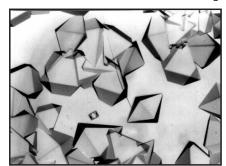


Educational Products		
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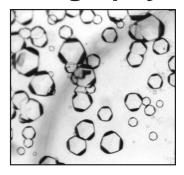
EB-2000-10-183-MSEC

## **Educational Brief**

## A NASA Recipe For Protein Crystallography







**Glucose Isomerase Crystals** 

**Lysozyme Crystals** 

**Excelsin Crystals** 

Protein crystals can be very difficult to grow. Even protein crystallographers are often unable to produce crystals of the high quality that is required to determine the molecular structure of many proteins. As part of NASA's educational outreach activities, we have prepared a simple 'recipe' for growing protein crystals from Brazil nuts. Please let us know how your attempts at growing these protein crystals turn out. You may contact us at:

#### microgravitynews@msfc.nasa.gov

For more up-to-date information about NASA's macromolecular crystallography program, see: <a href="http://crystal.nasa.gov">http://crystal.nasa.gov</a>

Note: all **bold face** words are referenced in the glossary in the back of this document.

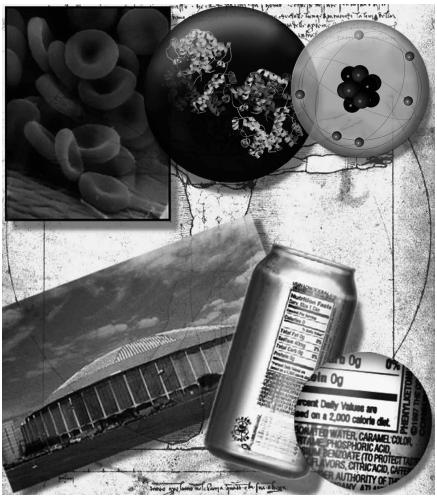
## Introduction

Many hours of scientific study and investigation are invested in growing protein crystals – crystals that, when nearly perfect in form, are very highly prized. The information they reveal about a protein's molecular structure makes them very sought after and important to science. Proteins, macromolecules involved in everyday functions of the body such as transporting oxygen and chemicals in blood, forming major components of muscle and skin, and fighting disease, come in over 100,000 varieties. Active sites on molecules of proteins, when inappropriately triggered or absent, can cause disease or an unwanted function. Scientists seek to locate those active sites so drug designers can understand their function and then, in some cases, work to block them or render them inactive. For example, the anti-inflammatory drug ibuprofen works on a specific protein, which is involved early in the signaling process that tells your body that inflammation should occur. Blocking the active site on this protein prevents or reduces the inflammation.

Infortunately, protein molecules are so small that humans can't see them individually, let alone find a specific site on a molecule or determine its molecular structure. If one cell in the human body were the size of a football stadium, one protein molecule would be approximately the size of a can of soda, and one of the atoms making up that protein molecule would be about the size of the fine print on the can.

A protein crystal is a three-dimensional array of molecules in which every molecule or specific group of molecules has the same orientation and relationship to its neighbors, as long as the chemical environment of the solution surrounding the crystal remains the same. When a protein crystal forms, protein molecules or groups of molecules align to produce a repeating three-dimensional pattern or array. The effect of bringing these molecules together in this arrangement is called amplification Amplification can be thought of in this way: image

together in this arrangement is called amplification. Amplification can be thought of in this way: imagine a football stadium full of people. If only one person stands up and yells, the sound produced is not easily heard. But if everybody in the stadium stands up, faces the same direction and yells at the same time, that sound can be heard from a great distance. A very similar thing happens during the analysis of a protein crystal. One protein



Comparative sizes of proteins and the atoms making up proteins

molecule, by itself, would produce a signal so weak it would be undetectable. But if all the molecules in the crystal produced the same signal at exactly the same time, then that signal would be strong enough to be recorded and decoded. The more closely oriented or aligned the molecules making up the protein crystal are, the better the signal, and the more accurate the molecular information.

