LARGE SCALE CRYSTALLIZATION OF PROTEIN PHARMACEUTICALS IN MICROGRAVITY VIA TEMPERATURE CHANGE*

Presented by Marianna M. Long, Ph.D.
Center for Macromolecular Crystallography
University of Alabama at Birmingham

ABSTRACT

The major objective of this research effort is the temperature driven growth of protein crystals in large batches in the microgravity environment of space. Pharmaceutical houses are developing protein products for patient care, for example, human insulin, human growth hormone, interferons and tissue plasminogen activator or TPA, the clot buster for heart attack victims. Except for insulin, these are very high value products; they are extremely potent in small quantities and have a great value per gram of material. It is feasible that microgravity crystallization can be a cost recoverable, economically sound final processing step in their manufacture.

Large scale protein crystal growth in microgravity has significant advantages from the basic science and the applied science standpoints. Crystal growth can proceed unhindered due to lack of surface effects. Dynamic control is possible and relatively easy. The method has the potential to yield large quantities of pure crystalline product. Crystallization is a time honored procedure for purifying organic materials and microgravity crystallization could be the final step to remove trace impurities from high value protein pharmaceuticals. In addition, microgravity grown crystals could be the final formulation for those medicines that need to be administered in a timed release fashion. Long lasting insulin, insulin lente, is such a product. Also crystalline protein pharmaceuticals are more stable for long-term storage. Temperature, as the initiation step, has certain advantages. Again, dynamic control of the crystallization process is possible and easy. A temperature step is non-invasive and is the most subtle way to control protein solubility and therefore crystallization. Seeding is not necessary. Changes in protein and precipitant concentrations and pH are not necessary. Finally, this method represents a new way to crystallize proteins in space that takes advantage of the unique microgravity environment.

The hardware design for the Protein Crystallization Facility (PCF) entails four polysulfone cylinders (500, 200, 100, 50 ml total volume) of different heights and same diameters. The aluminum cap of each cylinder is apposed to the heating element of the RIM and the temperature is decreased from 40°C to 22°C early in the mission for insulin, the sample used for STS-37 and STS-3. The four cylinder sizes resulted in four different temperature gradients. On STS-37, the temperature was ramped down in four steps over 23 hours, while on STS-43 the ramp was in one step.

The results from these two flights showed that the hardware performed perfectly, many crystals were produced and they were much larger than their ground grown controls. Morphometric analysis was done on over 4,000 crystals to establish crystal size, size distribution and relative size. Space grown crystals were remarkably larger than their earth grown counterparts and crystal size was a function of PCF volume. For example, for the largest volume PCF (500 ml) from STS-37, the space crystals were 10 times bigger than controls while for the smallest volume (50 ml) they were 2 times bigger. That size distribution for the space grown crystals was a function of PCF volume may indicate that ultimate size was a function of temperature gradient. Since the insulin protein concentration was very low, 0.4 mg/ml, the size distribution could also be following the total amount of protein in each of the PCFs. X-ray analysis showed that the bigger space grown insulin crystals diffracted to higher resolution than their ground grown controls. When the data were normalized for size, they still indicated that the space crystals were better than the ground crystals.

*Funded by NASA grant NAGW-813 to Charles E. Bugg
CENTER FOR
MACROMOLECULAR CRYSTALLOGRAPHY

A NASA CENTER FOR THE COMMERCIAL DEVELOPMENT
OF SPACE

PROTEIN CRYSTALLIZATION
FACILITY
(PCF)

Presentation by Marianna M. Long

Space Station Freedom
Utilization Workshop

Huntsville, Alabama
August 4-6, 1992
PROTEIN CRYSTALLIZATION FACILITY
(PCF)

OBJECTIVES

• To grow protein crystals in large batches

• To use temperature as the means to initiate and control protein crystal growth

PROTEIN CRYSTALLIZATION FACILITY
(PCF)

ADVANTAGES OF LARGE SCALE PROTEIN CRYSTAL GROWTH

• No surface effects to interfere with crystal growth

• Easy dynamic control is possible

• Yields large quantities of large, very pure crystals
  • Purity of protein is achieved
  • Uniformity of size of protein crystal is possible
  • Patentability is enhanced and this stimulates commercial interest
PROTEIN CRYSTALLIZATION FACILITY (PCF)

ADVANTAGES OF TEMPERATURE IN PROTEIN CRYSTAL GROWTH

- Dynamic control is possible
- Non-invasive, most subtle way to control protein solubility
- No seeding is necessary
- No changes in [protein], [precipitant], or pH are necessary
- Represents a new way to crystallize proteins that takes advantage of microgravity environment

PROTEIN CRYSTALLIZATION FACILITY (PCF)

HARDWARE DESIGN

- Polysulfone with neoprene O-rings
- 4 cylinders: 500ml, 200ml, 100ml, 50ml
- All have the same diameter with different heights
- The metal cap of each cylinder is apposed to the heating element of the R/IM and the temperature is decreased from 40C to 22C early in flight
- Insulation is around each cylinder
- The 4 sizes will result in 4 different temperature gradients
PROTEIN CRYSTALLIZATION FACILITY
(PCF)
TEMPERATURE STEPS
STS-37

