displayed more uniform morphologies, and/or yielded diffraction data to significantly higher resolutions (as much as 0.3 Å) than the best crystals of these proteins produced on earth. More recent shuttle missions utilizing the VDA hardware have produced even more dramatic enhancements in resolution. On STS-42 (IML-1, January, 1992) several large crystals of human serum albumin were produced using the VDA. Although there was clearly a variation in crystal quality, several crystals (by visual examination) showed clear evidence of diffraction to d-spacings beyond the solvent ring, which had not been previously observed. In addition, one crystal data set exhibited diffraction statistics clearly superior to all other data including data from gel-grown human serum albumin crystals (figure 6). Furthermore, analysis of the combined and average data sets resulted in 15% more observations than all other previous data sets including a synchrotron data set. What is particularly striking is that the $I / \sigma (I)$ ratio of 2 for a cut-off of the higher resolution data is extended from 3.8 Å to 3.0 Å (when compared to the best gel-grown crystals). In addition, the enhancement at the 2σ level for the space crystal compared to the best solution crystal is more than 1.0 Å.

USML-1:

For the USML-1 mission, both the VDA apparatus and the glovebox hardware specifically designed to allow on orbit preparation of the crystallization solutions were flown. The VDA apparatus (60 vapor diffusion experiments) was used for proteins crystallized at 22° C in one incubator, and at 4° C in a second incubator (total of 120 experiments). The

glovebox hardware was used to optimize crystal growth conditions for 24 different proteins, the majority of which once prepared, were stored in a 22° C incubator during the crystallization period. Approximately 25% of the proteins flown in the glovebox required between 10 and 14 days for crystal growth in ground-based experiments. In most cases, these proteins took even more time in microgravity for nucleation and/or crystal growth. As a result, small crystals, if seen at all, were not observed for almost all of these proteins until day 11 of the 14 day mission. By mission end, although still growing, none of these proteins produced crystals of sufficient size for diffraction analysis. In spite of this, the glovebox was invaluable for a number of proteins. Seventy-nine percent of the proteins used for the glovebox experiments produced crystals and 42% of these produced crystals that were large enough for diffraction experiments to be performed.

Four proteins that were prepared using the glovebox hardware had flown on one or more previous shuttle missions using the VDA hardware. VDA results for these proteins did not produce crystals on return and it was suspected that this was due to inadequate mixing of the protein and precipitant solutions (all four of these proteins required high molecular weight polyethylene glycol as a precipitant - a highly viscous solution). Utilizing the glovebox hardware, it was possible to thoroughly mix the protein and precipitant solutions by stirring or by withdrawing and re-extruding the solution from a Hamilton syringe. As a result, all four proteins produced crystals and two proteins, malic enzyme and factor D, produced high quality data.

Malic enzyme (figure 7), (M.W. = 260 kilodaltons, malic enzyme is an NAD+-dependent enzyme isolated from a parasitic nematode. It is being studied to exploit structural differences from the human form of this enzyme to aid in the development of an antiparasitic drug.) was flown on two previous shuttle flights using the VDA. For both of these missions, no crystals were observed at the conclusion of the flight. This lack of success was attributed to inadequate mixing of the viscous precipitating agent utilized (polyethylene glycol 4000). On the USML-1 mission using the glovebox crystallization hardware, thorough mixing was achieved and, as a result, many small crystals were observed within 5 days. Based on observations made through a binocular microscope on orbit, new conditions were set up which ultimately produced the best crystals. Earth-grown crystals of malic enzyme diffract weakly to 3.2 Å resolution. Using crystals obtained in microgravity, an ultimate resolution limit of 2.6 Å was achieved. Figure 8a shows the comparison of diffraction intensity data for one space-grown crystal and the best earth-grown crystal. It should be noted that the earth-grown crystal was 4 times the volume of the space-grown crystal used for this comparison. Although significantly smaller in volume, the space-grown crystal of malic enzyme produced higher quality (increased signal to noise ratio) data at all resolution ranges, with an appreciable enhancement in the ultimate resolution at which measurable data can be obtained. In addition, the statistics from a Relative Wilson plot comparing data from the space-grown crystal with that from the best earth-grown crystal revealed a significant difference in B values for data in the higher resolution range indicating better internal order for the space-grown crystal (figure 8b).

Bovine brain prolyl-isomerase (M. W. = 12 kilodaltons, this enzyme is the target for a new class of drugs designed to prevent transplant rejection) when crystallized on earth often forms clusters which exhibit extensive twinning and variability in diffraction quality. The crystallization solutions used for this shuttle mission were identical to those used on earth, yet the space-grown crystals (figure 9) were substantially larger (approaching 3.0 mm x 1.0 mm thick as opposed to 0.6 mm x 0.2 mm for earth-grown crystals) and did not exhibit any clustering, twinning, or large variations in diffraction quality, as do their earth-grown counterparts. It should be noted that the space-grown crystals were of a size sufficient to consider neutron diffraction experiments. The space-grown crystals also had sharper edges and were clearer than their earth-grown counterparts; the space-grown and earth-grown crystals were isomorphous. Unfortunately, the space-grown crystals suffered some degradation from the time they were retrieved from the flight hardware until they could be mounted in capillaries at the co-investigators laboratory. It is believed that this degradation may have affected the diffraction quality of the space-grown crystals, yet in spite of this, a

complete data set to 2.3 Å resolution was collected on the crystal judged to be of the highest quality. When the data from this crystal is compared to that from the best earth-grown crystal, it is clear that the space-grown crystal is superior in diffraction quality throughout the resolution range (figure 10).

Factor D (M. W. = 24 kilodaltons) is an essential enzyme for the initiation of the alternative pathway of the complement system. Two crystalline forms, triclinic (space group P1) and monoclinic (space group $P2_1$) can be produced using identical crystallization conditions. The monoclinic form was used to determine the three-dimensional structure, however, these crystals are extremely difficult to grow on a reproducible basis. In fact, using several different batches of protein over a ten month period including the batch used for the USML-1 microgravity experiments, this laboratory was unable to produce monoclinic crystals (this crystal form is needed to continue studies with this important protein). On USML-1, one monoclinic crystal was produced using the glovebox hardware. This crystal was the longest crystal ever grown of Factor D (although it was only 1/3 as thick as the best earth-grown crystal) and produced diffraction data comparable in intensity to the best earth-grown crystal with a slight improvement in resolution (0.1 Å). The Relative Wilson plot revealed a significant difference in B values for data in the higher resolution range indicating better internal order for the space-grown crystal.

Canavalin (M. W. = 11 kilodaltons) is the major storage protein of leguminous plants and a major source of dietary protein for humans and domestic animals. This protein has been crystallized on several shuttle flights using the VDA hardware. For USML-1, large crystals of recombinant canavalin were grown both in the VDA and glovebox hardware. However, canavalin crystals are extremely unstable and as a result, several exhibited severe degradation by the time the crystals were harvested after the shuttle flight. One VDA experiment contained crystals that, although somewhat degraded, were suitable for x-ray data collection. From this syringe, three large rhombohedral crystals were used for data collection on an SDMS area detector system. Comparison of average $I / \sigma (I)$ versus resolution for the two space crystals with the best earth-grown crystals demonstrated a marginal improvement for those crystals grown in space. However, Relative Wilson statistics were not conclusive, indicating that the data produced from microgravity were quite similar to those produced by equivalent crystals grown in the laboratory. In addition, no extensions of the resolution to higher limits was observed. On previous missions, native space-grown canavalin crystals showed more dramatic improvements in diffraction quality.[8]

Human α-Thrombin (M. W. = 36.5 kilodaltons) is a serine protease involved in the final step of the coagulation cascade cleaving soluble fibrinogen to produce insoluble fibrin. For the USML-1 mission, crystallization experiments on α-Thrombin yielded two large single crystals and some clusters of smaller crystals. The largest α-Thrombin crystal grown to date was grown on this space shuttle flight (1.0 mm x 0.5 mm x 0.45 mm). This crystal was rectangular in shape with a hollow area along one edge indicating possible rapid growth. A second crystal, similar in habit but slight smaller, was also produced. Unfortunately, both crystals severely degraded when transferred to a synthetic stabilizing buffer after return to the laboratory. As a result, x-ray data could not be collected on these crystals.

