	Experiment title: Von Willebrand Factor A1 domain	**Experiment number**: LS1923
Beamline: ID14-EH2	**Date of experiment**: 13 - 14 April 2001	**Date of report**: 8 August 2001
Shifts: 1/2	**Local contact(s)**: Dr. Stéphanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report: <u>For review purpose only.</u>

Von Willebrand factor (vWF) plays a crucial role in thrombus formation after vascular damage. It forms a bridge between collagen exposed at sites of injury and glycoprotein Ib (GpIb) located on the surface of blood platelets. Binding to GpIb is mediated via the A1 domain of vWF. Mutations in the A1 domain, so called vWD type 2B mutations, result in spontaneous binding of vWF to GpIb. Patients suffering from these mutations manifest bleeding episodes. In order to investigate an explanation for this spontaneous binding to GpIb, we solved the crystal structure of the A1 domain to compare with models of the A1 domain containing mutation R543Q.
Diffraction data were collected to 2.25 Å resolution on a 170 * 80 * 20 μm^3 crystal (Rmerge 6.5 %; redundancy 3.5). The crystal has space group P2(1) (a=76.83 Å, b=60.65 Å, c=87.66 Å, β=95.071) with 4 molecules in the asymmetric unit. The structure was solved by molecular replacement and refined to a R-factor of 20.7 % and a free R-factor of 27.5 %. Conformational changes were visible in the peptides flanking the A1 domain. Interpretation of this conformational change is complicated by the presence of Cd++ ions interacting with the flanking peptides in the R543Q mutant and not in the wt molecule.

ESRF	**Experiment title:** Von Willebrand Factor A1 domain R543Q mutant	**Experiment number**: LS1793/LS1923
Beamline: ID29 ID29	**Date of experiment**: 1) 3 - 5 February 2001 2) 30 June - 2 July 2001	**Date of report**: 8 August 2001
Shifts: 1/2 1/2	**Local contact(s)**: 1) Dr. Vivian Stojanoff 2) Dr. Bill Shepard	*Received at ESRF:*

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Report: <u>For review purpose only.</u>

Von Willebrand factor (vWF) plays a crucial role in thrombus formation after vascular damage. It forms a bridge between collagen exposed at sites of injury and glycoprotein Ib (GpIb) located on the surface of blood platelets. Binding to GpIb is mediated via the A1 domain of vWF. Mutations in the A1 domain, so called von Willebrand's disease type 2B mutations, result in spontaneous binding of vWF to GpIb. Patients suffering from these mutations manifest bleeding episodes. In order to understand spontaneous binding to GpIb, we solved the crystal structure of the A1 domain with mutation R543Q.
Diffraction data were collected to 2.2 Å resolution on a 40 * 40 * 20 μm^3 crystal (Rmerge 8.5 %; redundancy 14). The crystal has space group $P4_32_12$ (a=b=80.5 Å, c=125.349 Å) with 4 molecules in the asymmetric unit. The structure was solved by molecular replacement and refined to an R-factor of 20.8 % and a free R-factor of 25.0 %. Conformational differences with respect to wt-A1 were visible in the N- and C-terminal peptides flanking the A1 domain. Interpretation of these conformational changes is complicated by the presence of Cd^{2+} ions interacting with the flanking peptides in the R543Q mutant and not in the wt molecule. Therefore, we recently collected data on a crystal grown under different conditions. Analysis of this data is in progress.

ESRF	**Experiment title:** Glycoprotein Ibα	**Experiment number**: LS-1923
Beamline: ID14-EH2 ID14-EH2 ID29	**Date of experiment** 1) 03 - 05 March 2001 (scheduled first for ID14-EH3) 2) 13 - 14 April 2001 3) 30 - 02 July 2001	**Date of report**: 07 August 2001
Shifts: 1/2 1 2	**Local contact(s)**: 1) Dr. Stéphanie Monaco 2) Dr. Stéphanie Monaco 3) Dr. Bill Shepard	*Received at ESRF:*

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Report: Confidential! For review purposes only.

