**PROMISS 3 EXPERIMENT INTO THE SPACE STATION**

**(extract from DSM-PR3-207-Issue1 Rev.3 : Flight Safety Data Package)**

# 4. PROMISS Experiment Description

## *4.1.* Introduction and Scientific Objectives

The crystallization of proteins in microgravity conditions can lead to a better understanding of the fundamental processes underlying the crystallization process. The major objective of the present experiments is to produce a detailed analysis and quantitative interpretation of the relationship between the quality of the crystals and the environment in which they were produced.

The objectives are:

To further evaluate the effect of microgravity on crystal quality, as compared to experiments in gel. Can we confirm that the removal of convection by the use of gels has a similar effect as microgravity?

To quantify the relationship between position of growth of the crystal and crystal quality.

The PROMISS-1 experiment has already been performed during the Belgian Taxi Flight in November 2002. Preliminary results obtained show that the instrument was strongly affected by vibrations and the temperature profile of the mission. These factors had a bad impact on the quality of the results obtained. The PROMISS-2 experiment was developed in the frame of the SSM flight opportunity. An upgraded version of the FM hardware, called PROMISS-2 experiment has been uploaded with Progress 11P in August 2003. The PROMISS-2 experiment was performed successfully during the SSM: 6 PROMISS experimental cells were uploaded with the Soyuz 7S, analysed with the PROMISS-2 optical diagnostic payload into MSG during 10 days, and downloaded with the Soyuz 6S. The PROMISS-3 experiment that is described in this FSDP is thus a re-flight of the previous

PROMISS-2 experiment performed during the SSM, but with an investigation of the experimental cells into the Digital Holography Microscope of 30 days and then stowage of them in an incubator (AQUARIUS) during 2 months, to fit the Progress 13P and Soyuz 8S/7S operation timeline.

The PROMISS-3 experiment is an adaptation of a digital holography instrument developed by ULB-MRC for the observation of counter-diffusion experiments in microgravity. This instrument would provide a view of the crystallization reactions in term of density gradients in the solution and of the localization of the crystals. The observation of the density gradients should make it possible to evaluate models of the diffusion and residual convection in the crystallization process. This information, together with the localization of the crystals, and the observation of the timing of their growth, can allow the identification of the growth conditions of the crystals. This information can then be related to crystal quality and internal order, provided the crystals have not moved too much upon return journey.

## *4.2.* Samples and Experiment Principle

In this set-up, only a limited number of protein experiments can be performed during one single flight opportunity. In the current design, six different proteins can be crystallized in separated PROMISS experimental cells. These proteins need to comply with some criteria necessary for the analysis and for adaptation to the mission timeline and temperature profile:

The crystallization needs to be very well controlled, so as to be able to identify conditions where the right number of crystals with the right size grows within the optical field. Ideally, crystals should grow independently from each other, i.e. their depletion zones should not interfere with each other.
The physico-chemical parameters related to the diffusion processes and fluid motion should be known to allow for the simulation of the crystallization experiment.

Six samples among the 8 proteins listed in Table 4-1 will be selected. The final selection will be done considering results of ground experiments currently in progress.

| |
|---|
| Lysozyme protein |
| Catalase<br>Thermotoga maritima Triosephosphate isomerase protein |
| Ferritin |
| Lumazine synthase<br>Camel VHH antibody fragment protein |
| Pike Paravalbumin protein |

**Table 4-1: PROMISS 2 Candidate proteins**

Two types of experiments are envisaged at this stage. The first is the crystallization in capillaries by counter-diffusion. The second consists of the observation of density gradients around a crystal growing, which requires a large volume of solution around the crystal is therefore performed without capillaries.

Volumes and shapes:

The crystallization by counter-diffusion requires a device in which an elongated volume of protein solution is separated from a volume containing precipitant solution by a volume that contains a gel. The crystallization takes place in the volume solution, and this container shall be transparent for the laser light.

**Protein volume:**

The path length of the laser through the protein chamber is limited by the condition that the probability to have one, and only one, crystal along the path shall be maximized. The length of the protein solution volume needs to be adapted to the duration of the experiment, so that the precipitant wave reaches the end of the protein volume well before the end of the experiment. During one week small molecule precipitant will diffuse over 1.5 cm – 3 cm.

Protein volume: depth 0.5 mm - 3 mm, height minimum 1 cm.

**Gel volume:**

The length of gel the precipitant has to travel through before getting to the protein chamber determines the delay before the start of the crystallization process. This length should be variable, as it needs to be adapted to the conditions for filling the reactors.

The length of the gel between precipitant and protein should range from 0.5 cm to 1.5 cm.

**Precipitant volume:**

The major requirement of the precipitant volume is that it should be large enough, so that after full equilibration of the precipitant in all the volumes the precipitant concentration is high enough for typical crystallization conditions.

The precipitant volume should be at least three times larger than gel and protein volume together.

Due to the three-day time gap between the end of the filling on ground and the actual start of the experiment in the ISS, up to 1.5 cm gel path between precipitant and protein volumes is required. The microgravity experiment will last 30 days [AD1], duration during which the precipitant wave can travel between 1.5

and 3 cm in the capillary.

**Materials:**

The materials used for the manufacturing of the PROMISS experimental cell shall be resistant against the chemicals used in protein crystallization experiments, and should not contain any chemicals that could contaminate the crystallization solutions. Conditions and chemicals encountered in protein crystallization experiments are:

-pH 3-10

-Salt concentrations up to 5M or saturation

-Organic solvents up to 60% (mostly alcohols)

-Polyethylene glycol up to 45%

-Buffering agents like carboxylate, sulfonic acids, TRIS, etc.

-A variety of additives like metal cations, anions, and detergents.

The sample materials were submitted to toxicological assessment by NASA (see also detailed list attached to the “containment of materials” HR 01) and are rated Tox Level 1.

