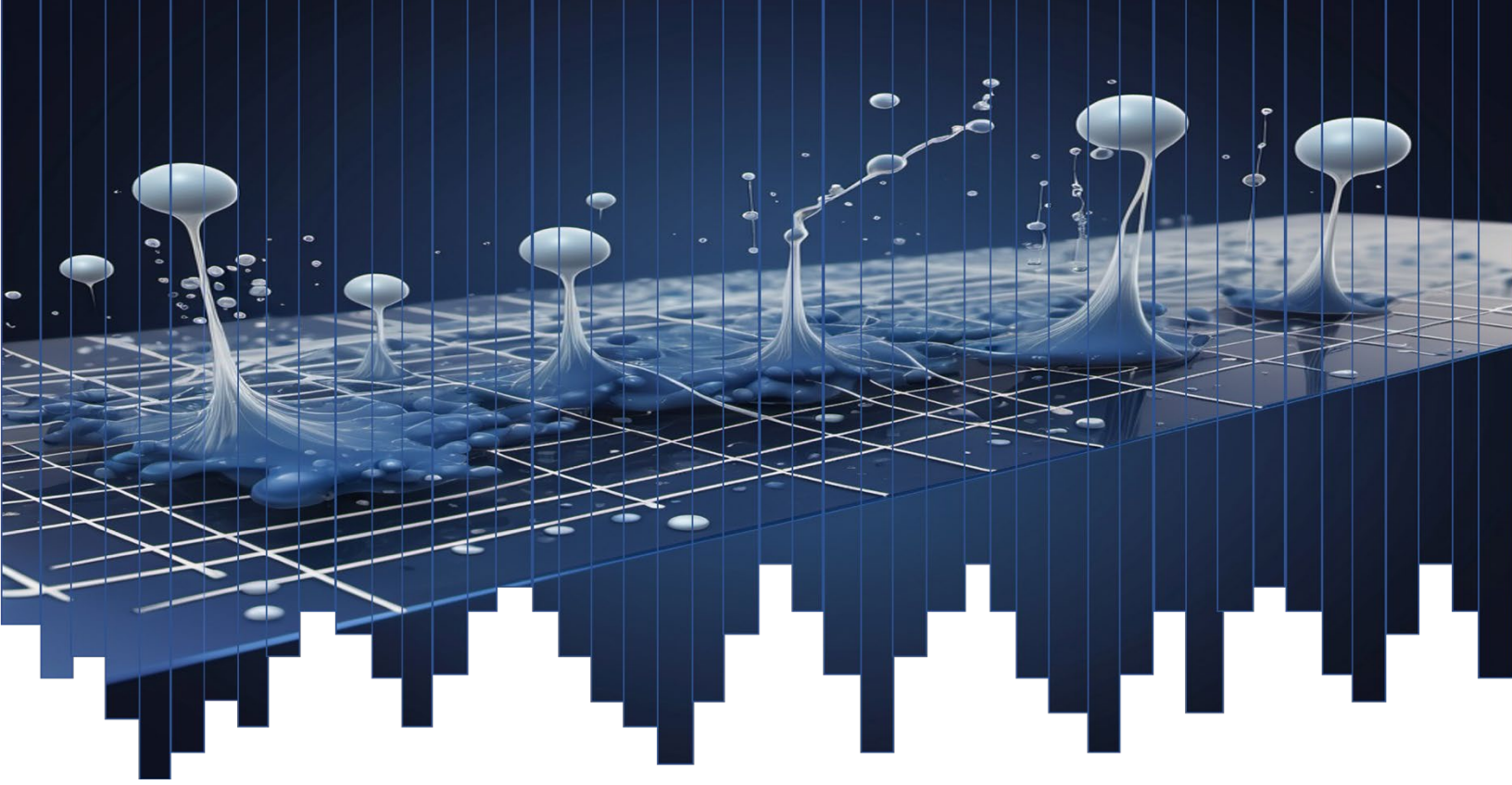
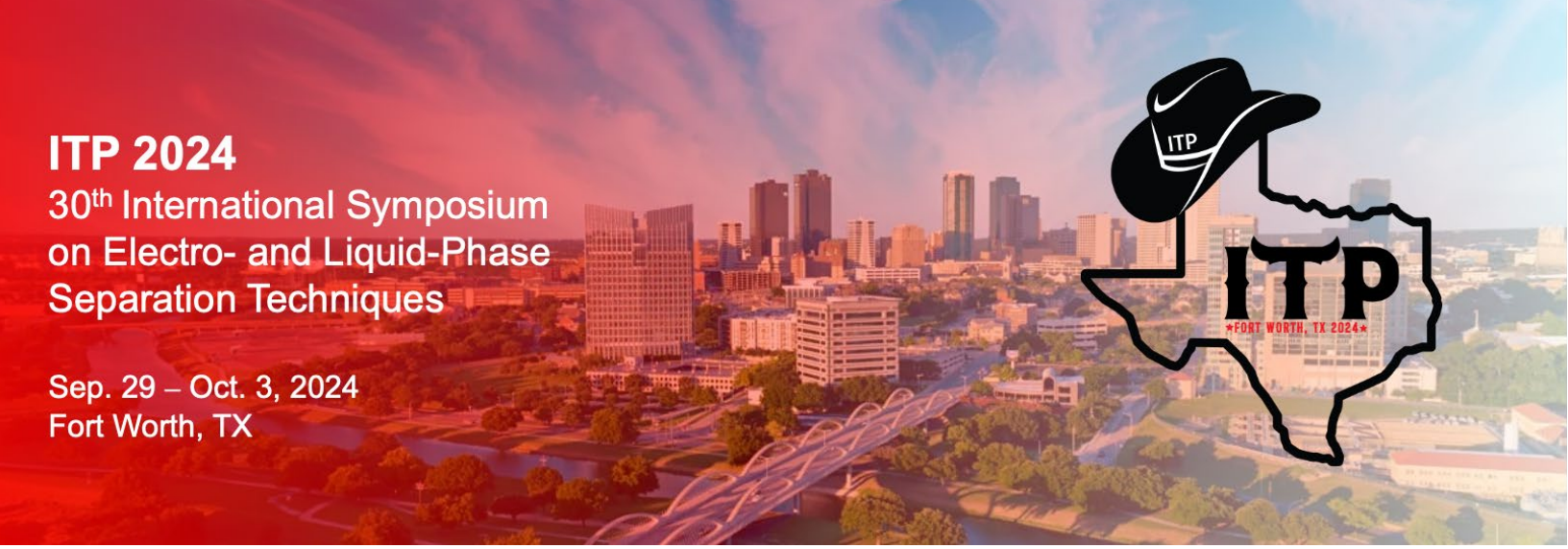


