SNBL	**Experiment title:** **CRYSTAL STRUCTURE DETERMINATION OF PROCTOLIN, AN INSECT NEURO-PEPTIDE.**	**Experiment number**: 01-02-351
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Shifts:	**Local contact(s)**: J.A. Beukes	*Received at UNIL:*

Names and affiliations of applicants (* indicates experimentalists):

*Silvia Capelli and *Phil Pattison
SNBL at ESRF, PO Box 220, F-38043 Grenoble

*Judith A.K. Howard and *Olga Chetina
Department of Chemistry, University of Durham, South Road, Durham DH1 3LE ,UK.

The problem of preparing oligo-peptide crystals suitable for diffraction studies is well known. Linear chains of amino-acids tend to be very flexible and as the chain length increases beyond 2 residues, the number of energetically favourable conformations a molecule may adopt in solution increases rapidly, hindering the crystallisation process. In addition, small peptide-chains are not long enough to fold into stable secondary structures in the same way as poly-peptides and proteins. Proctolin is an oligopeptide composed of five residues, and we were able to prepare only five individual crystals of suitable size for our diffraction study. All of them were grown from an ethanol solution directly in a glass capillary. The diffraction studies were performed using a MAR345 image plate at beamline BM1A, using monocromatic radiation of 0.80023 Å wavelength.

A first, well-diffracting crystal (~ 1.1 Å) was used to collect a room temperature dataset in two resolution ranges, but suffered radiation damage and did not survive the end of the first high-resolution shell measurement (60s was probably too long an exposure time). The on-line data processing revealed an orthorhombic cell with axes parameters 15.413(3) 35.337(7) 43.639(9) 90.0 90.0 90.0 and a unit cell volume of 23768.0 $Å^2$. Subsequent analysis showed the space group to be $P2_12_12_1$ with 6 (!) independent molecules in the unit cell, meaning 276 independent non-H atoms.

A second crystal, diffracting only out to ~ 4 - 3.5 Å, was used to test the behaviour of the material at low temperature. A nitrogen cryo-stream was used to reduce the temperature. A few imagess taken after flash-cooling the crystal to 120K showed the quality of the diffraction patter got worse with severe peak splitting. The simultaneous increase in the number of diffraction spots suggested also a phase-transition occurred. In fact, a single-image indexing of one diffraction patter at 120K with the program Denzo confirmed the crystal had undergo a phase transition to a monoclinic cell.

We went back to room temperature to try the quality of a third crystal and because it was diffracting out to ~1.0 Å with nice sharp spots, we decided to collect some room temperature high-resolution data before trying to cool it down. 360 images were collected at a sample-detector distance of 180 mm (max resolution: Å), with φ rotation width of 1º and exposure time of 15 s. The crystal did not show radiation damage and we cooled it down slowly, collecting images every 10º from 290 to 200K. The cell at 200K was still orthorhombic: (15.4902(3) 35.043(3) 43.138(8) 90.0 90.0 90.0) so, we proceed with a second data collection, using a sample-detector distance of 230 mm, with φ rotation width of 1º and exposure time of 6 s for a total of 90 frames. An attempt to collect an additional dataset at higher resolution on the same crystal at 200K failed because of the movement of the reservoir solvent in the capillary that touched and started dissolving the crystal.

The fourth crystal did not produce any diffraction at all but the fifth one was of the same good quality of #1 and #3. With the last crystal we had again the problem of contact between the crystal and the reservoir of solvent and this time also some wax slowly moved over to the crystal. We were able to collect only 60 images at sample-detector distance of 130 mm and exposure time of 20s before diffraction rings from the wax started appearing on the frames and at image 102 we had to stop because the diffraction from the wax was saturating the detector.

From this experiment we were able to retrieve only three datasets, none of them complete and for which the data processing is still in progress:

- in the high-resolution dataset at 290K, the low-resolution peaks are saturated and in addition the significant decay of the crystal imply a very difficult scaling process to bring all the collected intensities on the same scale

- the high-resolution dataset at 200K is also representing a problem because of the increasing background noise due to the diffraction from the wax

- the low-resolution dataset at 200K has been processed, resulting in an internal R factor of ~ 4%, but in this case the limited resolution is the main obstacle to the solution of the structure. Attempts to solve the structure have been made both with small-molecule dedicated packages using Direct Methods (SHELX, SIR92) and vector search methods (PATSEE), and with protein crystallography programs (SnB) but up to now with very modest results. The 6 independent molecules in the asymmetric unit are contributing to making the interpretation of the already poor electron density maps even more difficult.

Although we have not yet been able to solve the crystal structure of this peptide, we have been able to make considerable progress in characterizing the behaviour of the crystals both as a function of radiation dose and temperature (in only 24 hours of beamtime). We have been able to index successfully the diffraction pattern at low resolution, and to identify the optimal experimental conditions for an improved quality data collection. We are currently re-crystallizing the material in an effort to obtain suitable crystals for another data collection. We have been thwarted this time by the combination of the complexity of the crystallographic problem and the difficulties of finding the optimal experimental conditions. However, we are sure that we are now in a position to collect better quality data and a beamtime proposal will be submitted for the coming experimental period.