



National Aeronautics and  
Space Administration

December 22, 1997

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NRA-97-HEDS-02

# RESEARCH ANNOUNCEMENT

## Microgravity Biotechnology: Research and Flight Experiment Opportunities

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Letters of Intent Due: February 20, 1998

Proposals Due: ..... April 7, 1998



**MICROGRAVITY BIOTECHNOLOGY:  
RESEARCH AND FLIGHT  
EXPERIMENT OPPORTUNITIES**

NASA Research Announcement  
Soliciting Research Proposals  
for the Period Ending  
April 7, 1998

NRA-97-HEDS-02  
Issued: December 22, 1997

Office of Life and Microgravity Sciences and Applications  
Human Exploration and Development of Space (HEDS) Enterprise  
National Aeronautics and Space Administration  
Washington, DC 20546-0001



**NASA RESEARCH ANNOUNCEMENT  
MICROGRAVITY BIOTECHNOLOGY:  
RESEARCH AND FLIGHT EXPERIMENT OPPORTUNITIES**

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**NASA RESEARCH ANNOUNCEMENT**

**MICROGRAVITY BIOTECHNOLOGY:  
RESEARCH AND FLIGHT EXPERIMENT OPPORTUNITIES**

This NASA Research Announcement (NRA) solicits proposals to conduct scientific investigations in the discipline of microgravity biotechnology. These investigations may involve flight experiments or ground-based experimental and theoretical research intended to support the study of biotechnology using the low-gravity environment of space. Further descriptions of the microgravity biotechnology research activities are given in Appendix A.

Investigations selected for flight experiment definition must successfully complete a number of subsequent development steps, including internal NASA and external peer review of the flight experiment, in order to be considered for a flight assignment. NASA does not guarantee that any investigation selected for definition will advance to flight experiment status. Investigations selected for support as ground-based research under the Microgravity Research Division (MRD) generally must propose again to a future solicitation in order to be selected for a flight opportunity.

Participation is open to U.S. and foreign investigators and to all categories of organizations: industry, educational institutions, other nonprofit organizations, NASA centers, and other U.S. Government agencies. **Though NASA welcomes proposals from non-U.S. investigators, NASA does not fund principal investigators at non-U.S. institutions** (See Appendix A). Proposals may be submitted at any time during the period ending April 7, 1998. Late proposals will be considered if it is in the best interest of the Government. Proposals will be evaluated by science peer reviews and engineering feasibility reviews. It is planned for selections to be announced by September 1998.

Appendices A and B provide technical and program information applicable only to this NRA. Appendix C contains general guidelines for the preparation of proposals solicited by an NRA.

This Announcement will not comprise the only invitation to submit a proposal to NASA for access to the reduced-gravity environment and is part of a planned sequence of solicitations inviting proposals in the various disciplines of the microgravity program.

<b>NASA Research Announcement Identifier:</b>	<b>NRA-97-HEDS-02</b>
<b>NRA Release Date:</b>	<b>December 22, 1997</b>
<b>Letters of Intent Due:</b>	<b>February 20, 1998</b>
<b>Proposals Due:</b>	<b>April 7, 1998</b>

This NRA is available electronically from and Letters of Intent may be submitted electronically via the Microgravity Research Division Web Page at:

**<http://microgravity.msad.hq.nasa.gov/>**

Alternatively, Letters of Intent may be submitted via e-mail to **loi@hq.nasa.gov**  
If electronic means are not available, you may mail Letters of Intent to the address given below.

Proposals are to be submitted to the following address:

Dr. Steve Davison  
c/o Information Dynamics Inc.  
Subject: NASA Research Proposal (NRA-97-HEDS-02)  
300 D Street SW, Suite 801  
Washington, DC 20024  
Telephone for delivery services: (202) 479-2609

**NASA cannot receive proposals on Saturdays, Sundays, or federal holidays.**

Proposal copies required: 15

Proposers will receive a postcard confirming receipt of proposal within 10 working days of the due date.

Obtain Programmatic Information about this NRA from:

Dr. Steve Davison  
Enterprise Scientist for Biotechnology  
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Selecting Official: Director  
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Office of Life and Microgravity Sciences and Applications  
NASA Headquarters

Your interest and cooperation in participating in this effort are appreciated.

Arnauld E. Nicogossian, M.D.  
Associate Administrator  
Life and Microgravity Sciences and Applications

## Technical Description

### **MICROGRAVITY BIOTECHNOLOGY: RESEARCH AND FLIGHT EXPERIMENT OPPORTUNITIES**

#### **I. INTRODUCTION**

##### **A. BACKGROUND**

The Human Exploration and Development of Space (HEDS) Enterprise, one of four National Aeronautics and Space Administration (NASA) strategic enterprises, conducts a program of basic and applied research using the reduced-gravity environment to improve the understanding of fundamental physical, chemical, and biological processes. The scope of the program, sponsored by the Microgravity Research Division (MRD), ranges from applied research into the effects of low gravity on the processing of various materials, to basic research that uses low gravity to create test conditions to probe the fundamental behavior of matter. This announcement is part of an ongoing effort to develop research in a single specific scientific discipline, Microgravity Biotechnology. The Division last released a NASA Research Announcement (NRA) for Microgravity Biotechnology in 1996 and expects to continue to release NRA's in this discipline approximately every two years.

In the MRD, an extensive research program has been used to gain a preliminary understanding of phenomena, and to define experiments to be conducted in the extended low gravity test times available in spacecraft in low-Earth orbit. MRD is developing multiple instruments for the conduct of biotechnology research offering improved control and diagnostic capabilities relative to earlier experiments. MRD also anticipates limited near-term flight opportunities for investigations capable of making use of existing hardware where no or minor modifications would be required.

MRD is preparing for flight opportunities using International Space Station research instruments, including development of modular research instruments that can be configured (or reconfigured) to accommodate multiple experiments and multiple users. This is envisioned as an evolutionary program with the objectives of providing experimental data in response to increasingly sophisticated science requirements and of permitting the evolution of experimental approaches and technologies as needed for scientific investigations throughout the era of the International Space Station. This announcement is being released as part of a coordinated series of discipline-directed solicitations intended to span the range of the MRD program. Other MRD-supported solicitations planned for periodic release over the next several years encompass the areas of combustion science, fluid physics, fundamental physics, and materials science.

The scope of this research announcement does not include research dealing with the response of living organisms to weightlessness, an area which is the focus of an ongoing program in the Life Sciences Division.

## B. RESEARCH ANNOUNCEMENT OBJECTIVES

The biotechnology program seeks a coordinated research effort involving both space- and ground-based research. This NRA has the objective of broadening and enhancing the microgravity biotechnology program through the solicitation of:

1. Experimental studies which require the space environment to test clearly posed hypotheses, using existing or slightly modified instruments in space-based experiments to increase the understanding of biotechnology;
2. Experiment concepts which will define and utilize new instruments for space-based experiments in biotechnology; and
3. Ground-based theoretical and experimental studies which will lead to the definition or enhance the understanding of existing or potential flight experiments in biotechnology, with emphasis on research leading to technologies required by future human space missions.

Further programmatic objectives of this NRA include objectives broadly emphasized by the civil space program, including: the advancement of economically significant technologies; technology infusion into the private sector; and enhancement of the diversity of participation in the space program, along with several objectives of specific importance to the microgravity science research program. These latter objectives include the support of investigators in early stages of their careers, with the purpose of developing a community of established researchers for the International Space Station and other missions in the next 10-20 years, and the pursuit of microgravity research that shows promise of contributing to economically significant advances in technology.

In support of the HEDS Enterprise, individuals participating in the MRD Program are encouraged to help foster the development of a scientifically informed and aware public. The MRD Program represents an opportunity for NASA to enhance and broaden the public's understanding and appreciation of the value of research in the microgravity environment of Space. Therefore, all participants in this NRA are strongly encouraged to promote general scientific literacy and public understanding of the microgravity environment and microgravity biotechnology through formal and/or informal education opportunities. Where appropriate, supported investigators will be required to produce, in collaboration with NASA, a plan for communicating to the public the value and importance of their work.

## C. DESCRIPTION OF THE ANNOUNCEMENT

NASA's Biotechnology Program supports the development of new research and technology under the HEDS Enterprise. The biotechnology program promotes U.S. competitiveness and insures NASA leadership in providing cutting edge research and technologies for space missions. With this NRA, NASA is soliciting proposals to conduct research in microgravity biotechnology, including experimental efforts sufficiently mature to justify near-term flight development. The goals of the discipline along with several research areas of interest are described in Section II. Proposals describing innovative low-gravity biotechnology research beyond that described there are also sought.

The biotechnology program seeks a balanced research effort involving both space- and ground-based research. Ground-based research forms the foundation of this program, providing the necessary experimental and theoretical frameworks for development of rigorous understanding of basic biotechnological phenomena. This research can eventually mature to the point where it

becomes the focus of a well-defined flight experiment. Ground-based research efforts are generally supported for a period of four years. However, shorter periods of support may be appropriate for proposals that are more exploratory in nature.

NASA is currently developing several types of flight instruments for microgravity biotechnology research. Brief descriptions of the planned capabilities are given in Appendix B. NASA anticipates future flight opportunities for investigations with requirements which can be met by existing apparatus with only minor modifications. Successful proposals for use of the existing apparatus will be funded for advanced definition studies which will produce a detailed Science Requirements Document (SRD). Authorization to proceed into flight development is contingent upon successful peer review of the experiment and SRD by both science and engineering panels. NASA does not guarantee that any experiment selected for definition which plans to use existing hardware will advance to flight experiment status.

NASA also encourages submission of experimental proposals for which none of the existing flight instruments is appropriate. NASA anticipates the development of new biotechnology research experiment apparatus and diagnostic tools for the International Space Station. Descriptions of possible future capabilities can also be found in Appendix B. These hardware descriptions are included as examples to allow researchers to consider the type of capabilities under development that might meet their science requirements. Though researchers should not feel limited by these capabilities, it must be emphasized that experiments calling for equipment significantly outside these envelopes will involve considerable more expense to NASA, a factor which must be taken into consideration in funding decisions.

Selected proposals requiring development of new capabilities (both currently planned ones and ones outside the envelope), will be funded for flight definition studies to define flight experiment parameters and conditions and the appropriate flight hardware. The length of the definition phase will be based on the experiment requirements, but will normally range from 6 to 24 months and will culminate in the preparation of an SRD. Authorization to proceed into flight development is contingent upon successful peer review of the SRD by both science and engineering panels. NASA does not guarantee that any experiment selected for flight definition which requires new instrument development will advance to flight experiment status.

Investigations that do not proceed into flight development will normally be required to submit a proposal for continuation of support at the conclusion of a typical four-year period of funding.

Promising proposals which are not mature enough to allow development of a flight concept within two years of definition may be selected for support in the MRD Research and Analysis (R&A) Program. Investigations selected into the R&A program must generally propose again to a future announcement in order to be selected for a flight opportunity.

## **II. MICROGRAVITY BIOTECHNOLOGY RESEARCH**

### **A. INTRODUCTION**

Use of the microgravity environment has begun to increase our understanding of the biological sciences, and to enable us to develop innovative biotechnological processes that can exploit space. There is abundant practical motivation for advancing biotechnology; it plays a key role in health, agriculture, and many other important economic areas. Advances in biotechnology will benefit a wide range of applications and research areas which depend on biotechnology as a basis for their work.

The NASA biotechnology program has identified protein crystal growth, cell science, and fundamentals of biotechnology as areas which contain promising opportunities for significant advancements through low-gravity experiments. Therefore, under this announcement, NASA is requesting proposals for research in these areas. Innovative proposals in areas of biotechnology not specified in this announcement that show a clear indication that they are affected by low gravity will also be considered.

### **B. MICROGRAVITY BIOTECHNOLOGY GOALS AND DESCRIPTION OF PARTICIPATION**

The biotechnology program seeks a coordinated research effort involving both space- and ground-based research. The overall goal of the biotechnology program of the Microgravity Research Division (MRD) of NASA is to use the low-gravity environment to support novel biotechnology research. The individual goals are 1) to advance the scientific understanding of biotechnology processes affected by gravity, 2) to use low-gravity experiments for insight into the physical behavior of biotechnology processes, 3) to provide the scientific knowledge needed to improve these processes, 4) to contribute to Earth-based systems concerned with biotechnology, and 5) to develop technologies specifically supporting low-gravity experiments and practical aspects in biotechnology.

To accomplish these goals, this research announcement is soliciting two types of proposals for all areas of microgravity biotechnology research:

#### **I) Flight proposals to carry out experimental research in the space environment**

- Experimental studies which require the space environment to test clearly posed hypotheses, using existing or slightly modified instruments in space-based experiments to increase the understanding of biotechnology.
- Experiment concepts which will define and utilize new instruments for space-based experiments in biotechnology.

#### **II) Ground-based experimental and theoretical research proposals**

- Ground-based theoretical and experimental studies which will lead to the definition or enhance the understanding of existing or potential flight experiments in biotechnology.

This research announcement is also soliciting a third type of proposal in the cell science area:

#### **III) Multidisciplinary proposals that can support development programs in advanced rotating vessel bioreactors for tissue culturing research**

## C. CELL SCIENCE AND TISSUE ENGINEERING

### Introduction

Millions of Americans suffer tissue or organ loss from diseases and accidents every year, and the yearly cost of treating these patients exceeds \$400 billion. The major medical treatment for these losses is transplantation of tissues and organs; however, these transplantations are severely limited by donor shortages (2). The shortages of replacement tissue and organs have generated a substantial research effort on the development of alternative sources for transplantations. Improved cultivation of cells and tissues so that the processes of organ failure and organogenesis are better understood may yield better approaches to either avoid pathological organ failure or allow the creation of new replacement tissues and organs for transplantations. While molecular biology has provided critical insight into the pathological processes that cause organ failure, the capacity for renewal of organ function is limited not only by deficits in the knowledge of the molecular biology, but also in the ability to culture cells from normal adult organs and to make them function appropriately outside of the host from which they arose. A major advancement would be the ability to achieve three-dimensional cell propagation resulting in differentiated and functional tissue. Unfortunately, most present day culture systems provide only occasional evidence of cellular diversification and differentiation. Conventional culture techniques attach cells to a planar substratum that produces a two-dimensional monolayer over the surface. While this configuration may optimize mass transfer of oxygen and nutrients to cells, it only supports inadequate two-dimensional intercellular interactions. Recent data from three-dimensional cultures of cells in gels or spheroids have shown that important aspects of normal and neoplastic differentiation are controlled by three-dimensional interactions including the ability to form tubules and to induce resistance to chemotherapy by cancer cells. However, these three-dimensional systems are limited in the number of cells cultured, whereas recovery of cells and factors from gel systems is cumbersome. Thus, there is a need for growing batch cultures in three-dimensions in aqueous media.