ITP 2024

30th International Symposium on Electro- and Liquid-Phase Separation Techniques

30th International Symposium on Electro and Liquid-Phase Separation Techniques
Sep 29 – Oct 3, 2024|Fort Worth, Texas, USA

SCIENTIFIC PROGRAM & BOOK OF ABSTRACTS



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WELCOME MESSAGE

Welcome to ITP 2024!

Dear colleagues, friends, and fellow scientists,

It is with immense pleasure that I welcome you to the 30th International Symposium on Electro- and Liquid-Phase Separation Techniques (ITP 2024) here in Fort Worth, Texas. This symposium marks an important milestone in the ongoing exploration and development of separation techniques, an area that has been instrumental in advancing analytical science and its applications in various industries.

Hosting ITP 2024 in Fort Worth, a city rich in history and culture, allows us not only to continue the tradition of scientific excellence but also to provide attendees with a unique blend of academic inspiration and Texan hospitality. Our location offers easy access to the not-to-be-missed Fort Worth Stockyards, an incredible variety of modern attractions, and a vibrant city that has long been a hub of innovation and collaboration.

This year's symposium boasts an exciting format with three plenary lectures that span a wide range of cutting-edge topics. The plenary speakers will explore the limits of speed and resolution in liquid chromatography, the importance of D-amino acids in biological systems, and novel ion separation techniques in hollow tubes. These talks will set the stage for a series of stimulating discussions and provide insights into the future directions of our field.

Additionally, ITP 2024 will feature **13 Keynote Lectures**, **15 Lectures**, and a rich mix of both academic and industrial presentations, offering a balance of theoretical advancements and practical applications. Furthermore, I am pleased to announce that **15 Young Scientist Lectures** have been included in this year's program. These lectures allow emerging scientists to showcase their research and presentation skills, an essential part of their professional development. Additionally, young scientists will also be presenting their exciting work in **33 Posters**.

I would like to express my deep gratitude to the organizing and scientific committees, whose dedication and hard work have been essential in bringing this event to life. A special thanks go out to our sponsors for their generous support and to the speakers and attendees, without whom this meeting would not be possible.

To all attendees, thank you for being a part of ITP 2024. Your presence and contributions make this symposium an event to remember. I hope that you will find the program enriching and that you take full advantage of the opportunities to engage with fellow experts, exchange ideas, and explore the many facets of beautiful Fort Worth.

Welcome to ITP 2024 – I look forward to a fantastic meeting with all of you!

