ESRF	**Experiment title:** Human β-Hexosaminidase	**Experiment number**: LS1958
Beamline: ID14-EH1	**Date of experiment**: from: 23.03.01 to: 24.03.01	**Date of report**: 27.08.01
Shifts: 3	**Local contact(s)**: Steffi Arzt	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Norbert Straeter *
Timm Maier

FU Berlin
Institut für Chemie - Kristallographie
Takustr.6
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Report:

β-Hexosaminidase is a lysosomal hydrolase that cleaves terminal non-reducing β-N-acetylhexosamines from a broad range of substances e.g. glycolipids and glycoproteins. Mammalian β-hexosaminidases occur as two major isoforms: HexA, a heterodimer of the closely related α– and β– chains, and HexB, a homodimer (ββ). Mutations in the gene encoding the β-chain of β-hexosaminidase are the primary cause of Sandhoff disease, a fatal inborn error of metabolism.
Datasets were collected in high and low resolution shells for human β-hexosaminidase recombinantly expressed in insect cells as well as an inhibitor complex of the protein. We were not able to process 4 out of the 5 collected datasets: Though indexing using DENZO was fine, meaningful scaling with SCALEPACK was not possible. Discussion with the beamline scientist led to the conclusion that a beamline shutter failure was the source of the problem. The only processable dataset was a high resolution inhibitor-complex dataset to 2.3 Å. The diffraction of all crystals was strongly anisotropic. Data collection details are given in Table 1.

Table 1. Data collection statistics

Space group	$P3_121$
Unit Cell	a=b=163.9Å c=244.7Å, α=β=90°, γ=120°
Resolution	30 - 2.2 Å
Total Observations	2 083 018
Unique Observations	192 852
R_{sym}	0.069
I/σ (final shell)	4.7
Completeness	99.9 %
Mosaicity (°)	0.464

Phases to 4 Å resolution for this structure were obtained in a MIRAS experiment at BAMline (BESSY, Berlin) using a Pt- and a Hg-derivate. Exploiting a sixfold non-crystallographic symmetry density modification and phase extension using the inhibitor-complex dataset from the above measurement resulted in well interpretable maps. Refinement is on the way, the actual status is summarized in Table 2.

Table 2. Refinement Statistics

Resolution	30 - 2.25 Å
Solvent Content	55 %
R_{cryst} / R_{free}	21% / 26 %
rmsd $_{bonds}$	0.010 Å
rmsd $_{angles}$	1.54°
B_{aver}	40.5 Å²
No. of Protein Atoms	23232
No. of Non-Protein / Non-Solvent Atoms	261
No. of Solvent Atoms	1680

ESRF	**Experiment title:** Human β-Hexosaminidase	**Experiment number**: LS1958
Beamline: ID14-EH4	**Date of experiment**: from: 16.06.01 to: 18.06.01	**Date of report**: 27.08.01
Shifts: 6	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Timm Maier*
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Report:

β-Hexosaminidase is a lysosomal hydrolase that cleaves terminal non-reducing β-N-acetylhexosamines from a broad range of substances e.g. glycolipids and glycoproteins. Mammalian β-hexosaminidases occur as two major isoforms: HexA, a heterodimer of the closely related α− and β− chains, and HexB, a homodimer (ββ). Mutations in the gene encoding the β-chain of β-hexosaminidase are the primary cause of Sandhoff disease, a fatal inborn error of metabolism. Datasets were collected in high and low resolution shells for different crystals of human β-hexosaminidase (non-inhibitor bound, previous datasets were not processable, see accompanying report) recombinantly expressed in insect cells. Data collection statistics for one high resolution dataset are given in Table 1. Refinement of the structure is currently focussed on the inhibitor-complex (see accompanying report).