### *4.3.* Experiment Hardware Description

The PROMISS-3 hardware consists of:

| Item | Description | Up | Down |
|---|---|---|---|
| PROMISS-2 Experiment Box | Includes Digital Holography Interferometer | Already on-board ISS | Kept on-board ISS |
| PROMISS-2 Electronic Box (Item from PROMISS 2 Kit 1) | Including pig-tailed harness (3) | Already on-board ISS | Kept on-board ISS |
| PROMISS Wheel (Item from PROMISS 2 Kit 1) | Machined Aluminium plate | Already on-board ISS | Kept on-board ISS |

| | | | |
|---|---|---|---|
| PROMISS-2 Locking System Parts A & B (Item from PROMISS 2 Kit 1) | System to temporary fasten the PROMISS Wheel on MSG rear wall during the setup of the experiment; | Already on-board ISS | Kept on-board ISS |
| PROMISS-2 Clamp (Item from PROMISS 2 Kit 1) | Small additional fastener for experiment attachment in MSG. | Already on-board ISS | Kept on-board ISS |
| PROMISS-3 Sample Container (Item from PROMISS 3 Kit 2) | Container for the experimental cells, includes PCM for temperature regulation during upload. | Progress 13P (soft bag) | Destructive re-entry |
| PROMISS-3 Allen Key (Item from PROMISS 3 Kit 2) | Allen Key with spherical head to open/close the PROMISS 3 Sample Container | Progress 13P (soft bag) | Destructive re-entry |
| PROMISS-3 Return Container (Item from PROMISS 3 Kit 1) | Container for the experimental cells, passive container for the download. | Progress 13P (soft bag) | Soyuz 7S (soft bag) |
| Videotapes (12)(Item from PROMISS 3 Kit 1) | DV 52 min. for MSG Drawer Rack | Progress (soft bag) | Soyuz 7S (soft bag) |

**Table 4-2: List of hardware items**

A MSG provided camera is installed on top of the experiment facility cover box, a hole exists in the cover box, obtured by a polycarbonate window, allows the camera to view in the box – see Figure 4-1 and Figure 4-2.

