ESRF	**Experiment title:** BAG Barcelona	**Experiment number**: LS-1805
Beamline: ID14-4	**Date of experiment**: from: 4/12/00 to: 5/12/00	**Date of report**: 31-Aug-01
Shifts: 3	**Local contact(s)**: Sean McSweeney / Raymond Ravelli	*Received at ESRF:*

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Report:

PhoB is the response regulator of the two-component signal transduction system. It consists of two domains: an N-terminal phosphate receiver domain and a C-terminal DNA-binding and transactivation domain. Upon phosphorylation, PhoB binds to its specific DNA sequence called pho box and interacts with σ^{70} to activate transcription of the Pho regulon genes.

We have solved the structure of the C-terminal domain of PhoB domain at 2.0 Å by molecular replacement with AMoRe (Navaza, 1994). It was used as a searching model the minimized and averaged NMR structure of this domain (Okamura *et al.*, 2000).. Molecular replacement calculation was difficult because a low signal to noise ratio. The true solution was confirmed by analyzing the correct crystal packing and the quality of the density maps generated with CNS (Brünger,1991). Refinement was performed with the last program. Firstly, An initial working R factor value of 55% fall to 39% applying a simulated annealing step. Structure refinement was performed with cycles of minimization and temperature factors

attribution. Refinement cycles were combined with model building rounds performed with Turbo-Frodo (Rousel & Cambilleau, 1989).

The C-terminal domain of PhoB is constituted by an aminoterminal four stranded β-sheet followed by a three α-helix bundle and a caboxiterminal β-hairpin. This hairpin interacts with a short β-strand located beetwen α1and α2 forming a three stranded antiparallel β-sheet. There is also a 3_{10}-helix connecting α3 and the β-hairpin. The topology of the domain is β1-β2-β3-β4-α1-β5-α2-α3-3_{10}-β6- β7.

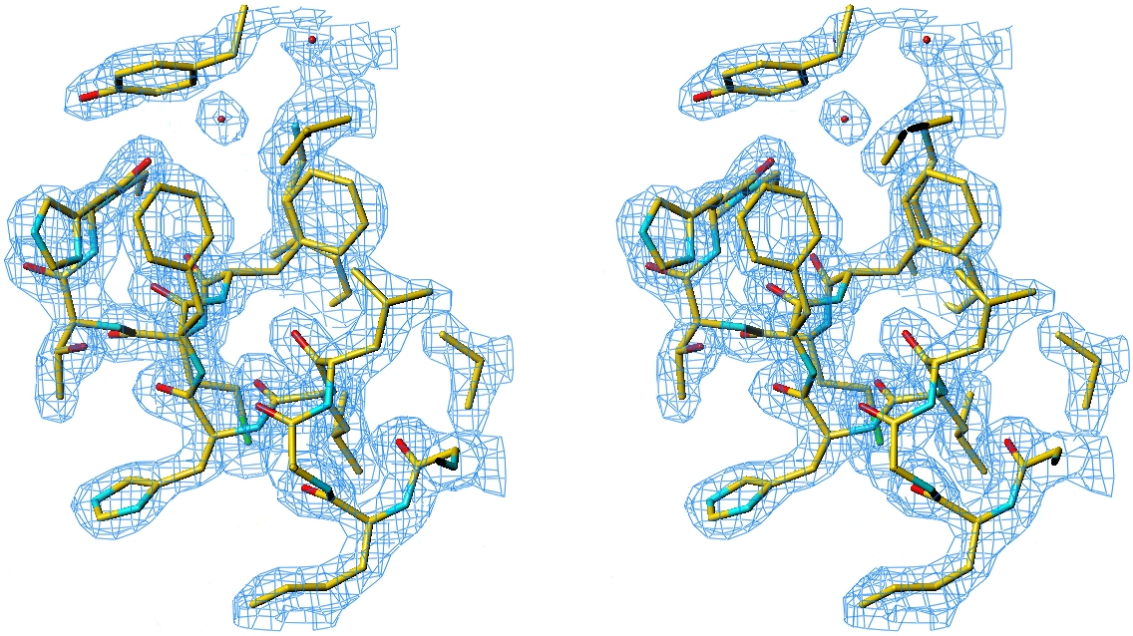

Figure 1.: Electron density map contoured at 1.5 σ of the C-terminal domain of PhoB.