Table 1. Data collection statistics

Space group	$P3_121$
Unit Cell	a=b=163.4Å c=244.7Å, α=β=90°, γ=120°
Resolution	30 - 2.25 Å
Total Observations	984283
Unique Observations	177699
R_{sym}	0.088
I/σ (final shell)	3.1
Completeness	99.9 %
Mosaicity (°)	0.318

ESRF	**Experiment title:** An inactive ζ(R171S) mutant of the plasmid addiction system ε,ζ encoded by pSM19035 from *Streptococcus pyogenes*	**Experiment number**: LS1958
Beamline:	**Date of experiment**: from: 16/06/01 to: 18/06/01	**Date of report**: 27/08/01
Shifts:	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Meinhart Anton

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Freie Universität Berlin
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Report:

The plasmid addiction system ε,ζ ensures stable inheritance of plasmids encoding the genes *ε* and ζ. Loss of these genes leads to programmed cell death. Both proteins form a non-toxic $\varepsilon_2\zeta_2$-heterotetramer. Comparison to structurally related proteins revealed ζ to be a phosphotransferase. Catalytically relevant residues were proved by site-directed mutagenesis. To demonstrate conserved tertiary structure, X-ray diffraction studies on protein crystals of mutated ζ protein were performed.

Collected data revealed dramatic radiation damage. A decrease in atomic occupancy of carboxylic groups from glutamate and aspartate side chains was observed. This phenomenon is in agreement with the results of Burmeister [1]. Therefore refinement converged at unusual high R-values (see Table 1), although the achieved resolution was much higher as observed up to now.

Data-collection statistics and refinement details are given in Table 1.

Table 1. Data collection and refinement statistics

Data collection	
space group	$P2_12_12_1$
unit cell (Å)	
a:	59.71
b:	80.03
c:	193.49
Resolution (Å)	20.00-1.55
Total observations	1 470 220
Unique reflections	134 527
R_{sym} [a)]	0.068
mean I/σ	16.7
R_{sym} (final shell)	0.493
mean I/σ (final shell)	3.97
Completeness	0.995
Completeness (final shell)	1.0
Model refinement	
R_{work} / R_{free}	0.218 / 0.216
R.m.s.d., bond length (Å)	0.004
R.m.s.d., bond angles (°)	1.07

[a)] $R_{lin.:}$ $(\Sigma(|I-\langle I\rangle|))/\Sigma I$ a

[1] Burmeister, W.P. (2000). Structural changes in a cryo-cooled protein crystal owing to radiation damage. Acta Cryst., D56., 328-341.

ESRF	**Experiment title:** Crystal structure at atomic resolution of HLA-B*2709 in complex with a defined nonameric peptide	**Experiment number**: LS-1958
Beamline: ID14-4	**Date of experiment**: from: 17.6.2001 to: 17.6.2001	**Date of report**: 22.08.01
Shifts: 1	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Martin Hülsmeyer and Roman C. Hillig, Charité, Institut für Immungenetik, Humboldt Universität zu Berlin, Berlin, Germany

*Timm Maier and Norbert Sträter, Institut für Kristallographie, Freie Universität, Berlin, Germany

Report:

The human MHC class I molecule HLA-B27 shows a strong association with spondyloarthropathies, a group of closely related inflammatory arthritic diseases, the most common of which is ankylosing spondylitis (AS). This connection has been recognized almost 30 years ago, but the underlying mechanism is still not understood. Within the last few years, a number of subtypes of B27 have been described and characterised concerning their association with disease. Remarkably, there are two subtypes, B*2706 and B*2709, who show no disease association. As B*2709 shows only one amino acid difference compared to the disease-associated subtype B*2705, we initiated a comparative structural analysis of B*2705 and B*2709.

We succeeded in refolding and crystallising B*2709 in complex with a defined nonameric model peptide (m9). Crystals were obtained by cross-seeding with crystals of subtype B*2705 obtained earlier, in complex with the same nonameric peptide. Data collection at beamline ID14-4 resulted in a native data set complete to 1.09 **Å** resolution (Table 1). The phase problem was solved by Molecular Replacement, and the structure has been refined to an R factor of 12.3% (R_{free} 14.8%).

