MSB 2009

23rd International Symposium on MicroScale Bioseparations

February 1-5, 2009
Boston Park Plaza Hotel & Towers
Boston, MA USA

Final Program & Book of Abstracts

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University of Illinois, Urbana, Illinois USA

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Registration

The MSB 2009 Symposium Registration Desk will be open according to the following schedule located on the Mezzanine Level of the Boston Park Plaza Hotel & Towers.

Saturday, January 31	13:00	-	17:30
Sunday, February 1	08:00	-	17:30
Monday, February 2	07:30	-	17:30
Tuesday, February 3	07:30	-	17:30
Wednesday, February 4	07:30	-	17:30
Thursday, February 5	07:00	-	12:00

Symposium Proceedings

The Journal of Chromatography A will publish original research contributions from this Symposium. In order to manage the manuscripts as fast and efficiently as possible, you are requested to submit your manuscript (please follow the instructions to authors which can be found on http://ees.elsevier.com/chroma) via the journal's online submission system, which is accessible via the journals' website at http://ees.elsevier.com/chroma. Once in the system, please select MSB 2009 as the Article Type when submitting to this symposium issue. Please mention in your cover letter that your paper is intended for the MSB 2009 special issue. Manuscripts must be submitted by March 6, 2009 to be considered for inclusion in the proceedings volume and will be subject to the usual stringent refereeing procedure of the journal. All registered participants will get 6 months free access to the on-line issue.

The MSB Symposium Series

Previous and future MSB Symposia are listed below together with their corresponding Chairs.

1st:	1989	Boston	B.L. Karger
2nd:	1990	San Francisco	B.L. Karger
3rd:	1991	San Diego	J. Jorgenson
4th:	1992	Amsterdam	F. Everaerts
5th:	1993	Orlando	B.L. Karger
6th:	1994	San Diego	S. Terabe
7th:	1995	Wuerzburg	H. Engelhardt
8th:	1996	Orlando	B.L. Karger
9th:	1997	Anaheim	W.S. Hancock
10th:	1997	Kyoto	S. Terabe
11th:	1998	Orlando	B.L. Karger, S. Fanali
12th:	1999	Palm Springs	E. Yeung
13th:	2000	Saarbrücken	H. Engelhardt
14th:	2001	Boston	B.L. Karger, W.S. Hancock
15th:	2002	Stockholm	D. Westerlund
16th:	2003	San Diego	A. Guttman, A. Paulus
17th:	2004	Salzburg	W. Lindner
18th:	2005*	New Orleans	J.M. Ramsey
19th:	2005	Kobe	Y. Baba, K. Otsuka
20th:	2006	Amsterdam	G. Rozing
21st:	2007	Vancouver	R. Kennedy
22nd:	2008	Berlin	A. Manz
23rd:	2009	Boston	J. Sweedler
24th:	2009	Dalian, China	H. Zou
25th:	2010	Prague	F. Foret

** HPCE becomes MicroScale Bioseparations (MSB)*

Student Travel Grants

The Organizing Committee is pleased to have been able to provide 11 students from the universities listed below with grants to help defray the costs of attending MSB 2009. The Committee wishes to acknowledge generous donations from our sponsors who helped fund these grants.

Georgia Institute of Technology
Institute of Analytical Chemistry, ASCR, v.v.i.
Institute of Microanalytical Systems, Zhejiang University
Kyoto University
Northwestern University
Oregon State University
University of British Columbia
University of California at Berkeley
University of Washington
University of Minnesota

Workshops

Two half-day workshops will be held on Sunday, February 1, 2009.

Location: Arlington Room – Mezzanine Level.

Sunday, February 1, 2009 – 09:00 – 12:30

Micro- and Nanofluidics: Fundamentals to Separations

J. Michael Ramsey, *University of North Carolina, Chapel Hill, NC, USA*
Stephen C. Jacobson, *Indiana University, Bloomington, IN, USA*

This workshop is geared toward scientists new to the fields of microfluidics and/or nanofluidics, but at any stage of their career, e.g., graduate students, postdocs, faculty, and industrial scientists. We intend to bring participants up to speed with terminology and basic principles and intersperse examples throughout the workshop to provide context, giving particular attention to liquid phase separations. Microfluidic-based separations benefit from the dexterity with which materials can be manipulated and the ability to fabricate microchannel structures having small volume interconnects. In fact, electrokinetically driven separations on microfluidic devices have generated efficiencies per unit length similar to or exceeding that of conventional capillary separations. We will review what makes microfluidic devices a preferred separations platform, including flow transport and control, electrokinetically- and pressure- driven separations, and multidimensional separation systems. Also, of particular interest is how device function scales when one or more of the conduit dimensions is on a nanometer length scale, i.e., nanofluidic devices, and what advantages, if any, there might be for separations. Some aspects of microfluidic transport and separation transfer directly to operation of smaller nanoscale channels, but nanofluidic systems can be significantly influenced by phenomena such as double layer overlap, surface charge, diffusion, and entropic forces, which are either insignificant or absent in larger microchannels.

Sunday, February 1, 2009 – 14:00 – 17:30

The Power of Coupling Capillary Electrophoresis with Mass Spectrometry: From Ions in Liquid Phase to Ions in Gas Phase

Jeff Chapman, *Beckman Coulter Inc., Fullerton, CA, USA*
David Y. Chen, *University of British Columbia, Vancouver, Canada*

For many, capillary electrophoresis is the separation technique of first choice when analyzing compounds which are highly charged and polar. In its most basic form, capillary zone electrophoresis (CZE), resolves analytes by differences in their size/charge with separations occurring in liquid phase under the influence of an electric field. CZE by its nature is a low flow technique (nl/min) driven by the ionic mobility of the analyte and the electroosmotic flow of the electrolyte. As electrospray is a concentration sensitive ionization technique, the low flow generated by CE provides an ideal match for interfacing to ESI-MS, maximizing sensitivity. This workshop is intended for those who wish to advance their understanding in coupling capillary electrophoresis with mass spectrometry, learning the most up-to-date techniques for interfacing these two technologies. We will describe the fate of an ion from its first introduction into the CE through its ultimate detection in the mass detector, in a simple and understandable way. Through a series of presentations and discussions you will learn how to apply CE-MS routinely in your laboratory, while discussing examples of the analysis of peptides, proteins, carbohydrates, basic drugs, enantiomers and metabolites.

Exhibition

An international exhibition of instruments, accessories, services and publications on genomics, proteomics, and other applications of microscale separations and analysis will be located in the Imperial Ballroom of the Boston Park Plaza Hotel & Towers. The exhibition will run from Monday through Wednesday during the hours listed below. Complimentary refreshments at morning and afternoon breaks will be served there on each of these days. All posters will be displayed in the exhibition area for the entire three days. An evening reception will be held in the Exhibition/Poster room on Tuesday.

Monday, February 2	14:00 – 15:30
Tuesday, February 3	14:00 – 15:30
Wednesday, February 4	14:00 – 15:30

2009 List of Exhibitors

Advanced Analytical
2901 S. Loop Drive
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Technical Program
MSB 2009

Sunday, February 1, 2009

08:00 – 17:30 *Registration on the Mezzanine Foyer*

PLENARY LECTURES in the Georgian Room

CHAIR: Jonathan Sweedler, *University of Illinois at Urbana-Champaign, Urbana, IL USA*

17:00 – 17:15 **Opening Remarks**
Jonathan Sweedler, *University of Illinois at Urbana-Champaign, Urbana, IL USA*

17:15 – 18:00 **Follow-On Biologics – New Challenges and Opportunities for LC/MS and CE/MS**
Barry Karger, *The Barnett Institute, Northeastern University, Boston, MA USA*

18:00 – 18:45 **Driving Biological Discovery Using Separations Coupled to Mass Spectrometry**
John Yates, *The Scripps Institute, La Jolla, CA USA*

18:45 – 19:30 **Microscale Separations: Past, Present and Future**
James W. Jorgenson, *University of North Carolina, Chapel Hill, NC USA*

Plenary Session One
Location: Georgian Room
Chair: Jonathan Sweedler

Sun. 17:15-18:00

Follow-On Biologics – New Challenges and Opportunities for LC/MS and CE/MS

Barry L. Karger, *The Barnett Institute, Northeastern University, Boston, MA USA*

At present, there is great interest in the regulatory and scientific issues involved in developing "generic" protein therapeutics. As in the case of generic versions of small molecule drugs, the goal is to reduce significantly the cost of biopharmaceuticals which can run as high as $100,000/patient per year. However, the problem in producing identical product to that of the innovator is very great, given the complexity of e proteins, e.g. monoclonal antibodies (MW 150 kDa) and the use of non-identical biomanufacturing conditions. One of the major challenges is to develop analytical methods that can characterize in detail follow-on products in order to assess the comparability to commercial products. In this presentation, we shall first overview the current scene for follow-on biologics and some of the critical analytical issues. We will then describe new developments from our laboratory in the areas of LC/MS and CE/MS that aid in the characterization of protein therapeutics. Specifically, we will describe LC/MS approaches using electron transfer dissociation (ETD) to determine disulfide linkages to assess proper protein folding. New methods for carbohydrate analysis of glycoprotein therapeutics will be presented using LC/MS and CE/MS. Rapid isoform separation and analysis of intact glycoprotein therapeutics using CE/MS will also be described. The presentation will be concluded with a discussion on future chromatographic/electrophoric MS methods that could enhance the detailed characterization of follow-on protein therapeutics.

NOTES:

Sun. 18:00-18:45

Driving Biological Discovery Using Separations Coupled to Mass Spectrometry

John Yates[1]; Bryan Fonslow[1]; Chtira Ratnayake[2]; Catherine Wong[1]

[1]The Scripps Research Institute, LaJolla, CA USA; [2]Beckman-Coulter, Fullerton, CA USA

A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional LC separations as well as mass spectrometers have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. We are exploring the use of capillary electrophoresis (CE) and multi-dimensional CE with fast scanning tandem mass spectrometers for separation of complex peptide mixtures derived from digested protein mixtures. Preliminary data from these experiments will be described.

NOTES:

Sun. 18:45-19:30

Microscale Separations: Past, Present and Future

James W. Jorgenson, *University of North Carolina, Chapel Hill, NC USA*

Since the introduction of the capillary column in gas chromatography by Golay more than 50 years ago, there has been a slow but persistent drift toward increasing use of microcolumns for analytical scale separations. In gas chromatography the capillary column has replaced packed columns for most analytical applications. In electrophoresis, capillaries have become a useful substitute for traditional tube and slab gels. CE is also an important alternative to HPLC for analysis of intact proteins. In liquid chromatography conventional scale packed columns remain dominant, while capillary LC columns are currently a niche technology for selected applications, such as LC-MS of peptides. Microfluidic device technologies (chips) are currently having a major impact on the direction of microscale separations in electrophoresis and liquid chromatography. A brief account of the history of microscale separations will be presented. The current status of microcolumns in GC, LC and electrophoresis will be described. The future of microcolumn separations will be governed by a tug-of-war between analytical needs and technological limitations, and some speculation about that future will be offered.

NOTES:

Monday, February 2, 2009

07:30 – 17:30 *Registration on the Mezzanine Foyer*

07:30 - 08:30 *Continental Breakfast in the Imperial Ballroom*

PLENARY LECTURES in the Plaza Ballroom

CHAIR: Norman Dovichi, *University of Washington, Seattle, WA USA*

08:30 - 09:15 **Optical Fiber Arrays for Chemical and Biochemical Analysis**
David R. Walt, *Tufts University, Medford, MA USA*

09:15 – 10:00 **Spatially Selective Measurements at Single Cells**
Andrew G. Ewing, *University of Gothenburg, Göteborg, Sweden*

10:00 – 10:30 *Break - **Visit the Exhibits and Posters: Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in the Plaza Ballroom
~CE-MS ~

CHAIR: Jeff Chapman, *Beckman Coulter, Inc., Fullerton, CA USA*

10:30 – 10:55 **CE-MS : New Possibilities for Profiling of (Bio)Pharmaceuticals**
Gerhardus J. de Jong, *Utrecht University, Utrecht, The Netherlands*

10:55 - 11:20 **Is CE-MS Ready for Commercialization? Development of a Simple CE-MS Technology for Metabolomics, Proteomics, and Single Cell Analysis**
Mehdi Moini, *Texas State University, San Marcos, TX USA*

11:20 – 11:45 **Coupling Capillary Electrophoresis with MALDI Mass Spectrometry for Enhanced Neuropeptide Detection**
Lingjun Li, *University of Wisconsin at Madison, Madison, WI USA*

11:45 - 12:05 **Capillary Electrophoresis-Electrospray Ionization Mass Spectrometry for Metabolomics at the Single Cell Level**
Theodore Lapainis, *University of Illinois at Urbana-Champaign, Urbana, IL USA*

12:05 – 12:25 **Towards Comprehensive Profiling of Polar Metabolites Using Advanced CE-ToF-MS Systems**
Govert W. Somsen, *Utrecht University, Utrecht, The Netherlands*

12:25 - 14:00 *Lunch Break – Attendees on their own ~OR~ Attend the **Technical Seminar sponsored by Agilent Technologies** in the Plaza Ballroom. Lunch is provided for the first 100 attendees only. The seminar runs for one hour - 12:45 to 13:45.*

14:00 – 15:30 ***Poster Session #1 in the Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~Microfluidics 1~

CHAIR: Juan Santiago, *Stanford University, Stanford, CA USA*

10:30 – 10:55 **Microfluidic Devices for Addressing Proteomic Measurements**
J. Michael Ramsey, *University of North Carolina, Chapel Hill, NC USA*

10:55 - 11:20 **Rapid Electrophoresis in Parallel for High-Throughput Analysis**
Robert Kennedy, *University of Michigan, Ann Arbor, MI USA*

11:20 – 11:45 **Hybrid Microfluidics for Pre-Processing and Separations**
Aaron R. Wheeler, *University of Toronto, Toronto, Ontario, Canada*

11:45 - 12:05 **Integration of Gel-Electrophoretic Separation in a Miniaturized Free-Flow Device for Continuous DNA Fractionation**
Dirk Janasek, *ISAS – Institute for Analytical Sciences, Dortmund, Germany*

12:05 – 12:25 **One- and Two-Dimensional Microchip Gel Electrophoresis**
Margaret A. Donoghue, *Indiana University, Bloomington, IN USA*

12:25 - 14:00 *Lunch Break – Attendees on their own ~OR~ Attend the* ***Technical Seminar sponsored by Agilent Technologies*** *in the Plaza Ballroom. Lunch is provided for the first 100 attendees only. The seminar runs for one hour - 12:45 to 13:45.*

14:00 – 15:30 ***Poster Session #1 in the Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in the Plaza Ballroom
~Interfacing to Nanoelectrospray~

CHAIR: Gary A. Valaskovic, *New Objective, Inc., Woburn, MA USA*

15:30 – 15:55 **Twenty Years of Nano-Electrospray**
Matthais Wilm, *UCD – Conway Institute, Dublin, Ireland*

15:55 – 16:20 **LC-MS Below Van Deemter Minima: Analysis of Signaling Cascades in Developmental Biology and Human Disease**
Jarrod Marto, *Dana-Farber Cancer Institute, Boston, MA USA*

16:20 – 16:45 **Flow Rate Limbo in Small Molecule Analysis: How Low Should We Go?**
Luke Utley, *Astra-Zeneca, Waltham, MA USA*

16:45 – 17:05 **Development and Validation of a New High Performance Mass Spectrometer for Fast Chromatography**
Darwin Asa, *Bruker Daltonics, Billerica, MA USA*

17:05 – 17:25 **Optimization of MRM-Based Protein Quantitation in Plasma: Lessons Learned from an Inter-Lab Study**
Susan E. Abbatiello, *The Broad Institute of MIT and Harvard, Cambridge, MA USA*

18:00 – 20:00 ***20th Anniversary/Welcome Reception – Georgian Room***

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~From Separation to Detection~

CHAIR: Aaron R. Wheeler, *University of Toronto, Toronto, Ontario, Canada*

15:30 – 15:55 **Novel Indirect Fluorescence Detection Methods using Isotachophoresis**
Juan Santiago, *Stanford University, Stanford, CA USA*

15:55 – 16:20 **Adventures in Attomole Capillary Isoelectric Focusing**
Norman Dovichi, *University of Washington, Seattle, WA USA*

16:20 – 16:45 **Applications of Gradient Micro Free Flow Electrophoresis**
Michael Bowser, *University of Minnesota, Minneapolis, MN USA*

16:45 – 17:05 **Traveling Wave Electrophoresis and Improved Microchip ESI/MS Interfaces for Proteome Analysis**
Aaron Timperman, West Virginia University, Morgantown, WV USA

17:05 – 17:25 **MicroPrep: On-Chip Subcellular Fractionation using Dielectrophoresis**
Meike Moschallski, *NMI Natural & Medical Sciences Institute, Reutlingen, Germany*

18:00 – 20:00 *20th Anniversary/Welcome Reception – Georgian Room*

Plenary Session Two
Location: Plaza Ballroom
Chair: Norman Dovichi

Mon. 08:30-09:15

Optical Fiber Arrays for Chemical and Biochemical Analysis

David R. Walt; Hans Heiner Gorris; Ragnhild Dragoey Whitaker, *Tufts University, Medford, MA USA*

We have used coherent imaging fibres to make fibre-optic chemical sensors and biosensors. Sensors can be made with spatially-discrete sensing sites for multi-analyte determinations. The arrays can be filled with living cells for creating functional biosensors. Such biosensors have been employed for detecting both geno and cytotoxicities. By confining single cells in each individual microwell, the responses of all the cells in the array can be monitored simultaneously. Each microwell, containing a single living cell, can be used to monitor several physiological and genetic responses simultaneously. Cells can be genetically engineered to express different reporter molecules and used in multiplexed screens. In addition, fundamental studies of cell processes such as cell to cell communication and the stochastic nature of cellular responses can be investigated with the arrays.

In another scheme, the arrays can be used for single molecule detection. In this format, individual molecules, such as enzymes, can be trapped in the microwells by sealing each microwell with a silicone gasket. The enzyme molecules catalyse the formation of a fluorescent product that can be detected readily. The kinetic properties of hundreds to thousands of single enzyme molecules can be monitored simultaneously using this format. Kinetic constants of enzymes can be determined by observing the distribution of individual enzyme substrate turnover rates. In addition, inhibition experiments with single enzyme molecules can be performed. By observing the stochastic nature of the single molecule responses, new mechanistic insights into the fundamental nature of the enzymes can be obtained.

NOTES:

Mon. 09:15-10:00

Spatially Selective Measurements at Single Cells

Andrew Ewing, *University of Gothenburg, Göteborg, Sweden; Penn State University, Univ. Park, PA USA*

We have been developing methods to measure molecules involved in neurotransmission and regulating neurotransmission at the single cell and subcellular level. Developments in three areas of technology have been realized. These include separations with capillary electrophoresis with on-chip detection, spatially selective measurements arrays of microelectrodes to measure and "image" release of transmitters across the surface of a cell, and mass spectrometry imaging of the lipid composition and domains across the cell surface.

Capillary electrophoresis has become an important tool in examining the chemistry of the cell and the separation of subcellular organelles. We have been developing a hybrid capillary-chip device to separate, lyse individual transmitter vesicles and to quantify the transmitters in each vesicle. These results are being used to compare to direct measurements of released transmitter in an effort to determine the fraction of vesicular transmitter released via exocytosis.

Carbon microelectrode arrays have been fabricated with micron size total probe tip and we have characterized and applied these to spatially and temporally resolve neurotransmitter release from single pheochromocytoma (PC12) cells. These carbon MEAs are composed of 7 individually addressable 2.5-mm-radius microdisks drawn to a total tip of under 20 um. The carbon MEAs have been characterized using scanning electron microscopy (SEM), steady-state and fast-scan voltammetry, and numerical simulations. Amperometric results show that subcellular heterogeneity in single-cell exocytosis occurs and can be electrochemically quantified. Concurrent events on different parts of the cell surface have also been shown to occur suggesting a cellular mechanism to coordinate exocytotic events.

We are interested in lipid microdomains and their involvement in regulating exocytosis. A central question here is if domains of lipids are formed to lower the thermodynamics of membrane fusion or if the process of fusion might drive formation of lipid domains – sort of chicken or the egg first type of question. To investigate when the lipid domain forms relative to fusion, we used a model system of membrane fusion – tetrahymena mating by membrane fusion and time-of-flight secondary ion mass spectrometry (ToF SIMS) imaging to spatially identify and relatively quantify the lipids. This is the only technique currently known to provide the chemical specificity and submicron lateral resolution required to directly visualize such domains. We have established that conjugation depends on these morphological changes but the phenomenon of lipid domain formation appears to be dependent on the thermodynamics of the bending of the membranes into pores.

NOTES:

AM Parallel Session: CE-MS
Location: Plaza Ballroom
Chair: Jeff Chapman

Mon. 10:30-10:55

CE - MS: New Possibilities for Profiling of (Bio)Pharmaceuticals

Gerhardus J. De Jong; Paul Hommerson; Rob Haselberg; Govert W. Somsen,
Utrecht University, Utrecht, The Netherlands

Capillary electrophoresis (CE) is a very efficient separation technique. In order to further extend its applicability, coupling to mass spectrometry (MS) is essential to allow sensitive detection and molecular structure characterization. Currently, CE–MS via electrospray ionization (ESI) is an accepted analytical approach, however, a major limitation of CE–ESI-MS is that only volatile CE background electrolytes (BGE) can be employed. Furthermore, effective ESI is limited to relatively polar compounds that can be (de)protonated easily. In order to gain insight into the specific features and usefulness of ionization techniques as atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI), we have performed a comprehensive study of CE-APCI-MS and CE-APPI-MS including comparison with CE–ESI-MS. Practical examples will be shown to outline the applicability of the CE-MS systems for drug profiling.
The production of pharmaceutical proteins has expanded enormously. Capillary electrophoresis (CE) is an attractive technique for purity and stability analysis of proteins offering efficient and fast separations and requiring only small sample amounts. Changes in protein charge and shape are reflected in the electrophoretic mobility and can thus be monitored. This presentation outlines a robust CE methodology for the characterization of pharmaceutical proteins. It will be shown that a bilayer or triple layer of charged polymers provides a fast and straightforward means to produce an effective capillary coating. Such a charged coating ascertains a strong and constant electro-osmotic flow and minimizes protein-wall interactions yielding high separation efficiencies. Special attention will be paid to the coupling with time-of-flight (TOF)-MS which offers high mass accuracy. Overall, the system shows high potential for the reproducible and quantitative profiling of complex protein mixtures. The applicability of CE-MS methods will be demonstrated by the stability monitoring of biopharmaceuticals such as human growth hormone (hGH) and insulin.

NOTES:

Mon. 10:55-11:20

Is CE-MS Ready for Commercialization? Development of a Simple CE-MS Technology for Metabolomics, Proteomics, and Single Cell Analysis

Mehdi Moini, Texas State University, San Marcos, *TX USA*

Recent advances in capillary electrophoresis mass spectrometry interfacing using a porous tip and CE capillary coating using self-coating background electrolyte have significantly simplified the utilization of sheathless CE/ESI-MS for the analysis of complex biological mixtures. Porous tip is a robust, reproducible, and single-step design that allows automated fabrication of the CE-MS interface. On the other hand, self-coating background electrolytes with ESI-MS transparent polyamines have eliminated time consuming and labor intensive capillary coating procedures for proteomics analysis. Elimination of these two major obstacles has raised the interest for commercialization of sheathless CE/ESI-MS not only as a complimentary technique to nano-LC-MS, but in many areas as a superior alternative to it. For example, in a proteomics study, low sample consumption, rapid analysis time, and facile change of the background electrolyte allow multiple injection-separation CE-MS cycles while changing experimental variables after each cycle to maximize sequence coverage. Experimental variables include mass range (gas phase fractionation), background electrolyte pH or composition (multi-dimensional analysis), sample concentration, ionization polarity, CE polarity, etc. Moreover, in the analysis of protein complexes, compatibility of CE with a variety of background electrolytes at a wide pH range allows separation of intact protein complexes under native conditions directly from cell lysates. By separating analytes not associated with the complex that could otherwise be attached to the complex during electrospray ionization process, CE-MS provides a more accurate representation of the constituents of the complex. In the analysis of the chemical contents of intact cells, CE-MS is an ideal tool, where different capillary sizes can be utilized to match with different cell sizes, thereby minimizing sample dilution for optimum sensitivity. In addition, cell contents from amino acids to peptides, proteins, and intact protein complexes can be detected with the same capillary. These advantages have significantly raised the interest level in commercialization of the sheathless CE/ESI-MS, and the availability of a commercial sheathless CE-MS product in the next year does not seem to be far fetched.

NOTES:

Mon. 11:20-11:45

Coupling Capillary Electrophoresis with MALDI Mass Spectrometry for Enhanced Neuropeptide Detection

Lingjun Li; Junhua Wang, *University of Wisconsin-Madison, Madison, WI USA*

MALDI MS is a powerful tool for neuropeptide analysis in direct tissue profiling and imaging experiments. However, interferences from high salt and lipid content in the tissue samples often lead to incomplete and reduced peptidome coverage. Here, we explore the coupling of capillary electrophoresis (CE) separation with high resolution MALDI Fourier transform mass spectrometry (FTMS) for improved detection of neuropeptides in decapod crustacean nervous systems.
Specifically, an off-line interface incorporating sheathless flow and counter-flow balance is developed. The new interface provides excellent performance due to the integration of three aspects: (1) A porous polymer joint constructed near the capillary outlet for the electrical circuit completion has simplified the CE interface by eliminating a coaxial sheath liquid and enables independent optimization of separation and deposition. (2) The electroosmotic flow at reversed polarity (negative) mode CE is balanced and reversed by a pressure-initiated capillary siphoning (PICS) phenomenon, which offers improved CE resolution. (3) The pre-deposited nanoliter volume DHB spots on Parafilm-coated MALDI sample plate offers improved substrate for effective effluent enrichment. Compared with direct MALDI MS analysis, CE separation followed by MALDI MS detection consumes nearly 10-fold less material while exhibiting 5 to 10-fold enhancement in S/N ratio. To further simplify the coupling of CE and MALDI FTMS, we employed a common MALDI matrix, 2,5-dihydroxybenzoic acid (DHB) as a novel background electrolyte (BGE) for CE separation. This concurrent use of DHB in CE separation buffer and subsequent MALDI MS detection as well as one-step extraction of neuropeptides has simplified peptide extraction and offered enhanced analytical sensitivity and spectral quality. In addition, via the characteristic migration behaviors in CE, some specific structural and chemical information of the neuropeptides such as posttranslational modifications and family variations can be visualized, making the off-line CE-MALDI MS a promising strategy for enhanced neuropeptidomic profiling.

NOTES:

Mon. 11:45-12:05

Capillary Electrophoresis-Electrospray Ionization Mass Spectrometry for Metabolomics at the Single Cell Level

Theodore Lapainis; Stanislav Rubakhin; Jonathan V. Sweedler,
University of Illinois at Urbana-Champaign, Urbana, IL USA

Capillary electrophoresis (CE) with electrospray ionization mass spectrometric detection (ESI-MS) is a technique that has much potential for metabolomics; CE provides high resolution separations of small charged molecules, and ESI-MS enables the detection and characterization of eluting analytes.

For metabolomic profiling to be successful, a robust system is required so that run-to-run variations reflect real differences between samples, not instabilities in instrumental performance. Here, we describe a sensitive and robust CE-ESI-MS system, and demonstrate its utility for metabolomics applications by using it to investigate the chemical make-up of individual neurons.

The system uses a home-built coaxial sheath-flow interface to complete the electrical circuit required for CE. A nebulizer-free format is used, and a Taylor cone is maintained at the outlet. The outlet end of the capillary is tapered in order to minimize dilution of eluting analytes within the Taylor cone. A 3-axis translation stage allows for the position of the CE outlet to be optimized in terms of signal intensity and electrospray stability.

After optimization and characterization of the CE-ESI-MS interface, individual identified neurons from the central nervous systems of *Lymnaea stagnalis* and *Aplysia californica* have been analyzed. The cells are isolated and analytes are extracted in a minimum volume of a mixed organic/aqueous solution. The samples are then injected hydrodynamically into the separation capillary. Formic acid is used as the background electrolyte for CE separation. Limits of detection for a number of relevant small molecules are in the low nanomolar range. Total ion electropherograms from our single cell samples exhibit numerous peaks. Tentative identifications use migration times and molecular mass. We detect many known signaling molecules including dopamine, serotonin, and acetylcholine. Future work involves interfacing the system to a mass spectrometer that provides tandem MS capabilities, and characterizing the neurotransmitters in cells of well-studied neuronal networks.

NOTES:

Mon. 12:05-12:25

Towards Comprehensive Profiling of Polar Metabolites Using Advanced CE-ToF-MS Systems

Govert W Somsen[1]; Rawi Ramautar[1]; Oleg A Mayboroda[2]; André M Deelder[2]; Gehardus J de Jong[1]

[1]Utrecht University, Utrecht, The Netherlands; [2]Leiden University Medical Centre, Leiden, The Netherlands

Metabolic profiling of body fluids provides essential insights into the physiological state of an organism and can reveal potential biomarkers of diseases. Analytical separation techniques currently used in metabolomics lack good coverage of highly polar metabolites. Coupled capillary electrophoresis time-of-flight mass spectrometry (CE-ToF-MS) is a very attractive tool for profiling of ionogenic compounds. Capillary electrophoresis (CE) offers fast and efficient separations requiring only minute amounts of sample. Time-of-flight mass spectrometry (ToF-MS) presents high mass accuracy and resolution at a speed compatible with narrow CE peak widths. Application of CE-ToF-MS, however, may be hindered by reproducibility and coverage problems.

In this presentation a novel strategy for reproducible and comprehensive metabolic profiling by CE-MS will be outlined. Very stable CE performance is accomplished by use of noncovalent capillary coatings of charged polymers. These easy-to-produce coatings provide high migration-time repeatabilities and good tolerance against sample matrix compounds. It will be demonstrated that coating and separation conditions can be selected such that fast and comprehensive coverage of all positively and negatively charged metabolites is achieved in only two CE runs. Incorporation of in-capillary preconcentration by pH-mediated stacking further aids the detection of low-level metabolites.

The applicability and usefulness of the developed CE-ToF-MS systems will be illustrated by the elucidation of metabolites involved in complex regional pain syndrome (CRPS) and urinary tract infection (UTI). CE-ToF-MS data acquired from urine of CRPS and UTI patients was compared with data from control subjects. Multivariate statistical analysis of the recorded profiles revealed biomarker candidates, which were subsequently identified by accurate mass and/or MS-MS using QToF-MS. In addition it will be shown that the CE-ToF-MS method also allows metabolic profiling of plasma from drug-treated mice.

NOTES:

AM Parallel Session: Microfluidics 1
Location: Arlington/Berkeley/Clarendon Rooms
Chair: Juan Santiago

Mon. 10:30-10:55

Microfluidic Devices for Addressing Proteomic Measurements

J. Michael Ramsey, *University of North Carolina, Chapel Hill, NC USA*

We have been developing one- and two-dimensional (2D) microfabricated fluidic separation devices that implement capillary electrophoresis (CE) and liquid chromatography (LC), in addition to including integrated electrospray ionization (ESI) for subsequent mass spectral analysis. Microchip-based separation devices have demonstrated speed advantages as great as two orders of magnitude over conventional approaches while generating equivalent numbers of theoretical plates. We have also demonstrated an integrated ESI source on our microfluidic devices that produces ion signal and stability equivalent to commercial nanospray sources. This ESI source has been monolithically integrated with microchip CE to generate nearly 300,000 theoretical plates for peptides and proteins. More recently we have coupled LC with CE devices to produce 2D in time LC-CE data, using two different strategies. One strategy utilizes a hybrid approach where a packed capillary is coupled to the microchip CE-ESI device. The second strategy involves monolithic integration of a packed LC channel with the CE-ESI architecture. The performance of these devices has been evaluated using various peptide and protein samples. Device design and performance will be discussed and potential applications discussed.

NOTES:

Mon. 10:55-11:20

Rapid Electrophoresis in Parallel for High-Throughput Analysis

Robert T. Kennedy, *University of Michigan, Ann Arbor, MI USA*

The throughput of electrophoretic analysis has typically been improved using two main approaches. Miniaturization of electrophoresis channels allows application of high electric fields over short distances to reduce separations times to a few seconds or less. This approach has been mostly used for "sensing" applications where concentration of separated and detected substances is monitored over time. Advances in fabrication and detection technology have also allowed development of systems with parallel channels for improved throughput. This latter technology has mostly been applied to genetic analysis. We have combined rapid separations on microchips with parallel architectures (up to 48 channels) for a substantial improvement in overall throughput, up to ~17,000 assays/hour. This high throughput allows a variety of new applications. In one example, 36 individual enzymatic reactions are monitored at 10 s intervals using rapid, serial electrophoresis in parallel. This system allows, for example, optimization of enzymatic conditions or determining rate constants in a single experiment. Another application is to monitor cellular secretions from discrete tissue samples over time in parallel. We have shown the utility of a 15-channel system for monitoring insulin secretion from 15 individual islets of Langerhans. Both of these cases monitor concentration changes that are generated within sample chambers on the chip. A more difficult problem is introducing discrete samples to a chip for high-throughput analysis. We are exploring use of segmented flows, where aqueous samples are manipulated as droplets within an immiscible carrier fluid, as a method of preparing and introducing discrete samples to electrophoretic channels. Such a system would allow samples to be pumped into the chip for injection with minimal carry-over for continuous introduction and separation of discrete samples. This system may have application to high-throughput screening.

NOTES:

Mon. 11:20-11:45

Hybrid Microfluidics for Pre-Processing and Separations

Aaron R. Wheeler; Michael W. Watson; Mohamed Abdelgawad, *University of Toronto, Toronto, Canada*

Microchannels have revolutionized analytical separations, facilitating fast analyses with higher resolution, higher efficiency, lower power, and lower reagent consumption than their column-based counterparts. Additionally, microchannels have been used for “on-chip” sample processing, a necessary (but tedious) step required for myriad applications. Unfortunately, integration of sample processing is challenging in microchannels due to the difficulty in maintaining spatial control over many reagents simultaneously. In contrast, the format of digital microfluidics (DMF), in which discrete droplets are manipulated on an open platform, is well-suited for carrying out sequential chemical reactions. Here, we report the development of a hybrid method for sample processing and separations, which leverages the advantages of chemical processing in DMF with the unmatched separations capabilities in microchannels.

The new device is formed from a poly(dimethyl siloxane) (PDMS) substrate containing a network of microchannels mated to an array of actuation electrodes for DMF. We have used this device in several proof-of-principle applications, including on-chip fluorescent labeling of amino acid standards and cell lysates and on-chip digestion of fluorescently labeled proteins. The separations generated using the new devices have identical characteristics (i.e., plate height, peak area/retention time reproducibility, etc.) as separations carried out in conventional devices.

In on-going work, we are applying the new method to proteomic processing – with on-chip precipitation, reduction, alkylation, and enzymatic digestion followed by separations of the processed peptides. Given the myriad applications requiring pre-processing and chemical separations, the digital-channel hybrid geometry has the potential to become a powerful tool for micro total analysis systems.

NOTES:

Mon. 11:45-12:05

Integration of Gel-Electrophoretic Separation in a Miniaturized Free-Flow Device for Continuous DNA Fractionation

Dirk Janasek; Cristina Pelaez-Lorenzo, *ISAS - Institute for Analytical Sciences, Dortmund, Germany*

A new approach of a continuous separation technique in free-flow employing size separation by means of gel-electrophoresis in a miniaturized device is presented for the first time.

In contrast to the discontinuous technique of capillary electrophoresis, in free-flow electrophoresis (FFE) the time domain is transferred to a spatial domain which allows a continuous separation. Miniaturized FFE has provided the unique opportunity to combine separation in preparative scale with fast data aquisition.

The feasibility of the simultaneous combination of µ-FFE with several modes of electrophoretic separation has been demonstrated previously [1,2].
Here, the integration of gel-electrophoretic separation with FFE at the microscale is reported for the first time. The most innovative challenge was the creation of a media for size separation inside the microstructure. Among different approaches assayed, a dynamic sieving matrix formed with the continuous supply of polyethylenoxide (PEO) was the most convenient. In contradiction to the recent published article of Zeng et al [3] in which voltage is applied for sample injection we demonstrate an injection by pressure.

The microdevice consists of a 12 mm × 4 mm chamber connected to the electrode reservoirs via microchannels. By the application of a voltage of around 7000 V across the compartment and a linear flow of sample and buffer of 0.8 µl/min and 3 µl/min, respectively, a separation of a low range DNA ladder was obtained.

Our studies are very promising in the investigation of sorting DNA samples as well as proteins.

1. Y. Xu, C.-X. Zhang, D. Janasek, A. Manz. Lab Chip 3 (2003) 231.
2. D. Janasek, M. Schilling, J. Franzke, A. Manz. Anal. Chem. 78 (2006) 3815.
3. Y. Zeng, M. He, D.J. Harrison. Angew. Chem. Int. Ed. 47 (2008) 1.

NOTES:

Mon. 12:05-12:25

One- and Two-Dimensional Microchip Gel Electrophoresis

Margaret A. Donoghue; Stephen C. Jacobson, *Indiana University, Bloomington, IN USA*

The analysis of biologically relevant molecules on microfluidic devices is of interest to many, as these devices offer a number of advantages over traditional separation techniques, including faster analysis times and better separation performance. We are interested in developing microfluidic devices for the high-performance separation of proteins, both in one and in two dimensions. Gel electrophoresis is incorporated on a glass microfluidic device with a spiral or serpentine analysis channel, and the electric field strength and buffer conditions are evaluated to determine the best performance. For detection, on- and off-chip methods for protein labeling are investigated to achieve highest detection sensitivity. After optimizing these separations in one dimension, they will be integrated onto microfluidic devices for two-dimensional (2D) separations. The basic design of these 2D chips is a planar format, consisting of a single first dimension channel intersected by an array of 32 parallel second dimension channels. Key design features, including electrokinetic flows adjacent to the sample flow and the close proximity of the orthogonal channels, minimize sample dispersion and leakage in the sample transfer region. The supporting electrokinetic flows are provided from 80 μm wide control channels placed to the left and right of the 1D and waste channels. Simple electrophoretic separations have been conducted independently in the two dimensions, showing good band shape in the transfer region and reproducible plug movement in the parallel channel array. To detect the separated components simultaneously, a laser beam is expanded in one dimension using a Powell lens to illuminate a narrow band on multiple parallel channels. Device design, sample transport in the first and second dimensions, as well as improved fluorescence detection, and separations on one- and two-dimensional chips will be presented.

NOTES:

Technical Seminar – 12:45-13:45
Location: Plaza Ballroom
Sponsored By: Agilent Technologies

Agilent in MicroScale Bioseparations: Recent Advances in CE and LC-MS-based Detection Capabilities & Chemistries

Determination of Impurities in Heparin by Capillary Electrophoresis

Todd Wielgos, Karalyn Havel and Nadia Ivanova, Baxter Healthcare Corporation,
Robert Weinberger, CE Technologies Inc., USA

The heparin contamination crises drove the development of emergency methods to ensure that contaminated material did not enter the world's supply. One of these methods employs capillary electrophoresis. Here, we will provide an update on how CE can provide most stringent information on heparin quality and purity.

HPLC-Chip: New Application Frontiers

Dayin Lin, Martin Vollmer, Stephan Buckenmaier and Tom van de Goor,
Agilent Technologies, Waldbronn, Germany

Two new applications will be discussed for this update seminar. With Ultra-high Capacity (UHC) HPLC-Chip, Pharma customers can now consider HPLC-Chip in DMPK studies. This new HPLC-Chip application offers comparable sensitivity and throughput with much lower injection volume. Another new product, Phospho-Chip, offers a Titanium Dioxide trapping column in the chip design. Phospho-Chip provides researchers a weapon in phosphorylated peptide analysis

Advances and New Products in Agilent's Electrophoresis Portfolio

Gerard Rozing and Tobias Preckel, Agilent Technologies, Waldbronn, Germany

Agilent Technologies is a premier provider for electrophoresis equipment and leads the market with respect to CE-MS technology. The talk will provide an update on CE applications and instrumentation. Achieving highest MS sensitivity when analyzing complex protein or peptide samples, often requires prefractionation of samples. Effective strategies will be presented here. In addition, new detection devices and chemistries for high sensitivity protein detection will be presented.

PM Parallel Session: Interfacing to Nanoelectrospray
Location: Plaza Ballroom
Chair: Gary Valaskovic

Mon. 15:30-15:55

Twenty Years of Nano-Electrospray

Matthias Wilm, *UCD-Conway Institute, Dublin, Ireland*

The nano-electrospray ion source became widely known because it allowed very sensitive sequencing of peptides and opened many practical applications in biological research. The motivation to build it however lay in its underlying scientific concept: a model of the electrospray ionization process. Experiments with the first nano-electrospray built for surface preparation in 1988/89 at the University of Münster in the department of experimental physics had demonstrated that this electrospray could generate vanishingly small particles even under electron-microscopic inspection. The accompanying theoretical model supported the idea that it should be possible to build an electrospray source that could generate droplets so small that they would contain on the average only one biomolecule. Interface with a mass spectrometer such an electrospray might release intact individual biomolecules as ions into the vacuum system as long as complete desolvation could be achieved.

Three years later the concept could be realized in form of the nano-electrospray ion source at the European Molecular Biology Laboratory (EMBL) in Heidelberg and let amongst others to the ability to identify and sequence peptides in a very sensitive way.
Many technical features that we see today realized on electrospray mass spectrometers can be explained by following the theory that large biomolecules are generated only from very small, highly charged droplets:

- the requirement to mount electrospray sources under atmospheric conditions,
- the off-center to orthogonal alignment of high-flow electrospray sources
- the tuned desolvation in the inlet-system of mass spectrometers
- the ability to see non-covalent complexes in a mass spectrometer
- the drive towards low-flow HPLC systems
- and finally the move towards direct quantitation without referring to isotopically labeled standards

The talk will give a short retrospect of the experiments that let to the construction of the nano electrospray source and will than show current efforts to bring one of its promises to life - systematic quantifications in biological experiments.

NOTES:

Mon. 15:55-16:20

LC-MS Below Van Deemter Minima: Analysis of Signaling Cascades in Developmental Biology and Human Disease

Jarrod A. Marto, *Dana-Farber Cancer Institute, Boston, MA USA*

Liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS), has become the technique of choice for rapid identification and characterization of proteins in biological systems. Current trends towards analysis of increasingly complex mixtures (biomarkers, signaling pathways, etc) drive innovation in various areas of biological fractionation, enrichment, and chemical modification, all with the goal of improved detection of low abundance proteins or rare post-translational modifications. Unfortunately biological samples enriched by cell type, protein class, or specific post-translational modification, often contain a concentration dynamic range that exceeds the capabilities of current LC-MS instruments.

We speculated that fabrication of assemblies that supported arbitrary combination of column dimension, effluent flow rate, and integrated emitter tip size would facilitate exploration of LC-MS parameters heretofore inaccessible and ultimately provide a viable path for improved detection limit and dynamic range in mass spectrometry-based proteomics. We recently developed a protocol for construction of fused silica capillary LC columns with integrated electrospray emitters. Our protocol readily supports various column/particle size combinations, to date down to 10 µm I.D. capillaries packed with sub-2 µm diameter particles. With minimal practice columns can be made in 30 minutes or less, and used for several weeks without failure.

Thorough characterization of these LC-electrospray assemblies yielded unexpected insight into the otherwise divergent trends in chromatographic resolution and electrospray ionization efficiencies expected in low flow regimes. In brief we observe a greater than 10-fold improvement in data quality for LC-MS-based proteomics analysis of complex biological mixtures at effluent flow rates below observed Van Deemter minima. Our results provide compelling evidence that improved electrospray ionization efficiency compensates for diminished chromatographic performance at effluent flow rates below 5 nL/min.

We are currently using these assemblies in conjunction with stable isotope labeling for LC-MS based comparative analysis of tyrosine signaling of biological pathways that drive normal development and human disease. For example we used quantitative proteomics and biochemical assays to identify focal adhesion signaling as a novel and conserved pathway for normal differentiation of both mouse and human embryonic stem cells. In separate studies, we used our miniaturized LC assemblies to identify specific points of divergent signaling downstream of common, constitutively active mutants of Flt-3 kinase that drive transformation in acute myeloid leukemia. Collectively our analytical and biochemical data suggest that a renewed focus on miniaturized LC coupled to ultra-low flow electrospray will provide significant gains in proteomics-based analysis of primary cells and rare post-translational modifications.

NOTES:

Mon. 16:20-16:45

Flow Rate Limbo in Small Molecule Analysis: How Low Should We Go?

Luke Utley, *AstraZeneca, Waltham, MA USA*

Until recently analytical chromatography flow rates of <100 uL/min had largely been confined to proteomic studies. Small molecule analysis seemed headed in the opposite direction with the development of sources that could accommodate flow rates of over 1 ml/min. This has changed in the last five years with the discovery of the nano-electrospray equimolar response phenomenon exhibited by closely related structures. Combined with improvements in low-flow pumping, this have caused small molecule researchers to reexamine the benefits of lower flow rates when incorporated into LC/MS studies. This talk will explore the advances and applications of nano-electrospray in small molecule research.

NOTES:

Mon. 16:45-17:05

Development and Validation of A New High Performance Mass Spectrometer for Fast Chromatography

Darwin Asa, *Bruker Daltonics, Billerica, MA USA*

In many analytical laboratories, fast chromatography with its increased throughput and resolution is becoming or on the way to becoming the norm. However, the state of the art in Mass Spectometry, especially with instruments designed to identify unknowns or de-convolute complex mixtures, suffers from a decrease in instrument resolution as the speed of the separation increases. This problem has limited the utility of high performance Mass Spec in many key functions.

To answer the challenge of retaining high performance, even at fast chromatography speeds, Bruker Daltonics has developed the maXis™. This instrument is unique, and is a revolution in high-resolution tandem mass spectrometry and delivers exceptional accurate mass (<1 ppm), high resolution (>50K), high sensitivity (attomole levels), and possesses a wide dynamic range (~5 orders of magnitude). Ideal for many analyses requiring high sensitivity and specificity, the maXis platform exceeds the performance of other hybrid mass spec instruments by a wide margin. Coupled with the unique SmartFormula 3D and the proprietary Target Analysis software package, the maXis is capable of achieving some of the most definitive, high resolution molecular formula determinations even at the fastest chromatography speeds.

Ideal for small molecule or biomolecule applications, the maXis is a game changing tool that will greatly expand the capabilities of any analytical group. This seminar will contain an explanation of the principles behind maXis that allow it to deliver such high performance, a catalog of the instruments many features, and a series of examples of data obtained with small molecules and biomolecules when using the maXis for fast chromatography. Results from the maXis will be compared and contrasted with today's state of the art in Mass Spectrometry performance.

NOTES:

Mon. 17:05-17:25

Optimization of MRM-based protein quantitation in plasma: Lessons learned from an inter-lab study

Susan E. Abbatiello[1]; Steven Hall[2]; Theresa Addona[1]; David Bunk[3]; Stephen Skates[4]; Lisa Zimmerman[5]

[1]The Broad Institute of MIT and Harvard, Cambridge, MA USA; [2]University of California San Francisco, San Francisco, CA USA; [3]NIST, Gaithersburg, MD USA; [4]Massachusetts General Hospital, Boston, MA USA; [5]Vanderbilt University, Nashville, TN USA

Isotope dilution mass spectrometry (ID-MS) has recently been coupled with nanoflow liquid chromatography (LC) and multiple reaction monitoring (MRM) for quantification of peptides in complex mixtures such as tissues, cell lysates and body fluids, and has particular relevance as a suitable approach for protein biomarker verification studies. Inherent to verification studies, a challenge exists to define quality metrics that demonstrate reproducibility and robustness in protein-based MRM assays. The Clinical Proteomic Technology Assessment for Cancer (CPTAC), a five-year initiative sponsored by the National Cancer Institute, was created to address these issues.

The CPTAC Experimental Design and Statistics Verification Studies working group has designed a three-part study to assess the reproducibility, robustness and potential sources of variation in an MRM SID-MS-based assay for quantification of seven proteins spiked into human plasma. This study was implemented across eight separate laboratories each containing a nanoflow HPLC system interfaced to a triple quadrupole mass spectrometer. The three experiments required construction of a standard curve representing ten signature peptide concentrations ranging from 1 to 500 fmol/µL in plasma digested with trypsin and their [13C/15N] stable isotope-labeled analogs spiked in at 50 fmol/µL, and ranged in complexity from synthetic peptides spiked into a background of digested plasma and prepared at a single site, to proteins spiked into neat plasma and processed separately at the individual sites. A detailed standard operating procedure (SOP) was incorporated for all aspects of sample handling and data analysis. Data were analyzed for reproducibility, limit of detection (LOD), limit of quantitation (LOQ), digestion efficiency, and recovery.

Results demonstrated the overall consistency in peptide recovery, digestion efficiency, LOD and LOQ for the ten peptides across the eight participating sites. However, each laboratory experienced a different "problem" peptide that did not perform ideally. Optimization of nanoflow LC and MS operating parameters will be discussed.