NASA's cell science program has focused on the development of rotating vessel bioreactors for the culture of cells using well-controlled process parameters and reduced levels of hydrodynamic stress, thus simulating the low gravity conditions of space to the extent possible on Earth. NASA's bioreactor program was instituted to investigate the problem of cell maintenance and viability in the space environment to support biological experiments. This research found that the stresses to which cells were subjected should be minimized in order to culture cells with a low rate of morbidity. The NASA biotechnology research group applied a clinostat, a cell maintenance system in which the container was rotated, to minimize the shear forces. This method of cell suspension creates shear stresses for small cell aggregates that are significantly smaller than those in conventional, stirred bioreactors. Mammalian cells cultured in this low shear environment aggregate and grow into relatively large masses, and the cultured cells display differentiation markers similar to those found in mammalian tissues (3). Cells have attained populations that are 2 to 4 times more dense than in conventional bioreactors. The advantage of this system is that tissue-like cell densities are suspended in a well-mixed aqueous medium that facilitates nutrient transfer, dispersion of wastes, and also makes possible the isolation of potentially novel factors. In addition, co-cultivation of cancer cells with normal cells produces tissues that mimic the structure of the cancer as it appears in the intact host. Similarly, bone and cartilage cultures have recreated the appearance and much of the strength of the normal tissue. These results suggest that the propagation of cells in a culture system are dependent on a low shear stress environment, spatial co-location of participating cell populations, and matrix materials that promote the morphology of the desired tissue.

In part, the microgravity simulation is due to the continuous free fall of cells through the growth media while the bioreactor rotates. Using the rotating vessel bioreactors, researchers have achieved three-dimensional tissue propagation in an aqueous medium. To further develop this technology and support research in this area, NASA is developing a program to understand the

role of reduced hydrodynamic stress and spatial co-location on mammalian tissue adhesion, proliferation, and eventual differentiation (1). In low earth orbit, the transport of mass to and from cells in culture is altered by the change to a microgravity environment. In addition, stresses on cells and tissues *in situ* due to the forces of buoyancy and fluid flows are altered in microgravity. In order to understand the changes in cell and tissue culture development occurring on orbit, it is necessary to quantitatively measure or estimate the changes in transport of material between the cells and media and to understand the changes in forces on the cells. Likewise in ground-based studies designed to identify how the microgravity environment may be used for cell and tissue cultures research a quantitative understanding of transport and stress is an important aspect of the overall program effort.

Ground-based research studies have demonstrated that both normal and neoplastic cells and tissues recreate many of the characteristics in the NASA bioreactor that they display *in vivo* (4-6). Proximal renal tubule cells that normally have rich apically oriented microvilli with intercellular clefts in the kidney do not form any of these structures in two-dimensional monolayer culture. However, when normal proximal renal tubule cells are cultured in three-dimensions in the bioreactor, both the microvilli and the intercellular clefts form. Similar results in recreating normal structures have occurred in bone and cartilage cultures. Cultivation of small intestinal enterocytes has been difficult in conventional culture systems, but human small bowel intestinal cells have been cultivated in the bioreactor successfully for over 2 months (7). In other studies with malignant cells, investigators have been able to recreate the morphologic appearance of metastases to liver by human colon carcinoma (8), to bone by human prostate carcinoma cells, or to various organs by other carcinomas. These studies have generally involved co-cultivation of the malignant cell with the normal cells that form the scaffolding of these organs and have recreated the three-dimensional shape of the cancerous tissue. This is important because, when the morphology is recreated, the function is more likely also to be rejuvenated. This is born out by related studies by Kerbel and co-workers (9, 10) who have recently found that three-dimensional cultures of many different types of malignant cells are resistant to chemotherapy that normally kills these tumor cells in two-dimensional monolayer culture. Thus, the ground-based studies with malignant cells that recreate the morphological appearance and behavior of metastases in an animal or man may provide a good test for the development of new therapeutic agents as well as improving our understanding of how prior chemotherapy might be made more effective.

Ground-based studies with the bioreactor have also achieved two other milestones. First, it is difficult to establish primary breast or prostate cancers in culture. However, investigators at the University of South Florida have achieved an impressive 80% success rate in establishing cultures of primary breast cancers. In addition, investigators have previously shown that when human colon cancer cells form masses within the bioreactor, they produce factors that stimulate the growth and differentiation of normal colon cells when the factors are placed back in the colon of an animal (11). Conventional cultures of these same colon cancer cells did not produce the same stimulatory activity. Thus, ground-based studies have shown that the bioreactor has achieved successful growth of fastidious malignant cells and that it permits the production of factors that are not detectable in conventional bioreactor cultures.

Research in this area will help establish the scientific basis for conducting culture experiments in the microgravity environment of space, contribute to the culture of functional and differentiated tissues for use in medical treatments, and will contribute to advances in developmental biology. The microgravity environment affords a unique opportunity to culture cells because they may be grown in three-dimensions in aqueous media without sedimentation. This provides the opportunity to recreate the three-dimensional relationships among cells that are extremely important to normal organ function. At this point, the research program has established a cadre of investigators who are involved in ground-based research on the feasibility and potential benefits of low gravity culture systems. Recently, Freed et al. (12) has reported on results from the first

long-duration tissue engineering experiment conducted in the microgravity environment of space; Saltzman (13) has commented on tissue engineering in space.

#### Objectives and Description of Participation

The program has three major goals concerning mammalian tissue culture: 1) to accelerate the development of a three-dimensional tissue culturing system using rotating-wall bioreactors, 2) to define and characterize mammalian cells and tissues that benefit from a low shear environment, and 3) to use the microgravity environment of space as necessary to surmount the obstacles to the propagation of complex tissues.

- **Ground-based Proposals**

Ground-based experimental and theoretical research proposals submitted under this announcement should address the above tissue culturing goals. Potential areas of research for investigators are as follows:

- a) Investigate the effect of reduced levels of mechanical and hydrodynamic shear, spatial co-location of participating cell populations, and the role of mass transport on cellular propagation and tissue assembly in rotating wall bioreactor systems.
- b) Research the effects of culture media (growth factors, etc.), cellular metabolism, and waste accumulation to facilitate the propagation of differentiated, functioning tissues in space and ground-based bioreactors.
- c) Assess the value of low shear and spatial co-location culturing by establishing functional tissue to do morphological analysis using rotating wall vessels. This research must be able to quantitatively measure determinable parameters such as tissue mass, tissue differentiation or diversification markers, tissue function, or production of biologically active materials.
- d) Research to support the development of technologies (biosensors for pH, glucose, and oxygen levels, etc.), techniques, and maintenance strategies for three-dimensional tissue culture to allow long-term automation and improve the tissue culturing process.
- e) Research that offers new tissue culturing methods and strategies that produce three-dimensional tissue propagation.
- f) Investigate the physical environment produced in the bioreactor as a model for understanding the cellular response to microgravity. This research may lead to a more expeditious use of the microgravity environment in tissue engineering and cell culture.

- **Multidisciplinary Proposals**

Research groups from the same or from different institutions may team and submit a joint research proposal. Proposals in this category must be formed through a cooperative arrangement between the research groups with one research group having, for example, comprehensive bioengineering capabilities and the other an outstanding background in the biological sciences and tissue culturing. The goals of the multidisciplinary proposals are to develop advanced tissue culturing technology, promote ground-breaking research, promote and support technology transfer, and allow investigators interested in using rotating-wall bioreactor technology to advance their research in tissue culturing and developmental biology. Because the growth of three-dimensional complex tissues is a major technical challenge, multidisciplinary proposals are being solicited under this announcement to produce research teams with a critical size to accelerate the development of advanced rotating-wall bioreactors for three-dimensional tissue culturing. These multidisciplinary proposals will allow the teaming of

science research groups practicing tissue culturing and bioengineering to address this complex problem.

Multidisciplinary proposals should identify key personnel and their expertise in the bioreactor development effort and tissue culturing research. It must be clearly stated who the Principal Investigator and the lead institution are and how the effort will be integrated (see Appendix C). A science team, for example, may wish to work with a strong bioengineering team at its own or another institution, but these proposals should state how teaming and cooperation between the engineering and science teams will be managed. Management structure, goals, and cooperation with the research community to facilitate the transfer of this technology must be evident in the proposal. Since a goal of these multidisciplinary proposals is to provide investigators using tissue culturing in their research access to the bioreactor technology, it must be evident in the proposal that there is a broad range of investigators who wish to use the technology as a research tool.

- **Flight Proposals**

Proposals in this category would involve tissue systems that have demonstrated enhanced growth and differentiation in ground-based rotating vessel research. NASA is carrying out preliminary development work on flight hardware capable of supporting short and long duration mammalian tissue culture studies (see Appendix B for instrument descriptions).

Investigations proposing flight experiments should be sufficiently mature from a scientific and technical standpoint to define a flight investigation and proceed to a review of their concept within two years of their initial funding. A clear need for reduced gravity should be described either theoretically or experimentally. Several possible levels of participation are envisioned for flight investigations: (1) proposers may offer to design and develop instruments under contract with NASA, (2) proposers may offer to use existing hardware, (3) proposers may offer to develop simple hardware that can be used within the capabilities of the glovebox, or (4) proposers may offer research to be performed in NASA developed or international instruments with the proposer providing scientific guidance to the development effort (see Appendix B for instrument descriptions).

#### D. PROTEIN CRYSTAL GROWTH

##### Introduction

NASA has demonstrated, through crystallization experiments conducted in space, that altering one crystallization parameter, gravity, can have a significant effect on the quality of certain protein crystals. In order to build on these results, NASA is supporting a research program to study the crystallization of biological molecules and assemblages. This research program will provide a framework for understanding microgravity protein crystallization results, optimize growth conditions for biological crystals formed in the microgravity environment of space, and provide a more rational approach to the growth of macromolecular crystals. NASA-sponsored ground-based research over the past several years has produced significant advances in our knowledge of the physical chemistry of protein crystallization and has contributed to evolving technologies for monitoring, controlling, and automating crystal growth of biological macromolecules. Under this research announcement, the protein crystal growth program will expand its ground-based and flight research efforts. The goals of this NRA are to encourage research in understanding the processes of biological macromolecular crystal growth, achieving improved crystallographic structural resolution of important biological macromolecules, and advancing technology development for achieving the former goals for both ground and flight research.

Structural determination of biological macromolecules is essential to progress in the biological sciences and pharmaceutical industry. The determination of accurate macromolecular structures is necessary for establishing the molecular mechanisms of biological reactions for rational drug

design (in which a molecule is designed to bind to a specific target protein) and for the design of proteins and nucleic acids with new activities and functions. During the past decade the methods of protein crystallography have been made faster and more accurate through the use of improved data collection methodologies and more powerful computers. Almost all proteins and nucleic acids can be made in sufficiently large quantities for crystallographic analysis using the methodologies of gene cloning or by direct chemical synthesis. This means that any biological macromolecule for which there is a gene sequence can, in principle, become a subject for study by crystallography if the growth of high quality crystals is possible. Currently, the growth of such crystals is a limiting step to progress on important structural problems.

Determination of crystal structure by diffraction techniques requires well-ordered, single crystals. X-ray diffraction studies typically require crystals whose minimum dimension is approximately 0.2 to 0.4 mm, and the accuracy of the molecular coordinates is directly related to the resolution to which the crystals diffract and, therefore, to the quality of the crystal. Proteins whose structures are refined to 2 Å or better and have coordinate errors of about 0.2 Å require very high quality crystals. These high quality crystals produce the increase in diffraction data necessary to get to higher resolution. Advances in neutron diffraction have led to the possibility of determining the location of solvent molecules within a crystal structure. Structural determination by neutron diffraction requires much larger, high quality crystals on the order of millimeters.

Biological macromolecular crystallization is greatly affected by convection in the solution surrounding the growing crystals and sedimentation of these crystals. Although the fundamental mechanisms of macromolecular crystal growth are the same as for small molecule crystal growth, the magnitudes of the underlying kinetic and thermodynamic parameters that govern the process differ dramatically. Compared to small-molecule crystal growth, macromolecular crystallization differs by orders of magnitude for the following: higher supersaturation levels required for nucleation, lower diffusion coefficients for macromolecules, lower kinetic coefficients of incorporation, and low purity levels for many proteins. Typically, macromolecular crystals are extremely small and fragile; degrade outside a narrow range of temperature, ionic strength, and pH; incorporate large amounts of solvent into their crystals; and diffract far short of the theoretical limit. The complexity and uniqueness of the macromolecular crystal growth process can make it more susceptible to convection and sedimentation effects than crystals of small molecules (1).

It is conjectured that improvements in the size and order of protein crystals grown in the microgravity environment of space are possible because crystal growth in an environment with greatly reduced convective mass transport limits local supersaturation by forming depletion zones around the growing crystals (2). This allows a more ordered and regulated delivery of molecules to the surfaces of the crystals. The depletion zone also serves as a filter that prevents the incorporation of disordered aggregates and larger impurities, and discourages secondary nucleation near growing crystals. A theory has been advanced that the coupling between the transport of molecules to the growing surface and the attachment kinetics of molecules to the surface of a crystal can be favorably altered by the microgravity environment such that transport to the surface is the rate limiting process (3). In addition, another effect of microgravity is the elimination of sedimentation which decreases contacts between crystals that might otherwise affect their morphology and degree of quality.

The resolution of the diffraction pattern from protein crystals formed in microgravity are often higher than the resolution achieved for the same protein crystallized on Earth. Thus, the microgravity environment affords an opportunity to study how crystals form and achieve better packing. This may, in turn, lead to obtaining higher resolution of structures or the solution of structures of more complex materials. Despite various theories which have been suggested, there is no consensus in the scientific community regarding the physical basis to explain the results from the Shuttle protein crystallization experiments. Developing and performing the experiments necessary to achieve such a consensus is a priority of the biotechnology crystal

growth program. NASA's goal of obtaining a better understanding of how gravity affects crystal growth processes is important for achieving quality crystal growth both in flight and on the ground.