Warm regards,

Yehia Mechref
Chair, ITP 2024
Robert A. Welch Endowed Chair in Chemistry
Paul W. Horn Distinguished Professor
Associate Vice President for Research and Innovation
Director, TTU Center for Biotechnology & Genomics
Spokesperson, TTU Horn Distinguished Professors
Chair of the Steering Committee of the Alliance of Glycobiologists for Cancer Research
Founding Editor-in-Chief of BioChem Journal
Texas Tech University
Department of Chemistry and Biochemistry
Lubbock, TX

ITP SYMPOSIUM HISTORY

Year	Chair(s)	Place (Country)
2023	A. Gentili	San Felice Circeo (Italy)
2022	D.S. Chung & F. Foret	Siem Reap (Cambodia)
2020	D. Chen	Virtual Conference
2019	H. Cottet	Toulouse (France)
2018	K. Otsuka	Kyoto (Japan)
2017	M. Markuszewski	Sopot (Poland)
2016	Z. El Rassi & B. Lapizco-Encinas	Minneapolis, MN (USA)
2015	M.L. Riekkola & H. Sirén	Helsinki (Finland)
2014	M. Tavares & E. Carrilho	Natal (Brazil)
2013	A. Cifuentes & J. Hernández-Borges	Puerto de la Cruz (Spain)
2012	Z. El Rassi	Baltimore, MD (USA)
2011	B. Chankvetadze	Tbilisi (Georgia)
2010	Z. El Rassi	Baltimore, MD (USA)
2008	V. Cucinotta	Catania (Italy)
2006	G. Peltre	Paris (France)
2004	S. Fanali & M.G. Quaglia	Rome (Italy)
2002	M.L. Riekkola	Helsinki (Finland)
2000	D. Kaniansky & E. Kenndler	Bratislava (Slovak) – Vienna (Austria)
1998	P.G. Righetti	Venice (Italy)
1996	B. Gas	Prague (Czech)
1994	F. Kilar	Budapest (Hungary)
1992	S. Fanali	Rome (Italy)
1990	D. Kaniansky	Tatranska Lomnica (Czechoslovakia)
1988	E. Kenndler	Vienna (Austria)
1986	F.M. Everaerts	Maastricht (The Netherlands)
1984	Z. Prusik	Hradec Kralove (Czechoslovakia)
1982	C.J. Holloway	Gosslar (Germany)
1980	F.M. Everaerts	Eindhoven (The Netherlands)
1979	A. Adam & C. Schots	Baconfoy (Belgium)

COMMITTEE MEMBERS

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Robert A. Welch Endowed Chair in Chemistry
Paul W. Horn Distinguished Professor
Associate Vice President for Research and Innovation
Director, TTU Center for Biotechnology & Genomics
Spokesperson, TTU Horn Distinguished Professors
Chair of the Steering Committee of the Alliance of Glycobiologists for Cancer Research
Founding Editor-in-Chief of BioChem Journal

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GUIDELINES FOR SPEAKERS

- Plenaries: 45' (40' for presentation + 5' for discussion) **(PL)**
- Keynotes: 30' (25' for presentation + 5' for discussion) **(KL)**
- Lectures: 20' (17' for presentation + 3' for discussion) **(L)**
- Young Scientist Lectures: 15' (12' for presentation + 3' for discussion) **(YSL)**

Some Remarks on Oral Presentations

- Each session has multiple presentations.
- Please prepare your presentation in PowerPoint.
- Do not forget to bring an additional copy of the presentation (on USB or by emailing it to yourself).
- All presentations should be uploaded at least one hour before the related session starts.
- If you wish to incorporate videos into your presentation, be sure to check that they are working on the venue computers well in advance.
- Technical assistance will be available in the presentation room.
- Session chairs will introduce each speaker.
- Session chairs can notify you when you have a few minutes to spare.

Please respect your fellow presenters and their allotted time by staying within the scheduled time and respecting the session chairs if they ask you to finish.

Some Remarks on Poster Presentations

- Posters must be adjusted to size 3 ft H X 4 ft W.
- The poster must have a title, name of author(s), and affiliation (to be placed at the top of the poster).
- Posters must be in English.
- Posters will be placed in the poster area in the Exhibition room.
- According to the schedule, posters will be presented from the first day of the conference until the last day: Monday (September 30) 12:05-13:05pm, Tuesday (October 1) 12:45-13:45pm, and Wednesday (October 2) 12:00-13:00pm.
- Presenters must attend to their poster board each day during the poster session.
- The posters must be put up before the morning oral session (before 9.00) on Monday, September 30, and removed at the end of the conference.