Large crystals of HIV-1 Reverse Transcriptase (this enzyme is responsible for copying the nucleic acid genome of the AIDS virus from RNA to DNA) complexed to a monoclonal antibody and one 19/18 base-paired double stranded DNA helical fragment were grown (M. W. = 180 kilodaltons) in the 4° C R/IM. Both space and ground control crystals were subjected to x-ray oscillation photography at the F1 station of the Cornell High Energy Synchrotron Source (CHESS) with an x-ray wavelength of 0.91 Å. All exposures for both the space crystals and the ground control crystals were of 6 to 8 seconds duration with an oscillation range of 0.7°. Fuji storage phosphor imaging plates were employed as the x-ray detecting medium with a crystal to detector distance of 340 mm. The crystals were large enough (typically 0.8 mm in length) to permit translation within the beam so that photographs could be taken from multiple positions of the crystals. A total of 101 exposures from 23 crystals were obtained from which 88 exposures from 22 crystals proved satisfactory

and therefore were included in the final data set. For the ground control crystals, 27 exposures from 5 crystals were collected, with 22 of these exposures included in the final control data set. Plots of intensities greater than various σ cutoffs versus resolution as well as average $I / \sigma(I)$ versus resolution indicate that there is very little difference between the space and ground crystals. However, a Relative Wilson plot indicated that the space-grown crystals had a significantly lower B value than the ground control crystals, particularly at the high resolution end of the plot (figure 11). This is an indication that the space-grown crystals are better ordered (beyond 4 Å) than are the ground control crystals. The differences in the post refined unit cell parameters were less than one-half of one percent. The post refined vertical and horizontal mosaic spread parameters were 0.2545° and 0.2322° for the space data set respectively. The corresponding values for the earth control data set were 0.2492° and 0.2911° respectively.

Other proteins which produced diffraction sized crystals showed diffraction data equal in quality or less than the best crystals for these proteins produced on earth. In all of these cases, the crystals grown in space were significantly smaller than the earth-grown crystals.

The USML-1 glovebox hardware was extremely successful in that: 1.) it demonstrated the ability to use crystal optimization to improve the quality of crystals that are grown on orbit; 2.) for the first time, mixing of viscous solutions could be carried out to completion; 3.) seeding and crystal mounting techniques were investigated and proved to be straightforward and quite useful; and 4.) high magnification microscopy with real time video downlink was successfully utilized.

Temperature Induced Crystallization:

The PCF was flown on four recent space shuttle flights: STS-37 (April, 1991), STS-43 (August, 1991), STS-49 (May, 1992) and STS-52 (October, 1992). Bovine insulin (M.W. = 6 kilodaltons) an enzyme important in glucose metabolism was flown on the first three shuttle flights and α-interfereon (M.W. = 18 kilodaltons) is a multifunctional enzyme of the immune system, playing an inhibitory role in viral and parasitic infections as well as certain tumors) was flown on STS-52. The analysis consisted of the following: 1.) photographic documentation of crystal location within each cylindrical container; 2.) high magnification photographic documentation of representative crystals from each container; 3.) morphometric analysis [11,12] on a statistically valid number of crystals (greater than 3,000 crystals) to establish crystal size, distribution within the containers, and relative size for crystals obtained from each container; 4.) x-ray diffraction analysis.

For the morphometric analysis, a statistically valid number of crystals from each crystallization chamber was analyzed with a GRAF/PEN sonic digitizer interfaced with a Hewlett Packard 9825A computer at approximately 250x magnification on a CRT interfaced with an inverted phase microscope via video camera. The analysis was repeated to validate statistics and intra/inter observer variation. The length and width of each crystal was directly measured on a CRT screen and the x and y coordinates of successive points are transmitted to the computer. From these points, the following data are calculated; length, width, volume, length-width ratio, mean, medium, minimum and maximum, standard deviation, standard error of the mean, and histograms for each parameter.

A new x-ray analysis method was utilized for these experiments in an effort to collect data quickly and on a large number of crystals. Data are collected on a Siemens area detector system but only 20 to 50 frames of data (at 0.25° per frame) are collected per crystal. These data frames are merged into one frame by taking the maximum value observed at each pixel. Several crystals can be examined by this technique under identical conditions in a relatively short period of time. The summed data frames are divided into twelve bins of resolution based on equal increments of $1 / d$. The background of each bin is determined separately in two passes. The RMS and standard deviations are calculated and pixels with counts greater than 1 σ above the RMS counts are discarded. The remaining pixels are used

to recalculate the RMS counts and standard deviation of the background. The average intensity (counts) of probable reflection pixels is evaluated and those pixels greater than 3 σ above the RMS counts are summed and divided by the number of pixels in the bin. A plot of ln [(Ispace) / (Iground) versus $1/d^{]}$ is then analogous to the Relative Wilson plots used in the previous analysis. The intensity of several crystals is averaged to make the graph statistically more meaningful. This new method allows data from many more crystals to be measured for comparative analysis, which increases the statistical validity of comparisons.

For STS-37, STS-43, and STS-49, one protein, bovine insulin, was chosen and numerous ground based experiments were performed over a period of nine months prior to each flight to optimize the crystal growth conditions in the flight hardware. For STS-37, crystals were grown by slowly lowering the temperature of the R/IM from 40° C to 22° C in six equal steps over a period of 24 hours. For STS-43 a steeper temperature gradient was created by lowering the temperature from 40° C to 22° C in one step immediately upon achieving orbit. On STS-49, the new programmable C-R/IM was utilized to slowly decrease the temperature from 40° C to 22° C at a rate of 0.2° C per hour.

From the numerous ground based studies, a protein concentration that remains soluble at 40° C but becomes supersaturated at 22° C was chosen, thereby creating conditions conducive to nucleation and crystal growth. The hardware performed flawlessly for all three space shuttle flights producing many crystals which were significantly larger than their earth-grown counterparts. For example, morphometric analysis of crystals from the 500 ml container revealed average values for the length of the space crystals from STS-37 that were 10.26 times as long as crystals obtained from numerous ground control experiments (figure 12) utilizing the flight hardware. Figure 13 shows a comparison of earth-grown and space-grown insulin crystals obtained from the 500 ml bottle on STS-37. X-ray analysis of several crystals from space compared with an equal number of the best crystals obtained from numerous ground control experiments showed a significant improvement in resolution for the space crystals. Effective resolution improvements of about 0.4 Å were observed for the larger space-grown crystals. Figure 14 shows a comparison of 7 space-grown crystals with 7 earth-grown crystals where an attempt has been made to choose only those space-grown crystals that are of comparable volume to the earth-grown crystals. It is significant that, in spite of this, the space-grown crystals exhibited intensities that were 3 times that seen for the ground-grown crystals.

On STS-52, crystals of α-interferon were grown that were approximately 3 times the volume of the largest crystals ever grown on earth. The detailed x-ray analysis of these crystals is in progress and will be reported at a later date.

Conclusion:

In conjunction with co-investigators from universities and pharmaceutical companies and with scientists and engineers from the Marshall Space Flight Center, this laboratory has conducted protein crystal growth experiments utilizing vapor diffusion and/or temperature induced crystallization on 16 different space shuttle missions. These initial experiments have demonstrated that the microgravity environment present on the space shuttle can be beneficial for protein crystal growth experiments. From these few space experiments there are several examples where crystals were grown that were larger, displayed more uniform morphologies, and/or yielded diffraction data to significantly higher resolutions than the best crystals of these proteins grown on earth by any method. Resolution enhancements have ranged anywhere from 0.1 Å to as much as 0.8 Å for the space-grown crystals. It should be noted that in several cases where significant resolution enhancements occurred, the space-grown crystals were much smaller in volume than the earth crystals with which they were being compared. This strongly indicates that the space-grown crystals were more highly ordered at the atomic level. However, in those cases where space-grown crystals were larger, increases in size as much as an order of magnitude were observed with x-ray diffraction resolution enhancements in excess of 0.5 Å. The success rate in the VDA is approximately 20%, which is quite remarkable when it is realized that most investigators are allotted 5

experimental chambers for each protein. These space results are then compared with the best crystals ever produced on the ground using any crystallization method (usually thousands of crystallization conditions were tested on the ground to optimize the crystal growth conditions).

Temperature induced crystallization was performed on 4 separate shuttle flights with a 100% success rate. Crystals from all 4 shuttle flights were significantly (2 times to as much as 10 times) larger than the best of their earth-grown counterparts. In addition, x-ray diffraction data for the space-grown crystals showed significant improvements.

