2019

Global CESI-MS Symposium

La Jolla, California, USA | October 21-22, 2019

2019 Global CESI-MS Symposium

The Scripps Research Institute
La Jolla, California, USA | October 20-21, 2019

Hosts and Symposium Chairs:

Professor Jennifer Van Eyk
Cedars-Sinai Medical Center, USA

Professor John Yates III
The Scripps Research Institute, USA

Session Chairs:

Professor Neil Kelleher
Northwestern University, USA

Professor Herbert Lindner
Innsbruck Medical University, Austria

Assistant Professor Rob Haselberg
Vrije Universiteit, Amsterdam, NL

Dr. Spencer Walse
USDA, USA

Sponsored by SCIEX

October 21st – Pre-Symposium Workshop, Novel & Advanced Applications

Time	Description
07:00 – 17:00	**Registration**
07:00 – 08:00	**HealthNut Continental Breakfast Bar** – *Covered Patio*
08:00 – 12:00	**Pre-Symposium Workshop –** For laboratory researchers who want to familiarize themselves with the technique, related tips and tricks, in an environment open to questions and discussion.
08:00 – 08:45	**Theory of CESI-MS**
08:45 – 09:30	**Basic CESI Operation and Maintenance**
09:30 – 09:50	**Break**
09:50 – 10:20	**Peptide Analyses**
10:20 – 10:50	**Intact Protein Analyses**
10:50 – 11:20	**Small Molecule Analyses**
11:20 – 12:00	**Tips and Tricks for Success**
12:00 – 13:00	**Lunch**
13:00 – 13:15	**Welcome and Introduction** *Professor Jennifer Van Eyk, Cedars-Sinai Medical Center, USA* *Professor John Yates III, The Scripps Research Institute, USA*
13:15 – 16:45	**Novel & Advanced Applications** | *Session Chair: Professor Neil Kelleher*
13:15 – 13:45	**Imaging Laser Induced Fluorescence Detection at the Taylor Cone of Electrospray Ionization Mass Spectrometry** *Professor Andras Guttman, University of Debrecen, Hungary*
13:45 – 14:15	**Development of Capillary Electrophoresis Electrospray Ionization Mass Spectrometry Methods and Hardware for Spaceflight Missions of Exploration** *Dr. Fernanda Mora, NASA Jet Propulsion Laboratory, USA*
14:15 – 14:45	**CESI-MS of Small Molecules for Agricultural and Environmental Science** *Dr. Spencer Walse, USDA, Agricultural Research Service, USA*
14:45 – 15:45	**Poster Session**
15:45 – 16:15	**Metabolic Profiling of Low Numbers of Mammalian Cells by CESI-MS** *Assistant Professor Rawi Ramautar, Leiden Academic Center for Drug Research, The Netherlands*
16:15 – 16:45	**Single Cell Analysis by CESI-MS** *Dr. Jolene Diedrich, The Scripps Research Institute, La Jolla, USA*
16:45 – 16:55	**Concluding Remarks – Day One** *Professor Jennifer Van Eyk, Cedars-Sinai Medical Center, USA* *Professor John Yates III, The Scripps Research Institute, USA*

NETWORK WITH YOUR PEERS

October 21st, beginning at 18:00

Join us for a networking reception in the grove, at the beautiful La Jolla Sheraton Hotel.

13:15 – 13:45

Professor Andras Guttman, Mate Szarka,[1] Marton Szigeti,[1,2] Horváth Csaba[1]

1. Memorial Laboratory of Bioseparation Sciences, University of Debrecen, Hungary
2. Translational Glycomics Laboratory, University of Pannonia, Hungary

Imaging Laser Induced Fluorescence Detection at the Taylor Cone of Electrospray Ionization Mass Spectrometry

INTRODUCTION: Laser induced fluorescence detection (LIF) is a powerful tool for the quantitative analysis of fluorescent molecules, widely used in glycan analysis with fluorophore labeled carbohydrates where each species has a common response factor. Electrospray ionization mass spectrometry (ESI-MS), on the other hand, while revealing important structural information about individual analytes, generally can have different response factors for different species. Integration of CE and electrospray ionization (ESI) into a single dynamic process, i.e., within the same device (CESI) offers the capability of performing CE separation at ultra-low nanoliter flow rates, resulting in reduced ion suppression and improved sensitivity for MS detection; however, still missing a simultaneous optical detection option.

METHODS: All capillary electrophoresis analyses were performed on a CESI 8000 Plus system using simultaneous laser induced fluorescence (LIF) and mass spectrometry (QTRAP® 6500+) detection (all from SCIEX). CESI-MS separations were achieved using an OptiMS bare fused-silica capillary cartridge by the application of -30 kV and low pressure (2 - 5 psi). Acetic acid and low pH ammonium ammonium acetate buffers were used as background electrolytes (BGE). N-Glycans were released by PNGase-F digestion and labeled with aminopyrenetrisulfonate (APTS) for both CE-LIF and CE-MS analysis using the Fast Glycan Sample Preparation and Analysis kit (SCIEX, Brea, CA).

PRELIMINARY DATA: For simpler and improved quantitation with ESI-MS, laser induced fluorescent images were collected at the Taylor cone of the electrospray interface, enabling simultaneous and robust optical (quantitative) and MS (qualitative) detection of fluorophore labeled sugars. The performance of this universally applicable, interface design independent imaging laser induced fluorescent (iLIF) system was demonstrated using CE-ESI-MS in the analysis of labeled linear and branched glycans. The limit of detection (LOD) of the iLIF system was in this case 40 attomole. The intra- and inter-day quantitative (peak area) reproducibilities of the system (RSD) were 4.15% and 6.79%, respectively.