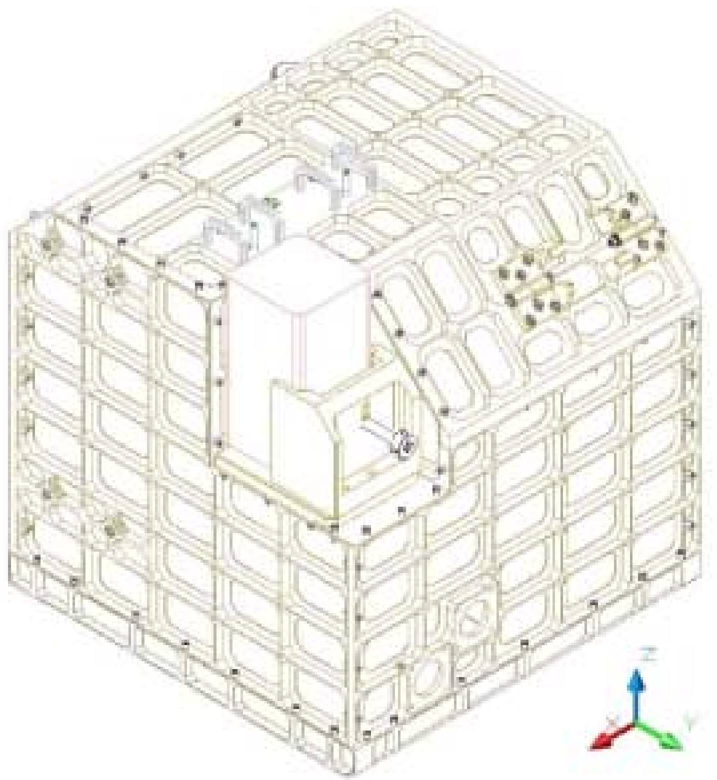

**Figure 4-1: PROMISS experiment facility with Cover Box and MSG camera attached**

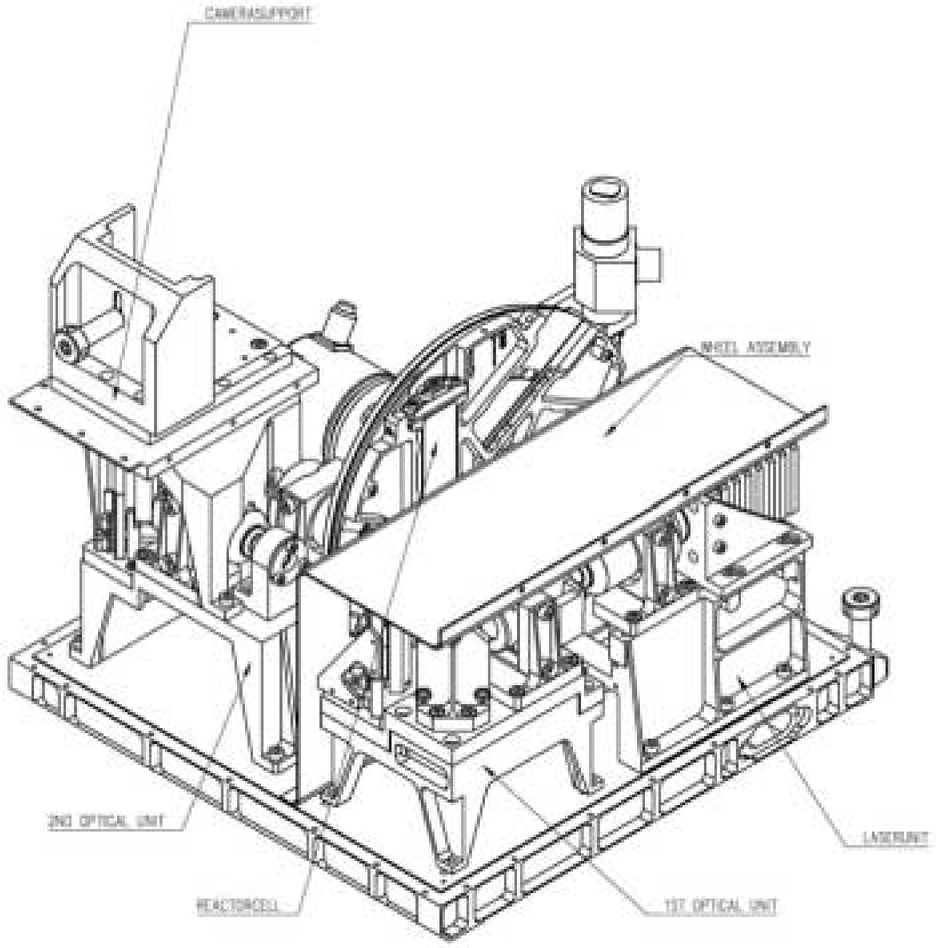


**Figure 4-2: PROMISS experiment facility with cover box removed**

#### *4.3.1. PROMISS Experiment Box*

As can be seen from the figures above, the components of the Experiment Box are mounted on a baseplate. The main components are:

- -The wheel assembly (to which the sample Wheel is fixed) with its DC motor drive,
- -The digital holographic interferometer,
- -The cover box, which has a lid (door) for the PROMISS Wheel insertion and removal.

The cover box has a polycarbonate window for allowing the camera to view inside the box (for collection of interferometric images). The door is fitted with two microswitches (redundant) for cutting off both laser power and wheel assembly motor when the lid is open.

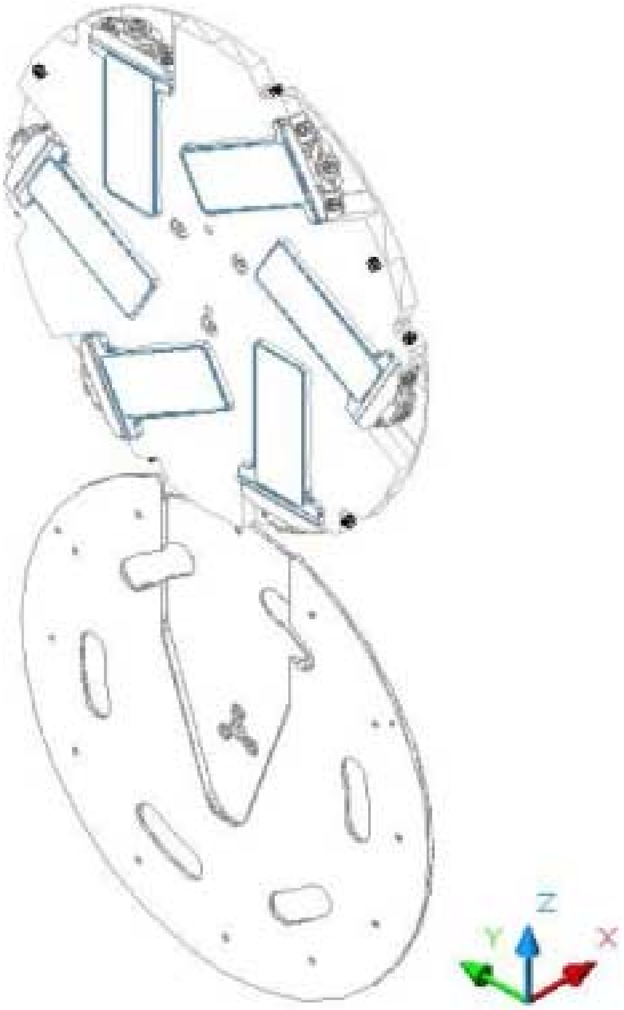


**Figure 4-3: The PROMISS Wheel with 6 experimental cells and its receptacle, the wheel assembly into the PROMISS 2 Experiment Box.**

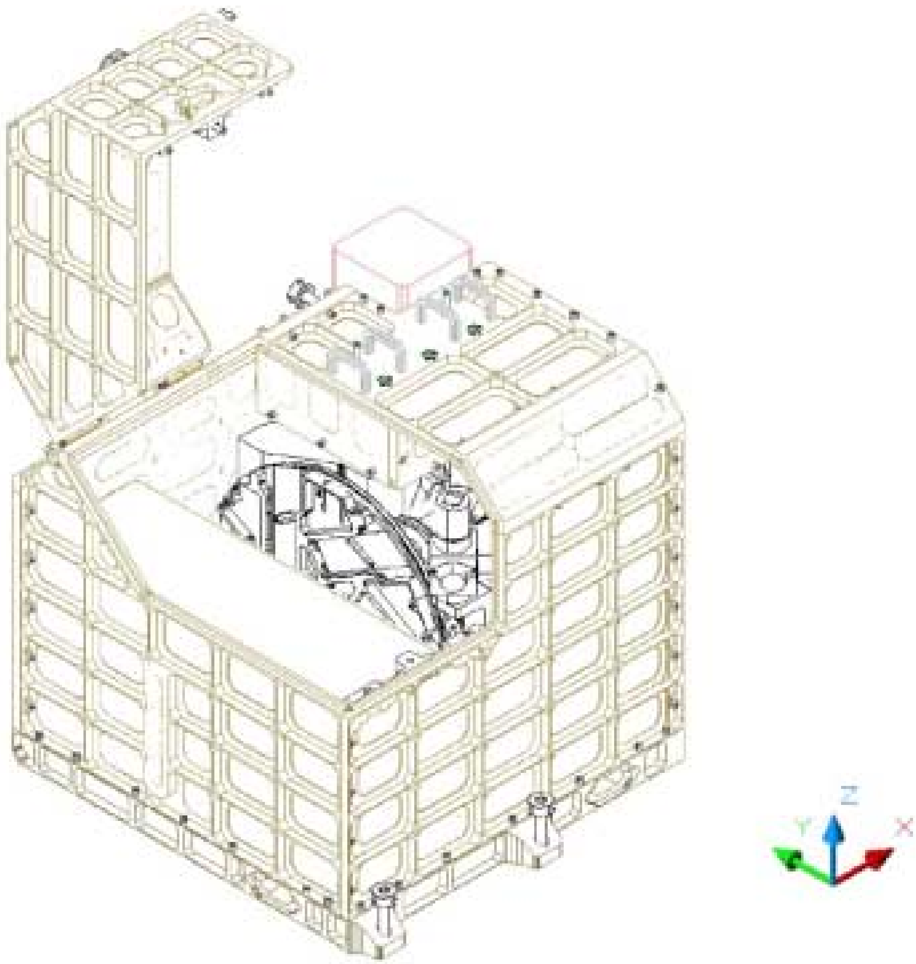

**Figure 4-4: The PROMISS 2 Experiment Box with cover box lid open**

The cover box of the PROMISS 2 Experiment Box payload is not designed to be pressure tight but is vented through filters in its bottom plate. When closed, it assures thus a second containment of the sample liquids. An internal aluminium wall provides a mechanical protection for the optical setup during transportation and installation inside MSG.

