EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** EMBL Grenoble Outstation BAG (Membrane reorganisation group of Winfried Weissenhorn)	**Experiment number:** LS 1814/LS 1945
Beamline: various	**Date of experiment:** from: September 2000 to: August 2001	**Date of report:** 31/8/01
Shifts:	**Local contact(s):** various	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Winfried Weissenhorn, EMBL Grenoble Outstation

Dr. Andreas Bracher, Dr. Maria Sola

Report:

(1) Crystal structures of neuronal squid Sec1 implicate inter-domain hinge movement in the release of t-SNAREs.

Sec1 molecules associate with t-SNAREs from the syntaxin family in a heterodimeric complex that plays an essential role in vesicle transport and membrane fusion. Neuronal rat n-Sec1 has an arch-shaped three-domain structure, which binds syntaxin 1a through contacts in domains 1 and 3. In both rat nSec1 and homologous squid s-Sec1, a potential effector-molecule binding-pocket is shaped by residues from domains 1 and 2 and is localized on the opposite side of the syntaxin 1a interaction site. Comparison of several crystal forms of unliganded neuronal squid Sec1 indicates a hinge region between domains 1 and 2 which allows domain 1 to rotate along a central axis. This movement could release syntaxin 1a upon interaction with a yet unspecified Sec1 effector molecule(s). The binding of an effector protein may also directly affect the conformation of the helical hairpin of domain 3, which contributes the other significant syntaxin 1a binding sites in the rat nSec1/syntaxin 1a complex structure but adopts multiple conformations in the unliganded s-Sec1 structures reported here.

The structures have been solved by molecular replacement using data collected at beam lines ID14-1 ($P2_1$, 2.8 Å) and ID13 ($P3_121$, 3.2 Å) in the previous data collection period (10/ 1999 to 8/2000)

Publication: A. Bracher and W. Weissenhorn. (2001) **Crystal structures of neuronal squid Sec1 implicate inter-domain hinge movement in the release of t-SNAREs.** *J. Mol. Biol.*, 306, 7-13.

Manuscript in preparation: A. Bracher and W. Weissenhorn. Crystal structure of the N-terminal domain of the neuronal t-SNARE syntaxin from squid.

(2) X-Ray crystal structure of the trimeric N-terminal domain of gephyrin

Gephyrin is an ubiquitously expressed protein which, in the central nervous system, forms a submembraneous scaffold for anchoring inhibitory neurotransmitter receptors in the postsynaptic membrane. The N- and C-terminal domains of gephyrin are homologous to the *E. coli* enzymes MogA and MoeA, respectively, both of which are involved in molybdenum cofactor (Moco) biosynthesis. This enzymatic pathway is highly conserved from bacteria to mammals, as underlined by gehyrin's ability to rescue Moco deficiencies in different organisms. Here we report the X-ray crystal structure of the N-terminal domain (amino acids 2-188) of rat gephyrin at 1.9 Å resolution. Gephyrin (2-188) forms trimers in solution, and a sequence motif thought to be involved in molybdopterin (MPT) binding is highly conserved between gephyrin and the *E. coli* protein. The atomic structure of gephyrin (2-188) resembles MogA, albeit with two major differences. The path of the C-terminal ends of gephyrin (2-188) indicates that the central and C-terminal domains, absent in this structure, should follow a similar three-fold arrangement as the the N-terminal region. In addition, a central β-hairpin loop found in MogA is lacking in gephyrin (2-188). Despite of these differences, both structures show a high degree of surface charge conservation, consistent with their common catalytic function.

The structure was solved by molecular replacement using data collected at ID14-2 to 1.9 Å.

Publication: M. Sola, M. Kneussel, I. Heck, H. Betz and W. Weissenhorn. (2001) X-Ray crystal structure of the trimeric N-terminal domain of gephyrin. J. Biol. Chem, 276, 25294-25301.

(3) Crystal structures of an oligomeric form of the matrix protein VP40 from Ebola virus.

The matrix protein VP40 from Ebola virus exists in a monomeric soluble conformation and a membrane-bound oligomeric form. Here we show that the N-terminal domain of VP40 adopts an octameric ring-like structure as detrermined from two different crystal forms belonging to space groups P422 and P6222. The oligomeric structures show considerable flexibility compared to the N-terminal domain of the monomeric structure of VP40 which might be important for the assembly process. In addition, the oligomeric structures suggest that part of the N-terminus which is ordered in the monomeric structure has to unfold and the C-terminal domain has to move out of the way to allow the oligomerization contacts observed in the octameric VP40 structures. This oligomeric form of VP40 may constitute a building block for virus assembly.

The crystal form belonging to spacegroup P422 was solved using a native dataset collected at ID14-2 (1.9 Å) and two MAD data sets collected from a platinum derivative. The hexagonal crystal form (collected at a previous data collection period [12/1999]) was solved by molecular replacement using the model obtained by the tetragonal crystal form.

Manuscript in preparation: W. Weissenhorn, X. Gomis-Ruth, and H.-D. Klenk. Crystal structures of an oligomeric form of the matrix protein VP40 from Ebola virus.