These initial experiments have been instrumental in allowing this co-investigator group to better understand the possible benefits of this unique environment. Results from the VDA have led to the development of a second generation of hardware to perform vapor diffusion experiments. This hardware will provide a number of advantages over the existing hardware including: 1.) thorough mixing of the protein and precipitant samples on orbit; 2.) real time video monitoring capabilities; 3.) precise control over equilibration rates. In addition, results utilizing temperature induced crystallization in the PCF hardware are encouraging. New hardware is being developed that will accommodate microliter quantities of protein for both temperature induced and batch crystallization methods.

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Figure Legend:

Figure 1: Vapor diffusion experiment chamber - a.) The chamber consists of two polysulfone halves, one containing protein and one containing reservoir solution. b.) The two sections are positioned with respective opposing chambers aligned for activation of the experiment. The experiments are deactivated by rotating the upper half 180°, thereby sealing all chambers on a neoprene gasket.

Figure 2: Bottles and dispensing syringe (one syringe is shown with needle puncturing septum of bottle) - Two bottle and syringe sizes were used:
Bottles: 20 ml volume (for buffer & precipitating solutions
1 ml volume (for protein solution)
Syringes: 1 ml syringe (for preparation of reservoir solutions)
0.25 μl syringe (for preparation of protein droplet)

Figure 3: Refrigerator/Incubator module with containment tubes - The tubes are stored in a tray that slides out of the incubator to provide easy access.

Figure 4: x-y-z translation stage with micromanipulator - A fiber optic bundle provides back lighting for experiment preparation and crystal observation.

Figure 5: Protein crystallization facility (PCF) - Four polysulfone cylinders of different sizes are sealed at one end by an aluminum cap. The cylinders are surrounded by an insulating pocket of air and a second larger cylinder which provides a second level of containment.

Figure 6: Comparison of diffraction data for space-grown (▲) (in solution), earth-grown (♦) (in gels) and earth-grown (•) (in solution) crystals of Human Serum Albumin. The y-axis shows the data with average $I / \sigma (I)$ greater than various multiples of sigma.

Figure 7: Crystal of malic enzyme grown in space. This space-grown crystal, although only 1/4 the volume of the best earth-grown crystal, produced a significant enhancement in the quality and resolution limit of the diffraction data (see figures 8a and 8b).

Figure 8a: Comparison of diffraction intensity data for space-grown (▲) and earth-grown (•) crystals of malic enzyme. The y-axis shows the fraction of data with $I / \sigma (I) \geq 5$.

Figure 8b: Relative Wilson plot comparing space-grown and earth-grown crystals of malic enzyme. $s = \sum Fa^2 / \sum Fb^2$, where a = space-grown and b = earth-grown crystal.

Figure 9: Space-grown crystals of bovine prolyl-isomerase. Crystals as large as 3.0 mm long by 1.0 mm thick were obtained from the VDA used on the USML-1 mission.

Figure 10: Comparison of diffraction intensity data for space-grown and earth-grown crystals of prolyl isomerase. The y-axis shows the fraction of data with $I / \sigma (I) \geq 5$.

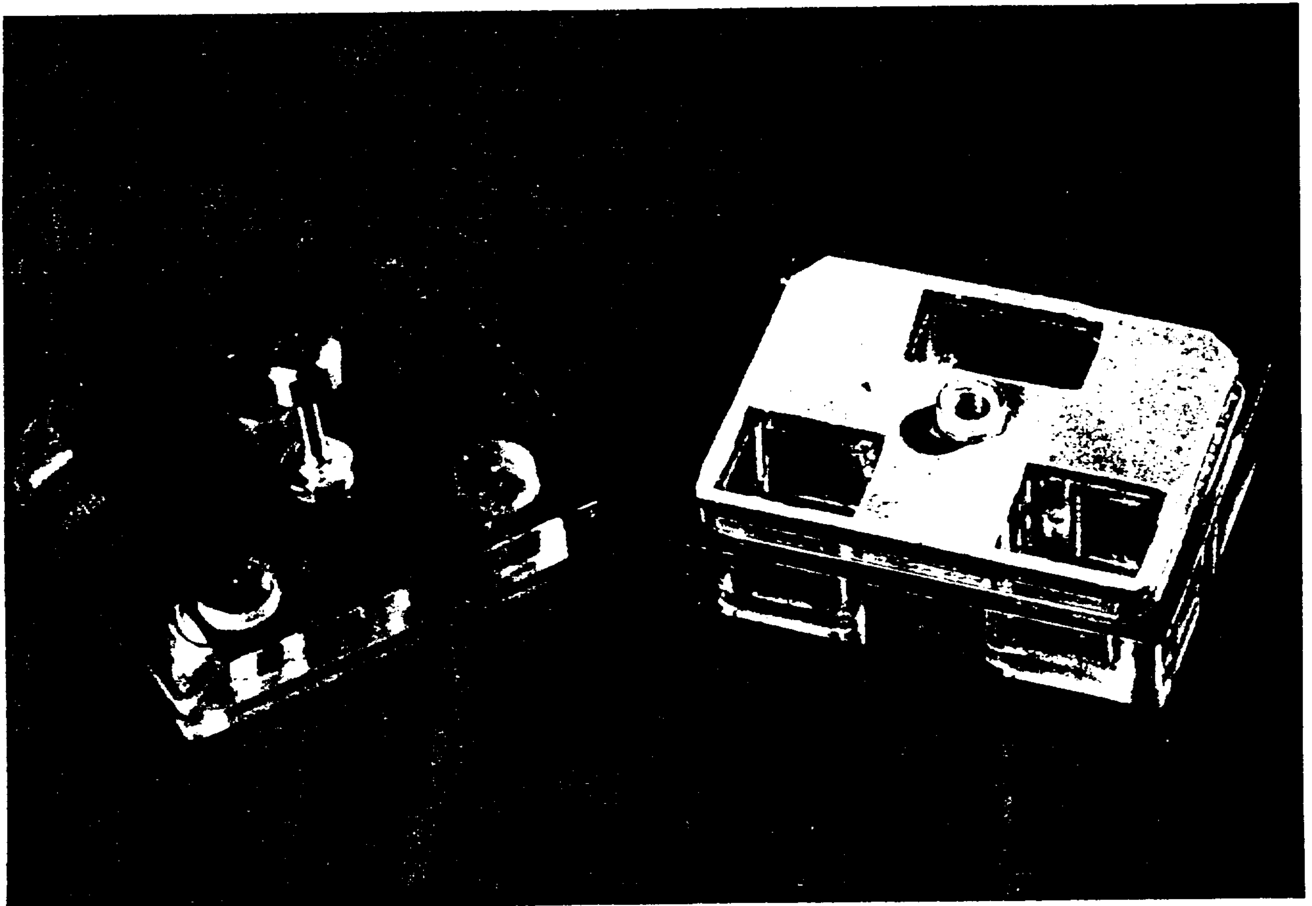
Figure 11: Relative Wilson plot comparing space-grown and earth-grown crystals of HIV-1 reverse transcriptase complexed to a monoclonal antibody and 19/18 base-paired DNA fragment. $s = \sum Fa^2 / \sum Fb^2$ where a = space-grown and b = earth-grown crystal.

Figure 12: Morphometric analysis comparing space-grown crystals and earth-grown crystals for shuttle flights STS-37, STS-43 and STS-49. Plot shows size of crystals as a function of PCF container volume (four different sized containers were used: 500 ml, 200 ml, 100 ml and 50 ml).

Figure 13: Comparison of earth-grown and space-grown crystals of bovine insulin using temperature induced crystallization technique. This photo is typical of the difference in size seen for the 500 ml container used in flight and ground experiments for STS-37.

Figure 14: Comparison of x-ray diffraction data for earth-grown crystals and space-grown crystals for STS-49. The mean intensity (•) for the earth-grown crystals is compared with the

mean intensity of space-grown (▲) crystals specifically chosen to be approximately equal in size. The difference in intensity is even more dramatic when larger space-grown crystals are used.



