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AES Newsletter

See you soon at the Annual AES Meeting to be held on Nov 5-9, 2007 at the Salt Palace Convention Center, Salt Lake City, UT

Inside this issue:

Many thanks to our Sponsors for contributions.

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Our traditionally strong meetings, with sessions strengthened by invited plenary speakers discussing state-of-the-art topics, would simply not be possible without help from sponsors. Their support is greatly appreciated.

News from our Meeting Organizers

The countdown is now in days instead of months toward the American Electrophoresis Society's annual meeting to be held in conjunction with that of the American Institute of Chemical Engineers (AIChE) November 5-9, 2007. The Salt Palace Convention Center promises to be a terrific venue.

Inside this issue you'll find a handy program grid detailing all the events sponsored by the AES. Also inside are two drink tickets for the poster reception to be held Tuesday evening (6:30 - 8:30 pm) in Exhibit Hall B. Poster set-up and take-down information will be emailed to presenters soon. Complete information including abstracts about our 14 sessions can be found at the AIChE link http://aiche.confex.com/aiche/2007/techprogram/D1199.HTM. Don't forget to sign up for the annual AES banquet to be held at Bucco de Beppo on Wednesday at 7:30 pm; $50 includes dinner, wine and a talk by ancestors expert Tim Bingaman on the Mormon family history library. Preceding the banquet on Wednesday, please attend the annual business meeting at 6:15 pm in Convention Center room 250B. The society needs your input. Ideas for session topics for next year will be welcome at the business meeting, along with volunteers to help with various items. See you soon!

Joe Biernacki
Tennessee Technical Collage
Chemical Engineering
JBiernacki@tntech.edu

Wayne Patton
Perkin Elmer
Life & Analytical Sciences
Wayne.Patton@perkinelmer.com

AES 2007 Meeting Co-Chairs

Blue Native Gel Electrophoresis – a valuable tool in studying membrane protein complexes

by Reiner Westermeier and Bob Marchmont, GE Healthcare

Blue-Native Polyacrylamide Gel Electrophoresis (Blue Native PAGE) was originally described by Schägger and von Jagow as a technique for the separation of enzymatically active membrane protein complexes under mild conditions (1). In this variation of gel electrophoresis, the anionic dye Coomassie Brilliant Blue is added to the sample prior to loading and binds to protein complexes during electrophoresis under physiological conditions. The technique has gained interest from researchers focused on functional proteomics in recent years, as it allows the study of protein-protein interactions, and the separation and analysis of very hydrophobic proteins, such as membrane proteins, their complexes, and even super-complexes.

In a further development, Blue-Native PAGE is a fully complementary method to high-resolution two-dimensional electrophoresis and also liquid chromatography of proteins, the most frequently used separation methods in proteomics. The technique is also useful for pre-fractionation of mg amounts of sample for subsequent analysis of smaller protein subsets. In most cases Blue-Native PAGE is combined with a second dimension, which is either a second Blue-Native PAGE after equilibration with a medium-mild detergent, or SDS PAGE for mapping of the related subunits. A comprehensive review of applications of this method has recently been published (2).

Hydrophobic proteins and complexes are first solubilized with a mild nonionic detergent, like Triton X-100 or digitonin. Digitonin, the preferred detergent as it is the mildest, allows the separation of intact super-complexes. Coomassie Blue is added to the sample and cathodal running buffer and remains bound to all hydrophobic proteins and to many water-soluble proteins by hydrophobic interactions even when an electric field is applied. Coomassie Blue is anionic so all protein-dye complexes become negatively charged in the pH 7.5 buffer used, and the complexes migrate towards the anode. Separation of protein complexes occurs according to size in the range 10 kDa to 10 MDa. These protein-dye-complexes are soluble in the absence of detergent, which minimizes the risk of denaturation. Aggregation of the proteins is also prevented because of their overall negative charge. Detection of the proteins and complexes is straightforward as the attached blue dye makes them visible. Porosity gradient gels from 4 to 16% T are employed: this allows large super complexes to enter the gel, prevents small complexes and single proteins from migrating out of the gel, and applies a band-sharpening effect.

After the first dimension electrophoresis is complete, the lanes containing the separated complexes are cut out with a sharp knife or ruler edge, equilibrated in SDS solution, and embedded into a stacking gel layer of a second dimension discontinuous SDS gel. During this process the complexes fall apart into their components (subunits) to form protein-SDS micelles that separate in the SDS gel according their molecular sizes. A Tris-tricine buffer system is preferred over the conventional Tris-glycine gel, because it offers an improved resolution of low molecular weight proteins. Gels containing non-labeled proteins can be stained after the separation with Coomassie Blue, silver stain, or with a fluorescent stains such as Deep Purple or Sypro Ruby.

A more recent development, Blue-Native DIGE, employing Blue-Native PAGE of fluorescent-labeled protein complexes with subsequent SDS PAGE, is perfectly suited for detecting biologically induced changes in proteomics experiments (3). It has the advantage of eliminating gel-to-gel variation. The CyDyes are added to the complexes prior to the Coomassie Blue dye. The different samples labeled with different fluorophores are combined and applied together in one lane. After the second dimension SDS PAGE, the gels are scanned with a multi-fluorescence imager. Results are shown in Figure 1.

