LS-1796
Marseille BAG

ID14-4
7 February 2001

Project	Responsible	B'line	Date	Method	Space Grp	Cell (A)	MW kDa	mol/au	Resol (A)	Rsym (%)	Comp. (%)	Mult.
CSP2+NaBr	Valérie Campanacci	ID14-4	07/02/01	SIRAS	P422	69.4 69.4 78.9	13	2	3.00	11.60	100.00	8.70
CSP2+Kr	Valérie Campanacci	ID14-4	07/02/01	test								
CSP2+Br-stearic	Valérie Campanacci	ID14-4	07/02/01	test								
CSP2 non ligandee	Valérie Campanacci	ID14-4	07/02/01		P422	68.6 68.6 79.0	13	2	2.00	5.00	99.10	5.60
Tlp NaBr ??	Yves Bourne	ID14-4	07/02/01	SIRAS	P21212	163 x 52 x 83	28	2	2.30	4.50	98.90	3.20
AChE-Fas2 R24T	Yves Bourne	ID14-4	07/02/01	MR	P6522	73 x 73 x 556	80	6	2.80	7.00		

ESRF	**Experiment title:** Acetylcholinesterase-fasciculin complex	**Experiment number**: LS 1796
Beamline: EH4	**Date of experiment**: 7-8 February 2001 from: 8 am to: 7 am	**Date of report**: Feb01
Shifts: 6	**Local contact(s)**: Sean MacSweeney	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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The acetylcholinesterase-Fasciculin 2 binding interface revisited

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Crystals of mouse acetylcholinesterase, an α/β hydrolase, in complex with the peptidic inhibitor fasciculin (Fas2-mAChE complex) belong to space group $P6_522$ 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 already 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 2θ arm for determination of the orientation matrix.

We have tested the feasibility of this system on ID14-EH4 beamline equipped with a Quantum 4 detector that can be lifted up. With this set-up, we collected a complete data set with resolution up to 2.7 Å with a total oscillation of 40° and a 1° oscillation range, and with the long c axis of the crystal being roughly aligned to the spindle axis (Fig. 1); the crystal-to-detector distance was 350 mm and the lift was 35 mm. A complete data sets were collected corresponding to a single Fas2 mutant aimed at a better knowledge of the hydrophobic *vs* electrostatic properties of the Fas2-mAChE binding interface. Data were processed with DENZO; unfortunately, this data set could not be merged due to twinning problems. However, this experience was successful and has shown that good quality crystals with such cell dimensions can be easily collected on ID14-EH4.

References

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

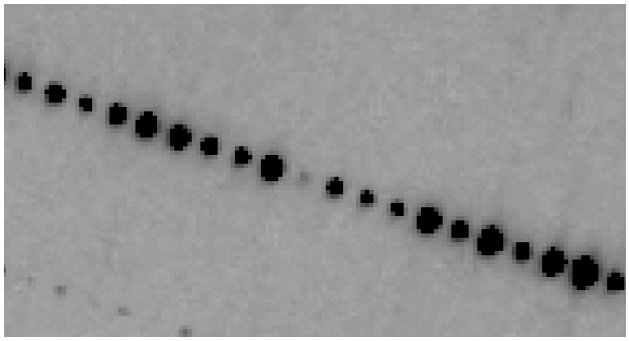

Figure 1: Diffraction pattern obtained at beamline ID14-EH4 from a Fas2-mAChE crystal with the long 560 Å c* axis horizontal.

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Two distinct carbohydrate binding sites having a different sugar specificity characterize a lectin from tulip bulbs

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We have obtained crystals of a new lectin from tulip bulbs, TxLCI, that possesses uncommon carbohydrate binding sites. The lectin is composed of two distinct domains, each domain possesses a different sugar specificity, one domain recognizes mannose while the other one binds N-acetyl-galactosamine (1). One domain possesses high homology with a lectin from snowdrop bulbs while the other shares only few homologies. A complete native data set has been already collected on beamine ID14-EH2 at 2.1 Å resolution. Attemps to solve the structure by molecular replacement using different homologuous structures failed.