Glycoprotein (Gp) Ibα is a member of the Gp Ib-IX-V adhesion complex located on the surface of blood platelets. This complex is involved in von Willebrand Factor mediated adhesion of platelets to collagen, an essential first step in the arrest of bleeding. We have expressed a 33 kDa N-terminal fragment of GpIbα in mammalian cells. This fragment contains a complete von Willebrand Factor binding site. Plate-like crystals were obtained with dimension of up to 0.3 mm x 0.3 mm and a thickness of about 10 _m. Most crystals suffer from disorder in one direction. About one out of 8 crystals is suitable for data collection. Unfortunately, crystal quality can not be judged from crystal morphology and no significant diffraction data can be measured on our in house equipment. Therefore a significant amount of data collection time at the ESRF must be spend on identifying suitable crystals. A first native data set to a maximal resolution of 2.0 Å was collected on ID14-EH2 (native 1 in Table). This data set did not process very well due to high mosaicity in one direction and considerable radiation damage. A second native dataset (native 2) collected on ID14-EH2 is of better overall quality, but has a somewhat lower resolution due to the use of a

shorter exposure time of 2 seconds/degree of oscillation. Exposing a different region of the same crystal for 10 seconds/degree revealed diffraction spots up to 1.8 Å resolution, but collection of a complete data set was not succesful due to radiation damage. A search for heavy atom derivatives was initiated. Fluorescenece scans recorded on ID29 suggested that K_2PtCl_4 and Na_3IrCl_6 were present in back-soaked crystals, while $HgCl_2$ was not. Peak data sets that are complete to 2.5 Å resolution were collected for Pt and Ir. For Ir data were also collected at the inflection point and at a remote wavelength. Data collection on a sodium bromide soaked crystal was hampered by insufficient insensity of the X-ray beam and will be repeated.

The Pt and Ir datasets did not contain an anomalous signal. No heavy atom positions could be identified from isomorphous difference-Pattersons. This could be due to binding of the metal-ions to a disordered hexa-histidine tag.

Since the expression system used for GpIb_ does not allow for Se-Met labeling and molecular replacement with partial homology models has not been succesful, the search for heavy atom derivatives will be continued.

	wavelength (Å)	**resolution** (Å)	**Rmerge** (%)	**<I>/<_>**	**completeness** (%)
native 1	0.9330	2.0	9.2 (36.8)	10.1 (3.2)	98.5
native 2	0.9330	2.2	7.5 (34.5)	14.3 (2.9)	89.9
Pt peak	1.0719	2.3	5.2 (17.0)	40.8 (7.5)	89.6
Ir peak	1.1053	2.3	7.3 (24.2)	15.7 (3.2)	93.5
inflection	1.1056	2.3	7.3 (29.8)	15.1 (3.0)	91.3
remote	1.1170	2.3	6.3 (29.7)	10.6 (1.7)	49.5*

Values in parenthesis apply to high resolution shell. *Incomplete data due to end of beam time.

	Experiment title: Combination of cryo-photolysis and temperature-controlled crystallography to study the reaction mechanism of acetylcholinesterase	**Experiment number**: LS1923
Beamline: ID14-EH3 ID29	**Date of experiment** 1) 03 - 05 March 2001 2) 30 - 02 July 2001	**Date of report**: 8 August 2001
Shifts: 1 1/2	**Local contact(s)**: 1) Dr. Stéphanie Monaco 2) Dr. Bill Shepard	*Received at ESRF:*

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Martin Weik*, Sjors Scheres*, Clasien Oomen*, Eric Huizinga*, Piet Gros and Jan Kroon (deceased 3 May 2001) Dept. of Crystal and Structural Chemistry, Utrecht University, The Netherlands

Dominique Bourgeois, ILL, Grenoble, France.

Joel L. Sussman, Weizmann Institute, Rehovot, Israel.

Report:

This long-term project aims at studying the reaction mechanism of acetylcholinesterase (AChE) by a combined use of cryo-photolysis of caged compounds (photolabile precursors of enzymatic substrates or products) and temperature-controlled protein crystallography. Caged compounds that are complexed to AChE are cleaved at 100 K by means of light from a laser or flash-lamp. Owing to the protein's rigidity at such a low temperature the photolysis products remain trapped within the active site of the enzyme. Subsequent application of an appropriate temperature profile is anticipated to result in trapping of putative enzymatic reaction intermediates. In this context dynamical transitions of both the protein and its surrounding solvent are of particular interest. They allow to define temperature ranges in which intermediates states may accumulate that can be studied by standard monochromatic X-ray crystallography.

