A Low-Temperature Device for Protein Crystallography

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A low-temperature device for use in the X-ray structural study of proteins has been designed and tested. It is easy to construct, portable, inexpensive to operate and extremely reliable in operation.

There are at least two considerable advantages in performing protein single-crystal X-ray diffraction studies at lower than ambient temperatures: significant reduction in radiation damage (Cucra, Singman, Lovell & Low, 1970; Haas & Rossman, 1970) and the possible improvement in the clarity of high-resolution electron-density maps due to a reduction in side-chain thermal disorder (Lipscomb, 1972). The enormous number of measurements required for a successful high-resolution protein structure determination (for example see Freer, Kraut, Robertus, Wright & Xuong, 1970) imposes several major criteria on a suitable low-temperature device:

(1) low long-term operational cost;
(2) operation for considerable periods, (> 4 days) without manual intervention;
(3) long-term temperature stability (± 0.5°C);
(4) ice-free operation over a wide ambient temperature range for long periods of time.

Nearly all previously described or commercially available devices fail to satisfy all these criteria, primarily because of their reliance on liquid nitrogen as a cryogenic material; the consumption of this fluid is usually a minimum of 0.5 l.h⁻¹, and apart from the cost this imposes serious problems arising from the need to monitor levels and manually refill the N₂ supply.

We describe here a device which meets all of the above requirements. Once assembled, its operational cost is practically nil; it has been run continuously for over 500 hours without any attendance; it is stable to ±0.1°C, and can produce temperatures from 30°C to −20°C without any icing problems.

Design

A schematic diagram of the design is shown in Fig. 1. A photograph of the assembled device is shown in Fig. 2; the numbering system below refers to both illustrations.

1. Compressed air source

We have found that ordinary laboratory compressed air at 60 p.s.i. is adequate for the cooling gas stream. A compressor capable of delivering 1.7 ft³/min at 60 p.s.i. would be an adequate substitute. The air is passed through two coarse filters (Fig. 1, 1a and 1b) to remove dust and large droplets of water and oil. Delivery at point 1d is 1.7 ft³/min at 30 p.s.i., pressure reduction being accomplished by valve 1c.

![Fig. 1. Schematic drawing of the low-temperature device. The numbers refer to the text.](image-url)
2. Regenerating gas drier

This commercially available unit will dry 1.7 ft³/min of air at 30 p.s.i. to a dew point of -100°F. Regeneration of the dessicant is controlled by a timing motor linked to solenoid valves. These allow the passage of 0.7 ft³/min of dried air through the column of dessicant not in use, purging it of moisture. Delivery at point 2a is therefore 1.0 ft³/min of dry air at 30 p.s.i. We have found that the drier operates most effectively with a flow control valve at point 2a to maintain a more uniform pressure across the drier.

3. Refrigeration unit and heat exchanger

A commercially available refrigeration unit 3a with a capacity of 450 W is used to cool a heat-exchange bath (3b) containing seven litres of ethylene glycol. Temperatures as low as -30 °C may be obtained in this way; the bath is insulated with expanded polystyrene and is stirred constantly (3e); this is essential both to prevent frosting and to ensure good heat exchange. A 500 W heater (3c) driven by a Variac provides a coarse temperature control for the entire system by regulating the bath temperature. The dried air at 30 p.s.i., 1 ft³/min, is cooled by direct heat exchange with the bath through a 9 in helix made from 20 ft of ¼ in o.d. copper tubing (3d).

4. Fine temperature control

Temperature stability and fine adjustment over a 10°C range are provided by a closed-loop electronic system (4a), which supplies heat to a completely insulated box (4b). The box contains 30 resistors of 130 Ω each, rated at ½ W; its construction is described below. Fig. 3 is a schematic diagram of the control system.

Circuit operation. The negative voltage at the junction of the potentiometer formed by resistor R3, and thermistor RV2, is added to the positive voltage from the SET AIR TEMPERATURE potentiometer RV1. When air heating is required, RV1 is adjusted until the sum of these two voltages is negative, and this voltage is applied to an amplifier whose output, via a compound emitter follower, is used to provide current for the air heater. The output, or heater, voltage is also displayed on a meter.

The negative temperature coefficient thermistor RV2 is placed in the outlet from the air heater, its resistance decreasing as the air is heated, thus taking the junction of R3 and RV2 more positive and reducing the negative voltage to the amplifier, and hence the heating. The thermistor operates as a feedback element so for any position of the SET AIR TEMPERATURE potentiometer the circuit will rapidly settle to equilibrium, heating the air to the temperature required and maintaining this temperature within very close limits.

The entire control system with the exception of the heater box may now be obtained commercially.

