FIP	**Rapport d'expérience** **Utilisation du temps de faisceau** **Run 2001**	**BM30A**

Identification de l'expérience date début-fin 02/06/2001 au 04/06/2001

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nom du laboratoire ou nom du responsable du projet D. MORAS

nom des utilisateurs présents

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Temps de faisceau	mode / intensité
temps alloué	48H
temps utilisé	48H

Statistique d'utilisation

MAD	oui [X]	seuil: Se	non [ ]	$\lambda = 0.9793$
cryo	oui [X]		non [ ]	

Rapport d'expérience et commentaires

3 projects.
Project: Estrogen receptor (ER). ER ligand-binding domain (LBD) in complexes with ligands: (16 hours of beam time used)

Steroid hormones regulate the transcription of target genes in the cells by binding to transcription factors which belong to the nuclear receptor superfamily. These receptors display a modular structure composed of six domains. Two human nuclear receptors specific for estrogen are known: estrogen receptor (ER) alpha and ERbeta. They are pharmaceutical targets in several diseases such as breast cancer.
Several crystal structures of ER alpha and beta ligand-binding domain (LBD) have been solved in complex with estradiol or different molecules. Our aim is to determine the structural basis of the specificity of synthetic ligands acting as agonists (activating gene transcription) or as antagonists (repressing transcription) for the ERalpha LBD and for the ERbeta LBD.

Space group P6(5)22, a=b=58.0, c=277.0

Name	**Resolution (Å)**	**Rmerge (%)**	**Complet. (%)**	**Multipl.**	**Time used**	**Current Status**	**Users**
ERalpha LBD RU 78850	2.3	6.3	97.7	11.9	8h	Structure solved	V. Cura
ERalpha LBD RU 80637	2.4	6.8	94.4	8.0	8h	Structure solved	V. Cura

Project: Structures of the catalytic core of HIV integrase in complex with several potential inhibitors. (23 hours of beam time used)

Integration of a DNA copy of the retroviral RNA genome into a host chromosome is a crucial step in viral replication. As such, HIV integrase, is a promising target for the design of antiviral drugs. The determination of its structure in complex with several drugs should be useful to design new inhibitors.

Space group: P3(1)21, a=b=72.7 c=66.0, a=b=90° g=120°
5 full datasets were collected.

ligand	no	FZ55	FZ41	FZ114	I33
Résolution (Å)	50-2.4	50-2.35	50-2.3	50-2.35	50-2.2
Rsym	4.1%	5.9%	5.2%	5.1%	5.8%
Completeness	93%	94%	94.%	94.5%	98.7%
Molecular replacement	CC=76% R=29.3	CC=75% R=29.6	CC=77% R=29.1	CC=76% R=29.2	CC=71% R=31.9

After refinement none of the ligands has been seen in the structures.

Project: ***Streptococcus mutans*** **Antigen I/II : Cterm Domain**

(9 hours of beam time used)

SA I/II proteins (molecular mass between 180 to 210 kDa) can be divided into seven distinct regions: (1) a signal peptide (38 residues), (2) an N-terminal region (~80 residues), (3) an alanine-rich repeat domain (A-region, ~320 residues), (4) a central domain (V-region, ~360 residues), (5) a proline-rich repeat domain (P-region, ~180 residues), (6) a C-terminal domain (~500 residues) and (7) a cell-wall-anchoring motif.

We have cloned, purified and crystallized the C-terminal domain (residues 998-1489) which has been shown to interact with the salivary receptors. Native and Se-Met derivative crystals has been obtained. Those crystals display only low resolution diffraction (5 Å) using a rotating anode.

Data collected on BM30 in June 2001 have shown that two crystal forms have been obtained under the same crystallization conditions and in the same drop. Crystals Form I belong to the space group $P2_1$ (unit cell parameters, a=73.Å, b=162Å, c=120Å, β=94°). Crystals form II belong to space group $P4_x2_y2$ (unit cell parameters: a=b=193Å, c=61Å). Partial native (or cryo-soaked crystals with halides) and SAD (Se-Met) data sets have been collected at 3.0 Å resolution on BM30. Thirty crystals have been used during this experiment. However, data analysis have shown that crystals of both forms present severe defects which can not be detected by eyes before data collection. Crystallization conditions are being improved in order to overcome this problem.

Name	Resolution (Å)	Rmerge (%)	Completeness (%)	Multiplicity	Time used	Current Status
Native 5 crystals	2.7	4.0	95.	4.0	4h	Phases problem to be solved
Se-Met 18 crystals	3.0-3.5	7.0	90.	3.0	4h	Only 1 SAD complete data set. Others data sets not completed
Soaking NaBr, 7 crystals	3.0-3.5				1h	Data sets not completed