Numerous data sets at various temperatures were collected in the past two years on AChE complexed with caged arsenocholine that was photolysed under different conditions. All experiment were performed at a fixed wavelength of 0.93 Å which corresponds to an energy just above the K-absorption edge of arsenium (11.8 keV), the anomalous signal of which may facilitate the location of the choline molecule at higher temperatures in putative intermediate states. Structural changes associated with the caged compound suggested that i) cleavage at cryo-temperatures is possible and that ii) a jump or increase of temperature in between two data-set collections induces minor, yet reproducible positional changes of the cleavage products. The data

sets collected in the framework of LS1923 are important control experiments that addressed the influence of X-ray irradiation on the observed structural changes.

In a first control experiment, two subsequent data sets were collected at 100 and 170 K on a single crystal of AChE complexed with UNphotolysed caged arsenocholine. No structural changes of the caged compound were detected if structures based on both data sets were compared. However, its electron density in both cases is reminiscent of the one that was attributed to a cleaved and structurally relaxed compound in our earlier studies. A second control consisted in comparing structures of AChE complexed with photolysed caged arsenocholine that were determined from data sets with and without excursion to 170 K prior to data collection at 100 K. Again, changes indicated a cleaved and relaxed compound in both structures. These experiments strongly suggest that irradiation-induced changes represent a major contribution to changes that were previously attributed exclusively to the application of a temperature profile and that these changes occur in the very first instances of data collection. In a last control we showed that data collection with a X-ray energy below the K-absorption edge of arsenium (1.07 Å, 11.6 keV) did not prevent or reduce the previsouly observed damage.

We conclude that photolabile compounds are extremely sensitive to highly intense synchrotron X-irradiation and that future studies should be done involving X-ray beams other than the one from an undulator source.

	Experiment title: Lipase of *Bacillus subtilis*	**Experiment number**: LS-1923
Beamline: ID14-2 ID29	**Date of experiment**: from: 13-4-2001 to: 14-4-2001 from: 30-6-2001 to: 2-7-2001	**Date of report**: Aug. 2001
Shifts:	**Local contact(s)**: Dr. S. Monaco and dr. R. Ravelli	*Received at ESRF:*

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Lab. Of Biophysical Chemistry
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Report:

The structure of *B. subtilis* lipase has recently been solved with data collected earlier at the ESRF synchrotron (van Pouderoyen et al. (2001) J. Mol. Biol. 309, 215-226).
We are interested in obtaining more insight in the broad specificity of this lipase and the enantioselective properties of the hydrolysis reaction of this enzyme. We therefore covalently bound a pair of enantiomeric substrate-mimicks into the active site.
A data set of one bound enatiomer was collected on ID14-2 (14-4-2001). The other on ID29 (1-7-2001). The data characteristics and statistics are as follows:

Space group $P2_12_12_1$
Unit cell 40 x 83 x 96Å^3

Data collection	ID14-2	ID29
Resolution (Å)	1.8	1.8
Completeness 40-1.8Å	88.4%	99.6%
Completeness 1.84-1.80Å	56.2%	94.5%
I/sigma(I) 40-1.8Å	19.4	10.1
I/sigma(I) 1.84-1.80Å	3.8	3.0
R-merge 40-1.8Å	5.5%	17.0%
R-merge 1.84-1.80Å	21.1%	37.9%
Wilson B-factor (truncate):	14.6	11.5
Wilson scale (truncate):	38.9	25.1
Refinement		
current R-factor	17.8%	20.9%
current R_{free}-factor	21.3%	24.3%

Electron density calculated with both these data sets clearly showed the two different enantiomeric inhibitors bound in the active site. The detailed interactions of the inhibitor with the lipase are being analysed at the moment. The data collected on ID29 has however too bad data statistics and higher R-factors to be able to use it for a publication.

ESRF	**Experiment title:** Esterases for drug synthesis	**Experiment number**: LS1793 LS1923
Beamline: ID29 ID14-2 ID29	**Date of experiment**: from: 3-february-2001 to: 5-february-2001 13-april-2001 14-april-2001 30-june-2001 2-july-2001	**Date of report**: 9-august-2001
Shifts: 3	**Local contact(s)**: Andrew Thompson, William Shepard, Julien Lescar	*Received at ESRF:*

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Report: FOR REVIEW PURPOSES ONLY

The application of enzymes as catalysts provides a convenient way to prepare synthetic chemicals in an ecologically more responsible fashion than is usually the case with conventional chemical methods. Moreover, enzymes are capable of synthesizing chiral compounds in an enantioselective way, which is crucial for the production of countless drugs.