NASA first flew protein crystal growth experiments in 1985 using a hand-held device operated by an astronaut to test the adaptation of the hanging drop technique to the space environment. This device was succeeded by the more automated Vapor Diffusion Apparatus (VDA) which was housed in a thermal enclosure and flown in the Shuttle middeck (4). Since this time, a number of devices for performing crystal growth have flown. These include the Diffusional Crystallization Apparatus for Microgravity (DCAM) which performs liquid-liquid diffusional studies, the Protein Crystallization Apparatus for Microgravity (PCAM) which is a sitting drop vapor diffusion device (5), and the Gaseous Nitrogen Dewar (6) which involves cryogenically preserved samples which undergo liquid-liquid diffusion on thawing. These include, in many cases, the growth of crystals to sizes and degrees of quality that surpass samples grown in conventional laboratories, and the formation of useful crystal morphologies which had not been previously observed in ground-based experiments. (7-12). Recent work has extended the use of microgravity to the growth of crystals of such diverse and complex biological materials as viruses, nucleic acids, and the nucleosome core particle. Proposals for further studies to determine novel biological materials appropriate for crystallization in the microgravity environment is encouraged by this NRA.

The previously described flight investigations have relied on equipment primarily designed to grow protein crystals for the purposes of diffraction studies conducted post-flight. These *in situ* observations of the protein crystals grown in flight experiments have for the most part been limited to photography. Descriptions of the flight hardware are contained in Appendix B. Hardware currently under development will provide the capability of performing *in situ* studies of nucleation via laser light scattering and observations of the concentration fields around macroscopic crystals via interferometry. Next generation flight equipment will provide investigators the opportunity to dynamically control the crystal growth process from the ground by remote observations and operations. New PCG techniques should consider the advantages and limitations of 3 month duration experiments typical for space station. Development of cryopreservation techniques for both solutions and crystals will be an important enabling technology for space station crystal growth experiments.

Recent ground-based studies by NASA have made great strides in better understanding the crystal growth of proteins and other biological materials. For example, maintaining the second Virial coefficient of a protein molecule in solution within a certain narrow range has been shown to be a necessary condition for formation of large, single crystals for a number of proteins (13). Currently NASA is funding ongoing studies involving atomic force microscopy (14), laser light scattering, fluorescent probes within molecules, holography, and interferometry to better observe the process of crystal growth and to correlate observations with various measures of crystal quality such as mosaicity, diffraction resolution, signal to noise ratios, Wilson plots, and rocking curves. Continuation of such studies is anticipated as a result of this NRA.

To complement this experimental work NASA is also conducting studies to model crystal growth processes. Modeling of crystal growth processes in both 1-g and the microgravity environment of space flight are of interest. Critical to such modeling is the determination of the properties of crystal growth solutions such as crystal structure, phase diagrams, solubility curves, second Virial coefficients, etc. are necessary to develop a number of proteins or other biological materials whose growth processes can be quantitatively modeled. Advances in purification techniques to assure reproducibility of results and consistency with measured physical data are another important aspect in the development of such model systems.

Evidence of the effects of the microgravity environment on macromolecular crystallization obtained from flight investigations, coupled with new data on the mechanisms and influences that

govern such growth, are converging to some hypotheses that can be evaluated experimentally. Currently, NASA is in the process of developing a new generation of flight hardware that will allow more macromolecular samples to be flown, automated control of the crystallization process, and will provide means for the direct observation of the crystal growth phenomenon as it occurs in space. This will allow the quantification of essential kinetic parameters, delineation of relevant mechanisms, and the identification of optimal macromolecular samples.

#### Objectives and Description of Participation

NASA has the following goals concerning protein crystal growth: 1) to form an integrated effort using the space environment for the advancement in understanding of the fundamental factors influencing macromolecular nucleation and growth, 2) to elucidate which of these factors provide the observed benefits in diffraction performance when protein crystal nucleation and growth is conducted in microgravity, 3) to contribute to the structural knowledge of biological macromolecules and macromolecular assemblages (viruses, etc.) through the growth of crystals suitable for x-ray diffraction analysis by utilizing the space environment, 4) to determine the roles protein crystal nucleation and growth in microgravity may play in extending crystallographic analyses to more complex and challenging systems, such as glycoproteins, lipoproteins, and integral membrane proteins, and 5) to develop technologies and quantitative methodologies that will improve the protein crystallization process on Earth as well as in space.

- **Ground-based Proposals**

Under this announcement, NASA is soliciting ground-based proposals for scientific research focusing on understanding the fundamental processes that create and order protein crystals; NASA's eventual goals are to reproducibly grow protein crystals of improved quality and allow the growth of previously uncrystallized proteins. This announcement is also soliciting research that will lead to technological developments that will improve protein crystal growth techniques.

- **Flight Proposals**

Investigations proposing flight experiments should be sufficiently mature from a scientific and technical standpoint to define a flight investigation and proceed to a review of their concept within two years of their initial funding. A clear need for reduced gravity should be described either theoretically or experimentally. Several possible levels of participation are envisioned for flight investigations: (1) proposers may offer to design and develop instruments under contract with NASA, (2) proposers may offer to use existing hardware, (3) proposers may offer to develop simple hardware that can be used within the capabilities of the glovebox, or (4) proposers may offer research to be performed in NASA developed or international instruments with the proposer providing scientific guidance to the development effort (see Appendix B for instrument descriptions).

- **Guest Investigator Flight Program**

Flight experiments on the Space Shuttle frequently have the capacity within the flight hardware to fly crystal growth samples for Guest Investigators. Researchers who are not seeking financial support but who are solely interested in opportunities to use the space environment to improve the quality of a particular protein crystal may take advantage of the Guest Investigator Flight Program.

Guest Investigators may perform proprietary work or work for the open scientific literature. Guest Investigators who agree to publish scientific flight results in an appropriate time frame are selected through a review committee by submitting a short description of their investigation which justifies the use of the flight to accomplish their goals. Guest Investigators performing proprietary work must submit a plan which is satisfactory to NASA's Space Development and Commercial Research Division indicating that their firm is taking a financial risk in undertaking the investigation.

The NASA point of contact for the Guest Investigator Flight Program is Dr. Marcus Vlasse of the Marshall Space Flight Center (205) 544-7781. He will provide the specific information on how to apply to the program and will match guest investigations with available flight opportunities and hardware.

Guest Investigators have flown on past flights in VDA, PCAM, DCAM, and Dewar hardware described in Appendix B. Flight hardware which is currently being developed for which Guest Investigations may be possible includes the Enhanced Dewar, the Dynamically Controlled Protein Crystal Growth apparatus, and the Observable Protein Crystal Growth device.

## E. FUNDAMENTALS OF BIOTECHNOLOGY

### Introduction

Expansion of biological technologies is required for the United States to retain a competitive advantage in biotechnology. This expansion requires an understanding of the fundamental processes on which these technologies are based. NASA is currently defining a program in fundamentals of biotechnology which will study those processes which are affected by buoyancy driven convection and sedimentation, and that can be gainfully studied using the low-gravity (diffusion controlled transport) environment of space. Gravity's effect on these processes can be virtually eliminated in space; thus allowing space-based experiments, coupled with ground-based experimental and theoretical research, to provide insights into biotechnological processes. NASA's goal is to exploit the unique microgravity environment of space to advance the understanding of basic phenomenon, and use the information gained through space experimentation on a wide range of biotechnology applications.

The Fundamentals of Biotechnology area of NASA's Biotechnology Program has historically been composed of research on electrophoresis, isoelectric focusing, and phase partitioning. Significant progress has been made in understanding the physics of these separation technologies. For example, knowledge gained from these experiments regarding the role of transport in separation processes is now applied directly to the design of commercial electrophoresis units. Space research has allowed the study of separation processes without the effects of buoyant flow and the sedimentation of suspended material caused by gravity. NASA-sponsored model samples showed the importance of electrohydrodynamics, (EHD), as a dominant fluid disturbance in wide-gap electrophoresis chambers. A series of isoelectric focusing experiments conducted in free fluids or gels on two Shuttle missions clearly showed EHD destroyed the desired high resolution focusing. This research is currently investigating the coupling of fluid flows and the applied electric fields, thus providing insight into phenomena such as electrohydrodynamic instabilities. Phase partitioning is limited on Earth due to sedimentation of both cells and phase droplets, so several small experiments were done on the Shuttle to explore the benefits of this technique in space.

The focus of this announcement is to solicit new concepts in fundamentals of biotechnology which may mature to flight research. Potential research areas include 1) molecular aggregation, 2) diffusion studies on macromolecules, 3) separation and purification studies, 4) the behavior of electrically-driven flows as related to biological separations, 5) capillary and surface phenomena as applied uniquely to biological systems, and 6) membrane transport phenomena affected by diffusion controlled conditions in microgravity. Proposals for theoretical research in this area which are connected to, or have an enabling role for investigations which seek to ultimately use the space environment, will be considered for support through this announcement.

### Objectives and Description of Participation

This announcement is intended primarily to solicit experimental investigations in Fundamentals of Biotechnology which will establish the scientific foundations for future flight experiment development. Proposals for flight experiments which are mature and could proceed to flight

using simple hardware designed to work inside the glovebox or using existing hardware with little modification are also solicited under this announcement.

- Ground-based Proposals

Experimental and theoretical research proposals are requested that define and characterize biotechnological phenomena and processes for which microgravity represents an enabling environment. Ground-based proposals submitted in this area should be focused on understanding biotechnological phenomena which require the low gravity environment to test a scientific hypothesis or which can be advantageously studied in the space environment. The program has as its near-term objective the development of a knowledge base sufficient to assess the scientific value of fundamental experiments in biotechnology under low gravity conditions, where diffusion controlled conditions dominate and sedimentation is essentially eliminated. The primary intent of this announcement is to select investigations which will form a coherent effort in understanding the scientific utility of microgravity for fundamental research and to begin to define simple middeck flight experiments.

- Flight Proposals

Investigations proposing flight experiments should be sufficiently mature from a scientific and technical standpoint to define a flight investigation and proceed to a review of their concept within two years of their initial funding. A clear need for reduced gravity should be described either theoretically or experimentally. Several possible levels of participation are envisioned for flight investigations: (1) proposers may offer to design and develop instruments under contract with NASA, (2) proposers may offer to use existing hardware, (3) proposers may offer to develop simple hardware that can be used within the capabilities of the glovebox, or (4) proposers may offer research to be performed in NASA developed or international instruments with the proposer providing scientific guidance to the development effort (see Appendix B for instrument descriptions).

## F. ENTERPRISE FOR HUMAN EXPLORATION AND DEVELOPMENT OF SPACE (HEDS)

The Microgravity Research Division of the Office of Life and Microgravity Sciences and Applications (OLMSA) is an integral element of the Human Exploration and Development of Space (HEDS) Enterprise. The HEDS Strategic Plan defines four major activities: (1) prepare to conduct human missions of exploration to planetary and other bodies in the solar system; (2) use the environment of space to expand scientific knowledge; (3) provide safe and affordable human access to space, establish a human presence in space, and share the human experience of being in space; and (4) enable the commercial development of space and share HEDS knowledge, technologies, and assets that promise to enhance the quality of life on Earth.

One of the HEDS Enterprise's major goal's is contributing to the opening of the space frontier and expanding the human experience into the far reaches of space. The focus of the MRD program in the HEDS Strategic Enterprise is to foster fundamental understanding of physical, chemical, and biological processes, building a foundation of knowledge that can be applied to Earth- and space-based technologies. Specifically, understanding of the fundamental role of gravity in the space environment in these processes is needed to achieve breakthroughs in science, and to develop enabling technology for exploration and colonization of space. The need for improved understanding of biotechnology phenomena to enable future space technologies and operations should be recognized as one of the opportunities of the discipline. As part of the HEDS objectives, the current NRA will be used to solicit research on biotechnological processes that support long-duration space flight. Future proposals are not limited to the topic areas discussed in this Appendix; extension to biotechnology topics not currently included in the biotechnology program is strongly encouraged to permit us to broaden the program scope. Research areas that may have potential to contribute to the HEDS goals include the following:

## **Separation and Purification Methods**

Separation and purification methods to clean and recycle water are critical technologies needed to reduce the costs of exploration in long-duration space flight research. For example, cell and tissue systems require significant amounts of pure water for long-duration space studies. Purification methods must be specific for toxic molecules, reliable, inexpensive, and make small demands on spacecraft resources such as power, mass, and volume.

Phase Partitioning and Emulsions: A technique for separation and purification involves emulsions to separate pollutants from an aqueous phase followed by a separation of the emulsion phase from the aqueous. The emulsion phase often contains chemical constituents which are designed to bind or react with impurities and contain them within the phase of the emulsifier.

## **Biotechnology Cell Science**

Cells are the basic units of living systems. Many microbial and mammalian cell systems are well characterized in Earth's gravity and we understand some of their responses to environmental changes. These cell systems can afford a basis for analysis of the impact of non-terrestrial environments on basic life forms. The Biotechnology Cell Science Program can provide the cell systems and the technology to operate such life-based systems that relay findings from remote unmanned missions or operate in an alert/warning mode for manned spacecraft.

Selective Pressures on Cell Populations: critical for humanity's long-term occupation of space is the assessment of selective pressures on mammalian and microbial cell populations. The induced phenotypic and genotypic changes need to be assessed through generations of cell populations sustained in the space environment (low gravity, etc.). The development of unique technologies and methodologies to enhance research on selective pressures will apprise us of the risks to our biological integrity and to our life-based support systems.

Development of Biosentinel Technologies: biosentinel systems to monitor radiation effects and other space environmental factors (magnetic fields, atmospheres, gravitational fields, etc.) on cell replication and DNA for manned and unmanned robotic science missions are needed. These biosentinel systems may incorporate biomolecules in their detection systems.

MicroBiosensor Monitoring Devices: micro- and nano- technology based sensors for use in biological systems and experiments that may have unique application to long-duration space missions. The development of extremely stable and small biosensor devices is central to their use in support of long-duration space missions and advancement of many biotechnological processes.

