ESRF	**Experiment title:** BAG Barcelona- Hydroperoxidase I (HPI)-Native	**Experiment number**: ls1805
Beamline: ID14-4	**Date of experiment**: from: 10/02/01 to: 11/02/01	**Date of report**: 1-Aug-01
Shifts: 3	**Local contact(s)**: Germaine Sainz	*Received at ESRF:*

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Report:

The bacterial heme-containing catalase peroxidase HPI encoded by the katG gene from *Mycobacterium tuberculosis* is responsible for the sensibility to INH (isonicotinic acid hydrazide), one of the principal antituberculosis drugs. The INH susceptibility of *M.tuberculosis* results from the conversion of the drug into bioactive compounds which interfere with a number of processes involved, in particular, in the mycolic acid synthesis. Clinical mutations which alter catalase activity result in high levels of resistance to INH.
The HPI subunit consists of about eight hundreds aminoacids organised in two domains that each should bear resemblance to plant peroxidases. Crystals from the C terminal and from the intact homologous HPI protein from *B.stearothermofilus* have been obtained

Protein crystals

Native crystals of an average size of 0.4x0.2x0.05 (mm) were obtained.

Figure 1) Native crystals of HPI

Data collection and processing

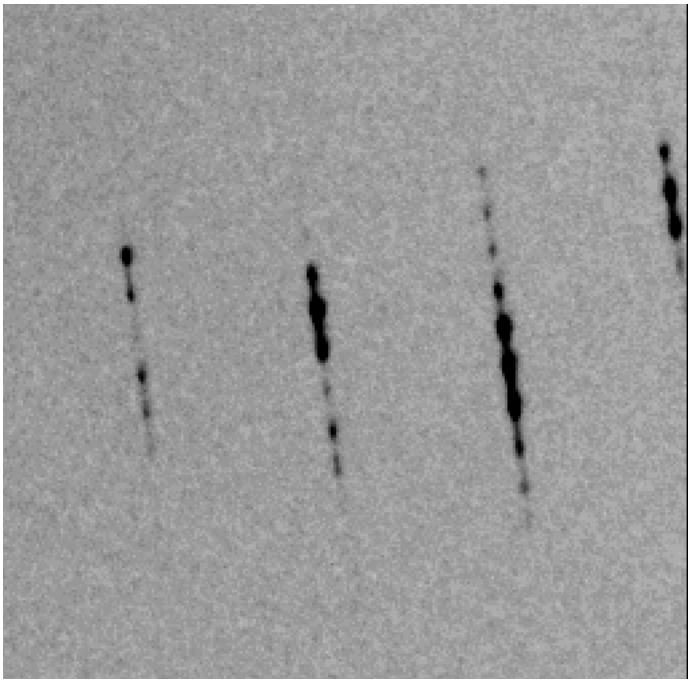
λ= 0.9739 Å
ΔΦ= 0.5º
d= 180 mm
R_{max}= 1.9 Å

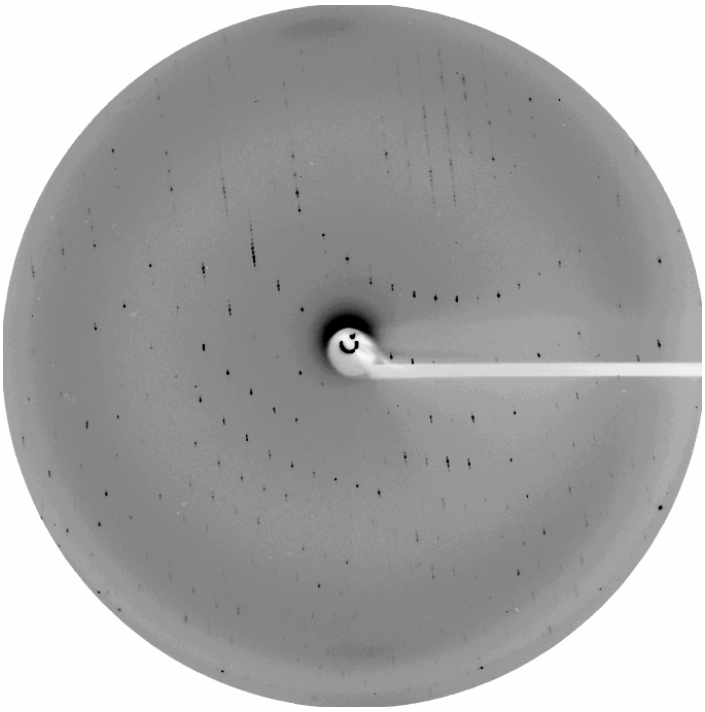
In this case, and accordingly to what we had previously tested, the determined c cell parameter was twice as big as the one obtained in the last data collection (SeMet crystal, see exp_report_HPI-2.doc).

