EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** Stuctural Studies of Neurotransmitter:Symporter Family	**Experiment number**:
Beamline:	**Date of experiment**: from: 31-07-2012 to: 01-08-2012	**Date of report**: 30-08-2012
Shifts: 1	**Local contact(s)**:	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The signal transmission between neurons in the brain is mediated by transporters of the neurotransmitter-sodium symporter (NSS) family that perform active reuptake of neurotransmitters from the synapse into the presynaptic neuron, thus terminating the action potential and recycling neurotransmitters. These transporters are targets for a variety of medicaments used in the treatment of several psychiatric and neurological disorders, as well as for psychostimulants (1).
A major breakthrough in the investigation of the NSS structure-function relationship came with revealing the crystal structure of LeuT from *Aquifex aeolicus*, which showed the location of the binding sites for the substrate (L-leucine) and the two sodium ions in the center of the transporter (2). A recent structure of a LeuT quadruple mutant, stabilized by antibody Fab fragment, in an intracellular open substrate-free conformation revealed major structural changes, which lead to the release of ligands into the cell (3). However, LeuT is a very slow transporter and the available functional information is primarily obtained through binding studies (4; 5). Furthermore, detergents interfere significantly with LeuT function (6; 7).
We are currently studying the multi-substrate hydrophobic amino acid transporter (O6) from *Bacillus halodurans* (5) – an alternative homologous model system – to gain insight into the structure-function interplay in the NSS family. Like LeuT O6 has about 25% sequence identity to human NSS proteins.
We have collected a dataset of O6 bound with substrates in presence of lipids diffracting to 2.3 Å resolution at ESRF ID23-2 microfucus beamline. We are at the final stages of refinment (R-work .18.3%, R-free 22.2%). The structure describes important features of the release of substrates.

1. Wang CI, Lewis RJ (2010). ***Biochem Pharmacol*** 79(8),1083-91
2. Yamashita, A, Singh, SK, Kawate, T, Jin, Y, and Gouaux, E (2005). ***Nature*** 437, 215-23
3. Krishnamurthy, H, Gouaux, E (2012). ***Nature*** 481, 469-74
4. Piscitelli CL, Krishnamurthy H, Gouaux E (2010). ***Nature*** 468, 1129-32

5. *Quick, M, and Javitch, JA* (2007). ***Proc Natl Acad Sci USA*** 104, 3603-8
6. Quick M, Winther AM, Shi L, Nissen P, Weinstein H, Javitch JA (2009) ***Proc Natl Acad Sci USA*** 106, 5563-8
7. Quick M, Shi L, Zehnpfennig B, Weinstein H, Javitch JA (2012). ***Nat Struct Mol Biol*** 19, 207-11