



**Experiment title:**  
**The acetylcholinesterase-Fasciculin 2 binding interface revisited**

**Experiment number:**  
LS1657

**Beamline:**  
ID14-2

**Date of experiment:**  
from: 3-3-00 to: 4-3-00

**Date of report:**  
Aug00

**Shifts:**  
3

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*Received at ESRF:*

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## Report:

Crystals of mouse acetylcholinesterase, an  $\alpha/\beta$  hydrolase, in complex with the peptidic inhibitor fasciculin (Fas2-mAChE complex) belong to space group  $P6_322$  with cell dimensions  $a=b=75$ ,  $c = 550$  Å (1). Separation of the resulting overlapping spots usually requires a very large crystal-detector distance, but the maximal resolution achieved is not sufficient to permit detailed interpretation of the structures. High quality and rapid X-ray data were collected at the ESRF beamline ID14-EH3 equipped with a large image plate camera (LIPS device), in addition to a 2k x 2k MarCCD detector mounted on a 2 $\theta$  arm for determination of the orientation matrix.

With this set-up, we collected complete data sets with resolution up to 2.6 Å (Table 1) with a total oscillation of 36° and a 3° oscillation range (2x6 images), and with the long  $c$  axis of the crystal being roughly aligned to the spindle axis (Fig. 1); the crystal-to-detector distance was 480 mm. Three complete data sets were collected corresponding to three different Fas2 mutants aimed at a better knowledge of the hydrophobic vs electrostatic properties of the Fas2-mAChE binding interface. Unfortunately, only two data sets out of three could be processed. Data were processed with DENZO, and scaled and merged with SCALA.

Refinement of these two structures is in progress using new 2.5 Å resolution, native Fas2-mAChE coordinates as a starting model. The current electron density maps unambiguously revealed the presence of the Fas2 mutations (Fig. 2).

**Table 1. Data collection and refinement statistics**

	Mutant A	Mutant B
Resolution (Å)	2.6	2.65
No. observations	340 066	253 322
No. unique	27 372	27 060
R <sub>sym</sub> (%)	7.9 (41)	9.4 (42)
I/σ(I)	8.4 (1.8)	6.8 (1.7)
Redundancy	4.7	5.2
Completeness (%)	96.1 (95.7)	99.2 (99.8)
Resolution (Å)	20 – 2.6	20 – 2.65
R-factor - R free (%)	22.4 – 27.7	25.7 – 28.1

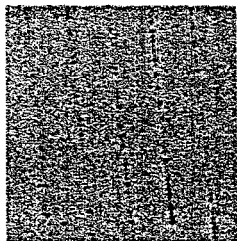


Figure 1: Diffraction pattern obtained from a Fas2-mAChE crystal with the long 550 Å c\* axis vertical.

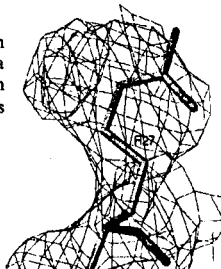


Figure 2: 2Fo-Fo and Fo-Fo electron density maps around the mutated Fas2 R27 residue contoured at 1  $\sigma$  and 3.5  $\sigma$ , respectively.

## References

- 1 Bourne, Y., Taylor, P. & Marchot, P. (1995) *Cell* **83**, 503-512.