NOVEL ASPECT: A novel and universally applicable system is introduced capable to support imaging laser induced fluorescence detection at the Taylor cone of ESI-MS.

13:45 – 14:15

Dr. Fernanda Mora, A. Noell, K. Zamuruyev, M. S. Ferreira Santos, E. A. Kurfman, P. Willis

NASA Jet Propulsion Laboratory, USA

Development of Capillary Electrophoresis Electrospray Ionization Mass Spectrometry Methods and Hardware for Spaceflight Missions of Exploration

INTRODUCTION: Planetary exploration. A powerful approach in this search involves seeking biochemical signatures of life at the molecular level, as distributions of organic molecules. To date, *in situ* organic analysis on extraterrestrial settings has relied on gas-phase chromatography methods, which have limitations for the detection of organic molecules in samples containing water and/or high concentration of salts. Capillary electrophoresis coupled to electrospray ionization mass spectrometry (CESI-MS) overcomes the limitations of gas-phase techniques and holds unique promise in the search for signatures of life on other worlds.

METHODS: Separations were performed on a CESI 8000 High Performance Separation - ESI Module from SCIEX, Brea, CA, USA using a silica surface cartridge (PN B07367) with a 30 µm ID x 90 cm total length capillary. We are developing chemical methods that would allow the detection of organic biosignatures in samples containing high amounts of salts like the ones expected to be found on future missions to ocean worlds. In particular, we focus on salts relevant to a possible mission to Europa, sodium chloride and magnesium sulfate. A standard mixture containing amino acids, di- and tri-peptides, nucleobases, and nucleosides was used for the optimization of the methods.

PRELIMINARY DATA: We evaluated the performance of a set of background electrolytes (BGE) based on acetic acid. In all cases the signal of a sample containing salt was compared to the signal for a sample without added salt. Concentration of salts varied from 0.1 M to up to 3 M, depending on the salt. It was found that the loss in signal due to the presence of salts was lower when using acetic acid 5 M as the BGE. Also, the reduced signal was independent of the identity of the salt. We have also developed a miniaturized and automated CE instrument that was coupled to MS and contactless conductivity detection (C4D). The system was optimized and validated by analyzing relevant natural samples and was operated successfully at a feld location, demonstrating its portability and robustness.

14:15 – 14:45

Dr. Spencer Walse,[1] Erik Rivera,[1] Jonathon Powell,[1] Wiley Hall[2]

1. USDA, Agricultural Research Service, USA
2. Safe Food Alliance, USA

CESI-MS of Small Molecules for Agricultural and Environmental Science

INTRODUCTION: This work addresses the need to quantify residues of highly polar pesticides, which is of great interest for a number of reasons including: highly publicized concerns about health effects (i.e. – glyphosate, sulfites), MRL violations caused by biogenic and anthropogenic interferents (i.e. – chlorate), and the large amounts of these compounds that are used each year. Moreover this work addresses the need to characterize the signaling molecules used by agricultural pests with as little sample handling and manipulation as possible. Often, the analytical processing of natural products changes the form and function of the chemical signal.

METHODS: Tree nut matrices, fortified with highly polar pesticides, such as glyphosate, sulfite, and chlorate were extracted using a modified version of the QuPPe and analyzed with CESI-MS/MS using a variety of different background electrolytes as well as bare and neutral coated capillaries. CESI-MS/MS was also used to characterize the chemical constituents in the secretion of the sugar cane spittlebug, with little to no sample preparation.

PRELIMINARY DATA: CESI-MS/MS has been found to be highly effective for the analysis of charged pesticides in "difficult" tree nut matrices including glyphosate, AMPA, glufosinate, fosetyl-Al, phosphonic and phosphoric acids, ethephon, paraquat, diquat, mepiquat, chlormequat and most recently sulfite, sulfate, and chlorate - all within a single run (3 different methods with 3 different columns are required to quantitate each of these compounds with LC-MS/MS).

NOVEL ASPECT: CESI-MS/MS has revolutionized the spectrometric analysis of highly polar small molecules of agricultural and environmental significance.

15:45 – 16:15

Assistant Professor Rawi Ramautar

Leiden Academic Center for Drug Research, The Netherlands

Metabolic Profiling of Low Numbers of Mammalian Cells by CESI-MS

INTRODUCTION: The analytical techniques commonly used in metabolomics require relatively large amounts of biological material, in particular for sample preparation and injection. To address these questions with a metabolomics approach, the development of new microscale analytical techniques and workflows is needed. Here, the utility of capillary electrophoresis (CE) hyphenated to mass spectrometry (MS) via a sheathless porous-tip nanospray interface is shown for the global profiling of basic and acidic metabolites in low numbers of mammalian cells.

METHODS: Sheathless interfacing of a CESI 8000 instrument (SCIEX) and a high-end TOF-MS instrument was achieved via a capillary of which the outlet section was etched with hydrofluoric acid, providing an outer terminus porous to the electric transport of small ions. The separation capillary with this porous tip was placed in a stainless steel ESI needle filled with static conductive liquid. ESI was performed in positive and negative ionization mode (1.4 kV) and 10% acetic acid (pH 2.2) was used as background-electrolyte.