<table>
<thead>
<tr>
<th>TIME</th>
<th>TEMP STEP</th>
</tr>
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<tbody>
<tr>
<td>L+3</td>
<td>40→36°C</td>
</tr>
<tr>
<td>L+9</td>
<td>36→32°C</td>
</tr>
<tr>
<td>L+19</td>
<td>32→28°C</td>
</tr>
<tr>
<td>L+26</td>
<td>28→22°C</td>
</tr>
</tbody>
</table>
Length and Width of "Rosette" Crystals

- Flight Length
- Ground Length
- Flight Width
- Ground Width

all n=150 except flight 50 (n=100)
Comparison of Flight to Ground "Rosette" Crystals

by: \[
\frac{\text{flight crystals}}{\text{ground crystals}}
\]

<table>
<thead>
<tr>
<th>PCF</th>
<th>Length</th>
<th>Width</th>
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<tbody>
<tr>
<td>500</td>
<td>5.94</td>
<td>4.13</td>
</tr>
<tr>
<td>200</td>
<td>4.71</td>
<td>3.43</td>
</tr>
<tr>
<td>100</td>
<td>2.40</td>
<td>1.74</td>
</tr>
<tr>
<td>50</td>
<td>2.76</td>
<td>2.34</td>
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</table>

STS-37
Comparison of Flight to Ground "Rosette" Crystals

STS-37
"Rosette" Crystal Size on Side of 500ml PCF Relative to Location Down Long Axis

STS-37

- Flight Length
- Ground Length
- Flight Width
- Ground Width

Millimeters

Top mid bot top mid bot top mid bot top mid bot top mid bot top mid bot
Length and Width of Single Crystals

![Graph showing the relationship between PCF volume (ml) and STS-37 with various data points for flight and ground length and width.](image)
Comparison of Flight to Ground Single Crystals

by: $\frac{\text{flight crystals}}{\text{ground crystals}}$

<table>
<thead>
<tr>
<th>PCF</th>
<th>Length</th>
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<tr>
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<td>10.26</td>
<td>3.96</td>
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<tr>
<td>200</td>
<td>8.66</td>
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<tr>
<td>100</td>
<td>2.42</td>
<td>1.84</td>
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<tr>
<td>50</td>
<td>2.42</td>
<td>2.24</td>
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STS-37
Comparison of Flight to Ground Single Crystals

![Bar chart showing comparison of flight to ground single crystals.](image)

STS-37
Single Crystal Size on Side of 500ml PCF Relative to Location Down Long Axis

STS-37

<table>
<thead>
<tr>
<th>Millimeters</th>
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<tbody>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>0.3</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.0</td>
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Flight Length
Ground Length
Flight Width
Ground Width
PROTEIN CRYSTALLIZATION FACILITY
(PCF)
TEMPERATURE STEPS
STS-43

<table>
<thead>
<tr>
<th>TIME</th>
<th>TEMP_STEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>L+4</td>
<td>40-&gt;22C</td>
</tr>
</tbody>
</table>
Length and Width of Single Crystals

- Flight Length
- Flight Width
- Ground Length
- Ground Width

PCF volume (ml)

STS-43
Comparison of Flight to Ground Single Crystals

by: \( \frac{\text{flight crystals}}{\text{ground crystals}} \)

<table>
<thead>
<tr>
<th>PCF</th>
<th>Length</th>
<th>Width</th>
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<tbody>
<tr>
<td>500</td>
<td>4.58</td>
<td>2.55</td>
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<tr>
<td>200</td>
<td>4.65</td>
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<tr>
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<td>2.68</td>
</tr>
<tr>
<td>50</td>
<td>2.76</td>
<td>1.65</td>
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</table>

STS-43
Comparison of Flight to Ground Single Crystals

STS-43
Single Crystal Size on Side of 500ml PCF Relative to Location Down Long Axis

STS-43

- Flight Length
- Ground Length
- Flight Width
- Ground Width

millimeters

top mid bot top mid bot top mid bot top mid bot top mid bot
<I> in bin (peaks>5\sigma, \sigma units)

Ground Based  
Space Crystals  
Giants of Space  

Resolution

12.00 7.12 5.07 3.93 3.21 2.71 2.35
log<\textit{I}> in bin (peaks>5\textit{sigma},raw units)

Ground Based
Space Crystals
Giants of Space

Resolution

-6.21
-1.24
3.74
12.00 7.12 5.07 3.93 3.21 2.71 2.35
PROTEIN CRYSTALLIZATION FACILITY

(PCF)

PROTEIN TO BE CRYSTALLIZED

• Insulin, at 0.4 mg/ml, in phosphate buffer, will be the first protein to fly in the PCF

• The temperature is decreased from 40C to 22C early in the flight