ESRF	**Experiment title:** Structural bases for allosteric regulation of acetylcholinesterase catalysis. Project 1: Search for another conformation of acetylcholinesterase	**Experiment number:** LS-601
Beamline: D2AM	**Date of Experiment:** Feb. 1997 from: Feb 2, 7:00 am to: Feb 3, 7:00 am	**Date of Report:** Feb. 28, 1997
Shifts: 3	**Local contact(s):** Richard KAHN	*Received at ESRF :*

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Report:

The crystals used in this experiment were grown in high salt concentrations. They belong to the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 139 Å, b = 173 Å , c = 225 Å ($\alpha=\beta=\gamma=90°$). Processing of the preliminary data obtained in-house on a MarResearch imaging plate mounted on a Siemens rotating anode (50kV x 100 mA) suggested the presence of 4 molecules of our protein in the asymmetric unit, with a Vm of 5.5 $Å^3$/Da or 77% solvent. In spite of the growth conditions and the high percentage in solvent in the crystals, suitable conditions for cryo-freezing could be set up in-house prior to data collection at ESRF. Crystals were transported in plate at 4°C and stored in a cold room nearby the hutch upon arrival. They were kept on ice (available at EMBL) when transferred to the hutch, and were cryo-frozen on line.

Several different problems were encountered. First, the beam was lost after reinjection and had to be realigned; thus, our experiment started later (about 3 hr) than expected. Second, our flash-cooling conditions could not be fully reproduced at the beam line, possibly because of a lower temperature of the nitrogen flux (90 K), compared to that available in-house (100 K); the conditions had to be re-adjusted. Third, a large number of crystals were tested but the resolution achieved was always lower than expected, compared to that achieved in-house; the crystals, that are very fragile, may have been damaged during transportation; alternatively, the new flash-cooling conditions that we have determined on-line may not be optimal.

However, a data set could be collected on a crystal that looked promizing, starting Feb. 3, -1:00 am. Experimental conditions were: λ, 0.978 A; crystal-to-detector distance, 245 mm; oscillation steps, 0.5°; exposure time, 60 sec. On-line partial processing of the initial frames with the local version of XDS suggested that the selected crystal and experimental conditions (crystal-to-detector distance, in particular) were tine. A total of 250 frames, or 125°, were collected.

The data were reprocessed at home with DENZO after they were converted with IMAC (developed by Michel Roth). Detailed analysis evidenced severe overlapping of the spots in several of the frames, suggesting that the choosen crystal-to-detector distance was too short; in addition, the low intensity of the spots indicated insufficient exposure time for the diffraction quality of the crystals. The overall Rsym is -15% at 3.3 Å resolution for a 82% completness, and the overall $I/\sigma I$ is -10 (-2 in the last shell). Refinement is underway, that will allow us to estimate the quality of these data more precisely.

In summary, we could not achieve the resolution, completness, and data quality that were expected based on the preliminary data collected in-house. Therefore, further beam time will be requested for this project. To increase the probability of obtaining better results upon next ESRF experiment, optimization of our flash-cooling conditions is underway, and we will try carrying some of the crystals in liquid nitrogen. At the beam line, both the crystal-to-detector distance and the exposure time will be increased.

Full-time assistance from Richard Kahn is much appreciated.