USSBE USGEB

UNIVERSITÄT BERN

41ST ANNUAL MEETING OF THE SWISS SOCIETIES FOR EXPERIMENTAL BIOLOGY (USGEB/USSBE)

Addendum

Additions as of January 27, 2009

http://www.usgeb2009.ch

Late Posters

L1-01
Improving the quality of CitS crystals

Kurz, M; Briand, C; Frey, D; Huber, T; Bosshart, Honegger, A; A; Plückthun, A; Grütter, M

Universität of Zürich, Biochemisches Institut, Winterthurerstrasse 190, CH-8057 Zürich

One of the largest functional transporter families are the secondary transporters, which characteristically drive transport of solutes across the membrane in an energy-requiring process, employing the free energy stored in ions and/or solutes. To understand the complex mechanism transporters are involved, three-dimensional structures are required. So far only a few crystal structures of secondary transporters have been determined. Furthermore, no three-dimensional structure of any 2-hydroxy-carboxylate transporter (2HCT), a subfamily of secondary transporters, has been described so far.
2HCT transporters are exclusively found in bacteria and use citrate, malate and lactate as substrates (Sobczak and Lolkema, 2005). The best-studied example is the integral membrane protein CitS (Na^+/citrate symporter) originating from *Klebsiella pneumoniae* (Bott, 1997, van Geest et *al*., 2000). Crystals of CitS from *Klebsiella pneumoniae* have been successfully grown. However, despite the use of several methods (e.g. selenomethionine derivative crystals) the structure could not be solved yet.
A new approach to obtain crystals suitable for structure determination is to work with homologues from different species or to co-crystallize the protein with specially designed binding proteins (DARPins) to enhance the soluble region of CitS and increase the probability to obtain better diffracting crystals (Huber et *al*., 2007). Here we also present the initial work of successful co-crystallization of DARPins (containing a modified Hg-binding site) with CitS suitable for structure determination.

References:
Bott, 1997; Arch. Microbiol. 167: 78-88
Huber et *al*., 2007; J Structural Biol 159(2): 206-221
Sobczak and Lolkema, 2005; Microbiol and Mol Biol, reviews 69: 665-695
Van Geest et *al*., 2000; Biochemica and Biophysica Acta 1466: 328-338

L1-02
Crystal structure of the *P. aeruginosa* multidrug transporter MexB

Gaby Sennhauser[1], **Magdalena A. Bukowska[1]**, Christophe Briand[1], Markus G. Grütter[1]

[1]*Institute of Biochemistry, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland*

Three-component efflux systems play a major role in the intrinsic and acquired capability of Gram-negative bacteria to extrude a plethora of compounds including clinically relevant antibiotics [1]. We expressed and purified the inner membrane protein MexB of *Pseudomonas aeruginosa*, an intensively studied member of the resistance-nodulation-cell division (RND) family of secondary transporters. Here we describe the crystal structure of MexB at 3.0 Å resolution. The transporter forms an asymmetric homotrimer where each subunit adopts a different conformation representing three snapshots of the transport cycle similar to the recently determined structures of its close homologue AcrB from *Escherichia coli*, the sole structurally characterized member of the superfamily so far [2-4]. The conformation of one subunit is remarkably altered compared to AcrB, indicating conformational flexibility of the whole system. Furthermore, a molecule identified as dodecyl maltoside (DDM) bound in the internal multidrug binding cavity is consistent with the binding principles seen in other multidrug-binding proteins. Additionally, we obtained crystals of MexB in the presence of its substrates. Analysis of the substrate binding might shed more light on a drug extrusion mechanism.
As the only missing piece of the puzzle in the MexAB-OprM system, the X-ray structure of MexB completes the molecular picture of the major responsible pump mediating drug-resistance in *P. aeruginosa*.

1. Nikaido, H., *Multidrug efflux pumps of gram-negative bacteria.* J Bacteriol, 1996. **178**(20): p. 5853-9.
2. Murakami, S., et al., *Crystal structures of a multidrug transporter reveal a functionally rotating mechanism.* Nature, 2006. **443**(7108): p. 173-9.
3. Seeger, M.A., et al., *Structural asymmetry of AcrB trimer suggests a peristaltic pump mechanism.* Science, 2006. **313**(5791): p. 1295-8.
4. Sennhauser, G., et al., *Drug export pathway of multidrug exporter AcrB revealed by DARPin inhibitors.* PLoS Biol, 2007. **5**(1): p. e7.

L1-03

Surface regulation of the ubiquitous sodium/proton exchanger isoform 1 (NHE1) involves both lysosomal and proteasomal pathways

Simonin A [1, 2], Fuster DG [1, 2]

[1] *Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland*
[2] *Division of Nephrology and Hypertension, University Hospital of Bern, Switzerland*