PRELIMINARY DATA: The use of CESI-MS with an injection volume of circa 42 nL resulted in subnanomolar detection limits for a wide range of basic metabolites, including amino acids, amines, nucleosides and small peptides. A unique feature of the proposed method is that it allows the profiling of both basic and acidic metabolites using a single capillary/buffer combination by only switching the polarity of MS detection and of the CE separation. CESI-MS could be effectively used for global metabolic profiling of HepG2 cells when using a starting amount of 10,000 down to 500 cells only, which corresponded to the injection content of 5 HepG2 cells to less than one cell.

NOVEL ASPECT: CESI-MS method for highly efficient and sensitive profiling of acidic and basic metabolites in low numbers of mammalian cells.

16:15 – 16:45

Dr. Jolene Diedrich, Professor John Yates III

The Scripps Research Institute, USA

Single Cell Analysis by CESI-MS

INTRODUCTION: As interest in single cell analysis increases, performing single cell analysis by MS still remains a challenge. We propose the use of CESI as an alternative to LC to increase sensitivity of the system and provide a more in-depth analysis.

METHODS: Analysis was carried out on a CESI 8000 which was coupled to a Bruker timsTOF and to a Thermo Fusion Lumos, both operating in data dependent modes. Hela lysate was injected at decreasing amounts to determine the sensitivity of the systems and provide information on the feasibility of single cell analysis by CESI-MS by label free analysis vs. TMT analysis.

PRELIMINARY DATA: CESI-MS with the integrated sheathless interface allowed increased sensitivity relative to LC-MS. Lower sample consumption for CE vs. LC while maintaining overall signal intensity by MS detection allows analysis to be performed with decreasing sample loads. The sensitivity of the Thermo Fusion Lumos and the Bruker timsTOF allows analysis of samples that are in the ng sample range. We demonstrate here the coupling of the CESI further increases the sensitivity of the analysis and we can obtain data with sample loading that is comparable to single cell analysis. Further optimization with TMT and a carrier channel can increase sensitivity further.

NOVEL ASPECT: Single cell analysis by CESI-MS

October 22nd – Biologics and Proteomics

Time	Description
07:00 – 13:00	**Registration**
07:00 – 08:00	**Del Mar Breakfast** – *Covered Patio*
08:00 – 08:15	**Welcome and Introduction** *Professor Jennifer Van Eyk, Cedars-Sinai Medical Center, USA* *Professor John Yates III, The Scripps Research Institute, USA*
08:15 – 09:45	**Biologics** | *Session Chair: Professor Herbert Lindner*
08:15 – 08:45	**Is the Signature Peptide for Quantification a Thing of the Past? Using CESI-MS to Quantify mAbs with a Pseudo Intact or Intact Approach** *Richard Snell, GSK, UK*
08:45 – 09:15	**Charge Variant Analysis by CESI-MS** *Dr. Fang Wang, SCIEX, USA*
09:15 – 09:45	**CESI-MS for the In-depth Characterization of (Heavily) Glycosylated Proteins** *Assistant Professor Rob Haselberg, Vrije Universiteit, Amsterdam, NL*
09:45 – 10:30	**Poster Session**
10:30 – 12:30	**Proteomics** | *Session Chair: Assistant Professor Rob Haselberg*
10:30 – 11:00	**Sheathless Capillary Zone Electrophoresis For Top-down and Bottom-up Proteomics** *Professor John Yates III, The Scripps Research Institute, USA*
11:00 – 11:30	**Enhancing Proteomic Throughput by CESI-MS** *Dr. Klaus Faserl, Innsbruck Medical University, Austria*
11:30 – 12:00	**Ultra-Sensitive Glycomic and Proteomic Profiling of Limited Biological and Clinical Samples Using CZE Coupled to Mass Spectrometry via CESI Interface** *Associate Professor Alexander Ivanov, Northeastern University, USA*
12:00 – 12:30	**Ultrasensitive Determination of Modified Nucleotides by Sheathless Interfaced Capillary Electrophoresis-Tandem Mass Spectrometry** *Professor Xin-Xiang Zhang, Peking University, China*
12:30 – 13:30	**Tapatio Lunch and Poster Session**
13:30 – 15:30	**Proteomics** | *Session Chair: Dr. Spencer Walse*
13:30 – 14:00	**Native Capillary Electrophoresis Top-down Mass Spectrometry for Characterization of Proteoforms and Their Complexes** *Professor Neil Kelleher, Northwestern University, USA*
14:00 – 14:30	**Advances in the Separation of Co- and Post-translational Protein Modifications Using CE-MS** *Professor Herbert Lindner, Innsbruck Medical University, Austria*
14:30 – 15:00	**Uncovering Proteomic Changes During Embryonic Neural Induction Through Ultrasensitive Mass Spectrometry** *Dr. Aparna B. Baxi, University of Maryland, USA*
15:00 – 15:30	**Next Stage Personalized Medicine: Automation and High Content Proteomics** *Professor Jennifer Van Eyk, Cedars-Sinai Medical Center, USA*
15:30 – 16:00	**Break & Poster Teardown**
16:00 – 16:30	**Poster Awards –** *Assistant Professor Rob Haselberg, Vrije Universiteit, Amsterdam, NL*
16:30 – 16:45	**2020 Announcement**
16:45 – 17:00	**Closing Remarks and Adjourn** *Professor Jennifer Van Eyk, Cedars-Sinai Medical Center, USA*

08:15 – 08:45

Richard Snell

GSK, UK

Is the Signature Peptide for Quantification a Thing of the Past? Using CESI-MS to Quantify mAbs with a Pseudo Intact or Intact Approach

INTRODUCTION: TOF-MS can provide more than just small molecule quantitation to the modern bioanalytical lab, in a time when molecules are becoming more diverse, and the needs of project are moving beyond what standard approaches can supply, can HR-MS provide a solution? TOF-MS is a common tool in peptide mapping and characterisation, but coupled with capillary electrophoresis (CE), HR-MS offers a robust and sensitive solution for quantitative bioanalysis of monoclonal antibodies (mAbs) using a pseudo intact approach rather than using signature.