NOTES:

PM Parallel Session: From Separation to Detection
Location: Arlington/Berkeley/Clarendon
Chair: Aaron Wheeler

Mon. 15:30-15:55

Novel Indirect Fluorescence Detection Methods Using Isotachophoresis: Minding the Gaps and Steps

Juan G Santiago; Robert D. Chambers; Tarun Khurana, *Stanford University, Stanford, CA USA*

Isotachophoresis (ITP) techniques date back at least 60 years, and yet there remain significant opportunities in the innovation of novel, widely applicable ITP assays. In ITP, analyte ions focus and self-segregate into distinct zones between leading and trailing electrolytes (LE and TE). The zone order is determined by analyte effective mobilities, and high (over one million fold) sample preconcentration is possible. At Stanford, we are developing methods to focus, separate, detect, and identify unlabeled (non-fluorescent) analytes using surrogate fluorescent molecules. The talk will summarize two novel ITP techniques which accomplish this. In the first, we mix analyte ions with fluorescent mobility markers. Upon application of an electric field, both the sample species and fluorescent markers self-segregate into distinct, high concentration zones between the LE and TE. The process creates a train of ions where unlabeled sample analytes are detected unambiguously as “gaps” in the fluorescent marker signal. In the second method, we use a single surrogate fluorophore for indirect detection. The fluorescent species is a counterion in the LE buffer. Upon application of a field, the counterion electromigrates toward the anode and incorporates itself into all ITP zones. The emitted intensity of the fluorescent counterion adapts itself according to the local electric field in ITP zones. Analyte zones are then detected and can be identified as “steps” in counterion intensity as it flows from LE to TE. Our goal is the development of on-chip ITP assays with unprecedented sensitivity and new functionality.

NOTES:

Mon. 15:55-16:20

Adventures in Attomole Capillary Isoelectric Focusing

Norman J Dovichi; Jane Dickerson; Yihan Li; Lauren Ramsay, *University of Washington, Seattle, WA USA*

We have coupled capillary isoelectric focusing with a laser-induced fluorescence detector that is based on a post-column sheath flow cuvette. We employed Chromeo P503 as a fluorogenic reagent to label proteins before analysis. This reagent reacts with the ε-amine of lysine residues, preserving the cationic nature of the residue; labeled proteins generate extremely sharp peaks in capillary isoelectric focusing. A set of four standard proteins generated a linear relationship between migration time and pI. A protein homogenate prepared from a Barrett's esophagus cell line resolved over 100 components in a 40-minute separation. Detection limits for Chromeo P503 labeled β-lactoglobulin were 5 amol injected onto the capillary. Fluorescent impurities present in the ampholytes generated a large background signal that degraded the detection limit by four orders of magnitude compared to other forms of capillary electrophoresis with this detector.

NOTES:

Mon. 16:20-16:45

Applications of Gradient Micro Free Flow Electrophoresis

Michael T. Bowser; Ryan T. Turgeon; Bryan R. Fonslow, *University of Minnesota, Minneapolis, MN USA*

In free flow electrophoresis (FFE) a thin stream of sample is continuously introduced into a planar flow chamber. An electric field is applied perpendicularly to the flow through the separation chamber. Analytes are deflected laterally in the electric field according to their electrophoretic mobility giving rise to individual stream paths. FFE has recently been miniaturized into a microfluidic format (uFFE), requiring less sample and reagents, a simplified flow profile and better heat dissipation.

The continuous nature of uFFE separations suggests a number of novel analytical applications. A single separation can be monitored over long periods of time for high sensitivity measurements. We have demonstrated a 20-fold improvement in signal to noise by averaging 500 images over a 2 minute period. Further improvements in limit of detection are possible if issues regarding peak position stability are addressed.

uFFE can also be used to continuously monitor a dynamically changing sample. We have demonstrated how introducing a buffer gradient into the uFFE device can be used to efficiently optimize a range of separation conditions or estimate dissociation constants. The separation of a group of amino acids was monitored as cyclodextrin concentration was increased from 0 to 50 mM. This gradient was performed in 5 minutes, allowing complete measurement across a range of buffer concentrations in less time than a single capillary electrophoresis separation.

We have also explored introducing a gradient at the sample channel. For example, we have titrated a fluorescently labeled aptamer with increasing concentrations of its protein target. This is analogous to a standard affinity capillary electrophoresis experiment where the intensity of the peak for the unbound ligand decreases and that for the complex increases as binding increases. Due to the continuous nature of gradient uFFE, complete coverage of the binding curve is possible in as little as five minutes.

NOTES:

Mon. 16:45-17:05

Traveling Wave Electrophoresis and Improved Microchip ESI/MS Interfaces for Proteome Analysis

Aaron Timperman; Kyoo D. Jo; Xiuli Mao; Brent R. Reschke; R. Lloyd Carroll; Boyd E. Edwards,
West Virginia University, Morgantown, WV USA

For the full potential of proteomics to be realized, new analytical tools are needed that will provide improved separations and reduced limits of detection. Currently, we are developing, traveling wave electrophoresis with a sandwich architecture, which has the potential to provide improved separations with enhanced control of band elution. Additionally, a new approach for coupling microchip ESI-MS is being characterized. Although the characteristics of these devices are intended to be compatible with proteomic samples, it is anticipated that they will be broadly applicable to numerous sample types.
A new technique for microchannel separations, termed traveling wave electrophoresis (TWE), is theoretically modeled and characterized experimentally. A distinguishing aspect of TWE is that charged species are pulled through the microchannel by a locally defined electric field wave. Our design, called sandwich TWE, uses a microchannel with one set of interdigitated electrodes channel top and another set of interdigitated electrodes on the channel bottom. A working prototype has been fabricated and used to achieve two exciting results: 1) analyte band, and 2) excellent agreement between theoretical and observed band velocities as function of the speed of the traveling electric field wave. Two important characteristics of TWE are the potential to reduce dispersion by trapping the analyte within nodes of electric field waves, and the ability to switch in real-time between band velocity while modulating between separative and non-separative transport.
Additionally we are comparing different approaches for the combining microchip electrophoresis with ESI-MS. The interfaces under development are designed to operate with very low electroosmotic flow in the separation channel to allow the use of coatings that minimize surface charge and protein adsorption. One interface uses voltage switching and hydrodynamic restrictors, and the amount of band broadening and sample transfer efficiency associated with this design will be reported.

NOTES:

Mon. 17:05-17:25

MicroPrep: On-Chip Subcellular Fractionation using Dielectrophoresis

Meike Moschallski[1]; Monika Hausmann[2]; Anton Posch[2]; Aran Paulus[2]; Nancy Kunz[2]; Dieter Stoll[1]; Thanh Tu Duong[3]; Gert Blankenstein[3]; Heiko Steuer[1]; Simon Werner[1]; Kai Fuchsberger[1]; Brigitte Angres[1]; Martin Stelzle[1]

[1]NMI Natural and Medical Sciences Institute, Reutlingen, Germany; [2]Bio-Rad Laboratories GmbH, Munich, Germany; [3]Boehringer Ingelheim microParts, Dortmund, Germany

In proteome analysis scientists face large numbers of different protein species per cell or tissue at a dynamic concentration range of 10^9.
Sample fractionation reduces the overall complexity of the protein sample, and enriches low abundance proteins relative to the original sample. In this context, subcellular organelle fractionation is of particular importance, since functional units are isolated providing additional information with respect to the cellular localization of a particular protein.
We have developed a microfluidic system with integrated electrode arrays and a dedicated high-throughput channel layout to isolate mitochondria from cell homogenate based on dielectrophoretic sorting. Sorting of particles according to size and dielectric properties is achieved by the competition between hydrodynamic friction and dielectrophoretic forces.
The following means to efficiently prevent electrode fouling and clogging of the channel were successfully tested: the inner surfaces are coated with bovine serum albumin. Mechanical actuators generate minute vibrations of the channel cover inducing periodic flow superimposed on the sample flow. Chip and sample are cooled below 10 °C to maintain the integrity of mitochondria during the fractionation. A chip material with high specific heat conductance ensures efficient transport of Joule heat generated in the vicinity of the deflector units.
MicroPrep separation yields micrograms of mitochondria. Western blots showed significantly less contaminants such as lysozomes or endoplasmatic reticulum when compared to samples prepared by gradient density centrifugation. Mitochondrial proteins were successfully characterized and identified by 2D gel electrophoresis and mass spectrometry.
In conclusion, a chip-based subcellular fractionation scheme was developed which provides mitochondrial samples of superior purity compared to standard enrichment technologies. Further applications of this technology are envisioned for sample enrichment in medical diagnostics. Chip coating, cooling and mechanical actuation for the first time provide for compatibility of dielectrophoresis chips with complex biological samples and enable robust long term operation without electrode fouling.

NOTES:

MONDAY POSTER SESSION ABSTRACTS
Monday, February 2, 2009
14:00 – 15:30
Imperial Ballroom and Stanbro Room

Advances in Capillary Separations 1

P-101-M

In-Capillary Reduction and Enzymatic Digestion of Nanogram Quantities of Disulfide Containing Proteins Using Capillary Electrophoresis

Robin Abel, *University of Western Ontario, London, Canada*

In this work, a method of open capillary reduction and digestion of disulfide containing proteins by CE, without reagent immobilization, is presented. In prior work, a sharp junction of rapidly dropping pH at the interface of two buffers of different pH, (~pH 4 and ~pH 10) was generated in a capillary. Using the junction as a trap, extremely dilute samples (0.0001 mg/mL) of protein were stacked as zwitterions, with concentration factors of up to 2000 (Nesbitt, et al., J. Chromatogr. A, 2005). When added trypsin was mobilized to the concentrated sample zone, rapid digestion was effected without significant autolysis (Nesbitt, et al., Electrophoresis, 2008). However, only proteins without disulfide bonds were suitable for analysis by this technique. A technique for reducing and digesting disulfide containing proteins in-capillary is investigated with sample loadings in the range of low picomoles to high femtomoles. Disulfide containing samples are reduced, digested and characterized by MALDI mass spectral detection without any need for cysteine alkylation. Using BSA as a model protein, a sequence coverage of over 90% was obtained from an amount of 700 femtomoles. The entire procedure is performed in one capillary with a total duration of approximately three hours, representing a significant improvement in sample requirement, flexibility, and experiment time over traditional digestion methods.

P-102-M

Capillary Electrophoresis Coupled With Mass Spectrometry Applied in the Determination of Biomarkers of Hepatic Porphyrins

Nilson Antonio Assunção; Michele Gotelipe de Souza Corrêa; Etelvino José Henriques Bechara, *Universidade Federal de São Paulo, São Paulo, Brazil*

Reliable methodologies to determine accurate and unambiguous procedures of metabolites from gene or epigenetic events have become a challenge in clinical biochemistry. The determinations of these products have increasingly been required. The species monitored were: 5-aminolevulinic (ALA), porphobilinogen (PBG), serotonin, GABA, tryptophan and creatinine. Analyses were carried out on a CE system model P/ACE MDQ (Beckman Coulter), using silica capillary of 50 um d.i. and 70 cm length. The separation occurred at 300 V cm-1 and pressure of 3 psi. The buffers used in the CE analysis were ammonium formiate and acetate 50 mM. The best conditions of pH value were studied. The MS system used was LCQ Advantage MAX of Thermo, with ionization electrospray in the positive and ion trap mode, analyzer of ions such as "ion trap". A sheath-liquid type interface was used. The MS-ESI analysis conditions used were: 4.5 kV, temperature = 275oC, N2 = 20 units.

Separations time was less than 5 min, indicating the high efficiency and speed of CE analysis, associated to the ability of identification by MS. The methodology was possible the determination of both ALA and PBG, biomarkers of hepatic porphyrins. The quantification of Tryp, Gaba and Serotonin were possible too. The analysis of these compounds is important because they may be involved in neurological manifestations of these porphyrins. The determination of creatinine is required for normalization of urinary concentrations of metabolites released. The methodology described allows simultaneous reliable and direct determination of metabolites of important research in the molecular mechanisms of porphyrins disorders, leading to possibilities for application in clinical diagnosis. The use in human urine samples has confirmed their usefulness in the characterization and determination of porphyrins as well as to elucidate clinical aspects of iron metabolism.

Acknowledgments
Fapesp, CNPQ, Milênio Redoxoma.

NOTES:

P-103-M

Two-Phase Single Drop Microextraction and Large Volume Stacking for Nonaqueous Capillary Electrophoresis of Weakly Acidic Compounds

Doo Soo Chung ; Yoo Gon Jin; Kihwan Choi; Jihye Kim, *Seoul National University, Seoul, South Korea*

In order to improve the concentration sensitivity of capillary electrophoresis (CE), two sample preconcentration techniques before and after injection, two-phase single drop microextraction (SDME) and large-volume stacking using the electroosmotic flow pump (LVSEP), were in-line coupled using a commercial CE instrument. By simple programming of liquid handling sequences of the instrument, a pentanol drop was prepared at the tip of a fused silica capillary over which a Teflon tube had been sleeved to serve as a hydrophobic support. After extraction of the analytes from an aqueous donor solution to the drop, the whole capillary column was filled with the enriched pentanol solution and LVSEP, in which the sample matrix is automatically removed by the EOF, was carried out using a methanolic run buffer. The overall enrichment factors for the analytes PCP, 3-BBA, and 4-IBA, from the combination of 30 min SDME and LVSEP using a 27 cm capillary, were about 7000 even without agitation of the donor solution. Since no modification of the existing CE instrument is necessary and a bare capillary is used for LVSEP, this scheme can be adapted quite easily for many CE applications requiring high concentration sensitivity.

P-104-M

Free-Solution Electrophoretic Sequencing of Over 250 Bases of Ssdna using Protein Polymer Drag-Tags

Jennifer A. Coyne; Jennifer S. Lin; Thomas P. Niedringhaus; Matthew B. Kerby; Annelise E. Barron, *Stanford University, Stanford, CA USA*

The list of organisms whose genomes have been sequenced continues to grow, resulting largely in part from advances made in electrophoresis materials and methodology during the Human Genome Project. Next-generation sequencing methods are beginning to impact the field, but capillary electrophoresis in a polymer matrix continues to be a workhorse technology for sequencing human DNA. Significant research has been done to translate these capillary separations onto microfluidic devices, resulting most recently in successful sequencing of 600 bases in 6.5 minutes by chip electrophoresis. The advantages of microfluidic separations (faster read length, smaller sample volume, potential integration into lab-on-a-chip devices), however, are dampened by the difficulties associated with loading the viscous polymer networks necessary to achieve size-based separations of sequencing fragments. Elimination of the polymer separation matrix is not an option for traditional DNA electrophoresis separations because DNA mobility in free-solution electrophoresis is size-independent due to linear scaling of both the charge and friction of DNA with length. Free-Solution Conjugate Electrophoresis (FSCE) enables size-based separations in free-solution with no entangled polymer network through the specific conjugation of exactly identical mobility modifiers to each DNA sequencing fragment. In FSCE, completely monodisperse, mostly charge-neutral mobility modifiers (“drag-tags”) are chemically conjugated to the 5’-end of ssDNA oligomers or sequencing primers. Drag-tags used for FSCE include peptoids and genetically engineered, highly repetitive protein polymers. Recent advances in FSCE drag-tags and separations have improved the sequencing length to over 250 bases of DNA separated in free-solution, which is almost a 40% increase in read-length over the previously published FSCE sequencing results and is comparable to the read-length of next-generation sequencing technologies. The transition to microchip electrophoresis is expected to go smoothly, and longer drag-tags also show promise of further increased read-lengths.

NOTES:

P-105-M

Purifying Proteins by CE with Hydrophobically Modified Lpas: Dependence Of Protein Removal Efficiency on Copolymer Structure

Ryan E Forster[1]; Annelise E Barron[2]

[1]Northwestern University, Evanston, IL USA; [2]Stanford University, Stanford, CA USA

High-efficiency methods and materials to purify genomic material from proteins in crude biological samples are necessary for point-of-care devices. We previously demonstrated that water-soluble block copolymers of acrylamide and hydrophobic N,N,-dialkylacrylamides can trap proteins with hydrophobic surfaces during CE, but allow genomic material to pass through. Hypothesizing that we could enhance protein removal further, we explored the effects of increasing the hydrophobic block length in the copolymers. To test this idea, we synthesized four copolymer matrices, each with an average molar mass of ~ 500 kDa, and average block sizes of 0 (polyacrylamide homopolymer), 3, 5, and 7 hydrophobic monomer units. Each copolymer contained ~ 0.5 mol% dihexylacrylamide, and was dissolved in 1x TTE at 5% (w/w). A fused silica capillary with a 20-cm effective length, dynamically coated with poly(N-hydroxyethylacrylamide), was used as the separation chamber . We found that the copolymers' ability to remove proteins increases substantially with hydrophobic block size. We used two model proteins, bovine serum albumin and beta-lactoglobulin, as well as goat serum for these studies. The proteins in all three samples were first fluorescently labeled so that they could be excited at 488 nm and detected at 630 nm. The elution time for the primary protein peak always increased with an increase in block size. These new polymer materials will be quite useful for biosample purification purposes, since smaller DNA molecules typically elute much more quickly than proteins (esp. serum albumin, which often is an important protein to remove from a complex biosample). The slowed protein migration provides a larger separation zone for DNA collection and therefore greater purity in the collected sample. The result is a copolymer system that enhances electrophoretically driven nucleic acid purification, which can be interfaced with on-chip PCR and DNA detection for rapid, integrated sample analysis.

P-106-M

Multichannel Capillary Electrophoresis with Fluorescence Detection of Tagged Peptides

Graham T. T. Gibson; Benjamin Rogers; Richard Oleschuk, *Queen's University, Kingston, Canada*

One approach to improving the performance of CE is to reduce the inner diameter (i.d.) of the capillary. Smaller capillaries are associated with much less Joule heating allowing more control by increasing the range of voltage that can be used before heating deteriorates the efficiency. Very small capillaries (~5 micron i.d.), however, have some technical difficulties such as very high flow-induced back pressure, which makes buffer loading and pressure injections difficult, and extremely small analysis volumes, which are especially demanding for detection techniques. As an alternative, a microstructured fibre (MSF) can be used for CE. Originally designed for photonics applications, MSFs feature an array of channels within a capillary format that form from heating and pulling a bundle of capillaries. In this case, the MSF is ~320 micron in diameter with an array of 30 4-5- micron channels, with 6-7 micron between them. Their multiple channels are able to deliver a larger amount of sample to the detector while increasing efficiency with respect to a single channel capable of delivering the same volume (~30 micron i.d.).
Electrophoretic separation of the Cy5-labelled peptides leucine enkephalin, leucine-valine and bradykinin using a MSF was compared with that using a traditional 30 micron i.d. fused silica capillary. Over the 10 cm length of fibre, the three isoelectric tagged peptides were separated in less than 3 minutes with ~1.8

times better efficiency than the 30 micron capillary. Evidence suggests that potentials up to 400 V/cm can be used before the effects of Joule heating are observed, even in the absence of a cooling system.
The multichannel MSF presented here represents a novel CE vessel capable of highly efficient separations of biomolecules with LIF detection.

NOTES:

P-107-M

Reagent-Release Capillary (RRC)-Based Isoelectric Focusing as a Simple, Disposable, and Sensitive Method for Bioanalysis

Hideaki Hisamoto; Masaki Kataoka; Hiroki Yokoyama; Toshio Yao,
Osaka Prefecture University, Osaka, Japan

A simple capillary isoelectric focusing (CIEF) is performed using a reagent-release capillary (RRC) to solve the problems of complicated reagent mixing and preconcentration procedure for bioanalysis.

CIEF is a well-known method for separation and preconcentration of proteins. On the other hand, we have been developed a novel microchip called, capillary-assembled microchip (CAs-CHIP), which allowed multi-analyte detection by using different chemically-modified square capillaries.1-5 Among these works, we recently developed RRC which releases the necessary analytical reagents from capillary wall upon introduction of sample solution by capillary force, and the reagents react with sample to give fluorescence response. Therefore development of a novel capillary immobilizing necessary reagents for IEF and other molecular recognition reagents such as antibody or labeling reagents is expected to be applied for simple, disposable, and sensitive analysis of proteins.

Here we prepared a new RRC possessing double layer structure comprised of EOF suppression layer (covalent bonding) and reagent release layer (physical adsorption) for IEF. RRC was prepared by coating a poly(dimethylacrylamide) by covalent bonding for suppressing EOF and further coated by carrier ampholyte and other reagents by physical adsorption. An introduction of sample solution by capillary force allowed not only a simple introduction of sample, but also a spontaneous release of necessary reagents for IEF from the inner wall of capillary, so that the capillary is ready to use for IEF.

Here, preliminary results for the preparation of RRC and the performance of RRC-based IEF for on-line labeling and immunoassay will be presented.

References

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[2] H. Hisamoto et al., Anal. Chem., 77 (2005) 2266-2271.
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[4] T.G. Henares et al., Anal. Chem., 79 (2007) 908-915.
[5] T.G. Henares et al., Anal. Bioanal. Chem., 391 (2008) 2507-2512.

P-108-M

Aptamer-facilitated Protein Isolation from Cells (AptaPIC)

Sahar Javaherian; Michael U. Musheev; Mirzo Kanoatov; Maxim V. Berezovski; Sergey N. Krylov,
York University, Toronto, Canada

Functional genomics requires structural and functional studies of a large number of proteins. While the production of proteins through over-expression in cultured cells is a relatively routine procedure, the following protein purification from the cell lysate often represents a significant challenge. The most direct way of protein purification from a cell lysate is affinity purification using an affinity probe to the target protein. Antibodies, classical affinity probes, can hardly be developed to a protein in the cell lysate, their development requires a pure protein. Thus, isolating the protein from the cell lysate requires antibodies, while developing antibodies requires a pure protein. Here we resolve this loop problem. We introduce AptaPIC, Aptamer-facilitated Protein Isolation from Cells, a technology that integrates (i) development of aptamers for a protein in cell lysate using Non-equilibrium Capillary Electrophoresis of Equilibrium Mixtures (NECEEM) as a separation mode and (ii) utilization of the biotinylated developed aptamers for protein

isolation from the cell lysate using streptavidin coated magnetic beads. Using MutS protein as a target, we demonstrate that this technology is applicable to the target protein being at an expression level as low as 0.8% of the total protein in the lysate and following purification of the protein from the cell lysate to the purity of 95%. AptaPIC has the potential to considerably speed up the purification of proteins and, thus, their structural and functional studies.

NOTES:

P-109-M

Evaluation of Carrier Ampholyte-Based Capillary Electrophoresis for Separation of Peptides and Peptide Mimetics

Dušan Koval[1]; Jean-Marc Busnel[2]; Jan Hlaváček[1]; Jiří Jiráček[1]; Václav Kašička[1]; Gabriel Peltre[3]

[1]Institute of Organic Chem. & Biochemistry, ASCR, Praha , Czech Republic; [2]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [3]Ecole Supérieure de Physique et Chimie Industriel., Paris, France

Carrier ampholyte-based capillary electrophoresis (CABCE) has recently been introduced as an alternative to CE (CZE) in the classical buffers based on inorganic and organic weak electrolytes and salts. In this study, isoelectric BGEs were obtained by fractionation of Servalyt pH 4-9 carrier ampholytes to cuts of typical width of 0.2 pH unit. CABCE feasibility was examined on a series of biologically active peptides, such as insect oostatic peptides, i.e. proline-rich di- to decapeptides, and phosphinic pseudopeptides - tetrapeptide mimetics synthesized as a mixture of four diastereomers having the -P(O)(OH)-CH_2- moiety embedded into the peptide backbone.

Basically, selectivity turned out to be identical in CABCE and CE in the salt-based BGEs. Further, the separation efficiency of CABCE proved to be as good as classical CE for the insect oostatic peptides and better for diastereomers of the phosphinic pseudopeptides. In addition, despite the numerous species present in the narrow pH cuts of carrier ampholytes, CABCE seems to be free of system zones that could hamper the analysis. Peak symmetry was good for moderately to low mobile peptides, whereas some peak distortion due to electromigration dispersion, was observed for short peptides with rather high mobility.

Reference: Koval, D., Busnel, J.M., Hlaváček, J., Jiráček, J., Kašička, V., Peltre, G. *Electrophoresis*, 2008, 29, 3759-3767

The authors acknowledge a support by the Grant Agency of the Czech Republic, grant nos. 203/06/1044, 203/08/1428, by the Research Project Z40550506 of the Academy of Sciences of the Czech Republic, and by the project no. 13 of the cooperation between ASCR and CNRS in the years 2007-2008.

P-110-M

Sensitivity Enhancement of Heavy Metals by Field Amplified Sample Stacking and Task Specific Ionic Liquids Extraction with Capillary Electrophoresis

Sam F. Y. Li, *National University of Singapore, Singapore, Singapore*

Capillary electrophoresis separation of four toxic metal ions (Cr3+, Pb2+, Hg2+ and Ni2+) was achieved by optimizing the composition of the histidine/ tartaric acid BGE. An on-column preconcentration technique, Field Amplified Sample stacking (FASS), was performed to improve the sensitivity. This method afforded an enhancement factor of up to 91800 folds and the LODs were ranged from 0.005 to 2.32 mg/L which were well below the maximum contaminant levels (MCLs) set by the United States Environmental Protection Agency. In order to further enhance the detection sensitivity of Hg2+, hydrophobic task-specific ionic liquids (TSIL) designed to extract Hg2+ from water was prepared with microwave-assisted irradiation and was used as extractant in Liquid-Phase Micro-Extraction (LPME) before analysis with the optimized CE-C4D conditions for toxic metals analysis. The preliminary results showed that sensitivity enhancement of 75 folds could be obtained. The robustness of this method was observed when it was applied to the analysis of real samples including tap water, reservoir water and drain water with recoveries between 90%

and 120%.

NOTES:

P-111-M

Functionalized Carbon Nanotube as Pseudostationary Phase for Capillary Electrophoretic Separation of Nsaids

Chuen-Ying Liu; Yi-Jin Huang, *Department of Chemistry, National Taiwan Universit, Taipei, Taiwan*

A functionalized multiwalled carbon nanotubes (c-MWNTs) as a pseudostationary phase for the capillary eletrophoretic separation of non-steroidal anti-inflammatory drugs (NSAIDs) was described. In order to increase hydrophilicity of the multiwalled carbon nanotubes (MWNTs) in an aqueous electrolyte, a sonochemical process was used to treat MWNTs in concentrated nitric/sulfuric acid mixture. Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and electron spectroscopy for chemical analysis (ESCA) were employed for the characterization of the oxidized MWNTs. The c-MWNTs afforded sieving mechanism, π-π interaction, hydrophobic interaction, hydrogen bond, and electrostatic interaction to separate NSAIDs, providing a different separation mode from sodium dodecyl sulfate (SDS) micelles. The effect of important factors such as pH and concentration of running buffer, separation voltage, organic modifiers and injection temperature were investigated. Complete separation of indoprofen, ketoprofen, suprofen, naproxen, flurbiprofen and fenoprofen could be achieved by using a mixture of borate buffer (75 mM, pH 10) / methanol (95:5, v/v) containing 0.02 mg/mL c-MWNTs and an applied voltage of +12 kV with UV detection at 214 nm. The average number of theoretical plates was 8.6×10^4 plates/m. The established method was also applied to analyze NSAIDs spiked in urine sample with satisfactory results.

P-112-M

A Platform Method for cIEF at High pH

Scott T. Mack; Ingrid D. Cruzado Park; Chitra K. Ratnayake, *Beckman Coulter, Fullerton, CA USA*

The use of cIEF for characterization of therapeutic monoclonal antibodies (MAbs) is being increasingly adopted. The determination of pI adds a critical dimension to establishing identity, purity, post-translational modification and stability of therapeutic MAb preparations. Many MAbs are basic in nature and have charge isoforms with pI values in the 7-10 range of the pH gradient. The analysis of basic compounds present the greatest challenge in cIEF due to the inadequate focusing and buffering by ampholytes comprising the basic region. The use of extremely long focusing times (>25 min) to ensure complete formation of the pH gradient is detrimental to cIEF resolution because the pH gradient is distorted over time by isotachophoresis. Incorporation of cathodic and anodic stabilizers in the cIEF sample can help overcome these obstacles. Optimization of stabilizer solution volumes, focusing times and ampholyte concentration is critical in the development of a single universal cIEF method having high reproducibility in the basic pH range. This work summarizes the results of an intermediate precision study carried out with three unique MAbs over six days using two instruments and multiple lots of neutrally coated capillaries. The results illustrate the exceptional reproducibility and robustness of cIEF.

Note: For Research Use Only; not for use in diagnostic procedures.

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P-113-M

Establishing cIEF Separation Conditions for Highly Acidic Proteins

Scott T. Mack; Ingrid D. Cruzado Park; Chitra K. Ratnayake, *Beckman Coulter, Fullerton, CA USA*

Highly acidic proteins with isoelectric points (pI) between 3.0 and 5.5 can be particularly challenging to characterize by capillary isoelectric focusing (cIEF). We have developed a method to overcome many of the obstacles faced by analysts attempting to perform these difficult separations. This approach implements both an inverted polarity setting of the power supply and a high concentration of iminodiacetic acid (IDA) in the sample solution. This strategy results in the formation of the acidic portion of the pH gradient adjacent to the capillary window, dramatically reducing detection times. Following sample focusing, sample peaks are detected using anodic chemical mobilization with a weak base, thus eliminating issues of broad or absent sample peaks due to stalling chemical mobilization. Characterization studies determining pI values for synthetic peptides have shown that this extreme acidic cIEF separation method is capable of generating a highly resolved and linear gradient between pH 3.4 and 5.5. Separation of recombinant human erythropoietin (rhEPO) samples resulted in baseline separations of eight charge isoforms in less than thirty minutes. Additionally, intermediate precision studies of rhEPO separations indicate that performance is consistent and reliable using this new method.

Note: For Research Use Only; not for use in diagnostic procedures.

P-114-M

A Novel High Sensitivity Porous Sprayer for CE-MS

Chitra K. Ratnayake; Jerald S. Feitelson; Jeff D. Chapman, *Beckman Coulter, Fullerton, CA USA*

The key to effectively coupling capillary electrophoresis (CE) to mass spectrometry (MS) is a robust interface capable of maintaining the high resolving power of CE. At the heart of the interface is the sprayer that introduces the sample for ionization before the mass spectrometer. Because flow rates in CE are typically well below that of high performance liquid chromatography (HPLC), the capillary effluent is classically compensated by the addition of volatile sheath liquid which also serves to close the circuits for electrophoresis and the sprayer. This approach was developed to help stabilize electrospray into the mass spectrometer, but it has been at the expense of the ultra-high resolving power generally associated with CE.

A novel interface, the high sensitivity porous sprayer (HSPS), has been developed as an alternative to the sheath liquid design. This approach addresses the issues of analyte dilution and loss of resolution due to the band broadening generally associated with conventional sheath liquid sprayers. With this design, the electrical connection to the outlet is achieved by making the tip of the fused silica capillary porous. The electrical connection is achieved simply by inserting the capillary outlet, containing the porous tip, into an ESI needle or metal sheath and then filling the needle with a conductive liquid. The interface design uses the distal end of the separation capillary itself as the sprayer tip and, therefore, does not add any dead volume to the CE-MS interface. In addition, bubble formation at the high-voltage electrode is outside the separation capillary and therefore does not affect analyte stability.

Note: For Research Use Only; not for use in diagnostic procedures.

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P-115-M

Applicability of sheathless CE-MS interface with porous sprayer for metabolic profiling of biological fluids.

Oleg A. Mayboroda; Alegría Carrasco-Pancorbo; Ekaterina Nevedomskaya; Crina Balog; André M. Deelder, *LUMC, Leiden, The Netherlands*

CE-MS is a powerful hyphenated technique for the separation and identification of a wide range of compounds. Due to the unique analytical qualities of CE such as high separation efficiencies, fast analysis times, low sample and reagent consumption, it has a potential to develop into one of the important tool for metabolic profiling. However, all benefits of CE-MS can be balanced by one important drawback, namely the lack of concentration sensitivity.
In the current study, we evaluate a sheathless interface with a porous sprayer and address its applicability for metabolic profiling. We present here two different applications: analysis of amino acids and small metabolites in mouse urine by using a bare fused silica capillary and formic acid as BGE, and analysis of peptides in human urine by using a coated capillary and acetic acid-based buffer. To summarize, we demonstrate the advantages of CE-MS interface with porous tip sprayer for metabolic profiling of biological fluids, especially for “challenging cases” when sample amount is limited.

P-116-M

Diffusion as a Tool of Measuring Temperature inside a Capillary

Michael Musheev; Viktor Okhonin; Sahar Javaherian; Sergey Krylov, *York University, Toronto, Canada*

Application of capillary electrophoresis (CE) to temperature- sensitive biomolecular interactions requires knowledge of the temperature inside the capillary. The simplest approach to finding temperature in CE employs a molecular probe with a temperature-dependent parameter. Up until now only spectral parameters of molecular probes were utilized for temperature measurements in CE. The arbitrary nature of spectral parameters leads to several inherent limitations that compromise the accuracy and precision of temperature determination. This paper introduces the concept of finding temperature in CE through the measurement of a nonspectral parameter of the molecular probesits diffusion coefficient. Diffusion is a fundamental property of molecules that depends only on the molecular structure of the probe, the nature of the environment, and the temperature. It is ideally suited for temperature measurements in CE if an approach for measuring the diffusion coefficient in a capillary with high precision is available. This work first develops an approach for measuring the diffusion coefficient in a capillary with a relative standard deviation of as low as 2.1%. It is then demonstrated that such precise measurements of the diffusion coefficient could facilitate accurate temperature determination in CE with a precision of 1 °C. This new method was used to study the effect on temperature of different amounts of joule heat generated and different efficiencies of heat dissipation. The nonspectroscopic nature of the method makes it potentially applicable to nonspectroscopic detection schemes, for example, electrochemical and mass spectrometric detection.

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P-117-M

Capillary Electrophoresis Mobility Modeling of Peptides with Particular Emphasis on Proteomics Applications

Marcell Olajos[1]; Stefan Mittermayr[1]; Péter Hajós[2]; Tibor Chován[2]; András Guttman[1]

[1]Horvath Laboratory of Bioseparation Sciences, Innsbruck, Austria; [2]University of Pannonia, Veszprem, Hungary

Rapid recent developments in proteomics require high resolution separation of a large number of peptides for their downstream identification by mass spectrometry. Capillary zone electrophoresis offers a high resolution option for peptide separation. Application of model-based approaches to predict peptide mobilities from known physicochemical properties can shorten the otherwise tedious experimental separation optimization process. This endeavor requires specification of structural descriptors followed by selection of appropriate modeling methods. Our approach focused on the investigation of structure-mobility relationship of small peptides to understand the influence of the alteration of several separation parameters on the electromigration in capillary zone electrophoresis (CZE). This parameter driven approach included exploration of the effects the pH and concentration of the background electrolyte, organic additive (methanol and acetonitrile) content, separation temperature and applied separation voltage. Two variable semi-empirical (TVSE) and artificial neural network (ANN) approaches were utilized to predict the electrophoretic mobilities of model peptides with non-polar, polar, aromatic, positively charged and negatively charged R group characteristics. The conventionally applied Offord's model was chosen for the TVSE model, while two ANN models were derived having either gradient descent with variable learning rate using momentum or Levenberg-Marquardt algorithm as training algorithms. Both ANN models showed superior performance over the TVSE approach, especially when such non-linear phenomena were simulated as the influence of organic additives and separation temperature.

P-118-M

Methods to Improved Sensitivity for Capillary Isoelectric Focusing with Laser-induced Fluorescence Detection

Lauren Ramsay; Jane Dickerson; Norman Dovichi, *University of Washington, Seattle, WA USA*

The study of the protein expression in complex biological samples has shown much promise to help predict disease progression. In order to properly exploit the potential of protein expression as a prognostic tool, the proteins must be quickly separated and detected at very low concentrations. We performed Capillary Isoelectric Focusing (cIEF) on a post-column sheath flow cuvette system with ultra-sensitive laser-induced fluorescence detection. Proteins were labeled with Chromeo P503, and we obtained detection limits in the low attomole (10-18 mol) range. A standard solution of four proteins was separated with high resolution; we also achieved a correlation coefficient of 0.9 for the relationship of pI and molecular weight. A protein homogenate from a Barrett's esophagus biopsy was also analyzed; over 150 components were resolved within 35 minutes. However, due to background fluorescence produced by trace impurities within the ampholytes used in the separation, detection limits for cIEF were four orders of magnitude poorer thenCE methods. In order to improve detection limits, we explored different method to decrease the fluorescent background such as photobleaching, oxidation reaction, and carbon treatment. Shifting the excitation and emission wavelengths to 532/ 580 nm was also explored. Improved detection limits in cIEF will have important implications for applying this technology to single cell analysis.

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P-119-M

Investigation of Intercellular Signaling Peptides at the Cellular and Subcellular Levels using Analytical Microanalysis

Stanislav S. Rubakhin; Jonathan V. Sweedler, *Beckman Institute & Department of Chemistry, UIUC, Urbana, IL*

Intercellular signaling peptides (SPs) play key roles in the modulation and integration of nervous system activity. Analysis of SPs in cells and tissues is challenging due, in part, to their broad range of concentrations, the large variety in physicochemical properties among SPs, and the dynamic changes in their expression which is dependent on the physiological state of an organism. Moreover, investigation of the function-related behavior of SPs in the nervous system requires methods of analytical microanalysis that allow the monitoring of synthesis, transport, and release of these analytes at the single cell and even subcellular level.

We have developed a broad range of approaches that allows detection and characterization of SPs in vertebrate and invertebrate cells and tissues. These approaches consist of optimized sample preparation techniques, analyte separation and detection methods, and specialized data analysis with in silico prediction of individual SP structures. The sample preparation techniques allow the isolation and preparation of individual organelles, neuronal processes, and cells. Preselected structural elements of mammalian and invertebrate nervous systems can be targeted using these techniques. Depending on sample and analyte properties, different separation and detection techniques are implemented including CE-LIF, CE-MS, CE-radionuclide detection, SPE-CE, SPE-MS, and microfluidic-MS. As proof of concept, these approaches are successfully applied to investigate SP content in individual secretory vesicles, release of SPs from different areas of a single cell, and spatial profiling of the localization of these compounds along a single neuronal process.

Several strategies are developed and applied to characterize unknown SPs including tandem MS approaches and hybrid approaches using bioinformatics. Multiple SPs are usually encoded by an associated prohormone gene. The prohormone is enzymatically processed into the bioactive SPs. Because of the number of processing steps, it becomes difficult to predict SPs from a novel gene. We have developed an approach for the in silico prediction of SP structure which is utilized in the Web-based tool Neuropred (neuroproteomics.scs.uiuc.edu/neuropred.html). Neuropred generates a list of the most likely SP molecular masses from a given prohormone gene. These masses are used in conjunction with the mass spectral data to discover and characterize novel SPs. Here, we describe the use of this suite of approaches for the characterization and functional assaying of SPs.

P-120-M

Entangled mesh size optimization for high performance protein separation by SDS capillary polymer electrophoresis

Keiko Sumitomo[1]; Koichi Mayumi[2]; Hiroyuki Minamikawa[3]; Mitsutoshi Masuda[3]; Hideaki Yokoyama[2]; Kohzo Ito[2]; Toshimi Shimizu[3]; Yoshinori Yamaguchi[4]

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The “mesh size” or “pore size” of sieving polymer networks is an important parameter for protein separation on SDS capillary electrophoresis. Although the mesh size has been estimated from the polymer

theory with the viscosity measurement of sieving polymer solutions, the estimation of average mesh size assumes the uniform networks in overall solution, which ignores the local conformations.

Dynamic light scattering (DLS) directly measures the mesh size of polymer networks including the mesh size distribution and polymer-solvent interaction. In this work, we directly evaluated the mesh size in the entangled polymer solution by DLS. The characteristic of polymer networks in solution, especially the mesh size, was related to the electrophoretic mobility or the resolution of protein separation.

In the presented work we directory measured the mesh size of linear polyacrylamide (LPA) with molecular masses of 600 000 and 5 000 000, hydroxyethyl cellulose (HEC) with molecular masses of 24 000, 90 000 and 720 000 and polyethylene oxide (PEO) with molecular masses of 35 000, 100 000, 300 000, 600 000, 1 000 000, 3 000 000 and 8 000 000 by DLS. The electrophoretic mobility and the resolution of fluorescein isothiocyanate (FITC) labeled standard proteins, whose masses range from 14 300 to 97 200 Da, was examined during the electrophoretic migration in capillary electrophoresis with these polymer solutions. We demonstrate the electrophoretic mobility of proteins is controlled only by the mesh size, but not by chemical compositions, molecular masses or local conformations of polymers. Meanwhile, the homogeneity of polymer network would influence the resolution of protein separation. We will discuss the details of the polymer networks and the optimized conditions for size-based separation of proteins by capillary electrophoresis.

NOTES:

P-121-M

Rolling Circle Amplification Combined with Capillary Electrophoresis for Detection of Small RNA

Wenwan Zhong; Ni Li, *University of California, Riverside, CA USA*

Our lab has developed a RCA-CE assay to detect DNA and has revised the assay for its application in small RNA detection.1 In the original assay design, sequence recognition is achieved by hybridizing the 5' and 3' termini of a padlock probe onto the DNA target and then joining them by a DNA ligase to form the circular template for amplification. Since the ligation efficiency of a DNA ligase using an RNA strand as template is very low, which would severely affect the detection sensitivity,2,3 and miRNAs have free hydroxyl group on their 3' ends and can serve as the primers for the DNA polymerase, Phi29, we removed the ligation step and started the reaction with a pre-formed circular probe which contained matching sequence to the target miRNA. The miRNA hybridized onto the circular probe and initiated the growth of the long RCA product upon the addition of the Phi29 enzyme. The circular probe also contained a sequence recognizable by a restriction enzyme, e.g. HhaI, so that the long product could be digested into monomers for CE analysis. The revised assay had a detection limit of 25 attomole, and the method was applied to detect the enhanced expression of the nat–siRNAATGB2 siRNA in only 90 ng of the total plant RNA extracts. We went on to test the specificity of this assay using several mutated RNA strands from the wild-type nat–siRNAATGB2, and significantly lower amounts of products were detected for the mutated targets, indicating the capability of our revised assay in discriminating sequence difference. The method can be adopted in a capillary array system to perform high-throughput analysis, and can be used as a fast and cost-effective assay for large-scale initial screening of small RNA expression in plants or other biological samples.

NOTES:

P-122-M

Progress Towards Highly Sensitive Detection of Biomolecules using Labeled Magnetic Nanoparticles in Microfluidic Devices

Esha Chatterjee; Simon Ghionea; Albrecht Jander; Pallavi Dhagat; Vincent Remcho, *Oregon State University, Corvallis, OR USA*

We report here on the progress made towards the development of a lab-on-chip sensor for fast and sensitive detection of biological and chemical agents.
The assay is analogous to a heterogeneous, sandwich immunoassay .The sample to be analyzed is mixed with nanoparticles conjugated with primary antibodies, which bind the analyte of interest. This mixture is then flowed over the detection area, functionalized with secondary antibodies, where the nanoparticle-analyte conjugate is immobilized via highly specific antigen-antibody interactions. Immobilized nanoparticles will be detected inductively at the ferromagnetic resonance (FMR) frequency.

Cobalt-silica core-shell nanoparticles have been synthesized by borohydride reduction of cobalt chloride in presence on citrate ions. A silica coat was introduced by the hydrolysis of TEOS (Tetraethyl orthosilicate). The effect of the order of addition of reactants on particle morphology was investigated. The particles were characterized by TEM and generation of a magnetization curve. For functionalization of the nanoparticles, the avidin-biotin pair was used as a surrogate for antigens and antibodies. Standard silanization chemistries were employed for the immobilization of biotin and avidin on the nanoparticles. The sensor contact patch, at which the nanoparticle-analyte conjugate is trapped, was selectively patterned by microcontact printing of avidin. This poster will describe investigations into sensor design and optimization.

P-123-M

Parallel Botulinum Neurotoxin/A Immuno- and Enzyme Activity Assays using the Versatile Rapidx Platform

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We present a novel approach for measuring the activity of Botulinum Neurotoxin Type A (BoNTA) in biological samples. BoNTA consists of a heavy chain, facilitating translocation into neuronal cells, and a light chain, which cleaves the SNAP25 protein of the SNARE complex within the cell thereby inhibiting neuronal function. The enzyme activity assay is readily incorporated in a multiplexed diagnostic platform that includes specific immunoassay detection of heavy and light chain toxin epitopes in parallel. Quantifying the level of toxin likely to enter nerve cells in conjunction with measurements of activity provides a more accurate diagnostic assessment. Measurements of enzyme activity are based on cleavage of a fluorescent synthetic substrate consisting of GST-SNAP25-eGFP (SNAG). Upon cleavage of the sandwiched SNAP25 sequence by active toxin, the SNAG construct (77 kDa) divides into non-fluorescent GST-SNAP25 and fluorescent eGFP (28 kDa) products that can be separated by gel electrophoresis.

Samples are first pre-treated and electrophoretically concentrated at an in situ photopolymerized polyacrylamide size-exclusion membrane. Intact SNAG is then loaded into the same membrane where it is

cleaved in the presence of active toxin. Intact and cleaved substrates are then PAGE separated and quantified by single-point LIF detection. The probe reaction products are difficult to resolve by native PAGE but are readily separated by SDS-PAGE on-chip. However, SDS inhibits enzymatic activity and we therefore designed a dual-membrane format to isolate reagent incubation from SDS denaturation steps prior to SDS-PAGE quantification.

The assay's configuration enables facile parallel incorporation with other assays on the multiplexed device used in the "RapiDx" – a portable, fully-automated diagnostic tool being developed at Sandia for the detection of toxins and host response markers (including BoNT, Shiga, SEB, Ricin, and several cytokines) from human samples with total assay time of ~10 minutes and low pM sensitivities.

NOTES:

P-124-M

Ultra-Trace Micro Gas Analysis System Utilizing Gas/Liquid Micro Flows

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Modern semiconductor industry demands ultra-clean environment in which not only particles but also chemicals should be removed from cleanroom air. Ammonia is a basic gas that affects reactivity of some photoresist and deteriorates quality of photo-patterning. In the next decade, control of ammonia concentration in 0.1 ppb level is required. For this purpose, a portable and continuous monitoring system with 0.1 ppb sensitivity is highly required. However, conventional methods do not meet these demands. In this work, we developed a microchip combining an ammonia extraction, gas-liquid phase separation and colorimetric reaction-detection with a thermal lens microscope (TLM) for practical application (ammonia detection) in semiconductor industry. The micro gas analysis system comprised of a gas pump with baking at 120 °C, a liquid pump, an annular gas-liquid microflow, ammonia extraction and concentration, gas-liquid phase separation, colorimetric reaction, and TLM detection. For sensitive detection, gas-liquid concentration factor is very important. For this purpose, large ratio in Vgas/Vliq (V: volumetric flow rate) is critical. However, the phase separation at the large flow rate ratio is very difficult because of an unstable flow. Here, we utilized gravity for the phase separation. In the downstream part, the gas-phase flowed out through the hole having a diameter of 2 mm, in which the Bond number is 1. As a result, Vgas/Vliq of 100000 (Vgas: 100 mL/min, Vliq: 1 μL/min) was achieved. Under this condition, 100 % extraction and 100 times concentration were realized for 6 min liquid-contact-time. We examined two-step colorimetric reaction for 10 min and limit of detection of 0.9 ppb ammonia solution in a microchip. By considering the concentration, 10 ppt ammonia gas can be detected within 16 min, and requirements for this system are almost met. Our method can be a powerful tool for on-site sensitive and fast gas analysis.

P-125-M

Multiplexed Microfluidic Immunoassays for the Detection of Biomarkers in Saliva

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Interest in integrated microfluidic systems for performing rapid biological analyses has increased in recent years as these platforms allow for sample pretreatment, separation, and detection with minimal external interaction. The application of such platforms to single analyte immunoassays has been demonstrated; however, simultaneous analysis of multiple analytes using a microarray format offers more information while reducing analysis time, cost, and reagent consumption. A novel PDMS-glass hybrid device incorporating an antibody-based microarray for the detection of proteins in saliva has been developed. Biomarker proteins in saliva can be used to diagnose and monitor the progression of pulmonary diseases, including cystic fibrosis and asthma. Protein detection is based on sandwich-immunoassay principles where sample, biotin-labeled secondary antibodies, streptavidin-labeled dye molecules, and the appropriate wash solutions are sequentially administered to the array. Due to the large number of interfering mucins and proteins present in saliva samples, various surface passivation reagents and methods were investigated to minimize non-specific protein adsorption to the PDMS and glass surfaces. Of the methods tested, the addition of a dynamic hydrophilic polymer coating to the buffer and reagent

solutions proved most effective in reducing non-specific adsorption. In addition to the surface passivation conditions, reagent concentrations, assay temperature, and sample and reagent incubation times were optimized using VEGF as the model analyte. Based on the optimized parameters for VEGF, additional proteins (IL-8, TIMP-1) important as markers for pulmonary diseases were investigated in both buffer and spiked saliva supernatant solutions. Results from these studies show that the highly sensitive assay can be completed in less than 60 minutes using reagent and sample volumes of less than 20-µL each. In addition, quantitative studies using VEGF and IL-8 proteins showed limits of detection in the low femto-molar range when measured in both buffer and spiked saliva supernatant solutions.

