An Open Flow Helium Cryostat for Synchrotron X-ray Diffraction Experiments

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Abstract. Open flow Helium cryostats, directly blowing a stream of cold gas over the sample, remain an attractive alternative to closed cryostats in a number of cases despite the high Helium cost and often unstable and difficult operation including the growth of ice crystals on the sample: they offer open access to the sample which may be rotated over large angles allowing for large diffraction angles and simultaneous visible optical access for spectroscopy techniques. We have designed and built a new open flow Helium cryostat, making use of the paraphernalia of a commercial cryostat as much as possible. The cryostat has a temperature range from 4.5 up to 100 Kelvin for a liquid Helium consumption of around 2 l/hr when cycled between 5 K and 50 K at constant flow. The use of the new cryostat allows data collection and structure determination below 10 K; at these temperatures it is possible to trap reaction intermediates in proteins in a frozen state, previously only identified spectroscopically. We have successfully carried out X-ray diffraction data collection of murine neuroglobin at 10 K.

1. Design considerations and construction

The operating principles of open flow Helium cryostats designed over the last 20 years vary a lot. Cold gas generation in the He supply dewar [1] or a closed cycle cryocooler [8,9] which cools a flow of Helium gas typically deliver relatively high output temperatures above 20K; lower temperatures down to 4.5K [2-7] are obtained using an intermediate Helium dewar or direct delivery through a syphon. Icing on the sample is reduced through the use of a large nozzle or a screen of warm Helium gas.

An open flow system has to provide a sufficiently large cold working volume to allow placing the sample into this region of stable temperature and aligning it. The working volume diameter is directly proportional to the nozzle diameter, while the working volume length depends on the flow rate and temperature. The flow rate has to be increased as the temperature goes down to compensate for the increasing density of the gas which reduces the cold volume and to compensate for increasing losses through heat exchange with ambient temperature. Liquid Helium consumption thus depends on the cold volume size necessary for the sample size and diffraction geometry.

The design of the cryostat is based on an in-house developed flow cryostat [10, 11] which was modified to incorporate an exit nozzle perpendicular to the main axis. It was designed to use as much as possible of the paraphernalia of a commercial open flow Helium cryostat. Liquid Helium from a
pressurised 100 liter transport dewar is directly delivered to the cryostat through an Oxford Instruments LLT syphon which incorporates a cold return gas shield (see figure 1). The syphon shield return gas is fed back to the cryostat as sample screening gas flow. The system needs a small quantity of Helium gas from a B50 bottle to pressurize the liquid Helium dewar. Helium arriving in the cryostat passes through the primary heat exchanger where it is brought to the setpoint temperature by a heater. The nozzle is mounted to the heat exchanger with an Indium seal, the room temperature side is sealed with a Viton O-ring allowing for the movement of thermal contraction upon cooling. The 5mm nozzle is constructed out of thin walled stainless steel; it is equipped with a Pt100 thermometer and a Manganine wire wound heater at the nozzle exit. The temperature of the tip of the nozzle is regulated at 30 °C to prevent ice growing on the nozzle. The siphon backflow is pumped through the secondary heat exchanger cooling the radiation shield. The cryostat has a temperature range from 4.5 up to 100 Kelvin for a liquid Helium consumption of around 2 l/hr when cycled between 10K and 50K at constant flow. Above 50K the flow can be lowered to 0.5 l/hr.

![Figure 1a: the cryostat under test, gas screen not yet mounted. 1b: scheme of the working principle.](image)

2. Operation and testing

For all of the temperature measurements in the flow a Lake Shore bare chip Cernox thermometer CX-1050-BC was used. These thermometers can be considered small with respect to the 5mm nozzle and the cold volume. The thermometer was calibrated down to 2K in the cryostat described in [11], wired with 40 micron Nichrome wire to avoid heat input. As visible in figure 2 the wire was wound in a pigtail to increase the length of thermalisation in the Helium flow; this was found essential to avoid false temperature readings. A spacer mounted below the thermometer serves as mechanical stabilisation and separation of the wires. The thermometer was placed in the flow at a small angle to optimise cooling of the sensor surface and avoid turbulence and ice growth which occurred with the sensor perpendicular to the flow.

![Figure 2a: temperature offset and 2b: heater output versus temperature](image)
The longitudinal and lateral temperature gradients were checked with a spatial resolution of the size of the thermometer. Close to the nozzle, the central cone of the flow was found to be homogeneous over a diameter of about 3mm. The temperature on the axis remains constant over 5 to 6 mm, but the temperature goes up much quicker in the lateral direction. The offset between the temperature controller setpoint and flow temperature was verified between 10K and 50K at three different arbitrary flow rates. Two counteracting mechanisms cause an offset. At low flow rate the thermalisation of the gas passing through the heat exchanger brings it very close to the setpoint temperature but it will warm up passing though the nozzle giving a positive offset. At high flow rate the gas does not have the time to thermalise in the heat exchanger, but it will warm up less in the nozzle, giving a negative offset. For a given temperature range the flow can be regulated to minimise the offset.