METHODS: CESI-MS together with a neutrally coated capillary was used to analyse samples injected electrokinetically. Background electrolytes containing acetic acid combined with a stacking injection approach, which used ammonium acetate, were used to separate proteins under a split pressure normal phase separation. Proteins were detected using TOF-MS.

PRELIMINARY DATA: Using a pseudo intact approach rather than using signature peptides CESI-MS has been shown to provide representative quantitative data, as well as potentially providing information about post-translational modifications (PTMs) in tox samples, that may affect activity.

By adjusting the ammonium acetate stacking concentration and injection conditions CESI run times have been reduced to less than 18 minutes. Separation of mAb fragments has been maintained by using a twostep pressure approach in the separation. Migration times and peak areas have been shown to be very reproducible aided by using mineral oil which eliminated evaporation effects on the sample.

NOVEL ASPECT: CESI-MS of Intact Protein Quantitation

08:45 – 09:15

Dr. Fang Wang

SCIEX, USA

Charge Variant Analysis by CESI-MS

INTRODUCTION: Comprehensive characterization of the charge variants in a mAb population is crucial as these variants reportedly affect the safety and efficacy of the biotherapeutics. However, there are several challenges using traditional RP-LC-MS for charge variant analysis of intact mAbs. A single peak is usually observed and the MS spectrum and deconvoluted MS spectra is dominated by the major species, so minor variants with critical post-translational modifications (PTMs) cannot be differentiated and identified in the RP-LC-MS workflow. The CESI-MS allows separation and identification of charge variants in a single workflow.

METHODS: CESI 8000 equipped with Neutral OptiMS cartridge and connected with SCIEX TripleTOF 6600 allows the separation of intact mAb charge variants and identification of the proteins at the intact level in one assay.

PRELIMINARY DATA: We were able to use a single workflow that not only provides separation and allows direct identity assignment of charge variants peaks, but also allows detection of low molecular weight (LMW) fragment impurities in the sample. We were also able to demonstrate the capability of the workflow using two different intact mAbs that have basic and neutral isoelectrical points.

09:15 – 09:45

Assistant Professor Rob Haselberg,[1] Valeriia O. Kuzyk,[1,2] Guinevere S.M. Kammeijer,[2] Gabriella Krištapavičiūtė,[1] Robert L. C. Voeten,[1] Manfred Wuhrer,[2] Govert W. Somsen[1]

1. Vrije Universiteit Amsterdam, Division of Bioanalytical Chemistry, Amsterdam, The Netherlands
2. Leiden University Medical Center, Center for Proteomics and Metabolomics, Leiden, The Netherlands

CESI-MS for the In-depth Characterization of (Heavily) Glycosylated Proteins

INTRODUCTION: Glycosylation is probably the most common, but also most complex, post-translational modification. In the biopharmaceutical field glycosylation is considered a critical quality attribute; changes in the protein's glycan composition might significantly impact the product's pharmacokinetics and –dynamics. On the other hand, protein glycosylation is known to rapidly change in certain diseases and can, subsequently, be a marker of progression. Not surprisingly, in-depth profiling of glycosylation has gained significant attention over the years. In this playing field, capillary electrophoresis coupled to mass spectrometry (CE-MS) has established itself as a key technology. It offers integration of the separation and detection required to distinguish structurally related protein glycoforms on the intact, glycopeptide, and released glycan level.

In this presentation we will show our efforts to bring CESI-MS to the desired performance characteristics, enabling detailed biopharmaceutical analysis and characterize potential disease ambassadors. For example, it will be shown that low flow (~5 nL/min) CESI-MS provides significantly increased ionization efficiencies and decreased ionization suppression for intact proteins, leading to unprecedented limits of detection down to 200 pM. Low-flow CESI-MS showed particularly useful for detailed glycolprofiling of intact pharmaceutical proteins, like the heavily glycosylated recombinant human erythropoietin. Over 70 different glycoforms and more than 300 proteoforms could be assigned in a single run.

Our work into disease ambassadors focused on the glycopeptide analysis of carcinoembryonic antigen (CEA). This protein, and especially its glycosylation profile, might be related to progression of colorectal cancer. Due to the heavy glycosylation, a combined protease digestion that resulted in a single N-glycosylation site per peptide was required to enable proper data interpretation. Analyses were performed with CESI-MS and with the addition of a dopant enriched nitrogen gas enabling us to reach to reach biologically relevant detection limits (250 ng/mL prior to capturing from matrix). The overall profile revealed known cancer related signatures, such as high level of branching in complex N-glycans and abnormally high fucosylation. Moreover, the distribution of the N-glycan species appeared to be site-specific with some glycosylation sites offering more microheterogeneity than others and some potential N-glycosylation sites appearing to be non-glycosylated. Overall, these complex profiles could bear a potential diagnostic value.

As a final point, the addition of trapped ion mobility mass spectrometry, or TIMS, to the CESI-MS workflow will be discussed. Practical considerations will be shared next to showing some initial results. For example, using the gas phase trapping and separation power of TIMS allowed us to investigate the stability of individual glycoforms on the intact protein level. Moreover, released labeled glycans could be separated and collisional cross sections derived.