## **History**

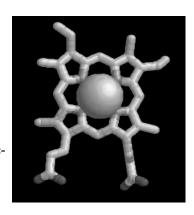
he first known published observation of the crystallization of a protein was made by F.L. Hunefeld in 1840 at Leipzig University in Germany. While working with hemoglobin, Hunefeld obtained flat, plate-like crystals of this protein when he pressed the blood of an earthworm between two slides of glass and allowed the blood to dry very slowly.

In 1851, Otto Funke, another German researcher, published a series of articles in which he described growing hemoglobin crystals by successively diluting red blood cells with a solvent such as pure water, alcohol or ether, followed by slow evaporation of the solvent from the resulting protein solution.

Early on, scientists grew crystals solely to purify proteins. Not until the 1930's did researchers begin to focus their attention on crystals as a source of structural information about protein molecules. They turned to **X-ray diffraction**, a procedure in which a pencil-lead-sized X-ray beam is directed at a crystal. The X-ray beam is scattered by the crystal, producing a signal that results in tiny pinpoints that can be recorded on film. Data from this recorded X-ray diffraction pattern has a direct relationship to the protein's molecular structure and can be used to help reveal the structure of a molecule of the particular protein under investigation. By the 1960's, scientists were investigating the molecular

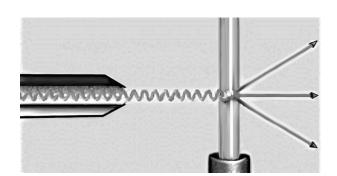
structures of an abundance of crystals grown by biochemists. Further, there was a century-long backlog of crystals to be investigated. By the 1970's, however, the

X-ray crystallographers had become more selective in determining which proteins needed to be crystallized for analysis, and production of the desired crystals was no longer meeting demand. New methods for growing higher-quality



Molecular structure of the heme ring in the hemoglobin molecule

crystals were needed. The inability of scientists to produce crystals useable for successful analysis of some proteins had become a bottleneck in the process of determining the three-dimensional structures of the molecules of many important proteins. Typically a protein crystal must be structurally well ordered and from about 0.2 to 0.5 mm in size. With a high water content, protein crystals are usually quite fragile and somewhat difficult to handle. Protein crystals can be described as "soft" in contrast to a "hard" salt crystal.



X-ray beam penetrates a protein crystal



3

## Enter Microgravity

During the 1970's, Walter Littke, a space research pioneer and professor of chemistry at the University of Freiburg, Germany, was using a common method of growing protein crystals: placing a salt **solution** together with the protein solution. When two such solutions come in contact, the salt becomes associated with some of the water molecules in which the protein is dissolved. This causes the protein molecules to move closer together and to begin to crystallize. However, many of the crystals produced by this method are fragile, and small or broken.

By 1980, Littke suspected the culprit in his unusable crystals to be convection, or fluid flow, in the solution surrounding the growing protein crystals. This phenomenon occurs in normal gravity, also known on Earth as one-g. Convection takes place during crystal growth in one-g as protein molecules move from the surrounding solution and assemble in an orderly way to become a part of the growing crystal lattice. As protein molecules in the solution move toward a crystal and become a part of the crystal, the solution bordering the crystal then contains a lower protein concentration than the remainder of the solution and, therefore, it has a lower density. This less-dense solution tends to rise, and the denser solution sinks under the influence of gravity, creating fluid flows or convection, next to the crystal. These convective currents can have a negative effect on the quality of the crystal being formed, because they can alter the orientation and position of the protein molecules as they become a part of the crystal lattice. This can cause disorder in the lattice structure of the protein crystal. These imperfections in the crystal lattice, in turn, adversely affect X-ray diffraction analysis results, or

the clarity with which a crystallographer can "see" the precise position each atom occupies in the three-dimensional structure of the protein molecule.

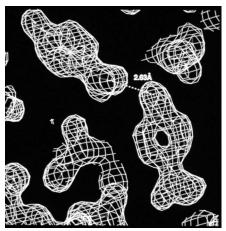
Another adverse effect of gravity on growing crystals is **sedimentation**. Crystals drift to the bottom of a drop of the solution when they have grown to a mass larger than can be supported by suspension in the drop. When this happens, partially formed crystals fall on top of one another and continue growing into each other. Since X-ray diffraction analysis requires single crystals, sedimentation renders potentially high-quality crystals unusable for data collection.