In our group we study the α-amino acid ester hydrolases (AEH's) of *Xanthomonas citri* and *Acetobacter turbidans*. These enzymes transfer the acyl group of an α-amino acid ester to water, but are also capable of transferring it to a β-lactam nucleus. The enzymes can thus be used for the production of synthetic penicillins and cephalosporins.

The AEH from *Xanthomonas citri* crystallises into rectangular blocks with a typical size of 0.1 x 0.1 x 0.05 mm. Though all crystals have the same shape, they exhibit three different lattice symmetries : primitive tetragonal, primitive orthorhombic and primitive monoclinic. Of these, the monoclinic crystals (space group $P2_1$) are the most amenable to X-ray analysis.

A selenium derivative has been prepared and crystallised. Part of the selenium oxidises readily, so that careful reduction with 10 mM DTT in mother liquor just before freezing is necessary. From the selenium-labeled crystals, three datasets could be collected :

A full three-wavelength MAD dataset was collected on ID29 from a crystal of space group $P2_12_12_1$. The statistics are in table 1. These data were collected from a crystal that showed two

peaks in the fluorescence spectrum which were attributed to reduced and oxidised selenium. No selenium positions could be found from these data. Subsequent crystals were subjected to reduction as described above. A single wavelength SAD experiment was done on ID14-2 with a crystal of space group $P2_1$. A 2.0 Å dataset was collected (statistics in table 1) but the anomalous signal was to weak for structure solution. Recently, a SAD dataset was collected from another $P2_1$ crystal at the selenium peak wavelength on ID29, which shows clear anomalous signal and structure solution is under way.

Crystallization experiments with the AEH from *Acetobacter turbidans* gave two crystal forms: non-birefringent rhombic dodecahedra (0.1x0.1x0.1 mm) and birefringent prisms (0.4x0.15x0.1 mm). The dodecahedra belong to either space group $I23$ or $I2_13$ and diffracted to 3.0 Å on the ID14-2 beamline. Native data and three heavy atom soaks have been collected (table 1), but difference Pattersons show no peaks. The prisms have primitive orthorhombic symmetry, and diffract to 3.5 Å on a home X-ray source. These crystals have not yet been measured on a synchrotron source. We are preparing a selenium derivative.

The naproxen esterase from *Bacillus subtilis* is another enzyme that is studied in our group. It hydrolyzes esters of arylpropionates with high enantioselectivity, which makes it useful in the synthesis of anti-inflammatory drugs like naproxen. Crystals of this enzyme have been grown as early as 1993. A mercury and a platinum derivative have been identified. We are also preparing a selenomethione labeled sample. We have collected a dataset from a crystal soaked for 30 s. in a 1.0 M sodium bromide solution on the bromide edge, but no anomalous signal could be detected.

Protein	Beamline	_ (Å)	**Space group**	**unit cell dim. (Å)**	**max. res. (Å)**	**Compl. (high res.)**	**R_{merge} (high res.)**
X. citri AEH, MAD, Semet peak	ID29	0.979	$P2_12_12_1$	a=89.1 b=123.8 c=128.5	3.0	94% (85%)	0.062 (0.119)
inflection point	ID29	0.979	$P2_12_12_1$	a=89.3 b=123.6 c=128.4	3.0	93% (79%)	0.073 (0.129)
high energy remote	ID29	0.915	$P2_12_12_1$	a=89.4 b=124.3 c=129.1	3.0	93% (71%)	0.094 (0.339)
X. citri AEH SAD data	ID14-2	0.933	$P2_1$	a=90.1 b=125.8 c=132.1 β=91.0 °	2.0	94% (71%)	0.094 (0.218)
X. citri AEH SAD data on peak	ID29	0.979	$P2_1$	a=90.3 b=126.13 c=132.2 β=91.0 °	3.0	100% (100%)	0.103 (0.215)
A. turbidans AEH native	ID14-2	0.933	$I23$ or $I2_13$	a=b=c=343.2	3.0	100% (100%)	0.065 (0.132)
A. turbidans AEH U soak	ID14-2	0.933	$I23$ or $I2_13$	a=b=c=342.8	3.0	100% (100%)	0.061 (0.148)
A. turbidans AEH Hg soak	ID14-2	0.933	$I23$ or $I2_13$	a=b=c=343.5	3.0	100% (100%)	0.056 (0.127)
B. subtilis NE Br soak	ID29	0.920	$P3_121$ or $P3_221$	a=b=46.9 c=211.7	3.5	97.3% (100%)	0.096 (0.116)