LS-1796
Marseille BAG

ID14-2
4-6 November 2000

Project	Responsible	B'line	Date	Method	Space Grp	Cell (A)	MW kDa	mol/au	Resol (A)	Rsym (%)	Comp. (%)	Mult.
AGX1 + Mgcl2	Caroline Peneff	ID14-2	04/11/00	MR	p 21	85.829 70.904 96.180 β= 95.253	60	2	1.90	5.60	99.60	3.80
CSP2+Xe	Valérie Campanacci	ID14-2	04/11/00		P422	71.7 71.7 78.4	13	2	3.80	2.80	90.60	2.40
CSP2-Br	Valérie Campanacci	ID14-2	04/11/00		P21	33.4 53.9 56.2 β=86.1	13	2	1.30	5.60	91.20	7.30
CSP2-Br+Xe	Valérie Campanacci	ID14-2	04/11/00		C2	61.5 54.6 33.2 β=117	14	1	1.80	3.80	81.30	1.60
pig OBP +AMA	Florence Vincent	ID14-2	04/11/00	MR	P212121	42.2 87.7 93.2	15	2	1.50	7.20	98.60	3.10
HMC	Mirjam Czjzek	ID14-2	04/11/00	MR	P62	108.4 108.4 102.8	16	1	2.40	5.07	99.30	6.20
XylUR72M CBM Fam6	Mirjam Czjzek	ID14-2	04/11/00	MAD	P6122	60.1 60.1 157.5	17	1	2.20	8.10	98.70	4.50
Riba6	Marie-Pierre Egloff	ID14-2	04/11/00	MR	P3121	112.4 112.4 56.47	18	1	2.60	6.70	92.70	2.00
GpppA	Marie-Pierre Egloff	ID14-2	04/11/00	MR	P3121	108.6 108.6 55.88	19	1	2.30	4.40	99.40	3.30
NiR d1 heme reduction intermediates	Mariella Tegoni	ID14-2	04/11/00	test								
NiR mixed valence CO complex	Mariella Tegoni	ID14-2	04/11/00	test								
AChE complexed to PAS inhibitors	Yves Bourne	ID14-2	04/11/00	MR	P212121	79 x 112 x 226	80	2	2.40	5.00	98.80	3.60
TxLCl	Yves Bourne	ID14-2	04/11/00	MAD	P21212	163 x 51 x 83	28	2	2.10	5.20	90.80	3.20

ESRF	**Experiment title:** Acetylcholinesterase	**Experiment number**: LS 1796
Beamline: EH2	**Date of experiment**: 4-6 November 2000 from: 8 am to: 7 am	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Stéphanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Yves Bourne*

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The peripheral anionic site of Mouse Acetylcholinesterase

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We used a new crystal form of mouse acetylcholinesterase (AchE) that permits more accurate studies of the peripheral anionic site of this enzyme. This new crystal form diffracts up to 2.2 Å resolution and contains two molecules in the asymmetric unit, with the catalytic gorge entrance being freely solvent accessible in each subunit. This differs from the previous 3 Å resolution structure (1) for which only one molecule out of two possessed a solvent-accessible gorge entrance. In addition, the previous crystal form grew with a high salt concentration compared to a low salt concentration required for the new crystal form, a critical parameter to study ligands directed to the peripheral anionic site. We used a new compound that displays high affinity for this site and performed co-crystallization experiments with AChE using large excess of this compound. Indeed, previous data from similar crystals soaked with similar compounds showed only partial occupancies. Data obtained from these crystals are of excellent quality. For these two structures, rigid-body refinement was then performed on each subunit with CNS using data between 30 Å and 3 Å and gave an R-factor and R-free value of 23.7. For 2% of the reflections against which the two models were not refined, R-free was 23.1%. Refinement of this two structure is underway at 2.4 Å resolution. The final model will be used for a comparative study with other peripheral ligands for which data have been recently collected at ESRF.

References: (1) Bourne, Y., Taylor, P. & Marchot, P. (1999) *J. Biol. Chem*. **274**, 2963-70.