NOTES:

P-126-M

Determination of Cyanobacterial Cyclic Peptide Hepatotoxins in Drinking Water using Capillary Electrophoresis.

Sam F. Y. Li, *National University of Singapore, Singapore, Singapore*

Four cyanobacteria hepatotoxins, namely Microcystin LR, Microcystin RR, Microcystin YR and Nodularin were simultaneously determined in tap water using Capillary Zone electrophoresis (CZE) and Micellar Electrokinetic Capillary Chromatography (MECC) coupled with UV detection. The four toxins were clearly separated in the CZE and MECC modes using formic acid or phosphoric acid as the back ground electrolyte (BGE). Detection limits in the range of 0.77 to 4.77 µg/mL were obtained.

P-127-M

A Fritless, EOF Microchip Pump for µ-HPLC

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In the last two decades tremendous effort has been directed toward the research and development of miniaturized total analysis systems (µ-TAS) or lab-on-a-chip. Ideally, a µ-TAS should be capable of performing all the analytical steps that comprise an analysis, including sample preparation, separation of multiple components from a complex sample matrix, and detection of each individual analyte. The accurate transportation of fluid within a microfluidic network via an on-chip micropump is a critical and limiting component to future advancement of µ-TAS. For example, there is growing interest in expanding the separation capabilities of capillary electrophoresis and electrochromatography microchip devices with a complementary approach, micro-high pressure liquid chromatography (µ-HPLC). The incorporation of high back pressure, packed bed microcolumns onto lab on a chip platforms presents a challenge for the integrated micropump. Electroosmotic flow (EOF) micropumps are a potential, on-chip solution to the pumping requirements for µ-HPLC because of their high pressure compatibility, reasonable flow rate generation, ease of integration and operation, and low fabrication cost. We will discuss the characterization and performance of a fritless, high pressure microchip EOF pump that is comprised of a densely packed microchannel containing 800 nm silica particles, a design which results in considerable improvements in high pressure generation for more effectively meeting future µ-HPLC requirements on a microchip platform. The operation capabilities, such as maximum flow rate, maximum pressure, and flow rate against a back pressure, of this pump for µ-HPLC-like applications, are assessed with pumping fluids such as aqueous buffer (CHES, pH 9), pure organic solvent (acetonitrile), and isocratic mixtures (water/methanol and water/acetonitrile). We will also present the on-chip integration of an EOF pump with a liquid chromatography (LC) microcolumn packed with C8 – C18 beads as the stationary phase for the separation of fluorescently labeled amino acids via liquid chromatography.

NOTES:

P-128-M

Cge-Lif Based Glycan-Analysis in Influenza Vaccine Production: Influence of Host Cell and Virus on the Glycosylation Pattern of Viral Hemagglutinin

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Mammalian cell culture processes, are commonly used for production of recombinant glycoproteins, antibodies and viral vaccines. Since several years there is an increasing interest in cell culture-based influenza vaccine production to overcome limitations of egg-based production systems, to improve vaccine supply and to increase flexibility in vaccine manufacturing. With the switch of the production system several key questions concerning the possible impact of host cell lines on antigen quality, passage-dependent selection of certain viral phenotypes or changes in hemagglutinin (HA) conformation have to be addressed to guarantee safety and efficiency of vaccines. In contrast to the production of recombinant glycoproteins, comparatively little is known regarding glycosylation of HA derived from mammalian cell cultures. Within this study, a capillary DNA-sequencer (based on CGE-LIF technology), was utilized[1] for N-glycan analysis of three different influenza virus strains, which were replicated in six different mammalian cell lines. Detailed results concerning the influence of the host cell line on complexity and composition of the HA N-glycosylation pattern, are presented. A strong host cell dependence of HA N-glycosylation could be shown. Clear differences were already observed, by simple fingerprint comparison. Further structural investigations of the N-glycan pools turned out, that the host cell dependence of HA N-glycosylation mainly results in variations in composition of the attached N-glycans and less in principle changes in N-glycan type. In contrast to this, a significant change in N-glycan type
attached to HA was observed, comparing different virus types and subtypes.

[1] J. Schwarzer, E. Rapp, U. Reichl, *N-glycan analysis by CGE–LIF: Profiling influenza A virus hemagglutinin N-glycosylation during vaccine production*, Electrophoresis, 2008, in press.

P-129-M

A Microfluidic Platform to investigate Structure and Function of Small Arteries

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The peripheral vascular resistance built up by small arteries with diameters between 60 – 250µm is a primary factor contributing to the regulation of blood pressure. Under pathophysiological conditions, inadequate regulation of these small arteries' diameter can result in high blood pressure (hypertension), which is a major risk factor for many cardiovascular diseases. Presently available experimental methods to study the 1-2 mm long segments of animal-derived small arteries are time-consuming, dependent on highly-trained personnel and rather expensive. We have designed and fabricated a polymer-based microfluidic device which overcomes several of these limitations and provides for the first time a high-throughput compatible solution for microvascular studies.
Our device has a number of key features. First is the organ bath, which is designed to deliver various drugs via superfusing flow. This is the region which must mimic physiological conditions, and is the region imaged during experiments. The next key feature is the four fixation points at each end of the vessel.

These serve a dual purpose, first is to provide a location to hold the vessel firmly in place via the application of a negative hydrostatic pressure. This suction also serves to separate the perfusion and superfusion flows required to maintain the local environment as close to physiological conditions as possible. During the course of a typical experiment, the artery will be introduced into the chip via pressure driven flow from a loading well. It is then fixed in place with suction at the fixation point, and the microenvironment is adjusted to physiological conditions via perfusion through the lumen, superfusion around the outside to control differential pressure across the vessel wall, and heating to maintain a temperature of 37°C. The flowrates, pressures, and compositions of the superfusing/perfusing streams can also be independently adjusted.

To investigate dynamic changes of the artery diameter (tone) in response to altered drug concentrations two individual feed streams, “B1” and “B2”, were initially mixed on the chip. The concentration of active substances in the resulting superfusing stream “B” could then be adjusted by changing the relative flow rates of the two computer-controlled syringe pumps. The time-dependent evolution of the artery tone was recorded on an inverted microscope.
The scalable device enables routine long-term culture and analysis of small blood vessels with a wide range of applications in microvascular research and drug discovery. Future work will involve the downstream integration of analytical tools, e.g. mass spectrometry.

NOTES:

P-130-M

Detection of Antibiotics Residues by Microcantilevers

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Development of sensitive and reliable assays for simultaneous detection of veterinary drug residues in milk is of high interest. Here we present and discuss the preliminary results of a label-free immuno-assay based on silicon microcantilevers (MCs) that we developed for detecting Penicillin and Tylosin in milk.
In biosensing platforms, MCs are functionalized with a probe that can selectively bind the target species. Specific adsorption of the target species induces a nanomechanical response of the MC. On the basis of the actuation mode, the response transduces (part of) the energy involved in the formation of the ligand-receptor complexes (MC deflection, static mode) or the ligand mass load (changes in the MC eigenfrequencies, dynamic mode). Microcantilevers are thus label-free and may reach sensitivities spanning from picograms to attograms, comparable with those of more mature biosensors, such as Surface Plasmon Resonance (SPR) spectroscopy and Quartz Crystal Microbalances (QCM). They also feature fast and real time detection, small size, implementability for multiplex analysis. In particular, thanks to direct nanomechanical transduction of the complex formation energy, when actuated in static mode MCs constitute a promising alternative for detection of low molecular weight species, including antibiotics.
For the detection of Tylosin and Penicillin we implemented MC immuno-assays made of arrays of eight silicon MCs (1x100x500 um3 each), prepared for static mode actuation. They were functionalized by a thin coating of a copolymer that bears the ability to react with the silanols onto the silicon MC surface as well as with the nucleophilic species displayed by biomolecules. The upper faces of the coated MCs were then decorated with polyclonal anti-Tylosin and anti-Penicillin antibodies and used to detect Tylosin and Penicillin in buffer solution. Performance, limits and perspectives of the proof-of-concept experiments will be discussed.
Aknowledgements:
FP7 – Capacities research grant NASPE (Nanomechanical Screeneing of Pharmaceutical Entities)

P-131-M

Analysis Of Citrinin and Monacolin K by Sweeping Micellar Electrokinetic Chromatography

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We have applied sweeping micellar electrokinetic chromatography (sweeping-MEKC) to the simultaneous determination of citrinin and monacolin K. Citrinin and monacolin K are the bioactive secondary metabolites produced by Monascus. Citrinin is one of mycotoxin found nephrotoxin and hepatotoxin effects on animal and human health and the quantity must be lower than 0.2 ppm regarded as safety when taking the related foods. Monacolin K is found to be effective as hypocholesterolemic agent to reduce serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol. Hence, the determination of citrinin and monacolin K appears to be of major importance for the analysis of Monascus products such as red mold rice. We monitored the effects of several of the sweeping-MEKC parameters, including the proportion of organic modifier, the concentration of sodium dodecyl sulfate (SDS), the pH, and the sample injection volume, to optimize the separation process. We also combined the sweeping-MEKC method with solid-phase extraction to successfully detect trace analytes with acceptable repeatability. Therefore, this

sweeping-MEKC method is useful for determining, with high sensitivity, the amounts of citrinin and monacolin K in real samples.

NOTES:

P-132-M

Determination of Phenolic Compounds in Distilled Beverages using CE Field Amplified Sample Stacking

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Reproducible and straightforward analytical methods are required by trading standards and trading authorities to confirm the authenticity of distilled beverages such as whiskey. This leads to characteristic analytical profiles for various congeners, which can be used as reference points in authenticity analysis.

The phenolic acid content of distilled beverages, including Irish, Scotch and American whiskeys was measured using CE with UV detection. Utilising Field Amplified Sample Stacking (FASS), whiskey samples were injected directly onto the capillary and preconcentrated online, with a total analysis time of less than 20 minutes. The Irish whiskeys and Scotch blended whiskeys were found to contain comparable concentrations of phenolic acids vanillin, gallic acid, syringic acid and vanillic acid. The single malt Scotch Glenfiddich was found to a contain a 3-fold increase in concentration in vanillin and gallic acid. Jack Daniel's, and American whiskey, had a comparable total phenolic acid concentration, but a lower gallic acid and a higher syringic acid concentration. The phenolic acid content of whiskey was found to depend primarily on the maturation process, namely the length of maturation, and the type of casks used. The type of grain used to produce whiskey may also affect the concentration and type of phenolic acids detected in the final product, along with processes such as charcoal mellowing, which are carried out prior to maturation.

1. Aylott et al., Analyst, 1994, 119, 1741-1746

P-133-M

Application of recA and rpoB Sequence Analysis on Phylogeny and Molecular Identification of Geobacillus Species

Shang-Shyng Yang; F. Y. Weng, *National Taiwan University, Taipei, Taiwan*

Some Geobacillus species have highly similar 16S rRNA gene sequences, making 16S rDNA sequence analysis-based identification problematic. To overcome this limitation, recA and rpoB sequence analysis was evaluated as an alternative for distinguishing Geobacillus species. The phylogram of 16S rRNA gene sequences inferred from the neighbour-joining method showed that nine clusters of Geobacillus species were characterized with bootstrap values >90%. The recA and rpoB sequences of 10 reference strains in clusters V, VIb, and VIc were amplified and sequenced using consensus primers. Alignment of recA sequences in clusters V, VIb, and VIc revealed three types of recA genes, consistent with the putative amino acid sequences and in vivo recA splicing analysis. The phylogram constructed from rpoB sequences showed more divergence than that constructed from 16S rRNA gene sequences. recA and rpoB sequence analysis differentiated closely-related Geobacillus species and provided direct evidence for reclassifying some species dubiously categorized as Geobacilli. Additionally, this study revealed three types of recA genes in the different Geobacillus species. This study highlights the advantage of recA and rpoB sequence analysis to supplement 16S rRNA gene sequence analysis for efficient and convenient determination of Geobacillus species. Various other genes, including the sodA and hsp65, are being evaluated for their abilities to enhance discrimination among currently recognized Geobacillus species.

NOTES:

P-134-M

Rapid Analysis of Melamine in Infant Formula by Capillary Electrophoresis with On-Line Concentration Techniques

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Melamine (2,4,6-triamino-1,3,5-triazine) is a nitrogen-rich organic base usually used in the field of plastics manufacturing. In 2008, more than 47,000 infants and young children in China suffered from kidney damage caused by consuming melamine-contaminated infant formula. The outbreak of this calamity pushed the governments worldwide to set the limit of detection of melamine in infant formula. Following this, developing a rapid and sensitive analytical method to detect melamine in infant formula is an urgent task.

Two on-line preconcentration techniques in capillary electrophoresis, namely, field amplified sample stacking (FASS) and sweeping, were developed and compared in this study. Parameters affecting concentration efficiency of each technique were optimized. The two methods were compared for their limits of detection (LOD) and the enhancement factor (EF). Under optimum conditions, the concentration LODs of melamine were found to be 1.2 ppb (ng/mL) and 8.7 ppb for the FASS-CZE (capillary zone electrophoresis) and the sweeping-MEKC (micellar electrokinetic chromatography) methods, respectively. The enhancement factors of the two on-line concentration methods compared with the conventional CZE and MEKC methods were 316 and 111 , respectively. In order to minimize the matrix effect, a solid-phase extraction (SPE) procedure was employed.

Validation and the application of the developed method will be reported later. Preliminary test for the application potential was demonstrated in this study by spiking different concentrations of melamine into several products of infant formula obtained from the markets. It should be able to be used in quality control of infant formula.

P-135-M

Determination of Heroin and its Metabolites in Human Urine by Capillary Electrophoresis

Shou-Mei Wu, *Kaohsiung Medical University, Kaohsiung, Taiwan*

Heroin (H) and its metabolites including morphine (M), codeine (C), 6-acetylmorphine (6-AM) were determined in urine by micellar electrokinetic chromatography. The optimal conditions were investigated and validated. The baseline separation could be done within 10 minutes. During method validation, the calibration curves were linear over a range of 50-500 ng mL-1 (r □ 0.994). The RSD and RE values in intra-day and inter-day assays were all below 18.4%, which showed good precision and accuracy. The limits of detection were 10 ng mL-1 (S/N = 3, 20 kV, 300 s). This method was applied to determine real urine samples from addicts. These samples were confirmed by mass spectrometry.

NOTES:

P-136-M

On-line, continuous nanofiltration for isolation of gold eleven nanoparticles synthesis produced in microreactors

Taehyeong Kim; Vincent T. Remcho, *Oregon State University, Corvallis, OR USA*

Nanofiltration membranes embedded in microfluidic structures enable the separation of gold eleven nanoparticles (Au11(PPh3)8Cl3, m.w. 4371) when coupled with continuous pump driven-flow synthesis system. An organic solvent resistant nanofiltration membranes, STARMEM™ 122 (Membrane Extraction Technology Ltd., London, UK) and Ceramic membrane (Sterlitech Corporation, Kent, WA), with molecular weight cut-off values of 220 and 1000, respectively, were used in the experiments. The rejection of gold eleven nanoparticles is 93% using the STARMEM™ 122 membrane; the byproducts, which permeate through membrane, can be determined by uv-vis and NMR data. The continuous synthesis system coupled with the filtration step results in a significant reduction in synthesis time and higher yield than batch scale can be achieved.

P-137-M

Detection of Metal Nanoparticles by Optical Microscopy Based on Localized Surface Plasmon Resonance (LSPR)

Wei Sun; Ning Fang; Edward Yeung; Changbei Ma, *Iowa Sate University, Ames, IA USA*

Metal nanoparticles show intense absorption and scattering of light in UV-VIS range. The scattering and absorption happen when the photon frequency of incident light is resonant with the collective oscillation of conduction electrons confined in the metal nanoparticles. It is known as localized surface plasmon resonance (LSPR). In this work, we tried three modes of optical microscopy to detect gold and silver nanoparticles. They are bright field, dark field and differential interference contrast (DIC) microscopy. Different from normal operation of optical microscopy, we changed the incident light by using different wavelength bandpass optical filters. The reason to change incident light wavelength is to make use of the LSPR of metal nanoparticles. By using incident light with wavelength at around LSPR peak, bright field microscopy can detect as small as 30nm gold nanoparticle and 30nm silver nanoparticle. It is based on the strong absorption of metal nanoparticles at LSPR peak. Dark field microscopy can have enhanced signal when operated under wavelength a little larger than LSPR peak. According to Kramers-Kronig relationship, metal nanoparticles increase refractive index much at around LSPR peak. Since DIC microscopy utilizes the refractive index difference between metal nanoparticles and surrounding medium, DIC can detect as small as 20nm gold and 30nm silver nanoparticles when using wavelength a little larger than LSPR peak. The result can be further applied to other metal nanoparticles that have LSPR.

NOTES:

P-138-M

Dielectrophoretic Fractionation of a Mixture of Bacteria and Yeast

Blanca Lapizco-Encinas;[1] Hector Moncada-Hernández[1]; Blake A Simmons[2];

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This work presents fractionation and separation of yeast (Saccharomyces cerevisiae) and bacteria (Escherichia coli) employing insulator-based dielectrophoresis (iDEP) and direct current (DC) electric fields. Numerous reports exist in the literature describing the manipulation of yeast and bacteria with the use of microelectrodes and alternate current (AC) electric fields. An array of insulating structures and DC electric fields were employed in this work, instead of an array of microelectrodes and AC electric fields to fractionate a mixture of bacteria and yeast. Dielectrophoresis (DEP) is described as the motion of particles resulting from polarization effects induced by non-uniform electric fields. This technique has been successfully employed for the manipulation of a wide range of bioparticles. The use of microelectrodes in the generation of non-uniform electric fields has some disadvantages compared with the use of insulating posts: higher cost of fabrication, less durability. The present study focuses on the potential of iDEP for yeast and bacteria cells fractionation. A microdevice made from the polymer Zeonor was employed. This device contained eight microchannels that were 10.2-mm long, 1-mm wide and 75-μm deep. Each microchannel contained an array of 12 columns, each with 5 rows of cylindrical insulating structures, 150-μm in diameter and 200-μm center-to center. Different electric field intensities across the post array were achieved by applying DC field between the inlet and outlet reservoirs of the microchannel. Several buffer solutions with different pH and conductivity were employed as a suspending medium. After a sample mixture of bacteria and yeast was injected in the inlet reservoir, the electric field was applied, and the presence of cylindrical posts formed zones of higher and lower intensity. The fractionation of bacteria and yeast was recorded in video and photo files. The results obtained demonstrate the potential of iDEP for sample concentration.

P-139-M

Insulator-Based Dielectrophoretic Concentration of Microalgae

Blanca Lapizco-Encinas; Jose I. Martínez-López; Roberto C. Gallo-Villanueva; Ileana Hernández-Mireles; Sergio O. Martínez-Chapa; Mario M. Álvarez, *Tecnologico de Monterrey, Monterrey, Mexico*

Dielectrophoresis (DEP) is an electrokinetic transport mechanism of particles produced by polarization effects in presence of a non-uniform electric field. In electrodeless dielectrophoresis (iDEP) such field is achieved by the inclusion of insulator structures between two electrodes, which creates zones of higher and lower intensity of the field, producing dielectrophoretic traps.

In this work, iDEP was employed to concentrate and fractionate microalgae in a microchannel containing an array of cylindrical insulating posts. The channel used was 10.12-mm-long, 1-mm-wide, 10-um-deep, and the posts had an array of 8 columns x 4 rows, with 200-um in diameter arranged 250-μm center-to-center.

Microalgae are photosynthetic, heterotropic organisms, which comprise the base of the food pyramid in the oceans. Recently, its study has increased for their role in the CO2 equilibrium in our planet, and their culture has been suggested as an alternative to attenuate or control global warming through CO2 capture.

They are also cultured to produce fuels and as a food source. They can be collected from fresh and sea water to be grown in closed-culture systems, but one limiting factor of this method is the isolation and concentration of each strain. Traditional isolation techniques may require 3 or 4 weeks of laboratory work, and cell growth processes may consume much time if the culture starts from only a few cells. Therefore, having samples of concentrated microalgae can significantly accelerate growth.

Microalgae (Chlorella kessleri) having an average diameter of 5 μm were dielectrophoretically concentrated employing DC electric fields. Results showed the immobilization and concentration of microalgae in bands across the cylindrical post array as a function of the applied electric field. By increasing the field, the concentration factor of microalgae increased. These results demonstrate the potential of this novel technique for rapid microalgae concentration, as a great alternative to traditional methods.

NOTES:

P-140-M

Development of an Enzymatic Microreactor Based on Microencapsulated Laccase Coupled to CE for On-line Measurement of Oxidation Reactions

Karen C. Waldron; Georgiana Gusetu-Roman; Dominic Rochefort, *University of Montreal, Montreal, Canada*

Microencapsulation as a means of enzyme immobilization provides an interesting alternative to enzyme attachment on solid phases because the interior cavity of the capsules creates an aqueous microenvironment that protects the enzyme from the external medium to thereby maintain enzyme activity and stability. We have been studying microencapsulated laccase, an oxidase enzyme, using o-phenylenediamine (OPD) as a model substrate. A capillary electrophoretic method with UV absorbance detection (CE-UV) was optimized to separate the substrate and products and to quantify the enzymatic reaction for both free and immobilized enzyme. Microcapsules were then packed into a capillary-sized microreactor and conversion of substrate to product was followed by CE-UV in an off-line manner. We are now coupling the microreactor to the CE-UV system on-line via a tee and micro-injection valve. The preliminary results of this system compared to the off-line and batch-wise experiments will be presented. Our long-term goal is to develop an integrated system based on CE that can be used to evaluate the efficiency of microencapsulated enzymes in biosensor and bioreactor applications.

P-141-M

Mobility and Band Broadening in Capillary and Microchip Electrophoresis: Theoretical Considerations and Computer Simulations

Mykyta V. Chubynsky[1]; Christopher P. Fredlake[2]; Daniel G. Hert[2]; Jean-Francois Mercier[1]; Annelise E. Barron[3]; Gary W. Slater[1]

[1]University of Ottawa, Ottawa, ON, Canada; [2]Northwestern University, Evanston, IL USA; [3]Stanford University, Stanford, CA USA

Based on experimental data for both ssDNA [1,2] and dsDNA [2], we show that the electrophoretic mobility μ in strong fields in entangled polymer solutions has a simple dependence on the DNA size N: $\mu=C_0\exp(-N/N_0)+C_1$, where C_0, C_1 and N_0 are constants. This dependence is also observed in computer simulations of the electrophoretic motion of a chain in a disordered array of obstacles, except for very short chains. In this simple computational model, we study both the case when the obstacles are immobile and the case of obstacles that can be dragged by the chain reflecting suggestions from recent videomicroscopy experiments [1,3] that matrix polymers can be dragged by the DNA even in well-entangled solutions. Parameter N_0 has a simple power-law dependence on the polymer concentration. In this model we also study the dependence of the band broadening on the "draggability" of the obstacles. We also suggest an additional source of band broadening in capillaries and microchips: Taylor-Aris dispersion due to the reduced mobility of DNA near the wall, which is expected since both the matrix polymers and the coating brush are present in this layer. The strength of the effect depends on the thickness of the coating layer, the amount of the mobility reduction and the transverse diffusion coefficient, but is independent of the capillary width. We show that if the thickness of the coating is on the order of 100 nm, this effect alone can produce the dispersion comparable to that observed experimentally.

[1] C. P. Fredlake et al., PNAS 105, 476 (2008)

[2] C. Heller, Electrophoresis 20, 1962 (1999)

[3] T.N. Chiesl et al., Anal. Chem. 79, 7740 (2007)
NOTES:

P-142-M

Experimental evidence for concentration polarization-based stacking/destacking phenomena in electrochromatography

Ivo Nischang[1]; Ulrich Tallarek[2]

[1]University of California, Berkeley, CA USA; [2]Philipps-Universitaet, Marburg, Germany

We identify electrical field-induced concentration polarization (CP) as the key physical phenomenon determining both the migration and zone dispersion of charged analytes in electrochromatography (EC) and related electrical field-assisted processes. Prerequisite of the used hierarchically structured packed beds is the coexistence of quasi electroneutral macroporous regions between the micrometer sized particles and the ion-permselective intraparticle pores. It is caused by coupled mass and charge transport normal to the charge-selective external surface of the particles, which leads to concentration gradients of ionic species in the adjoining interparticle electrolyte solution. Charged analytes traversing the conductivity "mountains" and "valleys" locally on the local particle scale are affected by CP, which on macroscopic scale influences their effective migration behavior and zone propagation (stacking/destacking). We present a detailed analysis based on quantitative confocal laser scanning microscopy, demonstrating the dependence of CP on the ionic strength of the background electrolyte (which modulates the intraparticle ion permselectivity) and electrical field strength (which modulates local transport). These results are complemented by zone sharpening phenomena observed in EC. Under conditions which depend on the mass/charge ratio of the analytes and the intensity of the developed local concentration gradients, we observe stacking phenomena, accompanied by peak shape anomalies. These stacking phenomena, which cannot be related to the injection histories, are elucidated in the framework of electrical field-induced CP. Further, they are discussed in a context with recently published results, reporting unreproducible observation of efficiencies of several million plates per meter for charged analytes.

P-143-M

Molecular Dynamics Simulation of the Effect of Polymer Brushes on EOF

Gary W. Slater; Owen A. Hickey, *University of Ottawa, Ottawa, Canada*

Typically the materials which are used to make micro- and nano-fluidic devices become charged when placed in contact with water. The thin charged layer of fluid subsequently drags the bulk of the fluid when an electric field is applied; this type of flow is called the electro-osmotic flow (EOF). In many cases, most notably capillary electrophoresis, the EOF is often suppressed by way of polymer coatings. In this talk we will present Molecular Dynamics simulation results which investigated neutral polymer brushes. The simulations were able to confirm scaling relationships in both the mushroom and brush regimes. For adsorbed polymer coatings, our results indicate that maximum EOF supression is obtained for weakly adsorbed polymers. We also present results for charged polymer brushes which confirm that the electroosmotic mobility of the polymer brush is equal to the electrophoretic mobility of the polymers which make up the brush for sufficiently thick brushes. In the case of charged brushes similar scaling results are more difficult as electrostatic interactions cause the brush to swell. Interestingly the EOF reverses direction before the net charge on the wall and the coating changes from negative to positive.

NOTES:

P-144-M

Computer Assisted Investigation into Principles of Enantiomeric Separation in Capillary Electrophoresis

Ying S. Sun; David D.Y. Chen, *University of British Columbia, Vancouver, Canada*

While chiral separation in chromatography is based on the difference in the affinity of the enantiomers towards the immobilized chiral selectors, chiral separations in CE depends on more complex factors. Computer simulation can be used to elucidate the determining factors that result in the separations of the enantiomers, and predict the efficiency of the chiral selectors when used in chromatography. We developed a JAVA based program CoSiDCCE (computer simulation of dynamic complexation capillary electrophoresis) to investigate the migration behaviors of different species involved in the competitive dynamic complexation interactions during a chiral CE process. In this work, three chiral selectors, α-cyclodextrin (α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin (γ-CD), were used as chiral selectors for the separation of amino acids enatiomers. The binding constants and the complex mobilities were estimated using nonlinear regression methods. The accuracy and the prediction capability of the CoSiDCCE were evaluated through the comparison of the experimental and simulated results. With the displayed concentration profile of the species involved in the interaction, the migration behaviors and separation mechanism of each species in the capillary can be well understood.

NOTES:

PLACE B&W AD HERE

Tuesday, February 3, 2009

07:30 – 17:30 *Registration on the Mezzanine Foyer*

07:30 - 08:30 *Continental Breakfast in the Imperial Ballroom*

PARALLEL SESSION in the Plaza Ballroom
~Carbohydrates~

CHAIR: Ed Yeung, *Iowa State University, Ames, Iowa USA*

08:30 – 08:55 **MALDI-TOF/MS and CE-LIF Glycomic Mapping in Cancer Diagnostic and Prognostic Measurements**
Milos Novotny, *Indiana University, Bloomington, IN USA*

08:55 – 09:20 **Integrated Microanalytical Strategies to Study the Glycosylation Pattern of Human Plasma Glycoproteins in Various Cancer Types**
András Guttman, *University of Innsbruck, Innsbruck, Austria*

09:20 – 09:45 **Cancer Detection Based On Glycoprotein Profiling Of Plasma**
Fred Regnier, *Purdue University, West Lafayette, IN USA*

09:45 – 10:05 **Monosaccharide Composition Analysis of Glycoproteins through Multiple Reaction Monitoring Mass Spectrometry**
Yehia Mechref, *Indiana University, Bloomington, IN USA*

10:05 – 10:25 **Rapid Analysis and Characterization of Intact Glycoproteins using CE-MS with LTQ – FT Instrument**
Tomas Rejtar, *Northeastern University, Boston, MA USA*

10:25 – 10:55 *Break - **Visit the Exhibits and Posters: Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~Sampling and Concentration~

CHAIR: Michael Bowser, *University of Minnesota, Minneapolis, MN USA*

08:30 – 08:55 **Modifications of Surface Chemistry of Porous Polymer Monoliths Enhancing their Performance**
Frantisek Svec, *Lawrence Berkeley National Laboratory, Berkeley, CA USA*

08:55 – 09:20 **Droplet Compartmentalization of Chemical Speciation**
Daniel Chiu, *University of Washington, Seattle, WA USA*

09:20 – 09:45 **Enhancing the Detectability in Microscale Electrophoretic Separations**
Koji Otsuka, *Kyoto University, Kyoto, Japan*

09:45 – 10:05 **Gradient Elution Isotachophoresis-Capillary Zone Electrophoresis: Rapid Enrichment Meets Rapid Separation**
Jonathan G. Shackman, *Temple University, Philadelphia, PA USA*

10:05 – 10:25 **Non-Protein Antibodies against Proteins, Viruses, Bacteria … Applications: (1) Biomarkers (2) Enzyme Reactors, Biosensors, etc. and (3) "Negative Purification"**
Stellan V. E. Hjertén, *Uppsala University, Uppsala, Sweden*

10:25 – 10:55 *Break - **Visit the Exhibits and Posters: Imperial Ballroom & Stanbro Room***

PARALLEL SESSIONS in the Plaza Ballroom
~ Bio-omics~

CHAIR: András Guttman, University of Innsbruck, Innsbruck, Austria

10:55 – 11:20 **Direct Imaging of Metabolites in Plant Tissues by Mass Spectrometry**
Ed Yeung, *Iowa State University, Ames, Iowa USA*

11:20 – 11:40 **Characterization of Membrane Proteins Using Nanoflow Liquid Chromatography-Tandem Mass Spectrometry**
Xiaoying Ye, *SAIC, Frederick, MD USA*

11:40 – 12:00 **Microfluidic LC Chips with PQD-Ion Trap MS Detection for iTRAQ-Based Protein Differential Expression Analysis**
Iulia Lazar, *Virginia Bioinformatics Institute, Blacksburg, VA USA*

12:00 – 12:20 **Capillary Electrochromatography and Its Applications in Pharmaceutical and Biochemical Analyses**
Chao Yan, *School of Pharmacy, Shanghai Jiaotong University, Shanghai, China*

12:20 – 12:40 **Challenges in Developing a 2D Gel on a Capillary Based System**
Jane A. Dickerson, *University of Washington, Seattle, WA USA*

12:40 – 14:00 *Lunch Break – Attendees on their own ~OR~ Attend the **Technical Seminar sponsored by Beckman Coulter** in the Plaza Ballroom. Lunch is provided for the first 100 attendees only. The seminar runs for one hour - 12:50 to 13:50.*

14:00 – 15:30 ***Poster Session #2 in the Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~Microfluidics 2~

CHAIR: Robert Kennedy, *University of Michigan, Ann Arbor, MI USA*

10:55 – 11:20 **Particle Manipulation Strategies in Microfluidic Devices**
Elisabeth Verpoorte, *University of Groningen, Groningen, The Netherlands*

11:20 – 11:45 **Microfluidics and Cellular Diagnostics from Blood**
Mehmet Toner, *Harvard Medical School, Cambridge, MA USA*

11:45 – 12:05 **Polymer Microchip with Integrated Magnetic Tracks for Multi-Plug Magnetic Bead Capture: Characterization, Advantages and Applications.**
Mélanie Abonnenc, *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

12:05 – 12:25 **Cell Electrophoresis**
Ferenc Kilár, *University of Pecs, Institute of Bioanalysis, Pecs, Hungary*

12:25 – 12:45 **Microfluidic Immunophenotyping of Endothelial Progenitor Cells**
Shashi Murthy, *Northeastern University, Boston, MA USA*

12:45 – 14:00 *Lunch Break – Attendees on their own ~OR~ Attend the* ***Technical Seminar sponsored by Beckman Coulter*** *in the Plaza Ballroom. Lunch is provided for the first 100 attendees only. The seminar runs for one hour - 12:50 to 13:50.*

14:00 – 15:30 ***Poster Session #2 in the Imperial Ballroom & Stanbro Room***

PARALLEL SESSIONS in the Plaza Ballroom
~Single Cells~

CHAIR: Lingjun Li, *University of Wisconsin at Madison, WI USA*

15:30 – 15:55 **What is a Neuron from a Genomic Standpoint? Unbiassed Molecular Dissection of Single Cells and Cell Compartments**
Leonid Moroz, *University of Florida, St. Augustine, FL USA*

15:55 – 16:20 **Twenty Years of Single Cell Analysis**
Jean Rossier, *ESPCI, Paris, France*

16:20 – 16:45 **Bioanalysis of Single Skeletal Muscle Fibers**
Edgar Arriaga, *University of Minnesota, Minneapolis, MN USA*

16:45 – 17:05 **On-Chip Flow Cytometry Coupled to Single-Cell Imaging for Studying Signal-Transduction Pathways**
Thomas Perroud, *Sandia National Laboratory, Livermore, CA USA*

17:05 – 17:25 **Cell-Based Assays on a Chip With a Cell Bank**
Yan Xu, *University of Tokyo, Tokyo, Japan*

17:30 – 19:00 ***Exhibitor & Poster Reception – Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~ Nanoscale~

CHAIR: Elisabeth Verpoorte, *University of Groningen, Groningen, The Netherlands*

15:30 – 15:55 **Electokinetic Transport and Trapping in Planar Nanofluidic Devices**
Stephen Jacobson, *Indiana University, Bloomington, IN USA*

15:55 – 16:20 **Catalytic Transformations Of Biological Macromolecules In Arrays Of Nanopores**
Paul Bohn, *Notre Dame University, Notre Dame, IN USA*

16:20 – 16:45 **Separation Mechanisms of DNA in Ordered Nanoporous Microfluidic Arrays**
Jed Harrison, *University of Alberta, Edmonton, Alberta Canada*

16:45 – 17:05 **Impact of Nanotechnologies on Bioanalytical Chemistry and Molecular Diagnostics**
Karel Kleparnik, *Institute of Analytical Chemistry, Brno, Czech Republic*

17:05 – 17:25 **Towards Understanding Biomolecule Behavior in Nanofluidic Channels**
Maria Teresa Napoli, *University of California, Santa Barbara, CA USA*

17:30 – 19:00 ***Exhibitor & Poster Reception – Imperial Ballroom & Stanbro Room***

AM Parallel Session: Carbohydrates
Location: Plaza Ballroom
Chair: Ed Yeung

Tues. 08:30-08:55

MALDI-TOF/MS and CE-LIF Glycomic Mapping in Cancer Diagnostic and Prognostic Measurements

Milos V Novotny; Yehia Mechref; Ahmed Hussein; Marwa M Saleh; William R Alley; Pilsoo Kang; Milan Madera; John A Goetz, *National Center for Glycomics and Glycoproteomics, Bloomington, IN USA*

In spite of numerous research efforts to identify cancer biomarkers through modern bioanalytical methodologies, reliable diagnostic indicators of cancer diseases are still a rare occurrence. Simultaneously, patients´ prognosis and progress of therapies are based more on gross clinical observations rather than biochemical measurements. The recent advances in glycomic high-sensitivity profiling techniques provide considerable hope for the availability of relatively non-invasive and rapid measurements of this type in not-so-distant future. In our laboratory, several dozens oligosaccharides can now be quantitatively recorded as mass/charge-based profiles from the blood serum samples as small as 10 µL. The isotope-aided quantitative measurements are ensured through microscale permethylation following reproducible cleavages of either N- or O-linked oligosaccharides from serum glycoproteins and microscale sample cleanup. Different cleavage enzymes can further provide complementary diagnostic information. To accommodate the cases where different glycan isomers may be biologically pertinent, CE-LIF can provide additional analytical information. Different applications of high-sensitivity glycomic profiling will be exemplified in the areas of prostate, breast, liver, ovarian, and esophageal cancers, where the biochemical individuality of different patterns has been thoroughly evaluated through the use of chemometric and clinical/statistical criteria.

NOTES:

Tues. 08:55-09:20

Integrated Microanalytical Strategies to Study the Glycosylation Pattern of Human Plasma Glycoproteins in Various Cancer Types

András Guttman; Marcell Olajos, *Horvath Laboratory for Bioseparation Sciences, Innsbruck, Austria*

Separation of complex carbohydrates by high performance capillary electrophoresis is a rapidly developing field. Applications of microanalytical strategies enable high throughput analyses of glycosylation changes in the extent and/or nature of oligosaccharide distribution (profiling) and exoglycosidase mediated carbohydrate analysis (sequencing). In this presentation we introduce and integrated microanalytical strategy for glycosylation pattern profiling by combining an automated multipipettor tip based sample preparation approach with multicapillary gel electrophoresis based analysis. We address such sample preparation issues as the very large molar access of the labeling reagent in the derivatization reaction mixture by using DPA-6S resin; elimination of the glucose content from human plasma prior to capillary electrophoresis based glycan profiling using 5K desalting resin and the introduction of a volatile buffer system (acetate) for exoglycosidase digestion mediated carbohydrate analysis also enabling glycan sequencing. To evaluate our integrated microanalytical strategy, glycan profiles of normal control human plasma samples have been compared to patient samples with different cancer types in order to identify glycosylation differences of biomarker potential.

NOTES:

Tues. 09:20-09:45

Cancer Detection Based On Glycoprotein Profiling Of Plasma

Fred Regnier; Wonryeon Cho; Qiang Gao; Kwanyoung Jung; Dawn Watson,
Purdue University, West Lafayette, IN USA

The focus of this presentation is on the use glycan targeting antibodies to capture cancer-associated glycoproteins from the blood proteome. As tumors progress, surface glycoproteins play a prominent role in the loss of cellular adhesion, metastasis, tumor cell binding at remote sites, and secondary tumor colonization. Lewis-x (Lex) antigen and sialylated Lewis-x (s-Lex) antigen are among the more important glycans involved in these processes and are shed into blood and lymph. Immunoaffinity chromatography (IAC) columns targeting the Lex and s-Lex antigens were used to isolate and identify potential cancer biomarker glycoproteins. Plasma of breast, ovarian, prostate, and colon cancer patients was applied directly to IAC columns. After extensive washing to remove abundant proteins, the selected glycoproteins were eluted from the IAC column with an acidic mobile phase and identified by mass spectrometry (MS) based proteomics. The degree of simplification obtained with IAC was sufficient to allow quantitative differentiation between cancer biomarker candidates in these various types of cancer within an hour using a 30 uL sample and RPC alone, without proteolysis or mass spectrometry. The potential of these candidates as cancer signatures is promising but remains to be validated in much larger, more diverse populations of cancer patients.

NOTES:

Tues. 09:45-10:05

Monosaccharide Composition Analysis of Glycoproteins through Multiple Reaction Monitoring Mass Spectrometry

Yehia Mechref; Loubna Hammad; Milos V. Novotny, *Indiana University, Bloomington, IN USA*

Glycosylation is one of the most common and diverse post-translational modifications of proteins. Carbohydrate composition analysis of glycoproteins is a challenging problem in glycobiology. Better understanding of the glycosylation of proteins is commonly achieved through an accurate determination of the monosaccharide composition. Currently, monosaccharide composition analysis is attained using different techniques, including capillary electrophoresis with laser induced fluorescence detection (CE-LIF), High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), and high-performance liquid chromatography with fluorescence detection. Quantification through mass spectrometry is currently attained using multiple reaction monitoring (MRM) approach in which quantification is based on specific transitions in tandem mass spectrometry. In this presentation, high-sensitive and reliable monosaccharide compositional analysis of glycoprotein is developed using chromatographic separation of native monosaccharides formed through acid hydrolysis in conjunction with MRM mass spectrometry. This method allows the quantification of all monosacharides commonly observed in glycoproteins, including galactose, mannose, N-acetylglucosamine, N-acetylgalactosamine, xylos, fucose, N-acetylneuraminic acid and N-glycolylneuraminic acid which are all resolved in a single LC run. Quantification of these monosaccharides through MRM mass spectrometry is achieved at 1-10 pg limit of quantification. Additionally, this method permitted for the first time the reliable monosaccharide compositional analysis of glycoproteins at microgram to sub-microgram levels. The figures of merits of this method will be described and discussed, while the advantages of this method over the other methods, which are commonly utilized in monosaccharide composition analysis, will be presented.

NOTES:

Tues. 10:05-10:25

Rapid Analysis and Characterization of Intact Glycoproteins using CE-MS with LTQ –FT Instrument

Tomas Rejtar; Dipak Thakur; Barry L. Karger, *Northeastern University, Boston, MA USA*

Analysis of intact glycoproteins with multiple glycosylation sites and evaluation of the abundance of individual glycoforms presents a significant analytical challenge, especially for the biotech industry. The focus of this presentation is on evaluation of the performance of CE coupled to FT ICR mass spectrometry for qualitative and quantitative analysis of intact glycoproteins. A lab-built CE MS instrument with a robust, pressurized liquid junction based, MS interface was developed. The alpha-subunit of human chorionic gonadotropin (α-hCG) was chosen as a model glycoprotein for study. α-hCG is known to possess two glycosylation sites. Over 50 different forms with up to 8 sialic acids could be resolved using CE with a separation time under 20 minutes. This high resolution allowed separation of not only glycoforms with different numbers of sialic acids but also glycoforms differing by the number and extent of neutral glycans. Masses of individual α-hCG forms were calculated using the high mass accuracy of the FT instrument based on the direct determination of the charge state. In addition, FT MS permitted the assignment of glycoforms with similar masses, which would be difficult to achieve with lower resolution MS instruments. Samples of α-hCG obtained from different manufacturers as well as different batches from the same manufacturer were analyzed and compared. It was found that there are significant differences in composition as well as glycoform abundance among different manufacturers. On the other hand, for triplicate analysis of the same sample showed that individual glycoforms could be quantitated with a relative standard deviation of less than 10% using CE-MS. The intact glycoprotein analysis was complemented with analysis of glycopeptides and glycans to assign glycans to individual glycosylation sites. The developed method has the potential to be applied in the biotech industry to monitor consistency of glycosylation of therapeutic proteins.

NOTES:

AM Parallel Session: Sampling & Concentration
Location: Arlington/Berkeley/Clarendon
Chair: Michael Bowser

Tues. 08:30-08:55

Modifications of Surface Chemistry of Porous Polymer Monoliths Enhancing their Performance

Frantisek Svec[1]; Jana Krenkova [1]; Yan Xu[2]

[1]Molecular Foundry, Lawrence Berkeley National Lab, Berkeley, CA USA; [2]Department of Chemistry, University of California, Berkeley, CA USA

Since their birth in the late 1980s and first application in the separations of proteins, porous polymer monoliths were used in numerous other applications such as solid phase extraction, enzyme immobilization, capillary electrochromatography, gas chromatography, ion chromatography, and HPLC. Their advantages include ease of the preparation, robustness, high permeability to flow, mass transfer via convection, and a vast variety of chemistries. Recently, most of our efforts are focused on capillary and chip formats for micro- and nanoscale applications. These small formats are advantageous for the separations of samples which volumes are small. Surface chemistry of the monoliths can be easily controlled by copolymerization with monomers bearing the desired functionality. However each new monomer requires optimization of the conditions to obtain monolith with desired porous properties. Therefore, we developed a more universal photografting of pore surface with polymer chains bearing a variety of functionalities. This approach enables the preparation of different monoliths in which both porous structure and chemistry are optimized separately. Its success is demonstrating on the preparation of a monolithic support with significantly suppressed non-specific interactions with proteins and its use for immobilization of proteolytic and glycolytic enzymes. This capillary device enables characterization of large proteins such as immunoglobulins. Recently, we have introduced surface modification with nanoparticles. For example, gold nanoparticles prepared in situ within the pores of the monolith appear to be a very interesting new ligand for the isolation of thiol containing peptides from complex digests.

NOTES:

Tues. 08:55-09:20

Droplet Compartmentalization of Chemical Separation

Daniel T. Chiu, *University of Washington, Seattle, WA USA*

This presentation will describe our recent efforts to integrate our droplet generation and handling capabilities with sensitive methods of chemical separation. In particular, this talk will focus on the use of droplets to compartmentalize species separated using sensitive methods of microscale separation and chromatography.

NOTES:

Tues. 09:20-09:45

Enhancing the Detectability in Microscale Electrophoretic Separations

Koji Otsuka; Kota Hashiba; Fumihiko Kitagawa, Kenji Sueyoshi, *Kyoto University, Kyoto, Japan*

To enhance the detectability in microchip-based micellar electrokinetic chromatography (MCMEKC), a novel on-line sample preconcentration and separation technique named “transient-trapping (tr-trapping)” was developed. In this technique, efficiencies of both the preconcentration and separation can be improved by using a partially injected short micellar plug. A longer separation length often provides a better resolution of closely-migrating analytes in conventional MCMEKC, while in tr-trapping applied MCMEKC the trap-and-release mechanism enables the short micellar zone to work as an effective preconcentration and separation field according to the theoretical model.
An application of tr-trapping was performed on a newly fabricated 5 way-cross microchip with sodium dodecyl sulfate (SDS) and rhodamine dyes as the test micelle and analytes, respectively. Typically, almost 400-fold improvement of the detectability was achieved in tr-trapping–MCMEKC compared with conventional MCMEKC. The resolution obtained in tr-trapping–MCMEKC was better than the conventional mode. We can conclude that tr-trapping in MCMEKC provides a rapid, high resolution, and high detectability analysis even in the short separation channel on the microchip. Fundamental characteristics of tr-trapping will be discussed along with an extended study of this technique to capillary based MEKC.

NOTES:

Tues. 09:45-10:05

Gradient Elution Isotachophoresis-Capillary Zone Electrophoresis: Rapid Enrichment Meets Rapid Separation

Jonathan G. Shackman; Nejea I. Davis; Manasa Mamunooru; Chandni A. Vyas, *Temple University, Philadelphia, PA USA*

Gradient elution isotachophoresis (GEITP) is a recently described technique for ITP enrichment and separation. The general approach utilizes rapid electrophoretic focusing at a discontinuous ionic interface within a sample well generated through combined electroosmotic (EOF) and hydrodynamic flows. The interface and enriched analytes are then pulled into a capillary or microchannel as the counter-flow is reduced for on-column detection. GEITP has demonstrated several hundred to over 100,000-fold enrichment of analytes as diverse as small dyes and amino acids to proteins and DNA in a few minutes. As ITP is initiated off-column, separations can be performed in lengths as small as 30 micrometers. Previously, GEITP utilized absorbance or fluorescence single wavelength detection. Zone resolution was achieved through addition of non-detectable spacing components, typically relying on carrier ampholyte mixtures.

The spacing technique severely limits the separation power of GEITP, as the spacers must be added to the sample in large concentrations or long enrichment times are required. We have developed a multi-dimensional method to address the issue of resolution in GEITP through addition of a secondary stage of capillary zone electrophoresis (GEITP-CZE). The sequence of steps for GEITP-CZE in a single microcolumn are: GEITP enrichment until the sample zones are introduced on-column; replacement of the sample and terminating electrolyte mixture with leading electrolyte; and application of counter-flow to negate EOF during CZE. These steps are analogous to performing extreme volume-coupled ITP with transient ITP but performed on a single capillary. The single solution switch and lack of polarity inversion allows for highly reproducible separations (typically <6% relative standard deviation in peak heights and <0.05% in migration times). As a first demonstration of the applicability of GEITP-CZE, amino acids that comprise the Mars7 standard, a mixture to be assayed for on future extraterrestrial missions as indicators of biological life, were studied.