## **Extremophile Research**

Certain bacteria and archea have been found to flourish in extreme environments: high and low temperatures, high and low pH, etc. The unique and robust enzymes produced by these organisms, or the organisms themselves, can have applications in industry and may be of substantial benefit for certain chemical processes involved in long duration space flight or colonization of the Moon and Mars. The specificity of enzymes is superior to conventional catalysts and can serve to eliminate multistep reactions and unwanted side products, making the overall chemical process much more efficient. Research may establish unique application of extremophiles or their enzymes in support of HEDS goals.

## **Biomolecular Self-Assembling Materials**

Research on biomolecular self-assembling materials lies at the interface between molecular biology, the physical sciences, and materials engineering. A key feature of biomolecular

materials, such as biological membranes, is their ability to undergo a self-assembly process forming a hierarchical structure without external intervention. Understanding nature's principles and mechanisms used in forming self-assembled structures can lead to their application in the development of new processes and formation of unique materials with significant technological impact. Examples of research in the areas of biomolecular self-assembling materials include: polymer biosynthesis, self-assembled monolayers and multilayers, decorated membranes, mesoscopic organized structures, and biomineralization (1). Biomolecular self-assembling materials may provide novel properties and applications in life support or other areas central to HEDS goals.

While basic research is still of major importance to our program, there is shift of emphasis toward "mission-oriented" research, that is, research aimed at specific problems in biotechnology applications on Earth as well as in the space environment. Thus, it is important that firmer links be developed between the research in support of the exploration of space and practical applications on Earth. In the future, more weight will be placed on relevance of the proposed research to attainment of the HEDS goal of contributing to the opening of the space frontier and expanding the human experience into the far reaches of space.

- **Ground-based Proposals**

Experimental and theoretical research proposals are requested that define and characterize biotechnological phenomena and processes which support HEDS goals. Ground-based proposals submitted in this area should be focused on understanding biotechnological phenomena which can be advantageously studied in the space environment.

- **Flight Proposals**

Investigations proposing flight experiments should be sufficiently mature from a scientific and technical standpoint to define a flight investigation and proceed to a review of their concept within two years of their initial funding. A clear need for the space environment should be described either theoretically or experimentally. Several possible levels of participation are envisioned for flight investigations: (1) proposers may offer to design and develop instruments under contract with NASA, (2) proposers may offer to use existing hardware, (3) proposers may offer to develop simple hardware that can be used within the capabilities of the glovebox, or (4) proposers may offer research to be performed in NASA developed or international instruments with the proposer providing scientific guidance to the development effort (see Appendix B for instrument descriptions).

### **III. EXPERIMENTAL APPARATUS AND FLIGHT OPPORTUNITIES**

#### **A. EXPERIMENTAL APPARATUS**

In order to address aspects of the research described in Section II, a number of pieces of flight hardware are being developed by NASA. NASA also contemplates the development of new research capabilities for biotechnology experiments, also described in Appendix B. In addition, Appendix B lists the ground-based facilities that are available to support definition studies.

#### **B. FLIGHT OPPORTUNITIES**

Limited early flight opportunities under this NRA are expected to include the Space Shuttle and the International Space Station, as well as other spacecraft, such as retrievable satellites. For the Shuttle opportunities, the experimental hardware will be located in the middeck, allowing astronaut interaction, or in the cargo bay, which does not permit such interaction. Residual acceleration levels on the order of  $10^{-4}$  g are available in the Shuttle for limited periods of time. The Space Acceleration Measurement System (SAMS) is expected to be available to measure and record accelerations near the apparatus for both Shuttle and ISS experiments. Flight

durations range from 7 to 16 days for the Space Shuttle. A high-capacity communications network supports Shuttle and payload operations. Downlink channels enable users to monitor their instrument status and science data streams in real time. An uplink channel enables them to act on that information. Considerable additional information on the Shuttle accommodations and capabilities can be found in the STS Investigators' Guide (see Bibliography). Experimental apparatus for the early utilization of the International Space Station will be primarily in facilities such as the Glovebox and Express Rack (ISS versions of Shuttle middeck class experiments) with the Biotechnology Facility anticipated to be available in the 2003 time period. Flight experiment protocols should also consider the additional benefits that could be derived from skilled crew interaction with experiments available during many of these flight opportunities.

### C. EXPERIMENT DEFINITION AND FLIGHT ASSIGNMENT PROCESS

Ground-based research is usually necessary to clearly define flight experiment objectives. Successful proposals for flight opportunities will be supported for a ground-based definition phase before review for flight assignment. The amount of support (technical, scientific, and budgetary) and the length of the definition period (usually from 6 months to 2 years) will depend on the specific investigator needs and the availability of resources from NASA. However, in preparing their budget plan for this research announcement, all respondents should estimate their annual costs for four years.

1. Near-Term Flight Opportunities. Successful proposals for use of existing instruments will be funded for a period of advanced definition work, after which time the investigator will generate a detailed Science Requirements Document (SRD). The SRD, a detailed experiment description outlining the specific experiment parameters and conditions, as well as the background and justification for flight, must be prepared jointly by a NASA-appointed Project Scientist and the Principal Investigator and submitted for peer review. This formal review by both science and engineering panels will determine if space flight is required to meet the science objectives and if instrument capabilities can be provided to meet the science requirements. Following approval by the review panels, subject to program resources, continuation support will be awarded and the hardware will be modified or developed to meet the science requirements. NASA does not guarantee that any experiment selected for definition will advance to flight experiment status. Investigations with unresolved science or engineering issues at the review of the SRD may be placed in ground-based status with support of limited duration (normally from one to three years), and the investigator will be asked to submit a proposal to a subsequent solicitation for further support.

2. Future Flight Opportunities. Successful proposals for the development of new apparatus will be funded for a period of definition. The length of the definition period will be based on the experiment requirements, but will generally be from 6 to 24 months. At the end of the experiment definition phase, the investigator will generate a detailed SRD. Following successful peer review of the SRD by science and engineering panels, the experiment will proceed into flight development and be considered for flight. As with opportunities for existing instruments, NASA does not guarantee that any experiment selected for definition will advance to flight development status, and the possibility exists that investigations may be placed in ground-based status, with continuing support from NASA for a limited period.

3. Ground-Based Definition Opportunities. Promising proposals for experimental research which are not mature enough to allow development of an SRD after two years of definition, and proposals for development of theory in areas of current or potential microgravity experiments, may be selected for support in the MRD Research and Analysis (R&A) Program. R&A studies are funded for periods of up to four years. A new proposal to a future announcement is required in order to be selected for a flight opportunity or to continue R&A studies if appropriate.

#### **IV. UNDERGRADUATE STUDENT RESEARCH OPPORTUNITIES**

Active research experience is one of the most effective techniques for attracting talented undergraduates to, and retaining them in, careers in mathematics, science, and engineering. The undergraduate years are critical in the educational sequence, as career-choice points and as the first real opportunities for in-depth study. MRD is endeavoring to foster the career development of undergraduate students by offering optional supplements of approximately \$5,000 per student to approved research tasks for undergraduate student research projects. This supplement may be requested for each year of the proposed research. These projects should involve undergraduate students in a meaningful way in ongoing research programs or in related sub-projects specifically designed for this purpose.

The proposals for the undergraduate student research projects should include the nature of the student activities, presenting plans that will ensure the development of student-faculty and student-student interaction and communication; a concise description of the experience and record of the Principal Investigator and any potential advisors of students; and the criteria for evaluating the success of the project. Proposals for up to two students should constitute a separate section (see information on proposal formatting in Section V, Subsection B) of about two pages per student and will not be counted against the maximum page limit. This effort should be shown as a separate line in the budget summary for each year. The review criteria to determine whether NASA will fund this activity if the proposal is selected are the following:

1. The value of the educational experience for the student participants, particularly the appropriateness of the research projects for and the nature of student participation in these activities
2. The quality of plans for student preparation, student mentoring, and follow through designed to promote continuation of student interest and involvement in research
3. The proposed arrangements for managing the project and how the project will be evaluated.

If selected for involvement in this program, investigators are required to submit reports on these activities in conjunction with reporting on the primary grant. In particular, reports should include information on the activities of each student, the degree of interaction with their mentors, the future career plans of the student (if known), and an evaluation of the project progress.

#### **V. PROPOSAL SUBMISSION INFORMATION**

This section gives the requirements for submission of proposals in response to this announcement. The research proposal submitted under this announcement consists of a Principal Investigator who is responsible for all research activities and one or more Co-Investigators. Proposers must address all the relevant selection criteria in their proposal as described in Section VI and must format their proposal as described in this section. Additional general information for submission of proposals in response to NASA Research Announcements may be found in Appendix C.

##### **A. LETTER OF INTENT**

Organizations planning to submit a proposal in response to this NRA should notify NASA of their intent to propose by electronically sending a Letter of Intent to the following e-mail address:

loi@hq.nasa.gov

If electronic means are not available, you may mail Letters of Intent to the address given for proposal submission in the following section.

The Letter of Intent, which should not exceed two pages in length, must be typewritten in English and must include the following information:

- The potential Principal Investigator (PI ), position, organization, address, telephone, fax, and e-mail address.
- A list of potential Co-Investigators (Co-I's), positions, and organizations.
- General scientific/technical objectives of the research.
- The equipment of interest listed in this NRA, if appropriate.

The Letter of Intent should be received at NASA Headquarters no later than February 20, 1998. The Letter of Intent is being requested for informational and planning purposes only, and is not binding on the signatories. The Letter of Intent allows NASA to better match expertise in the composition of peer review panels with the response from this solicitation. Investigators may also request more detail on the capabilities of the specific equipment that might be utilized to accomplish their scientific objectives.

#### B. PROPOSAL

The proposal should not exceed 20 pages in length, exclusive of appendices and supplementary material, and should be typed on 8-1/2 x 11 inch paper with a 10- or 12-point font. **Extensive appendices and ring-bound proposals are discouraged.** Reprints and preprints of relevant work will be forwarded to the reviewers if submitted as attachments to the proposal.

In preparation of proposals, the standard forms and certifications at the back of this research announcement should be used. Proposers should prepare cost estimates by year for a period not greater than four years in preparing budget plans in response to this research announcement.

**Fifteen copies of the proposal must be received at NASA Headquarters by April 7, 1998, 4:30 PM EDT to assure full consideration. Treatment of late proposals is described in Appendix C. Send proposals to the following address:**

**Dr. Steve Davison  
c/o Information Dynamics Inc.  
Subject: NASA Research Proposal (NRA-97-HEDS-02)  
300 D Street, S.W.  
Washington, D.C. 20024  
Telephone number for delivery services: (202) 479-2609**

**NASA cannot receive proposals on Saturdays, Sundays, or federal holidays.**

**Proposals submitted in response to this Announcement must contain at least the following information in the format shown below:**

- Title Page
- Table of Contents
- Executive Summary (replaces abstract) (1-2 pages)
- Research Project Description
  - Statement of the hypothesis, objective, and value of this research
  - Review of relevant research
  - Justification for new or further microgravity research
  - Description of Experimental or Analytical Method
  - Data Analysis
  - References
- Appendices
  - Proposed Costs (see Appendix C)
  - Current and Pending Support
  - Management Approach
  - Complete vitae for the PI and all Co-investigators
  - Facilities and Equipment (see Appendix C)
  - Signed Certifications
- **3.5 inch computer diskette containing electronic copy of principal investigator's name, address, complete project title, and executive summary**

The title page must clearly identify the research announcement to which the proposal is responding; the title of the proposed research, the Principal Investigator name, institution, address and telephone number; the total proposed cost and proposed duration; and all required signatures.

The executive summary should succinctly convey, in broad terms, what it is the proposer wants to do, how the proposer plans to do it, why it is important, how it meets the requirements for microgravity relevance, and how it relates to outlined HEDS objectives. The executive summary replaces the proposal abstract.

Each proposal should include the Solicited Proposal Application (Form A). Those requesting financial support should also include: Detailed Budget for 12-Month Period (Form B) for each year of funding; Budget for Entire Project Period (Form C); signed Certifications regarding Drug-Free Workplace Requirements (Form D); Debarment, Suspension, and other Responsibility Matters (Form E); and Lobbying (Form F). Copies of these forms may be found at the end of this document.

Proposal Cost Detail Desired. Sufficient proposal cost detail and supporting information will facilitate a speedy evaluation and award. Dollar amounts proposed with no explanation (e.g., Equipment: \$58,000, or Labor: \$10,000) may cause delays in evaluation or award. The proposed costing information should be sufficiently detailed to allow the Government to identify cost elements for evaluation purposes. Generally, the Government will evaluate cost as to reasonableness, allowability, and allocability. Enclose explanatory information, as needed; each category should be explained. Offerors should exercise prudent judgment, as the amount of

detail necessary varies with the complexity of the proposal. **The Proposers are strongly urged to be realistic as to the time-phasing of their funding requirements, rather than simply “straight-lining” the costs over four years. Very often, funds will be expended at a relatively low level during the first few months of a project, with higher than “straight-line“ expenditures during the remaining grant period; if the proposer anticipates this being the case, he/she should indicate this in the proposed funding profile, since “carry-over” funding from year to year is strongly discouraged by NASA management.**

## **VI. GUIDELINES FOR INTERNATIONAL PARTICIPATION**

NASA accepts proposals from outside the United States, although this program does not financially support Principal Investigators in countries other than the U.S. Accordingly, proposals from non-U.S. entities should not include a cost plan. Non-U.S. proposals and U.S. proposals which include non-U.S. participation, must be endorsed by the appropriate government agency in the country from which the non-U.S. participant is proposing. Such endorsement should indicate that:

1. The proposal merits careful consideration by NASA
2. If the proposal is selected, sufficient funds will be made available, from the country from which the non-U.S. participant is proposing, to undertake the proposed activity.

Proposals, along with the requested number of copies and Letter of Endorsement, must be forwarded to NASA in time to arrive before the deadline established for this NRA. All proposals must be typewritten in English. All non-U.S. proposals will undergo the same evaluation and selection process as those originating in the U.S.

Sponsoring non-U.S. agencies may, in exceptional situations, forward a proposal directly to the address given in the first section of this announcement if review and endorsement is not possible before the announced closing date. In such cases, an accompanying letter should indicate when a decision on endorsement can be expected.