ESRF	**Experiment title:** Acetylcholinesterase	**Experiment number**: LS 1796
Beamline: EH2	**Date of experiment**: 4-6 November 2000 from: 8 am to: 7 am	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Stéphanie Monaco	*Received at ESRF:*

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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. Because this protein cannot be over-expressed in bacteria, we plan to use the new method consisting of a rapid soak of crystals in the cryo solution supplemented with highly concentrated halide salts to solve the phase 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.

ESRF	**Experiment title: Dengue capping enzyme : soaks with different cap analogs and RNA oligonucleotides**	**Experiment number**: LS1796
Beamline:	**Date of experiment**: from:04 11 2000 to: 06 11 2000	**Date of report**: 01 03 2001
Shifts: 6	**Local contact(s)**: Stéphanie Monaco	*Received at ESRF:*

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Egloff Marie-Pierre *

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Selisko Barbara

Romette Jean-Louis

Canard Bruno

Report:

Please refer to the previous report (06-08 10 2000) for general introduction to this project.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a broad-spectrum antiviral agent whose exact mode of action remains controversial. Many viruses are inhibited by ribavirin. It is currently used for treatment of infections such as HCV as well as of co-infections due to human immunodeficiency virus (HIV) and HCV. The inhibition mechanism is still elusive and may result from both direct and indirect effects. Since ribavirin is a guanosine analogue, NS5 is a candidate to be a target of ribavirin. It was also shown that ribavirin-triphosphate is a direct inhibitor of the viral MTase of vaccinia virus.

GpppA is a cap analogue which is commercially available. It has already been used for the characterization of several capping enzymes. The three dimensional structure of the complex between CEF and GpppA would be of great help in localizing the active site and understanding the mecanism of the mRNA transcript recognition.

CEF crystals were soaked in either GpppA or Ribavirin triphosphate, flash frozen and collected during this experiment. The data collection statistics are given below:

	Space group	Resolution (A)	cell	Rmerge (%)	completeness	redundancy
GpppA	P3121	2.3	108.6 108.6 55.9	4.4	99.4	3.3
Ribavirin triphosphate	P3121	2.6	112.4 112.4 56.5	6.7	92.7	2.0

ESRF	**Experiment title: AGX1-MgCl2**	**Experiment number**: LS 1796
Beamline: ID14-2	**Date of experiment**: from: 04.11.2000 to: 06.11.2000	**Date of report**: 26.02.2001
Shifts: 6	**Local contact(s)**: Stephanie Monaco	*Received at ESRF:*

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Crystal structures of the human UDPGlcNAc (UDPGalNAc) pyrophosphorylases AGX1 and AGX2

Caroline Peneff[1], Véronique Zamboni[1], Florence Fassy[2] and Yves Bourne[1].

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[2] Aventis Pharma-Hoechst Marion Roussel, Infectious diseases group, 102 Route de Noisy, 93235 Romainville Cedex, France

UDPGlcNAc is a ubiquitous and essential metabolite in eukaryotes as well as in prokaryotes. AGX1 and AGX2 are the human enzymes responsible for UDPGlcNAc biosynthesis from UTP and GlcNAc-1P in presence of Mg2+. These enzymes are isoforms of the same gene and differ by the insertion in AGX2 of an additional 17-residue segment near the C-terminus. Substrate specificity studies showed that these two enzymes have both UDPGlcNAc and UDPGalNAc synthesis activity at a ratio of 1:3 for AGX1 and 8:1 for AGX2, suggesting that the 17-residue insert is responsible for a change in the enzyme activity[1].

To better understand the catalytic mechanism of these enzymes as well as the 17 residue-induced substrate specificity change, we have solved the structures of AGX1 and AGX2 complexed with UDPGlcNAc. The structure of AGX1-UDPGlcNAc was solved by Se-Met MAD at beamline BM14 and refined it at 1.9 Å resolution, whilst that of AGX2-UDPGlcNAc was obtained by molecular replacement using the AGX1 model and data collected on beamline ID14-3. (in first part of the year 2000).