ESRF	**Experiment title:** BAG Barcelona- Hydroperoxidase I (HPI)-Semet	**Experiment number**: LS1805
Beamline: ID14-4	**Date of experiment**: from: 4/12/00 to: 5/12/00	**Date of report**: 1-Aug-01
Shifts: 3	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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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

A SeMet derivative was prepared after re-transforming into the met$^-$*E. coli* B834 (DE3) strain. Crystals were obtained with similar conditions to native crystals, but they diferred in its size, since SeMet crystals were smaller than native ones (0.1x0.1x0.02) .

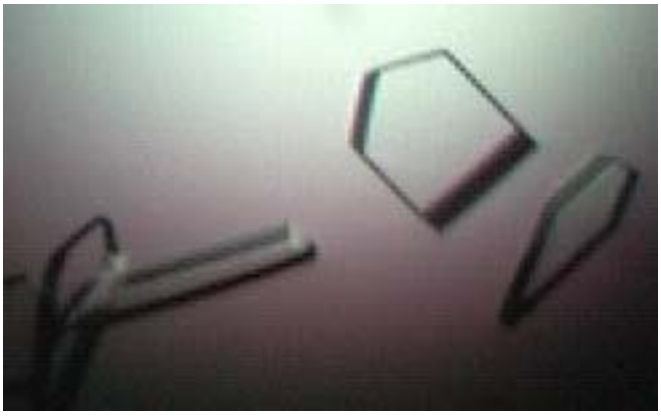


Figure 1) SeMet crystals of HPI

Data collection and processing

λ= 0.9793 Å
ΔΦ= 1º
d= 260 mm
R_{max}= 2.8 Å

A complete dataset was collected at the wavelength that yield the highest anomalous signal after a fluorescence scan of the K Se edge. Due to crystal decay, a second dataset could not be collected.

Data was processed using the DENZO package yielding the following cell parameters:
C2221 **a**: 101.61 **b**: 151.90 **c**: 84.30 90 90 90

Data scaling was performed with SCALEPACK, obtaining a final file with 14816 reflections, a 99.2 % of overall completeness, and an Rfac of 5%

Crystal packing

In order to understand the crystal packing, we analysed its selfrotation function with SFCHECK. No other axis except the crystallographic binary axis seemed to appear.

There might be 1 molecule per asymmetric unit, with a solvent content of 75 % and a matthews coefficient of 4.86

A native patterson analysis was performed and only the peaks corresponding to the cell symmetry could be observed.

Structure Factor Check
Semet_pic

Title: MPI_3D crosslinkDec00-green
Data Processed/final Feb01
PDB code: Semet_pic

Crystal

Cell parameters:

a: 101.61 Å b: 151.90 Å c: 84.30 Å
α: 90.00° β: 90.00° γ: 90.00°

Space group: C 2 2 21

Structure Factors

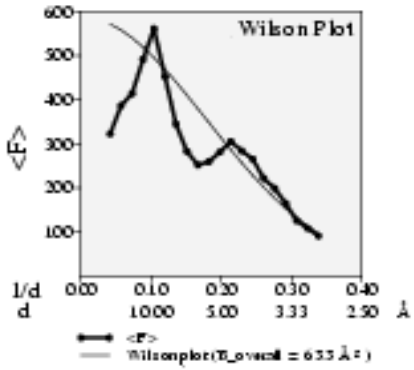
Input:

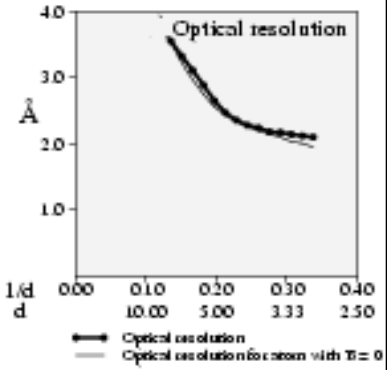
Nominal resolution range:		20.83 – 2.89 Å
Reflections in file:		148116
Unique reflections:	above 0:	148116
	above 1σ:	145351
	above 3σ:	110933

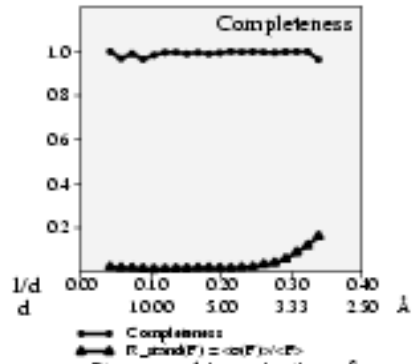
STCHECK:

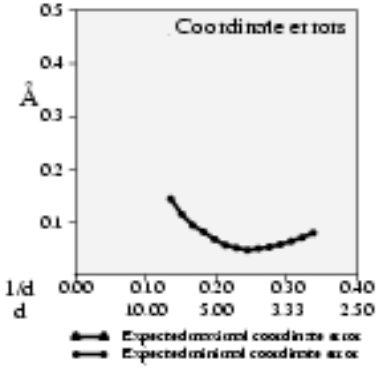
Nominal resolution range: (max from input data, min from anisotropy)	20.83 – 2.89 Å
Used reflections:	148116
Completeness:	99.2 %
R_stand(F) = <σ(F)>/<F> :	0.043
Anisotropic distribution of Structure Factors: ratio of eigenvalues:	1.0000 0.6688 0.7349
B_overall (by Patterson):	44.8 Å²
Optical resolution:	2.10 Å
Expected optimal resol. for complete dataset:	2.10 Å
Estimated minimal error:	0.080 Å

Structure Factor Check
Semet_pic

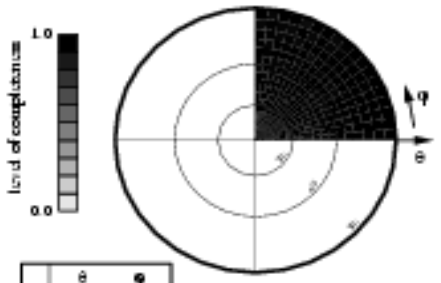






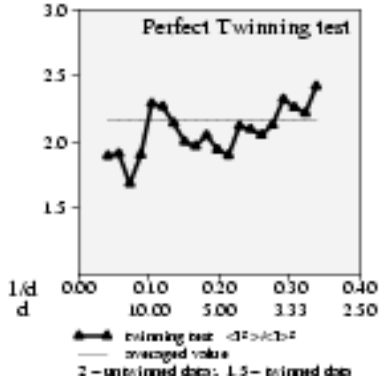


Stereographic projection of
the averaged radial completeness



	θ	φ
h	90.00	0.00
k	90.00	90.00
l	0.00	0.00

pole coordinates of the crystallographical axes

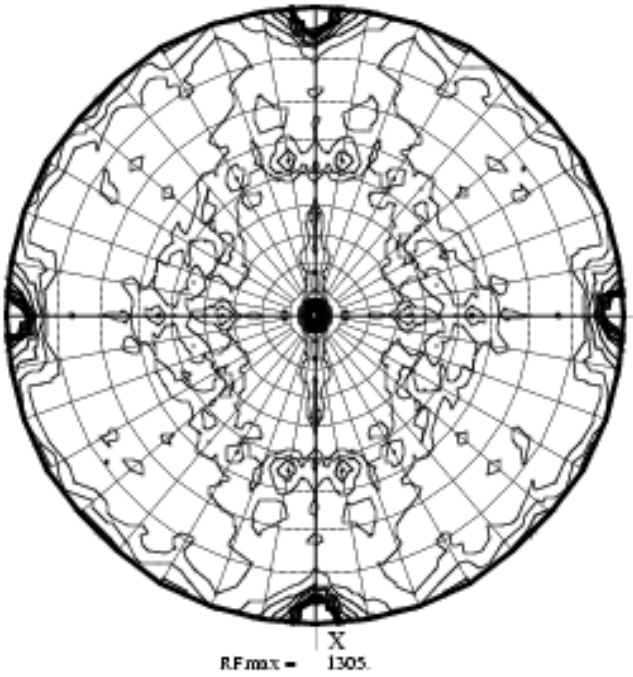


MOLREP

Self Rotation Function

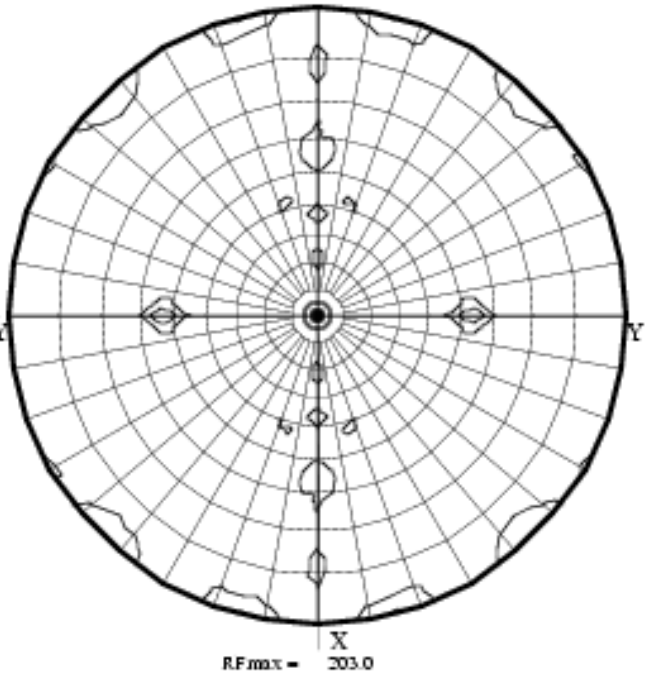
RF(theta,phi,chi)_max : 1305. rms : 86.36 Rad : 30.00 Resmax : 3.00

