ESRF	**Experiment title:** BAG Barcelona - Human Rhinovirus 2-LDL-Receptor complex (1)	**Experiment number:** LS1805
Beamline: ID14-3	**Date of experiment:** from: 23-Sep-00 to: 25-Sep-00	**Date of report:** 28-Aug-01
Shifts: 6	**Local contact(s):** Hassan Belrhali	*Received at ESRF:*

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Report:

The attachment of a virus to specific cell surface receptors is a key event in the life cycle of animal viruses. It determines the host range and tropism of infection, and initiates delivery of the genome into the cell.
Human rhinovirus serotype 2 (HRV2) belongs to the minor group of HRVs that bind to members of the LDL-receptor family including the very low density lipoprotein (VLDL)-receptor (VLDL-R). We have been crystallised and preliminary analysed the structure of the complex between HRV2 and the first three ligand binding repeats of the VLDL-Receptor
Crystals of about 0.1x0.05x0.05 mm in size diffracted to 3 Å resolution, but were stable on the X-ray beam for only 1-2 exposures. The unit cell, characterized using 0.3º rotation diffraction images analyzed with the MOSFLM package was consistent with a orthorrombic $P2_12_12$ space group with parameters: a= 313. b= 348., c= 381 Å. From the unit cell dimensions , half virus particle was expected to be found in the symmetric unit (30-fold non crystallographic symmetry).
A partial data set was collected at from 116 crystals, mounted on sealed capillaries. Data evaluation statistics is shown in table I.
The structure was solved by molecular replacement using the structure of native HRV2 (1) as a model.
Examination of the electron density maps showed some extra density, located around the five-fold axes, which was consistent with the presence of the receptor in the crystals. However more data will be necesary in order to refine the structure
The structure of this complex will provide an explanation about the basis of receptor specificity in minor group rhinoviruses

Table I
Data collection statistics from the HRV2-VLDLR crystals

• Resolution	3 Å
• Number of crystals	116
• Number of images	120
• Number of reflections	236576
• Number of independent reflections	194574
• R-merge %	19
• Completeness %	31.4

References

1. N.Verdaguer, D.Blaas and I.Fita
"Structure of Human rhinovirus serotype 2 (HRV2)"
J.Mol.Biol. **300** (2000), 1181-1196.

ESRF	**Experiment title:** BAG Barcelona- Hydroperoxidase I (HPI)	**Experiment number**: ls1805
Beamline: ID14-3	**Date of experiment**: from: 23/09/00 to: 25/09/00	**Date of report**: 1-Aug-01
Shifts: 6	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

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Report:

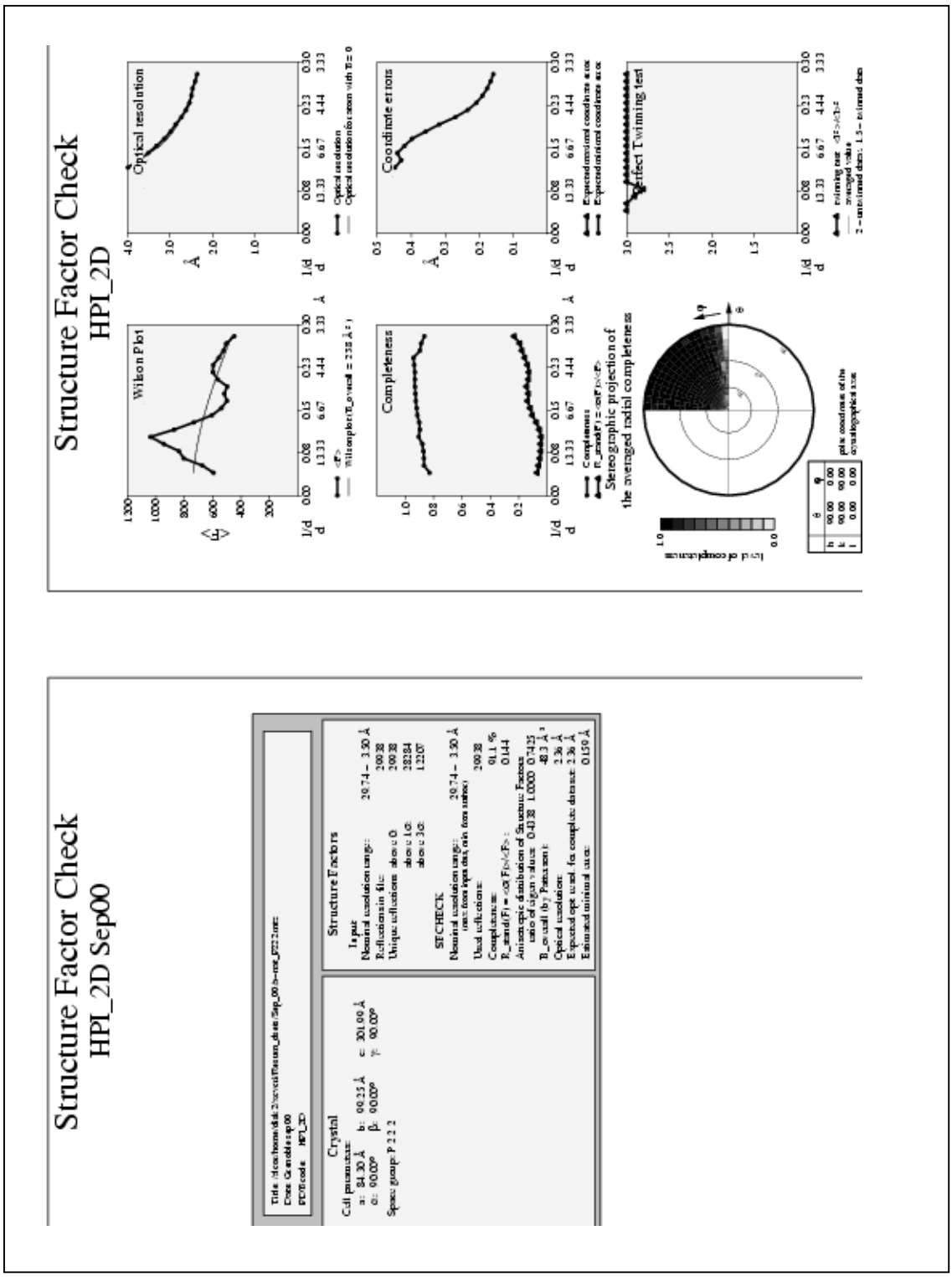
The bacterial heme-containing catalase peroxidase HPI encoded by the katG gene from *Mycobacterium tuberculosis* is responsible for the sensibility to INH (isonicotinic acid hydrazide), one of the principal antituberculosis drugs. The INH susceptibility of *M.tuberculosis* results from the conversion 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 hundred 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

Data collection of native crystals

λ= 0.9310Å
$\Delta\Phi$= 1.0º
d= 220 mm
R_{max}= 3.5 Å

Data was processed using the DENZO package yielding the following cell parameters:
P222 **a**: 84.30 **b**: 99.25 **c**: 301.99 90 90 90

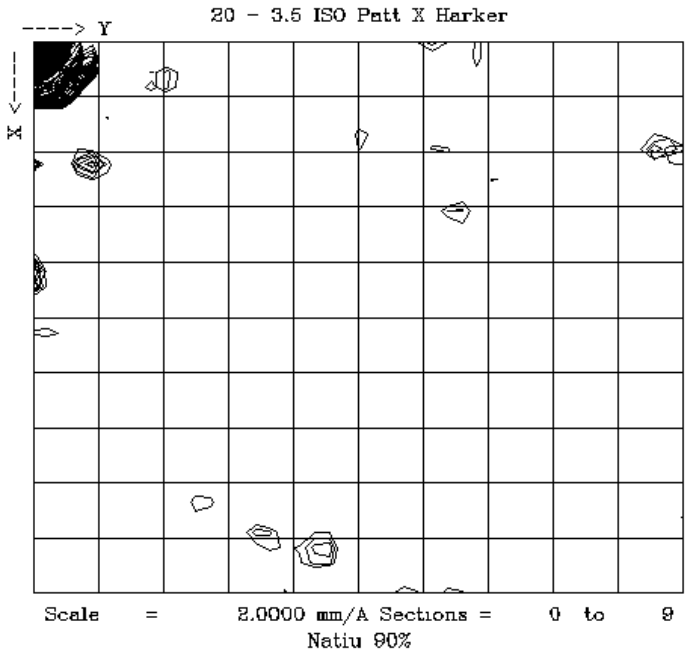
Data scaling was performed with SCALEPACK, obtaining a final file with 29938 reflections, a 91.1 % of overall completeness and an Rfac of 14.4%