A comparative analysis of the structures of B*2709 (obtained from the data collection described here) and of B*2705 (data collected in June 2000 at beamline ID13, ESRF, exp. no. TC-88) is in progress and is expected to shed light on the role of HLA-B27 in arthritic diseases. As the only structure of a HLA-B27 subtype available so far contains a mixture of peptides, our structures with defined peptides will also provide more detailed insights into the characteristic peptide binding behaviour of HLA-B27.

Table 1 Diffraction data statistics

Data collection	**HLA-B*2709 : m9**
X-ray source	ID14-4 (ESRF)
Space group	$P2_12_12_1$
Unit cell	a = 50.8 Å b = 82.5 Å c = 110.7 Å
Resolution (Å)	**19.3 – 1.1**
Resolution outer shell (Å)	1.13 – 1.10
No. of unique reflections	182507
Multiplicity	4.7 (2.9)
Completeness (%)	96.7 (96.9)
I/σ	12.9 (3.1)
R_{sym}	0.091 (0.353)
Refinement	
Refelctions (work / test set)	176285 / 5660
No. of non-hydrogen atoms	4339
R_{cryst}	**0.123 (0.17)**
R_{free}	**0.148 (0.17)**

Values in brackets refer to the highest resolution shell.

ESRF	**Experiment title:** RepA DNA helicase from plasmid 1010 complexed with flavone inhibitors	**Experiment number**: LS 1958
Beamline:	**Date of experiment**: from: 16/06/01 to: 18/06/01	**Date of report**: 27/08/01
Shifts:	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Hai Xu *

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Germany

Report:

DNA helicases are ubiquitous enzymes required for DNA replication, recombination, transcription and repair, for RNA translation, splicing of mRNA and assembly of ribisomes. We have determined a high-resolution X-ray structure (2.4 Å) of an intact, full-length hexameric helicase - DNA helicase RepA. The hexamer shows an annular structure with 6-fold rotational symmetry and a ~ 17 Å wide central hole which suggests that ssDNA may be threaded during unwinding [1]. The elucidation of the structure information of RepA helicase can be used to select specific helicase inhibitors and further give rise to the development of antibiotics and drugs. By biochemical screening, we recently found potential inhibitors for RepA helicase. In order to understand both the mechanism by which duplex DNA is unwound and by which inhibitors block cellular function, structural information is needed to elucidate in detail the interaction between inhibitors or DNA and RepA**.**

Data of a RepA/inhibitor complex was collected to 2.4 Å. Data-collection statistics and refinement details are given in Table 1.

Table 1. Data collection and refinement statistics

Data collection	
crystal form	monoclinic
space group	$P2_1$

unit cell	a = 104.1 Å, b = 178.8 Å, c = 115.5 Å, β = 108.5°
Resolution (Å)	50.0 - 2.4
Total observations	485032
Unique observations	154278
R_{sym} [a)]	0.071
R_{sym} (final shell)	0.215
I/sigma (final shell)	8.4
Completeness (overall)	0.989
Completeness (final shell)	0.986
Model refinement	
R_{cryst} / R_{free} [b)]	0.284 / 0.303
Resolution range (Å)	30.0 - 2.4
R.m.s. deviations from stereochemical target values	
r.m.s.d., bond lengths (Å)	0.0091
r.m.s.d., bond angles (°)	1.37

[a)] $R_{sym} = \sum|I-\langle I \rangle| / \sum I$, where $\langle I \rangle$ is the average intensity over symmetry equivalent reflections.

[b)] R_{cryst} and $R_{free} = \sum||F_o|-|F_c|| / \sum|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes. For the calculation of R_{free}, 5% of the reflection data were selected in thin resolution shells and omitted from refinement.

Further refinement and structure analysis is underway.

References:

1. Niedenzu, T., Röleke, D., Bains, G., Scherzinger, E., and Saenger, W. (2001) *J. Mol. Biol. 306*, 479-487.

ESRF	**Experiment title:** Crystal Structure of human Pirin	**Experiment number**: LS 1958
Beamline:	**Date of experiment**: from: 16/06/01 to: 18/06/01	**Date of report**: 28/08/01
Shifts:	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Uwe Mueller et al.