Chi = 180.0



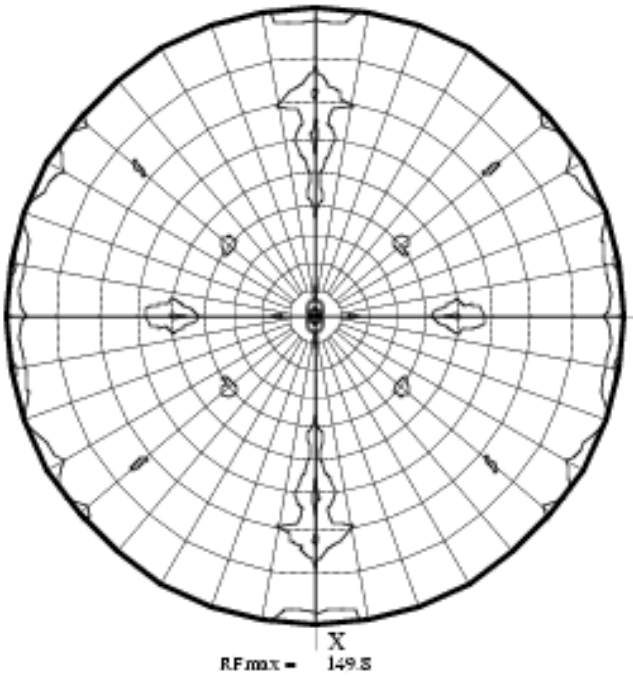
RFmax = 1305.