10:30 – 11:00

Professor John Yates III, Fabio Gomes, Dr. Jolene Diedrich

The Scripps Research Institute, USA

Sheathless Capillary Zone Electrophoresis For Top-down and Bottom-up Proteomics

INTRODUCTION: The comprehensive characterization of proteins is a difficult process. Proteins are large molecules with the potential for many types of post-translational modifications. In addition to the identification of modifications to proteins it is the patterns of modification that can be critical for understanding function and regulation. Seminal plasma is a critical and complex fluid that carries sperm to eggs to initiate the fertilization process. The identification and characterization of proteins and post-translational modifications (PTMs) in this biological fluid is crucial to understand sperm function and fertilization process. Here, we present a top-down mass spectrometry (TDMS) strategy for identifying PTMs in seminal plasma from bulls and a bottom-up proteomics (BUP) approach for high-throughput analysis of seminal plasma proteins from bulls with contrasting frozen-thawed sperm. Our TDMS approach uses size exclusion (SEC) prior to capillary zone electrophoresis (CZE) and liquid-chromatography (LC), both assisted by EThcD and 213 nm UVPD while the BUP approach uses CZE and LC coupled with trapped ion mobility spectrometry (TIMS) and time of flight (TOF) for high-throughput analysis of seminal plasma proteins from bulls with contrasting frozen-thawed sperm viability.

METHODS: For TDMS, seminal plasma proteins from bulls were quantified and fractionated via SEC. Fractions were reduced, alkylated, and desalted for CZE-MS and LC-MS analysis. All experiments were performed on a Lumos mass spectrometer. Raw files were processed using ProSightPD 3.0 (Thermo Scientific).

For BUP, the percentage of viable sperm was determined in frozen-thawed semen samples from 10 Holstein bulls via flow cytometry. Seminal plasma proteins were precipitated and digested prior to CZE-timsTOF and LC-timsTOF analysis. All experiments were performed on a timsTOF (Bruker) mass spectrometer. A CESI-MS adapter (SCIEX) was used for interfacing the CZE with the timsTOF mass spectrometer. The Integrated Proteomics Pipeline Software was used for data analysis.

All CE experiments were performed on a CESI-8000 using a neutral OptiMS capillary cartridge (SCIEX).

PRELIMINARY DATA: Given the different separation mechanisms of CZE and LC, combining the two data set resulted in a high number of identifications. Both EThcD and UVPD produced satisfactory dissociation of intact protein ions. UVPD provided a higher number of identifications while EThcD returned better scoring metrics and sequence coverage. For BUP, over 200 proteins were identified where some were found to carry acetylated and phosphorylated groups as well as GG tags, which is a strong indicator of ubiquitinated proteins in this biological fluid. Unsurprisingly, we observed the separation between some unmodified and modified peptides as well as the satisfactory resolution between some isobaric peptides. The sheathless CZE-timsTOF approach resulted in tandem MS spectra with significant low background noise that facilitated the identification of modified peptides.

NOVEL ASPECTS: The first application of both TDMS for the characterization of PTMs and CZE-timsTOF via BUP for the identification of proteins in seminal plasma from bulls.

11:00 – 11:30

Dr. Klaus Faserl,[1] Bettina Sarg,[1] Andrew J. Chetwynd,[2] Herbert Lindner[1]

1. Innsbruck Medical University, Austria
2. School of Geography Earth and Environmental Sciences, University of Birmingham, UK

Enhancing Proteomic Throughput by CESI-MS

INTRODUCTION: With the rising demands on comprehensiveness, sensitivity and accuracy, modern proteomics studies have become very time-consuming endeavors often taking several weeks. Costs for operating mass analyzers together with the manpower needed are rising and it becomes timely to fully optimize and properly characterize the approach regarding the proteomic depth expected. In this context, the current work demonstrates the potential of sequential injection of samples in CE-MS for highly efficient and sensitive proteome characterization.

METHODS: Proteins were extracted from human lymphoblastic T-cells, enzymatically digested, and the resulting peptides fractionated by RP-HPLC. Twenty fractions were thereafter analyzed by CE-MS within a single MS analysis. The CE-MS method was designed so that every 10 minutes a new fraction was injected into the CE system. Without any rinsing or equilibration steps we were able to generate a continuous stream of peptides feeding the mass analyzer.

PRELIMINARY DATA: In 250 min, the total analysis time of a single sequential injection experiment, we were able to identify roughly 28.000 peptide sequences counting for 4.800 proteins. High total ion current was observed between 15 and 240 min, which indicates peptides continuously entering the MS for 3.75 h. The numbers of characterized analytes could be increased to 62.000 peptides and more than 6.100 proteins identified, when analyzing a total of 60 fractions, all within 12.5 hours. This result clearly shows the high separation efficiency during RP-HPLC prefractionation and reveals the possibility to boost proteome coverage by increasing the number of fractions analyzed when needed.

NOVEL ASPECT: Sequential sample injection in CE-MS allows balancing proteomic depth with rapid throughput which is essential for all complex proteomic studies.