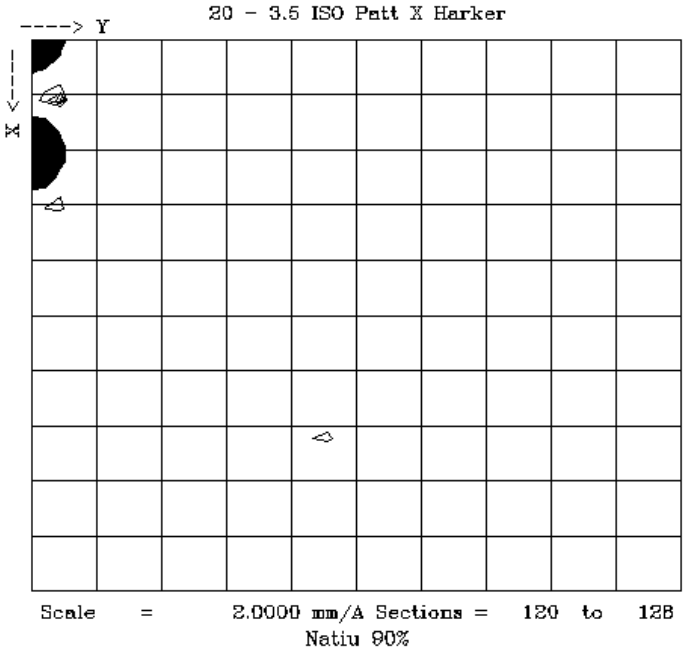


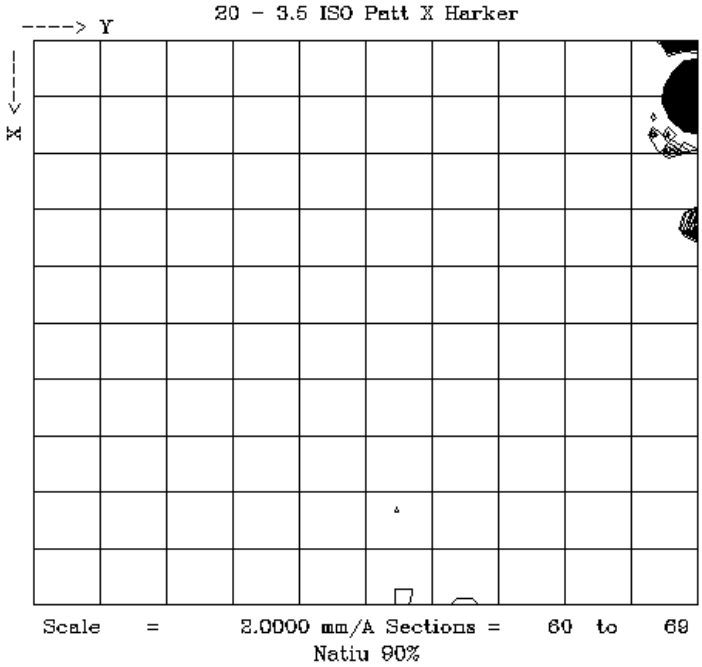
Molecular replacement

Due to its high homology to yeast ccp, we tried to solve our structure by means of molecular replacement using AMoRe program. Unfortunatedly we weren't unable to find any clear solution. We expect to have pseudosymmetries which may help us to understand its strange crystal arrangement derived from its native patterson map (sections Z= 0, Z=0.25 and Z=0.5).

ATOM	1	OW	WAT	X	1	0.000	0.000	0.000	160.22
ATOM	866	OW	WAT	X	2	4.379	49.625	75.520	67.92
ATOM	1379	OW	WAT	X	3	8.658	0.000	150.995	52.43
ATOM	1378	OW	WAT	X	4	0.000	0.000	150.995	44.06
ATOM	49	OW	WAT	X	5	2.495	3.458	2.321	7.41







ESRF	**Experiment title:** C2 domain of PKCα in complex with Ca^{2+} and anionic phospholipids	**Experiment number**: LS1805
Beamline: ID14.3	**Date of experiment**: from: 23/09/00 to: 25/09/00	**Date of report**: 29/08/01
Shifts: 6	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

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Report:

The structure of the ternary complex of PKCα-C2 domain with Ca^{2+} and 1,2-dicaproyl-*sn*-phosphatidyl-L-serine (DCPS) determined recently (Verdaguer et al., 1999) showed how one of the the Ca^{2+} ions is simultaneously coordinated by the top loops of the C2 domain and by the DCPS head group. In addition, DCPS is bounded by positively charged residues that surround the Ca^{2+} binding site in the C2 domain and part of the fatty acyl chains present hydrophobic interactions with residues from the CBR3 but remain partially exposed to the solvent. Now, we have determined the structure of this C2 domain crystallised in presence of Ca^{2+} and the short chain phospholipids 1,2-diacetyl-*sn*-glycerophosphatidyl-L-serine (DAPS) and 1,2-dicaproyl-*sn*-phosphatidic acid (DCPA).Choosing the head groups PS and PA, we would like to structurally analyse the preference of the PKCα-C2 domain for PS over PA. In addition, the choice of the shorter hydrocarbon chain for DAPS was in order to better accommodate the phospholipid ligand in the Ca^{2+} binding pocked.

Diffraction data to resolution of 1.9 Å for the DAPS complex and to 2.0 Å for the DCPA crystals were measured at 100K. The coordinates of the isolated PKCα-C2 domain were used as a model to determine the structure of the two complexes by

molecular replacement. The final model for the PKCα-C2_Ca^{2+}_DAPS complex includes 86 solvent molecules, three Ca^{2+} and one phosphate ions and two DAPS molecules . One DAPS molecule was located in the Ca^{2+} binding site in close contact with one of the calcium ions (Figure 1) ion in an equivalent positions to that found previously in the DCPS complex (Verdaguer et al., 1999). The second DAPS molecule was located at the conserved positively charged cluster which involves the lysine residues 197,199,211 and 213 from strands β3 and β4 suggesting a new site for phospholipid binding (Figure 2).

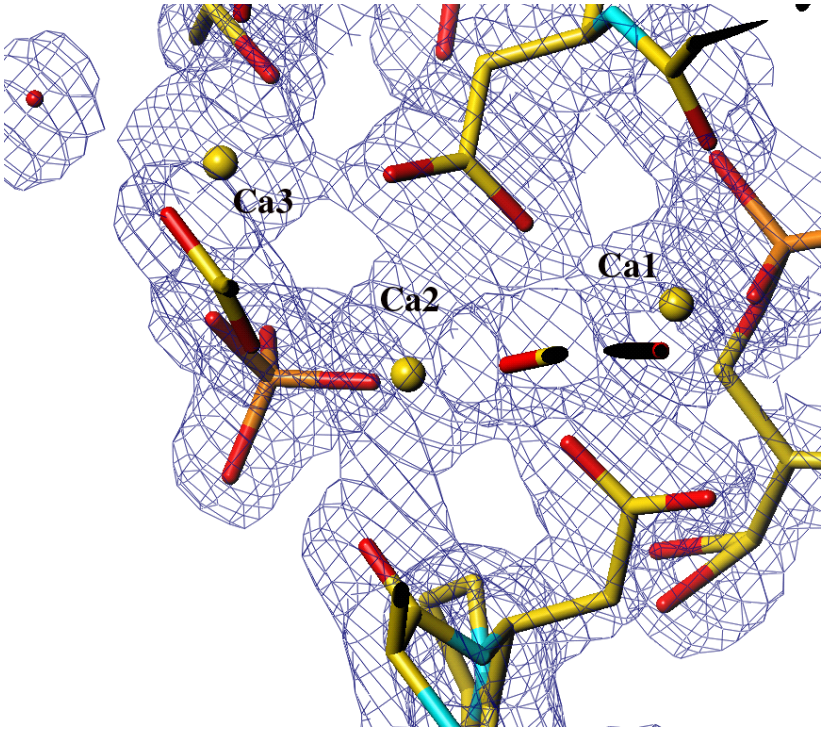

Fig1. View of the 2Fo-Fc map of the Ca2+ binding site of the PKCα-C2_Ca^{2+}_DAPS ternary complex.