Proteinstrukturfabrik c/o BESSY GmbH
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12489Berlin
Germany

Report:

N- and C-terminal tagged Human Pirin (Swiss-Prot acc. no. O00625)

could be expressed, overproduced, purified and crystallized using an integrated approach for three dimensional structure solution, which are developed and utilized within the German structural genomics initiative "Proteinstrukturfabrik" [1].

This protein crystallized as thin needles of dimensions of 30x30x200 μ^3 and diffracted at ESRF-Beamline 14-4 afte establishing suitable cryo-conditions to a maximum resolution of 2.2 Å. Since no derivate for MAD-phasing was available, using the optained data the structure solution is not possible. Futher attempts in order to produce an Se-Met-Derivative Crystal are currently performed by PSF scientists.

Table 1. Data collection and refinement statistics

Data collection		
space group	$P6_322$	
unit cell		a = 118.9 Å, b = 118,9 Å,
		c = 60.8 Å,
Resolution (Å)		50.0 - 2.2
Total observations		132641
Unique observations		12366
R_{sym} [a)]		0.064
R_{sym} (final shell)		0.11
I/sigma (final shell)		10,63
Completeness (overall)		0.929
Completeness (final shell)		0.663

[a)] $R_{sym} = \sum|I-\langle I \rangle| / \sum I$, where $\langle I \rangle$ is the average intensity over symmetry equivalent reflections.

References:

1. Heinemann, U., J. Frevert, et al. (2000). “An integrated approach to structural genomics.” Prog. Biophys Mol. Biol. **73**(5): 347-362.

ESRF	**Experiment title:** MAD-data collection from crystals of the ribosomal release factor RF1 from *Thermus thermophilus*	**Experiment number**: LS 1958
Beamline: ID 14-4	**Date of experiment**: from: 16.06.2001 to: 18.06.2001	**Date of report**: **27.08.2001**
Shifts: 1	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Uwe Müller at Proteinstrukturfabrik c/o Bessy GmbH, Albert-Einstein-Str.15, D-12489 Berlin

Yvette Roske at Crystallography Group, Prof. U. Heinemann, Max-Delbrück-Centrum, Robert-Rössle-Str.10, 13092 Berlin-Buch

Report:

Termination of protein biosynthesis and release of the nascent polypeptide chain is signaled by the presence of an in-frame stop codon at the aminoacyl (A) site of the ribosome. The process of translation termination is universal and is mediated by protein release factors. RF1 from *Thermus thermophilus* is unrelated in primary structure to the omnipotent, eukaryotic eRF1 suggesting the existence of two distinct protein families. Moreover, it is expected that these two families will adopt different protein architectures, since a secondary structure prediction indicated a pattern that would be inconsistent with eRF1 (Goldstein *et al*., 1970; Konecki *et al*., 1977; Frolova *et al*., 1994). To provide insight into the structure of the prokaryotic RF1 from *T. thermophilus* and for comparison to the eukaryotic eRF1 structure (Song *et al*., 2000) MAD data from crystals of *T. thermophilus* RF1 were collected in collaboration with the group of Prof. M. Sprinzl at the University of Bayreuth, Germany. Datasets were collected at the peak (0.9795 Å) and the inflection point (0.9797 Å) of selenomethionine (no time was left for high-energy remote). The crystal diffracted up to a maximal resolution of 2.2 Å and belongs to the rhombohedral space group R32 with cell parameters a = 109.2 Å and c = 152.1 Å. The peak dataset is 94.6% complete with Rsym of 6.3%, 914,868 total reflections and 32,408 unique reflections. 3 of 11 selenomethionine sites were found by SAD with the peak dataset. No further sites were found with MAD by using both datasets (peak and inflection point) and map resulting from this method also showed no improvement. After initial tracing of the electron density it appears as if the map represented

only a fragment of the protein. We cannot exclude the possibility, therefore, that partial degradation of the SeMet-RF1 protein occurred during the crystallization experiment. At this time it is not known which part or domain of the protein is seen in the map.