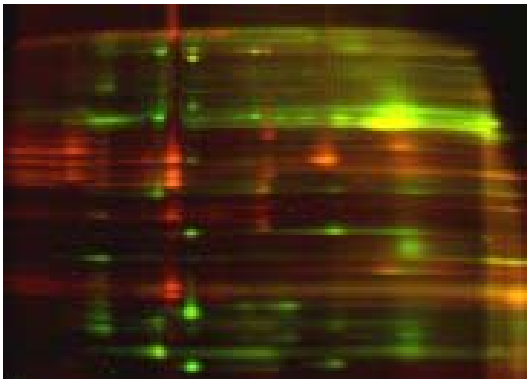

Figure 1: False color display of two fluorescent labeled samples separated with Blue Native DIGE. Complex proteins of Arabidopsis mitochondria (Cy3, green) respiratory system and chloroplasts (Cy5, red) photosynthesis system.

Blue native PAGE gels are interpreted in a different manner then conventional 2-D gels. Vertically aligned spots indicate the protein composition of a protein complex. Larger complexes and super complexes are located on the left hand side of the image.

Blue Native DIGE is a useful additional method for studying membrane proteins and protein complexes and offers some distinct advantages over conventional 2-D electrophoresis or liquid chromatography techniques.

References

1. Schägger, H. and von Jagow, G. (1991) Blue native electrophoresis for isolation of membrane protein complexes. *Anal Biochem*. **199**, 223-231.
2. Krause, F. (2006) Detection and analysis of protein–protein interactions in organellar and prokaryotic proteomes by native gel electrophoresis. *Electrophoresis* **27**, 2759-2781.
3. Perales, M. et al. (2005) Disruption of a nuclear gene encoding a mitochondrial gamma carbonic anhydrase reduces complex I and supercomplex. *J Mol Biol*. **350**, 263-277.
4. Further reading: Reisinger, V. & Eichacker, L. (2006) Analysis of membrane protein complexes by Blue native PAGE. *Practical Proteomics*. **1**, 6-15.

Dr. Reiner Westermeier
reiner.westermeier@ge.com
GE Healthcare

Dr. Robert Marchmont
robert.marchmont@ge.com
GE Healthcare

Origins of the Electrophoresis Society

By Robert C. Allen, AES Historian

President's note: Dr. Robert C. Allen, the first president of the Electrophoresis Society, is an emeritus professor of pathology at the Medical University of South Carolina in Charleston. Dr. Allen developed many techniques for the gel electrophoresis of proteins and nucleic acids and for isoelectric focusing and has written extensively about those topics. He currently resides in Olympia, WA, and serves as a consultant in the clinical investigation Division at the Madigan Army Medical Center in Tacoma. The following article is Dr. Allen's account of the founding of the Electrophoresis Society.

The genesis of the Electrophoresis Society was the "First Small Conference of Specialists of PAGE and PAGIF" convened by Dr. H. Rainer Maurer and me in Tübingen, Germany, October 6-7, 1972. There were 80 invited participants from Europe and the USA who were funded by the Erwin – Riesch – Stiftung non-profit organization and EMBO and hosted by the Max Planck Institute of Tübingen. This meeting followed a meeting the previous spring of the New York Academy of Sciences on isoelectric focusing. A potential founding group got together in Tübingen and gave some thought to starting an Electrophoresis Society, but little was done at the time toward this goal. The Tübingen conference came to be known as the "Blue Fingers Meeting," a name derived from a poster designed by Dr. Maurer's wife, Sigred. A participant list from the 1972 meeting is below.

K. Abraham	O. Kling
R. C. Allen	H. R. Maurer
N. Catsimpoolas	C. J. O. R. Morris
A. Chrambach	S. Nees
W. Dames	V. Neuhoff
K. Felgenhauer	G. Philipps
W. Giebel	P. Pogacar
D. Graesslin	E. G. Richards
A. Griffith	R. Rüchel
U. Grossbach	D. Rodbard
H. Haglund	H. Stegemann
H. Hoffmeister	L. Strauch
R. Hunter	J. Uriel
T. Jovin	G. Utermann
G. Kapadia	O. Vesterberg

Drs. Nick Catsimpoolas and Ann Griffith, also participants at the Tübingen conference, convened a meeting at MIT in Boston in 1978. At the MIT meeting, a core group came together and decided that a society for electrophoresis was a worthwhile pursuit.

I wrote to Drs. Andreas Chrambach and Nick Catsimpoolas in March 1979 to ask them if they would consent to be included on a form letter to sound out interest in an Electrophoresis Society. They agreed, and the three of us sent out a jointly-signed letter to 100 of the attendees from the previous meetings as well as to other colleagues in the field. With one exception, we received positive replies and subsequently a founding group, consisting of Drs. Olaf Vesterberg, Bertold Radola and Pier Giorgio Righetti from Europe and Nick Catsimpoolas, Andreas Chrambach and me, acted to formally set up the society. Thus, we announced the formation of the society at the Electrophoresis Forum convened in the Fall of 1979 by Dr. Radola in Munich, Germany. After the meeting Dr. Radola and I met with Dr. Grunewald of Verlag Chemie to draw up an agreement for the Journal *Electrophoresis*.