NOTES:

Tues. 10:05-10:25

Non-Protein Antibodies against Proteins, Viruses, Bacteria ...
Applications: (1) Biomarkers (2) Enzyme Reactors, Biosensors, etc. and (3) "Negative Purification"

Stellan V.E. Hjertén, Nasim Ghasemzadeh; Fred Nyberg, *Uppsala University, Uppsala, Sweden*

The synthesis of the "antibodies" is based on a non-conventional, molecular-imprinting method: the "antigen" (protein, virus, bacterium...) is added to a solution of acrylamide and bis-acrylamide. The gel formed upon polymerisation is granulated. When the antigen is washed out a cavity forms with a shape, which exactly fits only the antigen added to the monomer solution. Therefore, the gel particles will recognize only this antigen. The selectivity will, accordingly, become exceptionally high. This antigen can, therefore, be fished out from a solution (for instance, serum) in a very high purity, also when it contains, besides this antigen, a great number of different biopolymers and bioparticles.
This high selectivity paves the way for many applications, for instance the enrichment of a particular antigen, including a biomarker in the form of a protein, virus or a bacterium. For diagnosis and prognosis of a disease the concentration of the marker in a body fluid must often be determined, preferably rapidly and yet accurately, for instance in Doctor´s Office. To this end we have developed a method to design a standard curve, which permits such a determination from absorption measurements of the biomarker, selectively adsorbed to the gel antibody. The design of the standard curve was not straight forward, because the incident parallel light beam in the spectrophotometer becomes divergent due to light refraction, total reflection and light scattering, when it passes the cuvette with the gel granules. We will show how these optical disturbances, which indirectly cause deviations from Beer´s law, can be compensated. Using this approach we found, for instance, that albumin in both serum and cerebrospinal fluid is a biomarker for neurological disorders.
We will also emphasize the great advantages of designing enzyme reactors and biosensors, based on the artificial gel antibodies, as well as their use for "negative purification".

NOTES:

AM Parallel Session: Bio-omics
Location: Plaza Ballroom
Chair: András Guttman

Tues. 10:55-11:20

Direct Imaging of Metabolites in Plant Tissues by Mass Spectrometry

Edward S. Yeung; Sangwon Cha; Hui Zhang; Basil J. Nikolau, *Iowa State University, Ames, IA USA*

We describe novel mass spectrometric imaging techniques to map metabolite distributions within plant tissues. Mass spectrometry not only allows positive identification of the many metabolites but can also reveal the substrates and precursors involved in each metabolic pathway. Such information will provide unprecedented details on the distribution of metabolites from cell to cell, cooperative and antagonistic effects among the metabolites, and environmental influences on metabolism, and will ultimately lead to a predictive understanding of the mechanisms that multicellular organisms use to regulate metabolic processes.
We will present results on the cuticle, flower and stem of Arabidopsis, and on sections of apple and strawberry. By studying the diversity of the cuticular waxes, we obtained detailed insight into their biosynthesis as a function of genetics, tissue type, development, and environment. We were also able to monitor light-induced flavonoid accumulation in the stem. In analogy to matrix-assisted laser desorption ionization, a laser beam was used to interrogate sequentially micrometer areas of a plant by vaporizing the surface contents of the tissue into a mass spectrometer. Rastering of the laser beam over the tissue produces a laterally resolved image of the various substances within different structures of the plant. We were able to generate ions directly from the plant tissue by employing novel additives as pseudo-matrixes. By minimizing sample preparation, compositional integrity and spatial resolution of the analysis is guaranteed.

NOTES:

Tues. 11:20-11:40

Characterization of Membrane Proteins Using Nanoflow Liquid Chromatography-Tandem Mass Spectrometry

Xiaoying Ye; Timothy D. Veenstra; Josip Blonder, *SAIC-Frederick, Frederick, MD USA*

Aberrant expression of certain membrane proteins is associated with the development and progression of cancer and hence has great potential in cancer biomarker discovery. Nanoflow liquid chromatography coupled online with tandem mass spectrometry (nanoLC-MS/MS) plays a pivotal role in proteome analysis and biomarker search. However, proteomic profiling of membrane proteins is still challenging due to the difficulty of solubilizing these hydrophobic proteins in suitable solutions that permit efficient separation and downstream mass spectrometry analysis. Here we report the development of an organic solvent based solubilization method for qualitative and quantitative membrane proteomics using nanoLC/MS/MS. The effectiveness of this method is demonstrated by the complete sequence coverage of a model membrane protein bacteriorhodopsin characterized by nanoLC-MS/MS. In addition, this method is compatible with quantitative approaches such as chemical and enzymatic stable isotope labeling and their combination for improved quantitative measurements. Validation of this method is demonstrated using a variety of membrane specimens isolated from eukaryotic cells and tissues. Recently, mass spectrometry friendly detergents, such as acid-cleavable detergent, have been developed. We further investigated the efficiency of acid-cleavable detergent based solubilization compared to current organic solvent based solubilization and their combined use for effective analysis of mass-limited biosamples.

NOTES:

Tues. 11:40-12:00

Microfluidic LC Chips with PQD-Ion Trap MS Detection for iTRAQ-Based Protein Differential Expression Analysis

Iulia M. Lazar; Abdulilah A. Dawoud; Jenny M. Armenta, *Virginia Bioinformatics Institute, Blacksburg, VA USA*

Biomarker discovery using novel proteomic technologies is an area that is attracting increased attention in the biomedical community. Early detection of abnormal physiological conditions will be highly beneficial for diagnosing various diseases and increasing survivability rates. Microfluidics has emerged as a technology that could become essential in proteomics research as it enables the integration of all sample preparation, separation and detection steps, with the added benefit of enhanced sample throughput. The combination of these advantages with the sensitivity and capability of mass spectrometry (MS) detection to deliver precise structural information, makes microfluidics-MS a very competitive technology for biomarker discovery. The integration of microchip liquid chromatography (LC) devices with MS detection, and specifically, their applicability to biomarker screening applications in MCF-7 breast cancer cellular extracts, is reported in this work. The microfluidic chips rely on electroosmotic propulsion for eluent delivery and sample valving. Specifically, each microdevice integrates two multichannel electroosmotic flow (EOF) pumps, a serpentine mixer, a serial multichannel EOF valving system with a double-T injector/on-column pre-concentrator, an LC separation channel, and an electrospray ionization (ESI) interface. The multichannel EOF pumps are capable of delivering up to ~1,000-1,100 nL/min (RSD=8-10 %) eluent without a restrictor, and ~125-200 nL/min (RSD=2-7 %) eluent with a restrictor connected to the pump outlet (i.e., a ~20 μm i.d. x 1 m long fused silica capillary equivalent to a 2 cm long packed separation channel). Loading ~0.1-1 μg of crude protein extract tryptic digest on the chip has typically resulted in the reliable identification of ~40-100 proteins ($p<0.001$) and of a panel 5-8 putative biomarkers. The potential of this LC-ESI-MS chip for comparative proteomic analysis of iTRAQ labeled MCF-7 breast cancer cell extracts with pulsed Q dissociation (PQD) ion trap MS detection is explored for the first time.

NOTES:

Tues. 12:00-12:20

Capillary Electrochromatography and Its Applications in Pharmaceutical and Biochemical Analyses

Chao Yan; Xue Gu; Yan Wang, *School of Pharmacy, Shanghai Jiaotong University, Shanghai, China*

Capillary electrochromatography (CEC) combines the best features of capillary electrophoresis (CE) and high performance liquid chromatography (HPLC): high separation efficiency of CE and the versatile selectivity and large sample capacity of HPLC. However, in practice, when CEC was used without pressure, often on a commercial CE instrument, there were problems and difficulties associated with bubbles formation and column dry-out. These problems can be solved by a pressurized CEC (pCEC) system, in which a mobile phase is driven by both a pressurized flow and an electroosmotic flow (EOF). In such a system, a pressure can be applied to the capillary column to suppress bubble formation. Quantitative sample introduction in pCEC can be easily achieved through a rotary-type injector. The EOF can either be in the same direction as, or against, the pressurized flow. Therefore, the sample elution order may be manipulated by changing the ratio of pressure to voltage. Most importantly, it is amenable for a solvent gradient mode, similar to that in HPLC, by programming the composition of mobile phone. With pCEC, the promises of CEC can be fully exploited.

We will report our recent advances in pCEC in instrumentation, column and applications. An innovative pCEC instrument was developed, which can also be used for micro-HPLC and CE. This technology, coupled with UV/Vis, MS and LIF detectors, was successfully used in more than 100 projects including studies on pharmaceutical, biological, environmental analyses, as well as pharmarcokinetics and metabolomics.

NOTES:

Tues. 12:20-12:40

Development of 2D Capillary Isoelectric Focusing/ Capillary Sieving Electrophoresis with Laser-induced Fluorescence for the Analysis of Proteins in Biological Samples

Jane A. Dickerson; Lauren M. Ramsay; Norman J. Dovichi, *University of Washington, Seattle, WA USA*

Two-dimensional capillary electrophoresis (2D-CE) with laser-induced fluorescence (LIF) detection is used for separation of complex biological samples. Previous 2D CE separations from our lab employed a sieving dimension and a micellar electrokinetic chromatography (MEKC) dimension. These separation modes were not completely orthogonal for the separation of proteins. We have been developing a 2D CE system that would greatly improve the resolution by coupling capillary isoelectric focusing (cIEF) and capillary sieving electrophoresis (CSE). There are several challenges involved in coupling cIEF with CSE. Mobilizing and transfer of the focused pI zones to the second dimension is one issue being investigated. 2D cIEF/CSE has many advantages over traditional 2D-gel electrophoresis, including higher sensitivity, much faster analysis time, less labor intensive and potential for automation. Biogenic amines are labeled by chromeo-P503, a pyrilium compound that reacts with primary amines to produce a positively charged pyridinium ion. Fluorescently labeled biomolecules are separated by 2D-CE and detected inside a sheath-flow cuvette with a 473 nm solid state laser and fiber-coupled avalanche photo diode (APD). 2D-CE-LIF is capable of detecting zeptomole (10-21) quantities of P503-labeled proteins. In 2D-CE, two capillaries, with i.d.s of 50 and 30 &mum, are aligned at a buffer filled interface. The proteins are separated by capillary isoelectric focusing (cIEF) in the first dimension. Fractions are electrokinetically transferred to the second capillary, and separated by capillary sieving electrophoresis (CSE). This technique is used to separate proteins in cellular homogenates prepared from biopsy tissue from patients with Barrett's esophagus (BE). Barrett's esophagus is the only known precursor to esophageal adenocarcinoma; investigation of the differences in protein expression of cells associated with BE may be used as a prognostic indicator of disease progression.

NOTES:

AM Parallel Session: Microfluidics 2
Location: Arlington/Berkeley/Clarendon
Chair: Robert Kennedy

Tues. 10:55-11:20

Particle Manipulation Strategies in Microfluidic Devices

Elisabeth Verpoorte;[1] Laurens-Jan C. Jellema[1]; Anton P. Markesteijn[2]; Ralph Lindken[2]; Jerry Westerweel[2];

[1]University of Groningen, Groningen, The Netherlands; [2]Delft University of Technology, Delft, The Netherlands

Microfluidics technology offers the researcher an unparalleled opportunity to create and control flow and force gradients in microenvironments. This unique feature has been exploited by a number of researchers to develop flow-based particle separation and concentration methods. The key to our approach is the generation of bi-directional flow through application of opposing electro-osmotic (EOF) and pressure-driven (PF) flows in a narrow microchannel whose width increases at both ends. The resulting recirculating flow pattern may be used to separate particles based both on differences in size and zeta potential.

In the former case, the separation mechanism is hydrodynamic in nature, reminiscent of Taylor-Aris dispersion. Both large and small particles are trapped in the recirculating flow, but larger particles will occupy a smaller region of the channel and thus tend to drift towards the low-pressure end. In contrast, the net flux of smaller particles will be in the opposite direction with EOF, allowing these to escape the trapping channel. The net drift velocities of large and small particles depend on the balance between applied pressure and electric field. This can be appropriately tuned to achieve separation of different micrometer-sized particles having the same zeta potential.

We call the trapping phenomenon mentioned above flow-induced electrokinetic trapping (FIET). Trapping occurs under conditions where the average net velocity of the particles themselves approaches zero. Importantly, the electrophoretic mobility (zeta potential) of the particles determines the flow conditions required for trapping. In other words, this mechanism for separation and concentration can be tuned to the mobility of the particle type of interest, allowing its separation from other particles having different mobilities. This trapping effect has effectively been used to separate polymer beads having different zeta potentials.

Progress in the development of particle separations based on both these mechanisms will be reported in this presentation.

NOTES:

Tues. 11:20-11:45

Microfluidics and Cellular Diagnostics from Blood

Mehmet Toner, *Massachusetts General Hospital, Harvard Medical School, Boston, MA USA*

Biomedical applications of microfabricated devices is no longer limited to non-living systems as genes-on-a-chip or lab-on-a-chip, recent advances in the understanding of cellular behavior in micro-environments have started to pave the way toward living micro-devices. These emerging devices are expected to become key technologies in the 21st century of medicine with a broad range of applications varying from diagnostic, tissue engineered products, cell-based drug screening tools, and basic molecular biology tools. They will also include multiple cell types and/or genetically engineered cells to investigate complex interactions between cells from different tissues. These sophisticated devices will contain micro-engineered tissue units coupled to each other by complex microfluidic handling network. Microfluidic mixing systems will also precisely regulate the composition and concentration of drugs to be tested microchips to isolate rare cells from blood for diagnostics purposes. This presentation will briefly review the literature on the use of microtechnologies in cellular systems and then focus on a number of applications, especially to those in blood diagnostics in cancer, HIV/AIDS and global health, and burns and trauma.

NOTES:

Tues. 11:45-12:05

Polymer Microchip with Integrated Magnetic Tracks for Multi-Plug Magnetic Bead Capture: Characterization, Advantages And Applications.

Mélanie Abonnenc; Anne-Laure Gassner; Jacques Josserand; Hubert H. Girault, *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

The use of magnetic beads (MBs) in microfluidic devices has been receiving growing interest in the last years [Verpoorte, 2003; Gijs, 2004; Pamme, 2006]. Indeed, magnetic separation in microscale format strongly limits the consumption of samples. MBs were shown to be very suitable for fast immunoassays, protein digestion and isolation.
Herein, a passive magnetic separator polymer microchip with embedded magnetic tracks, which are activated by an external magnetic field (i.e. permanent magnets), is presented.

The microchip is engineered on a polymer substrate by UV-photoablation. It is composed of a microchannel (100 x 50 µm2) and on both sides of it, of rectangular grooves, spaced by 200 µm to each other and drilled perpendicularly to the microchannel on a distance of 4 mm. The grooves are filled with home-preparated magnetic ink. The designed magnetic tracks allow re-distributing and focusing the magnetic field in specific locations. The MBs are therefore organized along the microchannel in consecutive multi-plugs instead of a single one, enabling extended and more stable bead capture.

The study presents a characterization of the microchip by 1) numerical simulation of the magnetic field forces, 2) microscope imaging to visualize the MB trapping with or without integrated magnetic tracks, and 3) the hyphenation of the microchip after capillary electrophoresis to easily feed it with solutions under controlled pressure, and quantify the bead capture and release. In this last study, the pressure drop induced by the multi-plugs was investigated as well. Finally, on-chip protein digestion with MBs functionalized with trypsin was assessed and the digest was analyzed by mass spectrometry (MS).
As perspectives, this microchip can be upgraded by the addition of other MB plugs presenting different functionalities, and/or direct coupling to MS by using it as electrospray emitter.

NOTES:

Tues. 12:05-12:25

Cell Electrophoresis

Ferenc Kilár[1]; Dávid Szabó[1]; Péter Buzási[1]; Virág Farkas[1]; Béla Kocsis[2]

[1]University of Pécs, Institute of Bioanalysis, Pécs, Hungary; [2]University of Pécs, Institute of Microbiology, Pécs, Hungary

The behaviour of microorganisms are strongly dependent on their surface properties. The migration of bacteria and fungi in electric field provides good knowledge on the differences between them. A new microscope-based microchip system was developed and applied for the analysis of different bacteria and fungi. The system allows a fast determination of the mobility, as well as the changes in the migration upon the changes in the environment, such as pH, buffer additives, etc. The possible interaction and also the aggregation of the cells can be easily seen. An experiment takes only 2-3 seconds in an open, coated capillary. The hydrophobic behaviour of different microorganisms was studied in the presence of different additives, denaturants, and a parameter for hydrophobicity was formulated on the changes of the mobilities. An interesting and surprising phenomenon was followed, in which the bacteria and fungi move opposite directions (at a certain pH), while the interaction between bacteria and fungi will result in a change in the migration. The system allows the investigation of individual cells, or bigger groups of cells. The data are obtained by digitalization, as histograms, and a software is used for obtaining the mobilities with statistical evaluation. The syncronization of the life cycles of the cells was also followed by the technique.

NOTES:

Tues. 12:25-12:45

Microfluidic Immunophenotyping of Endothelial Progenitor Cells

Shashi Murthy; Brian D. Plouffe, *Northeastern University, Boston, MA USA*

Endothelial progenitor cells (EPCs) are present in very low concentrations (1 in 10-100 million cells) in blood and have unique regenerative abilities. These cells can be utilized to achieve repair of vascular tissue and blood vessels. While markers of these cells are known, relative binding affinities of adhesion ligands to these cells has not been characterized. We present a variable shear microfluidic device that can be functionalized with a broad variety of ligands and is capable of measuring cell adhesion over a range of shear stresses in a single experiment. Characterization of a range of EPC markers was achieved using this device and high affinities were observed for the markers CD34, VEGFR-2, and CD31. Microfluidic ligand-coated devices also offer a highly attractive alternative to current isolation techniques for EPCs. The current technique has several steps, including density gradient centrifugation and a series of plating steps to deplete undesired cell types. We will present preliminary work on a microfluidic cell capture device capable of direct EPC isolation from whole blood and a method to release captured cells.

NOTES:

Technical Seminar – 12:50-13:50
Location: Plaza Ballroom
Sponsored By: Beckman Coulter

Taking CE to New Heights

On the 20th Anniversary of MicroScale Bioseparations, Beckman Coulter, Inc. will preview the new PA 800 plus Pharmaceutical Analysis System, boasting easy operation, best-in-class robustness, high-resolution applications and modular detection. In this workshop we will present this new generation CE analysis system and its applications, including SDS-MW for protein sizing, carbohydrate profiling, and state-of-the-art capillary isoelectric focusing. The PA 800 plus Pharmaceutical Analysis System can also be coupled to a mass spectrometer, and we will discuss our new collaborative program on sheathless CE-MS.

This is also Beckman Coulter's 20th anniversary on the commercialization of capillary electrophoresis. We invite you to join us for the celebration, have lunch on us, and learn about the most advanced CE instrument on the market. All attendees will be entered into a drawing for an altimeter watch. Together, let's take CE to new heights!

PM Parallel Session: Single Cells
Location: Plaza Ballroom
Chair: Lingjun Li

Tues. 15:30-15:55

What is a Neuron from a Genomic Standpoint? Unbiassed Molecular Dissection of Single Cells and Cell Compartments

Leonid L. Moroz, *University of Florida, St. Augustine, FL USA*

What makes a neuron a neuron? What are the genomic bases of unique neuronal phenotypes? How different is the transcriptional profile of one neuron from another? Here, we attempt to identify and quantify nearly all RNA species present in a given neuron. Therefore, we have provided the first unbiased view of the operation of an entire genome from a single characterized neuron. First, we developed protocols for digital expression profiling of identified Aplysia neurons representing interneuron, sensory and motor neuronal classes. The generated single-neuron cDNA libraries accommodate the emPCR for two complementary massive parallel sequencing technologies (stating from pyrosequencing to the-sequencing-by-ligation-SOLiD) and allow assembly of the shorter sequence reads. In summary, >9 billion bases from just three identified neurons were obtained (~80 million sequences from each neuron). It is estimated that such coverage represents >99% of all RNA species in a single neuron. Second, using absolute real-time PCR we demonstrated that our method is fully quantitative with the dynamic range covering the entire neuronal transcriptome (from the rarest transcripts with only a few copies per cell to the most abundant RNAs with many thousands of copies). Q-RT-PCR and in situ hybridization further validated this method of digital profiling. Third, this type of analysis can be complemented by unbiased proteomic and metabolomic analysis from the same identified neurons providing nearly complete molecular portraits of functionally characterized neurons in a simpler neural circuits. Emerging data related to large-scale gene expression profiling at the level of individual identified neurons can be interpreted in the context of the hypothesis of polyphygenesis (independent origin of neurons) and lead to a simple scenario for both the origin and evolution of neurons. In addition, growing neurogenomic information from various lineages provides unique recourse for novel strategies in analysis of Learning & Memory mechanisms, NeuroSystematics and Neuronal Classification.

NOTES:

Tues. 15:55-16:20

Twenty Years of Single Cell Analysis

Jean Rossier, *ESPCI, Paris 5, France*

This conference will review the progress on single cell analysis. Cell diversity is large particularly in the nervous system. Each of the neurons of the brain has a specialized function and deciphering the content of a single neuron become an invaluable help to understand its function.
Our work has been focused on the neocortex. The complexity of the neocortical network composed by a highly heterogeneous population of neurons led us to design single cell RT-PCR after patch clamp to study cellular diversity. This technique is now widely used by numerous groups including the one of Bert Sakmann, the inventor of patch-clamp recording with Erwin Neher. In brief, after electro-physiological recordings of a cell, the cytoplasm of the cell is aspirated in the recording pipette and the content analyzed for the presence of few different mRNA species. With multiplex RT-PCR the expression of 100 different genes can be monitored simultaneously.
This work has led to new classification of neurons based on the analysis of multiple electrophysiological and gene expression parameters. The diversity at the gene expression level is associated with different functional properties. Some interneurons behave like coincidence detectors and are important in the generation of rhythmic activities. Another class of interneurons is central to drug addiction and reward mechanisms as these interneurons co-express opioïds and nicotine functional receptors. Other interneurons control brain metabolism and blood perfusion. The neurons controlling blood flow are diverse. They contain NO synthase and the neuropeptides VIP, somatostatin and NPY.
VIP or nitric NOS in interneurons induce dilatation whereas somatostatin and NPY interneurons elicit contraction. These results suggest that cortical GABA interneurons could control local blood brain perfusion.
The key idea behind the new technologies currently developped is to perform each step from cell handling to quantitative analysis of gene expression or protein detection at the nanoliter scale (0.1 to 100 nL). Preliminary work done by several groups including our laboratory have shown that many molecular biology assay such as mRNA extraction, reverse transcription and PCR are more efficient at high sample concentration in small volumes. Quantitative detection schemes are mostly sensitive to final concentrations and therefore at a fixed amount of analytes (the material from one cell), a diminution of 3 orders of magnitude of the sample volume will push the detection limit significantly enough to reach the single cell level over the whole transcriptome.

NOTES:

Tues. 16:20-16:45

Bioanalysis of single skeletal muscle fibers

Edgar A. Arriaga; Xin Xu; Marian Navratil; LaDora V Thompson, *University of Minnesota, Minneapolis, MN USA*

Skeletal muscle is composed of bundles of single fibers each with diameter cross sections ~ 10-100 µm and lengths in the mm range. The fibers of a bundle display parallel alignment that makes it possible to use microscopies to investigate longitudinal or cross sectional distributions of histochemical properties (e.g. ATPase activity) after tissue dissection. Unfortunately, this approach is cumbersome when measuring properties of viable fibers. Furthermore, conventional microscopies are unsuitable to measure functional properties in whole tissue bundles due its thickness.
We have implemented a tissue culturing procedure to prepare viable single fibers from the soleus and semimembranosus muscles with sustained viability for up to three days. We demonstrate that we can use fluorescence microscopy to monitor several properties in each fiber including mitochondrial membrane potential, phosphatidyl serine levels on the outer plasma membrane that is related to cell apoptosis, and the localization of mitochondria in the subsarcolemmal region. These properties are important parameters in investigating the role of mitochondria in aging and in mitochondrial related diseases.
Some properties such as the generation of reactive oxygen species (ROS) cannot be measured using fluorescent reporters and conventional fluorescence microscopy techniques because of the presence of non-specific fluorescent products. Here we describe an approach to sample, separate and detect specific and non-specific fluorescent ROS reporters of whole single skeletal muscle fibers by capillary electrophoresis, despite the incompatible dimensions of the fiber with those of the separation capillary. The first step consists of incubating viable fibers with triphenyl-phosphonium hydroethidine that reacts with superoxide to form the fluorescent compound hydroxy-triphenyl-phosphonium hydroethidine. The second step uses a home-built nano-pipettor to transport a chosen fiber to a nanoliter-volume well in the fiber is dissolved. Subsequently, the preparation is either frozen for storage or immediately analyzed using conventional capillary electrophoresis with laser-induced fluorescence detection.

NOTES:

Tues. 16:45-17:05

On-chip Flow Cytometry Coupled to Single-Cell Imaging for Studying Signal-Transduction Pathways

Thomas D. Perroud; Catherine S. Branda; Nicole H. Romano; Anup K. Singh; Kamlesh D. Patel,
Sandia National Laboratories, Livermore, CA USA

Lab-on-a-chip platforms are in concept capable of performing multiplexed assays on the same sample, a key enabler for microfluidic-based system biology. Among the desired functionalities, on-chip flow cytometry is capable of rapid, multi-parametric measurements on relatively large cell populations (~ 1,000); whereas, microscopy imaging can provide spatial information at high-resolution, but only on a limited number of cells (~ 10). These two techniques are fundamentally different, but the integration of the two into a single multiplexed assay represents a powerful approach for studying signal transduction in mouse macrophages. In this paper, we present our work on the tandem integration of these two functionalities for correlating the transcriptional activation kinetics of TNFα with the localization of RelA within the cell after a lipopolysaccharide challenge. On-chip flow cytometry is coupled to noninvasive optical cell sorting to rapidly (20 cells/s) identify and sort cells expressing TNFα through a mCherry-PEST transcriptional reporter. The sorting mechanism is based on optical tweezers, where strong gradient forces created by a powerful near-infrared laser (9.6 W at the sample) laterally displace the cell of interest into a neighboring laminar flow stream. In the downstream single-cell array, the sorted cells are individually trapped by 20-μm-wide, 6-μm-deep radial micropores using a combination of hydrodynamic confinement and hydrodynamic focusing. Once immobilized, each cell is imaged to determine the localization of GFP-RelA within the cell. In this configuration, we demonstrate the rapid sorting and immobilization of single cells at specific positions in the array with a near 100% trapping efficiency. We also confirm that mouse macrophages (N = 48) trapped at these micropores remain viable for at least 4 hours. Overall, this on-chip cellular assay provides a novel approach to combine different, but complementary, techniques for multiplexed measurements on single cells.

NOTES:

Tues. 17:05-17:25

Cell-Based Assays on a Chip with a Cell Bank

Yan Xu; Kae Sato; Tomohiro Konno; Kazuhiko Ishihara; Takehiko Kitamori, *Univerisity of Tokyo, Tokyo, Japan*

For cell-based assays such as cytotoxicity assays, drug discoveries and molecules diagnostics, recently we have developed a technique integrated with on-site encapsulation, long-term storage, and on-demand releasing of living cells on chip by a stimuli-sensitive hydrogel system, which is called a living cell bank by us. The technique can conquer the shortcoming of recent cell-based chips needing culturing cells off chip before assays, and would finally make cell-based assays totally on chip.
Cell suspension in a culture medium containing the dissolved polymer poly(2-methacryloyloxyethyl phosphorylcholine(MPC)-co-n-butylmethacrylate(BMA)-co-p-vinylphenylboronic acid (VPBA))(PMBV) was firstly introduced into a chip with a cell-container chamber. Then, the poly(vinyl alcohol) (PVA) solution was introduced. Through a spontaneously cross-linking gelation of the polymers, the cells were encapsulated in the formed hydrogel in the chamber of the chip without any physical process such as photo- and electro-treatments.
Viability of L929 cells encapsulated on a glass chip was confirmed by a LIVE/DEAD assay. Most L929 cells kept alive in the chip after eight days' encapsulation. The cell viability rate quantitatively estimated through the LIVE/DEAD fluorescence analysis was at least 92%. The experiments further indicated that the L929 cells encapsulated in the chip exhibited a very low proliferation rate of about 10%. The L929 cells encapsulated in hydrogel were exposed to a serials of toxins including saponin, triton X-100, methanol and tomoxifen. The results indicated that the encapsulated cells kept functional activity and exhibited high resolution to different toxins with different concentration. The encapsulated cells can be released from a cell encapsulation chamber (the so-called living cell bank) to an analysis chamber on demand by injecting the D-fructose solution, due to the dissociation of the hydrogel by the D-fructose, and thereby various cell-based assays can be done on the released cells.

NOTES:

PM Parallel Session: Nanoscale
Location: Arlington/Berkeley/Clarendon
Chair: Elisabeth Verpoorte

Tues. 15:30-15:55

Electrokinetic Transport and Trapping in Planar Nanofluidic Devices

Stephen C. Jacobson; John M. Perry; Kaimeng Zhou; Michelle L. Kovarik, *Indiana University, Bloomington, IN USA*

Nanofluidic channels integrated in microfluidic devices offer a unique platform for studying transport behavior and conducting various analytical and bioanalytical assays. Often, these nanoscale devices are influenced by phenomena such as double layer overlap, diffusion, surface charge, and entropic forces, which are insignificant or absent in larger microchannels. Using standard micro- and nanofabrication techniques, we can create a variety of nanoscale geometries, and fabricating these structures in plane allows simultaneous optical and electrical characterization to explore what happens along the nanoscale conduit. We manufacture the nanofluidic channels by casting a high modulus polymer onto a master, which is created by electron beam lithography in a negative tone resist. The nanochannels are mated with microchannels, which permit easy coupling of liquids and electrical potentials to the nanochannels. Small lateral dimensions and asymmetric geometries produce unbalanced fluxes in electrically driven systems causing enrichment and depletion zones to form. By analyzing the effects of transport phenomena on increasingly smaller features, we can determine the relative contributions of electrophoresis, dielectrophoresis, and electroosmosis within and near these nanochannels.

NOTES:

Tues. 15:55-16:20

Catalytic Transformations of Biological Macromolecules in Arrays of Nanopores

Paul W Bohn[1]; Sean Branagan[1]; Zhen Wang[1]; Travis L. King[2]

[1]University of Notre Dame, Notre Dame, IN USA; [2]University Of Illinois at Urbana-Champaign, Urbana, Illinois USA

Studies of macromolecular reactivity in confined environments are challenging, but the confined environment can affect molecular recognition and catalysis. Our work is aimed at comparing the reactivity of biomolecules in geometrically confined space, such as the interior of cylindrical nanopores, with that in free solution. Arrays of nanopores (1 – 1000) are fabricated in membranes containing a layer of poly (methyl methacrylate)/poly (glycidyl methacrylate) (PMMA/PGMA) sandwiched between two layers of PMMA by focused ion beam milling. The exposed glycidyl group is used to immobilize biomolecules through reaction with solvent-accessible primary amines. Horseradish peroxidase (HRP) is immobilized on the interior surface of PMMA/PGMA membrane, and laser induced fluorescence (LIF) is employed to monitor the enzymatic conversion of non-fluorescent amplex red to fluorescent resorufin in the presence of H2O2. The immobilized HRP exhibits very high activity, which is reflected by the strong evidence that we have achieved quantitative substrate conversion, i.e. all the substrate which enters the nanopore is converted prior to exiting into the receiving channel. The enzymatic reaction in single nanopores proceeds at a rate that is closely coupled to the transport mechanism and the residence time in the nanopore, and comparison to simulations of the coupled reaction-diffusion problem yield an Eley-Rideal rate constant 103 M-1s-1 < ktot < 104 M-1s-1.

NOTES:

Tues. 16:20-16:45

Separation Mechanisms of DNA in Ordered Nanoporous Microfluidic Arrays

D. Jed Harrison; Neda Neda Nazemifarad, *University of Alberta, Edmonton, Canada*

We have demonstrated that colloidal self-assembly offers a simpler and cheaper alternative to conventional nanofabrication of sieving structures, and can be used to create nanoporous arrays for biomolecule separation. We are able to self-pattern large-area, crack-free colloidal nanoarrays, creating a 2-D microsystem for use as a high-throughput DNA fractionator. The mechanisms of dispersion and the factors that control resolution in these structures are poorly understood. We have found that a simple model describes a large range of the observed performance, explaining pore size, molecular size and frequency dependent behaviors. We have been able to extend separations to longer DNA (> 100 kbases), which is of greater commercial relevance, through this understanding. By doping the array crystal structures with various particle sizes we have also been able to explore the role of defects in the ordered porous array and the difference between separations in random and ordered porous materials.

NOTES:

Tues. 16:45-17:05

Impact of Nanotechnologies on Bioanalytical Chemistry and Molecular Diagnostics

Karel Kleparnik[1]; Ivona Svobodova[1]; Vera Hezinova[1]; Jan Prikryl[1]; Marcela Liskova[1]; Zuzana Bilkova[2]; Frantisek Foret[1]

[1]Inst. Anal. Chem., Brno, Czech Republic; [2]University of Pardubice, Pardubice, Czech Republic

Unusual optical properties of nanomaterials offer an extraordinary high sensitivity to specially designed systems. The combination of specific probes including immunofluorescent probes with highly fluorescent labels and an advanced optical instrumentation allows even the single molecule probing of individual cells. The application of highly stable Quantum dots (QD) in laser-induced fluorescence detection (LIF) and surface enhanced Raman scattering (SERS), are two examples of methods where the advantages of nanoparticles are taken. While in LIF, the fluorophor with a high quantum efficiency must be conjugated with a high-affinity selector, an antibody, in SERS, the nanoparticles serve as centers where the electromagnetic energy of light is accumulated nonselectively in the form of surfce plasmon and transferred to the adsorbed molecules of analyte to excite them. The objective of this presentation is to show, the methods of the application and characterization of nanoparticles in analytical and diagnostic practice. Due to the broad excitation (350-500 nm) and narrow emission spectra (58nm),up to 6 probes excited by a single laser for parallel detection of several molecules and/or receptors in flow cytometry can be used. The examples of detection of important molecules and receptors in cells by high sensitivity fluorescence microscopy will be demonstrated. Namely, CD3 molecules connected with the TCR receptor of human lymphocytes and PCNA molecules in mouse embryo tissues. Another example of the preparation of detection probes is the nano CdTe probe for simultaneous electrochemical and fluorescence detection of oligonucleotides.

NOTES:

Tues. 17:05-17:25

Towards understanding biomolecule behavior in nanofluidic channels

Maria Teresa Napoli; Sumita Pennathur, *UC Santa Barbara, Santa Barbara, CA USA*

In recent years, there has been a remarkable increase of experimental investigations focused on biomolecule separations in nanofluidic channels. However, the physics behind these experimental results remains not well understood, since biomolecules can have complex interactions with the environment and an observed phenomenon might be the function of a variety of different parameters.

In this work, we theoretically and experimentally investigate the effective electrophoretic mobility and effective diffusivity of spherical polystyrene particles in both microfluidic and nanofluidic channels. Starting from a well-defined system, we uniquely identify and quantify the forces on the particles resulting from reducing the dimensions of the channel to the nanoscale. The case of small DNA molecules and globular proteins follows as a generalization of these results.

To quantify the behavior of the particles, we perform separation experiments on two different geometries of double-T isotropically-etched fluidic chips: a) 50μm wide by 20μm deep and b) 5μm wide by 1μm deep, with the larger channels serving as a reference for comparing and interpreting the results obtained in the smaller channels. In different sets of experiments, we inject buffered flow (1 and 10 mM of borate, pH 9, phosphate, pH 7 and acetate, pH 5) seeded with charged or uncharged polystyrene particles (50 and 100nm, fluorescently labeled) into a channel. Fluorescence intensity is measured 5mm, 10mm and 20mm downstream from the injection point using an inverted epifluorescent microscope.

Initial results show that the separation dynamics of particles in nanochannels match DNA separation better than small ion separation. We attribute this behavior to the finite size of the particles with respect to the channel. A quantitative investigation of this behavior will result in a model that can be extended to the characterization of biomolecules dynamics.

NOTES:

TUESDAY POSTER SESSION ABSTRACTS
Tuesday, February 3, 2009
14:00 – 15:30
Imperial Ballroom and Stanbro Room

Advances in Capillary Separations 2

P-201-T

1 Mm I.D. Poly(Styrene-Co-Divinylbenzene) Monolithic Columns for Fast and High Efficiency Protein Separations

Bas Dolman; Sebastiaan Eeltink; Remco Swart, *Dionex, Amsterdam, The Netherlands*

Due to the unique properties of monolithic stationary phases, monolithic columns have become an attractive alternative for packed columns, especially for the separation of complex protein samples. The porous monolith is covalently anchored to the capillary wall. This attachment increases the robustness of the column. The control that can be exerted over the preparation process facilitates optimization of the porous properties of the monolith, and consequently the chromatographic performance of the entire system. Furthermore, virtually no carryover effects are observed on monolithic stationary phases compared to frequent observation on silica materials, resulting in a more reliable identification and quantification of proteins, especially for the low abundant species.
In this study, the LC performance of a 1 mm I.D. monolithic column is demonstrated for the separation of proteins. The effects of gradient time and volumetric flow rate on peak capacity are demonstrated and loadability of the 1 mm I.D. monolithic column was determined for proteins. Furthermore, high efficiency separations of a complex E. Coli protein mixture are demonstrated. For these separations a volumetric flow rate < 100 μL/min was applied to ensure high detection sensitivity and maximum compatibility with ESI interfacing and MALDI spotting. Finally, the application of the 1 mm I.D. monolithic column used as a 2nd dimension RP column is demonstrated after an 1D ion-exchange separation.

P-202-T

Effect of capillary cross section geometry and size on chromatographic performance of monolithic poly(butyl methacrylate-co-ethylene dimethacrylate) columns

Ivo Nischang[1]; Frantisek Svec[2]; Jean M. J. Fréchet[1]

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Chromatographic separations are mostly carried out in columns with circular cross section. Ease of accessibility of tubes, their simple machining and threading, as well as straightforward connection to end fittings are the major technical reasons for popularity of tubular column formats. The rebirth of non-cylindrical conduits in chromatography emerged with the advent of microfluidic separation devices. The typical methods used for their fabrication such as micromachining, etching, laser ablation, embossing, and injection molding typically do not afford devices containing circular channels. Our study concerns porous polymer monoliths prepared in both cylindrical and non-cylindrical capillaries with the latter having square or rectangular cross section and varying lateral dimensions. Using this set of capillaries, we could explore

the effect of the size and shape on porous and hydrodynamic properties of poly(butyl methacrylate-co-ethylene dimethacrylate) monoliths. Permeability to flow, scanning electron microscopy, and liquid chromatographic separations of a model protein mixture were used to monitor the effects. As expected from the similarity of porous structures of chemically identical monoliths, the separation of standard proteins facilitated by the convectional mass transport is not affected by the flow rate that can be varied in a wide range thanks to the excellent permeability to flow. Our experiments also prove downwards scalability of the monolithic devices. We reached ultimate limits of miniaturization using polymer-based monoliths in capillaries having diameter in a single micrometers range. Our study for the first time demonstrates that conduit size and shape do not affect the morphology and the chromatographic performance of porous polymer monoliths prepared in situ. This result is important since model experiments can be now carried out safely with monoliths prepared in easily accessible capillaries with a circular cross section. Such experiments are then easily transferable to more expensive microfluidic chips, which channels have a shape vastly different from circular.

NOTES:

P-203-T

A Novel Approach to Solid Phase Extraction Employing Functionalized Magnetic Particles for Applications In Capillary And Microchip Electrophoresis

Yolanda H. Tennico; Myra T. Koesdjojo; Vincent T. Remcho, *Oregon State University, Corvallis, OR USA*

We report here a new approach of performing solid phase extraction employing magnetic particles and electrophoresis. Iron oxide magnetic particles were synthesized as a solid support to be further functionalized with C18 groups. Solid phase extraction of several hydrophobic compounds was performed using the synthesized magnetic sorbents. Capillary electrophoresis was used as a method to separate and detect the analytes. The extraction approach was fully integrated into the CE setup, which enable a significantly faster analysis time compared to an off-line approach. With this approach, extraction, elution, and detection of the analytes can be performed sequentially without interruption or need for sample handling. In this study, mixtures of parabens were successfully extracted from suspension using the synthesized magnetic sorbents. CE was able to completely separate the analytes within 10 minutes. Our results confirmed the applicability of using functionalized magnetic particles for performing extraction in the capillary setup. We have shown that the extraction approach is readily transferable to the microchip format. On-line SPE-microchip electrophoresis with fluorescence detection was used to extract, separate, and detect the target analytes on-chip. In our initial study, hydrophobic dyes were used as the analytes due to their interaction with the C18 functionalized magnetic particles and to facilitate detection. The analysis was carried out in a microfluidic device made in-house using a disposable plastic substrate, in this case poly(methyl methacrylate) (PMMA), via a hot embossing technique (for fabrication of the microchannels) and solvent welding (to bond the microchip).

P-204-T

Hydrophilic Interaction 10 µm I.D. PLOT Columns for Ultratrace Glycan Analysis by LC/MS

Quanzhou Luo; Dongdong Wang; Tomas Rejtar; Marina Hincapie; Barry L. Karger, *The Barnett Institute, Northeastern University, Boston, MA USA*

Glycosylation is one of the most important post-translational modifications (PTMs) of proteins, which plays a crucial role in numerous biological processes. MS has become the key tool for glycan analysis, mainly due to their high sensitivity and capability for structure determination. The sensitivity of LC-ESI-MS increases with the decrease of the mobile phase flow rate, accompanied by reduced ion suppression. Several issues must be addressed for the LC-ESI-MS to be a robust tool for sensitive glycan analysis, including (1) new chromatographic approaches to resolve glycans effectively and (2) minimization of the ion suppression of these compounds during ESI. To address these issues, we developed a new approach for the preparation of hydrophilic interaction chromatographic (HILIC) PLOT columns. The amine- and amide-HILIC PLOT columns are characterized by high resolving power for glycans at a flow rate of 20 nL/min. When coupled on-line with ESI-MS, the columns demonstrated high separation efficiency and high sensitivity for glycans. The applications of such columns in the characterization of immunoprecipitated glycoproteins will be shown.

NOTES:

P-205-T

Optimization of Protein Purification Using Small-Scale Separation Columns

Lisa Thurston; Lee Hoang; Chris Suh; Doug Gjerde, *PhyNexus, San Jose, CA USA*

Characterization of therapeutic candidates requires that proteins are well purified and enriched post expression. The process for adequate preparation requires that sufficient quantities of material be scaled up and processed in a time consuming manner using expensive chromatography equipment. As developments in functional and analytical assays increase throughput and reduce the amount of protein required for analysis, efficient small-volume protein purification would provide high-value information to researchers in earlier stages drug discovery and development.

Recent advances in the area of miniaturized high-throughput tools for purification, enrichment and desalting of proteins eliminate bottlenecks associated with traditional protein purification techniques. By performing high-performance functional protein separations on small samples in parallel, it is now possible to obtain more relevant data in a completely automated format.

Investigation of protein separations in small-scale extraction columns is presented along with optimized conditions enabling functional and analytical characterization of therapeutic proteins purified by this unique format.

P-206-T

Mathematical Modelling of the Trapping Zones in an Insulator-Based Dielectrophoretic Device

Blanca Lapizco-Encinas; Javier L Baylon-Cardiel; Sergio O. Martínez-Chapa, *Tecnologico de Monterrey, Monterrey, Mexico*

Insulator-based dielectrophoresis (iDEP) is an efficient technique with great potential for miniaturization. It has been successfully applied for the manipulation and concentration of a wide array of particles. When iDEP is applied employing DC electric fields, other electrokinetic transport mechanisms are present. In order to concentrate and immobilize particles iDEP has to overcome electrokinetics. This study presents the mathematical modeling of the performance of an iDEP microdevice, in order to identify the optimal conditions for particle concentration employing DC-iDEP.

The geometry of an iDEP microdevice was captured with COMSOL Multiphysics, the microchannel considered was 10.16-mm long, and 2-mm wide containing an array of 32 insulating posts, distributed in 4 rows of 8 posts each. The posts have a diameter of 440 um, arranged 520 um center-to-center. For this model, particles having a diameter of 1 um were considered.

Laplace equation was solved between the insulating posts employing suitable boundary conditions. It is assumed that the main contributions to the particle flux along the microchannel come from DEP and EK flow. A region for dielectrophoretic trapping is where the flux along electric field lines is equal to zero. Therefore, trapping of particles will occur where the dielectrophoretic velocity is greater than the electrokinetic velocity.

Several simulations with different electric potentials applied across the microchannel were performed, varying the properties of the suspending medium. Regions of higher and lower electric field intensity were generated, making it possible to predict where the regions for dielectrophoretic trapping are located. It was demonstrated that the results obtained with the mathematical model are in agreement with experimental

results. This mathematical model has the potential to be employed to predict the performance of dielectrophoretic devices and aid on the selection of operating conditions, thus providing with guidelines for the optimization of iDEP separations.

NOTES:

P-207-T

Online Preconcentration by Transient Isotachophoresis with Capillary Zone Electrophoresis for Monitoring the Activity of Ribonucleotide Reductase

Huey-Fen Tzeng; Shih-Wei Huang, *National Chi Nan University, Puli, Nantou, Taiwan*

One simple and rapid capillary electrophoretic method was developed for the simultaneous determination of micro-molar deoxyribonucleoside diphosphates and deoxyribonucleoside triphosphates in enzyme assays without using radioactive labeled substrates. The enzyme assay mixture was analyzed by capillary zone electrophoretic (CZE) coupled with isotachophoresis as online preconcentration technique to improve the sensitivity. This preconcentration method can be applied to the sample buffer even with high conductivity, so it is suitable for the direct injection of the enzyme assay mixtures. Several parameters including the concentration of leading electrolyte, the pH and the concentration of running buffer, and the concentration of EOF suppresser were optimized. Under the optimal condition, the injection volume could be increased to 150-fold of that used in the normal CZE mode without losing the resolution, and the detection limit could be down to sub-μM level, which is about 240-fold lower than that in the normal CZE mode. The good separation of dCDP, dCTP and dADP were achieved in 6 minutes. This method could be applied to increase the sensitivity of ribonucleotide reductase assays.

P-208-T

ssDNA Separations by Microchip Electrophoresis: Systematic Analysis of the Performance of Engineered pDMA and LPA Networks

Daniel G. Hert[1]; Christopher P. Fredlake[1]; Annelise E. Barron[2]

[1]Northwestern University, Chicago, IL USA; [2]Stanford University, Stanford, CA USA

Electrophoresis of DNA molecules through entangled polymer solutions continues to be an important tool for genetic analyses, including DNA sequencing and forensic genotyping. To date, a variety of polymer classes have been developed to perform such assays, but no comprehensive mechanistic understanding of these electrophoretic separations in microfluidic channels exists. Previous DNA sequencing studies by CE have reported the average read lengths obtained in polymer matrices, using specific polymer molar masses or concentrations for individual cases. Other work has focused on developing predictive models for DNA separation in entangled polymers in capillary-based systems. DNA separations in capillary systems have been shown by our group to be systematically different than separations in microfluidic chips, due to inherently unique injection schemes in each system. In microchip electrophoresis, the apparent dispersion coefficient of the ssDNA molecules in the matrix is a reliable measure of the influence of band broadening on the separation, which can greatly lower the resulting resolution of the separation. Utilizing the apparent dispersion coefficient and DNA sequencing read length as measures of the separation ability of the polymer matrix, we have synthesized a small library of linear polyacrylamides and poly(N,N-dimethylacrylamides) to systematically study the effects of dominant experimental parameters and the underlying separation mechanisms of ssDNA in microfluidic channels. Here, we describe the effects that the polymer molar mass, polymer concentration, polymer chemistry, electric field strength, and separation distance have on the electrophoretic migration behavior of single-stranded DNA fragments in microfluidic chips. We discuss the implications of the results in the context of previously described separation mechanisms, and also translate these results to inform polymer matrix choices for integrated sequencing and genotyping microfluidic chips.

NOTES:

P-209-T

New Microfluidic Chip for Phosphoproteomes

Dayin Lin; Martin Vollmer; Tom van de Goor, *Agilent Technologies, Inc., Waldbronn, Germany*

Protein phosphorylation is one of the most important post-translational modification (PTM) events among mechanisms of regulating protein function in cells. Myriad biological processes, including cell proliferation, migration, and apoptosis involve phosphorylation steps. One of the major efforts in proteomics is devoted to the identification and understanding of phosphoproteomes in cells. Nevertheless, comprehensive identification of sites of protein phosphorylation remains a challenge, best left to experienced proteomics experts. In order to achieve selective enrichment of phosphorylated proteins and peptides most commonly used technologies are currently immobilized metal affinity chromatography (IMAC), anti-phosphotyrosine antibodies, and titanium dioxide prior to LC/MS (liquid chromatography and mass spectrometry) analysis. Recent advances in HPLC chip technology have created an environment to allow automation of such a workflow with increased ease of use and confidence of analysis. The new microfluidic chip is a re-usable HPLC nano-flow rate chip with titanium dioxide particles (TiO2) based phosphopeptide enrichment. The chip is a multilayer polyimide laminate that contains an enrichment section with TiO2 beads flanked on both sides with C18 reversed phase material. The 3 section sandwich is separated from each other by micro-fabricated frits. This enrichment section is connected to a reversed phase separation column ending in an integrated electro-spray tip by a micro valve in direct contact with the chip surface providing a zero dead volume high pressure seal. The chip is used with a HPLC-chip/MS instrumentation using the HPLC-chip cube interface combined with a Mass Spectrometer. The unique sandwich configuration of the enrichment section provides researchers three modes of peptide analysis: (1) standard peptide analysis, (2) phosphopeptide analysis only, and (3) combined peptide and phosphopeptide analysis. This approach will offer non-expert proteomics researchers a reliable way in phophoproteome analysis.