11:30 – 12:00

Associate Professor Alexander Ivanov,[1] Anne-Lise Marie,[1] Kendall Johnson,[1] Marcia Santos,[2] Somak Ray,[1] Amanda Figueroa-Navedo,[1] James C. Kostas,[1] Helen Gandler,[3] Shulin LU,[4] John Tigges,[4] David A. Frank,[3] Ionita Ghiran[4]

1. Northeastern University, USA | 2. SCIEX, USA | 3. Dana-Farber Cancer Institute, USA
4. Division of Allergy and Inflammation, Beth Israel Deaconess Medical Center and Harvard Medical School, USA

Ultra-Sensitive Glycomic and Proteomic Profiling of Limited Biological and Clinical Samples Using CZE Coupled to Mass Spectrometry via CESI Interface

INTRODUCTION: Deep proteomic profiling of limited samples (e.g., extracellular vesicles (EVs), rare cells) and especially, characterization of post-translational modifications (PTMs) of such specimens have been a major challenge because of very low abundance and high heterogeneity in biological matrices. EVs are submicron membrane-enclosed blebs released from cells into extracellular space and biofluids. EV isolates appear attractive for the development of novel diagnostic and therapeutic strategies. Proteomic and glycomic profiling of rare cells such as circulating tumor cells is important to elucidate the biology of the cells and gain critical information about the disease status. To overcome the challenges associated with the analysis of such limited biological samples, we developed highly sensitive methods using CZE-MS and the CESI interface.

METHODS: CZE-MS analyses were performed using a commercial sheathless interface (CESI 8000 Plus, SCIEX), and QE or QE Plus mass spectrometers (Thermo Fisher Scientific). Released N-glycans were analyzed by CZE-MS using bare fused-silica capillaries (SCIEX). Key CZE and MS parameters were optimized using dextran ladder standards and subsequently evaluated for glycan profiling of human serum IgG, plasma EV isolates and HeLa and OCI-AML5 cancer cells. Digests of lysed HeLa and OCI-AML5 cells were analyzed by transient isotachophoresis-CZE coupled to MS using neutral-coated capillaries and positive ESI mode. CZE-MS data were processed using GlycReSoft, SimGlycan, and Proteome Discoverer programs.

PRELIMINARY DATA: To control the microenvironment of the nano-ESI interface, we evaluated activated carbon-purifed air and dopant-enriched nitrogen (DEN)-gas introduced into the CZE-ESI interface region. The combination of DEN-gas with optimized levels of in-source collision-induced dissociation (CID) and the optimized temperature of the ion transfer tube led to a ~70-fold improvement in the detection sensitivity for numerous glycans. The optimized CZE-MS conditions allowed for the separation of closely-related and isobaric glycan species. The analysis of total serum IgG isolates resulted in the detection of more than 200 N-glycans for an injected amount equivalent to ~3 nL of serum. The analysis of total EV isolates, using sample amounts equivalent to ~120 nL of plasma, led to the detection of over 110 non-redundant N-glycans. Approximately 50% of the detected N-glycans were structurally characterized by MS/MS using higher energy collision-induced dissociation (HCD). Diagnostic fragment ions derived from both glycosidic and cross-ring cleavages enabled identification of antennary structures and α-2,3- and α-2,6-sialic acid linkages. Initial N-glycan profiling of three EV subpopulations fractionated by nano-flow cytometry was conducted and showed quantitative differences in glycomic profiles, and also resulted in detection of low abundant species that could not be detected in total EV isolates. Initial N-glycan profiling of OCI-AML5 cancer cells from an injected amount equivalent to ~35 cells demonstrated qualitative and quantitative differences between cells exposed to cytokine treatments used to alter activation and differentiation states of the cells. In particular, variations in fucosylation levels were observed. Proteomic profiling of ~20 ng HeLa digests routinely yielded identification of >4,000 peptides and >1,000 protein groups. CZE-MS demonstrated an advantage over nanoLC-MS in detection of modified peptides, specifically phosphorylated and acetylated.

NOVEL ASPECT: Increased detection sensitivity and depth of glycomic and proteomic profiling of limited samples in comparison to conventional techniques.

12:00 – 12:30

Professor Xin-Xiang Zhang

Peking University, China

Ultrasensitive Determination of Modified Nucleotides by Sheathless Interfaced Capillary Electrophoresis-Tandem Mass Spectrometry

INTRODUCTION: A label-free ultrasensitive method for the simultaneous determination of RNA modified nucleotide was first established . The perfect performance of this system was demonstrated to identify and quantify eight important and functional RNA modifications, including N6-methyladenosine (m6A), N1-methyladenosine (m1A), 2′-O-methyladenosine (Am), 5-methylcytidine (m5C), 2′-O-methylcytidine (Cm), 2′-O-methylguanosine (Gm), pseudouridine (ψ) and 2′-O-methyluridine (Um). Furthermore, a series of hydrazine labeling reagents were discovered for the derivatization of rare modified nucleotides, such as 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), to further improve the detection sensitivity. Each of the labelling reagents harbors have good reaction activity and ionization efficiency.

METHODS: Sheathless CE-MS/MS system with the multiple reaction monitoring (MRM) mode.

PRELIMINARY DATA: All these eight RNA modifications could be easily detected by using about 500 pg total RNA samples and 5 ng mRNA samples without any sample pretreatment steps, derivation steps or signal enhancing methods, which was the most sensitive method compared to other reported methods. 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), to further improve the detection sensitivity with our designed new labeling reagent.

13:30 – 14:00

Professor Neil Kelleher, Kevin Jooss, Phil Compton, Jack McGee, Ben Des Soye

Northwestern University, USA

Native Capillary Electrophoresis Top-down Mass Spectrometry for Characterization of Proteoforms and Their Complexes

INTRODUCTION: With the advances in protein research, the demand for analytical tools within the field of proteomics is steadily increasing. In recent years, CE-MS has gained significant interest in the scientific and industrial community, due to its advantageous separation principle for intact protein analysis. Top-down mass spectrometry (TDMS), as an emerging proteomics approach, provides an in-depth characterization of post-translational modifications and proteoforms. In addition, native separation can also be achieved using CE, enabling the characterization of proteins and related interactions in their unaltered, biologically functional states. Therefore, native CE in combination with TDMS represents a promising concept to face and overcome current and future challenges associated with protein analysis.