Scientists began to consider the idea that in a microgravity environment, with reduced convection and sedimentation, protein molecules would move together more slowly, primarily by diffusion. It was expected that higher quality crystals could be produced in the microgravity environment, such as that of the orbiting Space Shuttle.

But just how do scientists grow a protein crystal in microgravity (or on Earth, for that matter), and what do they do with it once they have grown it? Researchers begin with a protein solution that is supersaturated. A protein solution is prepared by dissolving the protein, also called the solute, in a solvent. Proteins have a solubility, which is a measure of the amount of the protein (solute) that can be dissolved in a given solvent, under specific conditions. Growing a crystal, however, requires supersaturation, which is a less stable condition that results when somewhat more than maximum amount of protein that can be dissolved in a solvent under nominal conditions is, in fact, dissolved in the solvent.

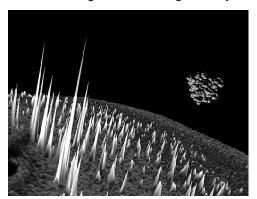
## Blueprint for Growing a Crystal

ost scientists don't know the precise solubility of the protein they may be investigating. A researcher tests many different solution conditions by varying such parameters as protein concentration, salt concentration and pH, in order to find the best conditions required to promote the formation of usable protein crystals. Once the researcher has defined an optimum range of solution conditions, he or she usually uses one of two methods for actually growing the crystal: vapor diffusion or liquid/liquid diffusion. Both methods involve changing the conditions of a protein solution to supersaturate the solution. In vapor diffusion, the crystallographer places a drop of protein solution in a chamber that also holds a solution called a **precipitant solution**, typically a salt solution. The salt in the precipitant solution is more concentrated than salt that is in the protein solution. This causes water vapor to diffuse through the air from the drop to the precipitant solution. As water is removed from the drop, the protein becomes more concentrated, causing protein molecules to move closer together and nucleate. In liquid/liquid diffusion, the researcher diffuses a salt solution or some other precipitant solution in one compartment of a two-chamber vial into a protein solution in the other compartment. As the salt concentration increases, some of the water from the protein solution becomes associated with the salt,



Electron Density Map

effectively raising the concentration of the protein in the solution. Under these conditions the protein molecules come together and begin to crystallize.



X-ray diffraction pattern from a protein crystal

To analyze a protein crystal, an X-ray crystallog-rapher shines an X-ray beam through the crystal. Unlike a single dental X-ray, which produces a shadow image of a tooth, these X-ray images have to be taken many times from different angles to produce a pattern from the scattered X-ray beam. This pattern is a map of the intensity of the X-rays after they diffract through the crystal. The X-rays actually bounce off the electron clouds that form the outer structure of each atom. A flawed crystal will yield a blurred pattern; a well-ordered protein crystal will yield a series of sharp, more distinct diffraction patterns.

From these patterns, researchers build an **electron density map**. With powerful computers capable of performing complex mathematical calculations, scientists can use the electron density patterns to determine the structure of the protein and make computer-generated models of the three-dimensional structure of a protein molecule.

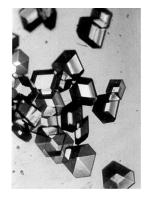
The models allow researchers to improve understanding of how the protein functions. In addition, the models allow scientists to look for **receptor sites** and active areas that control a protein's function and role in the progression of diseases.

## NASA Macromolecular Crystal Growth Results

icrogravity has been the chosen environment for dozens of fundamental science experiments growing macromolecular crystals. Since NASA's protein crystal growth program began, principal investigators and their research teams have flown samples of a total of 185 different proteins, RNA's, DNA's and viruses (as of August 1999). The macromolecules these scientists have studied range from insulin to lactate dehydrogenase ( a major enzyme in energy production and an important muscle protein in all animals) to thaumatin (a sweet tasting protein with potential as a sugar substitute.) Most of NASA's protein crystal growth experiments, conducted from 1985 to 1999, have been flown on Space Shuttle missions. The remainder were conducted on the Russian space station Mir.

Given the great strides NASA's protein crystal growth program has made since the first protein crystal growth experiments were conducted in microgravity, where is the field headed now? For many, it is to the International Space Station (ISS).