The ubiquitously expressed plasmalemmal NHE1 regulates cell volume and pH and has been shown to be an important regulator of cell adhesion, migration and proliferation. Here we investigated the mechanisms underlying NHE1 internalization and degradation. Pulse-chase experiments using surface biotinylation demonstrated that NHE1 membrane half-life is 26h in HEK293 cells and mouse fibroblasts. Membrane NHE1 half-life is increased using various lysosomal and proteasomal inhibitors, indicating contribution of both pathways. Half-life prolongation by proteasomal inhibitors cannot be explained by ubiquitin exhaustion since an E1 ubiquitin ligase inhibitor also prolongs plasmalemmal NHE1 half-live. Using wild-type and NHE1-deficient mouse fibroblasts as well as HEK cells we show that endogenous plasmalemmal NHE1 is directly poly- or multi-ubiquitylated in vivo.
Using different NHE1 C-terminal truncations (NHE1Δ747, NHE1Δ675 and NHE1Δ550) with sequential surface biotinylation and immune-precipitation we find that NHE1 ubiquitylation is lost at the plasma membrane upon truncation beyond amino acid 675, suggesting loss of ubiquitylation sites or loss of E3 ligase binding. However, NHE1Δ550 is strongly ubiquitylated when present on intracellular membranes, suggesting specific regulation of NHE1 ubiquitylation at the plasma membrane.
Unexpectedly, treatment of HEK293 cells or mouse fibroblasts with lysosomal or proteasomal inhibitors decreased the amount of membrane NHE1 compared to untreated cells. Membrane insertion assays showed diminished insertion of NHE1 during inhibition of lysosomes or the proteasome.
In conclusion, we demonstrate that NHE1 is ubiquitylated at the plasma membrane in vivo and that NHE1 surface abundance is tightly regulated by exo- and endocytotic pathways involving both lysosomal and the proteasome/ubiquitin pathways. The individual importance of these pathways under physiological and pathophysiological conditions and the underlying mechanisms remain to be determined.

Participants List

Michael Altmann
University of Bern
michael.altmann@mci.unibe.ch

Renato Baumgartner
University of Bern
r.baumgartner@students.unibe.ch

Karolina Blajecka
University Children's Hospital Zürich
Karolina.Blajecka@kispi.uzh.ch

Magdalena Bukowska
University of Zürich
m.bukowska@bioc.uzh.ch

Cristina Cartoni
Nestlé Research Center Lausanne
cristina.cartoni@rdls.nestle.com

Marco Celio
University of Fribourg
marco.celio@unifr.ch

Sami Damak
Nestlé Research Center Lausanne
sami.damak@rdls.nestle.com

Fabiola Dionisi
Nestlé Research Center Lausanne
shireen.sardar@rdls.nestle.com

Stephane Dupuis
University Medical Center, Geneva
stephane.dupuis@medecine.unige.ch

Jenny Eggenschwiler
University of Applied Science, Wädenswil
eggi@zhaw.ch

José Fahrni
University of Geneva
jose.fahrni@unige.ch

Antonia Fettelschoss
University Hospital Zürich
antonia.fettelschoss@usz.ch

Theresa Fitzpatrick
University of Geneva
Theresa.Fitzpatrick@unige.ch

Christian Flogaus
ETH Zürich
flogaus@mol.biol.ethz.ch

Maryse Girard
University Medical Centre, Geneva, Switzerland
Maryse.Girard@unige.ch

Alexandra Gisler
University of Geneva
algisler@ethz.ch

Christel Gumy
Glenmark Pharmaceuticals S.A., La Chaux-de-Fonds
christel.gumy@gmail.com

Heidi Hediger
University of Bern

Katrin Heuser
ETH Zürich
kheuser@mol.biol.ethz.ch

Sachiko Hirosue
EPFL Lausanne
sachiko.hirosue@epfl.ch

Francis Jacob
University Hospital Zürich
Francis.Jacob@usz.ch

Jan Kucera
University of Bern
kucera@pyl.unibe.ch

Mareike Kurz
University of Zürich
m.kurz@bioc.uzh.ch

Gaia Legnani
ETH Zürich
legnanig@student.ethz.ch

Maurus Locher
University of Fribourg
maurus.locher@unifr.ch

Victoria Makrides
Universtiy of Zürich
makrides@access.uzh.ch

Schneider Marlen
University Hospital Zürich
marlen.schneider@usz.ch

Swetlana Martell
Biozentrum, Universität Basel
swetlana.martell@unibas.ch

Cyril Moccand
University of Geneva
Cyril.Moccand@unige.ch

Pascale Ohnsorg
University Hospital Zürich
Pascale.Ohnsorg@usz.ch

Sylvie Pridmore-Merten
Nestlé Research Center Lausanne
shireen.sardar@rdls.nestle.com

Julius Rabl
ETH Zürich
julius.rabl@mol.biol.ethz.ch

Eric Raddatz
University of Lausanne
eric.raddatz@unil.ch

Lucia Rohrer
University Hospital Zürich
lucia.rohrer@usz.ch

Daniela Ross
University of Bern
daniela.ross@mci.unibe.ch

Sara Sabatasso
University of Fribourg
Sara.Sabatasso@unifr.ch

Magalie Sabatier
Nestlé Research Center Lausanne
shireen.sardar@rdls.nestle.com

Roger Schneiter
University of Fribourg
roger.schneiter@unifr.ch

Yechiel Shai
The Weizmann Institute of Science, Israel
yechiel.shai@weizmann.ac.il

Luca Simonelli
Institute of Research in Biomedicine, Bellinzona
luca.simonelli@irb.unisi.ch

Abhijeet Todkar
University of Zürich
abhijeet.todkar@access.uzh.ch

Carine Rosa Tsamo Nintedem
EPFL Lausanne
carinerosa.tsamomintedem@epfl.ch

Kacper Wojtal
University Hospital Zürich
kacper.wojtal@usz.ch

Xiushan Wu
Human Normal University, China
xiushanwu@yahoo.com

Joel Zumstein
University of Applied Science, Wädenswil
joel.zumstein@zhaw.ch