These structures reveal an AGX fold composed of 3 domains: a large central domain reminiscent of the Rossmann fold, as found in other nucleotidyltransferases, flanked by two smaller extra domains, the N-terminal domain which defines a new fold and the C-terminal domain which is mostly involved in dimerisation of AGX1. In order to get further insight into the catalytic mechanism and most precisely to determine the role of the divalent cation indispensable for the reaction, we crystallised AGX1 in presence of MgCl2 and collected diffraction data. Unfortunately, no extra electron density was observed in the active site suggesting that the magnesium cation was not present.

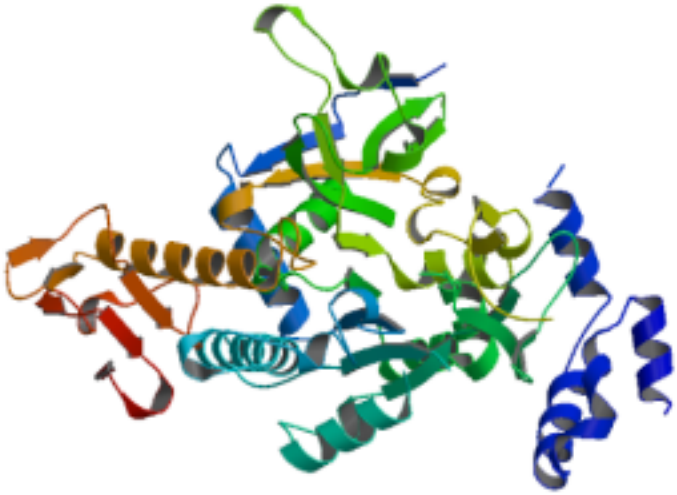

Figure1: Ribbon diagram of the AGX1 structure.

References

1 – A. Wang-Gillam, I. Pastuszak & A. D. Elbein. (1998), *J. Biol Chem* **273** (42), 27055-57.

Publication arisen from this work:

Peneff C., Zamboni V., Fassy F. and Bourne Y., Crystal structures of the human UDPGlcNAc (UDPGalNAc) pyrophosphorylases AGX1 and AGX2, *in preparation*.

ESRF	**Experiment title:** **BAG** Protein Crystallography at AFMB CNRS Marseille	**Experiment number**: LS1796
Beamline: ID14 EH2	**Date of experiment**: from: 4. Nov 2000 to: 6. Nov 2000	**Date of report**: Feb 01
Shifts: 6 shifts	**Local contact(s)**:	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Mirjam Czjzek, CNRS-AFMB Marseille

Bernard Henrissat, CNRS-AFMB Marseille
Julie Allouch, CNRS-AFMB Marseille

Report: High molecular weight cytochrome HMC from *Desulfovibrio vulgaris* Hildenborough

Multiheme cytochromes c are type III cytochromes, present in the periplasm of all sulfate and sulfur reducing anaerobic bacteria. The structures of tetra-heme, octa-heme and nine-heme containing cytochromes c are known, but no representant of the 16 heme containing, high molecular weight cytochrome is known to date. It seems however evident, that this high molecular weight cytochrome is composed of cytochrome c_3-like domains. Molecular replacement is therefore the adequate technic to solve the structure. The precise physiolgocal role of these different multiheme cytochromes have not yet been completely elucidated, the cytochrome HMC is supposed to interact with a transmembrane electron transporter.Recently, we obtained one single crystal of HMC and a native data collection in the laboratory was possible at 3.2 Å resolution. Diffraction spots were visible up to 2.8 Å. The molecular replacement in the resolution range 12Å-4.5Å gave a clear solution for "half" of the protein (correlation coefficient 25.1, R-factor 49.1), and density is clearly visible for the other half. A complete data set was collected on ID14 EH2 at 2.4 Å resolution. The data collection statistics are given below. The construction of the 16 heme containing molecule is under way.