ITP 2024 SCIENTIFIC PROGRAM

Following are a few things to note about the 30th International Symposium on Electro-Liquid-Phase Separation Techniques' Scientific Program:

Sunday, September 29, 2024

2:00 PM	**Registration open**
4:00 – 6:00 PM	**Welcome Reception – Crystal D (Hilton Fort Worth)** **Open to All Registrations and Accompanying Persons**

Monday, September 30, 2024

8:00 – 9:00 AM	**Symposium Registration Open – West Promenade**
8:30 – 9:00 AM	**Opening Remarks –** Dr. Yehia Mechref, Symposium Chairman **Venue** – **Crystal D**

Monday, September 30, 2024

Time	Conference Hall – Crystal D	Speaker
	Session I Chair: Dr. Dimitri Pappas *Professor & Department Chair, Texas Tech University*	
9:00 – 9:45	PL-01 **Exploring the Limits of Speed and Resolution in Liquid Chromatography**	**Robert Kennedy** *Hobart H Willard Distinguished University Professor of Chemistry, Pharmacology, University of Michigan*
9:45 – 10:15	KL-01 **New Approach to Lateral-Flow Immunochromatographic Assay**	**Richard Willson** *Huffington-Woestemeyer Professor of Chemical & Biomolecular Engineering, Biology & Biochemistry, and Biomedical Engineering, University of Houston*
10:15 – 10:45	KL-02 **Capillary Electrophoresis for Biomolecular Analyses Under Physiological Conditions**	**Lisa Holland** *Professor, West Virginia University*
10:45 – 11:15	Coffee Break	
	Session II Chair: Dr. Katarína Maráková *Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia*	
11:15 – 11:35	L1 **Can Automation Help you be a Better Separation Scientist?**	**Doug Carlton** *Shimadzu Scientific Instruments*
11:35 – 11:50	YSL 1 **A Multi-Omics Investigation Uncovering the Biological Mechanisms Underlying Pompe Disease and Muscle Myopathy**	**Vishal Sandilya** *Department of Chemistry & Biochemistry, Texas Tech University*
11:50 – 12:05	YSL 2 **Are Conventional Efficiency Measurements Reliable in Chiral Chromatography?**	**Ryan Jacob Burk** *Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX*
12:05 – 13:05	Exhibition/Poster	
13:05 – 14:00	Lunch – On Your Own	

Afternoon Session		
	Session III Chair: Dr. Lisa Holland *Professor, West Virginia University*	
14:00 – 14:30	KL-03 **Capillary Zone Electrophoresis in Quantitative Analysis of Intact Proteins in Pharmaceutical and Biomedical Samples**	**Katarína Maráková** *Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia*
14:30 – 15:00	KL-04 **Cell Separations in Sepsis and Cancer**	**Dimitri Pappas** *Professor & Department Chair, Texas Tech University*
15:00 – 15:20	L2 **Improve Chromatographic Performance Towards Metal-Sensitive Analytes using UPLC**	**Jorge Smith** *Waters Corporation*
15:20 – 15:50	Coffee Break	
	Session IV Chair: Dr. Adam Woolley *Dean, Graduate Studies, Brigham Young University*	
15:50 – 16:10	L3 **Applications of Micro Free Flow Electrophoresis**	**Michael Bowser** *Professor, University of Minnesota*
16:10 – 16:30	L4 **High Sensitivity Analysis of Biosignatures using Capillary Electrophoresis and Laser-induced Fluorescence**	**Laura Casto-Boggess** *Assistant Professor, The University of North Carolina at Charlotte*
16:30 – 16:45	YSL 3 **LC-MS/MS of Permethylated O-Glycans, Free Oligosaccharides, and Glycosphingolipid Glycans Using Mesoporous Graphitized Carbon Column**	**Oluwatosin Daramola** *Department of Chemistry & Biochemistry, Texas Tech University*
16:45 – 17:00	YSL 4 **Daidzein to Equol: Cyclodextrin-Based Chiral Separations of Key Metabolites**	**Amanda Razo-Smith** *Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX*
17:00 – 17:15	YSL 5 **Moving Towards a Proteomics Blood-Based Diagnosis of Alzheimer's Disease Using Human Brain Tissue, CSF, and Plasma Samples by Leveraging a nanoLC-FAIMS-MS/MS Approach**	**Andrew I. Bennett** *Department of Chemistry & Biochemistry, Texas Tech University*
17:15 – 17:30	YSL 6 **Unveiling Glycan Profiles to Study Breast Cancer Brain Metastasis**	**Joy Solomon** *Department of Chemistry & Biochemistry, Texas Tech University*