Successful and unsuccessful proposers will be notified by mail directly by the NASA program office coordinating the NRA. Copies of these letters will be sent to the sponsoring government agency. Should a non-U.S. proposal or U.S. proposal with non-U.S. participation be selected, NASA's Office of External Relations will arrange with the non-U.S. sponsoring agency for the proposed participation on a no-exchange-of-funds basis, in which NASA and the appropriate government agency will each bear the cost of discharging its respective responsibilities. Depending on the nature and extent of the proposed cooperation, these arrangements may entail:

1. A letter of notification by NASA
2. An exchange of letters between NASA and the sponsoring government agency
3. An agreement or memorandum of understanding between NASA and the sponsoring government agency.

## **VII. EVALUATION AND SELECTION**

### **A. EVALUATION PROCESS**

The evaluation process for this NRA will begin with a scientific and technical external peer review of the submitted proposals. NASA will also conduct an internal engineering review of the potential hardware requirements for proposals that include flight experiments. The external peer review and internal engineering review panels will be coordinated by the NASA Enterprise Scientist for Biotechnology. Consideration of the programmatic objectives of this NRA, as

discussed in the introduction to this Appendix, will be factored in by NASA to ensure enhancement of program breadth, balance, and diversity; NASA will also consider the cost of the proposal. The MRD Director will make the final selection based on science panel and programmatic recommendations. Upon completion of all deliberations, a selection statement will be released notifying each proposer of proposal selection or rejection. Offerers whose proposals are declined will have the opportunity of a verbal debriefing regarding the reasons for this decision. Additional information on the evaluation and selection process is given in Appendix C.

## B. EVALUATION FACTORS

**This section replaces Section I of Appendix C.** The principal elements considered in the evaluation of proposals solicited by this NRA are: relevance to NASA's objectives, intrinsic merit, and cost. Of these, intrinsic merit has the greatest weight, followed by relevance to NASA's objectives, of slightly lesser weight. Both of these elements have greater weight than cost. Evaluation of the intrinsic merit of the proposal includes consideration of the following factors, in descending order of importance:

1. Overall scientific or technical merit, including evidence of unique or innovative methods, approaches, or concepts, and the potential for new discoveries or understanding, or delivery of new technologies/products with economic benefit or applications to health care.
2. Qualifications, capabilities, and experience of the proposed Principal Investigator, team leader, or key personnel who are critical in achieving the proposal objectives
3. Institutional resources and experience that are critical in achieving the proposal objectives
4. Overall standing among similar proposals available for evaluation and/or evaluation against the known state-of-the-art.

Evaluation of the cost of a proposed effort includes consideration of the realism and reasonableness of the proposed cost, and the relationship of the proposed cost to available funds.

## C. SELECTION CATEGORIES, PERIOD OF SUPPORT, AND FLIGHT-DEFINITION PROCESS

Proposals selected for support through this NRA will be selected as either ground-based or flight-definition investigations. Investigators offered support in the ground-based program normally will be required to submit a new proposal for competitive renewal after no more than four years of support. Investigators offered flight-definition status are expected to begin preparing detailed experiment requirements and concepts for flight development shortly after selection in cooperation with the assigned representative from the Marshall Space Flight Center or Johnson Space Center. The selected investigations will be required to comply with MRD policies, including the return of all appropriate information for inclusion in the MRD archives during the performance of and at the completion of the contract or grant.

Commitment by NASA to proceed from flight-definition to the execution of a flight experiment will be made only after several additional engineering and scientific reviews and project milestones have established the feasibility and merit of the proposed experiment. Investigations that do not pass these reviews will be funded for a limited period (generally no more than four years from the initial award date) to allow an orderly termination of the project.

The Principal Investigator in flight-definition must prepare a Science Requirements Document (SRD) early in the development of a flight experiment to guide the design, engineering, and integration effort for the instrument. The SRD describes specific experiment parameters, conditions, background, and justification for flight. Ground-based, normal, and reduced-gravity experimentation, as well as any necessary parallel modeling efforts, may also be required to prepare an adequate SRD. The amount of support (technical, scientific, and budgetary) provided to investigators by NASA will be determined based upon specific investigator needs and the availability of resources to NASA and MRD.

It should be noted that while a proposer can propose multiple flights, in general NASA will not commit to more than one flight without a reflight peer review after the first flight.

### **VIII. NRA FUNDING**

The total amount of funding for this program is subject to the annual NASA budget cycle. The Government's obligation to make awards is contingent upon the availability of appropriated funds from which payment for award purposes can be made and the receipt of proposals which the Government determines are acceptable for an award under this NRA.

For the purposes of budget planning, we have assumed that the Microgravity Research Division will fund 4 to 6 flight experiment definition proposals. These efforts are typically funded at an average of \$175,000 per year. It is also anticipated that approximately 35 ground-based study proposals will be funded, at an average of approximately \$125,000 per year, for up to 4 years. In addition, one or two multidisciplinary proposals for the cell science area will be funded at an average cost of approximately \$700,000 per year. The initial fiscal year (FY) 1999 funding for all proposals will be adjusted, if required, to reflect partial fiscal year efforts.

**It is particularly important that the proposer realistically forecast the projected spending timeline rather than merely assuming an equal amount (adjusted for inflation) of requirements for each year.** The proposed budget for ground-based studies should include researcher's salary, travel to science and NASA meetings (for a flight investigation, roughly eight meetings per year with NASA should be anticipated, though travel activity will vary over the development of the experiment), other expenses (publication costs, computing or workstation costs), burdens, and overhead. During subsequent years, NRAs similar to this NRA will be issued, and it is planned that funds will be available for additional investigations.

### **IX. BIBLIOGRAPHY**

The *Microgravity Science and Applications Program Tasks and Bibliography for FY 1996*, which contains a searchable data base on currently funded research, may provide useful information to proposers and can be viewed at the following web location:

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## HARDWARE AND FACILITY DESCRIPTIONS

### MICROGRAVITY BIOTECHNOLOGY: RESEARCH AND FLIGHT EXPERIMENT OPPORTUNITIES

NASA's Microgravity Research Division (MRD) is currently pursuing a program for the development of payloads capable of accommodating multiple users. This program is expected to meet the science requirements of microgravity investigations and to support the development of technologies for microgravity biotechnology research. In the interest of minimizing project cost and complexity, NASA encourages the use of existing U.S. or Internationally developed space hardware whenever consistent with experiment requirements.

The equipment described is either planned for development or has been developed by NASA for flight on the Space Shuttle and other space platforms. International equipment described here may be offered for flight on a cooperative basis with agencies from various countries. The specific availability of certain flight hardware is subject to coordination with the Space Development and Commercial Research Division prior to the finalization of arrangements for flight opportunities. The availability of the hardware described in this section is contingent upon the availability of funds, flight manifest opportunities, and, for international hardware, cooperative agreements between NASA and the appropriate foreign space agency.

#### I. CURRENT FLIGHT HARDWARE

The experimental hardware described in this section is available with or without modification contingent upon the availability and allocation of resources.

##### A. MIDDECK GLOVEBOX (MGBX) (NASA/MSFC)

The Middeck Glovebox is a multi-user and multidiscipline facility that provides an enclosed working space for experiment manipulation and observation. Glovebox Investigations which have flown include: protein crystal growth, fluid physics, combustion, and materials science experiments.

The MGBX occupies two standard lockers in the Space Shuttle middeck. The MGBX door opening to insert or retrieve investigation hardware is 20.3 cm by 19.4 cm, with a working volume of 35 liters. Forced air cooling can withdraw a maximum of 60 W of investigation generated heat. Up to 60 W of 24,  $\pm$  12, and 5 VDC power is available for experimenter apparatus. The MGBX can be used in various modes of pressure and air circulation. The working area can serve as a sealed environment that is isolated from the crew cabin atmosphere, as a constantly recirculating atmosphere that is maintained at a pressure slightly lower than the middeck ambient, or as a working area open to the middeck. Multipurpose filters exist to remove particles, liquids, and reaction gasses from the recirculated air.

Due to limitations of the Space Shuttle middeck, there is no standard data or video downlink. There is the possibility of some near real-time video downlink (from the Shuttle Camcorder), but this will be decided on a mission-by-mission basis. Four video recorders provide data storage, with digital data stored in the audio channels; an additional connector records eight channels of data to the Interface frame data recorder. An adjustable light switch, video port plugs, a backlight panel, a halogen flashlight, redlight filters, and cutout window covers provide illumination.

## B. THERMAL ENCLOSURES

Use of the Single-locker Thermal Enclosure System (STES) or the Thermal Enclosure System (TES) is encouraged for investigations requiring experiment temperature control. The Gaseous Nitrogen Dewar/Freezer (GN2 Dewar) can be used for samples that need to be frozen. Because the STES, TES, and GN2 Dewar are frequently manifested in the middeck of the Shuttle Orbiter, the equipment can be installed shortly before a launch. Current timetables provide late access at approximately 24 hours before launch and early removal at three-to-eight hours after Shuttle landing. A thermal enclosure to meet the more demanding requirements of payloads on the International Space Station is under development.

Ideally, equipment built to be accommodated in the TES or STES should be fully automated. However, some crew time may be available for tending of experiments (e.g., activation or deactivation of protein crystal growth process) on some flights. The proposer should recognize that crew time and communication channels are subject to mission priorities that place experiment management behind crew health, safety concerns, and accomplishment of the primary mission.

### 1. Single Locker Thermal Enclosure System (STES)

The STES is the size of a single middeck or Spachab locker and provides a controlled temperature environment within plus or minus 0.5° C of a set point in the range from 4 to 40° C; the set point must be within 24° C of ambient temperature. The STES time/temperature profile is programmable within the thermal capability of the hardware. Internal STES heat is transferred primarily by conduction. The STES has nine sensors which are placed at various locations to record temperature history and to provide temperature control. Temperature data is available post-mission. A payload assembly, consisting of an STES and an experiment apparatus, must meet interface, operational, and safety requirements of the vehicle or space platform used. Section III below provides information on selected experiment apparatus accommodation. A small amount of power is available for use by an experiment apparatus; use of this resource may impact the temperature control capability of the unit. Experiment duration, available crew time, and air-to-ground communication capability for STES payloads is mission dependent. Periodic monitoring of STES operation is required. The STES door can be opened easily to accommodate experiment operations.

### 2. Thermal Enclosure System (TES)

The TES is the size of two vertically adjacent middeck lockers and provides a controlled temperature environment within plus or minus 0.2° C of a set point in the range from 4 to 40° C; the set point must be within 24° C of ambient temperature. The TES time/temperature profile is programmable within the thermal capability of the hardware. Internal TES heat is transferred primarily by convection. The TES has nine sensors which are placed at various locations to record temperature history and to provide temperature control. Temperature data is available post-mission. A payload assembly, consisting of a TES and an experiment apparatus, must meet interface, operational, and safety requirements of the vehicle or space platform used. Section III below provides information on experiment apparatus accommodation. A small amount of power is available for use by an experiment apparatus; use of this resource may impact the temperature control capability of the unit. Experiment duration, available crew time, and air-to-ground communication capability for TES payloads is mission dependent. Periodic monitoring of TES operation is required. The TES door is not normally removed for experiment operations: mechanical and electrical/electronic functions are accomplished by use of "feed throughs" in the TES door. An experiment-unique TES door may be proposed.

### 3. Gaseous Nitrogen Dewar (GN2 Dewar)

The GN2 Dewar is a device used to transport and maintain samples at cryogenic temperatures. Units can be flown in a standard Shuttle middeck locker and are available by agreement with the Johnson Space Center. These existing units are capable of maintaining a cryogenic temperature for six-to-13 days. Passive cooling is provided by absorbed liquid nitrogen which slowly evaporates from the dewar. Depletion rate for the nitrogen is specific to the unit used, and the units cannot be recharged on orbit. Samples may be launched in a GN2 Dewar and then allowed to thaw in a microgravity environment. Sample holders or experiment devices may be designed to be housed in a GN2 Dewar, but must meet certain size and design requirements.

## C. PROTEIN CRYSTAL GROWTH HARDWARE

### 1. Protein Crystallization Apparatus for Microgravity (PCAM)

The PCAM is a protein crystal growth device that has been developed to provide a large number of protein crystallization experiments in a single middeck locker flight. Six cylindrical PCAM units can be mounted in an STES unit. Each cylindrical PCAM contains nine crystallization plates, each having seven sample chambers, for a total of 63 chambers per cylinder. Thus, the total number of samples that can be flown in an STES is 378. The crystallization plates are modified "sitting drop" vapor diffusion devices based on the commercial CrysChem design. In the center of each chamber is a pedestal with a depression on its top which can contain up to 40 microliters of pre-mixed protein sample solution and precipitant solution. The pedestal is surrounded by a toroidal reservoir of absorbent material capable of containing approximately one milliliter of precipitant solution. The protein solution is isolated from the reservoir prior to activation and after deactivation. Activation occurs simultaneously for all chambers in a cylinder, as does deactivation.

### 2. Vapor Diffusion Apparatus (VDA)

The VDA and the improved VDA (or VDA-2) are protein crystal growth devices based on a syringe assembly design for mixing protein solutions and precipitant solutions in microgravity. VDA trays are designed to fit inside the STES units. An STES can accommodate three VDA trays and four VDA-2 trays. Each VDA tray consists of 20 double-barreled syringes, and each VDA-2 tray has 20 triple-barreled syringes. One barrel of each syringe can be loaded with up to 30 microliters of protein solution, and a second barrel with up to 30 microliters of precipitant solution. Absorbent reservoir material containing approximately one milliliter of precipitant solution surrounds the drop in each chamber. The experiment is initiated by deployment of the solutions onto the tips or the syringe assemblies to form drops. Mixing of drops is effected by moving the droplet solutions into and out of the syringes. In the case of the VDA-2, improved mixing is effected by moving the droplets into and out of the third barrel. The experiment is deactivated by moving the drops back into the syringes. Prior to drop deployment and after deactivation the solutions in the syringes are sealed with plugs that press against the syringe tips.

### 3. Diffusion-controlled Protein Crystallization Apparatus for Microgravity (DCAM)

The DCAM hardware, which was designed for long duration protein crystal growth on the Mir Space Station, combines liquid-liquid diffusion and dialysis methods to effect protein crystal growth. Each DCAM tray assembly consists of 27 DCAM experiment chambers containing precipitant solutions and protein sample solutions. These chambers are arranged in nine rows of three units each and are mounted on an aluminum tray. No crew activation or deactivation of the hardware is required: the DCAM does not employ mechanical activation/deactivation. Crystallization conditions are approached very slowly. Each DCAM self-activates as the precipitant solution slowly diffuses through a control plug from a larger solution chamber into and

across a smaller solution chamber until it reaches a small volume of protein sample solution separated from the chamber by a dialysis membrane.

