ESRF	**Experiment title:** Molecular and crystal structure of amylose complexed with α-naphthol	**Experiment number**: SC-3191
Beamline: ID13	**Date of experiment**: from: 24/09/11 to: 27/09/11	**Date of report**: 20/05/12
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Names and affiliations of applicants (* indicates experimentalists):
Jean-Luc PUTAUX* - Centre de Recherches sur les Macromolécules Végétales, Grenoble
Yoshiharu NISHYAMA* - Centre de Recherches sur les Macromolécules Végétales
Mateus B. CARDOSO* - Brazilian Synchrotron Light Laboratory, Campinas - Brazil

Project outline

We have investigated the molecular structure of model crystalline complexes of a major biopolymer, amylose, with α-naphthol. Thin lamellar amylose V-α-naphthol single crystals yield exceptional base-plane electron diffraction patterns, up to a resolution close to 1 Å. We have prepared complexes in the form of $10 \times 10 \times 0.4$ μm^3 crystals that constitute some of the largest and most crystalline polymer single crystals ever prepared. X-ray microdiffraction datasets have been collected from individual fast-frozen solvated crystals embedded in ice and mounted on a microgoniometer. The molecular model calculated from the datasets should allow locating the guest ligands within the crystal lattice and clarify their interaction with amylose.

Experimental method

Preparation of the single crystals. Dilute solutions of 0.3 wt% synthetic amylose with a degree of polymerization of about 100 and α-naphthol were heated up to 140°C for 15 min, maintained at 85°C for 1 h and allowed to slowly cool down to room temperature. Large square single crystals *(Fig. 1a)* were obtained and their "quality" was assessed by recording base-plane electron diffraction patterns from the thinnest crystals *(Fig. 1b)*.

Microdiffraction set-up. Experiments have been carried out on the "microfocus" section of the ID13 beamline. In order to ensure that the initial crystallinity was preserved and that radiation damage was minimized, the crystals were probed at low temperature. Glycerol was added to the aqueous suspension as a cryoprotectant. A thin liquid film of the crystal suspension was formed in the upper compartment of a Kapton cryoloop *(Fig. 1c)* and quenched in the N_2 stream of a cryoflow system. The specimens, maintained at 100 K throughout the experiments, were probed at an energy of 12.46 keV ($\lambda \approx 1$ Å) with a 600×350 nm^2 beam. The diffraction patterns were collected on a Frelon 1024×1024 CCD detector. Preliminary mesh-scans were recorded from the crystals embedded in vitreous ice *(Fig. 1d)*. A strong spot was then chosen to determine the crystal position with respect to the beam. The target crystal was centered on the beam and then eucentrically rotated on the microgoniometer in order to explore the reciprocal space. Typically diffraction images were recorded each 0.2° step with a typical exposure time of 0.5 s so that one diffraction spot appears on at least in five diffraction diagrams. The sample was translated vertically by 1.5 μm at the end of each line-scan to avoid excessive influence of beam damage.

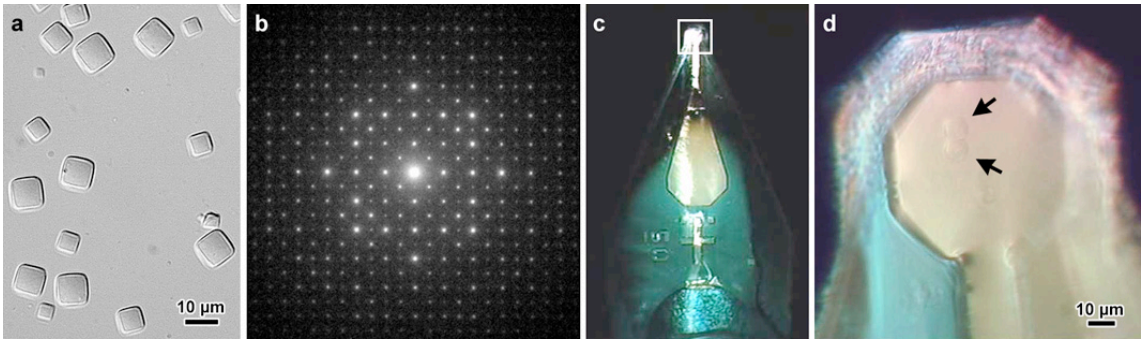

Figure 1: *a) Optical micrograph of amylose V-α-naphthol single crystals; b) corresponding base-plane electron diffraction pattern; c) part of a Kapton cryoloop allowing to hold a thin vitreous film of aqueous crystal suspension; d) detail of the top of the Kapton loop indicated by the white square in c. the black arrows point to two single crystals embedded in vitreous ice.*

Results

We could successfully record series of diffraction data extending to a 3 Å resolution from three single crystals. The base-plane spots *(h k 0)* were the less sampled in the diffraction data. This is because the flat crystals tend to align parallel to the vertical axis on the loop, and the sample rotation could be achieved only around the vertical axis. The data analysis is in progress to obtain molecular details of the crystal structure.

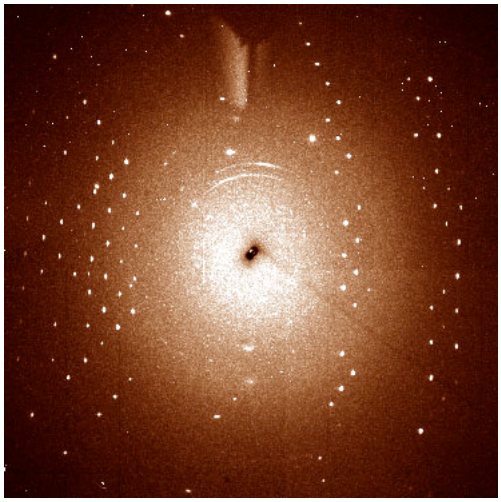

Figure 2: *Example of a diffraction diagram with merged data collected from a single crystal of amylose V-α-naphthol.*