	Experiment title: Templated Solid State Reaction in TrpGly·H_2O	**Experiment number**: CH1007
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Report:

The purpose of the present experiment was to investigate the high temperature behavior of TrpGly·H_2O. We have previously shown that the crystal structure of this dipeptide monohydrate consists of peptide nanotubes held together by hydrogen bonding [1], see the Figure (top left). These tubes enclose disordered water molecules. Below room temperature, the thermal expansion along the tube axis is negative [1]. The tube axis coincides with the *c*-axis of this tetragonal system (space group $P4_1$). Above room temperature, we found, by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), that the water molecule is lost between about 317 and 420 K. At higher temperatures, 420-440 K, a mass equivalent to an additional water molecule is lost and the DSC shows sign of a chemical reaction and/or melt/recrystallization. This additional water molecule, one per peptide molecule, must stem from a chemical reaction. The goal of the present experiment was to follow this behavior by powder diffraction and to solve the structure of the product of the high temperature reaction. We followed the evolution of the powder patterns above room temperature using a MAR345 imaging plate detector and a hot air blower. Data points were taken every 10ºC from 30ºC to 180ºC. Several conclusions could be made directly from the raw data:

1. The structure remains tetragonal and crystalline upon loss of water.
2. At the reaction, there is a loss of intensity in the existing Bragg peaks, growth of new broad Bragg peaks and an increase in the overall background. This points to a recrystalization mechanism, most likely going through a melt phase, though this needs further independent confirmation.

These points are illustrated in the Figure (top right), that compares raw spectra at several temperatures.

Lattice constants were extracted as a function of temperature using Le Bail fits as implemented in GSAS after integrating the 2D images to 1D powder patterns with FIT2D. The results are show in the bottom left part of the Figure. Until the loss of the structural water, the *a*-axis expands uniformly while the *c*-axis is almost constant. Upon loss of the water molecule, at ~100ºC, the thermal expansion along *a* increases (change of slope). The value of *c* drops and the *c*-axis contracts strongly until the highest temperatures accessible.
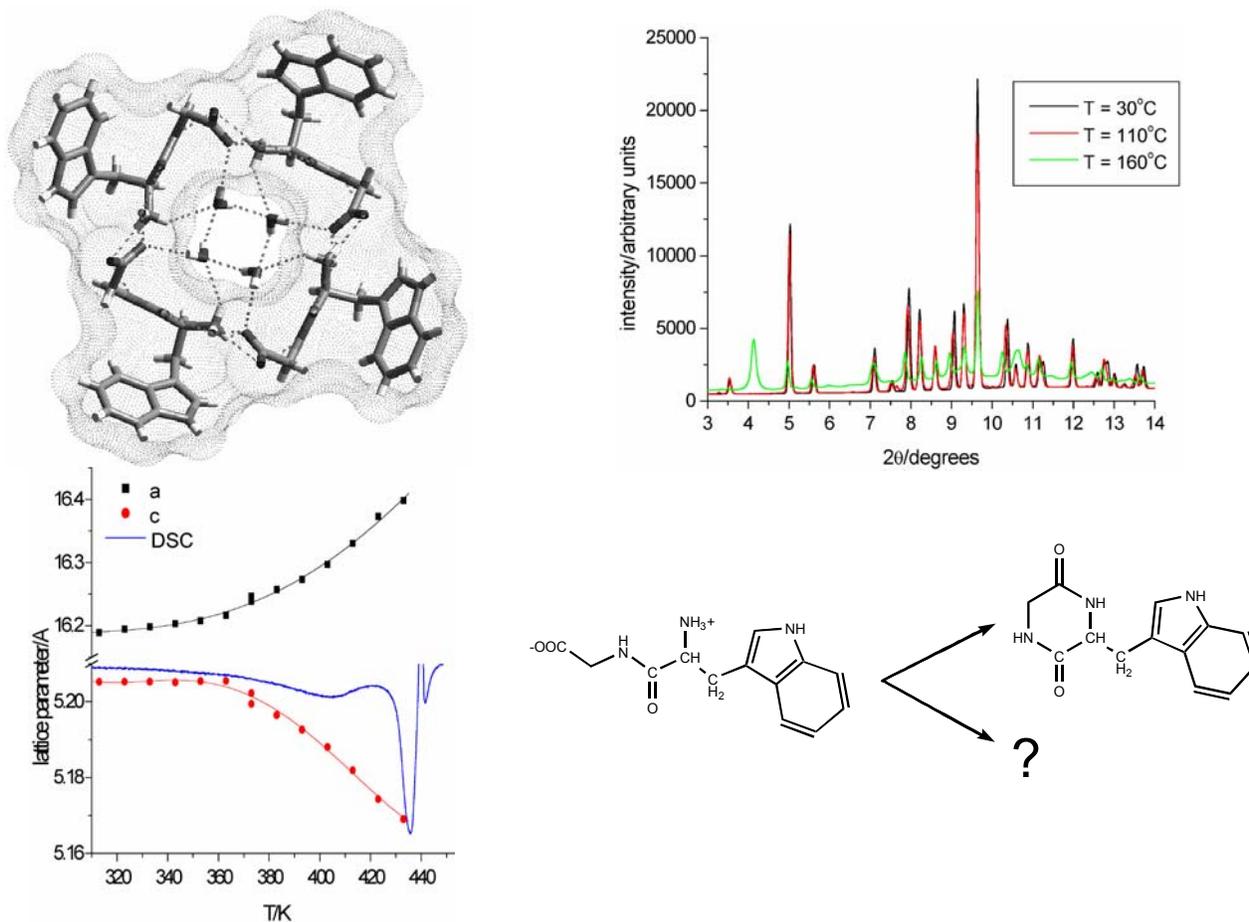
Figure: Top left: Structure of TrpGly·H$_2$O. The dotted surface shows the van der Waals surface of the peptide column and reveals the water channel. Only the water site with highest occupation is shown. Hydrogenbonds are drawn as dashed line. Top Right: comparison of powder data taken at three temperatures. Bottom left: evolution of lattice constants compared with DSC profile in blue, lines are rough guides to the eye. Bottom right:High temperature reaction, the minor product, cyclic TrpGly, was identified by powder diffraction.