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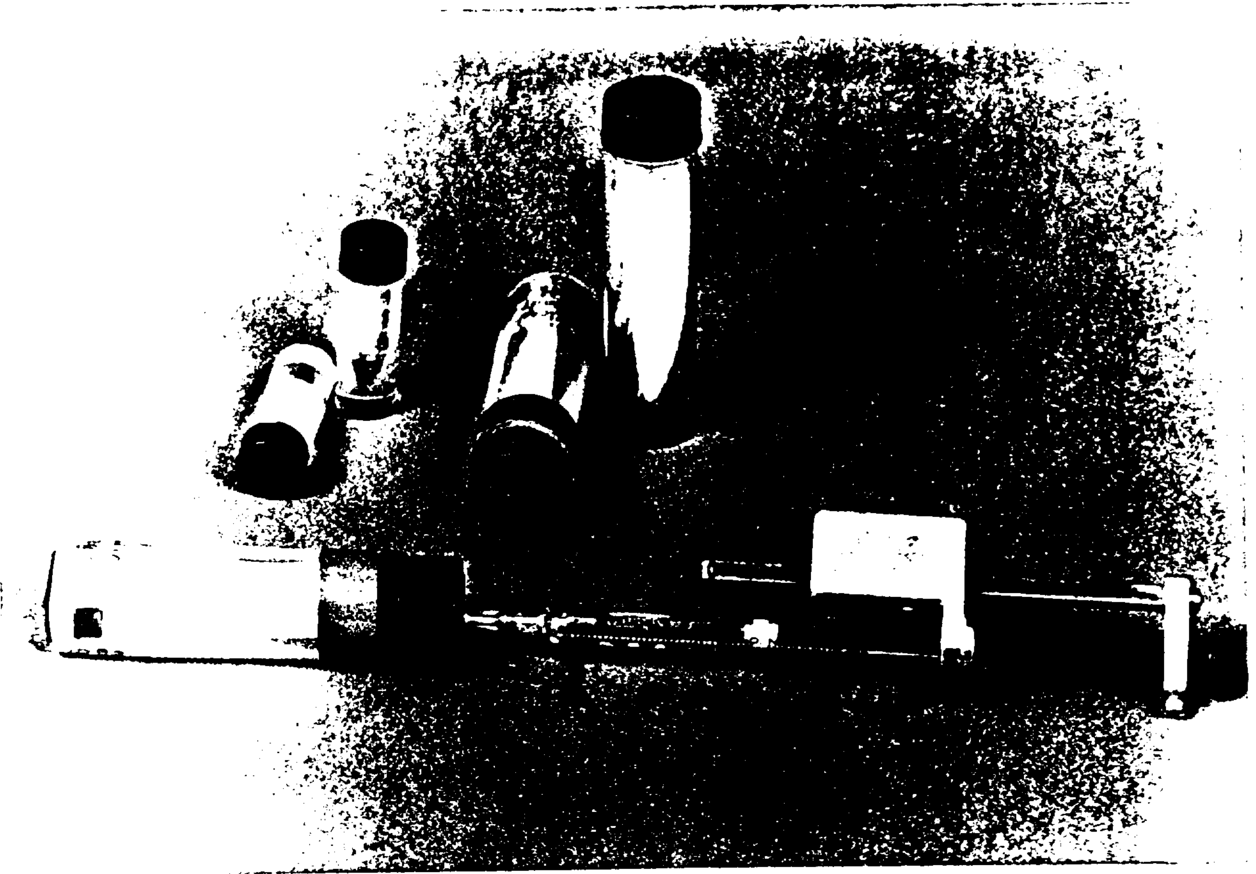

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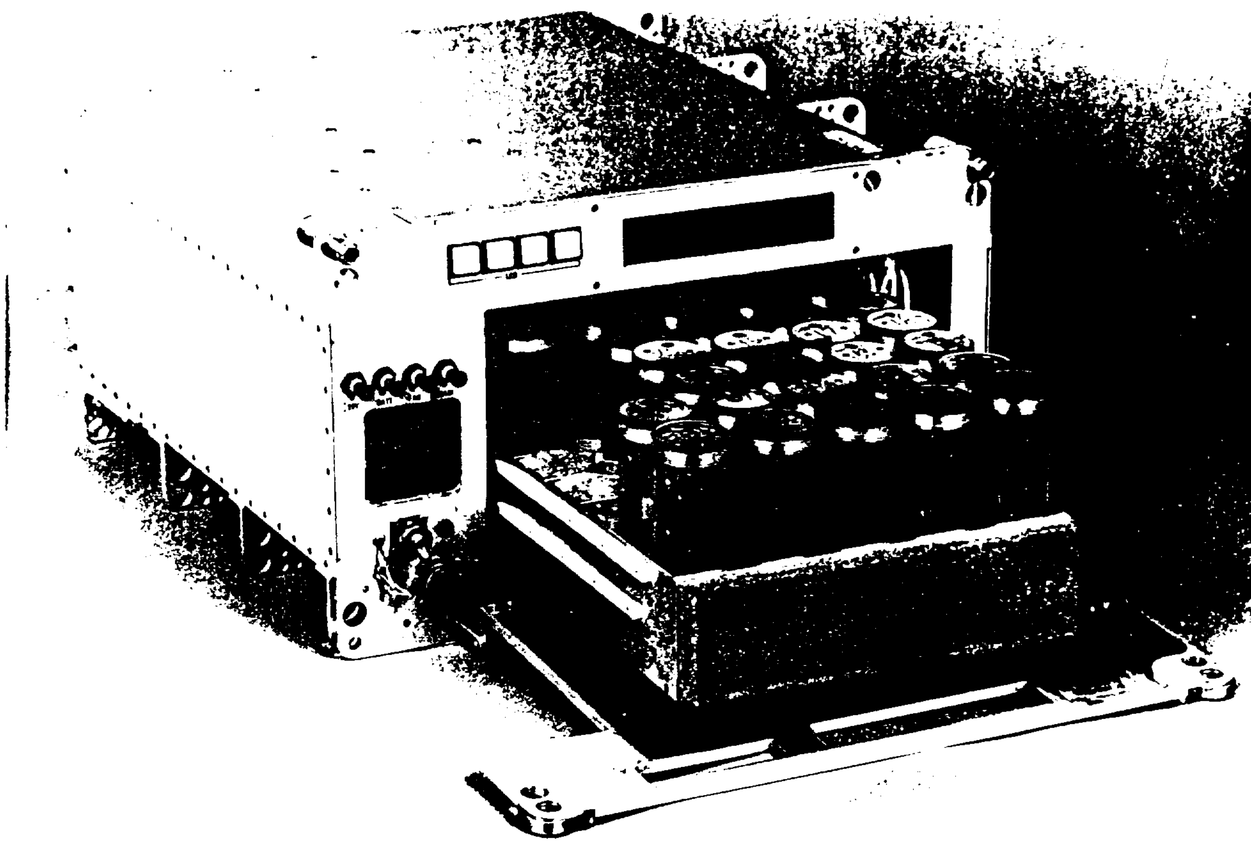