Tuesday, October 1, 2024

Time	Conference Hall – Crystal D	Speaker
	Session V Chair: Dr. Firas Kobaissy *Professor, Morehouse School of Medicine, Associate Director, Center for Neurotrauma, MultiOmics & Biomarkers (CNMB)*	
8:30 – 9:15	PL-02 **Importance of D-Amino Acids in Biological Systems: Detection and Analysis**	**Daniel Armstrong** *R.A. Welch Distinguished Professor, University of Texas at Arlington*
9:15 – 9:45	KL-05 **Specific Interactions in Liquid Phase Separations**	**Takuya Kubo** *Professor, Kyoto Prefectural University*
9:45 – 10:15	KL-06 **3D Printed Microfluidic Devices Integrating Solid-Phase Extraction and Electrophoretic Separation**	**Adam Woolley** *Dean, Graduate Studies, Brigham Young University*
10:15 – 10:45	Coffee Break	
	Session VI Chair: Dr. Kevin Schug *Shimadzu Distinguished Professor of Analytical Chemistry, University of Texas at Arlington*	
10:45 – 11:15	KL-07 **Promise of MitoQuinone in TBI Therapeutics: Lessons from Clinical TBI Trajectories**	**Firas Kobeissy** *Professor, Morehouse School of Medicine, Associate Director, Center for Neurotrauma, MultiOmics & Biomarkers (CNMB)*
11:15 – 11:35	L5 **Bruker Daltonics – 4D Proteomics Update De-risking Discovery**	**Shourjo Ghose** *Bruker Daltonics*
11:35 – 11:55	L6 **Investigation of Chiral Separation of α-Hydroxy Acid Biomarkers via SPP-Teicoplanin**	**Saba Aslani** *Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX, USA*
11:55 – 12:10	YSL 7 **Characterizing Insect-Mediated Microbial Degradation of Insecticides Through LC Methodologies**	**Maria Olds** *Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX, USA*
12:10 – 12:25	YSL 8 **Identifying Dysregulated Lipids in Human Brain of Alzheimer's Disease and their Effects on Biological Pathways**	**Akeem Sanni** *Department of Chemistry & Biochemistry, Texas Tech University*

12:25 – 12:40	YSL 9 **LC-MS/MS-Based Metabolic Profiling: Investigating Serum and CSF in TBI Patients**	**Sarah Sahioun** *Department of Chemistry & Biochemistry, Texas Tech University*
12:45 – 13:45	Exhibition/Poster	
14:00 – 17:00	Visit to the Stockyard	

Wednesday, October 2, 2024

Time	Conference Hall – Crystal D	Speaker
	Session VII Chair: Dr. Takuya Kubo *Professor, Kyoto Prefectural University*	
8:30 – 9:15	PL-03 **Ion Separations in Hollow Tubes: Mirage or Oasis?**	**Sandy Dasgupta** *Professor Hamish Small Chair in Ion Analysis, University of Texas at Arlington*
9:15 – 9:45	KL-08 **Octadecyl Monolith for the Reversed-Phase Capillary Electrochromatography of Pre-column Derivatized Mono- and Oligosaccharides with Three Different Ultraviolet Absorbing Tags**	**Ziad El Rassi** *Regents Professor, Oklahoma State University*
9:45 – 10:15	KL-09 **Targeted and Untargeted Analysis of Psilocybin Mushrooms using LC-MS**	**Kevin A. Schug** *Shimadzu Distinguished Professor of Analytical Chemistry, University of Texas at Arlington*
10:15 – 10:45	Coffee Break	
	Session VIII Chair: Dr. Ziad El Rassi *Regents Professor, Oklahoma State University*	
10:45 – 11:05	L7 **Considerations and Capabilities of Ion Chromatography**	**Tom Cardwell** *Shimadzu Scientific Instruments*
11:05 – 11:25	L8 **Reducing Hepatitis C Diagnostic Disparities with a Point of Care Assay for HCV Antigen detection using a Handheld Microfluidic Device with Sequence Oscillatory Flow Controls**	**Hui Chen** *Associate Professor, Department of Chemistry & Biochemistry, Texas Tech University*
11:25 – 11:40	YSL 10 **LC-MS/MS-Based Proteomic Profiling of Small Extracellular Vesicle Alterations in Severe Traumatic Brain Injury**	**Mojibola Fowowe** *Department of Chemistry & Biochemistry, Texas Tech University*
11:40 – 11:55	YSL 11 **An Innovative Method for Analyzing IgG Glycosylation Significance in Traumatic Brain Injury**	**Sherifdeen Onigbinde** *Department of Chemistry & Biochemistry, Texas Tech University*
12:00 – 13:00	Exhibition/Poster	
13:00 – 14:00	Lunch – On Your Own	