The cover box is made of aluminium 7075 and is alodined. It is attached to the baseplate using M2.5 screws. There is a mechanical interface for camera fixation on the top of the cover box. In order to easily perform the PROMISS Wheel insertion, the cover box is fitted with a wide opening, i.e. a lid (see **Figure 4-4).** The cover box is also fitted with two sets of microswitches (two for the opening of the PROMISS lid and two for the camera) that disable the laser light emission, either if the camera is not in place or removed from its nominal position, or if the cover is opened by the crew. In these cases, the power of the motor Wheel will be cut off too.

During operation, the rotation speed of the PROMISS Wheel is about 1/60 rotation per minute (rpm), i.e. very slow. The wheel assembly is driven by a DC motor that is coupled to an endless screw. This endless screw drives a cogged wheel. The rotation of the DC motor at its gearhead level is 6 rpm.

#### *4.3.2. PROMISS Experimental Cells, Sample Container, Return Container and Wheel*

##### 4.3.2.1 Experimental Cell design

The reactor cell is designed to safely enclose the capillary tubes under investigation and the optical wedge present in the cell. It is made of the following parts (see **Figure 4-5, Figure 4-6** and **Figure 4-7):**

Experimental cell frame: made of ERTA Polycarbonate PC111,

Experimental cell windows; made of ERTA Polycarbonate PC111. The windows are glued to the reactor frame using Scotch Weld 2216.

Capillary tubes holder: made of ERTA Polycarbonate 111. There exists two different types of holder, but both are inserted in the reactor frame according to the same procedure: they slide vertically, and when in position, remain fixed.

The experimental cell is sealed by a silicone rubber layer.

The experimental cell cover is made of stainless steel and fixed to the reactor frame using two M2.5 passivated screws.

A volume compensation system to avoid overpressure due to temperature increase is placed in the top of the reactor thanks to silicon rubber layers that separate the reactor chamber and small chambers empty of liquid. The volume compensation is incorporated into the stainless steel reactor cover. It is shown in Figure 4-7. A second cap is fixed to the reactor cover with one M3 passivated screw.

For some experimental cells, it is possible to accommodate an optical wedge, in BK7. This wedge is inserted inside the cell and is totally enclosed.

Figure 4-5: PROMISS experimental cell (exploded view)

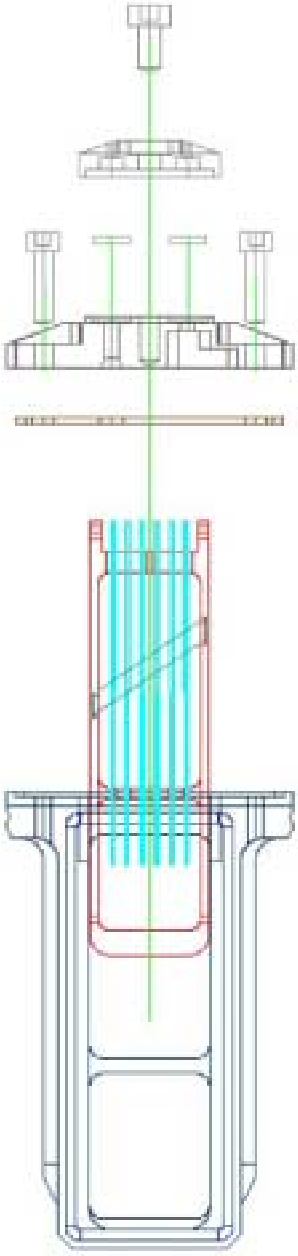

**Figure 4-6: PROMISS experimental cell (exploded view)**

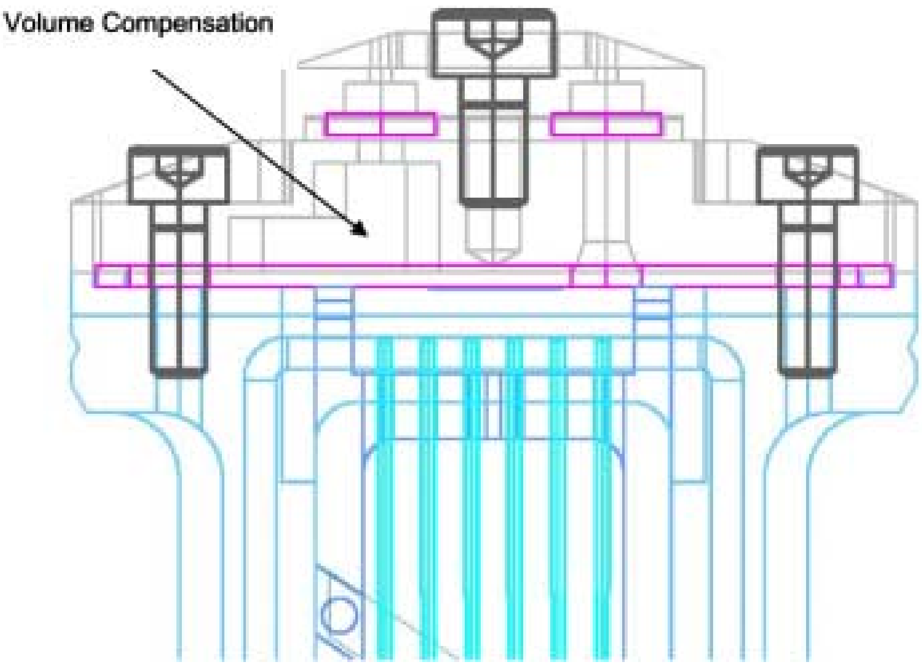


**Figure 4-7: PROMISS reactor cell - volume compensation device detail.**

#### 4.3.2.2 PROMISS Sample Container: Phase Change Material Transportation Box

The experimental cells are presenting a single containment and must therefore be packaged at any time in a second containment. This is achieved differently during transportation to/from the ISS, and during the PROMISS run inside the MSG.

