Beamline:BM30

## Project: PilW from Neisseria meningitidis

We had previously collected a 2.8A data set of native crystal, but it was not possible to solve the structure by molecular replacement. In order to identify a heavy metal that bounds to the protein, we analyzed on BM30 beamline the fluorescence of crystal soak with different heavy metal and wash in the free-metal cryo solution. We test 6 different heavy metals complexes (Hg, Pt, Ho, Sm,Os and Gd)

1) Crystal soaked O.N. in 1mM PhenylHgacetate: no fluorescence spectra, diffraction 6A
2) Crystal soaked O.N. in 1mM CNPt: no fluorescence spectra, diffraction 6A
3) Crystal soaked O.N. in 1mM $HoCl_3$: no fluorescence spectra, diffraction 6A
4) Crystal soaked O.N. in 1mM SmCl: no fluorescence spectra, diffraction 6A
5) Crystal soaked O.N. in 1mM $Cl_3OsS$: no fluorescence spectra, diffraction 6A

We collected at □=1.000A (pick of Hg)

NB: the crystal soaked in Os solution was black, but we don't see any fluorescence signal.

6) Crystal soaked 20min in 100mM GdHPDO3A and not washed: collected 120°, no fluorescence spectra, diffraction 6A

We collected at □=1.711A (usually used for Gd experiment)

NB: also in this case no fluorescence was measured, even if the 100mM of Gd was present in the loop because the crystal wasn't washed before collection. The same results were obtained using a loop containing only the 100mM Gd solution.

Data set with Gd-soak crystal was treated with XDS, the presence of an anomalous signal was not evident. We subsequently analyzed with Shelx, the Patterson confirmed the absence of Gd in the crystal.

We don't find an explication of the totally absence of fluorescence, in particular with the Os (black crystal) and the solution 100mM Gd.

## Project: human Lg CRD

We had previously collected a 2.3Å data set of native crystal, but it was not possible to solve the structure by molecular replacement.

The protein has Ca sites, so we tried to replace Ca by Gd (with $GdCl_3$ soaking) in the way to make heavy metal phasing.

In order to identify Gd that may bind in the Ca site of the protein, we analyzed the fluorescence of soaked crystals washed in free-metal cryo-solution. Analyze of fluorescence spectra reveal the occurrence of Gd in crystal.

We test 8 different crystals to find best diffraction profile (spots shape, mosaïcity and resolution).

We collected with the best crystal at λ=1.711Å. A set of 360 images (180 °) was recording and data where treated with XDS. We don't find anomalous signal, Gd seems not be fixed on protein.

## Turkey-egg white lysozyme and glucose isomerase

Diffraction data were collected to test the fixation of a europium complex on two proteins :turkey-egg white lysozyme and glucose isomerase from *Spectromyces rubiginosus*. SAD experiments at the Eu $L_{III}$ absorption edge were attempted. Anomalous signal, allowing the resolution of the structure, was found with the lysozyme crystals.

## Users : Eva Pebay-Peyroula, Hugues Nury, Aurélien Deniaud.

Samples tested and results :

- ANC : We tested two crystals which diffracted at low resolution (10-15A), not sufficient to analyse anything.

- AcrB : We tested eight crystals with the better which diffrated up to 4A. We just collected data on 20° to analyse th espace group.

- CR : We tested ten crystals, which didn't diffract at all.

- YedZ-GFP : We tested two small crystals, which didn't diffract.

## Thiol-oxydoreductase chez Neisseria meningitidis

DsbA3 : Diffraction data were collected on two native crystals. These crystals diffracted until 3.0 Å resolution. They belong to the hexagonal system 6/m (a=b=126.6 Å c=53.9 Å). We did not manage to determine the precise space group yet.

DsbA1 : a full dataset was collected on one single native crystal until 1.5 Å resolution Rsym=6%
These crystals belong to the orthorhombic system, space groupe P212121, with the following parameters :
a=44.05 Å b=46.40Å c=79.90Å