ESRF	**Title** Structural bases for allosteric regulation of acetylcholinesterase catalysis. Project 2: Search for the route for substrate entry into the fasciculin-acetylcholinesterase complex.	**Experiment number:** LS-896
Beamline: ID 14-EH3	**Date of experiment:** from: 6/5/98 to: 8/05/98	**Date of report:** 30 Aug 98
Shifts: 6	**Local contact(s):** Wim Burmeister	*Received at ESRF:*

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Report:

Four complete data sets were collected at 100°K on crystals of the mouse AChE which belong to space group $P2_12_12_1$ with cell dimensions a=141Å; b=178 Å and c=230 A. Four AChE molecules are present in the asymmetric unit (1). These crystals were co-cristallized with an inhibitor of the catalytic site (two data sets with each of the R and S eniantomer) and two potential inhibitors of the peripheral site (decidium and amyloid peptide β1-20). The resolution was in the 3 Å range with R-sym values ~ 12% . A detailed analyze of these four structures is underway: for the two structures with potential inhibitors of the peripheral site, no clear electron density maps is observed in this region of the protein probably due to the high salt concentration (2 M Na/K phosphate) needed for crystallisation experiments, whereas the two structures with the catalytic site inhibitor are still being refined. In this case we are more confident in the presence of such new inhibitor, because it is covenlty bound to the catalytic site Ser residue.

For the AChE-Fas2 complex, a good crystal, which diffracted up to 2.3 Å resolution, was obtained at the end of the experiment and was not collected. For this crystal form, the installation of the Large Imaging Plate detector (8000 x 4000 pixels) is necessary to allow data collection with a crystal-to-detector distance of 590 mm, and only few hours of beamtime were available. This crystal represents an « inhibited » form of the enzyme, in which a ternary inhibitor acting at the catalytic site of AChE was soaked. This crystal belongs to the hexagonal space group P6522 with cell dimensions:a=b=75A and c=550A and was stored in liquid nitrogen and will be available for data collection at ESRF.

A large number of crystals of the homologous AChE-Fasl complex were also tested; however, no data could be collected on these crystals, which all appeared to be twinned or of a poor diffraction quality (maximal resolution achieved was about 5 Å).

In addition, crystals of Civl, a protein kinase for which beamtime has been allocated on the non-operational beamline EH4 (LS-897) were tested using the Mar CCD. These crystals diffracted up to 3.5 Å and a 85% complete data set was collected. The overall R-sym is 9%. Molecular replacement experiments using homologue kinase structures are underway, although searches for heavy atom derivatives will be necessary for this project.

Finally, we collected a complete data set at 3.5 Å resolution (R-sym = 9.5%) with new crystal of the glycosyltransferase GalNAc-T2, benefits of these preliminary data will he used to prepare new crystals for the new proposal deadline Sept I 98.

Full-time assistance from Wim Burmeister is much appreciated.