For transportation (in Progress 13P), the experimental cells are enclosed in a leak-proof box (i.e. the PROMISS Sample Container), providing also a temperature control thanks to a volume of phase change material. For the download with the Soyuz 7S, the experimental cells are packed into the PROMISS Return Container, a lightweight structural container that provides one additional level of containment.

Figure 4-8 : PROMISS Sample Container Concept.

The PROMISS Sample Container has been developed in the frame of the SSM. It is fully qualified, and consists of two sealed cavities that are fastened together using 12 stainless steel M6 Captive Screws. One cavity, the top one in the Figure 4-8, is thus fitted with those screws, while the bottom cavity is fitted with corresponding M6 helicoils.

Each cavity is made by two Al6082 parts that are welded by qualified personnel. Figure 4-9 shows the two parts before welding process. Both parts are milled, one has only two welding paths for each of the two cavities.

**Figure 4-9 : Constitutive parts of a single cavity.**

After welding, each cavity is filled with the Phase Change Material, using a filling hole depicted also in figure 4.10. The filling is performed at 50°C, i.e. the maximum temperature the PROMISS Sample Container can face during its overall use (launch with Progress 13P, stowage on ISS, destructive re-entry with Progress). Each cavity is designed to contain 0.8 kg of Phase Change Material. For our application, we use Heptadecane $C_{17}H_{36}$, a paraffin substance compatible with Al6082. Its freezing point is 22°C, and it is characterized by a large latent heat of fusion, i.e. 213 kJ/kg.

After filling of the cavity, it is sealed using a M8 stainless steel screw, fitted with two viton o-rings (see **Figure 4-10** and **Figure 4-11).** The screw is then locked using structural glue ScotchWeld 2216. It is not foreseen to remove them during any period of the mission. This design is identical on both PCM cavities.

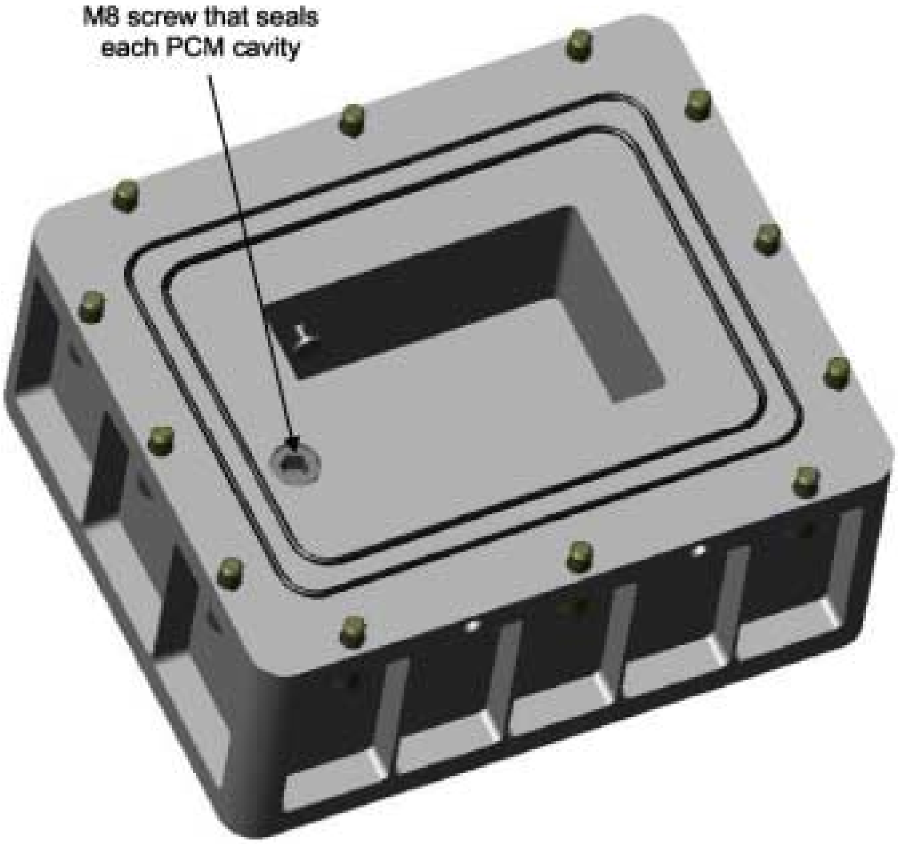


**Figure 4-10 : PCM cavity, with the M8 screw used as a permanent cap after filling. The two grooves where the viton O-rings ensuring the leak tightness of the box are also visible.**