Construction of heater box. This consists of a square tube containing thirty 130 Ω, ½ W metal oxide resistors arranged in ladder formation along the length of the box. At each end of the box are short lengths of ¼ in copper tubing to provide connections to the air delivery tube. Fig. 4 shows the construction of the ‘ladder’ from resistors and two sides of the box which are ¼ in fibreglass board, copper-clad on one side. The resistors are soldered into place, and the two remaining sides fixed with Araldite. These sides are of ¼ in plain fibreglass board.

The two end pieces are also of copper-clad board, fixed in place by Araldite with the copper on the outside, enabling a one inch length of ¼ in copper tubing to be soldered to each end.

Finally, the heater must be well-insulated. Eight pieces of 5 x 5 x 1 in expanded polyurethane board are drilled at the centre to provide a clearance hole, and the heater is slid into the middle of the stack. After the connexion of 1 ft of delivery tubing to each end, two more identical slabs of insulation are fitted at each end. The entire assembly is clamped between two 6 in squares of plyboard by means of 4 lengths of 2 B.A. studding, one at each corner.
5. Delivery tube

The cold dry air is delivered to the crystal through 9.0×2.5 mm silicone rubber tubing, insulated with flexible expanded polyurethane tubing ½ in thick. Under these conditions a total length of over 12 ft of delivery tubing may be used with only a 4°C increase in temperature from the heater box. We have found it advisable to mount the tube so that the cold air stream is coaxial with the quartz capillary tube in which protein crystals are routinely mounted. If the air flow is...
ensured to be laminar, and is of a diameter 5 mm wider than the capillary tube, thermal gradients across and along the capillary wall are undetectable.

For use with a conventional 4-circle diffractometer, a χ-circle mounting is most convenient. In particular, the Hilger–Watts 4-Circle Diffractometer has a slot in the counter-weight on the χ-circle, which provides for coaxial delivery of the cold air for all possible crystal orientations during data collection. Hilger and Watts Ltd. should be consulted before removal of this counter-weight to insert a clamp for the delivery tube. A photograph of this attachment is shown in Fig. 5.

For use with a precession camera, where the total motion of the capillary tube is much less, a simple transfer of the delivery tube to a fixed support is all that is required; a variation of this showing the device in use with a fibre camera is given in Fig. 6.

The bulk of the device is permanently mounted on a small trolley (cf. Fig. 2) and is completely portable. Transfer from one type of data collection device to another takes only a few moments.

Stability

The device is extremely reliable and stable. The use of a refrigeration unit and alcohol bath completely eliminates the need for refilling of liquid nitrogen dewars. The regenerating gas drier maintains a stream of completely dry air; in over 1000 h of testing at temperatures from +20 to −20°C no trace of ice or condensed moisture was ever observed on the crystal or its surroundings. In continuous runs of up to 500 h no manual intervention has ever been found to be necessary. Over this period of time, the temperature at the crystal, 1 cm from the end of the delivery tube, was not observed to vary by more than 0.1°C, despite variations of over 10°C in the ambient temperature. Total operational cost is only the cost of the electricity, less than 1 kW.

Results

This device has permitted the routine measurement of protein crystal X-ray diffraction intensities at temperatures down to −20°C in our laboratory. We have noticed considerable reduction in radiation damage for several different proteins, and this in turn has made possible the use of fewer crystals for each data set, thus minimizing errors due to scaling (Watson, Shotton, Cox & Muirhead, 1970). It has also been possible to increase counting times for each reflexion and thus to increase the precision. This device should also be helpful in the study of those proteins whose crystals are unstable at room temperatures or which undergo temperature-dependent conformational changes. We are currently exploring methods of increasing its useful range of temperatures to below −75°C.

Fig. 5. The low-temperature device attached to the χ-circle of a Hilger–Watts four-Circle diffractometer.
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APPENDIX

List of suppliers of major components

In the following list, the numbers are those used in the text and in Fig. 1.

(1b) and (2). Coarse air filter and regenerating gas drier. Model 4103-6 'Dry Pak' Gas Drier. Model 1137 - 3E Pre-Filter. Wilkerson Corporation, Englewood, Colorado 80110, U.S.A.


(4f). A compound emitter-follower which increases 1 mA to 3 A is quite suitable.

(4e). SET AIR TEMPERATURE Potentiometer. Type Multi-Pot 10K (10 turn helical potentiometer). R. S. Components, P.O. Box 427, 13–17 Lepworth St., London EC2 P2HA, England. The potentiometer may be calibrated with a counting dial; Type 10T from the above manufacturer is suitable.

(4f). The heater power supply is 12 V D.C. at 3 A.

(4). The entire control circuit may be obtained from C. Wilkins, 127 Park Road, Didcot, Berks, England.

(5). Delivery tubing. Type T.C. 156/4 (silicone rubber tubing). Esco Rubber Ltd., 14–16 Gt. Portland Street, London W1N 5AB, England. Flexible expanded polyurethane and rigid expanded polyurethane may be obtained from any commercial insulation engineer.

References


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