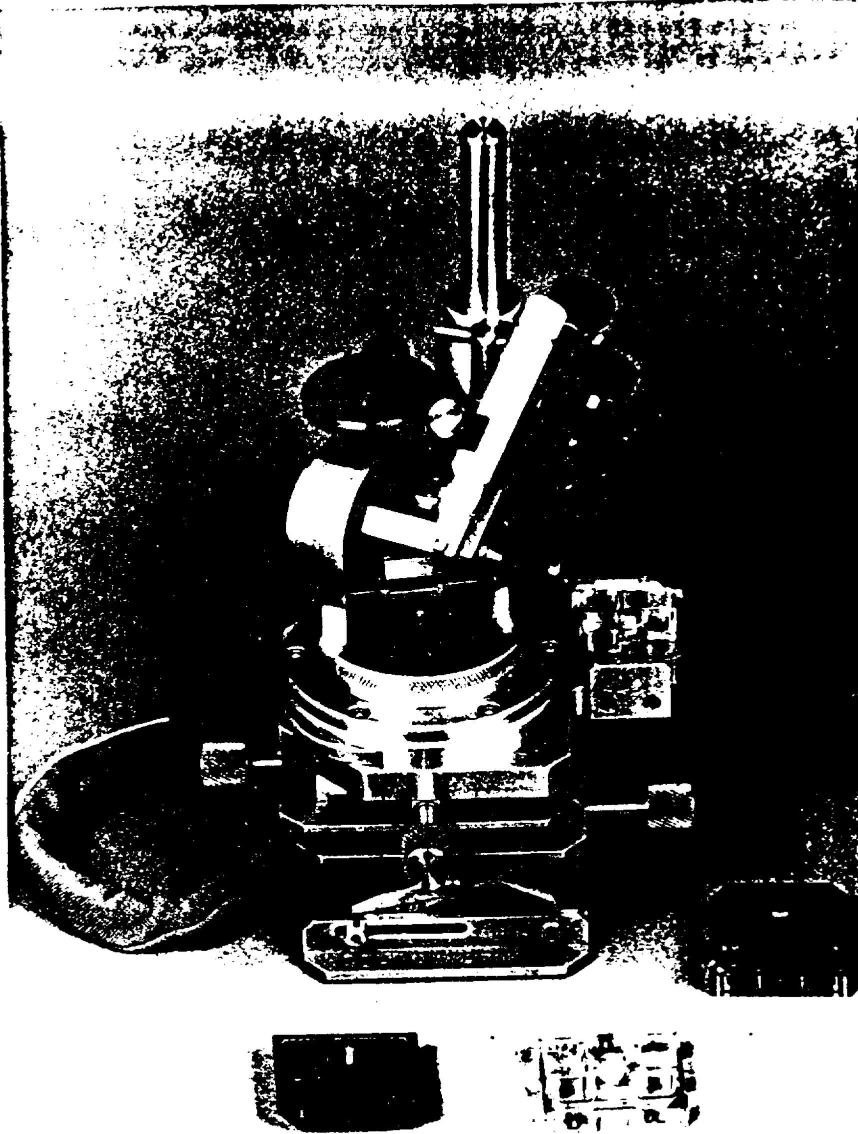

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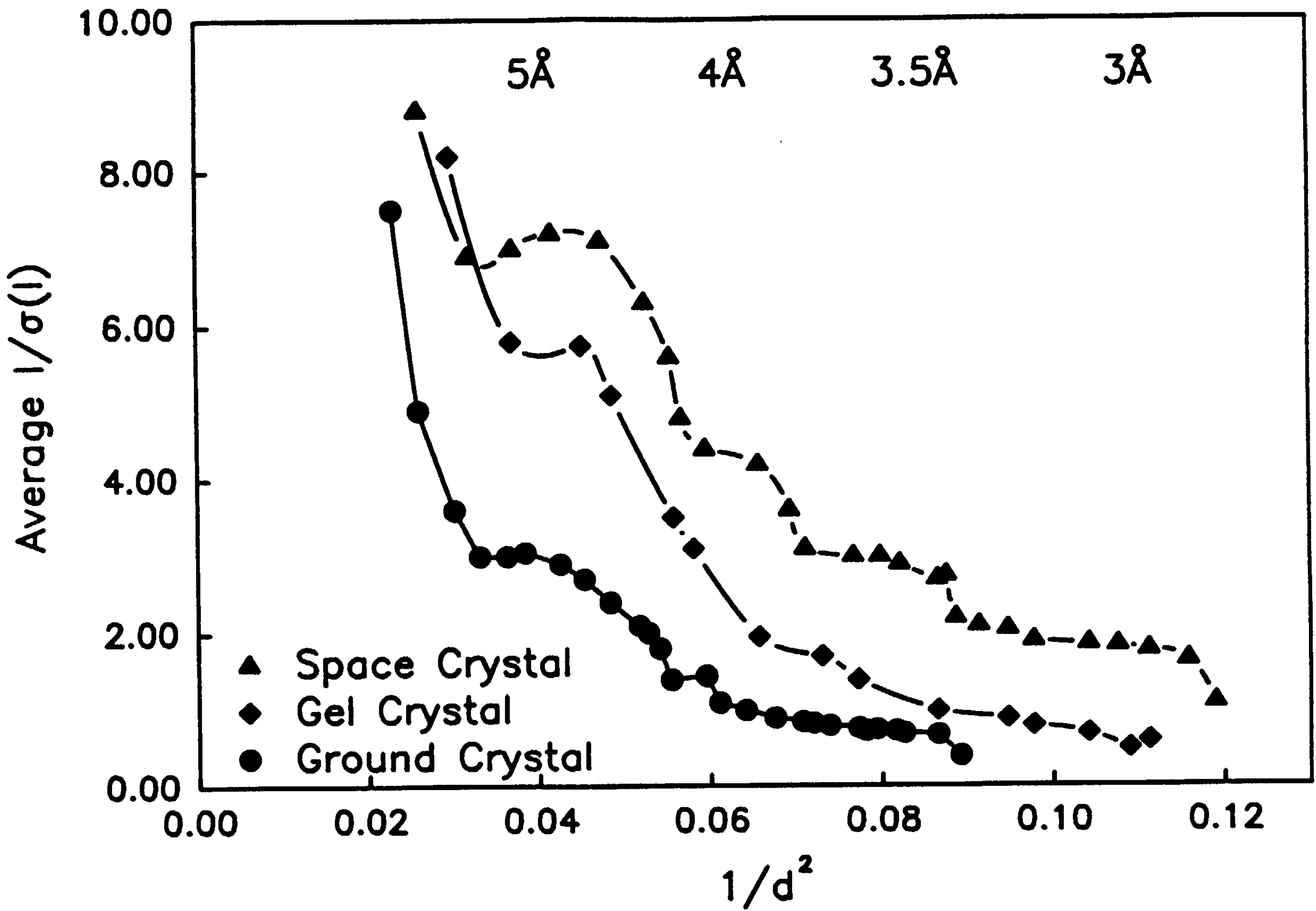
10.00
8.00
6.00
4.00
2.00
0.00
Average I/σ(I)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
1/d²
5Å
4Å
3.5Å
3Å
Space Crystal
Gel Crystal
Ground Crystal

