

Aim

The workshop provides researchers with the theoretical and practical know how to perform membrane proteomics by two dimensional Blue native / SDS polyacrylamide gel electrophoresis (2D BN / SDS PAGE). The workshop is also intended to be a platform for the exchange of information on membrane proteomics in general.

Topics

- Basics and principles of 2D Blue native / SDS polyacrylamide gel electrophoresis
- Sample preparation: solubilization of native membrane protein complexes, optimization of solubilization
- Casting of native gradient gels
- Blue native electrophoresis (first dimension)
- Transfer of protein complexes onto the second dimension (SDS-PAGE) and separation of the protein complex subunits
- Casting of SDS gels
- Protein detection: Coomassie / silver staining, fluorescence labeling
- 2D gel documentation

Training

Lectures, practices and demonstrations provide participants with the skills to understand and perform 2D BN / SDS-PAGE for the separation and identification of membrane proteins.

Tutorial

Teaching is based on a tutorial which will be sent to participants in advance upon request. If arranged with us in advance, participants may analyse samples from their own laboratory during the workshop.

Participants

12 persons

Venue

Institut für Pflanzengenetik
Leibniz Universität Hannover
Herrenhäuser Str. 2
30419 Hannover
Germany



Accommodation

The venue (orange circle) is close to the following hotels:

1. Hotel Gästehaus am Herrenhäuser Garten, Herrenhäuser Kirchweg 17, 30167 Hannover, Tel: +49 (0)511 700720
<http://www.mit-h.de/>
2. Hotel Körner, Körnerstrasse 24-25, 30159 Hannover, Tel: +49 (0)511 16360 (not on the map; about 2 km from the workshop location) <http://hotelkoerner.de/index.htm>
3. Hotel in Herrenhausen, Markgrafstr. 5, 30419 Hannover, Tel: +49 (0)511 7907600 (not on the map; about 1.5 km from the workshop location) <http://www.hotel-in-herrenhausen.de>

Expenses

€ 780.-
€ 680.- for members of the DGPF*

The workshop fee includes all laboratory and lecture material excluding accommodation and boarding.

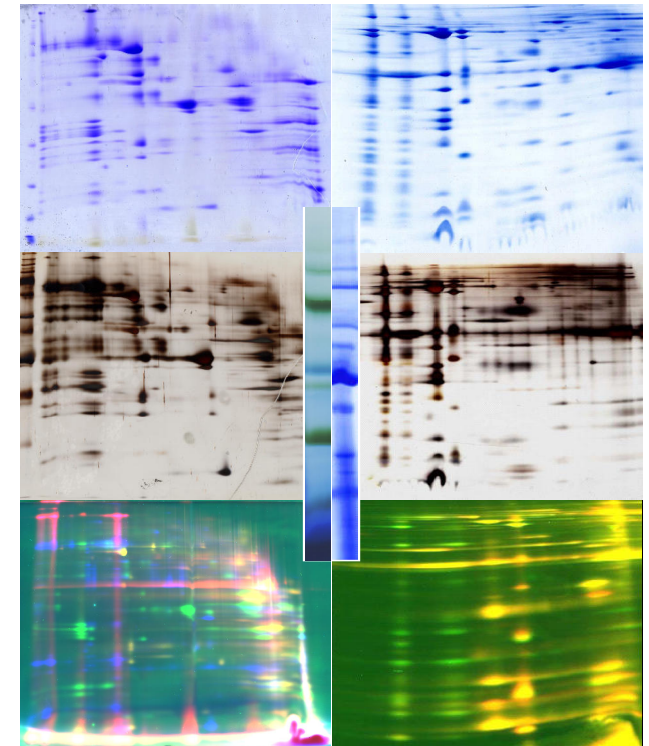
* DGPF members may apply for a partial refund of travel expenses



Deutsche Gesellschaft für
Proteomforschung e.V.