Afternoon Session		
	Session IX Chair: Dr. David Chen *Professor The University of British Columbia*	
14:00 – 14:30	KL-10 **Vibrating sharp-edge spray ionization: A new paradigm for coupling condensed-phase separations with mass spectrometry**	**Stephen Valentine** *Eberly Professor of Chemistry, West Virginia University*
14:30 – 14:50	L9 **LC Hardware Considerations for Better Biomolecule Separations**	**Joe Lopez** *Shimadzu Scientific Instruments*
14:50 – 15:10	L10 **The Power of PASEF in Lipidomics Research**	**Beixi Wang** *Applications Scientist, Small Molecule and Metabolomics, Bruker Daltonics*
15:10 – 15:45	 Coffee Break	
	Session X Chair: Dr. Peng Li *Associate Professor | West Virginia University*	
15:45 – 16:05	L11 **Advances in Multidimensional Mass Spectrometry for Resolving Complex Biological and Environmental Sample Mixtures**	**Touradj Solouki** *Professor, Baylor University*
16:05 – 16:25	L12 **Slow Your Flow for Improved Top-down Analysis of Proteins**	**William Russell** *Associate Professor, UTMB Mass Spectrometry Director, The University of Texas Medical Branch at Galveston*
16:25 – 16:40	YSL 12 **Glycoproteomics Profiling of Biofluids in Severe Traumatic Brain Injury Patients**	**Moyinoluwa Adeniyi** *Department of Chemistry & Biochemistry, Texas Tech University*
16:40 – 16:55	YSL 13 **Heart Cut 2D-LC in a Single Chromatograph for a Difficult Isomeric Separation**	**Siddharth Jaya Sajeevan J** *Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX, USA*
16:55 – 17:15	YSL 14 **Quantitation of Intact Proteins in Biological Fluids Using tITP-CZE-MS with Off-Line Microelution SPE Sample Pretreatment**	**Martina Opetová** *Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University Bratislava, Odbojárov 10, 831 04 Bratislava, Slovak Republic*
17:15 – 17:30	YSL 15 **LC-MS/MS Characterization of the N-glycosylation of Spike protein S1 Derived from 11 variants of SARS-CoV-2**	**Cristian D. Gutierrez-Reyes,** *Department of Chemistry & Biochemistry, Texas Tech University*
18:00	Conference Dinner	

Thursday, October 3, 2024

Time	Conference Hall – Crystal D	Speaker
	Session XI Chair: Dr. Stephen Valentine *Eberly Professor of Chemistry, West Virginia University*	
8:30 – 9:00	KL-11 **Advancing Proteomics: Efficient Mass Spectrometry Techniques for Analyzing Protein Interactions and Modifications**	**Saiful M. Chowdhury** *Associate Professor, University of Texas at Arlington*
9:00 – 9:30	KL-12 **Coupling 3D-Printed Microfluidic Devices with Mass Spectrometry for Advanced Liquid Chromatography Separation**	**Peng Li** *Associate Professor | West Virginia University*
9:30 – 10:00	KL-13 **Current State of Erythropoietin (EPO) Analysis in Doping Control and the Potential of Liquid Chromatography/Capillary Electrophoresis Mass Spectrometry as a Better Alternative**	**David Chen** *Professor The University of British Columbia*
10:00 – 10:30	Coffee Break	
	Session XII Chair: Dr. Saiful Chowdhury *Associate Professor, University of Texas at Arlington*	
10:30 – 10:50	L13 **Advance your LC Experience with SFC**	**A. Paige Wicker** *Shimadzu Scientific Instruments*
10:50 – 11:10	L14 **Digital Restoration of Chromatographic Data for Signal-to-Noise Enhancement**	**M. Farooq Wahab** *Research Engineering Scientist V | The University of Texas Arlington*
11:10 – 11:30	L15 **A Custom-made Autosampler for Capillary Electrophoresis**	**Giacomo Musile** *Department of Chemistry, University of Basel, Basel, Switzerland*
11:30 – 12:00	Closing Remarks	