P-210-T

Denaturing microChip Electrophoresis (DmCE): A New Approach to Rapid Genotyping and DNA Mutation Detection for Routine Clinical Diagnostics

Patrik Sekerka[1]; Barbora Belsanova[1]; Richard Chudoba[2]; Lucie Benesova[1]; Bob Gas[2]; Marcus Gassmann[3]; Fritz Bek[3]; Marek Minarik[1]

[1]Genomac International, Prague, Czech Republic; [2]Faculty of Science, Charles University in Prague, Prague, Czech Republic; [3]Agilent Technologies, Waldbronn, Germany

Contemporary medical genetics is fundamentally based on a wide range of PCR-based genetic tests for diagnostics of various forms of most frequent genetic disorders. In molecular oncology, a common diagnostics of the inherited cancers is complemented by a new family of tests directed at evaluation of cancer survival prognosis or in prediction of clinical response to targeted anticancer therapy. A common attribute of such clinical genetic testing is a low to medium throughput analysis of inherited DNA mutations (or SNP polymorphisms) in blood or detection of somatic DNA mutations in tissue samples of individually approached patients. A variety of experimental techniques is applied for such purpose, including SSCP, RFLP, or heteroduplex analysis performed on either traditional slab-gel or CE platforms. Since the arrival of routine quantitative (real-time) PCR technology in late 1990, many tests have been adopted on rtPCR-based platforms and more recently further refined with the extension of rtPCR by high-resolution melting option.

In the present report we introduce a revival of electrophoretic-based DNA mutation detection and genotyping application. We utilize a microchip electrophoretic platform based on commercial Agilent 2100 Bioanalyzer to perform rapid separation of DNA heteroduplexes under partial denaturing conditions. We employ temperature programming, including constant as well as cycling temperature approaches for detection of a wide range of DNA mutations and to perform genotyping of clinically relevant SNP polymorphisms in routine molecular testing. The main advantages of the technique include simplicity, low cost and mutation sensitivity.

The project is supported by the Czech Ministry of Industry research grant No. FI-IM3/215

NOTES:

P-211-T

Supported Lipid Membranes for Bioseparations on PDMS Microchips

K. Scott Phillips; Andrew K. Kang; Chris Sims; Nancy Allbritton, *University of North Carolina, Chapel Hill, NC USA*

A method for coating poly(dimethylsiloxane) (PDMS) microchips with supported phospholipid bilayer membranes is reported for high-performance electrophoretic separations of biomolecules. Fluorescent dyes, peptide kinase substrates, and proteins are separated succesfully. The method is inexpensive, rapid, and robust, and the results obtained are comparable to or better than many existing strategies for bioseparations. Coated chips can be continuously used for many hours with <10% change in migration time. The membranes can be tailored for different applications by changing the lipid headgroup or adding receptor/protein complexes. Greatly reduced non-specific adsorption allows for separation and detection of <1000 molecules with laser-induced fluorescence, while also preventing fouling by cells or cellular debris. This new alternative microchip coating is a biomimetic and versatile solution for challenging new bioanalytical applications such as single-cell cytometry.

P-212-T

Purification of DNA and RNA from Serum using a Polymer Capture Matrix

Brian E. Root[1]; Abhishek K. Agarwal[1]; David M. Kelso[1]; Annelise E. Barron[2]

[1]Northwestern University, Chicago, IL USA; [2]Stanford University, Stanford, CA USA

Purification of complex biological samples, such as serum and whole blood, has been a major obstacle in the development of automated biomarker detection systems. We demonstrate the electrophoretic purification of DNA and RNA from a 10% serum sample using an oligonucleotide capture matrix. This approach provides a one-stage, totally aqueous system capable of purifying both DNA and RNA for downstream PCR amplification. The advantages of utilizing the polymer capture matrix method in place of the solid-phase extraction method is that the capture matrix eliminates both the isopropanol wash (residual isopropanol may inhibit PCR downstream) and competition with proteins for the binding sites that can limit the capacity of the device. This method electrophoreses a biological sample (e.g. serum) containing the nucleic acid target through a polymer matrix with covalently-bound oligonucleotides. These capture oligonucleotide selectively hybridize and retain the target nucleic acid, while the other biomolecules and reagents (e.g. SDS) pass through the matrix to waste. Following this purification step, the solution can be heated above the melting temperature of the capture sequence to release the target molecule, which is then electrophoresed to a recovery chamber for subsequent PCR amplification. We demonstrate that the device can be applied to purify both DNA and RNA from serum. The gag region of HIV at a starting concentration of 37.5 copies per microliter was successfully purified from a 10% serum sample demonstrating the applicability of this method to detect viruses present in low copy numbers.

NOTES:

P-213-T

Field Amplified Sample Stacking in Nanofluidic Channels

Jess M. Sustarich; Sumita Pennathur, *University of California at Santa Barbara, Goleta, CA USA*

Nanofluidic channels provide a promising technology for single cell bioanalytical and diagnostic devices due to low sample consumption and unique biomolecule separation properties. One of the greatest difficulties of such nanoscale separations, however, is sensitivity of detection. Field amplified sample stacking (FASS) is a common sample pre-concentration technique used in separation assays at the microscale. FASS involves a nonlinear variation of electric field between the sample plug and background buffer solutions due to differences in conductivities of the two regions. While FASS allows for concentration enhancements up to 1000 times in microfluidic channels, the conductivity difference between the sample plug and background buffer induces an internal pressure gradient, which increases the sample dispersion, lowering the ultimate signal enhancement. In a nanofluidic channel, however, the Peclet number is so small that dispersion follows pure molecular diffusion and the nonuniform velocity profile induced by the thick electric double layer counters the internal pressure gradient that arises from FASS.

In this poster, we present results from an experimental investigation of FASS in nanochannels. Our custom designed and fabricated nanofluidic channels have a characteristic dimension of 180nm. We used sodium flourescein as the fluorescent analyte in a background buffer of sodium tetraborate, varying buffer concentrations from 1-100mM. After injecting a sample plug into a double cross separation channel, we collected intensity versus time data to measure the concentration enhancement as well as the plug width. Our preliminary experiments have thus far shown an increase of SNR by a factor of 20 and sample plug dispersion on the order of molecular diffusion, which allows for a dramatic concentration enhancement of the sample plug in a nanofluidic channel. Further investigation will yield optimum concentration enhancement conditions, which can then be applied to increasing the resolution of biomolecule separations.

P-214-T

Microchannel Electrochromatography for Gradient Peptide Elution on Reverse Phase Columns

Michael W.L. Watson; Aaron R. Wheeler, *University of Toronto, Toronto, Canada*

Reverse phase (RP) HPLC instruments capable of gradient elutions are a workhorse for separations in a wide range of applications. For example, shotgun proteome profiling, a method which has become the gold standard for clinical proteomics applications, is absolutely dependent upon repeatable gradient elutions on RP chromatographic media. Until now, gradient elution separations have been largely implemented in conventional HPLC instruments (macro or “nano” flow). Microchannel electrochromatography (MEC) is emerging as an alternative to HPLC for some applications; however, without the ability to perform gradient elutions, it is doubtful that MEC will ever become a widely used technique.

Here we present the first gradient elution separations over a stationary phase medium in a microfluidic device. Stationary phases were formed in planar glass microfluidic channels with a “double T” cross injection element and a “Y” element for buffer stream mixing. The stationary media used here are porous polymer monoliths (PPMs), which were fabricated in acryloxysilane pre-treated channels by UV photoinitiated free-radical polymerization of an acrylate monomer containing casting solution in an aqueous porogenic solvent. Charged moieties on the polymer surface facilitate electroosmotic flow (EOF) through the porous structure, while hydrophobic moieties yield reverse phase functionality. In isocratic mode, PPMs are capable of separating peptide standards with efficiencies surpassing 500,000 plates/m.

More importantly, when used to separate peptide standards and protein digests, devices operating in gradient mode are capable of resolving more peaks, in less time than isocratic mode. We anticipate that the capacity to implement gradient elutions by MEC is an important step forward for this technique that will eventually lead to microfluidic shotgun proteomics and other applications.

NOTES:

P-215-T

Microfluidic Spontaneous Picoliter-Scale Sample Introduction for High-Speed Chiral Separation of Amino Acid Enantiomers

Qun Fang;[1] Ting Zhang[1]; Wen-Bin Du[2]; Jing-Lin Fu[3]

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Herein, we developed a microfluidic high-speed capillary electrophoresis (HSCE) system based on a short capillary and spontaneous picoliter scale injection technique without the requirement of microfabricated devices. Using the novel system, high-speed chiral separation of a mixture of amino acid enantiomers was achieved within 6.5 s in a short capillary with an effective separation length of 1.5 cm, reaching separation efficiencies up to 2,880,000 theoretical plates per meter.

High-speed capillary electrophoresis (HSCE) has achieved rapid separation in a few seconds, using narrow sample plugs and high electric field strengths. Several approaches have been developed for achieving subnanoliter sample introduction. However, most of these methods suffered from complicated structures and sample biasing effects.

The HSCE system was composed of a short fused-silica capillary (3-cm-long, 50-μm i.d., 375-μm o.d.) and automated sample introduction system with slotted sample and buffer reservoirs and a computer-programmed translational platform. Translational sample introduction was performed by linearly moving the platform, allowing the capillary inlet to enter the sample solution, and then remove from it to achieve spontaneous sample introduction by capillary action. The platform was continuously moved to allow the capillary inlet into the buffer solution, and CE separation was performed by applying high voltage between the buffer and waste reservoirs.

The conditions for sample introduction and CE separation processes were optimized. The present system was applied in the chiral separation of amino acid enantiomers under optimized conditions. A mixture of FITC-labeled amino acid enantiomers were resolved in 6.5 s over a separation length of 15 mm, using β-cyclodextrin (β-CD) and sodium taurocholate (STC) as chiral selectors. The resolutions were higher than 1.55. High separation efficiencies ranging from 2,500,000 to 2,880,000 theoretical plates per meter were obtained, corresponding to 0.40 to 0.35 μm plate heights, respectively. The separation speed and efficiency is comparable to or even better than those reported in the microfluidic chip-based CE systems.

The advantages of the present system include simple structure, ease of operation and versatility in application. This work just showed that this picoliter-scale injection method was indeed feasible in chiral separation of amino acids. Preliminary studies revealed potentials for electrophoresis separation of other complex samples such as peptide, nucleic acid and protein in different separation modes

NOTES:

P-216-T

A Simple Way to use a 1D Split-Free Nano-HPLC System in an Automated 2D LC-MS/MS Setup

Michael Andersen[1]; Paul Taylor[3]; Michael Moran[2]; Ole Vorm[1]; Alexandre Podtelejnikov[1]; Thomas Kislinger[2]

[1]Proxeon A/S, Odense C, Denmark; [2]University of Toronto, Toronto, Ontario; [3]Hospital for Sick Children, Toronto, Ontario

Analysis of complex biological samples often requires several separation steps. One of the commonly used technologies in proteomics is automated two-dimensional liquid chromatography coupled with mass spectrometry (2D LC-MS) using on-line strong cation exchange (SCX) / reverse phase (RP) separations. This currently requires a sophisticated solution with ternary or quaternary gradient systems to deliver the stepped salt gradient to elute peptides from the SCX column onto the RP column and the linear gradient to elute peptides from the RP column into the MS/MS analysis. Here we describe a simple, automated SCX/ RP on-line 2D separation strategy achieved on a split-free 1D nano LC-MS system.
System performance was evaluated by analyzing the proteome of mouse placental cells. The cytosolic fraction was subjected to cysteine reduction and alkylation with iodoacetamide followed by trypsin digestion. The lysate was purified and analyzed by 1D and 2D methods on a split-free nano LC-MS system (EASY n-LC, Proxeon, Denmark) coupled to LTQ-Orbitrap (Thermo-Fisher, Germany). The first separation used a 10 step injected salt gradient varying from 0.05 to 0.5 M NH4acetate.
By using the autosampler component and standard sample injection programs within the EASY-nLC system for the first separation, we transformed this 1D system into a 2D system. The viability of this method was demonstrated by comparison of LC-MS data obtained from 1D and 2D separations. The 1D analysis yielded about 600 protein identifications whereas 2D analysis gave approximately 2000 proteins identified. As expected, much more information could be obtained from the 2D separation, but, in this set-up, we were able to avoid the complexity of using a conventional 2D LC system.
Novel aspects: these results clearly demonstrate the significant improvement which can be achieved by only slight modifications of a 1D nano LC-MS configuration.

P-217-T

Colloidal Crystals: First Step Towards Optical Chromatography in Microfluidic Devices

Aoife Morrin; Blanaid White; Jeremy Galineau; Malcolm R. Smyth, *Dublin City University, Dublin, Ireland*

Chromatographic columns or capillaries for liquid chromatography are currently moving to reduced dimensions. The formation of well ordered packing such as colloidal opals should provide an increase in columns efficiencies, thus reducing the separation time, and improving chromatographic resolution. Moreover, the use of capillary photonic colloidal crystals, optically active thanks exceptional diffraction properties [1] is of great interest as the column integrity and the liquid phase composition can be studied and might reach to a new range of detectors [2]. The growth of colloidal crystals, made with 200 nm negatively charged latex, in microfluidic channels devices is described. The quality and structure of the opals formed by different techniques ranging from simple drop coating to filtering, and printing are discussed. This first step is of primary importance, as the separation efficiency should be directly linked to the quality of the packing.

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NOTES:

P-218-T

A Reproducible Method for Heart-Cut Analysis Utilizing Online RP/RP 2D nanoLC/MS of Proteomic Samples

Martha Stapels; Keith Fadgen; James Langridge, *Waters Corporation, Milford, MA USA*

Two-dimensional chromatography is often used to separate peptides from proteomic samples in a biomarker discovery workflow. In order to validate a biomarker, many samples need to be analyzed to prove that the same peptides are reproducibly identified and are changing in a statistically significant manner due to a biological perturbation. Rather than running an entire 2D experiment, which is too time-consuming during validation, a better approach is to elute the targeted peptides in one fraction in a heart-cut manner. A highly reproducible method for performing on-line two-dimensional chromatography with mass spectrometry was developed. Peptides were separated by RP chromatography at high pH in the first dimension, followed by an orthogonal separation at low pH in the second dimension. An online dilution of the effluent was performed after the first dimension so that no hydrophilic peptides were lost in the second dimension. As peptides eluted from the second dimension, a hybrid quadruple time-of-flight mass spectrometer was used to detect the peptides and their fragments by alternating collision cell energy between a low and elevated energy state. All peptides were fragmented in this method, which takes out the irreproducible nature of typical MS/MS experiments. This fragmentation allowed for the identification of the peptides with a novel database searching algorithm that uses 14 physiochemical properties to score identifications and minimize false positives. Proteins had to be identified in two out of three replicate injections, which narrowed the list to the most confident identifications. Comparing the third fraction of a complete 2D experiment to the targeted run in the heart-cut analysis resulted in over 90% of the same proteins and peptides being identified. Comparisons of measured retention times and peak areas between the two methods will be made.

P-219-T

Coupled Column Capillary Isotachophoresis/Electrospray Mass Spectrometry for Large Volume Sample Analysis

Roman Tomas[1]; Marian Koval[2]; Frantisek Foret[1]

[1]Institute of Analytical Chemistry, Brno, Czech Republic; [2]Villa Labeco, Spisska Nova Ves, Slovakia

Modern instrumentation for capillary isotachophoresis with coupled columns provides an efficient means for rapid electrophoretic analysis of sample volumes of up to 30 microliters. While such instruments, which are now commercially available, are commonly equipped with conductivity and UV absorbance detectors, their on-line coupling with electrospray mass spectrometry is highly desirable. In this work we have designed a new coupled column electrophoresis system allowing direct connection to the ion trap mass spectrometer. A short capillary transfer lines, utilizing fused silica capillaries with different internal diameters, were tested for optimum sensitivity and zone broadening. The transfer line served also as the ESI tip of the sheathless electrospray interface. Optimization of the design with respect to liquid flows, pressure conditions and sample flow rates has been performed in this study. The system was applied for determination of proteins, peptides and metabolites in body fluids. During the analysis the first, wide bore preseparation capillary with 0.8 mm internal diameter served for removal of the bulk sample components and preseparation of the potentially interfering analytes. Final separation was then finished in the second narrow bore capillary after the electronic column switching. Besides the unequivocal identification of the selected sample components the MS analysis has also increased the instrument sensitivity by more than 4

orders of magnitude when compared to the on-column conductivity detection. Supported by the Czech grants: IAA 400310703, MSMT LC06023, AV0Z 40310501

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P-220-T

Accumulation and Lysis of Bacteria on a Microfluidic Chip

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Fast and reproducible on-chip sample preparation can make lab-on-a-chip systems viable for clinical applications.
We present a microfluidic chip that collects and accumulates bacteria from a sample by positive dielectrophoresis and subsequently lyses cells to retrieve DNA and RNA for further analysis. Suspensions of Escherichia Coli are pumped through a microchannel comprising an array of interdigitated electrodes at the bottom. An AC-voltage is applied to the electrodes resulting in an inhomogeneous electric field that extends into the channel and results in dielectrophoretic forces acting on the bacteria. Size and direction of these forces depend on the voltage amplitude and the frequency of the electric field. At a frequency of 1 MHz bacteria are attracted to areas of high electric field gradient at the edges of the electrodes (positive dielectrophoresis). When a volume of several mL of cell suspension is pumped through the chip, the bacteria are accumulated on the electrodes and are thus concentrated in a small volume of only 8 µL inside the channel.
In a second step the flow is stopped and the frequency of the electric field is changed to 10 kHz. At this frequency negative dielectrophoresis is observed, meaning cells are pushed away from the electrodes to positions of low electric field gradient. However, at the same time lysis of the bacterial cells starts if the electric field is strong enough. The lysis of E. coli is monitored using a live-dead staining that changes fluorescence color of bacteria.
The bacteria release DNA that is collected and analyzed further using real time PCR. PCR of the lysate retrieved from the chip showed a signal about 10 cycles earlier than in case of the original E. coli suspension. This confirms lysis of bacteria in the chip, the release of DNA and indicates a 1000-fold higher DNA concentration.

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P-221-T

Quantitative Analysis of Propofol Concentration in Patients Undergoing CABG with CPB Using Capillary Electrophoresis

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We have developed a micellar electrokinetic chromatography (MEKC) method to determine clinically relevant propofol concentrations in whole blood from patients undergoing coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB). 400 uL of whole blood was used in this procedure to minimize the sampling error but increase the operability. The preparative step involves the addition of thymol as an internal standard, followed by subsequent extraction into cyclohexane. The dried sample is re-suspended in 6% acetonitrile. The separation takes place in a bare fused silica capillary with a borax buffer containing an SDS pseudostationary phase. UV detection was used (wave length = 200 nm). The optimized separation conditions provide propofol and thymol peaks in less than 8 minutes, and was linear between 0.5 and 16 ug/mL of propofol (r2 = 0.9996). We are able to detect propofol in the ng/mL range, with a limit of detection for propofol of 0.11 ug/mL (S/N = 3) and the limit of quantitation of 0.28 ug/mL (S/N = 10). The method was successfully applied to evaluate propofol concentrations in thirty human patients.

P-222-T

Fast and Extremely Sensitive Detection of Bacterial Endotoxins in Microchip Electrophoresis

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Bacterial endotoxins (lipopolysaccharides, LPSs) are highly active components of the outer membrane of gram-negative bacteria and are released during growth, division and lysis. They have been recognized as the most potent stimulants of mammalian immune systems, causing a wide spectrum of pyrogenic and toxic reactions.
LPSs consist of a lipid region, termed lipid-A covalently attached to a polysaccharide region, which have extremely high variability in their structures.
Though several methods have been used for endotoxin analysis, much progress is still needed to separate and identify the many subclasses and structure-function relationship of LPSs from individual strains. The lack of strongly UV-active groups or chromophores in the LPS molecule and its strong tendency to aggregate in aqueous solution makes the detection of the underivatized substances difficult.
A comprehensive study was continued to explore the complex structure of the components with several unique sugar components and differences in the lipid part. The novel and fast methods using conventional capillaries and microchips with LIF detection developed especially for endotoxins allowed us 1) to differentiate between R and S endotoxins, 2) to monitor endotoxin-protein complexes and 3) to determine the molecular components of the toxic variants of LPSs. MALDI-TOF MS, GC-MS and CE-MS studies were conducted to prove the presence of the different molecular forms, including the “absolute R”, this form together with the “core”, which contained unusual heptose units, and also the repeating units that are responsible for toxicity and immunogenicity.

The techniques developed are usable to analyse and confirm the structures and types of LPSs directly from the cell cultures of the bacteria. This is of high importance, when fast analyses are necessary in infection.

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P-223-T

Label-Free, Dynamic and Quantitative Measurement of Biomolecular Interactions

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Microarrays have been used by the bioresearch community for a number of applications. The necessity of performing this detection in a label-free format has emerged due to the drawbacks of labeling target molecules. Over the past year, we have proposed a simple interferometric technique to be used for label-free microarray imaging. Spectral Reflectivity Imaging Biosensor (SRIB) measures the optical thickness change on a thermally grown oxide surface as a result of binding of target molecules to spotted immobilized probes. The mass precision of the system is ~10pg/mm2 for each spot of a 400 spot array, with the ability of producing real-time data. In our previous work, we have shown that this technique can be used for monitoring various types of biomolecular interactions that require a high throughput.
In our recent work, we show that SRIB can be used to monitor the presence of antigens in the test solution, such that it can be utilized as a high-throughput diagnostics tool. Using a novel polymeric surface coating, immobilized antibodies remained highly functional on the surface that led to successfully capturing the target antigens. As a proof of principle, human, rabbit and mouse proteins were captured by their corresponding antibodies, with a sensitivity limit ~100ng/ml. Capturing of Hepatitis B viral surface antigens was also demonstrated.
We have immobilized different types of biomolecules, and measured the height increase as a result of increasing mass which was known. The SRIB signal is highly quantitative and perfectly linear with the increasing mass, effectively with a limitless dynamic range. We use a common approximation that defines the optical refractive index of biomolecules as ~ 1.45, and 1 nm of height change corresponds to ~1 ng/mm2 mass density increase on the surface. We show that this approximation holds well for the interferometric detection techniques.

P-224-T

A Novel Method for Separating Diagnosing Y Chromosome Azoospermia Factor Microdeletions by No-Gel Sieving Capillary Electrophoresis

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Human Y chromosome deletions in occur frequently as de novo mutation events in men with idiopathic azoospermia or severe oligozoospermia. Yq11 is called Azoospermia Factor (AZF). About 15 Y genes have been reported in the Y chromosome AZF today and the method to diagnose is Y chromosome deletions multiple PCR. Due to low efficiency of some DNA template in multiplex PCR and low sensitivity of agarose gel electrophoresis, it is necessary to develop simple, fast, and sensitive method for the diagnosis of Y chromosome deletions.
Capillary electrophoresis in polymer solution has been successfully applied to various DNA analyses including DNA sequencing, mutation detection, separation of restriction fragments PCR products, and oligonucleotides analyses. In this study high weight mass Poly(vinylpyrrolidone)(PVP) is used to separate pUC19DNA/MspI(Hpa□) fragments through no-gel sieving capillary electrophoresis. The optimal separation conditions were made by adjusting PVP concentration, pH value of running buffer, voltage and capillary temperature. The pore size is increased which make the resolution of short DNA fragments

sufficient as temperature is increased, while viscous is decreased which make the resolution of long DNA fragments insufficient. The effect of temperature was studied between 15□ and 30□, maintaining the PVP concentration at 3%, the pH of running buffer 7.8, applied voltage 10 KV. Under optimal conditions, direct gene diagnosis of patients with Y chromosome azoospermia factor microdeletions was performed. pUC19DNA/MspI(Hpa□) fragments were completely resolved except of 110bp and 111bp which can not be resolved enough and good reproduction and linearity between DNA base pairs and migration times was obtained. Good resolution and sensitivity make diagnosis of Y chromosome azoospermia factor microdeletions rapidly and exactly. The results showed that PVP is an excellent medium and it would make diagnose of genetic disease rapidly, exactly, conveniently and sensitively.

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P-225-T

Nanoscale Determination of Conformation of a Polymeric Coating for Microarray Applications

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With microarrays becoming a main tool in genetics and proteomics research, advancement of microarray technology through optimization of surface chemistries and probe-target interactions has become a major research area. Ideally, surface chemistries should provide functional groups for probe attachment, minimal nonspecific adsorption, stability to environmental changes, and probe activity after immobilization for efficient target capture. Among existing surface chemistries, three-dimensional coatings are the most promising in meeting these criteria. One such 3-D polymeric coating, copoly(DMA-NAS-MAPS), has been introduced previously for use in DNA and protein microarrays. The polymer self adsorbs to the surface and forms a hydrophilic coating with functional binding sites for covalent probe attachment.
Earlier studies with copoly(DMA-NAS-MAPS) have shown an improved performance in DNA hybridization efficiency when compared to existing organosilanization-based surface chemistries. With the aim of understanding the effect of the conformation of the polymer on the obtained results, we use an interferometric technique, Spectral Self-Interference Fluorescence Microscopy (SSFM) for characterization of the conformation, specifically swelling, of the polymer on oxide surfaces. Using SSFM, direct observation of the axial position of fluorescent markers can be realized with sub-nanometer accuracy.
In this study, we covalently attach short strands of fluorescently labeled DNA (23mers) to the functional groups of the polymer and use them to probe conformational changes. Fluorophore heights obtained at single-stranded DNA spots indicate an axial increase of 8nm upon hydration. Control surfaces treated with epoxysilane show no swelling. Furthermore, we measure the swelling using different probe molecules, and report interesting results that reveal information about probe penetration in the polymer and the dependence of hybridization efficiency to the axial position of the probes with respect to the surface.

P-226-T

Determining Neurochemicals from the Non-Human Primate Brain by using Capillary Hydrophilic Interaction Chromatography-Mass Spectrometry

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Changes in the concentration of neurochemicals, including neurotransmitters, neuromodulators and metabolites, reflect the complex interactions within local and global neural networks. The assessment of such changes promises thus insights into neural communications; in particular if several neurochemicals can be studied at the same time. Here we report findings from chemical analysis of six polar neurochemicals, including acetylcholine (ACh), choline, glutamine, glutamate, lactate and pyruvate, by means of hydrophilic interaction chromatography (HILIC) coupled to tandem mass spectrometry (MS/MS). Extracellular fluid was withdrawn at 40 nl/min by nano push-pull perfusion (NFP3) and collected in vials for sequence analyses. We have successfully obtained and analyzed ECF samples from the primary visual (V1) and extrastriate visual (V4) cortex of awake or anesthetized non-human primates (macaca mulatta). The results showed that all six compounds can be determined by HILIC-MS with low sample treatment requirement. The sensitivity of our measuring system allows the simultaneous monitoring of these six compounds from a 200 nL in vivo sample. We further studied the effects of intracortical application of

cholinergic agents in the cortex on the concentration of these chemicals in the extracellular brain fluid. We observed significant increases in extracellular neurochemicals like glutamate and glutamine after the application of ACh or nicotinic agonists. During the ACh application experiments, the profile of exogenously applied ACh and its product choline were monitored, showing that ACh itself remained at low nM range 30 min after injection of 1 mM solution, due to the fast cholinergic metabolism in the brain. All in all, we conclude that capillary HILIC-MS combined with NFP3 is a sensitive technique for simultaneous monitoring of multi-class neurochemicals from extracellular brain samples. Future experiments will concentrate on the effects of the animal's internal state on the concentration of such neurochemicals.

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P-227-T

"Inject-Mix-React-Separate-and-Quantitate" (IMReSQ) Method for Screening of Enzyme Inhibitors in a Capillary Microreactor

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Many regulatory enzymes are considered attractive therapeutic targets and their inhibitors are potential drug candidates. Screening of large combinatorial libraries for enzyme inhibitors is pivotal to identifying large numbers of hit compounds for the development of drugs targeting regulatory enzymes. Here, we introduce the first inhibitor screening method that consumes only nanoliters of the reactant solutions and is applicable to regulatory enzymes. The method is termed Inject-Mix-React-Separate-and-Quantitate (IMReSQ) and includes five steps. First, nanoliter volumes of substrate, candidate inhibitor, and enzyme solutions are injected by pressure into a capillary as separate plugs. Second, the plugs are mixed inside the capillary by transverse diffusion of laminar flow profiles. Third, the reaction mixture is incubated to form the enzymatic product. Fourth, the product is separated from the substrate inside the capillary by electrophoresis. Fifth, the amounts of the product and substrate are quantitated. In this proof-of-principle work, we applied IMReSQ to study inhibition of recently-cloned protein farnesyltransferase from parasite Entamoeba histolytica. This enzyme is a potential therapeutic target for anti-parasitic drugs. We identified three previously unknown inhibitors of this enzyme and proved that IMReSQ could be used for quantitatively ranking the potencies of inhibitors.

P-228-T

Applications of CE-LIF for Carbohydrate Analysis Used in Characterization of Therapeutic Proteins

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The assessment of carbohydrate microheterogeneity and its batch-to-batch consistency are of utmost importance in manufacturing of therapeutic recombinant proteins. Therefore, there is an extensive need for high performance analytical techniques to provide structural characterization and routine analysis of closely related glycans derived form glycoproteins.

Applications of CE-LIF for quantitative N- and O-linked carbohydrate analysis in recombinant proteins will be presented. N-linked glycans have been released by digesting the protein with peptide-N-glycosidase F, PNGase-F, using a membrane for a rapid sample preparation step.

An Orela kit combined with microwave-assisted chemical reaction for release of glycans has been applied in characterization of O-linked glycans. The released N- and O-linked glycans have been derivatized with 8-aminopyrene-1,3,6-trisulfonic acid, APTS, prior to CE-LIF analysis. Details regarding assay performance for both type of analysis will be discussed.

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P-229-T

CE/Native-LIF of recombinant Antibodies

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CE-SDS is an important separation technique for biopharmaceutical manufacturing. The advantages of CE-SDS compared to other techniques such as SDS-PAGE and size exclusion chromatography are improved resolution, the ability for quantification and automation. CE-SDS-LIF has become a popular method for characterization and quantification of MAb's.
FITC, TAMRA and FQ are reported to be used for labelling of MAb's for fluorescence detection. A drawback with the analysis of labelled antibodies, is the chemical reaction which has to be run, and the purification of the labelled molecules, when using FITC and TAMRA. FQ is a fluorogenic dye which avoids the use of the purification step, but as with the other dyes several labelled species are present. Labelling of proteins can also result in inconsistent binding, especially at low protein concentrations. In addition, the label may cause artefacts and an increase in the baseline. The advantage of labelling is to be selective and to results in a very sensitive detection sensitivity.
In this work, we have compared the use of CE with native fluorescence to CE-LIF with different labelled antibodies.
The new ball lens-ellipsoid LIF optical arrangement was used inside a CE instrument (1). The analysis of FITC labelled IgG was optimized using a 14cm effective length capillary. The limit of detection of an FITC labelled IgG2 at an excitation wavelength of 488nm was 150 pg/mL, which was 10 times better than the detection limit recorded with slab gel silver staining. Using a TAMRA labelled IgG2 and a 532 nm excitation wavelength the LOD is 50 pg/mL. For the native fluorescence experiment, a 266nm pulsed laser was used. This allows excitation of the Trp and Tyr which are in these antibodies. Because it is a pulsed laser and because Trp and Tyr are much less fluorescent than FITC and TAMRA, the sensitivity is less than with the labelled molecules, 40 ng/mL. We will show different analysis of several commercial antibodies using CE-nativeLIF. It indicates that 266nm pulsed laser in well adapted to analyse low quantities of impurities. We demonstrate these results on many different commercial IgG samples. The integrated LIF detector provides an extremely powerful and convenient tool for antibody analysis for both labelled and native fluorescence analysis of therapeutic monoclonal antibodies in pharmaceutical facilities.

Reference: 1 Rodat A, Kalck F, Poinsot V, Feurer B, Couderc F. Electrophoresis. 2008, 29, 740-6.

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P-230-T

CE-Tof-MS using Non-Covalent Multi-Layer Coated Capillaries for the Analysis of Pharmaceutical Proteins

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Biopharmaceuticals play a more and more important role in current drug development, and a considerable number of therapeutic proteins have been registered as regular drugs. As a result there is a growing demand for separation-detection methodologies that allow analysis of intact proteins. The combination of capillary electrophoresis (CE) and electrospray ionization-mass spectrometry (ESI-MS) provides a powerful tool for the separation and characterization of intact proteins. Recently, we have shown that CE-MS analysis of intact acidic proteins can be performed efficiently by applying non-covalent multi-layer coated capillaries in combination with a sheath-liquid interface. However, as the mass resolution provided by the applied ion trap mass analyzer was relatively low, the information obtained on protein identity was limited. In the present study, a time-of-flight (ToF) mass analyzer, which presents high mass resolution and accuracy, was applied for the CE-ESI-MS analysis of intact proteins. Protein adsorption onto the capillary wall was prevented by applying non-covalent multi-layer coated capillaries. For the analysis of acidic proteins a negatively charged coating, consisting of Polybrene and poly(vinylsulfonic acid), was employed. For basic proteins, a positively charged coating of successive layers of Polybrene, dextran sulfate and Polybrene, was used. Optimal conditions for the analysis of proteins were determined. The influence of the concentration and pH of the background electrolyte on the separation and MS signal intensity was examined for a set of test proteins. To find stable spray conditions and optimum MS responses for proteins, the composition and flow rate of the sheath liquid was optimized. The applicability of the CE-ToF-MS systems was studied by the analysis of (degraded) pharmaceutical proteins. The developed CE systems allowed the separation and identification of degradation products (deamidations, oxidations) and glycoforms of both acidic and basic biopharmaceuticals. Examples include analyses of recombinant human growth hormone, oxytocine, interferon-β, and recombinant humanized monoclonal antibodies.

P-231-T

Rapid Enantiomeric Separation of Basic Drugs and Metabolites: A Universal Strategy for Ultra-Fast Chiral Method Development.

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Compared with other separation strategies, the application of highly-sulfated cyclodextrins represents a universal and ultra-fast approach to method development. This strategy was applied to a group of 101 drugs and metabolites of interest to scientists working in both the pharmaceutical industry and forensic science. Separation effectiveness of this strategy was confirmed in this study by achieving complete resolution of the enantiomers for over 94% of the tested compounds. An example of method development for purity analysis, highlighting a complex four-chiral center molecule, illustrates the rapid solution to a complex but routine problem.

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P-232-T

Assessing Biosimilars: Applying the European Pharmacopoeia Capillary Zone Electrophoresis Method for the Separation of Recombinant Human Erythropoietin (rhEPO)

Mark Lies; Marcia Santos, *Beckman Coulter, Fullerton, CA USA*

Erythropoietin (EPO) is a naturally occurring hormone that stimulates red blood cell production and release from bone marrow and was one of the first therapeutic recombinant glycoproteins commercialized for the treatment of anemia. EPO is known to have complex N- and O-linked glycosylation patterns, and can exist as numerous protein isoforms that play a critical role in the bioavailability, activity, potency and stability of rhEPO. Proper characterization of rhEPO is extremely important in order to ensure the comparability or efficacy of a biologic preparation. The European Pharmacopoeia first defined a method to qualitatively determine EPO isoform distribution using capillary electrophoresis in 1999. Subsequently, collaborative studies among pharmaceutical companies, government agencies and academic institutions have refined this method and also developed a suitability standard. We present a method for the analysis of isoform distribution in rhEPO using the ProteomeLab™ PA 800 Protein Characterization System and describe instrument setup, sample preparation and data analysis.

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P-233-T

Alternative Two Dimensional Electrophoresis - Offgel Electrophoresis Combined with High Sensitivity Microfluidic On-Chip Protein Detection

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Two dimensional gel electrophoresis (2D-GE) employs isoelectric focusing in the first dimension and a separation of the proteins according to their molecular weight in the second dimension. The gels are then stained using silver stain to visualize the protein pattern. This method is unrivalled in terms of resolution but is a tedious and time-consuming procedure. Here we present a combination of two easy methods that separate proteins in analogy to 2D-GE according to their isoelectric point (pI) and molecular weight (kDa). For the first dimension, OFFGEL electrophoresis was used. This newly developed method takes advantage of the impressive resolving power of immobilized pH gradient gel based isoelectric focusing (IPG IEF) but in contrast to conventional isoelectric focusing delivers sample in liquid phase thus avoiding sample recovery from the gel. For the second dimension, a microfluidic high sensitivity on-chip protein sizing method was employed. This method allows separating proteins from 5 to 250 kDa and offers a sensitivity equivalent or better than silver staining and a linear dynamic range across four orders of magnitude.
Our data demonstrates that it is possible to easily detect a 1 % change in protein expression.

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P-234-T

Sensitive DNA Fragment Analysis in Cell Culture Derived Influenza Vaccines by Capillary Electrophoresis

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Influenza A and B viruses cause up to 500000 deaths p.a. around the world and leads to up to US$ 167 billion in economic costs per year only in the USA. Therefore, a highly flexible vaccine production, which allows a simple and fast up-scaling, especially in case of epidemics or pandemics is urgently needed. Currently available influenza vaccines are produced in large part in embryonated chicken eggs. Although they were shown to be an extremely useful substrate for influenza virus propagation, e.g. their limited availability and selection of subpopulations differing from the naturally occurring virus, demand alternative vaccine production systems. At Novartis Vaccines and Diagnostics the continuous and licensed cell line Madin Darby Canine Kidney cells (MDCK) is used for influenza vaccine production. To exclude any risk for the patients, concerning the content of host cell DNA an analytical method for the determination of DNA fragments in the vaccine was developed. Additionally the method also meets the requirements of the food and drug administration (FDA), demanding the determination of remaining host cell DNA for cell cult derived vaccines in contrast to those produced in eggs. So far this patent protected method, being relevant for product release, is unique and allows the fast and sensitive determination and characterization of remaining host cell DNA fragments in the vaccine, regarding amount and fragment size. It includes the concentration and purification of the DNA fragments utilizing magnetic beads technology and their separation and detection by capillary electrophoresis coupled with a laser induced fluorescence detector. Within this study the developed method is applied to cell culture derived influenza vaccines. Samples were investigated concerning their contamination with host cell DNA fragments. The presented method is also able to demonstrate that during the vaccine production process at Novartis Vaccines and Diagnostics, only small host cell DNA fragments remain in the vaccine, known to have no oncogenic effect. In comparative trials we could show that the egg derived vaccine contained a much higher load with bigger fragments on remaining host cell DNA than the analyzed cell culture derived vaccines.
Recapitulatory, a powerful, sensitive and so far unique quality control tool for influenza vaccines was established as a more sensitive alternative to existing DNA content analysis methods like hybridization or the Threshold system. Further we could show that, considering the contamination with host cell DNA, cell culture presents an alternative to egg based vaccine production processes.

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P-235-T

Comparison of CE Formats for SDS-denatured Purity Analysis of an Immunoglobulin: Standard Capillary versus Microfabricated Device

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A comparison of format and operating parameters for quantitative analysis of purity for a recombinant momoclonal antibody under denaturing conditions is described. CE-SDS using commercially available kits were evaluated in terms of typical assay validation parameters as well as analysis cost and throughput. A standard capillary system using detection intrinsic protein absorbance at 280nm was compared to a highly automated, 96-well plate based microfabricated device using fluorescence detection of a protein specific dye. Good accuracy, precision and linearity for both analysis systems were demonstrated. Differences in selectivity and sensitivity for the techniques are also discussed.

P-236-T

Identification and Authentication of Panax Ginseng and Panax Quinquefolium by Chromatographic Fingerprints with Ultra Performance Liquid Chromatography-Diode Array Detector

Y. Jane Tseng; Ching-Hua Kuo; San-Yuan Wang, *National Taiwan University, Taipei, Taiwan*

Total of one hundred and two samples of Panax ginseng and Panax quinquefolium were collected from various source and analyzed with Ultra Performance Liquid Chromatography. Acquity UPLC BEH C18 column was used in the separation. Water and acetonitrile mixture were used as mobile phase with gradient elution. The analysis was completed within 20 minutes with flow rate kept at 0.4 ml/min. All analytes were detected at 203 nm.

Chromatographic fingerprints were defined as the chromatographic patterns of chemically characteristic constituents presented in the Ginseng extracts. Chromatographic fingerprints were analyzed with principle component analysis with calibration of internal standard. Chromatograms were normalized with internal standard intensity. Chromatograms peak of 0.01 second elution time were allowed and considered same peak.

Chromatographic fingerprints were successfully classified the “sameness” and “differences” between various Panax ginseng and Panax quinquefolium samples. Eleven samples originally labeled as Panax quinquefolium by the distributor but later confirmed closer related to Panax ginseng than Panax quinquefolium by the DNA analysis were also considered having similar chromatographic patterns to Panax quinquefolium on the PCA plot. Therefore, we demonstrate here an effective method for identification, and authentication of different Panax. Same method can be applied in the future for the authentication and identification or even quality control in different samples of herbal medicine.

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P-237-T

A Rapid HPLC Method to Quantify Chlorhexidine and its Salts in Human Urine by Protein Precipitation

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Chlorhexidine is a chemical antiseptic. It is a bactericidal to both gram-positive and gram-negative microbes. Chlorhexidine and its salts, such as acetate, and gluconate provide broad antimicrobial activities. Therefore, Chlorhexidine based products have extensively been utilized in medical devices. A quick reverse phase HPLC-UV method has been developed to determine chlorhexidine in human urine. The dynamic range is from 5.0 µg/mL to 500 µg/mL with a correlation coefficient $r \geq 0.998$. Chlorhexidine is eluted within 6 min using an Agilent Eclipse XDB-CN column. To protect the analytical column, an Agilent Eclipse XDB-CN guard column and a frit are connected prior to the analytical column. The samples were prepared by protein precipitation with a recovery greater than 90%. This method has been successfully applied to the quantification of chlorhexidine and its salts in human urine and other matrices.

P-238-T

Isoelectric Focusing on the Chip: Protein Separation being Faster and Easier

Jian Wen; Jacob Albrecht; Klavs F. Jensen, *Massachusetts Institute of Technology, Cambridge, MA USA*

Disposable, inexpensive microfluidic devices have the potential to become a robust new tool for proteomic research involving difficult proteins and protein complexes. In this work, a preparative scale free flow IEF isoelectric focusing (FF-IEF) device was designed, investigated, and optimized to address the needs of molecular biologists working with samples of milligrams in mass and milliliters in volume. Here we present a triangular-shaped preparative IEF device fabricated by soft lithography in PDMS and having 24 outlets. The triangular design of the separation channel facilitates the development of the pH gradient with a corresponding increase in separation efficiency and decrease in focusing time.

The unique design of a triangular separation channel required the electric fields across the central channel to be optimized. After the shaping of the PDMS prior to the device binding, a functionalized polyacrylamide gel region at the bottom of the device was selectively controlled to adjust the applied potential across the separation channel. The length of the polyacrylamide gel was found to have a critical effect on the electric fields and could block as much as 99% of applied voltage. The device is able to achieve constant eletric fields of from 75 ± 25 V/cm to 365 ± 50 V/cm (n = 5) through the entire triangle channel when the applied voltage changed from 500 V to 1800 V, enabling a powerful micro device for different separation environments. A very reproducible pH gradient across the 24 outlets from pH 10 to pH 4 was also observed.

The devices are able to process complex biological samples, and are capable of fractionating whole cell lysate and other complex protein mixtures at rates between 0.5-2 mL/hr while providing greater separation of traditionally difficult proteins. These findings show the promise of inexpensive, disposable microfluidic FF-IEF devices in proteomics research.

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P-239-T

Expanding Isoelectric Focusing (IEF) Applications to Difficult Pharmaceutical Samples by using Whole-Column Detection Capillary Isoelectric Focusing (cIEF)

Jiaqi Wu, *Convergent Bioscience Ltd, Toronto, Ontario, Canada*

In the biopharmaceutical industry, charge heterogeneity characterization is required by the regulatory authorities for the drug products since the heterogeneity may influence the products' potency. Currently, only quantitative methods are acceptable for the characterization. Ion exchange chromatography (IEC) and capillary isoelectric focusing (cIEF) are the only two available methods for this application. However, IEC suffers the problem of variability in resolution from column to column. In addition, IEC can not be used to develop and implement an reproducible generic method. Conventional cIEF with single point detection has the problems of long method development and sample analysis time, poor resolution and poor reproducibility due to its mobilization phase.

Recently, a new cIEF technology – whole-column detection cIEF (WCD-cIEF), has been adopted by leading biopharmaceutical companies as the platform technology of choice for quantitative protein charge heterogeneity characterization. The WCD-cIEF technology eliminates the mobilization phase, thus, it eliminates all problems associated with the mobilization. Since the IEF process in its separation column is continuously monitored by the on-line, whole-column detector, focusing time and other separation parameters can be optimized easily. Sample aggregation and precipitation can be largely avoided. These advantages of WCD-cIEF greatly reduce the method development time and make it possible to analyze samples that are difficult for IEC and for conventional cIEF. Generic methods have been developed for mAbs, which are applicable up to 80% of mAbs developed in the pharmaceutical industry. This presentation will show examples of WCD-cIEF applications to proteins with extreme pI values, PEGylated proteins, heavily glycosylated proteins, protein conjugates and viruses.

P-240-T

Novel LC-MS and Gel LC-MS Platforms for the Characterization of Therapeutical Monoclonal Antibodies

Yi Wang; Cheryl Lu; Shiaw-Lin Wu; Barry L. Karger; William S. Hancock, *Northeastern University, Boston, MA USA*

Detection and characterization of therapeutical monoclonal antibodies are critical in biotech industry. Particularly, detection of glycosylation, disulfides, and other modifications of recombinant monoclonal antibodies has great quality implications in the production of these drugs. Several analysis platforms have been developed to enable selectively used for different degrees of product characterization. Such as, an on-line LC-MS platform is developed for the detection of intact, reduced, and digested monoclonal antibodies. In addition, the intact and reduced monoclonal antibodies are further analyzed by the Gel LC-MS platform to further enhance the characterization of these drug modifications in trace level. Several examples, such as the detection of disulfide scrambling, chain cleavages, and glycosylation difference, will be presented. The LC-MS platform with an additional fragmentation capability in the mass spectrometer, electron transfer dissociation (ETD), to further enhance the detailed characterization of disulfides will also be used.

NOTES:

P-241-T

Development of Chemical Methods for the Global Analysis of Protein N-Homocysteinylation

Tianzhu Indi Zang; Joshua J Klaene; Suli Liu; Dajun Chen; Ketki Dhamnaskar; Zhaohui S Zhou, *Northeastern University, Boston, MA USA*

Elevated levels of homocysteine (Hcy) can be caused by oxidative stress and methionine rich diets and has been established as an independent risk factor for cardiovascular disease and neurodegenerative disorders such as Alzheimer's disease. Homocysteine-thiolactone (Hcy TL) is a metabolite of Hcy and reacts with the amino groups in proteins to form N-homocysteinylated proteins, which comprise a major pool of Hcy in humans. Protein N-homocysteinylation has been hypothesized as a contributing factor for the cytotoxic effects of excess Hcy. Thus, there is an increasing interest in the systematic characterization of N-homocysteinylation and the discovery of related protein biomarkers. Due to the relatively low abundance of N-homocysteinylated proteins, the detection of this post-translational modification remains a major challenge in bioanalytical chemistry. Currently, most proteomic analyses heavily depend on instrumental separation and antibody binding. Considering that the modified proteins possess different chemical properties than the native proteins, protein chemistry techniques should be particularly useful in the analysis of protein modifications. We have successfully developed several new biochemical and chemical methods to selectively derivatize N-homocysteinylated proteins with various chemical tags, therefore facilitating subsequent proteomic analysis. For instance, fluorescent or biotin tagging permits quantification and global profiling, and affinity enrichment drastically reduces sample complexity.

P-242-T

Capillary Electrochromatography and Its Applications in Pharmaceutical and Biochemical Analyses

Chao Yan; Xue Gu; Yan Wang, *School of Pharmacy, Shanghai Jiaotong University, Shanghai, China*

Capillary electrochromatography (CEC) combines the best features of capillary electrophoresis (CE) and high performance liquid chromatography (HPLC): high separation efficiency of CE and the versatile selectivity and large sample capacity of HPLC. However, in practice, when CEC was used without pressure, often on a commercial CE instrument, there were problems and difficulties associated with bubbles formation and column dry-out. These problems can be solved by a pressurized CEC (pCEC) system, in which a mobile phase is driven by both a pressurized flow and an electroosmotic flow (EOF). In such a system, a pressure can be applied to the capillary column to suppress bubble formation. Quantitative sample introduction in pCEC can be easily achieved through a rotary-type injector. The EOF can either be in the same direction as, or against, the pressurized flow. Therefore, the sample elution order may be manipulated by changing the ratio of pressure to voltage. Most importantly, it is amenable for a solvent gradient mode, similar to that in HPLC, by programming the composition of mobile phone. With pCEC, the promises of CEC can be fully exploited.

We will report our recent advances in pCEC in instrumentation, column and applications. An innovative pCEC instrument was developed, which can also be used for micro-HPLC and CE. This technology, coupled with UV/Vis, MS and LIF detectors, was successfully used in more than 100 projects including studies on pharmaceutical, biological, environmental analyses, as well as pharmarcokinetics and metabolomics.