METHODS: A CESI 8000 Plus system was hyphenated to a custom Q Exactive Orbitrap HF MS with Extended Mass Range for native CE-TDMS analysis. Neutral polyacrylamide-coated (PAM) capillaries were used in combination with ammonium acetate based background electrolytes to enable electromigration under native conditions. Capillaries were conditioned and equilibrated according to the standard protocols provided by SCIEX. High voltage of up to +20 kV was used for separation. Additionally, minor pressure (up to 5 psi) was applied during separation to ensure stable and robust ionization. TD analysis was performed using higher-energy collisional dissociation (HCD) for fragmentation.

PRELIMINARY DATA: Initially, a standard protein mix was characterized. The observed low charge states and protein complexes were similar compared to native direct infusion (DI)1. For example, only two major low charge states (9/10+) of carbonic anhydrase were observed and pyruvate kinase (PK) was detected as a non-covalent tetramer complex. The concentration sensitivity was similar if not better than native DI. For instance, a signal-to noise ratio improvement factor of ~11 was achieved for the PK tetramer1. In this way, absolute amounts of proteins in the amol range could be detected. Later on, the native CESI-(TD)MS system was expanded to analyze different mAb as well as immunoprecipitated protein samples.

NOVEL ASPECT: Analysis of high molecular weight proteins by native CE-MS including Top-Down data Combination of IP and CE-MS technology

14:00 – 14:30

Professor Herbert Lindner, Dr. Klaus Faserl, Bettina Sarg

Innsbruck Medical University, Austria

Advances in the Separation of Co- and Post-translational Protein Modifications Using CE-MS

INTRODUCTION: In higher eukaryotes a majority of proteins is post-translationally modifed and these modifcations are often essential for the function of a protein. The goal of PTM analysis is to localize the amino acid residues in a given protein that are altered *in vivo* to activate, inactivate, or modify the biological activity of proteins. Due to their biochemical importance, various analytical techniques for the characterization of PTM´s have been described. Especially HPLC coupled with high-resolution mass spectrometry is a well-established technique and in many cases the method of choice for the separation and identifcation of proteins and their modifcation sites. An alternative strategy, capillary electrophoresis coupled to mass spectrometry, seems to be gaining momentum especially for the analysis of protein modifcations.

METHODS: Post-translationally modifed peptides and intact proteins were investigated by CE-MS and LC-MS. For CE-MS analysis a CESI 8000 (SCIEX) equipped with a bare, positively or neutrally-coated fused-silica capillary with a porous tip acting as nanospray emitter (SCIEX) (total length: 90 cm, i.d.: 30 µm, o.d.: 150 µm) was coupled via an ESI module to a Thermo Scientifc Q Exactive Plus or LTQ Orbitrap XL ETD. For LC-MS analysis an UltiMate 3000 nano-HPLC system (Thermo) was coupled to the MS analyzers. A homemade fritless fused-silica microcapillary column (75 µm i.d. x 280 µm o.d.) packed with 10 cm of 3 µm reverse-phase C18 material (Reprosil) was used for separation.

PRELIMINARY DATA: This work describes the use of low-flow CE-MS/MS to determine post-translational modifcations, e.g. acetylation, phosphorylation, methylation, deamidation, glycosylation and citrullination in medium complex nuclear samples. Further, the suitability of CE-MS for the analyses of intact nuclear proteins and their multiply modifed forms was investigated to provide information regarding the extent of existing PTMs. Method inherent advantages of CE-MS e.g., highly efficient separations, low flow rates, reduced ion suppression, and greater sensitivity were investigated and will be discussed.

NOVEL ASPECT: Based on the results obtained CE-MS can be considered a complementary technique to conventional LC-MS and an alternative approach for PTM analysis.

14:30 – 15:00

Dr. Aparna B. Baxi,[1,2] Camille Lombard-Banek,[1] Sally A. Moody,[2] and Peter Nemes[1,2]

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Uncovering Proteomic Changes During Embryonic Neural Induction Through Ultrasensitive Mass Spectrometry

INTRODUCTION: Uncovering the molecular mechanisms that differentiate embryonic cells to form neurons is critical to understand neural development and disease. Recent technical advances in single-cell analyses have opened new possibilities to advance our understanding of embryonic cell differentiation. Our laboratory has designed and built specialized microanalytical instruments to analyze proteins (and metabolites) in limited populations of cells and single cells in early developing embryos of important biological models in cell/developmental biology and neuroscience, including the South African clawed frog (*Xenopus laevis*) and zebrafish. This presentation will explore the use of these technologies to characterize the proteomic changes that establish the first neural tissue of the embryo: the neural ectoderm.

METHODS: Cellular content (~ 10 nL) from single embryonic cells was aspirated using a borosilicate capillary. Newly formed neural cells were collected by microdissection using sharpened forceps. Proteins from cells/ cell extracts were precipitated and then digested with trypsin. Resulting peptides were separated using our custom-built capillary electrophoresis-electrospray ionization (CE-ESI) platform or by liquid chromatography (LC) and measured using orbitrap mass spectrometers (Q-Exactive Plus and Fusion Lumos, Thermo Scientific). MS data were searched using MaxQuant against a custom-built concatenated database with the mRNA derived PHROG database and the X. laevis SwissProt proteome database.