Earth (1g)

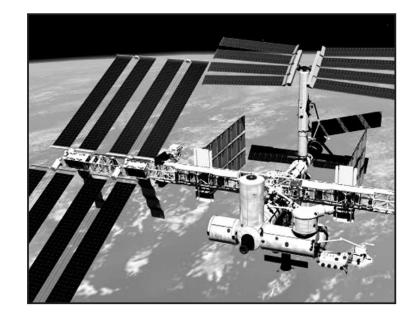
Space (µg)

Normal gravity vs. microgravity grown insulin crystals

The ISS will expand the opportunities for growing crystals in microgravity, enabling continued advances in understanding the fundamental science of the crystal growth process. Crystals, which usually grow more slowly in microgravity, will have more time to fully develop into usable specimens. Follow-up experiments, an important and common feature of all ground-based research, will be more feasible in space on the ISS.

The International Space Station (ISS) will provide a platform for long term microgravity experiments

http://spaceflight.nasa.gov/station





## Connections to Academic Standards

This NASA Educational Brief supports the following National Academy of Sciences Science Content Standards:

National Science Education Content Standards (Grades 9-12) supported by the NASA Recipe for Protein Crystallography.

Abilities Necessary to do Scientific Inquiry	<ol> <li>Identify questions and concepts that guide scientific investigations</li> <li>Design and conduct scientific investigations</li> <li>Use technology and mathematics to improve investigations and communications</li> <li>Formulate and revise scientific explanations and models using logic and evidence</li> <li>Recognize and analyze alternative explanations and models</li> <li>Communicate and defend a scientific argument</li> <li>Understandings about scientific inquiry</li> </ol>
Physical Science	1) Structure of atoms 2) Structure and properties of matter 3) Chemical reactions 4) Motions and forces
Life Science	1) The cell 2) Molecular basis of heredity 3) Matter, energy, and organization in living systems 4) Behavior of organisms
Science and Technology	Abilities of technological design     Understandings about science and technology
Science in Personal and Social Perspectives	Personal and community health     Science and technology in local, national, and global challenges
History and Nature of Science	1) Science as human endeavor 2) Nature of scientific knowledge 3) Historical perspectives



# Isolation, Crystallization & Purification of Excelsin From Brazil Nuts

Ground Brazil nuts can be extracted with 5% aqueous NaCl at 50° to 70° C, filtered, then dialyzed against distilled water to obtain crystals.

This activity takes approximately 60 minutes

(CAUTION: Glass wool should only be handled while wearing gloves.)

## **Key Words**

Aqueous	Dialysis Membrane (or tubing)	Morphology
Clarify	Centrifuge	Pellet
Decant	Opaque	Dialyze
Filtrate	Denature	Supernatant

#### **Materials**

Funnel	Styrofoam (for bottom of 500 ml beaker)
Water (distilled preferred)	Table Salt (sodium chloride)
Brazil Nuts (raw, organically grown)	Glass Rod
Centrifuge Tubes	Cheesecloth, Glass Wool, or Filter Paper
Dialysis Membrane (app. 20 cm in length)	Pipette or Medicine Dropper
50 ml graduated cylinder	Thermometer
500 ml and 150 ml beakers	Heating Plate
Small clamp(s)	Small Centrifuge (up to 3000rpm)(optional)
Blender or Coffee Grinder	Laboratory Balance



## Methods

#### I. Isolation of Excelsin

- Record the mass of ~ 8 or 9 raw Brazil nuts (preferably organically grown). Grind the nuts to granular size. 1 nut has a mass of approximately 2.8 grams.
- 2) Prepare a 5% sodium chloride (NaCl) solution by dissolving 10 grams of solid NaCl in 200 milliliters of distilled water. Measure 25 ml of 5% NaCl solution into a graduated cylinder and then pour into a 150 ml beaker. Record the volume of NaCl solution in the beaker. (Reserve remaining 5% NaCl solution for making 1% NaCl solution in Part II.3.)
- 3) Create a water bath by placing a flat piece of styrofoam (can be cut from the bottom of a styrofoam cup) into the bottom of a 500 ml beaker. Pour approximately 50 ml of water into the 500 ml beaker. Place the smaller 150 ml beaker (that contains the NaCl solution) in the larger 500 ml beaker (that contains the water). Place the beaker assembly on the heating plate and heat until the water is at 50°C.