3. Experiment

3.1. Sample preparation

Expression, purification, and crystallization of recombinant metNgb were carried out as described in [12]. In order to prepare the NgbCO derivative, metNgb crystals were transferred on a cover slip and were crosslinked by diffusing vapours of glutheraldehyde (25% solution, see [13]) in controlled conditions to stabilize them and to decrease deterioration and loss of crystal order, which could be due to the rearrangements observed in the CO-bound Ngb structure. After crosslinking, crystals were transferred into a glass container filled with mother liquor, which contained 25% glycerol as cryoprotectant and 4 mM sodium dithionite that had been previously purged with N₂, to achieve reduction of the heme iron. Then, the reduced crystals were soaked into the same solution, which contained 1mM CO to obtain the CO bound form (CO-Ngb), and then harvested with a cryo-loop and flash frozen into liquid nitrogen.

3.2. Data collection

X-ray data collection was carried out at ESRF, ID14-2 beamline, at a wavelength of 0.933 Å, using a ADSC 210 CCD detector. The temperature controller setpoint was set in a range between 15 and 40 K. CO-Ngb crystals were mounted in a cryo-loop and for each sample diffraction data sets were collected both at 15 K under illumination (‘light on’ state) and at 40 K (‘dark’ state, increased temperature to allow CO rebinding). In order to achieve photolysis of the CO-Fe bond we attempted to use two set-ups i) an optical fiber microscope white lamp, and ii) a laser focused and centered using the microspectrophotometer optics. We could not record spectra or utilize the laser to achieve photolysis, since it was incompatible with Helijet flow stability and it caused severe icing. Therefore “light on” data collection was carried out under illumination from the optical fiber microscope white lamp. Figure 3 shows the raw data from the detector at various moments during the data collection. For the best crystal, where we could actually observe partial photolysis of the CO-Fe bond diffracted at 1.9 Å resolution (light on) and 2.0 Å resolution (dark), data statistics are given in the table below.

![Figure 3a: Raw diffraction data (intermediate) and 3b the last image of the dataset. No data treatments were applied to the diffraction patterns. It is clearly visible that no ice rings develop during the data collection. The images were resized to remove unnecessary detail.](image-url)
### Table 1: CO-Ngb data collection statistics

| Carbonmonoxy Neuroglobin | dark (40 K) | light (15 K) |
|--------------------------|------------|-------------|
| Wavelength, Å             | 0.933      | 0.933       |
| Space group               | R32        | R32         |
| a, Å                      | 88.06      | 88.04       |
| b, Å                      | 110.16     | 110.16      |
| Resolution range, Å       | 44.63-2.0 (2.1-2.0) | 44.63-1.9 (2.0-1.9) |
| No of unique reflections  | 11287 (1614) | 12847 (1878) |
| Rmerge overall            | 0.053 (0.236) | 0.055 (0.373) |
| Completeness, %           | 99.9 (100.0) | 98.5 (99.9)  |
| I/σ(I)                    | 27.2 (7.5)  | 16.9 (3.0)   |
| Redundancy                | 7.1 (7.2)   | 3.7 (3.6)    |

### 4. Conclusion and further developments

The cryostat has been made available for the ESRF beamlines in the Sample Environment Pool. The use of the new cryostat allows data collection below 10 K, at these temperatures it is possible to isolate and characterize unstable reaction intermediates in protein structure, previously identified spectroscopically. We have successfully carried out X-ray diffraction data collection of murine neuroglobin at 10 K. The cryostat was tested up to temperatures of 100 K. The implementation of an automated needle valve to regulate the Helium flow would ease the user operation. Some adjustment will still be necessary to allow the insertion of additional devices such as a laser or microspectrophotometer during data collection.

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### References

[1] Greubel K H, Gmelin E, Moser N, Mensing Ch, Walz L 1990. Cryogenics 30, september supplement ICEC 13 proceedings 457
[2] Teng T-Y, Schildkamp W, Dolmer P, Moffat K 1994. J. Appl. Cryst. 27 133
[3] Hardie M J, Kirschbaum K, Martin A, Pinkerton A A 1998. J. Appl. Cryst. 31 815
[4] Hanson B L, Martin A, Harp J M, Parrish D A, Bunick C G, Kirschbaum K, Pinkerton A A, Bunick G J 1999. J. Appl. Cryst. 32 814
[5] Ribaud L, Wu G, Zhang Y, Coppens P 2001. J. Appl. Cryst. 34 76
[6] Agilent Technologies: Helijet http://www.chem.agilent.com
[7] Cryo Industries of America: Cryocool-LHe www.cryoindustries.com
[8] Nakasako M, Sawano M, Kawamoto M 2001. The Rigaku journal 18 47
[9] Nakasako M, Sawano M, Kawamoto M 2002. Rev. Sci. Instr. 73 1318
[10] Steinmann R, van der Linden P 2006. Proceedings of the International Workshop on Mechanical Engineering Design of Synchrotron Radiation - Equipment and Instrumentation 2006, Egret Himeji, Japan; Available on line: http://medi2006.spring8.or.jp/proc/50_2.pdf
[11] Martinez-Criado G, Steinmann R, Alén B, Labrador A, Fuster D 2007. Rev. Sci. Instr. 78 025106
[12] Arcovito A, Moschetti T, D’Angelo P, Mancini G, Vallone B, Brunori M, Della Longa S. 2008. Arch Biochem Biophys. 475(1) 7
[13] Lusty, C. J. 1999. J. Appl. Cryst. 32 106