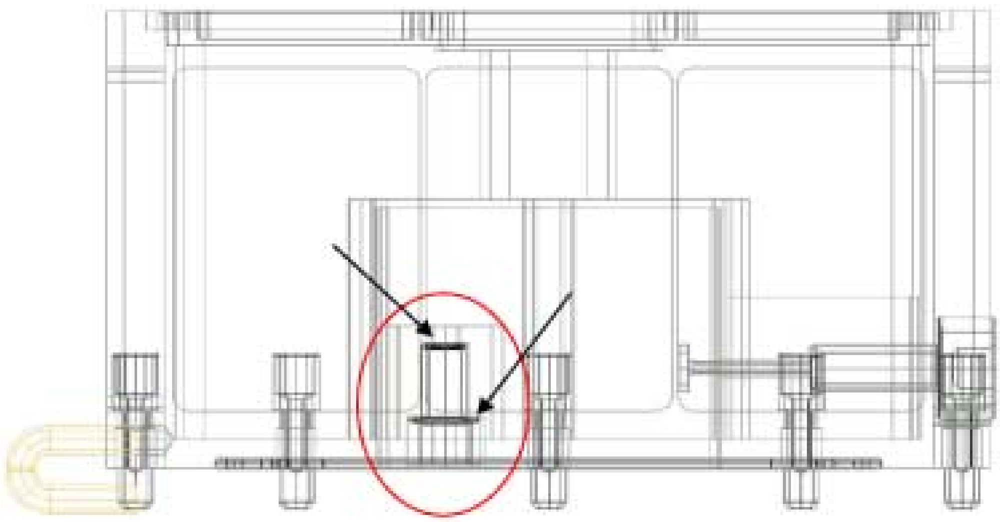

**Figure 4-11 : Detail view of the M8 screw. The arrows are showing the two o-rings.**

The PROMISS Experimental Cells are stowed into the internal cavity as depicted in **Figure 4-11.** There are 6 experimental Cells in total fitted into a DELRIN holder. The PROMISS Sample Container is providing 2 levels of containment with two separate viton o-rings (see **Figure 4-13).** Note that once opened, the two main cavities remains linked together, since two kevlar cords are linking them. Moreover, Teflon tape is wrapped around each Kevlar cord.

When outside the MSG, the PROMISS Sample Container remains closed, providing also two levels of containment for the PCM material, the filling holes being inside the two viton o-rings grooves (see **Figure 4-13).**

Prior to the Progress 13P launch, the PROMISS Sample Container is packed into a dedicated ziplock bag;

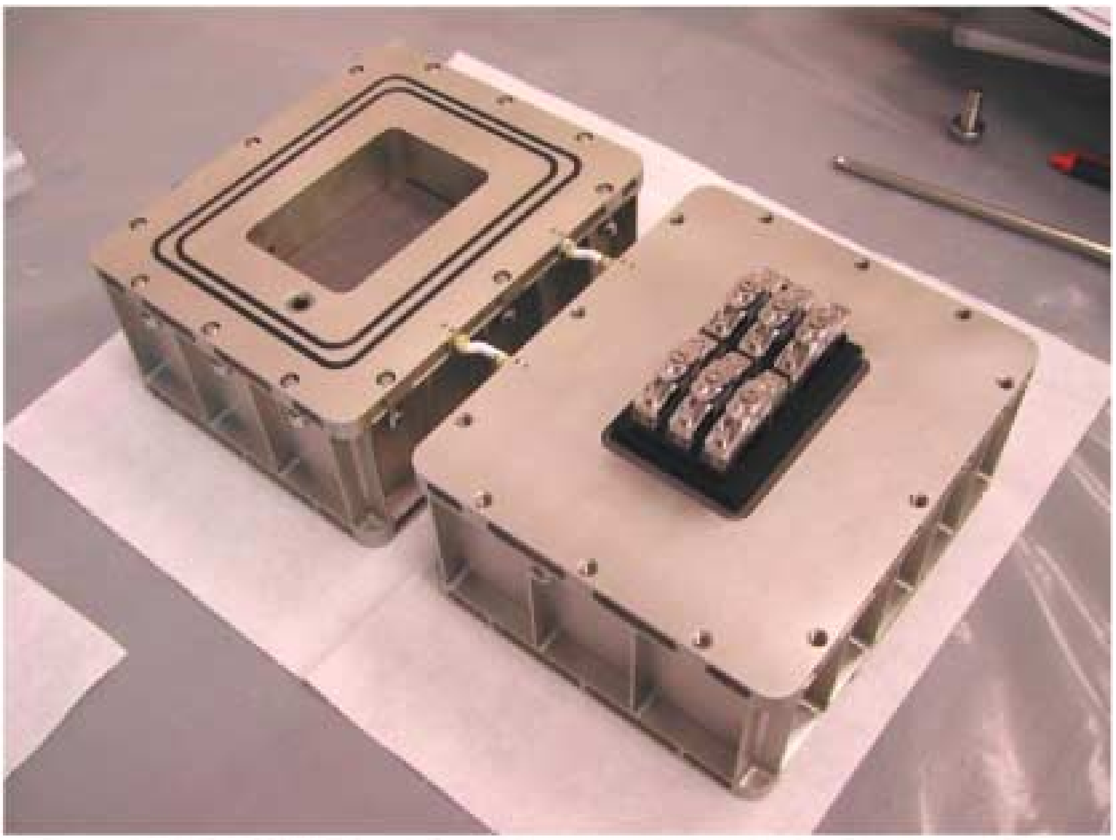

**Figure 4-12 : PROMISS Sample Container opened, showing the Experimental Cells accommodation (FM hardware during the SSM)**