We thus decided to test a new method consisting of rapid soak of crystals in the cryo solution supplemented with highly concentrated halide salts (2). Two crystals have been tested, each having different salt concentrations and soaking time. For each crystal, a single complete data set has been collected with the wavelength value chosen at 0.918 Å corresponding to the maximum f''. Data were processed and merged at the beamline using DENZO and SCALA. However, a rapid analysis of both the anomalous signal and the Patterson maps reveal that no clear halide positions could be detected. In addition, the curve of cumulative intensity distribution of one crystal reveals an anormal shape compared to a theorical curve, suggesting a twinning problem.

References: (1) ElsJ. M. van Damme et al. (1996) Molecular cloning of two different mannose-binding lectins from tulip bulbs. *Eur. J. Biochem*. **236**, 419-427. (2) Dauter, Z. et al. (2000) Novel approach to phasing proteins: derivatization by short cryo-soaking with halides. *Acta Cryst*. **D56**, 232-237.

ESRF	**Experiment title:** **CSP soaked in NaBr**	**Experiment number**: Ls1796
Beamline: ID14-EH4	**Date of experiment**: from: 7 Feb 2001 to: 8 Feb 2001	**Date of report**: Feb 01
Shifts: 3	**Local contact(s)**: Sean MCSWEENEY	*Received at ESRF:*

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Valérie CAMPANACCI*

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Report:

Crystals of MbraCSP were soaked in solutions containing different concentration of NaBr as described by Dauter *et al.* (1999+2000) and then frozen to 100 K with no cryoprotectant.
The space group and cell dimensions obtained were the followings: P422, 69.4x69.4x78.9 Å, $\alpha=\beta=\gamma=90°$.
One data set was collected to 100 K, with an exposure time of 5 sec per degree, see table bellow.

Data collection	
Total number of observation	135266
Number of unique reflections	6367
Overall % data > 1 sigma(I) (last shell)	100 (100)
Overall R merge (%) (last shell)	15.8 (35.2)
Overall I/sigma(I) (last shell)	2.5 (2.1)
Resolution (Å)	30.0 – 3.0
Redundancy	8.7

Using the technique described by Dauter *et al.*, we tested several crystals soaked in different concentration of NaBr and using different time of soaking.
Our results clearly showed the difficulty of the technique, that didn't allow us to obtain phases to solve the structure of the CSP.

Dauter, Z. and Dauter, M. (**1999**) Anomalous signal of solvent bromides used for phasing of lysozyme. *J Mol Biol*, **289**, 93-101
Dauter, Z., Dauter, M. and Rajashandar, K.R. (**2000**) Novel approach to phasing proteins: derivatization by short cryo-soaking with halides. *Acta Cryst* **D56**, 232-237

ESRF	**Experiment title:** **CSP unliganded**	**Experiment number**: Ls1796
Beamline: ID14-EH4	**Date of experiment**: from: 7 Feb 2001 to: 8 Feb 2001	**Date of report**: Feb 01
Shifts: 3	**Local contact(s)**: Sean MCSWEENEY	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Valérie CAMPANACCI*

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Report:

Crystals of unliganded MbraCSP were frozen to 100 K with no cryoprotectant.
The space group and cell dimensions obtained were the followings: P422, 68.6x68.6x79.0 Å, $\alpha=\beta=\gamma=90°$.
One data set was collected to 100 K, with an exposure time of 5 sec per degree, see table bellow.

Data collection	
Total number of observation	366182
Number of unique reflections	14314
Overall % data > 1 sigma(I) (last shell)	99.1 (99.1)
Overall R merge (%) (last shell)	6.4 (35.6)
Overall I/sigma(I) (last shell)	6.4 (1.6)
Resolution (Å)	30.0 – 3.0
Redundancy	5.6

Final comments on CSP project:

We actually have several data set of liganded or unliganded CSP with different space groups. Unfortunately, no phases were obtained using MAD or SAD method at the Br edge. However, we are currently expressing the seleno-methionine substituted protein in order to use the Se edge to solve the phases problem. We have also mutated the CSP in order to introduce a free cysteine to obtain heavy-atom derivatives and to use MIR method. Moreover, we also try to obtain phases by *ab initio* method.