Membrane proteomics by Blue-native PAGE

Prof. Dr. Hans-Peter Braun
Prof. Dr. Lutz Eichacker

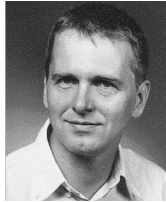


May 26th -28th, 2010
Centre of Applied Plant Biotechnology (ZAP)
Leibniz Universität Hannover, Germany

Contact

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Curriculum

Prof. Hans-Peter Braun is head of the plant proteomics group at the Institute for Plant Genetics of Hannover University. His research interest is focussed on protein complexes and protein supercomplexes of mitochondria and plastids in higher plants. Prof. Braun graduated in Molecular Biology in 1993 and teaches Plant Physiology and Plant Genetics at the Faculty of Natural Sciences of Hannover University.

Recent publications including Blue native PAGE:

- Heinemeyer et al. (2009) *J. Proteomics* 72, 539-544
- Braun et al. (2009) *Meth. Enzymol.* 456, 183-190
- Peters (2008) *Biochim. Biophys. Acta (Bioenergetics)* 1777, 84-93.
- Heinemeyer et al. (2007) *J. Biol Chem.* 282, 12240-12248
- Heinemeyer et al. (2007) *Methods Mol Biol.* 355, 343-52

Prof. Dr. Lutz Andreas Eichacker

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Curriculum

Prof. Dr. Lutz Andreas Eichacker is head of the Proteomics and Mass Spectrometry facility at the Centre for Organelle Research (CORE) of Stavanger University, Norway. His work is focussed on the assembly of membrane protein complexes. He graduated at Munich University in Plant Phytochemistry and teaches Plant and Protein Biochemistry since 1997.

Recent publications including Blue native PAGE:

- Plöschner et al. (2009) *Proteomics.* 9(3):625-35
- Reisinger V, Eichacker LA. (2009) *Methods Mol Biol.* 564:325-33.
- Reisinger V, Eichacker LA. (2008) *J Proteomics.* 71(3):277-83.
- Granvogel et al. (2008) *Anal Biochem.* 383(2):279-88

Program

Wednesday, 26. May 2010

- 13.00 Welcome, Introduction of the participants
- 13.30 Lecture I: Blue native PAGE.
Basic principles, practical aspects of 2D Blue native / SDS gel electrophoresis, advanced gel systems.
- 14.30 Practice 1: Casting of the first dimension blue native gel.
Assembly of the casting stand. Preparation of the gel for blue native electrophoresis.
- 15.30 Discussion: Sample preparation.
- 16.15 Break
- 16.30 Practice 2: Sample preparation.
Solubilization of membrane protein complexes.
CyDye labelling of protein complexes.
Casting of the stacking gel for the blue native gel.
Loading of the samples onto the blue native gel.
Start of blue native gel electrophoresis
- 19.15 Discussion

Thursday, 27. May 2010

- 9.00 Lecture II: Solubilization of membrane proteins.
Strategies for the solubilization of native membrane protein complexes. Sample preparation for electrophoresis.
- 10.00 Break
- 10:15 Practice 3: Transfer from first to second gel dimension. Dissection of the blue native gel into stripes.
In-gel denaturation of proteins.
Transfer of gel stripes onto glass plates for second gel dimensions.
- 11.00 Practice 4: Casting of the second dimension SDS gels.
Assembly of the casting stand for the "Dalt" multiple gel electrophoresis system. Casting of the separation gels.
- 12.30 Lunch
- 13.30 Practice 5: Casting of the sample gels embedding the blue native gel stripes. Start of electrophoresis.

Program

Thursday, 27. May 2010

- 14.30 Lecture III: Applications of Blue native PAGE
Presentation and discussion of data obtained by two dimensional blue native / SDS PAGE.
- 15.30 Discussion
- 18.30 Dinner

Friday, 28. May 2010

- 9.00 Practice 6: Visualization of proteins after 2D blue native / SDS-PAGE. Coomassie stainings and fluorescence detection.
- 9.30 Lecture IV: Protein visualization and identification.
Coomassie, silver, and activity staining, fluorescence labelling, immunoblotting and mass spectrometry.
- 10.30 Break
- 10.45 Discussion: Results.
- 13.00 End