I returned to the USA and drew up bylaws and a constitution for the new society based on those of the Histochemical Society, where much of the early starch and acrylamide electrophoresis of isoenzymes in the USA was presented in the middle and late1960s. By April 1980 the new society had some 100 members. As acting Secretary/Treasurer, I sent out ballots to choose a President, Vice President, and Council. Ballots were counted independently by Drs. Catsimpoolas and Griffith. I was elected President and Dr. Volker Neuhoff was elected Vice President. The council consisted of Bertold Radola, Pier Giorgio Righetti, Olaf Vesterberg, Phillipe Arnaud, Andreas Chrambach, and Nick Catsimpoolas.

First Meeting in 1981. From left to right: Berthold Radola, Technical Univ. Munich; Rainer Maurer, Free Univ. of Berlin; Robert Allen, Medical Univ. South Carolina; Hans Heidrich, Max Plank in Munich; and Hidimatsu Hirai, President of the Japanese Electrophoresis Society. Sand Dollars were used as badges for the officers.

From left to right: Jim Drysdale, Boston Univ., Olof Vesterberg, Natl. Board of Occupational Safety and Health -Solna Sweden; Bob Allen, Pier-Giorgio Righetti, Univ. of Milan; and Berthold Radola.

The Electrophoresis Society was incorporated as non-profit organization in South Carolina and I served as Secretary/Treasurer until 1988, with Mrs. Sue Haskill and later Mr. Mike Lack as administrative assistants. The first issue of *Electrophoresis* came out later in 1980 with Dr. Radola as Editor in Chief and me as Associate Editor.

Dr. Robert Allen
boballenion@comcast.net
AES Historian
One of the AES founders

CONTACT: MATT

Matt Hoelter, Executive Director
American Electrophoresis Society
Email: matt-aes@tds.net

Nancy Kendrick
Newsletter Editor
Email: nancy@kendricklabs.com

1202 Ann St
Madison, WI 53713
Phone: 608-258-1565
Fax: 608-258-1569

Web Master Change:

Dr. Erich Gombocz
Vice President, Chief Science Officer
IO-Informatics, Berkeley, CA

Dr. Gombocz resigned early this year as AES web master. He single-handedly set up our first web page on May 1, 2000, and was instrumental in the creation of the current one. The society owes Erich many, many thanks!

Dr. Adrienne Minerick

Assistant Professor, Chemical Engineering
Mississippi State University

Dr. Minerick, who volunteered for the position of AES webmaster, was approved by the AES council during the September 2007 teleconference. She will begin in November when her position as AES councilor expires. Many thanks to Adrienne as well!

Results of AES 2007 Councilor Election

The single councilor seat that was open for election has been filled. We are once again fortunate that the applicant, Dr. Tom Berkelman, from Bio-Rad Labs, approved by the standing council, is of the highest caliber. Tom will be formally approved at the Salt Lake City meeting in November.

Dr. Tom Berkelman
Thomas_Berkelman@Bio-rad.com

Education: University of California, Davis, Ph.D. in biochemistry, 1990
Cornell University, B.A. cum laude in biochemistry, 1984

Biosketch:

I have worked in the life sciences tools industry since 1995 developing reagents and instrumentation for electrophoresis and proteomics, and currently hold a position as Senior Staff Scientist in the Life Sciences Group at Bio-Rad Laboratories. Former scientific positions were at Lynx Therapeutics and Amersham Pharmacia Biotech.

My career has been focused on electrophoretic methods including working on projects to develop equipment and reagents for IEF and proteomics, and writing several book chapters on various aspects of IEF and 2-D electrophoresis, along with a widely used manual for the latter. I have conducted seminars and workshops in 2-D electrophoresis and preparative IEF. In all of my positions, I have served as a resource for solving technical problems and aiding researchers in learning and applying new techniques. More recently, my focus has been on research involving fluorescent reagents for detection and quantitation of proteins during and following electrophoretic separations. This has lead to three patent applications in the area. My ongoing interests include the development of novel separation techniques that allow greater depth of proteome coverage and the development of detection methods that discriminate on the basis of protein functionality or modification.

My goals as councilor include learning about and generating greater awareness of the diversity of electrophoretic techniques available to researchers and the various biological problems that can be addressed through them.

T3000 Field Trip to the Center of Excellence for Biomedical Microfluidics at the University of Utah: University of Utah professor, Bruce Gale, Director, writes "Our center is dedicated to the discovery, understanding, and commercialization of microscale and MEMS devices for application to biological, biomedical, and medical problems. Come along on the field trip and see what we're about." Sign up for item T2 ($15.00) on the registration form to reserve a slot.