- Measure the final temperature and record.
- 4) Add the ground nuts to the NaCl solution in the 150 ml beaker and stir with a glass rod to mix. Continue heating the water bath to maintain the temperature of the NaCl solution and ground nut mixture at 50°C to 70°C for 20 minutes, stirring occasionally. Record the temperature at regular intervals. (Be careful not to stir vigorously or to heat above the designated temperature range because this may cause the protein to **denature** or break down).
- 5) (Caution: Glass wool should only be handled when wearing gloves.) Place 1-2 layers of glass wool (or 10 layers of cheesecloth or filter paper) in a funnel and place over a beaker. Pour the NaCl/nut mixture through glass wool. Measure and record the volume of filtrate. (Note: Filtrate should be opaque.)
- 6) Option 1 For classrooms without a centrifuge: Skip steps 7-9 and go directly to the Crystallization of Excelsin in Section II.







- 7) Option 2 For classrooms with a centrifuge: Pour filtrate into two centrifuge tubes and weigh to make sure tubes are of equal weight. Record the mass of each centrifuge tube.
- 8) Centrifuge for 20 minutes at 3000 rpm. Referring to the diagram from the previous page, pipette off the oil (top layer) and discard. Carefully push the pipette through the nut meat
- layer into the **aqueous** layer and decant off the aqueous portion (located above the **pellet** and just below the oil). Discard the pellet and oil.
- 9) Centrifuge the supernatant to clarify the solution further. The supernatant should now be a fairly clear brown or brownish-yellow solution. Measure and record the volume of the extracted protein solution.

## II. Crystallization of Excelsin

- Soak the dialysis membrane (small molecular weight, preferably below 100,000 MWCO (molecular weight cut off)) in room temperature water for 5 minutes or longer. Take the membrane out of the beaker and tie a knot in one end. Refer to page 12 for procedures on the handling of dialysis tubing.
- Pour the extracted protein/filtrate solution from step 5 or 8 above into the dialysis membrane/tubing and tie the other end of the membrane.
- 3) Prepare 500 ml of 1% NaCl solution by combining 100 ml of 5% NaCl solution and 400 ml of distilled water. **Dialyze** the protein solution
- against the 500 ml of 1% NaCl solution. The volume of the 1% NaCl solution should be at least three times the measured volume of the extracted solution. Record the volume of protein filtrate/solution and 1% NaCl solution. Crystals should appear 4 10 hours later (They will appear as white powder in the bottom of the bag). If crystals do not appear in the 1% NaCl solution, lower the NaCl concentration to 0%.
- 4) When the white powder appears, view it under a microscope while the solution is still in the bag.

## III. Recrystalization and Purification of Excelsin

- 1) Cut one end of the **dialysis membrane** bag and **decant** the solution off of the crystals.
- 2) Add just enough 5% NaCl solution (1-3 ml) to get the crystals to go back into solution. Either retie the bag or seal the open end with a clamp. Place the bag into a fresh solution of 1% NaCl and allow to dialyze overnight, or until crystals appear. Record information about crystals: size, number, and morphology.
- 3) By repeating this dialysis process, a protein can usually be rendered essentially pure. To obtain larger crystals, slowly decrease the concentration of NaCl in solution. For example, instead of going directly to 1% NaCl, start with 4.5% and decrease in increments of 0.5%.



## Lab Record

Step	Procedure	Record
I(1)	Brazil nut mass (grams)	g
I(2)	Volume of NaCl solution (ml)	ml
I(3)	Temperature of water bath (°C)	°C
l(4)	Temperature of NaCl/nut solution (°C) – 1st recording	°C
l(4)	Temperature of NaCl/nut solution (°C) – 2nd recording	°C
I(4)	Temperature of NaCl/nut solution (°C) – 3rd recording	°C
l(4)	Temperature of NaCl/nut solution (°C) – 4th recording	°C
l(4)	Temperature of NaCl/nut solution (°C) – 5th recording	°C
I(5)	Volume of protein filtrate (ml)	ml
I(6)	Centrifuge tube #1 mass	g
I(6)	Centrifuge tube #2 mass	mg
I(8)	Volume of extracted protein solution (ml)	ml
II(3)	Volume of protein filtrate (ml)	ml
II(3)	Volume of 1% NaCl solution (ml)	ml
II(3)	Volume of 0% NaCl solution (ml) (if required)	ml
III(2)	Volume of 5% NaCl solution added to bag (ml)	ml
III(2)	Volume of 1% NaCl solution added for dialysis (ml)	ml
III(3)	Volume of 5% NaCl solution added to bag (ml)	ml
III(3)	Volume of 1% NaCl solution added for dialysis (ml)	ml