NOTES:

PLACE B&W AD HERE

Wednesday, February 4, 2009

07:30 – 17:30 *Registration on the Mezzanine Foyer*

07:30 – 08:30 *Continental Breakfast in the Imperial Ballroom*

PLENARY LECTURES in the Plaza Ballroom

CHAIR: Barry Karger, *The Barnett Institute, Northeastern University, Boston, MA USA*

08:30 - 09:15 **New Tools for Bioanalysis: The Development of Microsystems for Use in Cell and Molecular Biology**
George Whitesides, *Harvard University, Cambridge, MA USA*

PARALLEL SESSIONS in the Plaza Ballroom
~Applications of CE in the Biotech Industry~

CHAIR: Wassim Nashabeh, *Genentech, Inc., South San Francisco, CA USA*

09:20 – 09:45 **Practical Applications of High-Throughput Capillary Electrophoresis Methods**
Oscar Salas-Solano, *Genentech, Inc., South San Francisco, CA USA*

09:45 – 10:10 **Successful Applications of CE in Biotherapeutics Development**
Meg Ruesch, *Pfizer Global R&D, Chesterfield, MO USA*

10:10 – 10:35 **Capillary Electrophoresis, a Tool for Antibody-Drug Conjugate (ADC) Development**
Darren Allender, *Seattle Genetics, Seattle, WA USA*

10:35 – 11:05 *Break - **Visit the Exhibits and Posters: Imperial Ballroom & Stanbro Room***

11:05 – 12:20 ***Panel Discussion:*** **CE in the Regulated Pharmceutical Industry Over The Last Decade: The (Un) Fulfilled Promise**

12:20 – 14:00 *Lunch Break – Attendees on their own*

14:00 – 15:30 ***Poster Session #3 in the Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~Next Generation Sequencing~

CHAIR: Kevin Ulmer, *Genome Corporation, Providence RI*

09:20 – 09:45 **Looking at Genomes One Molecule at a Time: Optical Mapping and Beyond**
David C. Schwartz, *University of Wisconsin, Madison, WI USA*

09:45 – 10:10 **Single-Molecule Technology for Broadband Identification of Microbes**
Rudolf Gilmanshin, *U.S. Genomics, Woburn, MA*

10:10 – 10:35 **New Technologies in Biological Mass Spectrometry: Problems and Promises**
Lloyd Smith, *University of Wisconsin, Madison, WI USA*

10:35 – 11:05 *Break - **Visit the Exhibits and Posters: Imperial Ballroom & Stanbro Room***

11:05 – 11:30 **Needs and Solutions for Next Generation Sequencing**
Charles R. Cantor, *Sequenom, Inc., San Diego, CA*

11:30 – 11:55 **Microfabricated Integrated DNA Analysis Systems (MIDAS)**
Samantha A. Cronier, *University of California, Berkeley, CA USA*

11:55 – 12:20 **Integrated Sample Preparation for Sanger and Next Gen DNA Sequencing**
Stevan Jovanovich, *Microchip Biotechnologies, Inc., Dublin, CA USA*

12:20– 14:00 *Lunch Break – Attendees on their own*

14:00 – 15:30 ***Poster Session #3 in the Imperial Ballroom & Stanbro Room***

PARALLEL SESSION in the Plaza Ballroom
~ Heparin Contamination - Lessons Learned and Implications~

CHAIR: Ganesh Venkataraman, *Momenta Pharmaceuticals, Cambridge, MA USA*

15:30 – 15:55 **Identification and Detection of Impurities and Contaminants in Heparin Sodium**
Lucinda Buhse, *DPA/OTR/FDA, St Louis, MO USA*

15:55 – 16:20 **Heparin: Analytical Methods to Move Forward after the Contamination Crisis**
Robert J. Linhardt, *Rensselaer Polytechnic Institute, Troy, NY USA*

16:20 – 16:45 **Heparin Monographs and Standards – Current and Future Developments from the USP**
Tina S. Morris, *U.S. Pharmacopeia, Rockville, MD USA*

16:45 – 17:10 **Identification of a Contaminant in Heparin Preparations Through the Use of Orthogonal Analytical Techniques**
Zach Shriver, *Momenta Pharmaceuticals, Cambridge, MA USA*

17:10 – 18:00 **Panel Discussion: Regulating Supply Chain in the Flat World – Role of the Analytical Chemist**

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~Next Generation Sequencing 2~

CHAIR: Annelise Barron, *Stanford University, Stanford, CA USA*

15:30 – 15:55 **Honey Bees, Neanderthals, Transplant Patients, Genome Complexity, and Personal Genomes: The Creation and Future of Next Generation Sequencing**
Jonathan M. Rothberg, *The Rothberg Institute, Guilford, CT USA*

15:55 – 16:20 **Focused Clinical Sequencing**
Richard F. Selden, *Network Biosystems, Woburn, MA USA*

16:20 – 16:45 **From Personal Genomes and Enviromes to Traits**
George M. Church, *Harvard University, Cambridge, MA USA*

16:45 – 17:10 **Molecular Engineering Approaches for DNA Sequencing by Synthesis**
Jingyue Ju, *Columbia University, New York, NY USA*

17:10 – 17:35 **Helicos True Single Molecule Sequencing: Current Research and Our Path to the $1000 Genome**
Parris Wellman, *Helicos Biosciences Corporation, Cambridge, MA USA*

17:35 – 18:00 **Assembly and Validation of Next-Generation Sequencing by the Use of Optical Mapping**
Colin W. Dykes, *OpGen Inc., Madison, WI USA*

Plenary Session Three
Location: Plaza Ballroom
Chair: Barry Karger

Wed. 08:30-09:15

New Tools for Bioanalysis: The Development of Microsystems for Use in Cell and Molecular Biology

George M. Whitesides, *Harvard University, Cambridge, MA USA*

New tools enable new science. This talk will outline several types of microsystems intended for use in separations and analysis of molecules interesting in biology. It will include discussion of systems using or focused on (inter alia) closed microchannels, micropatterned paper, gels, and antibodies.

NOTES:

AM Parallel Session: Applications of CE in the Biotech Industry
Location: Plaza Ballroom
Chair: Wassim Nashabeh

09:20-09:45

Practical Applications of High-Throughput Capillary Electrophoresis Methods

Oscar Salas-Solano, *Genentech, Inc., South San Francisco, CA USA*

Since the introduction of first CE application in the routine control system for Rituxan a decade ago, CE has been a key analytical tool to support various areas of product development at Genentech, including formulation studies, process development, product characterization and validated lot release and stability testing of both commercial and clinical products. To meet the demands for even more CE applications, a strategy based on platform assay and high throughput formats has been implemented at Genentech. In this talk, various practical applications of those methodologies will be discussed along with future directions and needs for CE in the biopharmaceutical industry.

NOTES:

Wed. 09:45-10:10

Successful Applications of CE in Biotherapeutics Development

Meg Ruesch, *Pfizer Global R&D, Chesterfield, MO USA*

The biotherapeutics pipeline at Pfizer has increased dramatically over the past several years, including both monoclonal antibody (mAb) and non-mAb proteins. CE has been key in the success of a growing analytical group that supports the development of clinical and commercial formulations for a wide range of biologics product types. Over the past three years, we have moved from providing support solely using gels and HPLC methods to broadly utilizing CE for general charge and size based analysis of mAbs as well as for more unique stability indicating method needs for non-mAb proteins. In this talk, project case studies will be discussed. The associated successes and challenges of the application of CE in biotherapeutics development at Pfizer will be discussed.

NOTES:

Wed. 10:10-10:35

Capillary Electrophoresis, a Tool for Antibody-Drug Conjugate (ADC) Development

Darren E. Allender; Phillip Jones; Mary Wallace; Claudia Jochheim, *Seattle Genetics, Bothell, WA USA*

Antibody Drug Conjugates (ADC) are an emerging therapeutic platform. While some antibodies are capable of killing cells on their own, others are not effective or minimally effective unless conjugated with a highly potent drug. In the latter cases, the antibody acts as a targeted delivery vehicle. With Seattle Genetics' proprietary ADC technology, internalizing monoclonal antibodies are linked to cell-killing drugs, resulting in highly potent agents. The novel drug-linkers are stable in the bloodstream, but release drug payloads once bound and internalized by the target cancer cells. Coupling an auristatin drug linker to the cysteines that comprise the interchain disulfides of a mAb creates an ADC population with zero, two, four, and eight drugs per antibody (E0, E2, E4, or E8, respectively). During process development, highly resolving and quantitative capillary electrophoretic methods are being employed in place of more labor intensive and non-quantitative gel electrophoretic assays to ensure consistency of the mAb production and the conjugation process. Methods that can be used for quality control and stability testing of ADCs will be discussed.

NOTES:

AM Parallel Session: Next Generation Sequencing
Location: Arlington/Berkeley/Clarendon
Chair: Kevin Ulmer

Wed. 09:20-09:45

Looking at Genomes One Molecule at a Time: Optical Mapping and Beyond

David C. Schwartz; Shiguo Zhou; Steven Goldstein; Konstantinos Potamousis; Brian Teague; Jill Herschleb; Mohana Ray; Timothy Schramm; Kristy Kounovsky, *The University of Wisconsin-Madison, Madison, WI USA*

Technological advances broaden our understanding of genomes, and new approaches employing single molecule analytes offer unique advantages for the discovery and characterization of genomic alterations complementing discernment of single nucleotide polymorphisms (SNPs) and copy number variants (CNVs). CNVs commonly represent genomic events, such as amplifications and deletions usually found by DNA hybridization to either chip-synthesized oligonucleotide, or spotted arrays. Although such measures of genomic alteration are relatively comprehensive and have analyzed small populations, CNVs effectively flag broad classes of genomic alterations, but do not readily discern genomic structural alterations embodied as translocations, gene-fusion events, insertions, or rearrangements--both large and small scale (sub-genic). Consequently, physical mapping by fluorescence in situ hybridization or PCR laboriously characterizes a subset of findings providing structural detail on a per CNP basis. The optical mapping system, we developed at the University of Wisconsin-Madison, exploits the detection range afforded by restriction fragment length polymorphism analysis, but with high throughput and single-DNA molecule precision engendered by automated fluorescence microscopy. Optical mapping enables the construction of genome-wide physical maps (consensus maps) from ensembles of ordered, single-DNA molecule restriction maps developed from genomic sources, obviating clone libraries, PCR and hybridization. Comparison of the consensus map against a reference map reveals structural alterations as “differences,” in the form of novel restriction sites (missing or extra cuts; MCs or ECs) or indels (insertions or deletions), which are statistically assessed, in part, based on the number of single-DNA molecule optical maps collectively represented by the consensus map. Since high-resolution restriction maps intrinsically reveal genome structure, elusive differences such as indels are discoverable and physically characterized.

In this talk, I will present our single molecule platforms and their applications to a broad range of biological problems, both current and future.

NOTES:

Wed. 09:45-10:10

Single-Molecule Technology for Broadband Identification of Microbes

Rudolf Gilmanshin; Gene Malkin; Eric J. White; Nirupama Chennagiri; Sergey V. Fridrikh; Peiming Huang; Jess J. Shen; Douglas B. Cameron, *U.S. Genomics, Woburn, MA USA*

U.S. Genomics has developed an automated system to detect and identify pathogens, based on Direct Linear Analysis™. This proprietary technology employs isolation of genomic DNA, its digestion with restriction endonucleases, site-specific labeling with fluorescent tags, and detection of single DNA fragments. Only long (100-350 kb) DNA fragments are used for detection. The tags recognize short 6-8 bp sequences, so the measured fragments carry ~10-30 tags. Automated sample preparation system requires 3.5-4.5 hours to produce a DNA sample of the injected bacteria.
We detect single molecule traces, identify and group traces of the same DNA fragments, and combine them to produce the maps generated by the hybridized tags. The maps are patterns of fluorescence peaks of different intensity at the tags positions. The intensity variation is caused by unresolved tags and by tags bound to the mutated sites. Long fragments are chosen so a single copy carries enough information to discriminate it from the other fragments; some fragments are sufficiently unique to identify the host genome at the species level. Therefore, this approach can be applied to mixtures of microbes. No target-specific reagents are required; a single reagent set, including the restriction enzyme and tag, is used to process all samples. Evaluation of the technology with multiple bacteria demonstrated that it can discriminate not only between different microorganisms, but also between strains of a microbe as conservative as Staphylococcus aureus. Redundancy of the map information allows detection even of microorganisms that have mutated. Identification of a microbe generally requires a priori knowledge of its DNA maps. These maps can be obtained either theoretically by known genomic sequences, or experimentally by measuring a homogeneous microbial sample. Using pattern classification algorithms to identify commonality in DNA maps, the detection of unknown but genetically related microbes is also possible.

NOTES:

Wed. 10:10-10:35

New Technologies in Biological Mass Spectrometry: Problems and Promises

Lloyd M. Smith, *University of Wisconsin-Madison, Madison, WI USA*

Biological mass spectrometry is simultaneously amazingly powerful by virtue of what it CAN do, and amazingly limited by virtue of what it CAN'T do. Its main area of strength is in protein identification; well-developed tools exist that permit the identification of proteins using a now-standard sequence of tryptic digestion to peptides, chromatographic separation, tandem MS, and database searching. Various isotopic tagging strategies permit relative quantification of the proteins, and both the qualitative and quantitive variants of this approach have become relatively routine workhorses in biology.

Equally or more impressive are the important questions in proteomics that cannot be answered today. Here is one example: determine the nature of the protein variation that exists for a protein or proteins of interest – what variants are there (post-translational modifications; splice variants; alternative codon usage), in what amounts, and how do they change in response to a stimulus? This apparently straightforward query of a biological system is not generally possible to answer today. A second example: given a moderate complexity mixture of proteins varying in concentration over some several orders of magnitude, determine their identies and concentrations. Again, this problem, often referred to as the "dynamic range" problem of biological MS, is not a problem that is possible to address very effectively using today's technology, yet its resolution is critical to being able to provide the information needed to bring MS technology to the clinic.

The reasons for these limitations span all aspects of mass spectrometry: separations technologies, ionization strategies, mass analyzer designs, and ion detection approaches. Investment is needed in all of these areas to bring the enormous but largely still-latent promise of biological mass spectrometry to bear upon the critical unmet needs in research and medicine facing the world today.

NOTES:

Wed. 11:05-11:30

Needs and Solutions for Next Generation Sequencing.

Charles R. Cantor, *Sequenom, Inc., San Diego, CA USA*

As DNA sequencing becomes progressively cheaper a variety of commercially valuable applications become feasible. However the needs of these applications are somewhat different, and this must be borne in mind when evaluating and developing potential new approaches. Some applications require whole genome assembled sequence; some require just whole genome sequence tag information; some require a focus on much more limited targets, and some, the hardest of all, require characterizing the properties of mixtures of different genomes.

I will use some of the existing second generation methods, as well as those that SEQUENOM currently focuses on, mass spectrometry and nanopores, to illustrate a number of these issues. One major consideration is how precisely must one characterize the quantitative abundance of individual types of sequence fragments. Many commercially attractive applications like analysis of gene expression, epigenetics, analysis of somatic mutations in mixed tissue samples, and metagenomics all ask questions primarily about quantitative abundance while reconstructing genomic sequence is largely qualitative, save for copy number polymorphisms. The cost advantage of current second generation sequencing methods all depends strongly on the fraction of the genome of interest and the extent to which quantitative measurements are needed. The current advantage of mass spectrometry is the high intrinsic quantitative accuracy of abundance measurements. The major potential advantage of nanopore approaches appears to be great flexibility in the ways they can be implemented and the kinds of non-standard questions they can approach.

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Wed. 11:30-11:55

Microfabricated Integrated DNA Analysis Systems (MIDAS)

Samantha A Cronier; Palani Kumaresan; Nadia Del Bueno; Richard Mathies, *The University of California, Berkeley, Berkeley, CA USA*

Our goal is to develop a bioprocessor that integrates all the steps of Sanger DNA sequencing on a microfabricated device operating at the attomoles-of-template scale. In previous work using microfabricated devices, we demonstrated Sanger extension reactions in integrated thermal cycling reactors, followed by capillary electrophoresis (CE) sequencing from only one femtomole of DNA template [1]. To further improve this sensitivity, we developed an affinity capture CE injector that utilizes a photopolymerized capture matrix for inline purification and injection that replaces the traditional inefficient cross injector. The inline injection is nearly 100% efficient, allowing long, high quality reads from only 100-attomoles of DNA template [2]. We are developing a fully integrated hybrid glass-polydimethylsiloxane (PDMS) microdevice comprising a 200-nL thermal cycling chamber connected to a purification/capture region that is inline with an 18-cm electrophoresis microchannel. Following thermal cycling, the reaction products are hydrodynamically pumped out of the chamber [3], and electrokinetically driven through a crosslinked polyacrylamide gel containing capture oligonucleotides that hybridize to a universal sequence in the product. The purified and concentrated extension fragments are then thermally released from the gel for inline electrophoretic injection into the separation channel. Preliminary work with this system has demonstrated sequencing from half the amount of template compared to what was required in our previous cross injector-based device. With this level of performance, it should now be possible to sequence PCR products amplified from single molecules or single cells using the Single Copy Genetic Analysis (SCGA) methods recently introduced by Kumaresan et al. [4].

[1] Blazej, R.G. et al. PNAS 2006, 103, 7240-7245.

[2] Blazej, R.G. et al. Analytical Chemistry 2007, 79, 4499-4506.

[3] Grover, W. H., et al. Sens. Actuator B-Chem 2003, 89, 315–323.

[4] Kumaresan, P. et al. Analytical Chemistry 2008, 80, 3522-3529.

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Wed. 11:55-12:20

Integrated Sample Preparation for Sanger and Next Gen DNA Sequencing.

Stevan Jovanovich, *Microchip Biotechnologies Inc, Dublin, CA USA*

Microchip Biotechnologies Inc. (MBI) is developing sample preparation systems for both Sanger sequencing and Next Generation sequencing. For Sanger sequencing, MBI has developed the Apollo 100 SystemTM which integrates advanced microfluidic chips with a standard laboratory robot to perform Sanger sequencing reactions. The Apollo 100 System automates both dye-terminator cycle sequencing reactions at the sub-microliter scale and subsequent clean-up in microfluidic chips. This significantly reduces the cost of Sanger sequencing by decreasing the use of expensive reagents, minimizing 'hands on' time, and the potential for human errors.

For the Apollo 100 System, input DNA samples, either plasmid or PCR products, are loaded robotically from a standard 96 well plate into four 24-channel microchips. The pneumatically activated Microscale On-chip Valves (MOVeTM) and micropumps distribute, mix, and move the fluids and beads to perform cycle sequencing reactions and clean-up processes using standard chemistries. Output processed 'Ready-to-Inject' TM samples are transferred robotically to a microtiter plate ready for analysis in an AB capillary DNA sequencer. The microchips are then automatically reconditioned and can be reused 50 times. The Apollo 100 System can process a 96 well plate every 3.5 hours and substantially reduce the cost of Sanger sequencing in Core Laboratories. The basic operation of the Apollo 100 System is described, as is the function of the MOVe microchips and software system that runs the protocols. Performance data for PCR and plasmid templates are equivalent to full volume reactions for readlength and signal-to-noise. Protocol optimization, input sample concentrations, and system validation data are presented.

MBI is applying its unique ability to integrate workflows to cost effectively prepare samples for next-generation sequencers. Starting from DNA samples, MBI and its collaborators have been developing devices for bead-based library construction and amplification that are applicable to low cost de novo next generation sequencing.

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PM Parallel Session: Heparin Contamination – Lessons Learned and Implications
Location: Plaza Ballroom
Chair: Ganesh Venkataraman

Wed. 15:30-15:55

Identification and Detection of Impurities and Contaminants in Heparin Sodium

Lucinda Buhse, *DPA/OTR/FDA, St Louis, MO USA*

In January 2008, CDC and FDA began receiving reports of adverse events with heparin sodium causing a recall from Baxter Healthcare of heparin products. In early March, the agency provided two methods (NMR and capillary electrophoresis) that could be used to screen active pharmaceutical ingredients (APIs). Later in March, oversulfated chondroitin sulfate (OSCS) was identified as a contaminant in heparin sodium. High levels of dermatan sulfate, a natural glucosaminoglycan, were also present in some heparin sodium samples. The analytical methods and data used for identification of this impurity and contaminant will be presented along with new methods for rapid screening of APIs and detection of low levels of oversulfated contaminants.

NOTES:

Wed. 15:55-16:20

Analytical Methods to Move Forward after the Contamination Crisis

Robert J. Linhardt, *Rensselaer Polytechnic Institute, Troy, NY USA*

Heparin is a mixture of linear acidic polysaccharides with anticoagulant activities. Heparin is one of the oldest drugs still in widespread clinical use, a natural product, one of the first biopolymeric drugs, and one of the few carbohydrate drugs. Heparin is prepared in ton quantities from the mast cell-rich tissues, lung and intestine. Recently, certain batches of heparin have been associated with anaphylactoid-type reactions, some leading to hypotension and death. These reactions were traced to a contamination with a semi-synthetic oversulfated chondroitin sulfate (OSCS). Analytical methods used to identify OSCS are described. OSCS is prepared by chemical sulfonation of the structurally related chondroitin sulfate. OSCS exerts a profound effect on Factor XIIa resulting in enhanced bradykinin production leading to hypotension. Dermatan sulfate, a natural polysaccharide impurity with no known toxicity was also present in contaminated heparin. OSCS also carried over into low molecular weight heparins. The suspicious origin of the semi-synthetic OSCS has led pharmaceutical scientists to examine dozens of natural and synthetic heparinoids as potential heparin contaminants. Effective assays, to monitor the quality of heparin, for both known and unknown contaminants are described.

NOTES:

Wed. 16:20-16:45

Heparin Monographs and Standards – Current and Future Developments from the USP

Tina S. Morris, *U.S. Pharmacopeia, Rockville, MD USA*

In response to the public health crisis caused by the adulteration of unfractionated heparin, the USP has conducted a comprehensive modernization of both the documentary standards and reference materials relevant to this class of products. The presentation will describe the two-staged approach that was applied to the revision process, the scientific rationale driving the new tests and specifications that have been proposed and discuss additional paths forward for related products like low-molecular weight heparins. Special attention will be paid to the selection and evaluation of different separation methods that have been proposed as identity and purity tests for heparin.

NOTES:

Wed. 16:45-17:10

Identification of a Contaminant In Heparin Preparations Through the Use of Orthogonal Analytical Techniques

Zachary H. Shriver; Ram Sasisekharan, *MIT, Cambridge, MA USA*

Recently, certain lots of heparin have been noted to contain a contaminant previously unobserved in heparin preparations. Screening methods introduced by the FDA, using capillary electrophoresis and one-dimensional NMR, were effectively able to screen heparin preparations for the presence of this contaminant and allow for the removal of contaminated heparin from the supply chain. This talk outlines the detailed structural elucidation of the contaminant which serves to support these screening methods and suggest other methods that might be employed to ensure the future safety of the heparin supply chain. Detailed structural analysis, using multiple orthogonal analytical techniques, determined that the contaminant was oversulfated chondroitin sulfate (OSCS), containing a disaccharide repeat unit of glucuronic acid linked 1 3 to a -N-acetylgalactosamine. OSCS possesses an unusual sulfation pattern, where the 2-O and 3-O positions of the glucuronic acid as well as at the 4-O and 6-O positions of the galactosamine are sulfated. In addition to the development of analytical methods to detect and quantify the amount of OSCS, if any, present in heparin preparations, biological characterization of the contaminant suggests a simple bioassay that can screen for activity of OSCS. Taken together, these assays can act as "filters", effectively characterizing heparin preparations and providing additional assurance of the absence of potential contamination.

NOTES:

PM Parallel Session: Next Generation Sequencing 2
Location: Arlington/Berkeley/Clarendon
Chair: Annelise Barron

Wed. 15:30-15:55

Honey Bees, Neanderthals, Transplant Patients, Genome Complexity, and Personal Genomes: The Creation and Future of Next Generation Sequencing

Jonathan M. Rothberg, *The Rothberg Institute, guilford, CT USA*

Moore's law allowed 40 years of predictability in microelectronics. Moving sequencing to a chip, not through the miniaturization of Sanger sequencing, but by solid-phase massively-parallel sequencing has profoundly changed life science research. 454-Sequencing by being first to eliminate bacterial cloning, first to sequence in the miniature, and first to market, enabled this revolution and created the field of "next-gen" sequencing. What's next? 454 sequencing, was the first of the "next-gen" technologies, and has had a profound impact on the life sciences, including the first sequencing of an individual human genome (James Watson), the discovery of the virus leading to the disappearance of the Honey bees, the first elucidation of a virus causing human mortality through massive sequencing, and the decoding of the Neanderthal Genome. In this talk I will discuss this work, performed under my leadership at 454 life sciences, and a re-thinking of sequencing to enable personal genome sequencing and unleash the promise of personal medicine.

NOTES:

Wed. 15:55-16:20

Focused Clinical Sequencing

Richard F Selden; Eugene Tan; Greg Kellogg, *Network Biosystems, Woburn, MA USA*

The ability to rapidly generate DNA sequence data from patient samples at the point of care has the potential to change the treatment of a wide range of diseases, including bacterial, viral, and fungal infections and certain forms of cancer, and to play a role in a variety of pharmacogenomic applications. “Focused Clinical Sequencing”, defined as the real-time sequencing of a small number of clinically relevant human or pathogen genes, will allow the physician to make rapid clinical decisions based on real-time DNA sequence data. Genebench-Dx™ is a benchtop system designed to perform focused clinical sequencing in the hospital laboratory and at the point-of-care. Based on microfluidic principles, the system consists of three components. The Smart Cartridge (SC) will accept a clinical sample (e.g. blood, swabs, tissue) and extract and purify DNA in 20 µl volumes. The Integrated Biochip (IB) will perform highly multiplexed amplification, Sanger sequencing, and electrophoretic separation and laser-induced detection. The fully integrated instrument will accept the SC and IB and performs all required process steps. Our work is intended to challenge the current clinical paradigm by providing a sequencing system that will meet the following objectives: • Time to answer. Sequence data will be provided one hour following sample introduction, allowing prompt initiation of specific treatment to improve patient outcomes. • Ease-of-use. Operator functions will be limited to inserting sample into a tube and pressing a start button. • Sensitivity and specificity. With respect to pathogen sequencing, the system will have a limit of detection of 1-10 cfu/sample. Generation of sequence data from 3-10 loci per pathogen will minimize false positives. • Closed system. The system will be self-contained to prevent contamination and errors in sample handling. • Site of Operation. The instrument will be ruggedized to a military standard to allow routine transport. Integration of processing steps is currently in progress, and data demonstrating the functionality of individual steps (DNA purification, amplification, Sanger sequencing, and separation and detection) in a number of model systems will be presented.

NOTES:

Wed. 16:20-16:45

From Personal Genomes and Enviromes to Traits

George M. Church, *Harvard University, Cambridge, MA USA*

Relative to a reference human genome, a personal genome shows about 10,000 DNA variations which affect protein structure and 3 million which do not. While association studies of common DNA variants with diseases mostly yield, so far, weak predictive power and few causative alleles, most researchers expect that this will be soon remedied by genome-wide sequencing and aggregating alleles by system functions. Second-generation sequencing (e.g. Polonator.org -- open-source hardware, software, wetware) has brought costs down by over 10-fold per year for 4 years (from $100M to $5K), but this needs to improve further in cost and interpretability – e.g. by targeted sequencing of coding variants (~1% of the genome) plus analysis of regulatory variants via RNA quantitation by sequencing, and environmental components via microbiome and VDJ-ome. Haplotypes and allele specific expression should help establish causative links and improve association studies. PersonalGenomes.org is a unique effort to integrate personal genomes with comprehensive sets of medical and non-medical traits and environmental measures and share these in an open-access format. To assess personal variation in RNAs in a broad set of cell-types, we establish pluripotent stem cells from skin. We have IRB approval to expand our current cohort to 100,000 volunteers.

NOTES:

Wed. 16:45-17:10

Molecular Engineering Approaches for DNA Sequencing by Synthesis

Jingyue Ju, *Columbia University, New York, NY USA*

DNA sequencing by synthesis (SBS) on a solid surface during polymerase reaction offers a new paradigm to decipher DNA sequences. We are pursuing the research and development of this novel DNA sequencing system using molecular engineering approaches. In one approach, four nucleotides (A, C, G, T) are modified as reversible terminators by attaching a cleavable fluorophore to the base and capping the 3'-OH group with a small reversible moiety so that they are still recognized by DNA polymerase as substrates. DNA templates consisting of homopolymer regions were accurately sequenced by using these new molecules on a DNA chip and a 4-color fluorescent scanner. This general strategy to rationally design cleavable fluorescent nucleotide reversible terminators for DNA sequencing by synthesis is the basis for a newly developed, next generation DNA sequencer that has already found wide applications in genome biology. In another approach, we have solved the homopolymer sequencing problem in pyrosequencing by using non-fluorescent nucleotide reversible terminators (NRT). Finally, we have developed a new SBS approach using these NRTs in combination with the four cleavable fluorophore labeled dideoxynucleotides. DNA sequences are determined by the unique fluorescence emission of each fluorophore on the ddNTPs. Upon removing the 3'-OH capping group on the dNTPs and the fluorophore from the ddNTPs, the polymerase reaction reinitiates and the DNA sequence are continuously determined. Various DNA templates, including those with homopolymer regions were accurately sequenced by using this hybrid SBS method on a chip and with a four-color fluorescent scanner.

NOTES:

Wed. 17:10-17:35

Helicos True Single Molecule Sequencing: Current Research and Our Path to the $1000 Genome

Parris Wellman, *Helicos Biosciences Corporation, Cambridge, MA USA*

Helicos has developed a novel genetic analysis platform to efficiently and accurately determine the direct sequence of individual DNA molecules. Simplicity in sample preparation, development of novel surfaces, chemistry to enable incorporation of single nucleotides into DNA strands and finally the visualization of fluorophore addition to monitor real time sequencing by synthesis has been achieved on billions of molecules in parallel. Synthesis is directed in micron scale channels in a multi-channel flow cell through the sequential addition of the appropriate reagents. This platform will provide the opportunity for researchers to interrogate the genome on a new scale and provides a path to sequence individual genomes at a cost to make integration of genome knowledge and healthcare possible. To demonstrate the current power of the HelicosTM platform we will discuss the use of the True Single Molecule Sequencing Technology (tSMSTM) for candidate gene resequencing, RNA measurements and other analyses.

NOTES:

Wed. 17:35-18:00

Assembly and Validation of Next-Generation Sequencing by the Use of Optical Mapping

Colin W. Dykes[1]; Buffy L. Stahl[1]; Timothy J. Durfee[2]

[1]OpGen Inc., Madison, WI; [2]DNAstar, Madison, WI USA

Next-Generation technologies have advanced whole genome sequencing with speed and lower costs compared to Sanger, but produce single sequence reads that are very short. Paired-end sequencing can produce longer reads but at higher costs. The combination of single and paired-end sequencing is now being utilized to sequence entire bacterial genomes. However, finishing the genomes has been problematic due to the complexity of sequence assembly using data from Next-Generation sequencing, making genome closing an expensive and tedious process. Currently there is no method of confirming a finished genome other than to use the sequence data that produced it. Optical Mapping provides validation of a finished genome by utilizing a technology that is completely independent of sequencing. Data will be presented that utilizes short single reads and longer paired end reads along with whole genome ordered restriction maps that together can aid the finishing process of whole genome sequencing. Optical mapping can identify mis-assemblies, order contigs, locate gaps, as well as highlight inversions and repeated regions that may otherwise never be known. The implication of new sequencing technology can truly aid the field of genomics as entire bacterial genomes can be sequenced and finished quickly with lower costs when Optical Mapping is utilized to relieve the problems of genome assembly and validation.

NOTES:

POSTER SESSION ABSTRACTS
Wednesday, February 4, 2009
14:00 – 15:30
Imperial Ballroom and Stanbro Room

Applications 3: Systems Biology, Single Cell and Biomarker

P-301-W

Development of Reliable MRM Assays for Protein Quantification Using LC-MSE Information

Catalin E. Doneanu; Weibin Chen; Asish Chakraborty; Scott Geromanos; John Gebler, *Waters Corporation, Milford, MA USA*

Forty serum proteins from a Top20 depleted human serum digest were measured using two peptides per protein and two transitions per peptide for a total of 160 MRM transitions. The distribution of MRM ions monitored ranged from m/z 400 to1000 for precursors, and from m/z 500 to 1,400 for fragments. All 160 MRM transitions derived from the alternate scanning LC/MS data produced very strong MRM signals, confirmed by two transitions for each peptide, demonstrating a success rate of 100% for our experimentally-based strategy for deriving MRMs. Previous attempts to generate MRMs for serum proteins, based on combining in silico prediction tools with experimentally derived MRMs, were not as successful [1]. In that case, 53 out of 62 proteins were successfully measured, but half of the predicted peptides were rejected following the initial MRM assay.
Peptide retention times were found by running a "scouting" MRM method, when no retention time windows were employed, and all peptides were measured simultaneously using a very short (5 ms) dwell time. The MRM assay were performed using the same gradient conditions employed for the "scouting" run, but using a dwell time of 25 ms instead of 5 ms. The average number of data-points for a 6-7 wide peptide peak (at half-height) was around 15. Reproducible retention times with retention time coefficients of variation (CV's) of 0.2% or better for 90% of MRM transitions were achieved. More than 70% of MRMs had peak area CV's less than 15%, correlating well with previously reported CV's obtained when serum proteins were measured after immuno-depletion [1]. The results indicate that the matrix interference due to ion suppression is not a significant problem when using 30 min LC gradients.
1. Anderson L, Hunter CL. Mol. Cell. Proteomics 2006, 5, 573.

P-302-W

Temperature Effects in Boronic Acid Lectin Affinity Chromatography (BLAC)

Marcell Olajos[1]; Alexandre Monzo[1]; Péter Hajós[2]; András Guttman[1]

[1]Horvath Laboratory of Bioseparation Sciences, Innsbruck, Austria; [2]Dept. of Analytical Chemistry, Univ. of Pannonia, Veszprem, Hungary

Glycosylation is one of the most thoroughly studied co- and post-translational modifications of proteins. Investigation of the human serum glycoproteome, especially focusing on glycosylation changes due to various diseases, is of great recent interest, in particular respect of biomarker discovery. Selective glycoprotein enrichment is a crucial issue in the analysis of such complex biological samples as human serum. Automated boronic acid lectin affinity chromatography (BLAC) is an excellent tool to isolate and

enrich various classes of human serum glycoproteins for downstream processing such as N-linked glycan profiling by capillary gel electrophoresis. In this presentation, the temperature dependency of glycoaffinity enrichment will be discussed through a wide range of 5°C - 70°C. Wheat germ agglutinin (WGA) and boronic acid beads were used in the study with standards proteins of trypsin inhibitor (boronic acid specificity), ribonuclease B (WGA specificity) and myoglobin (negative control). Glycoprotein enrichment level was determined microLC analysis at the different temperatures. Our results suggested ambient temperature to be the best for BLAC enrichment of human serum glycoprotins. At higher temperature values non-specific interactions prevailed confirmed by the large amount of myoglobin in the eluate. At low temperatures, on the other hand, significant glycoaffinity loss was observed, probably due to the kinetically controlled elution step.

NOTES:

P-303-W

Development of Reproducible Proteomic Platform for Analysis of Laser Capture Microdissected Samples

Dipak A. Thakur[1]; Tomas Rejtar[1]; Quanzhou Luo[1]; Buffie Clodfelder-Miller[3]; Dennis Sgroi[2]; Barry Karger[1]

[1]Northeastern university, Boston, MA; [2]Molecular Pathology Unit, Mass. General Hospital, Charlestown, MA; [3]University of Alabama, Birmingham, AL

Advances in cell collection using laser microdissection (LCM) as well as advances in protein extraction, purification and identification have made microproteomics a reality. The aim of this study was to develop a reproducible proteomic platform to maximize the number of identified proteins from a small number of cells collected by LCM. In order to improve on current sample preparation methodologies, we have evaluated multiple parameters commonly used in sample preparation techniques in microproteomics including cell lysis, protein extraction, and separation. A set of model samples extracted from mouse liver tissue (5000 cells each) using Molecular Devices LCM instrument was employed to evaluate individual steps in the sample preparation. The collected cells were lysed using different buffers with and without the use of heating. The extracted proteins were next separated using SDS-PAGE with short or full-length gel separation. The gel lanes were cut into three sections (high, middle and low molecular weight), and proteins were digested using trypsin following an in-gel digestion protocol. Finally, the peptides were separated using 1D or 2D nano HPLC and/or using ultra-narrow PLOT columns [1] with a linear ion trap instrument. Variability in terms of the number of identified proteins as well as in the percentage of contaminants was evaluated and compared. The influence of various sample preparation steps on the total number of identified proteins as well as the summary of the results will be presented in this poster.

1) Luo, Q.; Yue, G.; Valaskovic, G. A.; Gu, Y.; Wu, S.-L.; Karger, B. L.
On-Line 1D and 2D Porous Layer Open Tubular/LC-ESI-MS Using 10-m-i.d. Poly(styrene-divinylbenzene) Columns for Ultrasensitive Proteomic Analysis Anal. Chem. 2007, 6174-6181.

P-304-W

An Accurate and Highly Sensitive Multiplex Gene Expression Analysis Method for Breast Cancer Biomarker Validation

Jeff Chapman; Yong Wu; Kathryn E. Sciabica; Handy Yowanto, *Beckman Coulter, Inc, Fullerton, CA USA*

Biomarker studies and signature validation panels often involve 20 or more genes. A multiplex panel, containing 21 genes of interest and 3 reference genes, was developed for quantitative validation of candidate breast cancer biomarkers. The target genes encode proteins that are involved in cell cycle, differentiation, apoptosis, DNA repair, angiogenesis and modulation of extracellular matrices and are also associated with breast tumor progression. Conventional approaches do not always accurately measure small changes in gene expression, therefore the significance of these subtle changes during tumor progression are poorly understood. Here we demonstrate a quantitative, multiplexed capillary electrophoresis assay to precisely measure these small changes in gene expression which may have potential biological relevance. Ten 0.5-fold increments in RNA concentration were accurately quantified for all 24 genes in this multiplex assay. An average correlation coefficient (R2) of >0.99 was determined between the amount of total RNA and the gene expression quantitation value for each gene within this panel. The standard curve approach demonstrated an average of 92% relative accuracy when microarray quality control (MAQC) RNAs were used. The ability to perform multiplex, sensitive, accurate and reproducible gene expression analysis provides researchers with a powerful tool to effectively explore subtle, yet biologically relevant changes, in cancer biomarkers.

Note: For Research Use Only; not for use in diagnostic procedures.

NOTES:

P-305-W

Modular Automated Processing System (MAPS) for Serum Processing for Biomarker Discovery

Gabriela Chirica; Dan Throckmorton; Mei Wu; Jim Brennan; Joe Schoeniger; Steve Branda; Marites Ayson,
Sandia National Laboratories, Livermore, CA USA

Microfluidic separations and mass spectrometry enable rapid and sensitive detection of potential biomarkers. Success in this approach depends upon meeting strict sample requirements: a limited number of components per sample; minimal amounts of salts, surfactants, and other contaminants; and significant enrichment of low abundance species. Current sample preparation practices involve complex, typically manual, multistep operations; these methods are too slow and too costly to adequately support comprehensive, high-throughput analysis of biological samples in clinical settings.

We have developed a novel modular automated processing system (MAPS) that enables reliable, high-throughput analysis as well as application- and sample-customized processing. This system is comprised of a set of independent modules that carry out individual sample processing functions: protein concentration, interferent removal, buffer exchange, enzymatic digestion, viral, bacterial concentration and cell lysis.

We are currently developing MAPS modules for mouse serum processing for comprehensive, high-throughput analysis of serum peptidome. The analysis of blood and its products is problematic, particularly in microfluidic devices, due to its viscosity, high salt (~150mM) and protein (~75mg/ml plasma) content. We developed a module that removes salt from 10-200 µl serum samples in a flow through format. Sandia-developed cartridges packed with inert, high capacity soft gel packing delivered rapid and very reproducible separations.

The high abundant proteins (HAP) present in serum, overshadow the signal of more informative proteins, precipitate and clog microfluidic channels. HAPs are typically removed using antibody-based resins in spin-column or HPLC format. We developed a module that immunodepletes HAP at low-pressure, low cost and with small foot print. This module was readily integrated in MAPS to enable fully automated serum processing. Ion-exchange and reverse phase modules were applied for fractionation of the desalted, HAP depleted pooled mouse serum. The fractions were digested and analyzed on a nanoLC-MS/MS system for proteins and peptides identification.

NOTES:

P-306-W

A Novel Procedure for Improving LC-MS Detection of Large Peptides with PTMs

Shujia Dai; Shiaw-Lin wu; Barry L. Karger, *Northeastern University, Boston, MA USA*

Enzymatic digestion of proteins often results to a distribution of peptide fragments with different sizes. The recovery of these peptides can be dramatically different depending on their hydrophobicity, which often causes major variation in LC-MS detection, particularly, the poor detection for peptides of large size (M.W. > 5 kDa). The detection of large peptides, however, is important because they often contain post-translation modification sites (PTMs) and contribute to high sequence coverage (ref 1-3). Here we present a novel procedure using a buffer with 4 M Guanidine HCl and 0.5% TFA solution to solubilize these large enzymatic peptide fragments prior to LC-MS analysis. Several examples, such as the detection of a sticky tetraphosphorylated peptide from β-casein, a 10 kDa peptide with two phosphorylation sites from epidermal growth factor receptor (EGFR), and a 13 kDa peptide with acetylation and phosphorylation sites from heat shock protein 27 (Hsp27), are presented. In particular, the detection of these peptides at low concentration (i.e. fmol / μL) is shown to be feasible by this approach. As a result, trace amounts of samples from immunoprecipitation of Hsp27 (HUVEC cell lysate) and EGFR (A431 cell lysate) are used by this approach to obtain valuable information for their biological function. This novel procedure enabled us to discover unknown PTM sites and obtain valuable structural information for proteins of interest.

1. S. -L. Wu, J.K. Kim, W.S. Hancock, and B.L. Karger. J. of Proteome Research 2005, 4, 1155-1170.
2. S. -L. Wu, A. Huhmer, Z.-Q. Hao, and B.L. Karger. J Proteome Res. 2007 Nov;6(11):4230-44.
3. S. Dai, Y. Jia, S.-L. Wu, J.S. Isenberg, L. A. Ridnour, R. W. Bandle, D. A. Wink, D. D. Roberts, and B. L. Karger. J Proteome Res. 2008, 7, 4384–4395.

P-307-W

A Hybrid Capillary-Microfluidic Device for the On-Column Separation and Quantification of Vesicles

Donna M. Omiatek[1]; Michael L. Heien[1]; Andrew G. Ewing[2]

[1]Pennsylvania State University, University Park, PA USA; [2]Göteborg University, Göteborg, Sweden

The primary method for neuronal communication involves the extracellular release of small molecules which are packaged in vesicles. Electrochemical measurements at single cells have previously been used to quantify the amount of redox active neurotransmitter released via exocytosis from both secretory cell lines and neurons from primary cultures. However, the question remains as to whether the total transmitter stored in vesicles corresponds to that detected upon stimulated release. The goal of this work was to develop a platform to isolate and electrochemically measure the contents of single vesicles. This study reports on a separations-based system developed to investigate sub-cellular components by capillary electrophoresis. Vesicles are isolated, and then separated on a fused-silica capillary which terminates onto a PDMS-based microfluidic device. This device provides an interface for chemical lysis of the vesicle and detection of its contents as it exits the separation capillary. Quantification of vesicular content upon lysis is achieved through end-column amperometry using carbon-fiber microelectrodes. The device was characterized using liposomes and loaded with an electroactive molecule. Then, measurements collected from numerous vesicles in the separation are plotted as quantitative distributions of neurotransmitter amount and compared to amounts reported previously from single cell release experiments.

NOTES:

P-308-W

Subcellular Analysis of D-Serine by Capillary Electrophoresis with Laser Induced Fluorescence Detection

Ting Shi[1]; Magalie Martineau[2]; Nobutoshi Ota[1]; Christine Cecala[1]; Jean-Pierre Mothet[2]; Jonathan V. Sweedler[1]

[1]University of Illinois At Urbana Champaign, Urbana, IL; [2]François Magendie Centre de Recherche INSERM, Bordeaux, France

A capillary electrophoresis method has been developed for the detection of D-Serine (D-Ser) in glia vesicles. D-ser, recently defined as a gliotransmitter, regulates synaptic function by acting on pre- and post- synaptic neuronal elements. D-Ser is synthesized from L-Ser by serine racemase and, in the brain is released exclusively by astrocytes. Understanding the molecular mechanism of the uptake and release of gliotransmitters and particularly D-Ser is important to understand the role of glia in the neuron-glia synapse. How are gliotransmitters packaged and released from astrocytes ? Are they present in astrocyte vesicles and released via a vesicular pathway ? Here we address these questions by directly testing the presence of D-Ser and other amino acids within glia vesicles. Vesicles are purified from astrocytes using magnetic beads coupled to vesicle specified antibodies. In order to determine the amino acids content in subcellular domains, a laboratory-assembled capillary electrophoresis system with laser-induced fluorescence (LIF) detection has been used. The system has been optimized for characterizing chiral amino acid pairs from chemically-complex, small-volume samples. Specifically, D/L-Ser is tagged with naphthalene-2,3-dicarboxaldehyde (CBI-D/L-Ser), which has many advantages in terms of product stability and fluorescence properties. Using a chiral selector system composed of beta-cyclodextrin (β-CD) and chiral micelles formed by sodium deoxycholate (SDC), the chiral resolution is achieve by micellar electrokinetic capillary chromatography. The limit of detection of Ser is 8×10-10 M for a 4 nanoliter injection. Our preliminary CE results suggest the presence of D-Ser, Glycine and Glutamate from purified vesicle samples. The ratio of D-Ser to L-Ser in vesicles is found to be greater than in astrocyte cell homogenate. Future work will confirm these results and examine mechanisms of packaging of D-Ser into vesicles.

P-309-W

Binding of Proteins to Model Membranes: Asymmetric Location of Anionic Phospholipids

Francisco Torrens[1]; Gloria Castellano[2]

[1]Universitat de Valencia-ICMol, Valencia, Spain; [2]Universidad Catolica de Valencia, Valencia, Spain

The role of electrostatics in the adsorption process of proteins to preformed negatively-charged (phosphatidylcholine/phosphatidylglycerol, PC/PG) and neutral (PC) small unilamellar vesicles (SUVs) is studied. The interaction is monitored, at low ionic strength, for a set of model proteins as a function of pH. The adsorption behaviour of lysozyme, myoglobin and bovine serum albumin (BSA) with pIs = 10.7, 6.9 and 5.5, respectively, with preformed SUVs is investigated, along with changes in the fluorescence emission spectrum of charged proteins, via the adsorption on SUVs. Significant adsorption of the proteins to negatively-charged SUVs is only found at pH values, where the number of positive charge moieties exceeds the number of negative charge moieties on the protein by at least 3 e.u. Negligible adsorption to SUVs composed of zwitterionic lipids is observed in the tested pH range (4-9), except for formally dianionic cardiolipin (CL). The fluorescence emission of positively-charged proteins increases after adsorption on negatively-charged SUVs. With increasing protein to phospholipid ratio, the increase in the fluorescence emission levels off and reaches a plateau; protein adsorption profiles show a similar shape. Analysis of the

data demonstrates that neutralization of the SUV charge, due to the adsorption of the positively-charged proteins, is the controlling factor in their adsorption. The reached plateau level depends on the type of protein and the pH of the incubation medium. This pH dependency can be ascribed to the mean positive charge of the protein. The effective charge of lysozyme, myoglobin and BSA (defined as the number of phosphatidylglycerol groups neutralized by one adsorbed protein molecule) is calculated from the charge differences between empty and protein-coated SUVs, using the Gouy-Chapman theory. With the Gouy-Chapman formalism, gamma is obtained as ln_gamma PROPORTIONAL_TO NU.sinh^(-1)(NU) APPROXIMATELY_EQUAL_TO NU^2. The activity coefficient goes with the square of the charge number. Thus, for an effective charge reduction by a factor of 3 (NU_eff = NU/3) as evaluated in the case of melittin, the corresponding reduction in the interaction energy is by a factor of 9. As GAMMA PROPORTIONAL_TO gamma, at constant NU it can be expected ln_GAMMA PROPORTIONAL_TO ln_gamma PROPORTIONAL_TO z_L.sinh^(-1)(z_L) APPROXIMATELY_EQUAL_TO z_L^2. It is shown the asymmetric location of the anionic phospholipid in the inner leaflet of the bilayer in mixed zwitterionic/anionic SUV for the lysozyme-PC/PG and myoglobin-PC/PG systems. This asymmetric location is in agreement with both experiments and molecular dynamics simulations.