Already at 150ºC, the first signs of the new phase appear in the spectra. At 180ºC all the material had transformed. The product of the high temperature reaction turned out to consist of two crystalline compounds. One of these could be isolated by recrystallization from a mixture of DMSO and acetone. It could be identified as cyclic TrpGly, *i.e.* the result of a diketopiperazine forming condensation reaction (Figure bottom right). This structure has been determined previously [2]. It is the minority product. The major product is still undentified but must also result from a condensation reaction since the TGA indicated loss of the equivalent of one water molecule per peptide molecule during the reaction. The lattice constants could be determined by calculating the difference between the product powder pattern and that of the minor phase: $a = 13.47$, $b = 14.20$, $c = 6.23$ Å, $\alpha = \beta = \gamma = 90º$. We are currently undertaking supplementary experiments in order to determine the nature of the major product. The presence of an increased background seems to indicate that the reaction proceeds in the liquid phase; this will be verified by optical studies.

In conclusion, the powder data collected showed that the nanotubular structure is stable at high temperature even after the structural water has been lost. This large structural stability shows promise for the possibility of inserting other molecules into the columns and/or using it for transmembrane transport. The thermal expansion of the water-free tubes is much larger than for the water-containing ones – and still negative along the tubes. The high temperature reaction was shown to yield two products one of which could be identified unequivocally.

[1] H. Birkedal, D. Schwarzenbach and P. Pattison, *Angew. Chem. Int. Ed.* **41**, 754-756 (2002); *ESRF Highlights 2000* pp. 18-19 (2001)
[2] Morris et al. *Cryst. Struct. Commun.* **3**, 145 (1974).