Frolova, L., Le Goff, X., Rasmussen, H.H., Cheperegin, S., Drugeon, G., Kress, M., Arman, I., Haenni, A.L., Celis, J.E., Philippe, M., et al. (1994) *Nature*, **372**, 701-703

Goldstein, J.L., Beaudet, A.L. and Caskey, C.T. (1970) *Proc. Natl. Acad. Sci. USA*, **67**, 99-106

Song, H., Mugnier, P., Das, A.K., Webb, H.M., Evans, D.R., Tuite, M.F., Hemmings, B.A. and Barford, D. (2000) *Cell,* **100,** 311-321

Konecki, D.S., Aune, K.C., Tate, W. and Caskey, C.T. (1977) *J. Biol. Chem*., **252**, 4514-4520

ESRF	**Experiment title:** Human Nicotinamide Mononucleotide Adenylyltransferase (NMNAT)	**Experiment number**: LS1958
Beamline: ID14-4	**Date of experiment**: 16.06.01	**Date of report**: 27.08.01
Shifts: 1	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Erik Werner, Crystallogrpahy Group, Max Delbrück Center for Molecular Medicine, Robert-Rössle-Str.10, D-13092 Berlin, Germany

Uwe Müller, Protein Structure Factory, c/o Bessy GmbH
Albert-Einstein-Straße-15, D-12489 Berlin, Germany

Report:

Human nicotinamide mononucleotide adenylyltransferase (NMNAT, EC 2.7.7.1) is an oligomeric enzyme consisting of identical 33 kDa subunits. It catalyses the final step in the biosynthesis of nicotinamide-adenine dinucleotide, the major coenzyme in cellular redox reactions (Magni *et al.*, *Meth. Enzymol*. **280**, 241 (1997)):

$$ATP + NMN \rightarrow NAD^+ + PP_i.$$

NAD^+ also appears to be an important constituent of several intracellular signalling pathways (Ziegler, *Euro. J. Biochem*. **257**, 1550 (2000)). NMNAT has been proposed as a chemotherapeutical drug target, since its activity is down-regulated in tumor cells (Emanuelli *et al*., *Bioch. Pharmacol*. **49**, 575 (1995))

Protein crystals containing SeMet as heavy atom derivative and NAD as ligand were measured at ESRF beamline ID14-4. The fluorescence scan showed a clear peak. The best crystal had satisfying statistics up to a resolution of 2.9 Å and a complete MAD dataset was measured at the wavelengths 0.9797 Å (peak), 0.9795 Å (inflection point) and 0.9393 Å (high energy).

Data collection statistics:

	λ1	**λ2**	**λ3**
wavelength [Å]	0.9795	0.9797	0.9393
resolution [Å]	20 – 2.9 (3.0 – 2.9)		
No. of observations	632,186	639,155	633,992
unique observations	62,691 (5937)	63,149 (6336)	62,822 (6276)
completeness [%]	98.3 (92.5)	98.9 (99.0)	99.1 (98.9)
average I/σ(I)	11.2 (1.1)	12.1 (1.7)	10.5 (1.2)
Rsym [%]	7.0 (49.2)	8.3 (72.5)	9.2 (96.5)

The spacegroup is $C222_1$ (#20, orthorhombic) and the cell parameters are a = 140.8 Å, b = 235.7 Å, c = 89.0 Å. Three monomers in the asymmetric unit are observed.
Although these three datasets were measured, phasing was only possible using the SAD method with the peak dataset. The program SOLVE (Terwilliger, *Acta Cryst*. **D55**, 849 (1999)) was not able to distinguish between peaks and noise with all three datasets but found all 9 non-terminal selenium sites when only the peak dataset was used. Phase improvement by running RESOLVE and NCS averaging improved the electron density in a way that allowed placing helices, strands and loops as well as identifying sidechains.
The biological unit, a hexamer built of two trimers on top of each other, is formed by a crystallographic two-fold rotation axis. However, the three-fold axis between the trimer could be used for non-crystallographic symmetry averaging.
Currently the structure is in the refinement state.