Poster Presentations

	Posters	Authors
P-01	Explore the Antileukemic Activity of the Synthetic Retinoid ST1926 in HTLV-1 Positive and Negative Malignant T Cells through LC-MS/MS Proteomics	Mona Goli
P-02	Exploring Tianeptine: Chiral Separation, Enantiomeric Determination and Biological Effects	Saba Aslani
P-03	LC-MS/MS Characterization of the N-glycosylation of Spike protein S1 Derived from 11 variants of SARS-CoV-2	Cristian D. Gutierrez Reyes
P-04	Exploring the Impact of Fasting on Biological Pathways through LC-MS/MS of Profiling Serum Proteome	Md Mostofa Al Amin Bhuiyan
P-05	Are Conventional Efficiency Measurements Reliable in Chiral Chromatography?	Ryan Jacob Burk
P-06	Moving Towards a Proteomics Blood-Based Diagnosis of Alzheimer's Disease Using Human Brain Tissue, CSF, and Plasma Samples by Leveraging a nanoLC-FAIMS-MS/MS Approach	Andrew I. Bennett
P-07	Glycoproteomics Profiling of Biofluids in Severe Traumatic Brain Injury Patients	Moyinoluwa Adeniyi
P-08	Daidzein to Equol: Cyclodextrin-Based Chiral Separations of Key Metabolites	Amanda Razo-Smith
P-09	Serum Proteome Profiling of Diabetic Patients Treated with Novel Antidiabetics Show Improved Cognitive Function, Cardiovascular Function and Reduce Inflammatory Responses	Md Abdul Hakim
P-10	Evaluation of LC-MS Mobile Phase Additives for Separation of Peptide Epimers/Isomers	Umang Dhaubhadel
P-11	A Multi-Omics Investigation Uncovering the Biological Mechanisms Underlying Pompe Disease and Muscle Myopathy	Vishal Sandilya
P-12	An Innovative Method for Analyzing IgG Glycosylation Significance in Traumatic Brain Injury	Sherifdeen Onigbinde
P-13	Chiral Separation of Synthesized Dihydropyridine Derivatives: A Chromatographic Approach for Potential Calcium-channel Blockers	Reza Salehi
P-14	LC-MS/MS of Permethylated O-Glycans, Free Oligosaccharides, and Glycosphingolipid Glycans Using Mesoporous Graphitized Carbon Column	Oluwatosin Daramola
P-15	Development of Simple and Rapid tITP-CZE-UV method for Salivary Lysozyme determination	Radovan Tomašovský
P-16	LC-MS/MS-Based Proteomic Profiling of Small Extracellular Vesicle Alterations in Severe Traumatic Brain Injury	Mojibola Fowowe
P-17	Quantitation of Intact Proteins in Biological Fluids Using tITP-CZE-MS with Off-Line Microelution SPE Sample Pretreatment	Martina Opetová
P-18	Identifying Dysregulated Lipids in Human Brain of Alzheimer’s Disease and their Effects on Biological Pathways	Akeem Sanni
P-19	N-Glycan Alterations in Kidney of Rats Chronically Exposed to Glyphosate-Based Herbicide	Favour Chukwubueze
P-20	Heart Cut 2D-LC in a Single Chromatograph for a Difficult Isomeric Separation	Siddharth Jaya Sajeevan J
P-21	Investigating the Effects of Alteration in Lipid Profile on Biological Pathways in Diabetic Patients Treated with Different Drugs	Waziha Purba
P-22	LC-PRM-MS/MS Reveals Significant Metabolomic Alterations in Parkinson’s Disease Frontal Lobe Tissue	Odunayo Oluokun

P-23	The Effect of Chiral Changes on Antibodies, Detection, and Sample Preparation: A Study of Epimeric Beta Amyloids	Arzoo Patel
P-24	In vivo Stable Labeling (GlyProSILC) of Mitochondria Glycans and Proteins	Judith Nwaiwu
P-25	Characterizing Insect-Mediated Microbial Degradation of Insecticides Through LC Methodologies	Maria Olds
P-26	LC-MS/MS-Based Approach for Examining Disease-Related N-Glycopeptides in Small Extracellular Vesicles: A Case Study on Traumatic Brain Injury	Ayobami Oluokun
P-27	Unveiling Glycan Profiles to Study Breast Cancer Brain Metastasis	Joy Solomon
P-28	Models, Metrics, and Methods for Greening Analytical and Preparative Chromatography	Troy Handlovic
P-29	LC-MS/MS-Based Metabolic Profiling: Investigating Serum and CSF in TBI Patients	Sarah Sahioun
P-30	Glycome Profiling of Small Extracellular Vesicle N-glycans as Disease Biomarkers: A Traumatic Brain Injury Case Study	Esther Oji
P-31	Understanding Protein Accumulation in Cleome Seeds Using NanoLC-MS/MS-Based Proteomics	Fang Chen
P-32	Proteomic Insights into Fusarium Oxysporum Adaptation and Host Interaction: A Study of Cotton Root Influence on Pathogen Protein Expression	Sarah Metwally
P-33	Exploring Metabolic Diversity: A Non-Targeted Metabolomics Study of Cleome gynandra and Cleome hassleriana	Sarah Metwally

ABSTRACTS: in order of appearance

Monday, September 30, 2024

PL-01: Exploring the Limits of Speed and Resolution in Liquid Chromatography

Robert Kennedy

University of Michigan, Ann Arbor, MI

Efficiency and throughput are of continual interest in HPLC analysis. In this talk we describe two different projects that attempt to push the limits of these figures of merit. For efficiency we describe columns that can generate peak capacity of 1000. For throughput, we describe systems that can yield HPLC separates at 1 s/sample. It is well-known that smaller particles and longer columns provide higher resolution; but also, require higher pressure for flow in LC. We have experimented with systems that operate up to 35 kpsi for lipid and other metabolite separations. We demonstrate that these systems allow higher peak capacity, especially with relatively long 50 cm columns packed with 1.0 to 1.7 um particles. These columns can generate peak capacity up to 1000 in analysis times under 4 h. When used to analyze plasma extracts, we find that the improved resolution increases the number of lipids that can be identified. We have also expanded this work from reversed-phase to HILIC columns. Regarding throughput, it has been demonstrated over the past few years that salient HPLC separations can be achieved in less than 5 s. However, potential throughput has been limited by autosamplers which typically take 15 s or more per injection. We demonstrate that a segmented flow input allows samples to be injected at a rate limited by the analysis time. Analysis of a complete 96 well titer plate is possible in ~100 s.