The PKCα-C2_Ca^{2+}_DCPA ternary complex includes 77 solvent molecules, three Ca^{2+} and two DCPA molecules . As in DAPS complex, the two DCPA molecules were located in the Ca^{2+} binding site and in contact with the lysine cluster respectively (Fig2)

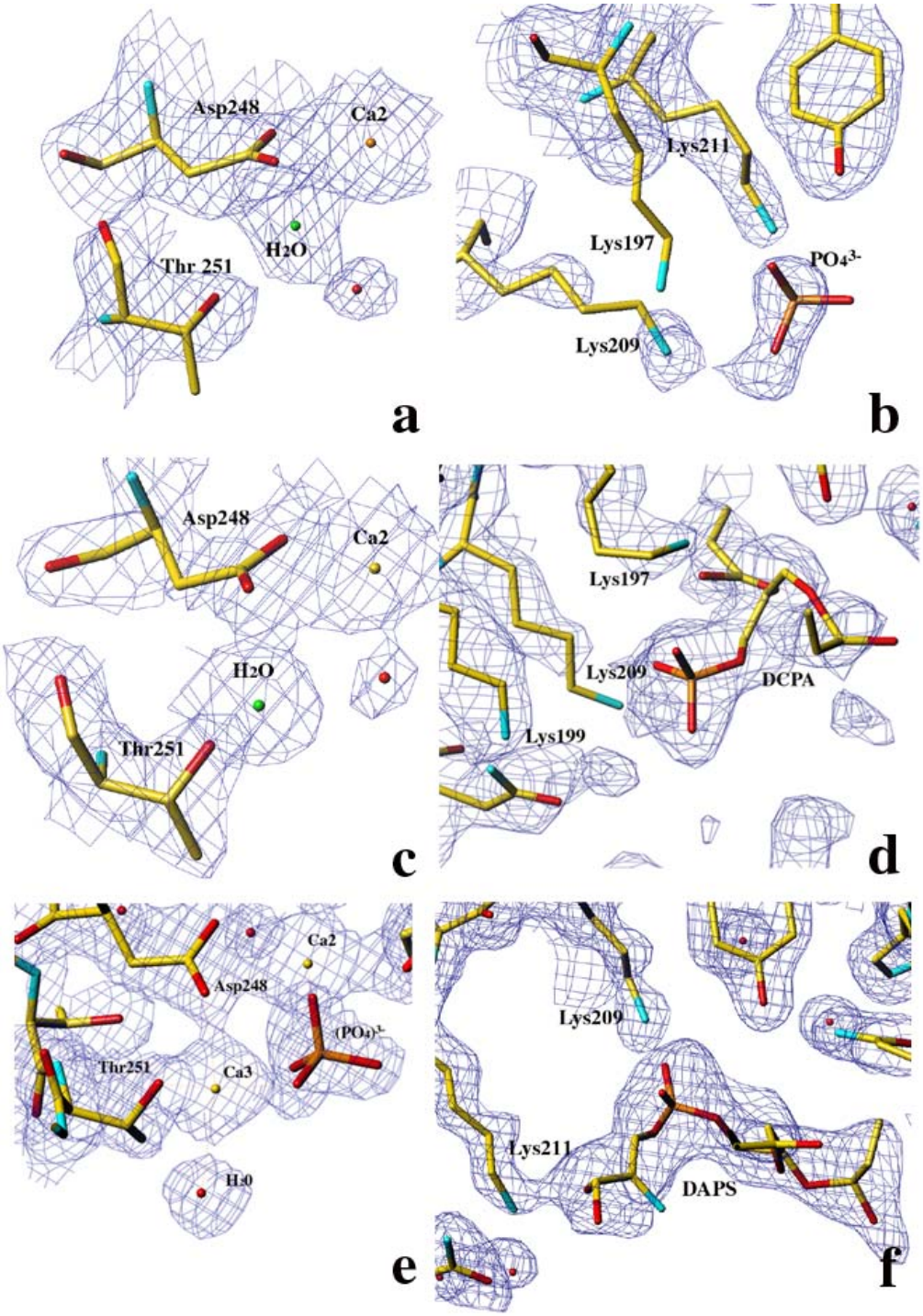

Fig. 2 2Fo-Fc electron density maps for the three and the lysines zone of the three ternary complexes, PKCα-C2_Ca^{2+}_DCPS (a,b), The PKCα-C2_Ca^{2+}_DCPA(c,d) and PKCα-C2_Ca^{2+}_DAPS (e,f) showing the regions corresponding to the Ca2+ binding pocket (a,c,e) and the lysine cluster (b,d,f).