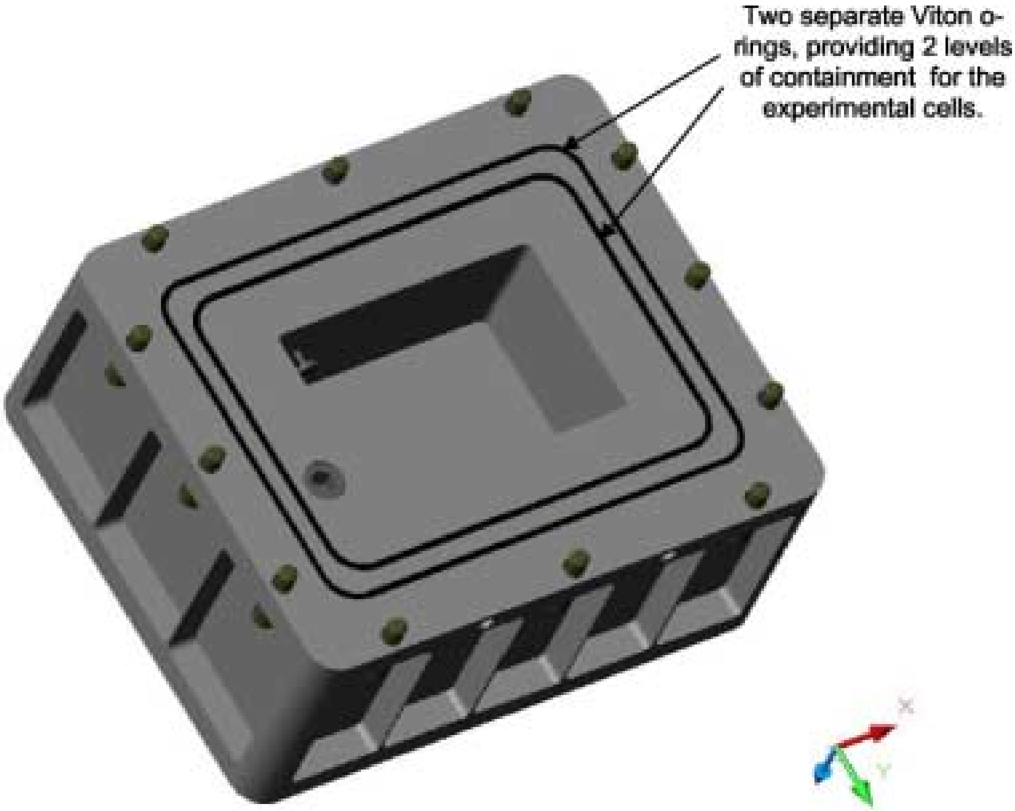


**Figure 4-13 : Detail view of the PCM Top part, showing the two separate Viton orings.**

In order to ease the opening procedure of the PROMISS Sample Container, it is fitted with a depressurization screw. It is captive and incorporates two separate o-rings, as depicted in **Figure 4-14** and **Figure 4-15.**


**Figure 4-14 : PROMISS Sample Container fitted with the depressurisation screw.**


**Figure 4-15 : Detail view of the depressurization screw. This screw is fitted internally with two separate viton o-rings. It is captive due to the presence of the locked second screw (small one below)**

From a basic computation, this transportation box concept, if surrounded by a soft packaging (like the PROMISS 3 Kit 2 proposed), is able to maintain an internal temperature of 22°C for the samples while facing a 30°C outside temperature for the soft bag kit during a period of 2 days. This represents the best compromise between mass load and thermal inertia for the PROMISS Sample Container.

#### 4.3.2.3 PROMISS Return Container

The PROMISS Return Container is designed to provide a sealed box for the transportation of the 6 PROMISS Experimental Cells during stowage of the experiment into the Russian AQUARIUS-B incubator and back to Earth with Soyuz 7S. It consists of a container made of Aluminium Al7075 with alodine surface treatment. Its overall envelope is 120mm x 90mm x 130mm.

The container provides one level of containment for the 6 PROMISS experimental cells with one viton seal on top of the container.

- The container consists of two metallic main parts that are bound by Kevlar cords, wrapped with Teflon tape.
- This container is compatible with the actual PROMISS black plastic holder for the experimental cells, which was used with the Sample Container previously developed

(see **Figure 4-16).**

- At the bottom of the container there will be a dedicated accommodation for 2 Sugar Cubes (temperature data loggers) enclosed in a separate cavity sealed with two viton o-rings (see **Figure 4-17).** The bottom cavity is not opened by the Crew during on-orbit operation
- The container is fitted with one depressurization screw (with one seal) that is captive (similar design as for the existing Sample Container).