NOTES:

P-310-W

Plasmid Purity and Heterogeneity Analysis by Capillary Electrophoresis

Cynthia L. Boardman; Hans A. Dewald, *Beckman Coulter, Inc., Fullerton, CA USA*

Plasmid DNA is often used as the gene-delivery vehicle for vaccinations and gene-therapy products. Federal regulations require purity testing for manufactured injectable plasmid products and recommend establishing a release criterion of a minimum supercoiled content of [greater than or more than] 80%. Capillary electrophoresis with LIF detection (CE-LIF) provides a rapid, sensitive, reproducible and automated method for the quantitative analysis of plasmid DNA isoforms. Advantages of CE-LIF over slab-gel electrophoresis include rapid analysis time, minimal sample handling, simpler set-up and prep time, greater sensitivity, more accurate quantitation and automated sample loading. This poster describes the development of a method for plasmid analysis by CE-LIF. The effects of gel composition and concentration on the migration behavior of the plasmid isoforms were explored and will be presented. A variety of fluorescent stains and run conditions were also evaluated, and the method was used to analyze plasmids ranging in size from 3 to 11 kilobases. A method developed for a 4.9 kilobase plasmid was found to be sensitive and reproducible and can provide baseline resolution of supercoiled, linearized and open-circular plasmid forms in less than 15 minutes.

P-311-W

Off-line Multidimensional RPxRP nanoLC for Separation and Tandem MS Detection of Complex Protein Digests

Bas Dolman; Evert-Jan Sneekes; Remco Swart, *Dionex, Amsterdam,The Netherlands*

Protein samples of biological origin are by nature highly complex, and require highly efficient separations to maximize protein identification. Although column and instrument technology have improved, the separation efficiency is still not sufficient to separate highly complex samples. More powerful separation tools are required like multidimensional liquid chromatography (MDLC). Various MDLC techniques have been described for proteomics studies, of which the combination of strong cation exchange (SCX) and reversed phase (RP) is most common for peptide profiling. An alternative for this approach is the use of ion-pair reversed phase chromatography, applying high pH eluents in the first-separation dimension and a low pH eluents in the second separation dimension.

In this study we discuss the application of this RPxRP 2D-LC method in proteomics. Automation of the method will be discussed. The performance of RPxRP is compared to SCXxRP in terms of protein identification, orthogonality, robustness and precision.

NOTES:

P-312-W

Optimization of Separation Power in One-Dimensional LC in Analysis of Proteomics Samples

Bas Dolman; Sebastiaan Eeltink; Remco Swart, *Dionex, Amsterdam, The Netherlands*

One of the main challenges in proteomics research with nanoLC-MS is to improve the quality of the separation of highly complex samples prior to MS detection. Digestion of proteins may lead to a very large number of peptides, e.g. it has been estimated that digestion of a cell lysate may produce up to 500,000 peptides.
Therefore, new column formats need to be developed e.g. increasing the column length to increase efficiency, and the LC conditions need to be adjusted accordingly. In this study the impact of the different parameters on the peak capacity, which were experimentally evaluated for the separation of peptides in nano-LC/MS/MS are described. In particular, we demonstrate the effects of gradient time and column length on the peak capacity. Finally retention time repeatability with long gradients is demonstrated.

P-313-W

Targeted Mass Spectrometry and Stable Isotope Dilution for Quantitation of Candidate Renal Cell Carcinoma Biomarkers

Agnes Rafalko[1]; William Hancock[1]; Marina Hincapie[1]; Othon Iliopoulos[2]

[1]Northeastern University, Boston, MA USA; [2]Massachusetts General Hospital, Boston, MA USA

The incidence of renal cell cancer (RCC) is increasing in the United States while novel targeted therapies present a new set of clinical questions. A reliable panel of blood RCC biomarkers of disease activity could guide therapeutic and preventive interventions and serve as surrogate end-points in clinical trials. Inactivation of the Von Hippel Lindau (VHL) tumor suppressor gene and the resulting constitutive up-regulation of hypoxia inducible factor (HIF) are hallmarks of the majority of clear cell RCC and the earliest known signal transduction defect in this cancer. We took advantage of the availability of human RCC cell lines deficient in VHL to interrogate the global gene expression changes linked to loss of VHL function as a first step in RCC biomarker discovery. We identified a number of specific genes that are upregulated by loss of VHL. We found that these genes or Cell line Derived Biomarkers (CDBs) are also up-regulated in all patient RCC tumors examined when compared to matched normal renal parenchyma obtained from the same individual. We have developed a proteomics platform to detect and validate the CDBs as circulating biomarkers of tumor activity in RCC patients before and after curative nephrectomy. The approach consists of an immunoaffinity enrichment step at the protein level followed by multiple reaction monitoring coupled with stable isotope dilution mass spectrometry (MRM/SID-MS). We demonstrated sufficient throughput, good recovery and reproducibility to enable robust detection and quantitation of the candidate biomarker proteins at the low ng/mL levels in human plasma.

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P-314-W

Characterization of Neuropeptides in the Zebra Finch Using RP-RP 2D LC and MS

Fang Xie; Suresh P. Annangudi; Andinet A. Wadhams; Sarah E. London; Xiaowen Hou; David F. Clayton; Jonathan V. Sweedler, *University of Illinois at Urbana-Champaign, Urbana, IL USA*

The zebra finch (*Taeniopygia guttata*) is an excellent model for studies underlying the evolution of vocal learning, communication and related cognitive processes. The zebra finch also holds an important place in understanding the functional significance and control of adult neurogenesis because of its well-defined circuits linked to specific behaviors. Neuropeptides, a complex set of cell-cell signaling molecules, play a vital role in many neuronal processes and so understanding the neuropeptides within the zebra finch will certainly aid many ongoing zebra finch studies. As a part of the zebra finch genome sequencing consortium, a concurrent neuropeptidomic study of zebra finch annotated neuropeptide genes annotation and aided studies on the influence of neuropeptides in this model system. Here, appropriate zebra finch brain samples are prepared via homogenization and extraction of the central nervous system (CNS) in acidified acetone. Microbore reverse-phase liquid chromatography (RP LC) is employed as the first stage separation to fractionate the complex samples. The resulting fractions are subjected to a second stage capillary RP LC; both electrospray ionization ion trap mass spectrometry (ESI-IT-MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) are used for peptide characterization. The two stage separation combined with two complementary MS techniques assures maximum coverage of neuropeptide profiles in the zebra finch central nervous system. At the same time, we created a list of known, expected, and predicted neuropeptide genes using the zebra finch EST and chicken databases; the tandem MS data has been searched using this list of putative neuropeptide genes. So far, more than 30 peptides processed from 12 prohormones have been identified and confirmed with tandem MS data. Additional unmatched spectra are being *de novo* sequenced to identify novel peptides. For some relevant peptides, in situ maps of peptide distributions are created. Knowledge of the complement of neuropeptides used by and released from specific neuronal systems increases our understanding of how complex suites of neuropeptides modulate neuronal network function to generate complex songbird behaviors.

P-315-W

RNA Size Separation by In-Capillary Denaturing Polymer Electrophoresis with 1,2,5-Thiadiazole as a RNA Denaturant

Yoshinori Yamaguchi; Keiko Sumitomo; Takashi Ogura, *ASMeW, Waseda University, Tokyo, Japan*

RNA size separation requires a strong denaturant to cleave the intramolecular hydrogen bonds that maintain the high-order structures of RNA. 1,2,5-Thiadiazole improved RNA separation dramatically in capillary polymer electrophoresis. 1,2,5-thiadiazole was synthesized as an extraction solvent substituted for a halogenated solvent such as chloroform or methylene chloride. While 1,2,5-thiadiazole is an excellent extraction solvent for both lypophilic chemical compound, 1,2,5-thiadiazole is an environmental friendly solvent.1 We suggested “in-capillary denaturing polymer electrophoresis” as the RNA separation in a small sample volume (<10 nL) that realizes the denaturing and separation simultaneously. We found that 1,2,5-thiadiazole was a strong denaturant for RNA and the RNA separation performance by in-capillary denaturing electrophoresis was dramatically improved with a running buffer containing 1,2,5-thiadiazole. Since 1,2,5-thadiazole is a neutral molecule, both conductivity and buffer pH was able to adjusted to a desirable strength for RNA separation by capillary polymer electrophoresis. In this paper, we report the resolution and number of plates of RNA separation peaks were larger than those of the RNA separation in a conventional capillary gel electrophoresis with sample preparation by 7.0 M urea and discuss the

chemical interaction dynamics between RNA molecules and 1,2,5-thiadiazole.
NOTES:

P-316-W

Characterization of Peptides in Glia: from Whole Cell Extracts to Single Cell Detection

Ping Yin; Ann M. Knolhoff; Jonathan V. Sweedler, *University of Illinois at Urbana-Champaign, Urbana, IL USA*

Neurons and glia are the two main cell types in the mammalian brain. A neuron becomes active when it receives appropriate input from other neurons across a synapse. The recent studies have demonstrated that glia, such as astrocytes, extend processes around synapses and regulate neuronal transmission. For example, stimuli that induce Ca^{2+} increases in astrocytes cause release of small molecules, such as glutamate, which contributes to the neuronal communication. Compared with the small molecules, the peptide signaling complement has not been well characterized in glia. To better understand the interactions between glia and neurons, we characterize the peptides of an astrocyte type I cell line from mouse cerebella, an enteroglial cell line from rats, and primary cultured glia from rat hippocampi using mass spectrometry. Following the whole cell extraction with boiling water and acetic acid, capillary liquid chromatography coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (CapLC-MALDI-TOF MS) is used for peptide profiling. The LC fractions are sequentially measured using both MALDI-TOF/TOF MS/MS and electrospray ionization ion trap tandem mass spectrometry (ESI-IT MS/MS) to characterize the observed peptides. To better focus on possible cell-cell signaling related peptides, Ca^{2+}-dependent secretion is studied as only a small fraction of the peptides in a cell are released after exposure. The conditioned cell media with various chemical stimuli known to cause Ca^{2+} waves in these cells is collected and then analyzed by mass spectrometry. Furthermore, individual cells are being characterized as this provides information on the cell-cell variability of peptides in heterogeneous cell populations. Our results show that astrocytes contain complex suites of peptides, several of which are released in an activity-dependent manner. Some of these peptides have been mass-matched to known neuropeptides, while many do not match, suggesting that novel peptides await discovery. Lastly, the combination of whole cell extraction and single cell characterization will provide new insights on glia-neuron networks.

P-317-W

Kinetic Capillary Electrophoresis-based Affinity Screening of Aptamer Clones

Diana Yunusov; Mandy So; Solmaz shayan; Victor Okhonin; Michael Musheev; Maxim Berezovski; Sergey Krylov, *York University, Thornhill, Canada*

DNA aptamers are single-stranded DNA molecules artificially selected from random-sequence DNA libraries for their specific binding to a certain target. DNA aptamers have a number of advantages over antibodies and promise to replace them in both diagnostic and therapeutic applications. The development of DNA aptamers involves three major stages: library enrichment, obtaining individual DNA clones, and the affinity screening of the clones. The purpose of the screening is to obtain the nucleotide sequences of aptamers and the binding parameters of their interaction with the target. Highly efficient approaches have been recently developed for the first two stages, while the third stage remained the rate-limiting one. Here, we introduce a new method for affinity screening of individual DNA aptamer clones. The proposed method amalgamates: (i) aptamer amplification by asymmetric PCR (PCR with a primer ratio different from unity), (ii) analysis of aptamer-target interaction, combining in-capillary mixing of reactants by transverse diffusion of laminar flow profiles (TDLFP) and affinity analysis using kinetic capillary electrophoresis (KCE), and (iii) sequencing of only aptamers with satisfying binding parameters. For the first time we showed that aptamer clones can be directly used in TDLFP/KCE-based affinity analysis without an additional purification step after asymmetric PCR amplification. We also demonstrated that mathematical modeling

of TDLFP-based mixing allows for the determination of Kd values for the in-capillary reaction of an aptamer and a target and that the obtained Kd values can be used for the accurate affinity ranking of aptamers. The proposed method does not require the knowledge of aptamer sequences before screening, avoids lengthy (3-5 hours) purification steps of aptamer clones, and minimizes reagent consumption to nanoliters.

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P-318-W

Glycoproteome Changes in Breast Cancer: Identification by Multi-lectin Affinity Chromatography (M-LAC) Combined with Digital ProteomeChip™ (dPC™) and Mass Spectrometry

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Protein glycosylation represents one of the major post-translational modifications and can have significant effects on protein function. Moreover, change in the carbohydrate structure is increasingly being recognized as an important modification associated with cancer. In this presentation we describe the development and application of a proteomics platform to measure changes in the serum glycoproteome of females with breast cancer. Diseased and healthy serum samples were processed by an optimized sample preparation protocol that partitions serum proteins based on glycan characteristics with the goal of identifying changes in either the concentration level and/or the carbohydrate structure of the glycoprotein(s). The method involves immunodepletion of abundant proteins, selective glycoprotein enrichment using M-LAC and fractionation by isoelectric focusing (IEF) using the digital ProteomeChip™ (dPC™). Peptide sequencing and protein identification were accomplished by LC-MS/MS and data was analyzed using the Mass Spec Results Analysis Tool™ (MSRAT™) bioinformatics software. A comparison of cancer and control samples revealed that this approach increased the information content of the analysis; both in terms of identifying changes in protein concentration of circulating glycoproteins, and detection of differences in glycoform profiles. We will present data that shows the utility of this proteomic platform for deeper mining of the serum glycoproteome and for the simultaneous profiling of glycosylation and glycoprotein abundance. In addition, we will discuss the identification of several glycoproteins that distinguish cancer from control samples and are candidates for future biomarker validation studies.

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P-319-W

Detection of Cellular Releasate from Single Neurons using a Novel Injection Apparatus and Capillary Electrophoresis with Laser-Induced Native Fluorescence Detection

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Understanding the signaling mechanisms that individual neurons use to communicate with each other is essential for elucidating the function of the neuronal network. One class of molecules used for neuron-to-neuron communication is the biogenic amines, which includes catecholamines and indolamines. A number of biogenic amines are neurotransmitters that are associated with a wide variety of higher order behaviors such as sleep, memory formation, feeding, and mood, but are low abundance analytes since they are present in localized regions of the nervous system in femtomole to attomole quantities. This prompts the use of technologies that enable single cell measurements, in order to reduce dilution of the analytes of interest and to simplify analysis. Single cell measurements also provide insight into cell-to-cell heterogeneity, as it has been demonstrated that adjacent neurons can have different complements of signaling molecules. The high sensitivity and low sample consumption of capillary electrophoresis (CE) combined with the sensitivity of laser-induced fluorescence detection (LIF) makes CE-LIF an ideal method to study single cells and even subcellular organelles; however, the isolation and loading of such small samples into the CE system is challenging. We address this issue by interfacing a single beam optical trap with a CE system that uses multi-channel laser-induced native fluorescence detection. The optical trap is formed by tightly focusing the output of a near infrared diode laser with a high numerical aperture objective. Once the cell is localized within the trap, the capillary inlet is moved adjacent to the trapped cell using a computer-controlled micromanipulator and microscope combination. The cell is pressure injected into the separation capillary; cell lysis occurs within the capillary and the cellular constituents are separated and detected. Detection takes place using multi-channel LIF which has been optimized for selective excitation and detection of biogenic amines. Briefly, a 248 nm NeCu hollow cathode ion laser is used in combination with a sheath-flow cuvette; the fluorescence emission is collected and measured using three channel detection with each photomultiplier tube having its own wavelength range selected with the appropriate dichroic mirror. This instrument allows unambiguous identification of a variety of catecholamines and indolamines based on differences in both their fluorescence emission profiles and migration times. The ability of the system to perform single cell manipulation and CE-LIF separations of analytes from individual neurons is highlighted, including the range of samples that the optical trap can accommodate and CE-LIF detector performance.

P-320-W

PDA/LIF-CE as the Central Analytical Tool in the Development of an Amine-Reactive Fluorophor

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Capillary electrophoresis with photodiode array absorbance (PDA) detection and argon ion laser-based fluorescence detection (LIF) proved to be an invaluable tool for the development of a multi-step synthetic method to obtain a tetra-substituted 3,6-diamino-9-acridinium fluorophor that carried a propylsulfonium-based tether between the fluorescent core and the amine-reactive N-hydroxysuccinimide ester group. Each step of the synthesis was monitored using, as appropriate, zone-electrophoresis, cyclodextrin-mediated zone electrophoresis, SDS-micellar electrokinetic chromatography, and isoelectric focusing. Some of the

separations were completed using pressure-mediated capillary electrophoresis (PreMCE) and yielded, very rapidly, complete impurity profiles as well as in-situ spectral characterization. Though CE is seldom used by synthetic chemists as their central analytical tool, this work demonstrates that carefully selected CE methods advantageously complement the more common HPLC methods.

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P-321-W

Automatic Dual-Color Variable-Angle Evanescence Field Microscopy (EFM)

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A new optical system for evanescent-field excitation fluorescence imaging has been constructed and evaluated. We have improved upon the variable angle EFM designs of Rohrbach (Biophys. J. 78 (5):2641-2654, 2000) and He and Yeung (J. Phys. Chem. B 109 (18):8820-8832, 2005). In the new design, the angles of the active beam deflector and the positions of the telecentric system are calibrated automatically to accomplish tens to hundreds of incident angles with a minimum angle step of 0.01°. At these incident angles, evanescent waves penetrate to different depths. A set of EFM images can be obtained by automatically scanning a large range of incident angles. Novel image reconstruction algorithms are being developed to display sectional images that are 1-10 nm thick over a range of several hundred nanometers. In addition to the improved z-resolution, the new EFM setup can utilize two lasers of different wavelengths for excitation of two types of fluorophores, and a dual-view filter for detection of two emission wavelengths simultaneously. This new system can be used to seek answers to a new set of problems at single-molecule level, such as competitive adsorption of multiple species at chromatographic surfaces and surface effects on single-enzyme activity.

P-322-W

Micro Solid Amalgam Electrodes (SAE) Array for Biomolecule Detection

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Sensitive methods amenable to automation and parallelization are needed to fullfil the growing demands in biotechnology and medicine. While separations coupled to mass spectrometry are irreplaceable especially in the discovery phases of the research, there is also need for smaller, selective and less expensive detection techniques for the screening and diagnostic purposes. At present this area is dominated by technologies based on fluorescence detection. Measurements based on direct monitoring of an electric quantity such as electrochemical methods have also shown their potential as tools for analysis of DNA, proteins and/or small molecules important in proteomics, biomedicine and, generally, in the biology related research. Recently we have shown that practically all proteins produce chronopotentiometric peak H at bare solid amalgam and hanging mercury drop electrodes at nanomolar and subnanomolar concentrations [1]. This peak, which is due to the ability of proteins to catalyze hydrogen evolution at mercury electrodes, reflects sensitively changes in the protein structure such as denaturation, and changes in redox states of peptides and proteins.
In this work we describe the preparation and characterization of simple micro SAE array for universal and/or selective biomolecule detection. The electrodes were prepared on a glass wafer substrate utilizing vacuum metal deposition and photolithography. The surface of the individual electrodes was separated by an insulating layer. Desired electrochemical activity/selectivity was achieved by proper surface modifications. The electrodes with the dimensions in the 0.1 mm2 range were characterized with respect to their size, stability and sensitivity. Due to the microfabrication methods the electrodes show excellent reproducibility allowing construction of simple disposable arrays. In comparison to the standard SAE, the

new devices provide easier and faster manipulation, consuming much less sample for comparable sensitivity.

[1]. Palecek, E; Ostatna, V. Electroanalysis 19, 2383-2403, 2007

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P-323-W

Highly Sensitive Analysis of Inorganic Ions Using Crown Ether Immobilized Quantum Dots

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A method for highly sensitive and selective analysis of inorganic ions was developed on the basis of a Förster-type excitation energy transfer between two quantum dots (QDs) with different sizes. QDs are semiconductor nanoparticles and their emission wavelengths depend on their sizes. In this study, mercaptoalkanoic 15-crown-5 ether (15C5) was introduced onto the surface of QDs with their diameters of 2.1 nm (15C5-QDs2.1) and 2.6 nm (15C5-QDs2.6) for the molecular recognition of potassium ion. When K^+ is added to a mixed suspension of 15C5-QDs2.1 and 15C5-QDs2.6, the efficiency of the excitation energy transfer will be enhanced since the recognition of K^+ provides a 1:2 sandwich-type complexation between the different sizes of QDs.
When K^+ was added to a mixed suspension of 15C5-QDs2.1 and 15C5-QDs2.6, the fluorescence intensity at longer wavelength derived from 15C5-QDs2.6 was increased, whereas that at shorter wavelength from 15C5-QDs2.1 was decreased. Upon increasing the concentration of K^+, such change became significant. Thus, the signal transduction could be achieved through the energy transfer resulting in a ratiometric change in the emission intensity. The limit of detection (LOD) of K^+ was evaluated to be 8 ppb under an optimal condition, so that highly sensitive detection of K^+ was succeeded. When Na^+ was added to the mixed suspension, the change in the fluorescence spectrum was not observed. Therefore, it was found that the 15C5-QDs was the K^+ selective fluorescent probe.
According to a similar strategy with the K^+ probe, mercaptoalkanoic 12-crown-4 ether (12C4) and 18-crown-6 ether (18C6) were immobilized onto the QDs surfaces to detect Na^+ and NH_4^+ ions, respectively. The prepared 12C4-QDs and 18C6-QDs showed selective responses toward corresponding cations. Good linear calibration curves were successfully obtained and the LODs of Na^+ and NH_4^+ ions were determined to be 2 and 11 ppb, respectively. Thus, the prepared fluorescent probes based on QDs will be applied to a simultaneous and sensitive analysis of inorganic cations, i.e., the analytes separated by electrophoresis or chromatography can be detected by post-column mixing with the QDs dispersions.

P-324-W

Development of Differential Interference Contrast Thermal Lens Microscope and Single Molecule Detection of Non-fluorescent Molecules in Liquid Phase

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We report a new detection method of single non-fluorescent molecules using Differential Interference Contrast Thermal Lens Microscope (DIC-TLM).
In micro and nanospaces, detection of single molecule is required due to small detection volumes. And it is also necessary to detect non-fluorescent molecules because most analytes have no or weak fluorescence. Therefore we developed Thermal Lens Microscope (TLM) for sensitive detection of non-fluorescent molecules. We have succeeded in detection of concentration corresponding to 0.4 molecules in 7 fL and counting of single metallic nanoparticle and λ-DNA so far. But counting of small molecule has not been achieved because its absorption cross section is two orders smaller than that of nanoparticle and conventional TLM has a problem of optical background.

Thermal lens is a refractive index change following absorption of excitation beam and thermal relaxation. And conventional TLM detects a small refraction of probe beam by thermal lens as a signal. In DIC-TLM, on the other hand, the intensity of probe beam becomes zero by polarization separation and interference using a pair of DIC prisms. And a phase contrast generated by a refractive index change between two separated probe beams is detected. As a result, background-free measurements are achieved and molecules passing the focal volume are counted as pulse signals. We fabricated new prisms because its shear value must be longer than thermal diffusion length to make high contrast.
First we verified this principle by examining background reduction, dependence on phase contrast and thermal diffusion length. Next we counted gold nanoparticles to check the performance of DIC-TLM. As a result, pulse signals proportional to the concentration was detected and their Signal-to-Noise ratio was improved 10 times than that of TLM. And finally, molecules of 5,10,15,20-Tetrakis-(2,6-dichlorophenyl)-porphyrin-Mn(III) chloride were counted. Signals were detected and verification is ongoing.

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P-325-W

Microsensors for Microchip Potentiometric Detection of NA^+, K^+ And NH_4^+ Ions

Adelina Smirnova[1]; Kazuma Mawatari[1]; Hiroko Takahashi[1]; Yoshikuni Kikutani[2]; Hiroaki Nakanishi[3]; Takehiko Kitamori[1]

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Ion-selective electrodes (ISEs) are one of the most common ion sensors employed in a wide variety of areas. Also many applications of electroanalysis require miniature electrodes due to small volumes of sample. In this research we developed capillary-based micro ISEs and integrated them on to the microchip for electrochemical detections of Na, K and NH4 ions in the fuel cell liquid after ion chromatography separation. Monolithic capillary-based ion-selective electrode was chosen as a working electrode for the determination of target ions due to its lower detection limit and significantly smaller size than conventional membrane electrodes. Besides, the size of a reference electrode should meet microchannel dimensions as well. So, we created the capillary-based micro reference Ag/AgCl electrode and applied it both as an inner reference electrode for working ISE and as a general reference electrode. Micro electrodes were introduced in to a PDMS microchip and detection of Na, K and NH4 ions was carried out with a detection limit of 20 ppb.

P-326-W

Recent Advances in UPLC for Hydrogen Exchange Mass Spectrometry: High-Speed & High-Resolution Separations at Zero Degrees Celsius

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Hydrogen/deuterium exchange mass spectrometry (HXMS) is one of the leading techniques used to probe protein conformational dynamics. However, there have been certain technical obstacles that have prevented wide-spread HXMS use. In order to maintain the deuterium label during LC/MS analyses, chromatographic separation must be done rapidly and at zero degrees Celsius. Traditional RP-HPLC generally has shown poor chromatographic performance under these conditions and thereby has been prohibitive for HXMS, particularly for larger proteins and complicated systems. Here we present a custom ultra performance liquid chromatography (UPLC) system designed to be a significant advancement in HXMS technology that should make HXMS more accessible and mainstream in the near future.

We have built and optimized a custom nanoAcquity UPLC system designed specifically for HXMS experiments. A peltier-cooled chamber housing the injection and switching valves, peptide trap, and analytical separation column was constructed and interfaced to the nanoAcquity control system. The online pepsin digestion column is housed in a heatable chamber within the cooled chamber. Single test proteins in excess of 80 kDa and a four-protein mixture in excess of 250 kDa were used to validate chromatographic performance. In each case, online digestion took less than one minute and separation of the resulting peptides was performed at 0 degrees Celsius in less than 10 minutes. Near baseline resolution was achieved with a median chromatographic peak width of ~2.7 sec at half height. Protein consumption with the UPLC system was approximately 20 fold less than with classical methodology. Deuterium recovery of heavily deuterated test peptides and peptides from partially deuterated protein digests were obtained with both the UPLC system and using conventional methodology. Test peptide

deuterium levels obtained with the UPLC system were similar to those obtained with conventional HPLC methods. Overall, this new system represents a considerable evolution in HXMS technology.

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P-327-W

A Polymer Microchip with an Integrated Microstructured Fibre for the LC-MS of Proteins

Graham T. T. Gibson; Samuel Mugo; Richard Oleschuk, *Queen's University, Kingston, Canada*

The need for small-scale separations with mass spectrometric detection in protein analysis has led to much interest in lab-on-a-chip systems with integrated separation channels and electrospray emitters. Herein we report the design, fabrication and use of a plastic microchip with a novel embedded nanoelectrospray ionization emitter.

The microchip is made from cyclic olefin copolymer (COC), with the 150 or 360 micron separation channel formed in the polymer by hot embossing. The cover plate is thermally bonded and sealed, and at the same time a capillary inlet is embedded at one end and a microstructured fibre (MSF) at the other end. MSFs, originally designed for photonics applications, feature an array of channels within a capillary format that form from heating and pulling a bundle of capillaries. In this case, the MSF is ~320 micron in diameter with an array of 168 4-5-micron channels, with 6-7 micron between channels. Such MSFs have been shown in our laboratory to behave as excellent nanoelectrospray emitters with multispray capability, so their incorporation into the chip can provide an integrated emitter to couple the LC and MS with minimal dead volume. Furthermore, the MSF can also assume the role of the frit being able to retain the 3 micron C18 or 3.5 micron C8 reversed-phase microspheres packed into the chip. Chips are able to withstand at least 600 psi (41 bar) without leaking, and are resistant to common LC solvents like water, acetonitrile and methanol. Preliminary results show that 1 picomole of peptide/proteins bradykinin, leucine enkephalin and insulin was successfully separated and detected in less than 15 minutes with a S/N ratio > 10. The LC-MS microchip presented here represents a step forward in the development of sensitive small-scale integrated protein separation and detection.

P-328-W

Development of a Robust Interface for Capillary Electrophoresis - Electrospray Ionization - Mass Spectrometry

E. Jane Maxwell; Hong Zhang; Xuefei Zhong; David D.Y. Chen, *University of British Columbia, Vancouver, Canada*

We have developed a novel interface for capillary electrophoresis - electrospray ionization - mass spectrometry. Design considerations for this interface have emphasized ease of use, and flexibility while maintaining excellent sensitivity. The unique features of this interface include the ability to modify the composition of the CE effluent with minimal sample dilution immediately prior to electrospray ionization using a precisely controlled flow of modifier solution. The composition of the modifier solution may be adjusted to optimize the stability and sensitivity of the electrospray process independent of the composition of the background electrolyte.

Another novel feature is the use of stainless steel electrospray emitters. Although this places the location of the electrical contact within the flow path of CE effluent and requires special care in managing bubble formation, it allows very precise and independent control of the CE separation and electrospray potentials, leading to increased sensitivity and reproducibility.

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P-329-W

Maximizing Performance of Sub-2 um Packed Nanobore LC Columns: Minimizing Extra-Column Variance

Amanda Berg; Gary A. Valaskovic, *New Objective, Inc., Cambridge , MA USA*

A junction-style high-voltage contact at the column inlet is a robust and facile configuration for nanoLC ESI-MS analysis. When seamlessly integrated onto the nanobore column, chromatographic performance is maintained with minimal contributions to band broadening. When the voltage is applied post-column, chromatographic performance is often compromised. Minimization–ideally, elimination–of all sources of dead volume within the LC systems must be effected. An electrically conductive, optically clear, zero-dead-volume union offers promising solutions for a post-column high-voltage junction contact. The performance of this union is compared to conventional post column high voltage connections is demonstrated by improvements in peak width and asymmetry in analyzing standards.

A 75 um ID column was slurry-packed with 1.9 um 175 Å HypersilGold™ C18 at a pressure above 5,000 psi. BSA tryptic digest peptides (Waters), 300 fmol/ul in 0.1% formic acid were loop injected (1 uL) on-column, using a 10-port valve. An elution gradient (25 minute) was delivered at 250 nL/min. by a direct flow pump (Eksigent). An uncoated SilicaTip™ (New Objective), 360 um OD x 20 um ID, with a 10 um ID tip was connected to the column via a variety of contact methods. Full-scan MS data was collected on a LCQ™ (Thermo) using a nanospray source.

All of the post-column, high voltage connection devices tested yielded stable spray throughout the gradient. Minimizing or eliminating post-column volume is a key consideration in the nanobore LC flow path. Maintaining a post-column flow path having an ID less than or equal to the column ID is critical. A PicoClear Conductive Union maintained optimal peak shape with a 75 um ID column packed with sub-2 um particles. The increase in peak area will be explored for quantitative peptide analysis in determining LOD, LOQ, and linearity.

P-330-W

Inline Electrospray Current Conductivity Detection for Gradient-Flow and Delay Characterization

Gary A. Valaskovic; Carla J. Marshall-Waggett; Amanda Berg, *New Objective, Inc., Cambridge , MA USA*

Due to introduction of undesirable swept volume, UV detection is typically eliminated as a viable method in combination with nanobore LC-MS. We herein employ a novel conductivity “cell” as an effective inline conductivity detector to measure gradient delay and mobile phase compositional changes at the column head. Two separate true-zero-dead-volume electrically conductive unions, seperated by a 100 to 250nL swept volume, were configured to enable mobile phase conductivity measurement. This method adds no post column volume, and does not negatively impact the quality of the chromatography. Each union contains a 150um diameter platinum wire electrode; typically separated by 5cm of 75µm-ID fused-silica tubing. The union closest the column was connected to the high-voltage power supply. The other union was connected to a PC interfaced micro-ammeter providing an electrical ground. A 10cm packed column containing a 75um tubing ID and 15um tip ID was employed in the analysis. Samples were injected onto a 2.5cm trap column via a 10-port automatic nano-valve. Analyses were conducted using a nanoLC pump, ion-trap mass spectrometer, and customized nanoelectrospray source; all current data were transmitted to a PC via serial port. Monitoring the ground current appear to be an effective means of monitoring the bulk mobile phase conductivity, and hence gradient composition performance. All analyses were executed at 500nL/min flow rates with a1.2kV applied voltage. A 98% water mobile phase composition was employed at the start of the gradient, and the recorded current was 8.1µA; at the conclusion of the gradient, the

mobile phase consisted of 98% acetonitrile, and the final current was recorded as 3.9μA. The current profile (uA versus seconds) exactly follows the programmed pump gradient profile.

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P-331-W

High-Speed CE-MALDI Instrumentation for Peptide Analysis

Michael W. Vannatta; Norman J. Dovichi, *University of Washington, Seattle, WA USA*

We have developed an inexpensive high-speed interface that couples capillary electrophoresis (CE) to matrix assisted laser desorption ionization (MALDI) mass spectrometry. The system employs a matrix sheath flow and produces drops that are approximately 2.5 nL in volume. The system captures analyte migrating from the electrophoresis capillary at rates up to 2 Hz. We have demonstrated rapid and highly efficient separations of standard peptides with nanomolar concentration and attomole mass limits of detection with ~0.5 s peak width. Additionally, we applied our technology to a complete tryptic digest of the standard protein α-lactalbumin. The separation was carried out in less than three minutes and 144 separate peptides were detected. Tandem MS experiments were carried out and used to identify the standard protein. The instrument's design, construction, and optimization are discussed. Furthermore, we demonstrate the versatility of this instrument by coupling it to two different mass spectrometers: Applied Biosystem's 4700 Proteomics Analyzer and Q-Star.

P-332-W

Design and Performance of a Novel Interface for Capillary Electrophoresis – Electrospray Ionization – Ion Trap Mass Spectrometry

Xuefei Zhong; E. Jane Maxwell; Hong Zhang; David D.Y. Chen, *University of British Columbia, Vancouver, Canada*

Hyphenation of the highly efficient and powerful separation technique - capillary electrophoresis (CE) and the sensitive and information rich detector – mass spectrometer (MS) has the potential to accomplish many challenging micro separation and detection tasks. However, several problems has impeded it from becoming a widely used analytical technique: the mismatch of the CE supplied flow rate and required flow rate for a stable electrospray, the mismatch of capillary electrophoresis current and electrospray current, bubble formation at the outlet of CE column that disturbs electrospray and fragility of the interface. To address these problems, a novel interface for capillary electrophoresis (CE) – electrospray ionization (ESI) – mass spectrometry (MS) has been developed in our group. It is robust, easy to construct and the sensitivity is high. Analysis of amino acids and peptides mixture standards are employed to demonstrate the performance of this CE-MS interface.

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P-333-W

Substrate-Assited Laser Desorption Inductively-Coupled Plasma Mass Spectrometry for Elemental Detection in Microcolumn Effluent and Biological Submicroliter Samples

Jan Preisler; Pavla Jungová; Ondřej Peš; Tomáš Vaculovič; Jarmila Navrátilová; Jan Šmarda; Viktor Kanický
Masaryk University, Brno, Czech Republic

A new method of sample preparation for elemental analysis using substrate-assisted laser desorption inductively-coupled plasma mass spectrometry (SALD ICP MS) is presented. Well-defined submicroliter volumes of analyzed samples are deposited onto polyethylene terephthalate glycol (PETG) plate and, after insertion into a commercial ablation cell, desorbed with pulses of 213-nm laser and analyzed with ICP MS. In contrast to conventional laser ablation (LA) ICP MS of solids, the analyzed compound is quantitatively desorbed during the PETG ablation. Furthermore, complete desorption is achieved at laser power density as low as ~10 MW/cm2, two orders below the levels typical in LA ICP MS. Model samples of metal salts in pg quantities were desorbed by less than 20 laser shots and relative spot-to-spot reproducibility was 3 - 10%. The limits of detection of Cr, Cu, Co, Fe, Ni, Sn and Zn deposited on the target were in the range of 50 – 500 fg. Selection of optimal conditions, such as laser fluence, number of laser shots, type of raster etc. will be discussed. The low limits of detection allow using SALD for off-line coupling of capillary electrophoresis (CE) to ICP MS. Moreover, a matrix for matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) can be added into the analyzed sample prior to deposition. In this case, PETG plate is fixed on a MALDI target for protein/peptide analysis followed by the elemental ICP MS analysis. An example of chromium speciation, 2-minute CE – SALD ICP MS speciation of 1pg of Cr(III) and Cr (VI) will be shown. Peak halfwidths of ~3 seconds were reached. In another application, SALD ICP MS was used for study of copper metabolism in disulfiram-treated myeloid leukemia U937 cells. Examples of copper determination in lysed and non-lysed U937 cells and in the extracellular media on nanogram scale are shown.

P-334-W

MicroPrep: On-Chip Subcellular Fractionation using Dielectrophoresis

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In proteome analysis scientists face large numbers of different protein species per cell or tissue at a dynamic concentration range of 10^9.
Sample fractionation reduces the overall complexity of the protein sample, and enriches low abundance proteins relative to the original sample. In this context, subcellular organelle fractionation is of particular importance, since functional units are isolated providing additional information with respect to the cellular localization of a particular protein.
We have developed a microfluidic system with integrated electrode arrays and a dedicated high-throughput channel layout to isolate mitochondria from cell homogenate based on dielectrophoretic sorting. Sorting of particles according to size and dielectric properties is achieved by the competition between hydrodynamic friction and dielectrophoretic forces.

The following means to efficiently prevent electrode fouling and clogging of the channel were successfully tested: the inner surfaces are coated with bovine serum albumin. Mechanical actuators generate minute vibrations of the channel cover inducing periodic flow superimposed on the sample flow. Chip and sample are cooled below 10°C to maintain the integrity of mitochondria during the fractionation. A chip material with high specific heat conductance ensures efficient transport of Joule heat generated in the vicinity of the deflector units.
MicroPrep separation yields micrograms of mitochondria. Western blots showed significantly less contaminants such as lysozomes or endoplasmatic reticulum when compared to samples prepared by gradient density centrifugation. Mitochondrial proteins were successfully characterized and identified by 2D gel electrophoresis and mass spectrometry.
In conclusion, a chip-based subcellular fractionation scheme was developed which provides mitochondrial samples of superior purity compared to standard enrichment technologies. Further applications of this technology are envisioned for sample enrichment in medical diagnostics. Chip coating, cooling and mechanical actuation for the first time provide for compatibility of dielectrophoresis chips with complex biological samples and enable robust long term operation without electrode fouling.

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P-335-W

Separation of Proteins using a Novel Two-depth Miniaturized Free-Flow Electrophoresis Device with Multiple Outlet Fractionation Channels

Marco Becker; Ulrich Marggraf; Dirk Janasek, *ISAS - Institute for Analytical Sciences, Dortmund, Germany*

An improved free-flow electrophoresis chip design for the separation and fractionation of proteins inside a miniaturized device is presented in this work.

The free-flow electrophoresis (FFE) technique is well suited for sample preparation in large scale since separation is performed continuously. An electric field is applied perpendicular to the hydrodynamic flow, and the time domain of separation is transferred to a local domain.

There are different approaches to apply the electric field to the separation chamber, e.g. using ion-permeable mebranes. The disadvantage of mebranes is the mechanical instability at high flowrates (>8 µl/min). To overcome this instability we used sidechannels to mimic membranes. To achieve a high flow resistance of the sidechannels, we fabricated a two-depth glass FFE-chip. The sidechannels are 1.5 µm deep and the separation compartement is 17 µm deep. So the depth of the chamber is 11-fold higher than the depth of the sidechannels, which leads to a 350-fold higher flow resistance in the sidechannels compared to the separation compartment. Because of this, no leackage through the sidechannels is observed.
We also integrated a novel interface for 9 outlet-channels. Each outlet channel is connected to a tube which allows collecting and further processing of the in-chip separated analytes.

We used 10 µM Myoglobin and 10 µM Trypsin labelled with fluorescein-isothiocyanate to test the separation capability of the novel FFE-design. The separation was performed in the modes zone-electrophoresis and temperature gradient focussing. When applying 3150 V to the electrodes at the end of the sidechannels, resulting in an electric field strength of 119 V/cm inside the separation compartment, the proteins were seperated and focussed to different outlet channels.

Our novel FFE chip design enabels the continuous high-flowrate separation combined with multi-outlet fractionation of analytes which is an essential step in the field of on-chip separation.

P-336-W

Quadrupole Magnetic Field-flow Fractionation for the Characterization of Magnetic Nanoparticles

Francesca Carpino; Maciej Zborowski; P. Stephen Williams, *Cleveland Clinic, Cleveland, OH USA*

Quadrupole magnetic field-flow fractionation (QMgFFF) is an analytical and characterization technique for magnetic nanoparticles such as those used for cell labeling and for targeted drug therapy. QMgFFF is capable of determining the mean magnetite mass per particle and the mass distribution of magnetite among the particles. Field-flow fractionation (FFF) is similar to chromatography in that different components of a small sample elute from a separation channel at different times. Chromatography exploits differences in partition between the mobile and stationary phases to separate sample components as they are carried along a column, but FFF separation is achieved within the flowing mobile phase and does not utilize a stationary phase. QMgFFF is a relatively new form of FFF and uses a helical channel mounted in an axisymmetric magnetic field gradient provided by a quadrupole electromagnet.

Magnetization of the particulate sample is induced by the applied magnetic field and the particles are driven toward the outer channel wall by their interaction with the field gradient. Due to viscous drag, the mobile phase velocity profile across the channel thickness is close to parabolic, with highest fluid velocity near the channel center and zero velocity at the walls. Particles that interact strongly with the field gradient form thin zones adjacent to the wall, and are confined to the slow moving fluid close to the wall. Particles that interact less strongly with the field gradient form more diffuse zones, and they sample faster fluid streamlines in addition to those close to the wall. A separation between the particles is thereby induced as they are carried along the channel. Particles are detected using a chromatographic UV-detector as they elute from the channel; the elution profile is mathematically transformed into a magnetite mass distribution. Examples will be shown of the characterization of magnetic nanoparticles from several commercial suppliers.

NOTES:

P-337-W

DNA Separation in Polyester-toner Electrophoresis Microchips

Gabriela Rodrigues Mendes Duarte; Juliane Cristina Borba; Wendell Karlos Tomazelli Coltro; Emanuel Carrilho, *University of Sao Paulo, Sao Carlos, SP - Brazil*

Microchip electrophoresis has become a powerful tool for DNA separation with all advantages of miniaturized techniques such as high speed, high resolution, easy automation, and great versatility for both routine and development applications. Different substrate materials have been used for producing these microchips for DNA separations including conventional (glass, silicon and quartz) and alternative (polymers) platforms. In this study we perform DNA separation in a simple and cheap microdevice in a polyester-toner (PT) electrophoresis microchip. PT devices were fabricated by a direct-printing process using a 600-dpi-resolution laser printer. The layout was drawn in a graphic software and laser-printed on a polyester film. The PT channel structure was defined by a double toner layer resulting in a channel with 12 μm deep and 200-μm wide. The sealing of the PT channels were obtained by thermal lamination at 130 ^{0}C. The injection and separation channels were 10 and 40-mm long, respectively. A sample aliquot was introduced into separation channel under the application of 300 V for 30 s. Laser-induced fluorescence detection was carried out at 34 mm from the injection point. The channel was filled with a solution of 0.5% hydroxyethylcellulose (HEC 90-105 kDa) by capillary action. DNA separations were performed under electric field ranging from 100 to 300 V cm^{-1}. The system allowed the separation of DNA fragments of size between 100 and 1000 bp with great correlation of the size of DNA fragments and mobility. The mobility increased with increase of electric field and separations showed the same profile regardless the electric field. The system showed a good efficiency (250,000 plates/m) and the separation was completed under 4 min. PT microchips are a promising new platform for DNA separation for cheap, simple and fast genetic analysis.

Acknowledgments:
FAPESP (05/04473-4) and CNPq.

P-338-W

Porous Nanocrystalline Silicon (pnc-Si) Membranes for Micro- and Nano-Fluidics Exhibit High Permeability and Sharp Protein Cut-offs

Thomas Gaborski; Christopher Striemer; Jessica Snyder; David Fang; Philippe Fauchet; James McGrath
University of Rochester/SiMPore, Rochester, NY USA

We recently introduced porous nanocrystalline silicon (pnc-Si) as a molecularly thin membrane material capable of size and charged based separation of proteins and other nanometer-sized particles (Striemer et al. Nature, 2007). The membranes can be produced in massive arrays with membranes freely suspended over millimeter support spacings. Mechanical tests indicate surprising strength with failure at or above 15 psi with no fatigue prior to rupture. Average membrane pore sizes can be tuned between 5 nm to 100 nm with porosities between 0.1-15%.

The structure of pnc-Si membranes suggests that they should display extraordinary permeability to water and gas under pressure. To test this prediction, we formatted membranes for easy assembly into pressure cells and centrifuge tube inserts. For membranes with mean pore sizes 10 - 20 nm and porosities 2 - 10%, we measured hydraulic permeabilities of nearly 5 – 30 cm^3/(cm^2 – min – bar). These values are at least 10 fold higher than the permeability values for commercial ultrafiltration membranes measured in side-by-side comparisons. The permeability to water is also more than one order higher than literature values for carbon nanotube/polymer composite membranes. Because pores can be directly imaged in electron

microscopy, we employ known pore sizes and distributions to test existing theories for water permeability of ultrathin membranes and find good agreement (Tong et. al. Nano Letters, 2004). Additionally, we have performed protein fractionation experiments showing that we can separate complex protein mixtures based on protein size. On-going work is investigating two-dimensional separations by utilizing charge-based separation characteristics of the membrane.

NOTES:

P-339-W

Microfluidic Control and Design Methods for Sequential 3-Step Gucha-Gucha Extraction

Akihide Hibara[1]; Ko Kasai[2]; Hajime Miyaguchi[3]; Takehiko Kitamori[1]

[1]The University of Tokyo, KAST, and JST, Tokyo and Kawasaki, Japan; [2]The University of Tokyo, Tokyo, Japan; [3]National Research Institute of Police Science, Kashiwa, Japan

One of the most distinctive features of multi-phase microfluidics is high specific interface area. By utilizing the feature, our group has demonstrated micro solvent extraction systems and continuous flow chemical processings. In these demonstrations, parallel two-phase flows of organic and aqueous phases have been used. Although the parallel two-phase flows are useful and effective for process design, flow control for multi-step extraction systems is slightly difficult. Therefore, versatile flow control method is desirable for large-scale integration. Recently, we have developed 'gucha-gucha' extraction chip. The gucha-gucha extraction chip is effective for fast solvent extraction and for multi-step extraction. (The word 'gucha-gucha' is Japanese state mimic word and means a state in disorder.) In this paper, we report a control method of the gucha-gucha flow and demonstrate an example of sample pretreatment in forensic toxicology, which consists of 3-step sequential extraction with different pH and different solvent compositions. The folloing GC-MS analysis showed good agreement in extraction efficiencies with bulk operation and 100-times shorter pretreatment operation time.

The gucha-gucha extraction chip was fabricated with a photolithographic wet-etching technique, where the lithography-etching procedure was repeated twice to obtain shallow and deep microchannel structure as desired. The chip also has a hydrophobic-hydrophilic patterned surface on the microchannels. By combining shallow-deep and hydrophobic-hydrophilic characteristics, we have successfully integrated organic droplet generation, droplet-to-plug conversion, and complete organic-aqueous separation. Furthermore, we would emphasize that these operations can be connected and repeated several times. For demonstration of the gucha-gucha extraction chip, selective sequential solvent extraction of caffeine (additive of stimulant), mephentermine (imitation of stimulant), and hydroxymethamphetamine (metabolite of stimulant). The conditions of each extractions are pH4 with chloroform, pH13 with chloroform, and pH9 with 3:1 mixture of chloroform and isopropyl alcohol. Each extraction phase was analyzed by GC-MS and we confirmed that each extraction proceeded as designed.

P-340-W

A Novel Technique for Microfabrication of Polymeric Microchips for Biomolecule Assays

Myra T. Koesdjojo; Kelsie E. Warner; Corey R. Koch; Vincent T. Remcho, *Oregon State University, Corvallis, OR USA*

Novel polymer microfluidic device fabrication methods are presented that use an SU-8 master in two-stage thermal embossing, followed by vaporized organic solvent bonding. The primary master is created by lithography using SU-8 photoresist on a silicon wafer. Microchannels were formed with a two stage hot embossing process. The process is faster, cheaper, and simpler than other approaches for fabrication of a master. These advantages, coupled with the ease and rapidity of hot embossing, provide a powerful, rapid prototyping method. Successful feature transfer from the SU-8 master to the final substrate was characterized by profilometry. To form a complete microchannel, the embossed chip was sealed to a blank polymer piece using a novel vaporized solvent welding technique. Various polymer materials were used and different designs were tested to demonstrate the feasibility of the fabrication process. Success in bonding was verified by SEM; the devices were leak-free when tested at a pressure of 1000 psi. The

fabrication method was applied in building a passive micromixer that contained high density microfeatures and required three polymer layers. To demonstrate the utility of the microchip fabricated via this new approach, the micro-mixer was used to perform a biomolecule assay.