Data was processed using the DENZO package yielding the following cell parameters:
P222 **a**: 84.21 **b**: 98.70 **c**: 302.82 90 90 90

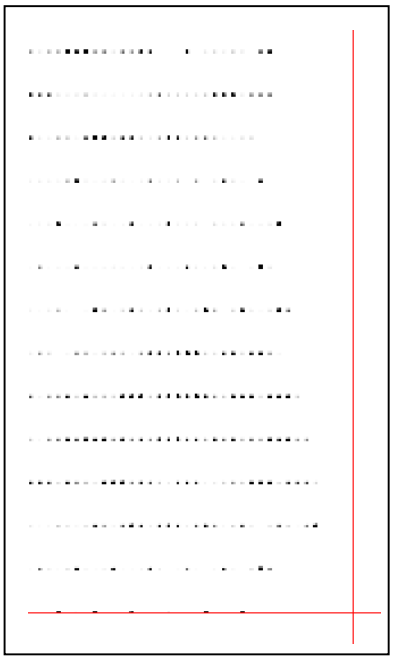
Data scaling was performed with SCALEPACK, obtaining a final file with 135569 reflections, a 91.6 % of overall completeness, and an Rfac of 9%.

It needs to be mentioned that this data collection requires special attention due to a high crystal mosaicity (around 0.8 when $\Delta\Phi = 0.5^{o}$) and its extremely long c axis, what makes data processing a tough task. Many of the discarded crystals gave a diffraction pattern which couldn't be processed by any means. Quite often the c axis gave overlaped reflections, what was not this situation.





What still puzzles us is the apparent extinctions that appear after looking the stucture factor file with HKLVIEW.



Such strange behaviour wll be discussed later when analysing its native patterson function.

Structure Factor Check
HPI_2D Feb01

Title:/alcor/home/disk2/xcveri/Resum_dsets/Feb_01/hpi_a+t.mtz
Date: Grenoble Feb01
PDB code: HPI_2D

Crystal

Cell parameters:

a:	84.21 Å	b:	98.70 Å	c:	302.82 Å
α:	90.00°	β:	90.00°	γ:	90.00°

Space group: P 2 2 2

Structure Factors

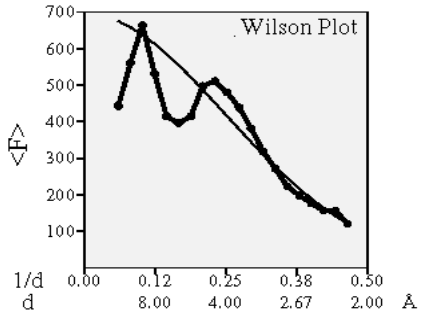
Input

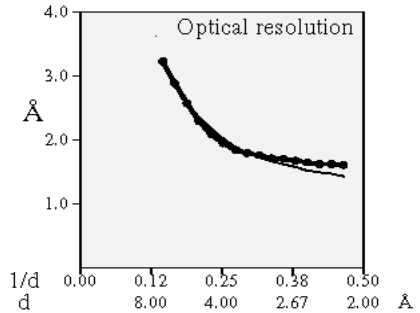
Nominal resolution range:		19.96 – 2.10 Å
Reflections in file:		135569
Unique reflections	above 0:	135569
	above 1 σ:	133057
	above 3 σ:	92413

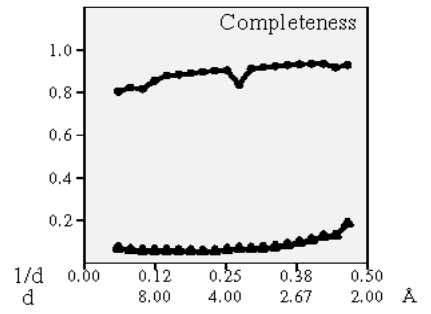
SFCHECK

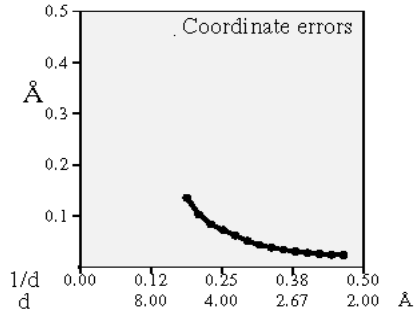
Nominal resolution range: (max. from input data, min. from author)	19.96 – 2.10 Å
Used reflections:	132602
Completeness:	91.6 %
R_stand(F) = <σ(F)>/<F> :	0.087
Anisotropic distribution of Structure Factors ratio of eigen values:	0.7363 0.6258 1.0000
B_overall (by Patterson):	28.8 Å²
Optical resolution:	1.61 Å
Expected opt. resol. for complete data set:	1.61 Å
Estimated minimal error:	0.023 Å