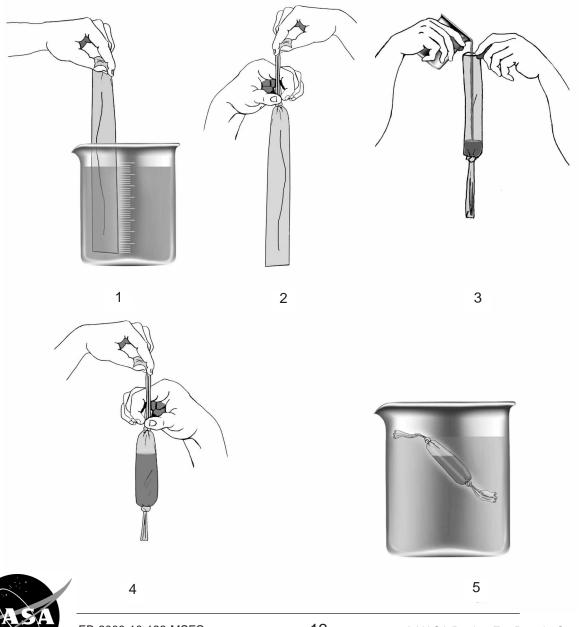
Record the size, number and **morphology** of the final Excelsin crystals produced.

Crystal	Morphology (check which box applies)	Number	Size
Excelsin	Oily		mm
	Spherical		mm
	Platelike		mm
	Hexagonal		mm
	Needles		mm
	Other		mm



# PROCEDURES FOR HANDLING DIALYSIS TUBING

- Soak the dialysis tubing in a beaker of distilled water.
- 2) Tie the dialysis tubing making sure to tie the knot from the top end toward the knot, ensuring that the area to contain the protein solution is not touched or handled. Touching the tubing may increase the pore size of the tubing and result in loss of protein.
- 3) Pour the protein solution into the dialysis tubing.
- 4) Tie the remaining open end of the tubing, leaving a small air pocket so the bag will float.
- 5) Place the dialysis bag in the NaCl solution (see Lab Method step II.3.)



## **GLOSSARY**

**Active site** the portion of a molecule that binds with a substrate molecule

Aqueous relating to, similar to, containing, or dissolved in water; watery

Clarify to make clear by removing impurities or solid matter

**Concentration** a measurement of the amount of solute that is dissolved in a given quantity of solvent

**Convection** heat transfer in a gas or liquid by the circulation of currents from one region to another

**Decant** to pour off gently

**Denature** to alter the structure of (a protein), as with heat, alkali, or acid, so that some of its

original properties, especially its biological activity, are diminished or eliminated

**Density** the ratio of the mass of an object to its volume

Dialysis/Dialyze the transfer of dissolved solids (solute) across a semipermeable membrane, which

permits or hinders diffusion of molecules according to their size

Dialysis membrane

(tubing)

a semipermeable casing used to dialyze a solution

**Diffusion** the spontaneous intermingling of molecules as a result of random thermal motion

Electron density map a graphical representation of the volume of space that an electron is most likely to

occupy; the smaller the volume of space occupied, the higher the electron density

Filtrate the liquid passing through the filter during filtration

**Gravity** the natural force of attraction of objects to each other due to their masses

**Hemoglobin** an iron-containing protein in red blood cells that carries oxygen from the lungs to the

rest of the body

Liquid/liquid diffusion the diffusion of a precipitant solution into a protein solution across their common

liquid/liquid interface

Macromolecule a very large molecule, such as a polymer or protein, consisting of many smaller

structural units linked together

Microgravity an environment in which the apparent weight of a system is small compared to its

actual weight (due to gravity)