NOTES:

P-341-W

Resistive Pulse Sensing at Single Nanopores in Microfluidic Devices

Michelle Kovarik; Kaimeng Zhou; Pamela J. Bonner-Brown; Yves V. Brun; Stephen C. Jacobson, *Indiana University, Bloomington, IN USA*

We report resistive pulse sensing on integrated nanopore/microchannel devices. On-chip resistive-pulse sensing is demonstrated using single track-etch nanopores isolated at the intersection of two poly(dimethylsiloxane) microchannels in a multi-layer device. Integrating microfluidic channels with the nanopores allows micro-scale handling steps to be performed prior to sensing and improves mass transit to the pore(s). On-chip resistive-pulse sensing is demonstrated using single conical poly(ethylene terephthalate) nanopores with tip diameters ranging from ~100 – 600 nm. These pores are used to sense polystyrene fluorescent microspheres ranging in size from 40 - 200 nm, and characteristic current signals are determined for particles of varying size and composition by determining the amplitude and duration of the current pulses resulting from their transport through the nanopore. Particles as small as 40 nm in diameter can be detected with a signal-to-noise ratio >10, corresponding to a 3% change in the measured current. Transit of the microspheres through the pore to the upper layer of the device is confirmed by simultaneous fluorescence detection via a CCD camera, validating current blockade signals as through-pore transfer events. Transfer efficiency to the opposite microchannel is investigated as a function of applied voltage and membrane surface chemistry, with transport of 100-nm microspheres through hydrophobically-modified nanopores approaching 100% for applied voltages as low as 4 V DC. Further development of the device will be used to study binding events by exploiting the precise fluid handling and mixing capabilities of microchannels and the sensitive detection provided by the integrated nanopore. Additionally, we are developing similar devices using larger cylindrical pores (1-2 μm diameter) to perform chemotaxis assays on *C. crescentus* and *E. coli* bacteria.

P-342-W

Integrated Microfluidic System for Immunocapture, Electrophoresis and Detection of Protein Biomarkers

Mohamad Reza Mohamadi[1]; Anne Le Nel[1]; Zuzana Bilkova[2]; Claire Smadja[3]; Jean Louis Viovy[1]

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Our group previously developed analytical microcolumns based on magnetic-beads self-assembled inside a microchannel. This microfluidic component was applied to the immunocapture of protein samples. We present here a new integrated microfluidic system in which this magnetic affinity column is combined with on chip electrophoresis.
The magnetic beads which were pre-coated with specific antibodies were self organized in the microchannel by applying an external magnetic field. Hydrodynamic flow was used to introduce the protein sample and to perform the washing steps afterward. The captured fluorescent sample was eluted by applying electrokinetic force and using an elution buffer (100 mM SDS). The eluted sample was then directed to an electrophoresis channel where free flow electrophoresis was performed and the separated samples were detected by LIF detection.
To overcome the problem of sample adsorption and also EOF which acts as a counter-flow to the elution, we developed a new double layer coating on PDMS using PDMA-AGE and cellulose derivatives. The coating successfully suppressed nonspecific adsorption of the sample and beads to the microchannels and reduced the EOF to a negligible value.

To validate the system, on-chip immunocapture, elution and electrophoresis of a model protein (Alexa labeled IgG) was conducted. The results showed a perfect reproducibility for successive elution and electrophoresis in the same chip. We detected 300 nM fluorescent labeled proteins and further improvement is going on to decrease the detection limit down to sub nM.
This microfluidic system is a preliminary prototype for detection of beta amyloid peptides. The aim of this project is to develop an automated microfluidic system capable of performing sequential capture, analysis and detection of beta amyloid peptides in biological samples of patients with Alzheimer disease. However the method has a great versatility which makes it applicable to detection of other biomarkers.

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P-343-W

Isolation of Tumor Cells using Size and Deformation

Hisham Mohamed; Megan Murray; James N. Turner; Michele Caggana, *Wadsworth Center, New York State Dept. of Health, Albany, NY USA*

Most cancer deaths are due to metastatic disease, not the primary tumor. As the primary tumor grows, it disseminates tumor cells into the circulation; this can happen even before it has been detected through routine screening or diagnosis. By the time the primary tumor is removed surgically, millions of circulating tumor cells (CTCs) have already entered the blood. A small portion of these CTCs can survive and seed a secondary, lethal tumor. The mechanism by which this occurs is not fully understood. Thus, the isolation and analysis of CTCs from blood are the subject of intense research.
Cancer cells morphology is modified mainly by increased nuclear size before becoming invasive, i.e., in dysplasia and carcinoma *in situ*. The nucleus of a dysplastic cell can be up to four times that of a non-dysplastic cell. Nuclear changes such as an increased size, deformation, and a change in internal organization are among the most universal criteria for detecting malignancy.
We have designed and micromachined a device to separate individual cells based on their mechanical characteristics. It has arrays of four successively narrower channels, each consisting of a 2-D array of columns. Current devices have channels ranging in width from 20 to 5μm, and are 10 or 20μm deep. When cells from nine different cancer cell lines were loaded into the device, all cancer cells were consistently isolated in one region of one specific device. Mixing experiments with human whole blood demonstrated that cancer cells could be isolated without interference from other blood cells. Additionally, either intact viable cells, or DNA, were extracted for molecular analysis.
These studies will lead to non-invasive methods to monitor patients, stage disease, and assess treatment. Furthermore, insights into the metastatic process and into gene expression profiles will be gained.

P-344-W

Microfluidic Isolation of Stem Cells by Negative Selection Depletion

Shashi Murthy;[1] James V. Green[1]; Milica Radisic[2];

[1]Northeastern University, Boston, MA USA; [2]University of Toronto, Toronto, Canada

Microfluidic cell separation systems have emerged as attractive alternatives to traditional techniques in recent years. These systems offer the advantages of being able to handle small sample volumes and at the same time achieve highly selective separation at low cost. Microfluidic devices with surface-immobilized adhesion molecules can achieve separation by exploiting ligand-receptor interactions. When ligands are immobilized on the microfluidic channel surfaces, the resulting cell capture devices offer the typical advantages associated with microfluidic systems, with the added benefit of not requiring complex fabrication schemes or pre-processing incubation. This presentation will describe how a microfluidic system of devices coated with peptides can be utilized to deplete a cell suspension of endothelial cells, smooth muscle cells, and fibroblasts in order to isolate a fourth cell type, adipose-derived stem cells, by negative selection. The peptides utilized are arg-glu-asp-val (REDV), val-ala-pro-gly (VAPG), and arg-gly-asp-ser (RGDS). Separation is achieved by continuous flow and by the provision of large surface areas for cell attachment. The significance of this approach is that it can be utilized to isolate stem and progenitor cell populations from digested tissue as a precursor to conventional tissue engineering on scaffolds or cell-based regenerative therapeutics. Furthermore, this approach could be an effective way to isolate stem/progenitor cells whose markers are not fully characterized.

NOTES:

P-345-W

Effect of Microchannel Geometry in a Cell-Affinity Chromatography Process

Shashi Murthy; James V. Green, *Northeastern University, Boston, MA USA*

Microfluidic channels coated with ligands are versatile platform for the separation or enrichment of cells from small sample volumes. This adhesion-based mode of separation is mediated by ligand-receptor bonds between the cells and channel surface and also by fluid shear stress. This paper demonstrates how aspects of microchannel geometry can play an additional role in controlling cell adhesion. With a combination of computational fluid dynamics modeling and cell adhesion experiments, u-shaped channels with sharp turns are shown to have regions with near-zero velocity at the corners where large numbers of cells adhere or become collected. Channels with curved turns, on the other hand are shown to provide more uniform and predictable cell adhesion provided the gap between vertical arms of the u-shaped channels is sufficiently wide. The magnitude of cell adhesion in these curved channels is comparable to that in straight channels with no turns.

P-346-W

Photoactivable Microfluidic Devices for Selective Extraction of Heavy Metals from Water

Jintana Nammoonnoy; Jeffrey R. Walker; Vincent T. Remcho*, Oregon State University, Corvallis, OR USA*

A new method has been developed for heavy metals extraction from drinking water using a spiropyran modified PMMA microchip as a photoactivable microfluidic device. Spiropyrans are metal chelating ligands used for photoreversible complexation.
Spiro compounds are a well-known class of dyes that exhibit interesting photochromic functionality in that their structure changes reversibly with UV/visible irradiation. The photoinduced reaction of spiropyran involves the cleavage of the spiro carbon-oxygen bond upon UV light irradiation and subsequent isomerization to the open form. Spiropyran chemistry is applicable to extraction of metal ions by first chelating the ions (after the spiro compound is activated using UV light), and subsequently exposing the metal-spiropyran complex to visible light to elute contaminants efficiently in a concentrated plug.
In this work the retention and release of metal ions on the spiropyran modified stationary phase are controlled by UV/visible irradiation. The extraction of Pb2+ ions from aqueous solution using spiropyrans immobilized in a PMMA microfluidic chip is studied. We demonstrate that spiropyrans retain their photochromic properties when immobilized on the surface of PMMA, and that useful extractions of Pb2+ from aqueous solution are possible.

NOTES:

P-347-W

Determining Binding Constants Using Gradient Micro-Free Flow Electrophoresis

Ryan T. Turgeon; Michael T. Bowser, *University of Minnesota, Minneapolis , MN USA*

A separation technique called micro-free flow electrophoresis (uFFE) has been developed as a continuous analytical separation technique. In uFFE, a sample stream is introduced into a planar separation chamber pumped continuously with separation buffer. An electric field is applied perpendicularly to the direction of flow and analyte streams are deflected based on their differences in mobility.
uFFE for analytical separations holds many advantages over other micro fluidic devices due to the continuous flow and detection of sample. Continuous flow and detection in uFFE allows the fast analysis of samples with changing concentrations. For example, a receptor can be titrated with a gradient of increasing ligand concentration to measure binding. Upon binding, the ligand-receptor complex assumes a larger size than the remaining unbound receptor. The bound complex and free receptor can then be continuously separated and detected using uFFE. The intensity of the bound complex increases as the titration gradient concentration of the ligand increases. Other methods for determining binding require the analysis of multiple individual samples, which are then mathematically fit to a curve. Gradient sample uFFE allows the determination of binding affinity over an entire range of concentrations. In our experiments, HIV reverse transcriptase (HIVRT) and a DNA aptamer selected to bind HIVRT were used to demonstrate ligand binding in gradient uFFE. Using gradient uFFE, 50 nM DNA aptamer was titrated with HIVRT concentrations between 0-500 nM in only five minutes. During the gradient, images were recorded at a rate of one per second thus resulting in 300 individual data points. Continuous monitoring of the titration gradient not only increased the sampling rate, but also revealed a complex 2:1 binding stoichiometry. Gradient uFFE was shown to more data and in a shorter time than other binding affinity methods.

P-348-W

Microchip DNA Electrophoresis Investigation of the Interplay Between the Nanoporous Gel Morphology and the Onset of Entropic Trapping

Nan Shi; Victor M. Ugaz, *Texas A&M University, College Station, TX USA*

Miniaturized DNA electrophoresis devices pose unique design challenges that arise from the need to exert increasingly precise and reproducible control over all aspects of the process so that separation performance can be maintained over ultra-short distances. The properties of the sieving gel matrix are particularly important because its nanoscale pore morphology plays a key role in directing DNA migration. Here we describe experiments aimed at exploring this interplay in photopolymerized crosslinked polyacrylamide gels by employing a unique combination of methods that enable both the mean pore size and pore size distribution of the gel to be quantified, and a versatile microfluidic platform that allows continuous monitoring of DNA separation progress so that the size dependence of mobility and diffusion coefficients can be established. This approach allows us to identify how polymerization conditions (i.e., UV intensity during photopolymerization) influence the gel pore size distribution, and ultimately shape overall separation performance. Analysis of double-stranded DNA separations in the size range below 1 kb reveals that varying the rate of photopolymerization induces a corresponding change in the physical mechanism of DNA migration between reptation and entropic trapping. We then develop an interpretation of these observations based on the distribution of pore sizes and their arrangement within the gel matrix. We hope these measurements can provide new insights into the interplay between the pore network architecture and electrophoretic transport of DNA through the gel that can help enable rational selection of optimal matrix materials and polymerization conditions that provide enhanced separation performance.

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P-349-W

Microchip Capillary Electrophoresis Cartridge with Contactless Conductivity Detection

Elwin Vrouwe; Johannes Oonk; Ronny Van 't oever, *Micronit Microfluidics, Enschede,The Netherlands*

A new concept is presented in this paper to interface capillary electrophoresis (CE) chips with external instruments. Today many CE chips are optimized for a particular separation without taking the costs of the chip into account. It is imperative that the cost of the CE chips is reduced to attract more users, particularly from outside the field of microfluidics. Here we present a CE cartridge consisting of a glass part, in which the CE separation takes place, and a plastic part to contain the sample and background electrolyte. This cartridge combines the advantages of glass which has stable surface properties with the low cost of injection molded plastics. The strongly suppressed or even absent electroosmotic flow in polymer chips can cause difficulties with quantitative analysis because the resulting sample stacking leads to peak sizes that are strongly affected by matrix components which have to be corrected using an internal standard. In the concept shown here the glass chip does not have any reservoirs. Instead, the channels terminate on the edge of the chip. The chip is mounted in plastic part which has compartments matching with the position of the channel inlets in the chip to hold the fluids. The advantage is that the separation is still performed in a glass chip. Hhigh voltage electrodes and optional conductivity detection electrodes can also be patterned on the surface of the chip. In this paper for example contactless conductivity detection is used to detect inorganic cations separated in the cartridge. Since the entire package is intended for single use there is no sample carryover, which could have been a high risk for use in diagnostic applications. Furthermore, the cartridge is simply plugged into the instrument to complete the electrical contacts providing a quick and user friendly means to change the chip.

P-350-W

Quantitative Immunological Detection of Proteins in Anisotropic Nanofluidic Sieving Structures

Masumi Yamada; Pan Mao; Jianping Fu; Jongyoon Han, *Massachusetts Institute of Technology, Cambridge, MA USA*

Nanometer-scale fluidic devices offer a promising potential for developing novel systems for biomolecule separation and manipulation. We have previously demonstrated the concept of a two-dimentional anisotropic nanofilter array (ANA) for continuous-flow separation of both DNA and proteins under native conditions (Fu et al., Nat. Nanotechnol., 2007). The nanoengineered ANA structure composed of perpendicularly-crossing deep (300 nm) and shallow (60 nm) nanofluidic channels allowed different-sized or -charged biomolecules to follow distinct trajectories, leading to efficient separation. In this study, we further demonstrate a quantitative study of native proteins by utilizing their immunological reactions and separating antibody-protein complex from non-reacting antibody inside the ANA structure. When molecules form relatively large complexes as a result of specific immuno-reaction, they can be effectively separated by the ANA structure from the non-reacting antibody, and this separation can be monitored by fluorescence detection. We investigated two proteins used as disease markers, human C-reactive protein (CRP) and human chorionic gonadotropin (hCG), by using fluorescent-labelled polyclonal antibodies. Our experimental results showed that the molecular ratio of target protein and antibody is a critical factor determining the size of the protein-antibody complex, and we successfully demonstrated the quantification of proteins in the range of 0.05 to 10 ug/mL. Detection sensitivity can be further improved by optimizing fluorescence readout methods, and the adaptation to sandwich immunoassay is also straightforward. The presented scheme will provide a useful tool for the quantification of target proteins, since the separation can be achieved continuously and rapidly without adopting laborious multiple mixing/washing procedures, and will be advantageous for processing multiple samples in series.

NOTES:

P-351-W

Counting Mitochondria by Electrophoretic Aligning

Yoshinori Yamaguchi; Keiko Sumitomo; Keisuke Natsui, *ASMeW, Waseda University, Tokyo, Japan*

Since mitochondria dominate the production of ATP and regulate cellular metabolism, it is critically important to count the mitochondria in cells as the first step to reveal the cell functions. The information of the number of mitochondria will also provide the diagnostication for many biological and clinical conditions, such as obesity, degenerative disease or aging from cellular metabolism. Unfortunately, the number of mitochondria in a single cell may range from one to over thousand and their morphologies are highly heterogeneous, it has been difficult to define the number of mitochondria correctly by using flow cytometry while the flow cytometry has been employed for the high-throughput analysis. Capillary electrophoresis was also employed for detection of individual mitochondrial event, and provided the morphological deference of individual mitochondrion based on the deference of electrophoretic mobility. However, both the flow cytometry and CE-LIF employed the fluorescence intensity based detection, it sometimes inadequate to determine the number of mitochondria in cells correctly. In addition, because the intensity of fluorescence labeling dyes to mitochondrion, especially the oxidative-reductive labeling dyes, strongly depend on the electron transfer process in the membrane, thus it depends on the activation of mitochondrion, the fluorescence intensity from the mitochondrion sometimes fluctuate.

In this paper, we discuss the mitochondria align system that realize precious mitochondrion counting by employing the capillary electrophoresis whose internal diameter is approximated to the size of mitochondrion, which is around 2 um. We have been succeeded to align the individual mitochondrion in the narrow capillary by electro osmosis with the modulation of electric field, and to estimate the number of mitochondria in single cell. We demonstrate the methodology to count the mitochondria and the core technology for one dimensional alignment system.

P-352-W

Development of PDMS Micro Check Valves

Yo Tanaka; Kae Sato; Takehiko Kitamori, *The University of Tokyo, Tokyo, Japan*

Introduction:
We fabricated micro check valves made of polydimethylsiloxane (PDMS) and demonstrated water pumping of a micropump incorporating the valves. Recently, various mechanical micro fluidic devices have been developed to make micro chemical processes more sophisticated. By contrast, we have developed a micropump exploiting beating cardiomyocytes requiring no externally energy sources or stimuli [1]. However, its flow rate was too poor to be applied for practical applications of micro chemical systems mainly because of the inefficiency of the check valves made of polyimide. As cardiomyocytes' force is weak, more flexible materials must be used. Here, we developed check valves made of PDMS that is more flexible than polyimide.

Experimental:
The valve design is a cantilever-type [1]. Firstly, check valves were fabricated. A thin (10 μm thickness) PDMS membrane was prepared by spin-coating, and it was cut into square pieces (5 mm × 5 mm). Then, a PDMS sheet was prepared and the PDMS square pieces were attached to both side of the sheet to cover holes made on the sheet by using tweezers and ethanol. Secondly, a reciprocating micropump incorporating the valves was assembled [1]. Finally, water pumping was demonstrated. Water was

dropped on the inlet of the microchannel, and diaphragm was oscillated using tweezers to prove the function of the valves.

Results and Discussion:
By the oscillation of the diaphragm, water was pumped into a chamber and get out from the outlet. From this result, the check valves were found to be successfully actuated. This check valve is different from previous PDMS valves that require mechanical instruments such as air pressure controllers [2]. By using this valve, a cardiomyocyte pump would be improved.

References:
1. Y. Tanaka, et al., Lab Chip, 6, 230 (2006)
2. S. R. Quake, A. Scherer, Science, 290, 1536 (2000)

NOTES:

P-353-W

Modeling and Experimental Validation of Dual Microfluidic Chamber, Infrared Laser-Mediated Polymerase Chain Reaction

Christopher R. Phaneuf[1]; Daniel C. Leslie[2]; James P. Landers[2]; Craig R. Forest[1]

[1]Georgia Institute of Technology, Atlanta, GA USA; [2]University of Virginia, Charlottesville, VA USA

The polymerase chain reaction (PCR), a technique used to amplify template DNA with a heat-stable polymerase enzyme and thermal cycling, is virtually universally used for sample preparation for genomic assays. Conventional PCR instrumentation utilizes conductive or convective heat transfer modes, requires relatively large volumes (e.g., 20 µL), and suffers from slow thermal cycling (e.g., 3˚C/s). In contrast, radiative heating by infrared sources enables rapid cycling (e.g., 30˚C/s) and smaller volumes (e.g., 500 nL), but is challenging for high throughput (e.g. several - hundreds) reactions. None of the existing techniques are amenable to high throughput PCR with independently-controllable temperature cycles for each reaction. This capability could be a boon for the emerging field of microfluidic genetic analysis instrumentation. We will report progress on modeling, design, fabrication, and testing of an infrared laser-mediated, dual microfluidic chamber thermal cycler for PCR. This work will serve as a stepping stone to independently-controlled multi-channel reactions.

Through modeling, we predict temperature ramp rates due to radiation absorption by aqueous reaction volumes. This model considers spectral, temporal, and spatial output of an infrared source as absorbed, transmitted, and reflected by the reaction volume. Heat transfer between the reaction volume, chamber, and environment are also considered.

Experimentally, we will present an infrared laser diode (LDX Optronics, LDX-2610-1550) coupled to a pair of sub-microliter, glass reaction chambers. Real-time temperature monitoring is achieved with a T-type thermocouple (Physitemp Instruments, T-240C) submerged in a reference chamber. Expected results include ramp rates on the order of 20˚C/s and thermal control using drive current pulsed-width modulation to 0.1˚C/s.

This system will demonstrate modular, optically-controlled PCR in multiple microfluidic chambers, a milestone towards high throughput independent PCR. Such instrumentation is extremely useful in areas such as genetic screening of pathogens, where rapid, small volume sample preparation is essential.

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Notes:

Thursday, February 5, 2009

07:00 – 12:00 Registration on the Mezzanine Foyer

07:00 – 08:00 *Continental Breakfast in the Imperial Ballroom*

PARALLEL SESSION in the Plaza Ballroom
~Fundamentals~

CHAIR: Frantisek Foret, *Institute of Analytical Chemistry, Brno, Czech Republic*

08:00 – 08:25 **Influence of Nano-HPLC-Column Conduit Shape and Column Aspect Ratio on Chromatographic Performance**
Gerard Rozing, *Agilent Technologies, Waldbronn, Germany*

08:25 – 08:50 **A Systematic Study of CIEF: Optimization of Critical Method Parameters**
Jeff D. Chapman, *Beckman Coulter, Inc., Fullerton, CA USA*

08:50 – 09:10 **CEC of Substances of Different Polarity with Neutral Monolithic Columns at Relatively Strong EOF in the Absence of Electrostatic Interactions**
Ziad El Rassi, *Oklahoma State University, Stillwater, OK USA*

09:10 – 09:30 **Surfactant Bilayers as Scaffolding for Hydrophilic Coatings in Capillary Electrophoresis**
Charles A Lucy, *University of Alberta, Edmonton, Alberta Canada*

09:30 – 09:50 **Approach to Development of Smart Small-Molecule Ligands by Kinetic Capillary Electrophoresis (KCE)**
Sergey Krylov, *York University, Toronto, Ontario Canada*

09:50 – 10:15 *Break*

PARALLEL SESSION in Arlington/Berkeley/Clarendon
~Detection~

CHAIR: Stephen Jacobson, *Indiana University, Bloomington, IN USA*

08:00 – 08:20 **Development of an Amine-Reactive Fluorophor Having Ph-Independent Fluorescence Properties and Mobilities in the 3 < Ph < 10 Range**
Gyula Vigh, *Texas A&M University, College Station, TX USA*

08:20 – 08:40 **Droplet Microfluidic Loading of Microcoil NMR: LC-MS+NMR Dereplication of Cyanobacteria Metabolites**
Roger Kautz, *The Barnett Institute, Northeastern University, Boston, MA USA*

08:40 – 09:00 **Development of Pseudo-SDS Dyes for Capillary SDS-PAGE**
Shaorong Liu, *University of Oklahoma, Norman, OK USA*

09:00 – 09:20 **Development of CE-Raman Spectrometry and –SPM for the Analysis of Nanofibers (Carbon Nanotube and B-Amyloid)**
Masaru Kato, *University of Tokyo, Tokyo, Japan*

09:20 – 9:40 **Optical Sensing in Microchip Capillary Electrophoresis by Femtosecond Laser Written Waveguides**
Rebeca Martinez Vazquez, *IFN-CNR, Milano, Italy*

09:40 – 10:15 *Break*

CLOSING PLENARY LECTURES in the Plaza Ballroom

CHAIR: Gerard Rozing, *Agilent Technologies, Waldbronn, Germany*

10:15 – 10:50 **Multi-Dimensional Capillary Liquid Chromatography-Tandem Mass Spectrometry for Phosphoproteome Analysis**
Hanfa Zou, *Dalian Institute of Chemical Physics, Dalian, China*

10:50 – 11:25 **Structured Nanoparticles For Sample Enrichment And Detection**
Frantisek Foret, *Institute of Analytical Chemistry, Brno, Czech Republic*

11:25 – 12:00 **Polymers in DNA Sequencing and Purification on Microfluidic Devices**
Annelise Barron, *Stanford University, Stanford, CA USA*

12:00 – 12:20 **Awards and Closing Remarks**
Jonathan Sweedler, *University of Illinois at Urbana-Champaign, Urbana, IL USA*

12:20 Conference Adjournment

AM Parallel Session: Fundamentals
Location: Plaza Ballroom
Chair: Frantisek Foret

Thurs. 08:00-08:25

Influence of Nano-HPLC-Column Conduit Shape and Column Aspect Ratio on Chromatographic Performance

Gerard Rozing[1]; Steffen Ehlert[2]; Ulrich Tallarek[2]; Stefanie Jung[2]; Monika Dittmann[1]; Jose Mora[1]; Lukas Trojer[1]

[1]Agilent Technologies, Waldbronn, Germany; [2]Philipps-Universität, Marburg, Germany

The influence of conduit geometry and particle-aspect ratio (conduit size/particle diameter) on the performance of packed beds in liquid chromatography has been a motif for many theoretical and experimental studies. So far these studies were limited to conduits with cylindrical shape and have mainly focused on the effect of the particle-aspect ratio on column permeability and separation efficiency (1). Very recently, Tallarek and co-workers (2-5) have expanded this study to conduits with non-circular perimeters such as square, rectangular, trapezoidal, and semi-circular shape. These data support the conclusion that particularly microchip-HPLC performance depends critically on the conduit geometry and achievable bed density.
A theoretical analysis of hydraulic permeability of HPLC columns with narrow, non-cylindrical perimeter will be provided and compared with experimental data from the manufacturing of cylindrical and non-cylindrical conduit, low-volume columns slurry-packed with 3, 5, and 10 µm-sized spherical, porous particles. Differences in interparticle porosity, permeability, and plate height data are analyzed and consistently explained by different microchannel-to-particle size ratios and particle size distributions. Intriguing results have been obtained when column performance data measured with UV-detection were compared with ESI-MS detection and will be discussed.

(1) S. Hsieh, J. W. Jorgenson, Anal. Chem. 1996, 68, 1212-1217.
(2) S. Khirevich, A. Höltzel, D. Hlushkou, U. Tallarek, Anal. Chem. 2007, 79, 9340-9349.
(3) S. Ehlert, K. Kraiczek, J.-A. Mora, M. Dittmann, G. P. Rozing, U. Tallarek, Anal. Chem. 2008, 80, 5945-5950.
(4) S. Khirevich, A. Höltzel, D. Hlushkou, A. Seidel-Morgenstern, U. Tallarek, Lab Chip 2008, in press.
(5) S. Jung, S. Ehlert, J.-A. Mora, K. Kraiczek, M. Dittmann, G. P. Rozing, U. Tallarek, J. Chromatogr. A 2008, submitted.

NOTES:

Thurs. 08:25-08:50

A Systematic Study of CIEF: Optimization of Critical Method Parameters

Jeff D. Chapman; Scott T. Mack; Ingrid D. Cruzado Park; Chitra K. Ratnayake, *Beckman Coulter, Fullerton, CA USA*

The biopharmaceutical industry has shown great interest in the analysis of therapeutic monoclonal antibodies (MAbs) by capillary isoelectric focusing (CIEF). Determination of charge heterogeneity adds an important dimension of measurement, useful for establishing the identity, purity and stability of a therapeutic protein preparation. However, issues with the repeatability and reproducibility of pI determination and sample peak profiles have limited the implementation of CIEF as a routine analytical tool. These obstacles include the effects of protein buffer components, pH gradient decay, poorly focusing carrier ampholytes, as well as sample precipitation and aggregation. Aided by computer simulations and a systematic study of CIEF inputs we have gained a better understanding into the mechanisms of protein focusing and mobilization. Using both synthetic peptides and a panel of therapeutic MAbs as a guide, we identify and optimize those experimental parameters having the greatest influence on method reproducibility and robustness. A critical factor in the analysis of basic proteins is the instability of the pH gradient which results from the presence of ITP and the need for longer focusing times. To address this we have adopted from isoelectric trapping [1], the practice of adding extreme anodic and cathodic spacer compounds into the sample solution to improve pH gradient stability. During our evaluation of these spacer compounds, we discovered that the cathodic spacer L-arginine was crucial for the proper implementation of CIEF, especially for those compounds with pI in the basic pH range. The results of intermediate precision studies on three different therapeutic MAbs with isoforms in the basic range demonstrate excellent performance with CV's less than 0.1% for pI, and from 0.6 to 3.0% for protein isoform percent composition.

[1] North, R.Y., Vigh, G. Electrophoresis 2008, 29, 1077-1081

NOTES:

Thurs. 08:50-09:10

CEC of Substances of Different Polarity with Neutral Monolithic Columns at Relatively Strong EOF in the Absence of Electrostatic Interactions

Ziad El Rassi; Samuel Karenga, *Oklahoma State University, Stillwater , OK USA*

In this talk, we will present our recent results on the introduction and characterization of novel, neutral acrylate based monoliths with various nonpolar ligands, e.g., alkyl and aryl functions, for achieving reversed-phase capillary electrochromatography (RP-CEC) separations of a wide range of polar and non polar compounds in the absence of secondary electrostatic interactions retention mechanism. The monolithic stationary phases are made of neutral acrylate monomers thus yielding monoliths without fixed charges on their surface. The neutral nonpolar monolithic columns exhibited cathodal electroosmotic flow (EOF) over a wide range of pH and acetonitrile concentration in the mobile phase despite the fact that the nonpolar monoliths were devoid of any fixed charges. It is believed that the EOF is due to the adsorption of ions from the mobile phase onto the surface of the monolith thus imparting the neutral column the zeta potential necessary to support the EOF required for mass transport across the monolithic column. Furthermore, the adsorption of mobile phase ions to the neutral monolith modulated solute retention and affected the separation selectivity. The wide applications of the neutral monolithic columns were demonstrated by their ability to separate a wide range of small and large solutes, both neutral and charged. While the separation of the neutral solutes was based on reversed phase retention mechanism, the charged solutes were separated on the basis of their electrophoretic mobility and hydrophobic interaction with the nonpolar ligands of the stationary phase. As a typical result, the neutral monolithic column was able to separate peptides quite rapidly with a separation efficiency of nearly 200 000 plates/m, and this efficiency was exploited in tryptic peptide mapping of standard proteins, e.g., lysozyme and cytochrome C, by isocratic elution.

NOTES:

Thurs. 09:10-09:30

Surfactant Bilayers as Scaffolding for Hydrophilic Coatings in Capillary Electrophoresis

Charles A. Lucy; Amy M. MacDonald, *University of Alberta, Edmonton, Canada*

Analyte adsorption and poor electroosmotic flow control compromise the robustness of CE methods. Capillary coatings can reduce or eliminate these problems. Such coatings can be covalent, semi-permanently adsorbed or formed by dynamic equilibrium with an electrolyte additive. Of these, semi-permanently adsorbed coatings are becoming increasingly popular as they are more cost-effective than covalent coatings and eliminate the need for buffer additives which may affect separation or detection.

However, preparation of coatings based on adsorbed hydrophilic polymers is challenging due to the conflicting demands that the polymer adsorb to the silica surface and yet not interact with analytes such as proteins. This seminar will discuss an alternate adsorption strategy where: a durable supported surfactant bilayer is formed on the capillary; and then the surface of the bilayer is modified with a diblock co-polymer possessing a hydrophilic block.

This approach will be illustrated using the double-chain surfactant dioctadecyldimethylammonium bromide (DODAB) and the diblock copolymer polyoxyethylene (POE) stearate [1]. The capillary is first equilibrated with DODAB vesicles to generate a supported bilayer coating which is stable for 12 days without regeneration [2]. The capillary is next flushed with the polyoxyethylene (POE) stearate, whose hydrophobic block anchors into the bilayer leaving the hydrophilic POE extending into solution. Such coatings are stable (<0.5% RSD over 28 consecutive runs) with a suppressed reversed EOF and efficiencies up to 1.2 million plates/m for model cationic proteins. The characteristics of the coating (e.g., magnitude of the EOF) can be modified by altering the length or nature of the hydrophilic portion of the diblock polymer.

1. A.M. MacDonald and C.A. Lucy, J. Chromatogr. A 2006, 1130, 265-271.
2. M.M. Yassine and C.A. Lucy, Anal. Chem. 2005, 77, 620-625.

NOTES:

Thurs. 09:30-09:50

Approach to Development of Smart Small-Molecule Ligands by Kinetic Capillary Electrophoresis (KCE)

Sergey N. Krylov; Andrei P. Drabovich; Maxim V. Berezovski, *York University, Toronto, Canada*

Small-molecule ligands (molecular weight less than 1000 Da) capable of binding therapeutic and diagnostic targets with high selectivity are used as leads to modern drug candidates and diagnostic probes. Such ligands can be selected from large combinatorial libraries of small molecules for their binding to a target using affinity methods. The development of drugs with predictable pharmacokinetics and diagnostics with desirable characteristics requires smart ligands – ligands with pre-defined binding parameters of interaction with the target. Selection of smart ligands is highly challenging and so far has been achieved only by methods of Kinetic Capillary Electrophoresis (KCE) and only for DNA ligands (aptamers) [1-3]. Selection of smart small-molecule ligands by KCE has been prevented by a fundamental problem associated with the structural identification of the selected ligands. Smart small-molecule ligands can only be selected from very diverse libraries in which every molecule is present in as few as a single copy. On the other hand, the most sensitive method for small-molecule structure determination, mass-spectrometry, requires at least millions of copies. Here we introduce a general approach for selection of smart small-molecule ligands that resolves the above problem. We prove the selection approach experimentally and determine key selection parameters: (i) the efficiency of ligand selection and (ii) the minimum number of steps required for completing the selection. This work further advances KCE and paves the way to the development and practical use of smart small-molecule ligands.

1. Drabovich, A.P.; Okhonin, V.; Berezovski, M.; Krylov, S.N. J. Am. Chem. Soc. 2007, 129, 7262–7261.
2. Drabovich, A.P; Berezovski, M.; Okhonin, V.; Krylov, S. N. Anal. Chem. 2006, 78, 3171–3178.
3. Drabovich, A.; Berezovski, M.; Krylov, S. N., J. Am. Chem. Soc. 2005, 127, 11224–11225.

NOTES:

AM Parallel Session: Detection
Location: Arlington/Berkeley/Clarendon
Chair: Stephen Jacobson

Thurs. 08:00-08:20

Development of an Amine-Reactive Fluorophor having Ph-Independent Fluorescence Properties and Mobilities in the 3 < Ph < 10 Range

Gyula Vigh; Roy Estrada; Ming-Chien Li, *Texas A&M University, College Station, TX USA*

Laser-induced fluorescence (LIF) detection is often used to improve the concentration detection limit in capillary electrophoresis (CE). Though there are many fluorophors that can be excited at the 488 nm line of the argon-ion laser, their fluorescence quantum yield, charge-state and electrophoretic mobility often change when the pH, ionic composition and organic modifier concentration of the background electrolyte is altered. The objective of the work reported here was to develop an amine-reactive fluorophor that (i) did not alter significantly the pI value of the labeled protein, (ii) had pH-independent fluorescence quantum yield in the 3 < pH < 10 range, (iii) had a pH-independent mono-cationic charge in the 3 < pH < 10 range, and (iv) had excitation maximum at 488 nm.

The desired fluorophore was synthesized from 3,6-diamino acridine by (i) varying the substituents of the 3,6-diamino groups, (ii) converting the heteroaromatic nitrogen atom in the 9-position of the acridine ring into a quaternary ammonium group by attaching a propyl sulfonate group, (iii) chlorinating the sulfonate group by oxalyl chloride, (iv) reacting the sulfonyl chloride group with the ethyl ester of N-methyl aminocaproic acid, (v) hydrolyzing the ester group and (vi) converting the carboxylate group into an amine-reactive N-hydroxysuccinimide ester.

The new fluorophore was successfully attached to small-molecule pI markers, amines, oligopeptides and proteins.

NOTES:

Thurs. 08:20-08:40

Droplet Microfluidic Loading of Microcoil NMR: LC-MS+NMR Dereplication of Cyanobacteria Metabolites

Roger A. Kautz[1]; Yiqing Lin[3]; Susie Schiavo[1]; Rose Gathungu[1]; Jimmy Orjala[2]; Paul Vouros[1]

[1]Barnett Institute, Boston, MA; [2]University of Illinois, Chicago, IL; [3]Biogen-Idec, Cambridge, MA

NMR is a challenge in microanalysis because of both its poor limits of detection, relative to mass spectrometry or fluorescence, and because the NMR detector must operate in the inconvenient location of the narrow bore of a high-field magnet. The sensitivity limitation is alleviated by microcoil NMR probes, with observed volumes of 1 µL down to 30 nL and limits of detection of 200 ng down to 30 ng, respectively. However, dead volumes of these NMR probes, with a flow path extending out of the magnet, are 5-30 µL in practice. Segmented flow or "droplet microfluidic" methods – moving samples as a drop of water in oil – can load small samples (1 µL or less) through several meters of capillary, without losses to flowpath surfaces or to dilution during flow injection loading. This "microdroplet NMR" approach has been implemented with a sample handler to transfer samples from 96-well plates into NMR. More generally, the system demonstrates the simplicity of segmented flow collection of a micro- or nano-sample, and the value of lossless transfer through macro-distances to a separate laboratory instrument or device.

An LC-MS-NMR platform was developed to best accommodate the disparity between the mass and time requirements of MS and NMR. Using a novel "nanosplitter" LC-MS interface, the higher sensitivity of nanospray MS is obtained from conventional 4 mm LC columns (100 µg capacity) and 99% of the eluant is collected and concentrated for microdroplet NMR. Good 1D NMR spectra of components at the 0.2% level (200 ng) were acquired in 1 hr/well automated acquisition of the fractions, using a commercially-available microcoil probe. The platform was used to dereplicate a cyanobacterial extract with antituberculosis activity. 4 previously-known compounds were identified; a fifth, unrecognized, compound was prioritized for followup studies.

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Thurs. 08:40-09:00

Development of Pseudo-SDS Dyes for Capillary SDS-PAGE

Shaorong Liu; Shuqing Wu; Joann J Lu; Shili Wang; Kristy L Peck, *University of Oklahoma, Norman, OK USA*

Abstract. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is one of the most commonly used methods for protein analysis. Usually, a staining and de-staining process is involved in order to properly detect the separated protein bands. Capillary electrophoresis (CE) is a miniaturized electrophoresis technique that has many advantages over traditional slab-gel techniques. Unfortunately, when SDS-PAGE is performed in capillaries the staining and de-staining process used in conventional slab gels cannot be simply adopted. In this work, we introduce a novel fluorescent staining dye for capillary SDS-PAGE. The dye consists of a long straight alkyl chain connected to a negative charged fluorescent head, and binds to proteins just as SDS (so-called pseudo-SDS dye). We add some of this dye in the solution when SDS-protein complexes are prepared, resulting in SDS-protein-pseudo-SDS-dye complexes. The number of dye molecules incorporated with a protein depends on the dye concentration relative to SDS in the sample solution, since SDS and dye bind to proteins competitively. The detection sensitivity will thus increase considerably because multiple fluorephors are bond to each protein. In this presentation, we will discuss the synthesis of several pseudo-SDS dyes, and report the results of their performances for capillary SDS-PAGE. Under optimized experimental conditions and using a laser-induced fluorescence detector, limits of detection (LOD) of as low as 0.13 ng/mL (bovine serum albumin) and dynamic ranges over five orders of magnitude in which fluorescence response is proportional to the square root of analyte concentration were obtained. The migration time correlated well with protein size. The dye was also tested for separations of proteins from crude extract of E. coli.

NOTES:

Thurs. 09:00-09:20

Development of CE-Raman Spectrometry and –SPM for the Analysis of Nanofibers (Carbon Nanotube and β-Amyloid)

Masaru Kato; Tatsuhiro Yamamoto, *The University of Tokyo, Tokyo, Japan*

We developed hyphenated analytical systems of CE-Raman spectrometry and CE-scanning probe microscopy (SPM). This system is a powerful tool for the analysis of nanofibers, such as carbon nanotube and -amyloid. The nano scale structures of separated nanofibers were identified by these methods.

Although capillary electrophoresis is an efficient separation method for small compounds, only few separation methods of nano-scale fibers except for nucleic acid chain were reported. A practical analytical method for the fiber structural nanomaterials is desired, because of the expectations for their excellent functions and anxieties for safety of these materials. We developed hyphenated analytical systems of CE-Raman spectrometry and CE-SPM. These two detection methods were powerful methods for the detection and identification of nanofibers. Single nanofibers were large enough for the observation by SPM and Raman spectrometry was often used for the identification of diameter of a single-walled carbon nanotube (SWNT).
b-Amyloid fiber and SWNT were chosen as typical nanofiber analytes. SWNTs are attractive for their electronic and mechanical properties, and b-amyloid is thought to be a pathogenic substance of Alzheimer's disease. Because SWNTs tend to form bundles (or ropes) structure by their strong van der Waals interactions, the individual dispersion of SWNT was required before separation. We found that triphenylene based surfactants were suitable compounds for the preparation of individually dispersed SWNTs solution.
We developed a separation method for SWNTs and b-amyloid. These nanofibers were separated within 30 minutes when Gly-NaOH buffer was used as a separation solution. For the CE-SPM system, a volatile solution, ammonium acetate solution, was used, because precipitation of the buffer solution became the trouble of the SPM observation. It was supposed that the elution order of these fibers were determined by their thickness.

NOTES:

Thurs. 09:20-09:40

Optical Sensing in Microchip Capillary Electrophoresis by Femtosecond Laser Written Waveguides

Rebeca Martinez Vazquez[1]; Roberto Osellame[1]; Marina Cretich[5]; Chaitanya Dongre[3]; Hugo Hoekstra[3]; Hans van den Vlekkert[4]; Roberta Ramponi[2]; Markus Pollnau[3]; Marcella Chiari[5]; Giulio Cerullo[2]

[1]IFN-CNR, Milano, Italy; [2]Politecnico di Milano, Milano, Italy; [3]University of Twente, Enschede, The Netherlands; [4]LioniX BV, Enschede, The Netherlands; [5]ICRM-CNR, Milano, Italy

Capillary electrophoresis separation in an on-chip integrated microfluidic channel is typically monitored with bulky, bench-top optical excitation/detection instrumentation. Optical waveguides allow confinement and transport of light in the chip directing it to a small volume of the microfluidic channel and collecting the emitted/transmitted radiation. However, the fabrication of optical waveguides or more complex photonic components integrated with the microfluidic channels is not a straightforward process, since it requires a localized increase of the refractive index of the substrate.
Recently, a novel technique has emerged for the direct writing of waveguides and photonic circuits in transparent glass substrates by focused femtosecond laser pulses.
In this work we demonstrate the integration of femtosecond laser written optical waveguides into a commercial microfluidic chip. We fabricate high quality waveguides intersecting the microchannels at arbitrary positions and use them to optically address with high spatial selectivity their content. In particular, we apply our technique to integrate optical detection in microchip capillary electrophoresis. Waveguides are inscribed at the end of the separation channel in order to optically excite the different plugs reaching that point; fluorescence from the labelled biomolecules crossing the waveguide output is efficiently collected at a 90° angle by a high numerical aperture optical fiber. The sensitivity of the integrated optical detection system was first evaluated filling the chip with a dye solution, obtaining a minimum detectable concentration of 40 pM.
After dynamic coating of the microchannels with an EPDMA polymer we demonstrate electrophoresis of an oligonucleotide plug with concentration down to 1 nM and wavelength-selective monitoring of on-chip separation of three fluorescent dyes. Work is in progress on separation and detection of fluorescent-labeled DNA fragments, targeting specific, diagnostically relevant regions of a template DNA, for application to the detection of chromosome aberrations.

NOTES:

Closing Plenary Session
Location: Plaza Ballroom
Chair: Gerard Rozing

Thurs. 10:15-10:50

Multi-Dimensional Capillary Liquid Chromatography-Tandem Mass Spectrometry for Phosphoproteome Analysis

Hanfa Zou; Fangjun Wang; Mingliang Ye; Guanghui Han; Xingning Jiang, *National Chromatographic R&A Center, Dalian 116023, China*

Phosphorylated peptides enriched from cancerous human liver are very complex and the amount is always limited. It is important to develop powerful fractionating method to increase the identification of low abundance phosphorylated peptides by decreasing sample complexity. A new approach was established by using off-line multi-dimensional capillary liquid chromatography-tandem mass spectrometry to increase the identification coverage of human liver phosphoproteome analysis. The tryptic digest of human liver extract firstly was pretreated with Ti –IMAC adsorbent, and then the enriched mixture was further fractionated by using a 9 cm × 200 μm i.d. strong anion-exchange (SAX) capillary trap column. The pass-through fraction was loaded onto C18 trap column for capillary liquid chromatography-tandem mass spectrometry analysis, and then the SAX trap column with adsorbed components was further coupled to C18 analytical column for multi-dimensional capillary liquid chromatography-tandem mass spectrometry analysis. The good complementarity for identification of phosphopeptides among the pass-through fraction and adsorbed components with SAX capillary trap column was observed and the total number of the identified phosphopeptides increased 105.6% by comparing to conventional method by directly loading Ti-IMAC enriched fraction onto C18 trap column for capillary liquid chromatography-tandem mass spectrometry analysis. When 500 μg tryptic digest of human liver extract was analyzed by this new approach with 300-min reversed phase gradient separation, totally 876 unique phosphorylated peptides (among which 224 were multiple phosphorylated peptides) and 926 phosphorylated sites were positively identified from 494 phosphorylated proteins at FDR 0.92%.

NOTES:

Thurs. 10:50-11:25

Structured Nanoparticles for Sample Enrichment and Detection

Frantisek Foret, *Institute of Analytical Chemistry of the ASCR, v.v, Brno, Czech Republic*

The classic talk that Richard Feynman gave on December 29th 1959 at the annual meeting of the American Physical Society at the California Institute of Technology (http://www.zyvex.com/nanotech/feynman.html) is often mentioned as one of the impulses for the development of nanotechnologies. Many of the ideas and envisioned processes have already materialized, especially in electronics and engineering, others, mainly related to the biosciences, are at the forefront of the current research. A number of relatively distinct research directions, ranging from chemical synthesis, colloid and polymer chemistry, self-assembled monolayers to semiconductors are now combined with new scientific knowledge into a variety of nanotechnology initiatives. The potential of nanotechnology related materials and processes is also often investigated for use in analytical and bioanalytical applications where the structures on the submicrometer scale bring unique properties providing basis for faster, more sensitive and/or more selective chemical analyses. In this work we have explored some of the metal and semiconductor based nanostructures for use in chemical sensors, specific binding for sample preconcentration and selective fluorescence tagging. In one part thin metal films with surface modifications were explored as selective electrodes capable of monitoring protein structural transitions. Microfabrication and colloid chemistry protocols were applied to prepare of multiphasic columns for peptide enrichment. Similarly, magnetic nanofilms were tested for formation of oriented beds of flow through sample reactors. Finally, semiconductor quantum dots were tested as universal and selective tags for electrophoretic separations and cell imaging.

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Thurs. 11:25-12:00

Polymers in DNA Sequencing and Purification on Microfluidic Devices

Annelise E. Barron; Daniel Hert; Ryan E. Forster; Brian E. Root; Jennifer A. Coyne, *Stanford University, Stanford, CA USA*

Water-soluble polymers play critical roles in DNA separations by microchannel electrophoresis. Genetically engineered protein polymers tethered to sequencing fragments serve as drag-tags that deliver > 250 contiguous DNA bases in free solution. Certain poly(N,N-dimethylacrylamide) (pDMA) matrices combined with a poly(N-hydroxyethylacrylamide) wall coating give 600-base read lengths on a microchip in < 7 minutes. This speed record for long-read sequencing is made possible by a hybrid separation mechanism that melds both reptation and transient entanglement coupling, giving extremely fast migration, narrow bands and good dependence of DNA mobility on size. Further optimized blends of high and low molar mass pDMAs reduce viscosity by 80% with no sacrifice in read length. We have also developed hydrophobically modified polyacrylamide (HMPAM) copolymers for on-chip DNA sequencing; these copolymers are 99.9% acrylamide and just 0.1% N,N-dihexylacrylamide, the latter in small isolated blocks. Physically crosslinked HMPAM networks provide a 10% increase in read length in a microfluidic device relative to linear polyacrylamide of matched molar mass (550 vs. 500 bases) in ~10 minutes. HMPAMs can also be used to purify DNA and RNA away from proteins such as serum albumin for the processing of a crude a biological sample. Hydrophobic blocks adsorb unwanted proteins during electrophoresis, more and more effectively as block size increases. Another way to purify genetic material away from unwanted elements of a complex biological sample such as serum or whole blood is to use a polymer “capture matrix” that selectively hybridizes genetic material, which is then eluted from the matrix and used downstream for efficient PCR. We show that capture networks can be used to purify both ssDNA and RNA from 10% serum; we detect purified PCR products by chip electrophoresis. These polymer and copolymer materials can be “mixed and matched” to add unique, modular functionalities to integrated microfluidic genetic analysis devices.

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