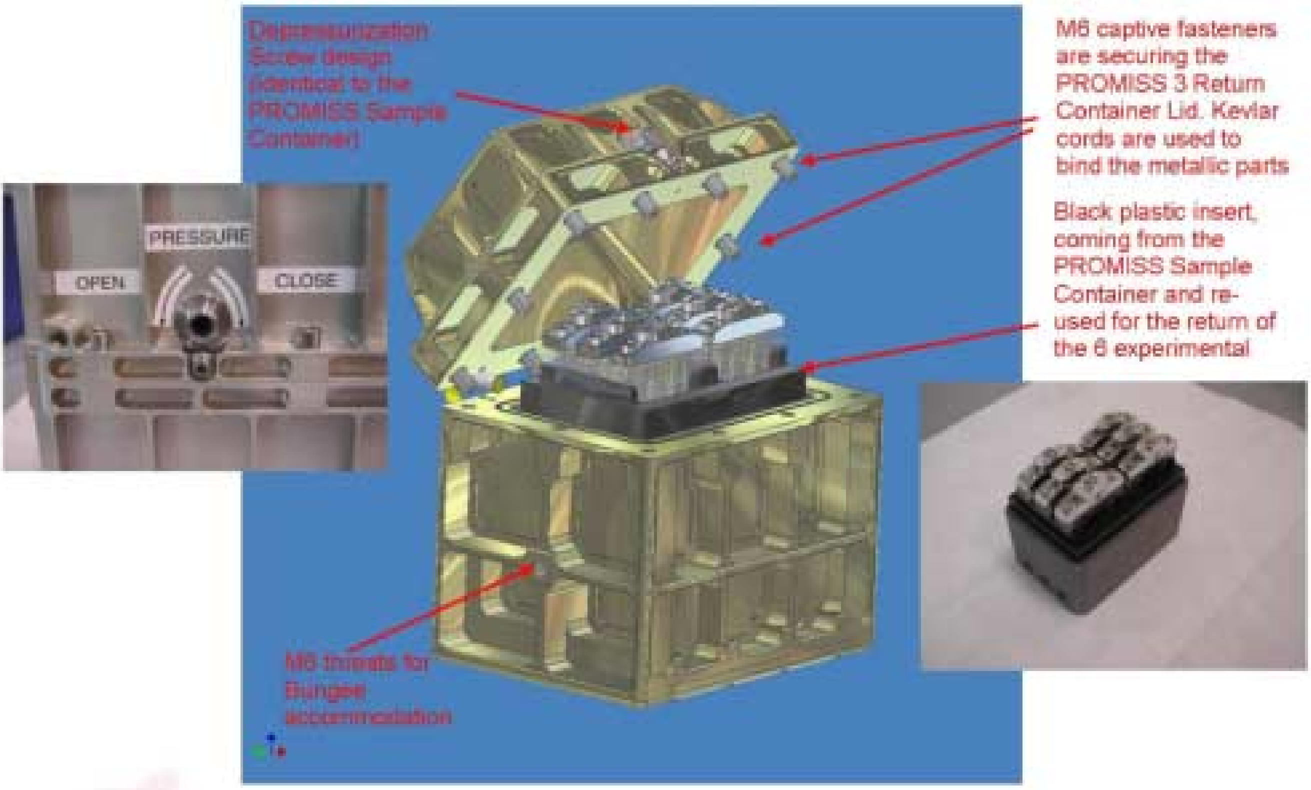


**Figure 4-16: The PROMISS Return Container Concept**

All the screws are made of stainless steel and are captive. All the screws that have to be operated by the Astronaut are requiring the same Allen M6 key. All the threads corresponding to those screws will be fitted with stainless steel helicoils. On the container sides, there will be 2 M6 threads fitted with helicoil, so that the Astronaut is able to use bungee straps to fix temporary the Box onto the Glovebox baseplate (similar design to PROMISS Sample Container).

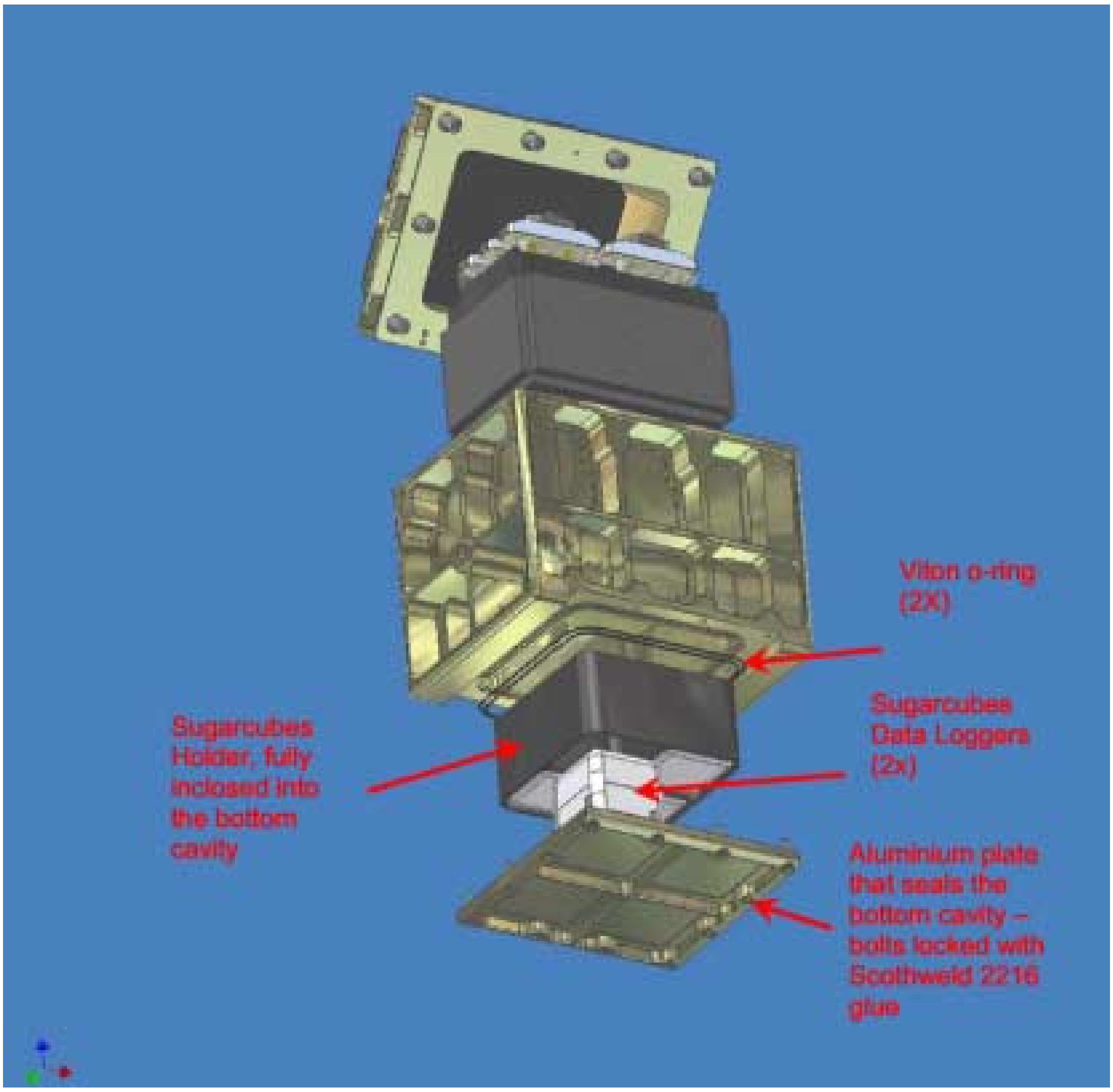


**Figure 4-17: The PROMISS 3 Return Container - Exploded view showing the Sugarcube Cavity**

The PROMISS 3 return Container is also fitted with Sugarcube Data Loggers. They are provided by the Meilhaus Cy. There are 2 temperature Data Loggers . The Sugarcubes will be tested according to the Unique Hazard Report PROMISS-05 presented in Attachment C3 of the present document.

Before the launch with Progress 13P, the PROMISS 3 Return Container will be packed into a dedicated ziplock bag, fitted with Velcro attachment points. This will facilitate its stowage not only into the MSG Work Volume during the optical investigation, but also into the AQUARIUS-B incubator (stowage for 2 months prior to the Soyuz 7S undocking).