Table 1. Data collection statistics

	HMC
Space group	$P6_2$
unit cell parameters	108.38Å 108.38Å 102.81Å
Resolution (Å)	2.4
No. observations	199 414
No. unique	26626
R_{sym} (%)	6.8 (38)
$I/\sigma(I)$	6.6 (1.9)
Redundancy	7.5
Completeness (%)	99.3 (99.2)

CBM module (family 6) from the xylanase U from *C. thermocellum*

Many glycoside hydrolase enzymes are composed of a catalytic domain coupled with one or several "carbohydrate binding modules" (further called CBM's). These modules, such as the catalytic domains can be grouped in families, having the same structural fold. Within a family the specificity may vary but the members are marked by highly conserved residues, responsible for the binding sites. The CBM from

xylanase U, 133 amino acids long, has been classified in family 6 of the CBM's and no structure is known to date. In order to understand the origin of different specifcities the structural model is a precious information that helps interpretate bichemical and enzymatic results. CBM6 from Xylanase U naturally does not contain methionine residues. Four mutants, each introducing a methionine residue, Y40M, R72M, W92M and Y112M, were therefore produced in conditions leading to selenomethionized proteins. Two of the mutants crystallized under the same conditions as the native protein. The crystals have the form of very thin needles (Figure 1) and diffraction was not detectable on a rotating anode. In order to prepare a MAD experiment on ID14 EH4, two "native" data sets were collected on ID14 EH2 prior to the available beamtime on ID14 EH4.
The data collection statistics are given below.

Table 1. Data collection statistics

	CBM6 R72M	**CBM6 Y112M**
Space group	$P6_522$	$P6_522$
unit cell parameters	60.42Å	59.86Å
	60.42Å	59.86Å
	158.14Å	157.34Å
Resolution (Å)	2.2 Å	3.0 Å
No. observations	41623	7224
No. unique	9183	3443
R_{sym} (%)	9.6 (44.6)	8.1 (14.1)
$I/\sigma(I)$	7.1 (2.2)	4.2 (3.8)
Redundancy	4.5	2.1
Completeness (%)	98.7 (99.0)	93.2 (96.0)

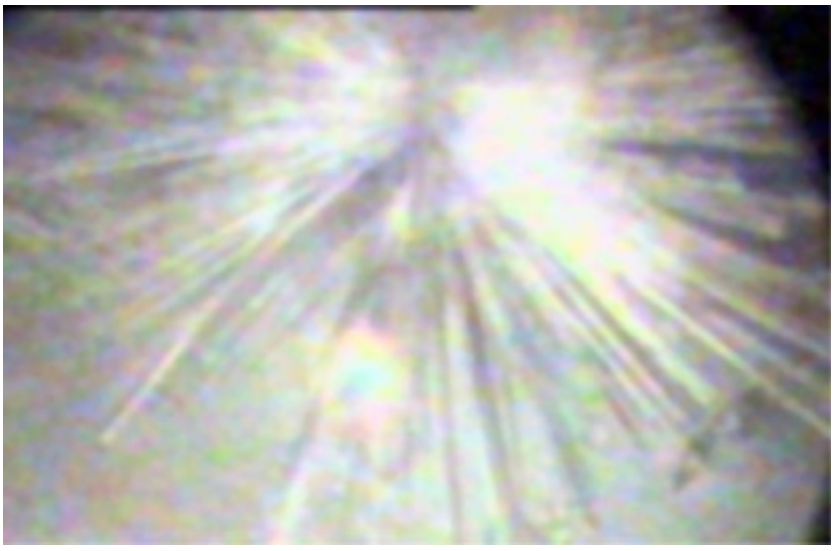

Figure 1: Needle formed crystals of CBM6-R72M. The needles are about 3 μm in cross-section and 1mm long. These crystals diffract to 2.2 Å on ID14 EH2.

ESRF	**Experiment title:** **Native Porcine Odorant Binding protein (pOBP), Complexed with Aminoanthracene**	**Experiment number**: LS1796
Beamline: ID14-2	**Date of experiment**: from: 04.11.01 to: 06.11.01	**Date of report**: Feb 01
Shifts: **6**	**Local contact(s)**: Stephanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Florence Vincent , AFMB laboratory, CNRS Marseille

Report:

Native Porcine Odorant Binding protein (pOBP), Complexed with Aminoanthracene

The porcine OBP belongs to the lipocalin family. The structure of pOBP is known. In this experiment we have complexed pOBP with aminoanthracene.
Crystals of pOBP was frozen to 100 K using 27% glycerol as cryoprotectant.
The space group and cell dimensions were confirmed from a preliminary exposure to be P212121, a=42.6Å b=87.72Å , c=93.05 Å.
One data set has been collected at 100 K, with an exposure time of 30 sec per degree.