#### 4. Interferometer for Protein Crystal Growth (IPCG)

The IPCG, which was originally designed as an experiment to be operated in the Mir Glovebox, comprises three major systems -- an interferometer, six fluid assemblies with test cells, and a flight data system. The IPCG interferometer system employs a Michelson-Morley phase-shift interferometer to produce images showing density changes in the solution as a protein crystal forms. The IPCG crystal growth cells are made of optical grade glass: Cells are 1 mm thick and contain 250 ml of solution. Each fluid handling system is a self-contained plastic assembly enclosing two pairs of 4-ml supply syringes (one containing protein solution and one containing precipitant solution), a waste receptacle, and a test cell -- plus mechanisms to inject fluids and to position the test cell. The crew operates the fluid system with a hand crank which depresses the syringe pistons. The flight data system includes a 486-based laptop computer and has video recording capability.

#### 5. Commercial Vapor Diffusion Apparatus (CVDA)

This experiment flight hardware was developed to allow commercial customers to grow protein crystals in a configuration that is similar to typical laboratory hardware using the vapor diffusion method. The CVDA provides a greatly improved thermal environment, sample capacity, and operational scenario over previous VDA flight hardware. The CVDA increases the sample capacity to 128 growth chambers within one thermal enclosure. The internal configuration of the growth chambers was designed to mimic a Limbro box. The protein and precipitating agent are separated in individual syringe barrels prior to activation.

#### 6. Protein Crystallization Facility (PCF)

This equipment is used for batch processing of proteins that show a temperature dependence on solubility. The PCF sample bottles range from 50 ml to 500 ml, accommodating four sample bottles in one thermal enclosure. This equipment has flown several times, performing with a high degree of predictability. Sample bottles are made from polysulfone with a Teflon coated aluminum lid.

#### 7. Protein Crystallization Facility - Light Scattering Temperature (PCF-LST)

The PCF-LST grows crystals using the same temperature induced growth process as the PCF. The PCF-LST incorporates a laser light scattering device that detects nucleation and displays the corresponding detector voltage level on a Macintosh Powerbook. This display allows a crew member to identify when nucleation has occurred in the sample and to adjust the temperature profile accordingly to control the growth period. This experiment hardware accommodates two sample bottles up to 50 milliliters in size, in one thermal enclosure. In addition, approximately one standard middeck locker of stowage is required for support equipment.

#### 8. Protein Crystallization Facility-Variable Gradient (PCF-VG)

This equipment is used to allow customers to process small amounts of protein using the temperature induced crystal growth process. Sample sizes for the PCF-VG include 1 ml and 5 ml. By varying thermal path configurations internal to the thermal enclosure used, investigators can obtain several temperature profiles for different samples. By allowing customers to screen a large number of conditions on a single flight, users are able to investigate a variety of growth parameters.

## D. CELL SCIENCE SYSTEMS

The cell science Biotechnology System (BTS) flight hardware provides the technological capability for addressing the potential of microgravity tissue and cell culturing. Major components of the BTS include the following: Experiment Module (BEM), Computer Module (BCM), Gas Supply Module (GSM), and the Biotechnology Refrigerator (BTR). The BTS offers increased levels of tissue culturing capability and automation allowing investigators to culture cells and tissues under the low mechanical shear environment of microgravity. BTS and ground-based rotating wall culture vessels suspend cells and tissue about a rotating horizontal axis creating a low fluid shear environment, and have successfully cultured suspension and anchorage-dependent mammalian cells. The vessels include features that allow addition of nutrients, removal of metabolic waste products, respiratory gas exchange, temperature control, and sample removal.

### 1. Slow Turning Lateral Vessel (STLV)

The STLV is a nonperfused, horizontally rotating bioreactor consisting of a fixed volume vessel (50 or 100 ml). The vessel is connected to a variable-rate motor and mounted on a fixed base. The STLV is autoclavable. The vessel has several separate sample ports for adding media or reagents and removing samples. The STLV has been optimized for to culture anchorage-dependent cells on microcarrier systems. It is commercially available through Synthecon, Inc., Houston, TX.

### 2. High Aspect Ratio Vessel (HARV)

The HARV is a nonperfused, horizontally rotating bioreactor consisting of a fixed volume vessel (10 or 50 ml) with a large radius and a short length. The vessel is connected to a variable-rate motor and mounted on a fixed base. The HARV is autoclavable. The vessel has several separate sample ports for adding media or reagents and removing of samples. The HARV has been optimized to culture suspension cells and anchorage-dependent cells with or without microcarriers. It is commercially available through Synthecon, Inc., Houston, TX.

### 3. The Rotating Wall Perfused Vessel (RWPV)

The RWPV is a perfused, horizontally rotating bioreactor consisting of a fixed volume vessel (250 or 500 ml), a silicone membrane oxygenator, a pH sensor, sample ports, and a pump for infusing or recycling fresh medium. The RWPV is sterilized with ethylene oxide in a specially designed apparatus. The vessel is secured to a support base and connected to two variable-rate motors that independently control the rotation of the vessel's outer wall and the hollow inner centerline spin filter. Rotation rates for the vessel's outer wall and spin filter can be varied in order to create different levels of fluid shear and turbulence. Samples are withdrawn through sample ports; the vessel's outer wall can be stopped temporarily during sampling. Fresh or recycled media can be perfused into the vessel at rates sufficient to support nutrient delivery, metabolic gas exchange, and waste-product removal. A version of the RWPV has been used to transition cell cultures to microgravity. It is commercially available through Synthecon, Inc., Houston, TX. Flight hardware is available through the Biotechnology Cell Science Program.

### 4. The Biotechnology Specimen Temperature Controller (BSTC)

The BSTC is flight equipment capable of transporting and maintaining biological and cell culture samples in a controlled temperature. The BSTC is a self-contained unit that can maintain a variety of specimen volumes up to 50 ml in as many as 4 independent temperature controlled blocks ranging from 7 to 37° C. The device can maintain target temperatures in this range during launch,

mission and reentry. The temperature profiles for the blocks can be maintained independently. Each block measures approximately 2.7 cm x 5.2 cm x 1.4 cm. Energy dependent cell functions can be investigated in microgravity by controlling the incubation temperature. The BSTC is a flight incubator and is used primarily for the definition phase of the experiment protocol.

## E. INTERNATIONAL BIOTECHNOLOGY EQUIPMENT

### 1. Cryostat (German Space Agency, DLR, Germany)

The Cryostat is a device developed for conducting experiments on the crystallization of proteins. The Cryostat has successfully flown on previous Shuttle missions and is available for future flights as a middeck payload. The equipment allows for a protein solution and a salt solution to be mixed through a buffer solution on orbit. When a shutter separating the protein and the salt solution from the buffer is slowly removed under microgravity conditions, the species diffuse from opposite sides into the buffer, where crystals of the protein can thus be formed. In the cryostat, two containers are accommodated, each allowing the processing of four such samples. Additionally, a 24 sample modified sitting drop container is available for use. U.S. investigators could plan on choosing up to half of the samples to be processed.

The thermal conditions during processing can be controlled  $\pm 0.5^\circ\text{C}$  between  $-10^\circ\text{C}$  and  $+20^\circ\text{C}$  by means of Peltier heating and cooling elements. The sample temperature can be held constant at  $20^\circ\text{C}$  during storage periods prior to and after processing. Temperature data is recorded during processing. The samples are returned for analysis along with data on the performance of the hardware. Late access to the facility for loading samples can be as late as two days prior to launch. Early removal of the samples from the vehicle can occur within several hours after landing.

### 2. Automated Protein Crystallization Facility (APCF) (European Space Agency, ESA)

The APCF is a multiuser facility dedicated to the growth of protein crystals. The APCF has successfully flown on previous Shuttle missions as a middeck payload and provides a wide range of experimental conditions. The following methods for crystallization can be employed: liquid/liquid interface diffusion, dialysis, or vapor diffusion.

An APCF occupies one Shuttle middeck locker space. A unit can contain up to 48 samples. Experimenters can choose different volumes for the solutions, typically 4-470  $\mu\text{L}$ . Temperature control within the APCF is  $\pm 0.3^\circ\text{C}$  for any preselected temperature between  $4^\circ\text{C}$  and  $20^\circ\text{C}$ . Ten crystal growth reactors can be monitored by B/W CCD video cameras and data from laser light scattering by micron size particles is available. The facility can be monitored for reactor status and temperature data. The process is fully automated, requiring crew intervention only to start (power on and initialize crystallization process) and to end (terminate process and power off) the experiment. The samples are returned with the Shuttle for analysis along with digitized video and imaging and hardware performance data. Late access to the facility for loading samples can be as late as two days prior to launch. Early removal of the samples from the vehicle can occur within several hours after landing.

### 3. Free Flow Electrophoresis Unit (FFEU) (National Space Development Agency of Japan, NASDA)

The FFEU is a multi-user Spacelab facility developed for the study of electrophoresis in space. The FFEU is a continuous flow electrophoresis unit which flew previously on Spacelab-J. Electrophoresis occurs in a sealed chamber containing the FFEU fluid components (buffer pumps, fluid pumps, etc.). Sixty (60) separation ports are available. Sample materials are stored in interchangeable cassettes and are installed in the FFEU on orbit. Each cassette hold 0.6 ml of

sample material. An adjustable electric field, maximums of 100mA and 100 V/cm, can be applied across the flow, causing the differently charged components to deflect into separate streams (fractions) which are monitored by a photometer using an ultra-violet light source. The maximum flow rate is 25 ml/min. One of three buffer solutions can be selected by the crew. The separation chamber can be cooled below 5° C. Ultraviolet absorbency monitoring of separation chambers is available. Samples are returned to earth for analysis. Late access to the facility for loading samples can be as late as one to two days prior to launch. Early removal of the samples from the vehicle can occur within several hours after landing.

4. Applied Research on Separation Methods using Space Electrophoresis (French Acronym: RAMSES) (French National Center for Space Studies, CNES, France)

RAMSES is a multi-user Spacelab facility designed to support basic and applied research on electrophoresis in space. It is a continuous flow zone electrophoresis unit. The sample material to be purified is continuously injected into a flowing buffer solution and carried across the separation chamber. The sample capacity is 10-20 ml. An adjustable electric field, 100 mA for 0-150V, 50 mA for 150-300V, can be applied across the flow, causing the differently charged components to deflect into separate streams (fractions) which are monitored by a photometer using an ultraviolet light source. When the photometer detects a significant amount of biological material in the output flow, each stream is individually collected in a total of 40 output ports. Otherwise the flow is diverted to a waste tank. Separation parameters – flow rates, electric field strengths, and buffer fluid temperature – can be altered to study a wide range of conditions. Separation experiments can also be monitored and photographed through a transparent window in the instrument front panel. A cross-illumination source provides a plane light sheet across the separation chamber that produces an image of the sample flow in cross section when viewed from the correct angle. Samples and cross-illumination photographs are returned to Earth for analysis. Other sensor data can be returned to Earth via the Spacelab data downlink. Late access to the facility for loading samples can be as late as one to two days prior to launch. Early removal of the samples from the vehicle can occur within several hours after landing.

5. Protein Crystallization Diagnostics Facility (PCDF) (European Space Agency, ESA)

The PCDF is a multi-user experimental facility capable of providing in-depth knowledge and understanding of protein crystal growth processes under microgravity. The crystal growth hardware consists of approximately six Dialysis Reactors and six Batch Reactors which have the mechanisms for optical focusing, zooming, and tracking of individual crystals. The scientific samples in the Reactors will be monitored by some or all of the following: high-resolution black and white video system, video microscope, static and dynamic light scattering, interferometer (straight and phase shift), pH sensors, and osmotic pressure sensors. Each Reactor is temperature controllable for a range of 4 to 40° C. The PCDF is designed to fly in an EXPRESS Rack (4 Middeck Locker Equivalents) on the International Space Station.

F. OTHER BIOTECHNOLOGY FLIGHT HARDWARE

1. Commercial Generic Bioprocessing Apparatus (CGBA)

The CGBA payload consists of a combination of temperature controlled locker replacement modules and fluid containment/mixing devices. It is compatible with Shuttle middeck, Spacehab, Spacelab and Mir interface requirements. Preparation of the CGBA payload allows samples to be loaded off-site and shipped to KSC for final integration, if so desired. Late access handover (L-24 hours) minimizes the time required between loading and launch for viable samples. Established protocol provides the opportunity to perform synchronized ground controls in flight-like hardware. Clinorotation in flight hardware is also available. The payload has flown on 8 STS missions since 1992 returning over 2000 cumulative biological and material samples at a better

than 99% success ratio. Experiments supported by CGBA have included: micro-organism growth, eucaryotic cell response, virus capsid formation, crystal growth, collagen and fibrin polymerization, and mammalian tissue development. The individual components of CGBA are described below.

- Fluid Processing Apparatus (FPA)

An FPA is essentially a "microgravity test tube". The first level of sample containment consists of a glass barrel (1.35 cm id x 11.7 cm) with movable rubber septa used to confine the fluids in separate chambers within the barrel. All components contacting the sample material are fully autoclavable allowing sterility to be maintained. The design provides initial isolation of 2 or 3 fluids and allows subsequent, on-orbit mixing. Fluid mixing is achieved as the fluid and septa are pushed forward until the fluid reaches a molded bypass in the glass barrel and flows around the forward septum into the adjacent chamber. The standard configuration provides a total liquid volume of 6.5 ml loaded as follows: 1.5 ml fixative / 1.5 ml initiator / 3.5 ml precursor. A sealed, Lexan sheath with a plunger handle encompasses the glass barrel providing an activation mechanism and a second level of containment. A positive pressure integrity test to 5 psi is performed on this preflight. Visual observation of samples in an FPA is possible and in-flight video or still photographs can be obtained.

Many variations of fluid volumes and configurations are possible. Several examples of modified FPAs include:

- a. Gas Exchange-FPA (GE-FPA): The GE-FPA has a gas permeable endcap and an O-ring Lexan insert is used in place of the distal rubber septum, thus allowing gas exchange between sample and entire GAP volume.