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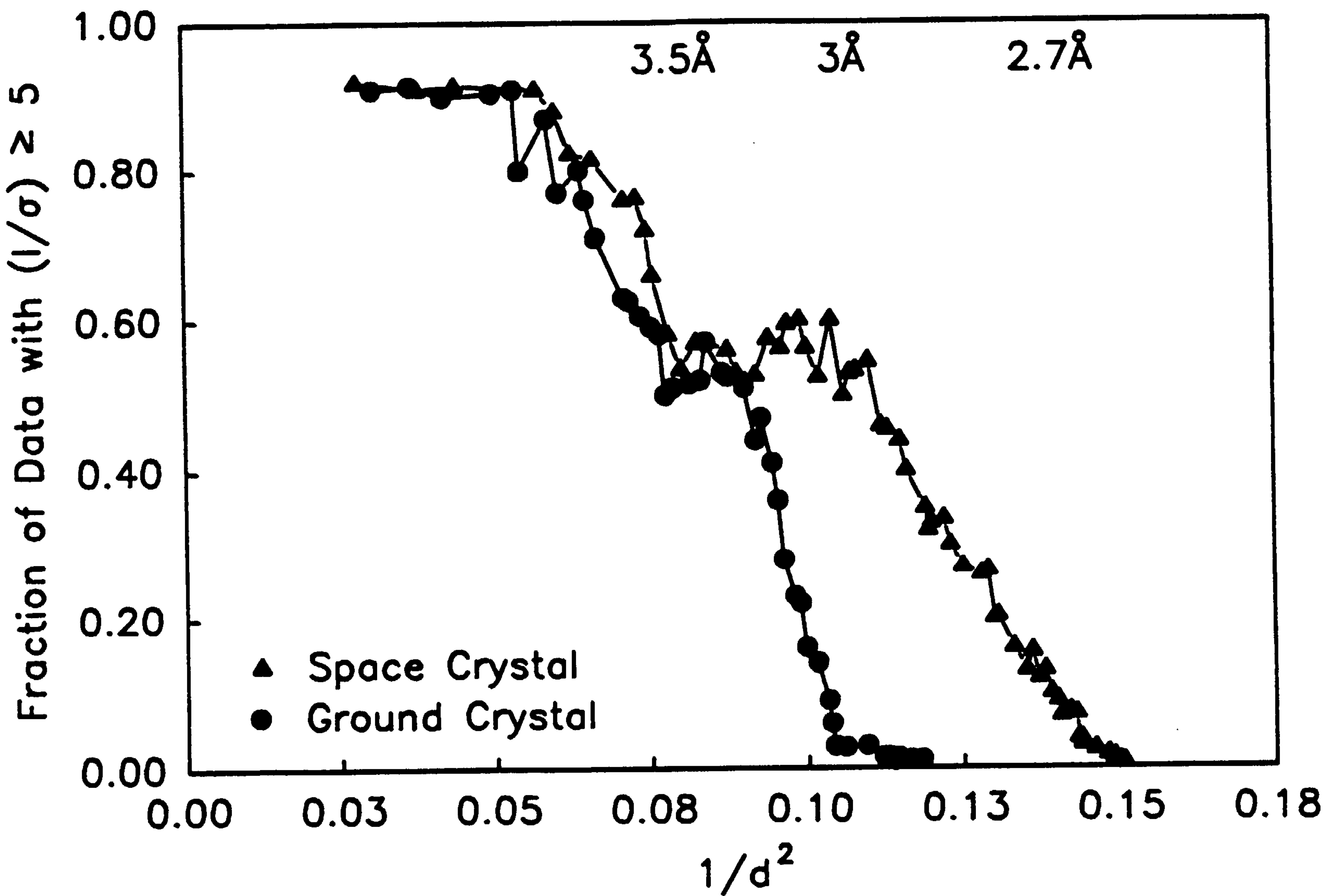
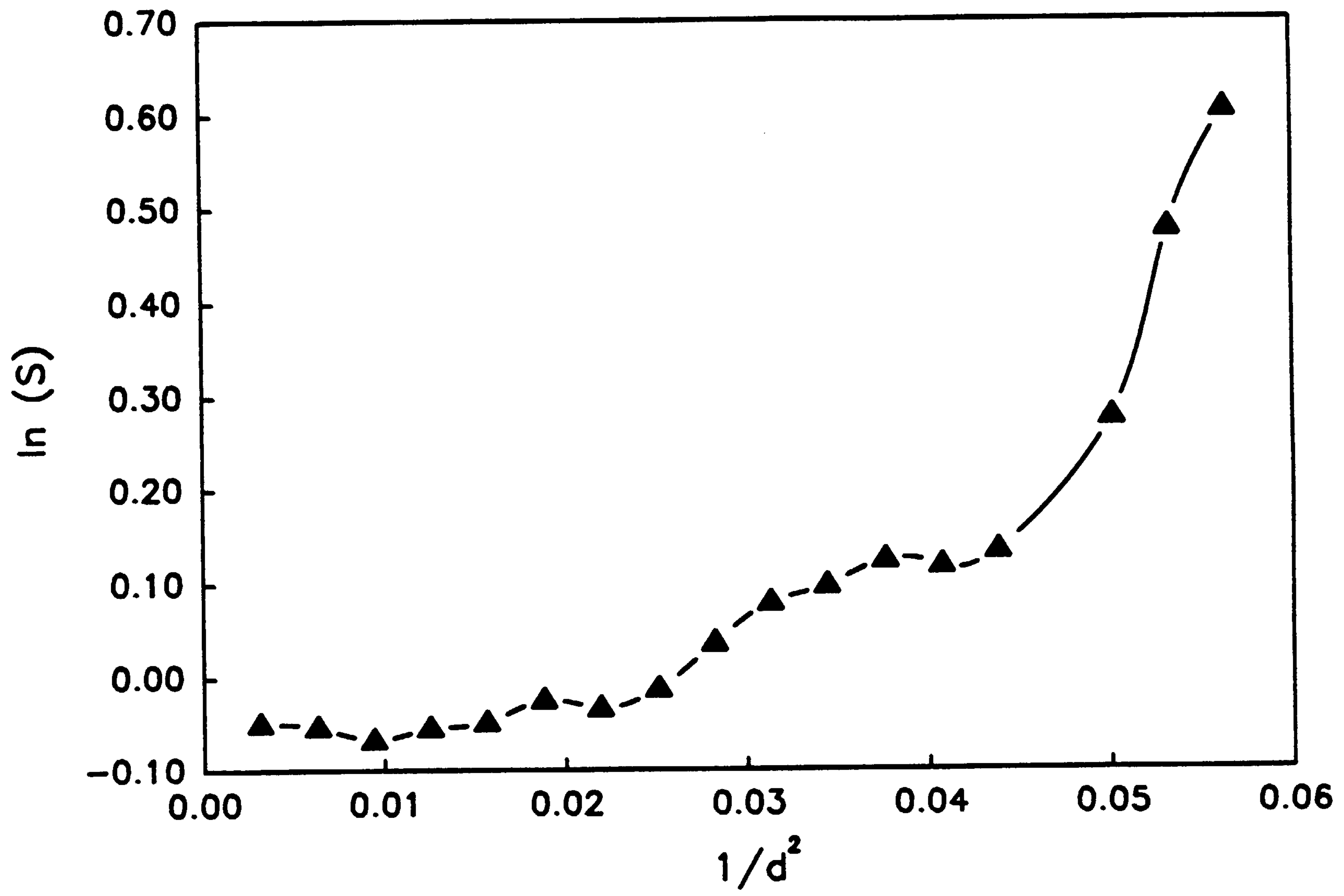

3.5Å
3Å
2.7Å
Fraction of Data with (I/σ) ≥ 5
1.00
0.80
0.60
0.40
0.20
0.00
Space Crystal
Ground Crystal
0.00
0.03
0.05
0.08
0.10
0.13
0.15
0.18
1/d²


0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
−0.10
ln (S)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
1/d²