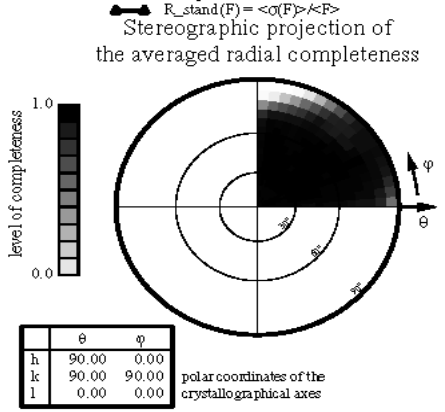
Structure Factor Check
HPI_2D Feb01

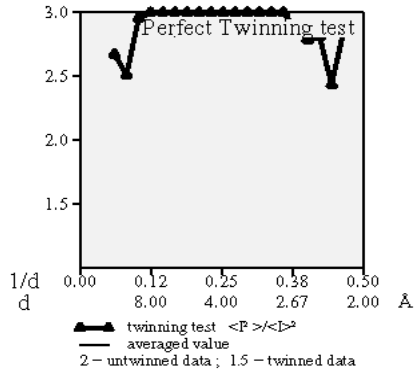






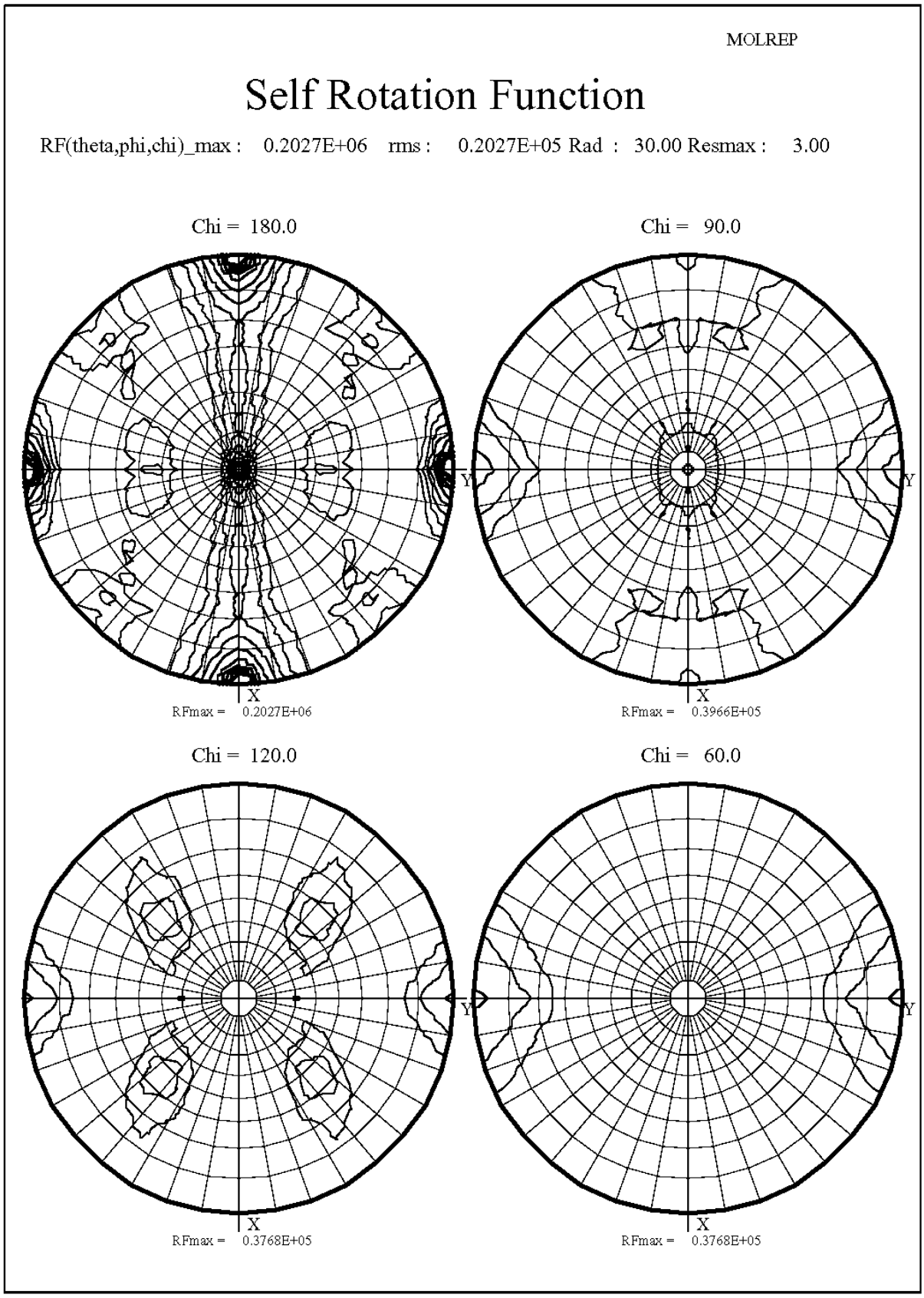






Molecular arrangement

Its selfrotation function (analysed with MOLREP) again, did not show any non crystallographic rotation axis.



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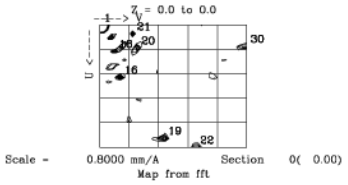
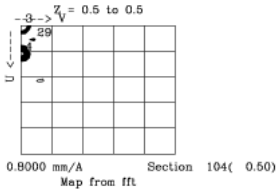
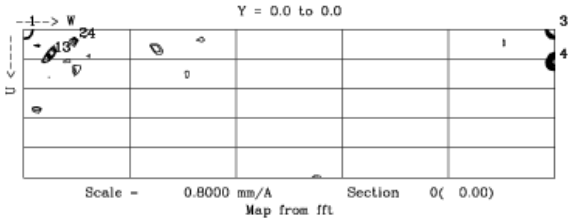
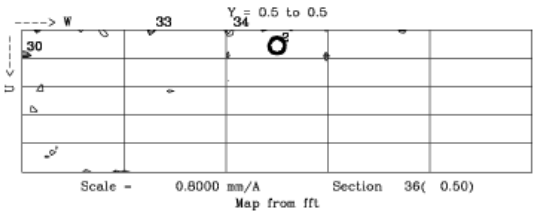
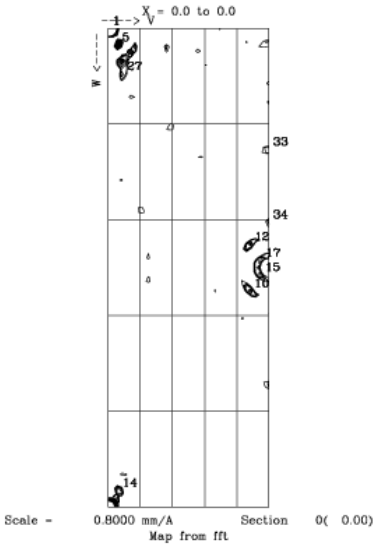
The number of molecules per assymetric unit may be either 3 or 4, what would give a 70 - 80% of solvent content and a matthews coefficient of around 5. There are three major peaks in the Harker sections of the native patterson map, a part from the origin peak.

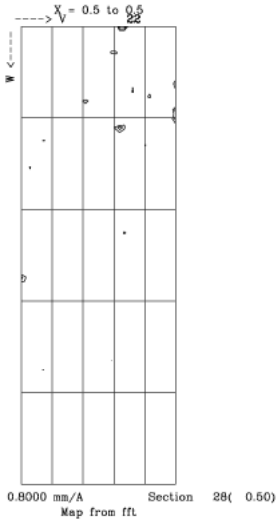












The fractional positions of those peaks are the following:

Peak	Fractional position	Relative Height
1.	(0.000 0.000 0.000)	2400
2.	(0.055 0.500 0.250)	100
3.	(0.000 0.000 0.500)	88
4.	(0.109 0.000 0.500)	44

It may seem that two of the three peaks would correspond to either a pseudorigin or to a non crystallographic binary axis which would be parallel to the crystallographic ones. The third one would be a consequence of the other two. The difference of height ot those three peaks could be explained thanks to the effect of the ripples seen in the map.