- b. Expanded Volume-FPA (EV-FPA): Provides up to 10 ml into 1 ml mixing capability.

- Group Activation Pack (GAP)

The GAP provides a third level of fluid containment composed of Lexan and aluminum. It allows simultaneous activation of 8 FPAs through attachment of a manual crank handle to a drive mechanism. (Used in GBA-INC or can be stowed in an ambient locker). A positive pressure integrity test to 5 psi is also performed on the GAPs preflight.

- Auto-GAP

Same as the GAP, but activated automatically by an external DC motor drive rather than a manual crank. (Used in GBA-ICM).

- GBA-INC

The GBA-INC is a middeck locker equivalent providing stowage for 9 GAPs(72 FPAs) at 37° C. Uniform temperature control is achieved using top and bottom strip heaters thermally coupled to the GAP aluminum endcaps. Optical density (565 nm) monitoring capability of 8 FPAs concurrently allows high resolution reaction rate data to be collected real-time.

- GBA-ICM

GBA-ICM is a middeck locker equivalent which provides temperature controlled stowage for 8 GAPs (64 FPAs) adjustable between 4° C and 37° C. Thermoelectric modules are used to transfer heat to/from active water loops distributed around all 6 sides to virtually eliminate thermal gradients. An accelerometer-based system is used to detect launch, thus allowing the GAPs to begin initiating experiments immediately upon entering orbit. Additionally, automatic GAP determination can be programmed to occur at any time during the mission, including just prior to reentry based on preplanned (or updated) end of mission time. Combined, these two capabilities allow an early-as-possible experiment initiation and a late-as-possible termination; periods when

crew availability for manual tasks is at a minimum. GBA-ICM also provides control versatility in light of launch delays, and can take advantage of mission duration extensions.

## 2. ADvanced SEPerations (ADSEP).

ADSEP is a fully-automated, processing unit that fits into a middeck or Spacehab locker. It is capable of separating living cells and cellular organelles using aqueous two-phase partitioning. The flight hardware contains three independently controlled processing modules, which can be programmed for totally automated operation or controlled via telemetry. Processing temperature can be independently controlled and regulated between 4-40° C in each of the three processing modules. Biological samples are loaded into a liquid-tight cassette assembly, which allows the cassettes to be installed and removed from the ADSEP modules on orbit. Processing consists of mixing with a programmable electromagnetic stirring system, and indexing the sample storage plates countercurrently. Each sample can be processed through up to 22 stages, employing a wide range of mixing, separation, and indexing parameters. In addition to separating cells, ADSEP has been employed for other fluids experiments where mechanical agitation, electromagnetic fields, and/or transfer of liquids from one well to the next is desired.

## 3. Materials Dispersion Apparatus (MDA) Minilab

The MDA is an automated laboratory which conducts approximately 100 fluid experiments within a brick-sized volume. Experiments which have been successfully conducted with this hardware include protein and other crystal growth, microencapsulation, thin film membrane formation, live cell culture studies, collagen formation, seed germination, and fluid science research. The heart of the MDA consists of a pair of blocks containing dozens of small test-tube like volumes of 20 to 500 uL each. Once in microgravity, the blocks are moved and the fluids which were separated are brought together to mix by either liquid-liquid diffusion, vapor diffusion, turbulent mixing, or wetting, depending upon the experiment design. As an option, the two liquids can be separated at a later time, or a third can be mixed in, such as a fixative for a cell culture experiment.

The MDA Minilab has to date successfully processed hundreds of biotechnology and other samples on the Shuttle. Four of the MDA Minilabs can be placed within a temperature-controlled middeck locker, for a total of up to 400 samples per locker. The MDA has flown on six Shuttle missions, along with six sounding rocket flights and the KC-135 low-g aircraft.

## **II. GROUND-BASED FACILITIES**

Investigators often need to conduct reduced gravity experiments in ground-based facilities during the experiment definition and technology development phases. The NASA ground-based reduced gravity research facilities that support the MRD program include two drop towers at the Lewis Research Center (LeRC), a DC-9 and KC-135 aircraft.

### A. 2.2-SECOND DROP TOWER (NASA Lewis Research Center, Cleveland, OH)

The 2.2-Second Drop Tower at LeRC provides 2.2 second of low-gravity test time for experiment packages consisting of up to 125 kilograms of hardware. The experiment package is enclosed in a drag shield and a gravitational acceleration of less than  $10^{-5}g$  is obtained during the fall since the experiment package falls freely within the drag shield. At the end of a drop, the drag shield and the enclosed experiment are decelerated in a 2.2-meter deep sand pit by the deceleration spikes. The peak deceleration rate can be as high as 70g's. Eight to twelve tests can be performed in one day. Data from experiments are acquired by high speed motion picture cameras

with rates up to 1,000 frames per second and by onboard data acquisition systems used to record data supplied by thermocouples, pressure transducers, and flow meters.

B. 5.18-SECOND ZERO-GRAVITY FACILITY (NASA Lewis Research Center, Cleveland, OH)

The 5.18-second Zero-Gravity facility at LeRC has a 132-meter free fall distance in a drop chamber which is evacuated by a series of pumpdown procedures to a final pressure of 1 Pa. Experiments with hardware weighing of up to 450 kilograms are mounted in a one-meter diameter by 3.4-meter high drop bus. Gravitational acceleration of less than  $10^{-5}g$  is obtained. At the end of the drop, the bus is decelerated in a 6.1-meter deep container filled with small pellets of expanded polystyrene. The deceleration rate is typically 60g (for 20 millisecond). Visual data is acquired through the use of high-speed motion picture cameras. Also, other data such as pressures, temperatures, and accelerations are either recorded onboard with various data acquisition systems or are transmitted to a control room by a telemetry system capable of transmitting 18 channels of continuous data. Due to the complexity of drop chamber operations and time required for pump-down of the drop chamber, typically only one test is performed per day.

C. PARABOLIC AIRCRAFT (NASA)

The aircraft can provide up to 40 periods of low-gravity for 25-second intervals each during one flight. The aircraft accommodates a variety of experiments and is often used to refine space flight experiment equipment and techniques and to train crew members in experiment procedures, thus giving investigators and crew members valuable experience working in a weightless environment. The aircraft obtain a low-gravity environment by flying a parabolic trajectory. Gravity levels twice those of normal gravity occur during the initial and final portions of the trajectory, while the brief pushover at the top of the parabola produces less than one percent of Earth's gravity ( $10^{-2}g$ ). Several experiments, including a combination of attached and free-floated hardware (which can provide effective gravity levels of  $10^{-3}$  for periods up to 10 seconds) can be integrated in a single flight. Both 28-volt DC and 100-volt AC power are available. Instrumentation and data collection capabilities must be contained in the experiment packages.

### **III. COMPUTATIONAL SUPPORT AND DATA MANAGEMENT**

NASA provides an advanced computational environment incorporating supercomputers, high performance mass storage, and software. NASA also provides an on-line, multidisciplinary directory of space science data sets of interest to the NASA-sponsored research community. NASA has chartered the NASA Science Internet (NSI) to provide transparent wide-area network connectivity to NASA researchers, computational resources, and data, worldwide. Each of these facilities and resources should be considered by an investigator to determine which are required for conducting biotechnology research. Investigators should include any requirements for these resources in their proposal.

**APPENDIX C  
NRA-97-HEDS-01**

**INSTRUCTIONS FOR RESPONDING TO  
NASA RESEARCH ANNOUNCEMENTS**

(JANUARY 1997)

A. General.

(1) Proposals received in response to a NASA Research Announcement (NRA) will be used only for evaluation purposes. NASA does not allow a proposal, the contents of which are not available without restriction from another source, or any unique ideas submitted in response to an NRA to be used as the basis of a solicitation or in negotiation with other organizations, nor is a pre-award synopsis published for individual proposals.

(2) A solicited proposal that results in a NASA award becomes part of the record of that transaction and may be available to the public on specific request; however, information or material that NASA and the awardee mutually agree to be of a privileged nature will be held in confidence to the extent permitted by law, including the Freedom of Information Act.

(3) NRA's contain programmatic information and certain requirements which apply only to proposals prepared in response to that particular announcement. These instructions contain the general proposal preparation information which applies to responses to all NRAs.

(4) A contract, grant, cooperative agreement, or other agreement may be used to accomplish an effort funded in response to an NRA. NASA will determine the appropriate instrument. Contracts resulting from NRA's are subject to the Federal Acquisition Regulation and the NASA FAR Supplement. Any resultant grants or cooperative agreements will be awarded and administered in accordance with the NASA Grant and Cooperative Agreement Handbook (NPG 5800.1).

(5) NASA does not have mandatory forms or formats for responses to NRA's; however, it is requested that proposals conform to the guidelines in these instructions. NASA may accept proposals without discussion; hence, proposals should initially be as complete as possible and be submitted on the proposers' most favorable terms.

(6) To be considered for award, a submission must, at a minimum, present a specific project within the areas delineated by the NRA; contain sufficient technical and cost information to permit a meaningful evaluation; be signed by an official authorized to legally bind the submitting organization; not merely offer to perform standard services or to just provide computer facilities or services; and not significantly duplicate a more specific current or pending NASA solicitation.

B. NRA-Specific Items. Several proposal submission items appear in the NRA itself: the unique NRA identifier; when to submit proposals; where to send proposals; number of copies required; and sources for more information. Items included in these instructions may be supplemented by the NRA.

C. Proposal Content. The following information is needed to permit consideration in an objective manner. NRAs will generally specify topics for which additional information or greater detail is desirable. Each proposal copy shall contain all submitted material, including a copy of the transmittal letter if it contains substantive information.

(1) *Transmittal Letter or Prefatory Material.*

- (i) The legal name and address of the organization and specific division or campus identification if part of a larger organization;
- (ii) A brief, scientifically valid project title intelligible to a scientifically literate reader and suitable for use in the public press;
- (iii) Type of organization: e.g., profit, nonprofit, educational, small business, minority, women-owned, etc.;
- (iv) Name and telephone number of the principal investigator and business personnel who may be contacted during evaluation or negotiation;
- (v) Identification of other organizations that are currently evaluating a proposal for the same efforts;
- (vi) Identification of the NRA, by number and title, to which the proposal is responding;
- (vii) Dollar amount requested, desired starting date, and duration of project;
- (viii) Date of submission; and
- (ix) Signature of a responsible official or authorized representative of the organization, or any other person authorized to legally bind the organization (unless the signature appears on the proposal itself).

(2) *Restriction on Use and Disclosure of Proposal Information.* Information contained in proposals is used for evaluation purposes only. Offerors or quoters should, in order to maximize protection of trade secrets or other information that is confidential or privileged, place the following notice on the title page of the proposal and specify the information subject to the notice by inserting an appropriate identification in the notice. In any event, information contained in proposals will be protected to the extent permitted by law, but NASA assumes no liability for use and disclosure of information not made subject to the notice.

**Notice**

Restriction on Use and Disclosure of Proposal Information

The information (data) contained in [insert page numbers or other identification] of this proposal constitutes a trade secret and/or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that it will not, without permission of the offeror, be used or disclosed other than for evaluation purposes; provided, however, that in the event a contract (or other agreement) is awarded on the basis of this proposal the Government shall have the right to use and disclose this information (data) to the extent provided in the contract (or other agreement). This restriction does not limit the Government's right to use or disclose this information (data) if obtained from another source without restriction.

(3) *Abstract.* Include a concise (200-300 word if not otherwise specified in the NRA) abstract describing the objective and the method of approach.

(4) *Project Description.*

(i) The main body of the proposal shall be a detailed statement of the work to be undertaken and should include objectives and expected significance; relation to the present state of knowledge; and relation to previous work done on the project and to related work in progress elsewhere. The statement should outline the plan of work, including the broad design of experiments to be undertaken and a description of experimental methods and procedures. The project description should address the evaluation factors in these instructions and any specific factors in the NRA. Any substantial collaboration with individuals not referred to in the budget or use of consultants should be described. Subcontracting significant portions of a research project is discouraged.

(ii) When it is expected that the effort will require more than one year, the proposal should cover the complete project to the extent that it can be reasonably anticipated. Principal emphasis should be on the first year of work, and the description should distinguish clearly between the first year's work and work planned for subsequent years.

(5) *Management Approach.* For large or complex efforts involving interactions among numerous individuals or other organizations, plans for distribution of responsibilities and arrangements for ensuring a coordinated effort should be described.

(6) *Personnel.* The principal investigator is responsible for supervision of the work and participates in the conduct of the research regardless of whether or not compensated under the award. A short biographical sketch of the principal investigator, a list of principal publications and any exceptional qualifications should be included. Omit social security number and other personal items which do not merit consideration in evaluation of the proposal. Give similar biographical information on other senior professional personnel who will be directly associated with the project. Give the names and titles of any other scientists and technical personnel associated substantially with the project in an advisory capacity. Universities should list the approximate number of students or other assistants, together with information as to their level of academic attainment. Any special industry-university cooperative arrangements should be described.

(7) *Facilities and Equipment.*

(i) Describe available facilities and major items of equipment especially adapted or suited to the proposed project, and any additional major equipment that will be required. Identify any Government-owned facilities, industrial plant equipment, or special tooling that are proposed for use. Include evidence of its availability and the cognizant Government points of contact.

(ii) Before requesting a major item of capital equipment, the proposer should determine if sharing or loan of equipment already within the organization is a feasible alternative. Where such arrangements cannot be made, the proposal should so state. The need for items that typically can be used for research and non-research purposes should be explained.

(8) *Proposed Costs.*

(i) Proposals should contain cost and technical parts in one volume: do not use separate "confidential" salary pages. As applicable, include separate cost estimates for salaries and wages; fringe benefits; equipment; expendable materials and supplies; services; domestic and foreign travel; ADP expenses; publication or page charges; consultants; subcontracts;

other miscellaneous identifiable direct costs; and indirect costs. List salaries and wages in appropriate organizational categories (e.g., principal investigator, other scientific and engineering professionals, graduate students, research assistants, and technicians and other non-professional personnel). Estimate all staffing data in terms of staff-months or fractions of full-time.

(ii) Explanatory notes should accompany the cost proposal to provide identification and estimated cost of major capital equipment items to be acquired; purpose and estimated number and lengths of trips planned; basis for indirect cost computation (including date of most recent negotiation and cognizant agency); and clarification of other items in the cost proposal that are not self-evident. List estimated expenses as yearly requirements by major work phases.