Data collection

Number of unique reflections	69515
Overall % data > 1 sigma(I) (last shell)	98.6(98.6)
Overall R merge (%) (last shell)	7.2(28.5)
Overall I/sigma(I) (last shell)	3.0 (2.2)
Resolution (Å)	38.3 - 1.5

The resoltuion of the structure has shown that the aminoanthracene is not in the cavity.

ESRF	**Experiment title:** **CSP/cetyl alcohol/Xenon complex**	**Experiment number**: **Ls1796**
Beamline: ID14-EH2	**Date of experiment**: from: 4 Nov 2000 to: 6 Nov 2000	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Stephanie MONACO	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Valérie CAMPANACCI

Kieron BROWN*

Mariella TEGONI*

Report:

Crystals of MbraCSP complexed with a pheromone-like compound (cetyl alcohol) were submitted to a xenon pressure in a Xcell pressure chamber and then frozen to 100 K with no cryoprotectant.
The space group and cell dimensions obtained were: P422, 71.3x71.3x78.4 Å, $\alpha=\beta=\gamma=90°$.
One data set was collected to 100 K, with an exposure time of 10 sec per degree, see table bellow:

Data collection	
Total number of observation	32507
Number of unique reflections	2257
Overall % data > 1 sigma(I) (last shell)	90.6 (90.6)
Overall R merge (%) (last shell)	20.8 (20.8)
Overall I/sigma(I) (last shell)	2.5 (2.0)
Resolution (Å)	40.0 – 4.20
Redundancy	2.4

ESRF	**Experiment title:** **CSP/bromo-dodecanol/Xenon complex**	**Experiment number**: **Ls1796**
Beamline: ID14-EH2	**Date of experiment**: from: 4 Nov 2000 to: 6 Nov 2000	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Stephanie MONACO	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Valérie CAMPANACCI

Kieron BROWN*

Mariella TEGONI*

Report:

Crystals of MbraCSP complexed with a brominated-pheromone-like compound (bromo-dodecanol) were submitted to a xenon pressure in a Xcell pressure chamber and then frozen to 100 K with no cryoprotectant. The space group and cell dimensions obtained were: C2, 61.5x54.6x33.2 Å, $\alpha=\gamma=90°$, $\beta=116.6°$.
One data set was collected to 100 K, with an exposure time of 10 sec per degree, see table bellow:

Data collection	
Total number of observation	35430
Number of unique reflections	8977
Overall % data > 1 sigma(I) (last shell)	81.3 (81.3)
Overall R merge (%) (last shell)	3.8 (8.5)
Overall I/sigma(I) (last shell)	11.8 (6.9)
Resolution (Å)	40.0 – 1.8
Redundancy	1.6

ESRF	**Experiment title:** **CSP/bromo-dodecanol complex**	**Experiment number**: **Ls1796**
Beamline: ID14-EH2	**Date of experiment**: from: 4 Nov 2000 to: 6 Nov 2000	**Date of report**: Feb 01
Shifts: 6	**Local contact(s)**: Stephanie MONACO	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Kieron BROWN*

Mariella TEGONI*

Report:

Crystals of MbraCSP complexed with a brominated-pheromone-like compound (bromo-dodecanol) were frozen to 100 K with no cryoprotectant.

The space group and cell dimensions obtained were the followings: $P2_1$, 33.4x53.9x56.2 Å, $\alpha=\gamma=90°$, $\beta=86.1°$.

One data set was collected to 100 K, with an exposure time of 10 sec per degree, see table bellow:

Data collection	
Total number of observation	700675
Number of unique reflections	38033
Overall % data > 1 sigma(I) (last shell)	96.4 (96.4)
Overall R merge (%) (last shell)	5.5 (32.0)
Overall I/sigma(I) (last shell)	7.3 (2.1)
Resolution (Å)	40.0 – 1.4
Redundancy	7.4

Comments:

The high resolution obtained with the crystals of CSP could allow us to obtain phases with ab initio method.