PRELIMINARY DATA: This work characterizes proteomic changes during differentiation of embryonic cells to neural cells. Our proteomic analysis spanned across the develoopment an identified single embryonic cell (D11) up to the formation of the neural ectoderm (NE). The D11 cell was chosen because it primarily contributes to the formation of the brain and the spinal cord. To enable proteomic analysis of single embryonic cells, we first developed a sample collection approach wherein ~10% of the cell volume was aspirated. We then extended the aspiration approach to collect proteins from subsequent progeny of the D11 cell. To collect NE cells, a lineage tracing approach was employed in which progeny of D11 were fluorescently labeled and selectively dissected when the NE was established (at gastrulation). Cellular proteomes were analyzed by developing specialized microanalytical approaches. First, protein extraction and sample preparation approaches were tailored to volume-limited cellular content aspirated from single-cells. For analyzing proteins from dissected tissue samples, a density-gradient centrifugation approach was used to deplete yolk proteins that are abundant in embryonic cells and interfere with the detection of lower abundance proteins. Next, separation of peptides was performed by our custom-built CE-ESI platform as well as by multidimensional LC to deepen proteomic coverage. Our approaches enabled reproducible proteomic measurements resulting in the identification of ~800 protein groups from single-cells and ~3,000 protein groups from dissected NE tissues. Hierarchical cluster analysis of our quantitative proteomic data distinguished D11 and its progeny based on their protein abundance profiles, suggesting stage-specific differential gene translation as neural fated cells divide. This novel information will now enable us to evaluate proteomic trends beginning at the neural fated precursor cells to the newly differentiated NE.

NOVEL ASPECT: Several technical advances were made to explore spatio-temporal remodeling of the proteome as neural tissues develop in the embryo.

14:30 – 15:00

Professor Jennifer Van Eyk, Andrea D. Matlock,[1*] Vineet Vaibhav,[1*] Farzin Gharahdaghi,[2*] Jose-Luis Gallegos-Perez,[2] Clive Svendsen[3]

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3. Regenerative Medicine Institute, Cedar-Sinai Medical Center, USA
** Equal contribution*

Next Stage Personalized Medicine: Automation and High Content Proteomics

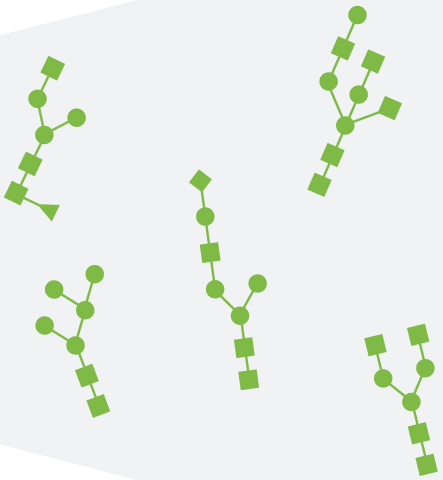
INTRODUCTION: Personalized medicine requires an ability to tailor therapy to the specific disease status and stage of an individual. To accomplish this will require i) having an array of therapies for each disease and ii) a means to identify which therapy (or combination) will be appropriate for a particular person. We propose that the use of iPSC-derived cultured tissue or organoids from the target individual could be beneficial in the identification of new and or the assignment of the appropriate drug treatment. On a practical level, our goal is to have in place an automated system that allows for the small-scale processing of iPSC-derived cells or organoid samples (in 96-well format) coupled with high-content and high-throughput proteomic screening capabilities with CESI potentially providing additional sensitivity to greatly reduce the amount of starting sample required. Today, sample preparation for protein quantification by mass spectrometry requires multiple processing steps including denaturation, reduction, alkylation, protease digestion, and peptide cleanup. Scaling these procedures for the analysis of numerous complex biological samples in a 96-well format can be tedious and time-consuming, as there are many liquid transfer steps and timed reactions where technical variations can be introduced and propagated.

METHOD: Our current pipeline design allows for the reproducible production of mass spectrometry samples initially from 96-well tissue culture plates where the entire sample processing is performed within the cultured wells under complete automation using the Beckman i7 automated workstation (liquid handler) and integrated use of Covaris sonication equipment. We have initially used HEK cells to assess methods for maximizing the depth of whole proteome identifications using CESI coupled to 6600 tripleTOF mass spectrometer The electrophoretic separation of the protelyzed peptides on the neutral capillary occured over 20-30 minutes therefore, complex whole proteome extracts were collected and analyzed as unfractionated or divided into 3 reverse phase fractions prior to CESI loading. We obtained >1400 protein identification with non-fractionated sample (45 ng on neutral capillary) and >2000 protein identifications with prefractionated samples (5ug of protein is separated into 3 fractions on C18 ZipTip; 45ng load onto CESI neutral capillary per analysis). Assessments of CESI stability and data quality were assessed using technical replicates of all ALS and HEK samples analyzed. Protein coefficient of variation on the technical replicates proved stable and highly reproducible in both ALS and HEK studies (1-5% CV)). Finally, Human motor neuron cell cultures of differentiated iPSC cell lines derived from ALS patients and non-disease control patients (n=4) were analyzed by nano-LC-5600 tripleTOF (DIA) and by CES-6600 tripleTOF(DDA) mass spectrometer. Maximum of 2622 and 1500 nonredundant proteins were quantifiable, respectively based on 45 ng of protein on column. 72 proteins were uniquely identified by CESI-MS contributing ~ 2% unique protein identifications in ALS motor neuron analyses.

NOVEL ASPECT: In conclusion, the value of decreasing the input material and fully automating cell processing from 96-well plates will provide cost saving benefit to large scale and costly iPSC differentiation protocols and make way to high throughput drug screens.

See how

CESI-MS



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