(iii) Allowable costs are governed by FAR Part 31 and the NASA FAR Supplement Part 1831 (and OMB Circulars A-21 for educational institutions and A-122 for nonprofit organizations).

(9) *Security*. Proposals should not contain security classified material. If the research requires access to or may generate security classified information, the submitter will be required to comply with Government security regulations.

(10) *Current Support*. For other current projects being conducted by the principal investigator, provide title of project, sponsoring agency, and ending date.

(11) *Special Matters*.

(i) Include any required statements of environmental impact of the research, human subject or animal care provisions, conflict of interest, or on such other topics as may be required by the nature of the effort and current statutes, executive orders, or other current Government-wide guidelines.

(ii) Proposers should include a brief description of the organization, its facilities, and previous work experience in the field of the proposal. Identify the cognizant Government audit agency, inspection agency, and administrative contracting officer, when applicable.

D. Renewal Proposals.

(1) Renewal proposals for existing awards will be considered in the same manner as proposals for new endeavors. A renewal proposal should not repeat all of the information that was in the original proposal. The renewal proposal should refer to its predecessor, update the parts that are no longer current, and indicate what elements of the research are expected to be covered during the period for which support is desired. A description of any significant findings since the most recent progress report should be included. The renewal proposal should treat, in reasonable detail, the plans for the next period, contain a cost estimate, and otherwise adhere to these instructions.

(2) NASA may renew an effort either through amendment of an existing contract or by a new award.

E. Length. Unless otherwise specified in the NRA, effort should be made to keep proposals as brief as possible, concentrating on substantive material. Few proposals need exceed 15-20 pages. Necessary detailed information, such as reprints, should be included as attachments. A complete set of attachments is necessary for each copy of the proposal. As proposals are not returned, avoid use of "one-of-a-kind" attachments.

F. Joint Proposals.

(1) Where multiple organizations are involved, the proposal may be submitted by only one of them. It should clearly describe the role to be played by the other organizations and indicate the legal and managerial arrangements contemplated. In other instances, simultaneous submission of related proposals from each organization might be appropriate, in which case parallel awards would be made.

(2) Where a project of a cooperative nature with NASA is contemplated, describe the contributions expected from any participating NASA investigator and agency facilities or equipment which may be required. The proposal must be confined only to that which the proposing organization can commit itself. "Joint" proposals which specify the internal arrangements NASA will actually make are not acceptable as a means of establishing an agency commitment.

G. Late Proposals. A proposal or modification received after the date or dates specified in an NRA may be considered if doing so is in the best interests of the Government.

H. Withdrawal. Proposals may be withdrawn by the proposer at any time before award. Offerors are requested to notify NASA if the proposal is funded by another organization or of other changed circumstances which dictate termination of evaluation.

I. Evaluation Factors.

(1) Unless otherwise specified in the NRA, the principal elements (of approximately equal weight) considered in evaluating a proposal are its relevance to NASA's objectives, intrinsic merit, and cost.

(2) Evaluation of a proposal's relevance to NASA's objectives includes the consideration of the potential contribution of the effort to NASA's mission.

(3) Evaluation of its intrinsic merit includes the consideration of the following factors of equal importance:

(i) Overall scientific or technical merit of the proposal or unique and innovative methods, approaches, or concepts demonstrated by the proposal.

(ii) Offeror's capabilities, related experience, facilities, techniques, or unique combinations of these which are integral factors for achieving the proposal objectives.

(iii) The qualifications, capabilities, and experience of the proposed principal investigator, team leader, or key personnel critical in achieving the proposal objectives.

(iv) Overall standing among similar proposals and/or evaluation against the state-of-the-art.

(4) Evaluation of the cost of a proposed effort may include the realism and reasonableness of the proposed cost and available funds.

J. Evaluation Techniques. Selection decisions will be made following peer and/or scientific review of the proposals. Several evaluation techniques are regularly used within NASA. In all cases proposals are subject to scientific review by discipline specialists in the area of the proposal. Some proposals are reviewed entirely in-house, others are evaluated by a combination of in-house and selected external reviewers, while yet others are subject to the full external peer review technique (with due regard for conflict-of-interest and protection of proposal information), such as by mail or through assembled panels. The final decisions are made by a NASA selecting official. A proposal which is scientifically and

programmatically meritorious, but not selected for award during its initial review, may be included in subsequent reviews unless the proposer requests otherwise.

K. Selection for Award.

(1) When a proposal is not selected for award, the proposer will be notified. NASA will explain generally why the proposal was not selected. Proposers desiring additional information may contact the selecting official who will arrange a debriefing.

(2) When a proposal is selected for award, negotiation and award will be handled by the procurement office in the funding installation. The proposal is used as the basis for negotiation. The contracting officer may request certain business data and may forward a model award instrument and other information pertinent to negotiation.

L. Cancellation of NRA. NASA reserves the right to make no awards under this NRA and to cancel this NRA. NASA assumes no liability for canceling the NRA or for anyone's failure to receive actual notice of cancellation.







**FORM B**

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: \_\_\_\_\_

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD DIRECT COSTS ONLY		FROM	THROUGH		
Duplicate this form for each year of grant support requested		DOLLAR AMOUNT REQUESTS <i>(Omit cents)</i>			
PERSONNEL <i>(Applicant Organization Only)</i>		EFFORT ON PROJECT	SALARY	FRINGE BENEFITS	TOTALS
NAME	ROLE IN PROJECT				
	Principal Investigator				
SUBTOTALS →					
CONSULTANT COSTS					
EQUIPMENT <i>(Itemize, use additional sheet if needed)</i>					
SUPPLIES <i>(Itemize by category, use additional sheet if needed)</i>					
TRAVEL	DOMESTIC				
	FOREIGN				
OTHER EXPENSES <i>(Itemize by category, use additional sheet if needed)</i>					
<b>TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD</b> <i>(Item 12a, Form A)</i>			\$		
<b>INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD</b>			\$		
<b>TOTAL COSTS FOR FIRST 12-MONTH BUDGET PERIOD</b> <i>(Item 12b, Form A)</i>			\$		

**FORM C**

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: \_\_\_\_\_

**BUDGET FOR ENTIRE PROJECT PERIOD DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS	1st BUDGET PERIOD	ADDITIONAL YEARS OF SUPPORT REQUESTED		
		2nd	3rd	4th
PERSONNEL ( Salary and Fringe Benefits ) ( Applicant organization only )				
CONSULTANT COSTS				
EQUIPMENT				
SUPPLIES				
TRAVEL	DOMESTIC			
	FOREIGN			
OTHER EXPENSES				
<b>TOTAL DIRECT COSTS FOR EACH BUDGET PERIOD</b>	\$	\$	\$	\$
<b>TOTAL INDIRECT COSTS FOR EACH BUDGET PERIOD</b>	\$	\$	\$	\$
<b>TOTAL DIRECT + INDIRECT COSTS FOR EACH PERIOD</b>	\$	\$	\$	\$
<b>TOTAL DIRECT + INDIRECT COSTS FOR ENTIRE PROJECT</b>				\$

JUSTIFICATION FOR UNUSUAL EXPENSES (Detail Justification in Cost Section of Proposal)

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CERTIFICATION REGARDING DRUG-FREE WORKPLACE REQUIREMENTS

This certification is required by the regulations implementing the Drug-Free Workplace Act of 1988, 34 CFR Part 85, Subpart F. The regulations, published in the January 31, 1989 Federal Register, require certification by grantees, prior to award, that they will maintain a drug-free workplace. The certification set out below is a material representation of fact upon which reliance will be placed when the agency determines to award the grant. False certification or violation of the certification shall be grounds for suspension of payments, suspension or termination of grants, or government-wide suspension or debarment (see 34 CFR Part 85, Sections 85.615 and 85.620).

I. GRANTEES OTHER THAN INDIVIDUALS

- A. The grantee certifies that it will provide a drug-free workplace by:
(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;
(b) Establishing a drug-free awareness program to inform employees about --
(1)The dangers of drug abuse in the workplace;
(2)The grantees policy of maintaining a drug-free workplace;
(3)Any available drug counseling, rehabilitation, and employee assistance programs; and
(4)The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;
(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);
(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will --
(1)Abide by the terms of the statement; and
(2)Notify the employer of any criminal drug statute conviction for a violation occurring in the workplace no later than five days after such conviction;
(e) Notifying the agency within ten days after receiving notice under subparagraph (d) (2) from an employee or otherwise receiving actual notice of such conviction;
(f) Taking one of the following actions, within 30 days of receiving notice under subparagraph (d) (2), with respect to any employee who is so convicted --
(1)Taking appropriate personnel action against such an employee, up to and including termination; or
(2)Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or Local health, Law enforcement, or other appropriate agency;
(g)Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e), and (f).

B. The grantee shall insert in the space provided below the site(s) for the performance or work done in connection with the specific grant: Place of Performance (Street address, city, county, state, zip code)

\_\_\_\_\_
\_\_\_\_\_

Check \_\_\_\_ if there are workplaces on file that are not identified here.

II. GRANTEES WHO ARE INDIVIDUALS

The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession or use of a controlled substance in conducting any activity with the grant.

Organization Name AO or NRA Number and Title

Printed Name and Title of Authorized Representative

Signature Date

Printed Principal Investigator Name Proposal Title

**FORM E**

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**CERTIFICATION REGARDING  
DEBARMENT, SUSPENSION, AND OTHER RESPONSIBILITY MATTERS  
PRIMARY COVERED TRANSACTIONS**

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This certification is required by the regulations implementing Executive Order 12549, Debarment and Suspension, 34 CFR Part 85, Section 85.510, Participants' responsibilities. The regulations were published as Part VII of the May 28, 1988 Federal Register (pages 19160-19211). Copies of the regulations may be obtained by contacting the U.S. Department of Education, Grants and Contracts Service, 400 Maryland Avenue, SW (Room 3633 GSA Regional Office Building No. 3), Washington, DC 20202-4725, telephone (202) 732-2505.

A. The applicant certifies that it and its principals:

- (a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;
- (b) Have not within a three-year period preceding this application been convicted or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State, or Local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;
- (c) Are not presently indicted for or otherwise criminally or civilly charged by a government entity (Federal, State, or Local) with commission of any of the offenses enumerated in paragraph A.(b) of this certification; and
- (d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State, or Local) terminated for cause or default; and

B. Where the applicant is unable to certify to any of the statements in this certification, he or she shall attach an explanation to this application.

C. Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lowered Tier Covered Transactions (Subgrants or Subcontracts)

- (a) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principles is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department of agency.
- (b) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

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Organization Name

AO or NRA Number and Title

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Printed Name and Title of Authorized Representative

---

Signature

Date

---

Printed Principal Investigator Name

Proposal Title

**FORM F**

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**CERTIFICATION REGARDING LOBBYING**

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As required by S 1352 Title 31 of the U.S. Code for persons entering into a grant or cooperative agreement over \$100,000, the applicant certifies that:

(a) No Federal appropriated funds have been paid or will be paid by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, in connection with making of any Federal grant, the entering into of any cooperative, and the extension, continuation, renewal, amendment, or modification of any Federal grant or cooperative agreement;

(b) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting an officer or employee of any agency, Member of Congress, an or an employee of a Member of Congress in connection with this Federal grant or cooperative agreement, the undersigned shall complete Standard Form - LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(c) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subgrants, contracts under grants and cooperative agreements, and subcontracts), and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by S1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

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Organization Name

AO or NRA Number and name

---

Printed Name and Title of Authorized Representative

---

Signature

Date

---

Printed Principal Investigator Name

Proposal Title

## NASA Research Announcement (NRA) Mailing List Update

This is the form to update information for the NASA Office of Life & Microgravity Sciences & Applications (OLMSA) NRA mailing list. Please fill out **CONTACT INFORMATION** completely. Check only those that apply in **INSTITUTION TYPE** and **PROGRAM AREAS/DISCIPLINES**. Fold the form, secure with tape (do not staple), and mail it back to the address on the reverse side. Proper postage must be applied.

~~Mailings will be sent electronically via E-Mail or World Wide Web to the following addresses:~~

**Check one:**

- |   |   |
|---|---|
| <input type="checkbox"/> 1. Please <b>add</b> my name to the mailing list.                                    | <input type="checkbox"/> 3. Please <b>change</b> my current listing (please attach mailing label).          |
| <input type="checkbox"/> 2. Please <b>remove</b> my name from the mailing list (please attach mailing label). | <input type="checkbox"/> 4. Please leave my current listing <b>unchanged</b> (please attach mailing label). |

<b>Contact Information</b>	
If your address has changed or your mailing label is incorrect, please provide COMPLETE contact information.	
Prefix: (Mr., Mrs., Ms., Dr., Prof., etc.)	Suffix: (M.D., Ph.D., Jr., III, etc.)
Name, First:	Last:
Position Title:	
Mail Code, Loc:	
Office, Dept, Div:	
Org/Agency/Ctr,	
Street or PO Box:	
City:	State:
Zip Code:	Country:
Telephone No:	Fax No:
Internet/E-Mail:	

**Institution Type**

(check all that apply)

- |  |   |   |
|--|---|---|
| <input type="checkbox"/> 1. College or University          | <input type="checkbox"/> 4. NASA Center             | <input type="checkbox"/> 7. Small Business    |
| <input type="checkbox"/> 2. Minority College or University | <input type="checkbox"/> 5. Other Government Agency | <input type="checkbox"/> 8. Private Industry  |
| <input type="checkbox"/> 3. Minority Business              | <input type="checkbox"/> 6. Nonprofit Corporation   | <input type="checkbox"/> 9. Foreign Addressee |

**Program Areas/Disciplines**

(check main area of interest)

- |   |  |
|---|--|
| <input type="checkbox"/> <b>1. Life Sciences</b><br><input type="checkbox"/> A. Advanced Life Support<br><input type="checkbox"/> B. Advanced Technology Development<br><input type="checkbox"/> C. Data Analysis<br><input type="checkbox"/> D. Environmental Health | <input type="checkbox"/> <b>2. Microgravity Sciences</b><br><input type="checkbox"/> A. Biotechnology<br><input type="checkbox"/> B. Combustion Science<br><input type="checkbox"/> C. Fluid Physics<br><input type="checkbox"/> D. Fundamental Physics<br><input type="checkbox"/> E. Materials Science |
|---|--|
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