ESRF	**Experiment title:** **BAG Barcelona.**	**Experiment number**: LS-1805
Beamline: ID 14-3	**Date of experiment**: from: 23.09.2001 to: 25.09.2001	**Date of report**: 31-Aug-2001
Shifts: 6	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

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***Pedro José Barbosa Pereira, postdoctoral, IBMB**

***Sandra de Macedo Ribeiro, postdoctoral, IBMB**

Report:

Biliverdin-IXβ reductase (BVR-B) catalyses the pyridine nucleotide-dependent production of bilirubin-IXβ. This is the major heme catabolite during early fetal development. BVR-B displays a preference for biliverdin isomers without propionates on either side of the C10 position, in contrast to biliverdin-IXα reductase (BVR-A), the major form of BVR in adult human liver. BVR-B displays also flavin and ferric reductase activities in the adult. The structure of human BVR-B in complex with NADP+ at 1.15 Å resolution has been solved (Pereira *et al*., 2001).

Human BVR-B is a monomeric enzyme, displaying an α/β dinucleotide-binding fold (see Fig. 1). The structures of ternary complexes with mesobiliverdin-IVα, biliverdin-IXα, FMN and lumichrome, also determined to high resolution (Pereira *et al*., 2001), demonstrate that human BVR-B has a single substrate binding site. Substrates and inhibitors bind to this site almost exclusively through hydrophobic interactions, explaining the enzyme's broad specificity. In the complexes, the reactive atom of biliverdin and flavin substrates is placed above the donor C4 of the cofactor, thus conveniently placed for direct hydride transfer. BVR-B discriminates against the biliverdin-IXα isomer through steric hindrance at the bilatriene side chain

binding pockets. The structures now obtained also explain BVR-B's preference for NADP(H) and its B-face stereospecificity.

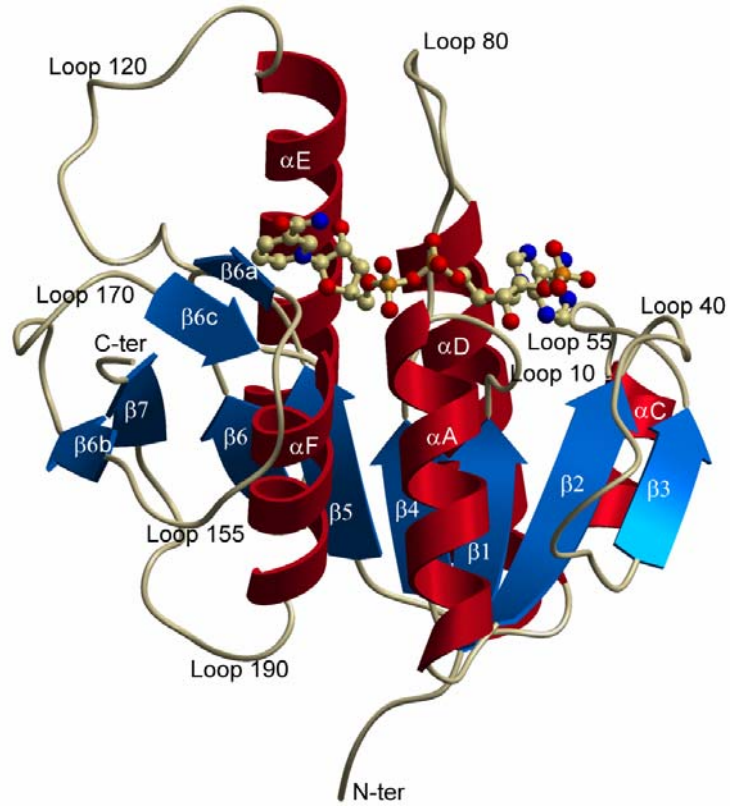

Figure 1 - Overall structure of human BVR-B in complex with $NADP^+$. The secondary structure elements are displayed in red (α-helices) and blue (β-sheets). Important loops, the C- and N-termini are labelled. The NADP molecule is shown as a ball-and-stick model with carbon atoms in yellow, oxygens in red, nitrogens in blue and phosphorous in orange.

Pereira, P. J. B., *et al*. (2001). "Structure of human biliverdin-IXβ reductase, an early fetal bilirubin-IXβ producing enzyme." *Nature Struct. Biol*. **8** (3): 215-220.