ORIGINAL PAGE IS
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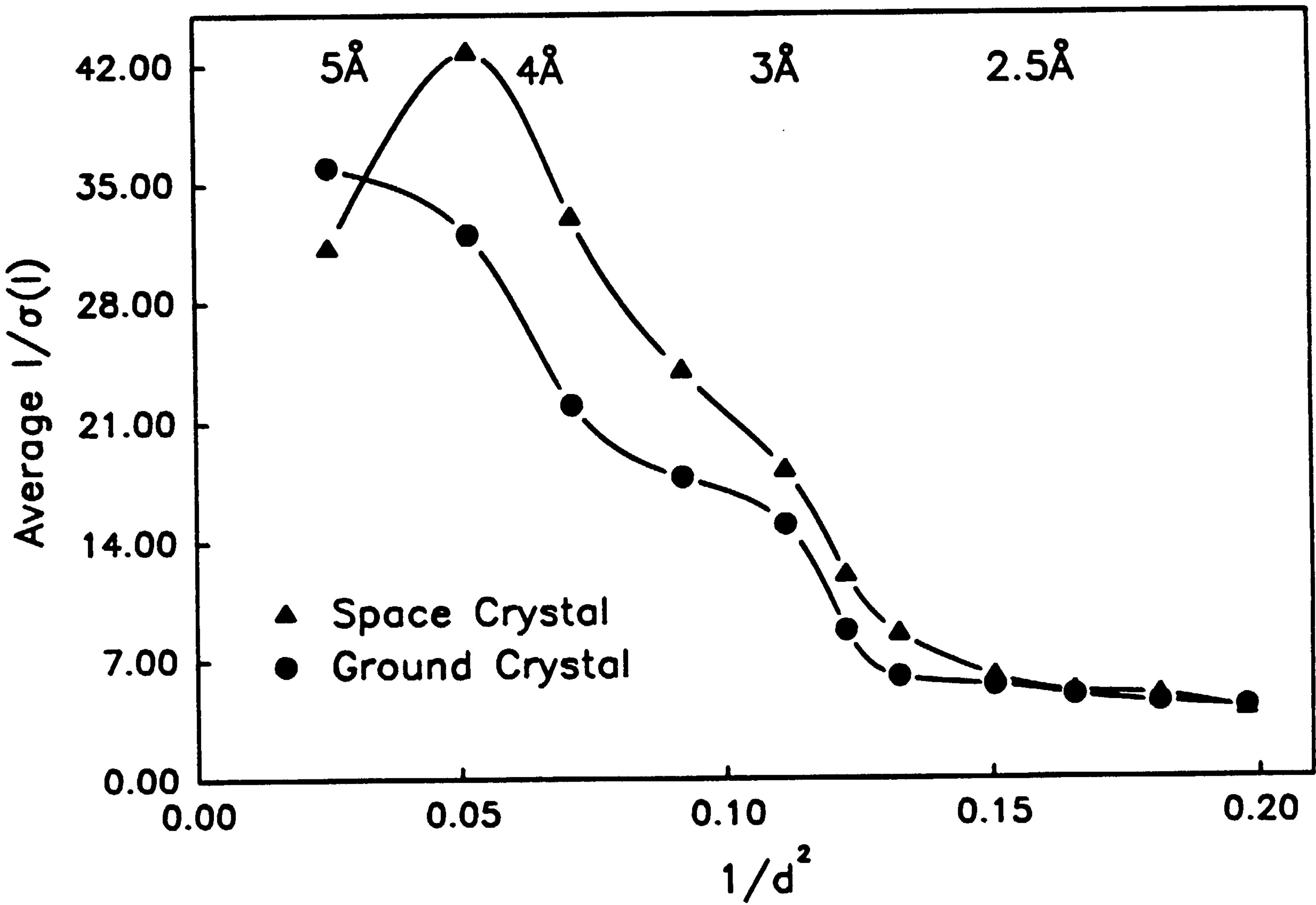
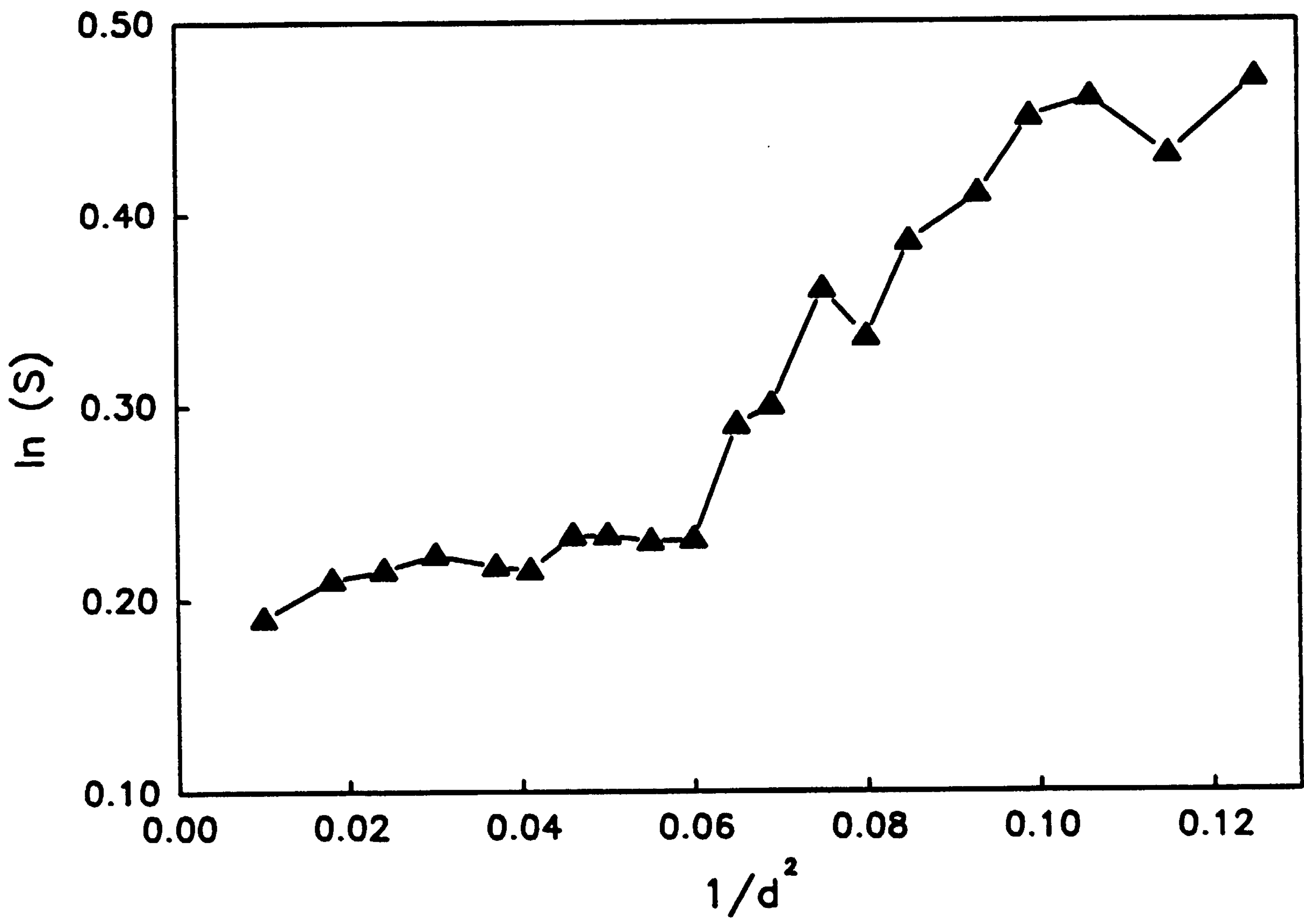
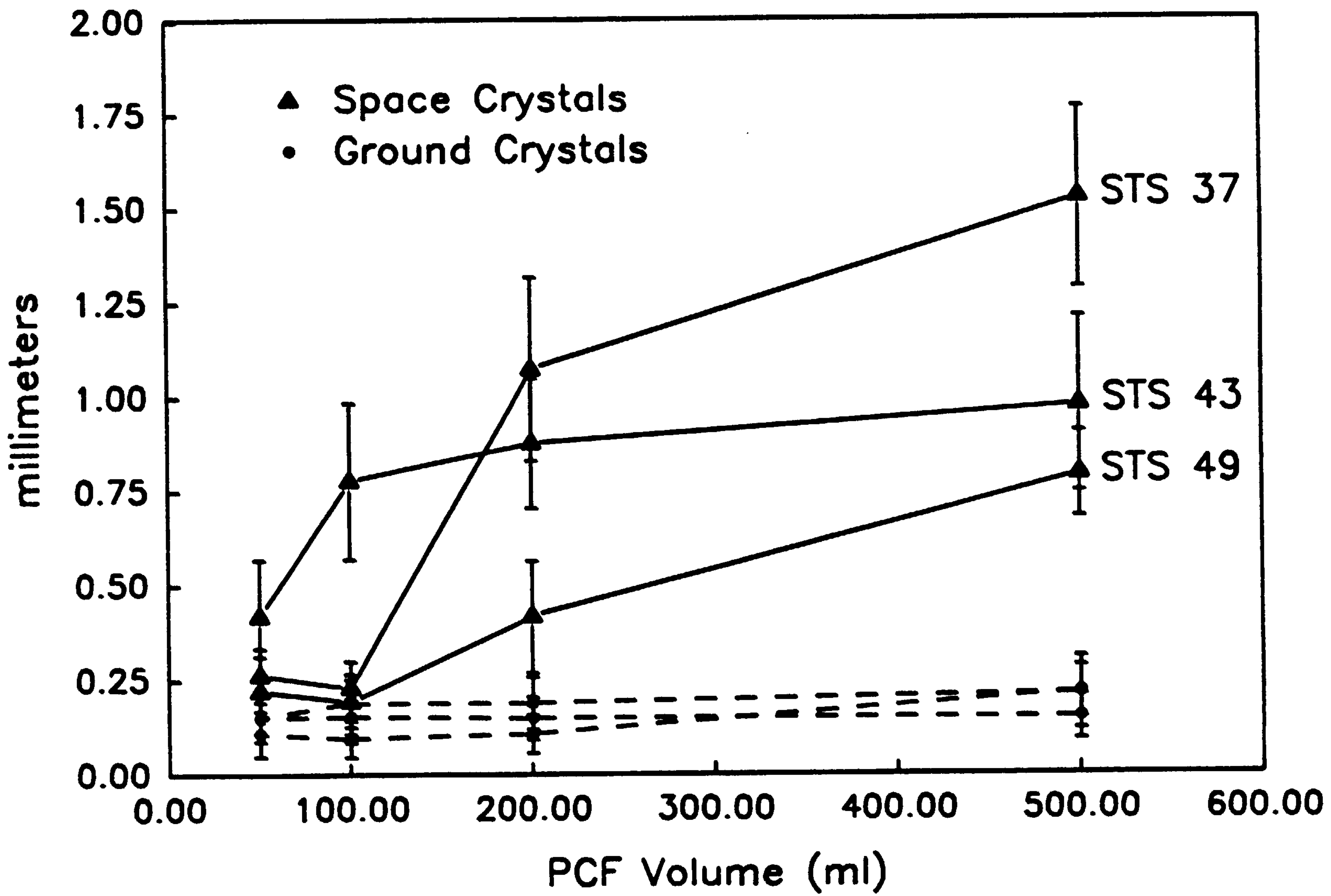
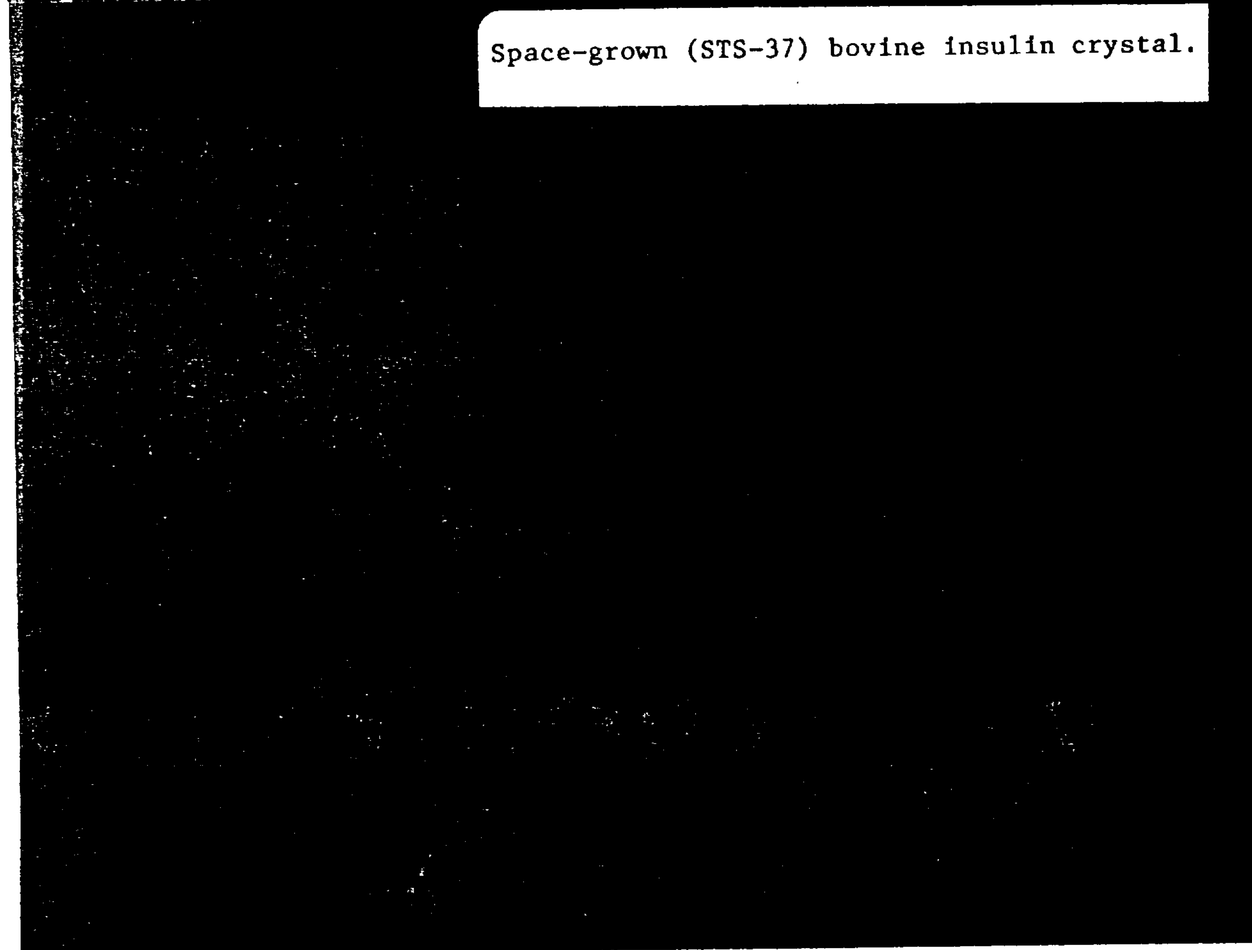

