FIP	**Rapport d'expérience** **Utilisation du temps de faisceau** **Run 2002/1**	BM30A

Identification de l'expérience date début-fin

3 shifts du 5/07/02-7:00 au 6/07/02-7:00

n° proposition 30-01-514

nom du laboratoire ou nom du responsable du projet **CNRS-Gif sur Yvette BAG**

nom des utilisateurs présents
Herman Van Tilbeurgh, Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), Bat. 34, CNRS, 1, avenue de la Terrasse, 91 198 Gif-Sur-Yvette

Nicolas Leuliot, LURE Orsay

nom du local contact Dr R Kahn

Temps de faisceau mode / intensité

temps alloué 3 shifts

temps utilisé 3

Statistique d'utilisation

MAD	oui [X]	seuil: Se	non [ ]	λ =
cryo	oui [X]		non [ ]	

Rapport d'expérience et commentaires

Using 1 shift allocated on BM30 with the proposal number LS2072 on 04-05/07/02 and the 3 shifts of proposal number 30-01-514 from 5/07/02 to 6/07/02 four data sets were collected for the structural genomics project on yeast target proteins (The systematic names of the genes are used. More details on every orf can be found on http://genomics.eu.org/targets.html)

YDR435c (target 182)

Spacegroup P6122 or P6522 a=112.7 c= 162.7
RESOLUTION = 2.0 A

This orf codes for a N-terminal methyl transferase involved in the regulation of a phosphatase complex. There are no structural models for this protein, so the structure has to be solved by MAD or MIR. Se met data were collected on beamline ID14 one week before and we are in the stage of phasing the protein. During this run, complete data sets on the native protein with and without a potential cosubstrate were collected. The good quality of the native data at 2.0A resolution will allow to refine the structure accurately.

YOR357c (target 190)

Spacegroup: P1 a=31.4 b=55.8 c=64.8 α=110.7 β=97.3 γ=99.4
Resolution: 2.3A
Completion: 97%
Rfac: 0.047

This orf codes for a PX domain that binds phosphatidylinositol-phosphates. These modules are involved in membrane trafficking through binding to phospholipids. PX domains are usually coupled to other functional domains, such as SH2 modules or catalytic domains. YOR357c only contains the PX domain and som extra peptide (30 aa). We have recently determined the structure of the YOR357c protein at 1.6A resolution. We observed that the active site was blocked due to crystal packing. Considerable effort was put into the cocrystallization of this PX domain with a specific ligand. New crystallization conditions were obtained in the presence of PtDIns-3P. These small crystals were tested during this run and showed very good diffraction, considering their size. A complete data set was colleted and the structure was solved using molecular replacement. Partial refinement revealed the presence of the ligand into the active site and the structure has now successfully been refined. This is the first case where liganded and non liganded structures are available for a PX domain.

COLLICIN D from E. coli

spacegroup P41212 a=62.8 c=148.7
resolution = 2.0 A
completion = 99.8%
R-fact_total = 0.06

Collicins are toxins that are used by bacteria to protect themselves against other microorganisms. They are secreted in the culture medium as a complex with the immunity protein, which protects the producing organism against toxic effects. Colicin D has a tRNA hydrolysing toxic activity. It was shown that ColD is proteolytically cleaved during entrance in the target cell. ColD has no sequence identity with collicins of known structure. We obtained crystals of the complex of the E. Coli ColD and its corresponding immunity protein. Native data have been collected at 2.6A and improvement of the crystals has now yielded data at 2.0A resolution (collected on the French CRG BM30). Biochemical analysis of the crystals revealed that they contain a proteolytic fragment of ColD that is generated during the crystallization process. Mass spectrometric analysis has been carried out to determine the exact sequence of the fragment. Se-Met production of these fragments is on the way to solve the structure of the fragment.

LicT-SeMet labelled crystals

Spacegroup P3221 a=48 c=166

Resolution 2.8A

	Remote	inflection	peak
Completion%	85	92	92
Rfac	6.1	4.3	6.1

LicT is a transcriptional antiterminator present in Bacillus subtilis and responsible for the regulation of transcription of some operons involved in carbohydrate metabolism. LicT becomes activated through the phosphorylation of well conserved histidines. Phosphorylation of these histidines causes dramatic conformational changes in the protein with a profound effect on RNA binding and antitermination activity. We have determined the structure of a constitutive active mutant of the regulatory domain, which gives structural explanations for the activation process. To complete the picture we also need the structure of the inactive protein however. Crystals were obtained of the native construct and 2.3A data did not allow to determine the structure by molecular replacement using the structure of the mutant. Therefore, the Se-met substituted protein was crystallized and a three wavelength data set collected. Although the anomalous signal is weak, two Se positions out of five could be determined with certainty and we try to use these to find the other positions and to phase the protein.