Molecular weight the sum of the weight of all the atoms in a molecule, also called

formula weight



Morphology the shape of a crystal

Nucleate to gather, as about a nucleus or center

Opaque impenetrable by light; neither transparent nor translucent

Pellet a small, solid or densely packed mass

pH a measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral

solutions, increasing with increasing alkalinity and decreasing with increasing acidity;

the pH scale commonly in use ranges from 0 to 14

**Precipitant solution** a solution which causes the formation of a precipitate

**Precipitant** a solid or solid phase separated from a solution

Protein any of a group of complex organic macromolecules composed of one or more chains

of amino acids; proteins are fundamental components of all living cells and include many substances, such as enzymes, hormones, and antibodies, that are necessary for

the proper functioning of an organism

Receptor site a region, often the exposed part of a membrane protein, that binds a substance but

does not catalyze a reaction in the chemical it binds

**Sediment** the material that settles to the bottom of a liquid

**Sedimentation** the settling of materials to the bottom of a liquid; this settling is due to gravity

**Solute** the dissolved substance in a solution; in salt water, salt is the solute

**Solution** a homogeneous or uniform mixture of two or more substances

**Solvent** a substance used to dissolve a solute to form a solution; in salt water, water is the

solvent

Supernatant the clear fluid floating above a sediment or precipitate

Supersaturated the state of a solution when it contains more solute (dissolved substance) than it can

theoretically hold

Vapor diffusion a process of diffusion in which a drop of protein solution is suspended above a precipi-

tant and sealed from the air, resulting in equilibration through the vapor phase

X-ray diffraction the scattering of x-ray beams by crystal atoms, producing a diffraction pattern that

yields information about the structure of the crystal



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Nasa's Education Home Page serves as a cyber-gateway to information regarding educational programs and services offered by Nasa for the American education community. This high-level directory of information provides specific details and points of contact for all of Nasa's educational efforts, Field Center offices, and points of presence within each state. Visit this resource at the following address: http://education.nasa.gov



## Spacelink & NASA Television

ASA Spacelink is one of NASA's electronic resources specifically developed for the educational community. Spacelink is a "virtual library" in which local files and hundreds of NASA World Wide Web links are arranged in a manner familiar to educators. Using the Spacelink search engine, educators can search this virtual library to find information regardless of its location within NASA. Special events, missions, and intriguing NASA websites are featured in Spacelink's "Hot Topics" and "Cool Picks" areas. Spacelink may be accessed at: http://spacelink.nasa.gov

NASA Spacelink is the official home to electronic versions of NASA's Educational Products. A complete listing of NASA Educational Products can be found at the following address: http://spacelink.nasa.gov/products

ASA Television (NTV) features Space
Shuttle mission coverage, live special events, interactive educational live shows, electronic field trips, aviation and space news, and historical NASA footage. Programming has a 3-hour block—Video (News) File, NASA Gallery, and Education File—beginning at noon Eastern and repeated five more times throughout the day. Live feeds preempt regularly scheduled programming.

Check the Internet for program listings at:
http://www.nasa.gov/ntv
For more information on NTV, contact:
NASA TV
NASA Headquarters
Code P-2
Washington, DC 20546-0001
Phone (202) 358-3572

NTV Weekday Programming Schedules (Eastern Times)

Video File	NASA Gallery	Education File
12-1 p.m.	1-2 p.m.	2-3 p.m.
3-4 p.m.	4-5 p.m.	5-6 p.m.
6-7 p.m.	7-8 p.m.	8-9 p.m.
9-10 p.m.	10-11 p.m.	11-12 p.m.

How to Access Information on NASA's Education Program, Materials, and Services EP-1999-06-345-HQ.

This brochure serves as a guide to accessing a variety of NASA materials and services for educators. Copies are available through the ERC network, or electronically via NASA Spacelink.

#### **Online Evaluation**

Please take a moment to evaluate this product at <a href="http://ehb2.gsfc.nasa.gov/edcats/educational\_brief">http://ehb2.gsfc.nasa.gov/edcats/educational\_brief</a>
Your evaluation and suggestions are vital to continually improving NASA educational materials.