5Å
4Å
3Å
2.5Å
42.00
35.00
28.00
21.00
14.00
7.00
0.00
Average I/σ(I)
Space Crystal
Ground Crystal
0.00
0.05
0.10
0.15
0.20
1/d²


0.50
0.40
0.30
0.20
0.10
ln (S)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
1/d²


Space Crystals
Ground Crystals
STS 37
STS 43
STS 49
millimeters
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.00
100.00
200.00
300.00
400.00
500.00
600.00
PCF Volume (ml)

Earth-grown bovine insulin crystals.



Space-grown (STS-37) bovine insulin crystal.

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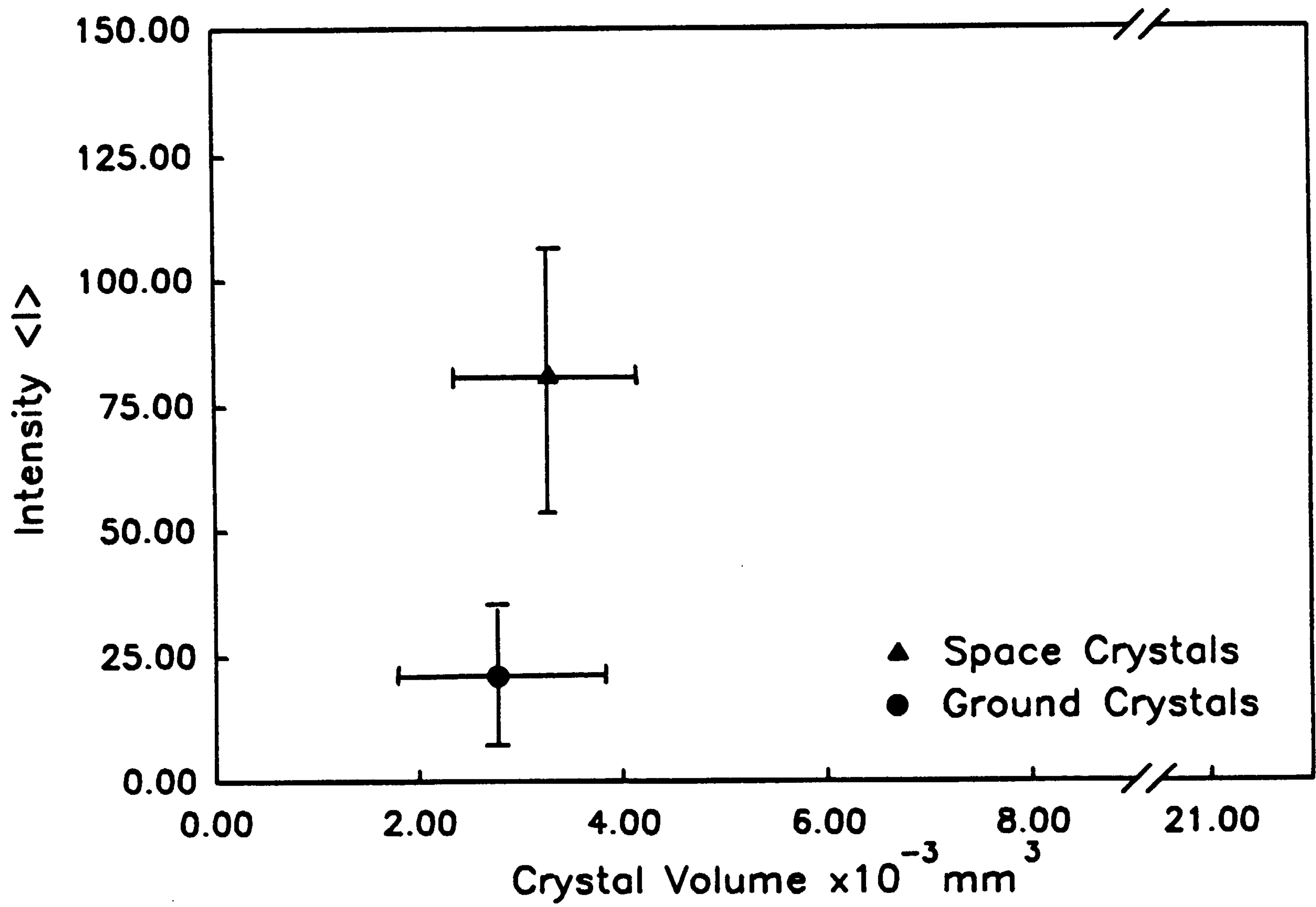
Intensity <I>
150.00
125.00
100.00
75.00
50.00
25.00
0.00
0.00
2.00
4.00
6.00
8.00
21.00
Crystal Volume $\times 10^{-3}$ mm^3
Space Crystals
Ground Crystals