Chi = 90.0



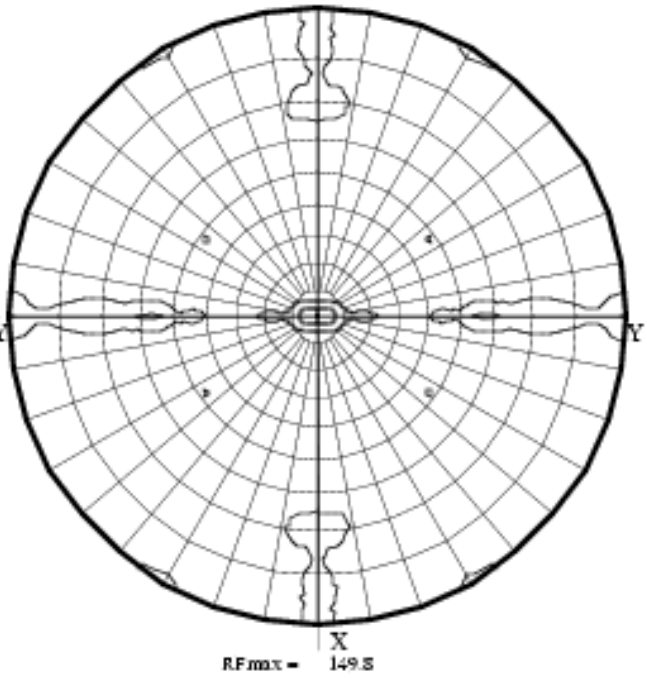
RFmax = 203.0

Chi = 120.0



RFmax = 149.8

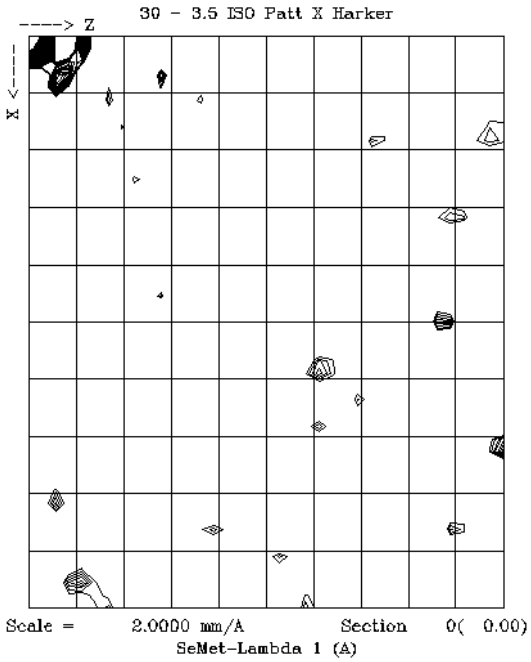
Chi = 60.0



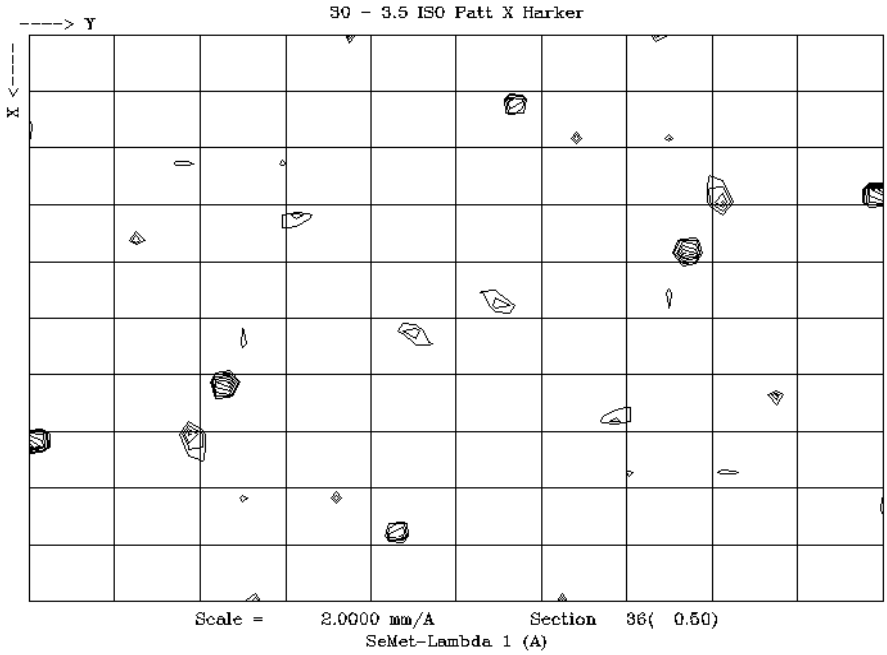
RFmax = 149.8

Phasing

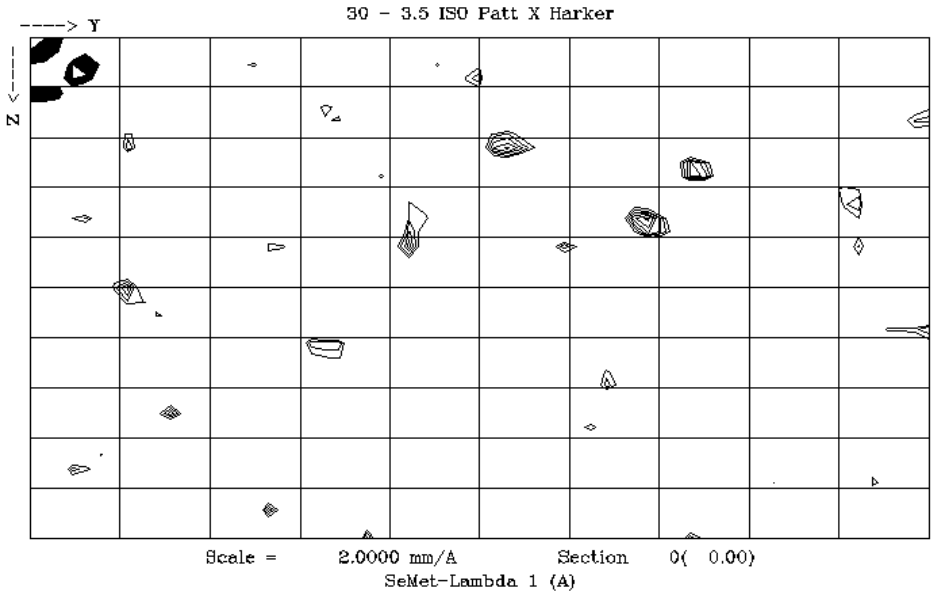
The lack of another wavelength dataset prevented us from doing a complete MAD experiment, hence we tried to obtain phases by the SAD method. However, the peaks obtained in the X= 0, Y= 0 and Z= 0.5 harker sections of a patterson calculated using anomalous signal didn't allow us to localize the expected 5 seleniums of the asymmetric unit. SOLVE attempts didn't take us any further.



Z= 0 Harker section



Y= 0.5 Harker section



X= 0 Harker section