KL-01: New Approaches to Lateral-flow Immunochromatographic Assays

Richard Willson

University of Houston, Houston, TX

Lateral-flow assays (LFAs) are fast, inexpensive and simple point-of-need diagnostic tools that can detect targets such as pathogens, drugs, and hormones. For many applications, however, LFA lacks sufficient analytical sensitivity or accuracy of quantitation. To address these limitations, we have been investigating the fundamental mechanisms of LFA and developing improved LFA reporter particles based on bacteriophage virus particles, phosphorescent nanoparticles, and chemical excitation of fluorophores. These approaches achieve much better sensitivity than conventional reporter particles, and in some cases also support accurate quantitation and/or color multiplexing. Applications including home detection of lupus nephritis by LFA (instead of kidney biopsy), fast identification of the one form of cancer that can be fatal if incorrectly treated within the turnaround time for molecular tests even in the TMC, and the (at least at one time) most-sensitive COVID-19 LFA will be discussed.

KL-02: Capillary Electrophoresis for Biomolecular Analyses Under Physiological Conditions

Lisa Holland

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Glycosylation is a non-templated post-translational modification that affects the physiological function of biomolecules and biological therapeutics. The structure of oligosaccharides is dependent upon enzymes that truncate or elongate these biopolymers. In this presentation technology is described that integrates enzymes into capillary electrophoresis separations. With this technology, enzymes can be used to modify or identify oligosaccharide structure. In addition, enzymes can be evaluated for activity. These capillary separations utilize nanogels which have a thermally-dependent viscosity. At temperatures below ~22°C nanogels have liquid-like viscosity. At higher temperatures nanogels have a gel-like viscosity. This property makes it easy to fill and pattern nanogels in narrow-bore capillaries at low temperature. Once the nanogel is loaded into the capillary, the fluids are then locked in place by raising the temperature to gel the material. Nanogels maintain enzyme function and are biocompatible. As a result, these matrices are modified to contain enzymes and then are introduced and patterned in a separation capillary. This enables the precise placement of 2-5 nanoliter enzyme reaction zones at the beginning of a capillary with a total liquid volume less than 1 microliter. Enzyme reactors of this low volume are mixed electrophoretically and then the substrate and products, or products, are separated, detected, and quantified. This approach is automated and reduces the time for enzymatic conversion from hours to minutes. The analyte resolution of biomolecules separated in nanogel yields efficient separation. Applications with hydrolase and transferase enzymes are demonstrated. This work is significant to separations because general separation methods are converted into sophisticated multifunctional separations that are programmed, erased, and repeatedly run.

L1: Can Automation Help you be a Better Separation Scientist?

Doug Carlton

Shimadzu Scientific Instruments

The more insight we gain as separation scientists, the more “check boxes” we realize we need to check before saying we have created a great method. Considerations such as column selection, mobile phase choice and gradients, detectors and data processing criteria become too important to be naïve to. Sample handling can be time consuming and introduce impurities or quantitative errors into the workflows. This presentation will present Shimadzu software solutions for generating and processing Design of Experiment data and absorbance peak deconvolution. LC hardware for automated sample pretreatment before separation will also be introduced.

YSL 1: A Multi-Omics Investigation Uncovering the Biological Mechanisms Underlying Pompe Disease and Muscle Myopathy

Vishal Sandilya[1], Favour Chukwubueze[1], Sarah Sahioun[1], Sherifdeen Onigbinde[1], Moyinoluwa Adeniyi[1], Stefania Mondello[2], Yehia Mechref[1*]

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Pompe disease, also known as glycogen storage disease II (GSD-II), is a rare genetic disorder resulting from mutations in the gene responsible for producing the enzyme acid-alpha glucosidase (GAA). GAA is crucial for breaking down glycogen into glucose. Mutations in GAA gene can reduce the amount of or completely eliminate the enzyme, leading to muscle deterioration. In contrast, myopathies encompass a broad category of muscle disorders, primarily affecting muscle structure, metabolism, or ion-channel function. Due to the overlapping symptoms between Pompe disease and myopathies, misdiagnosis or delays in diagnosis are common.

In this study, blood serum samples from 26 control, 14 Pompe, and 30 myopathy patients were analyzed to uncover the biological mechanisms underlying Pompe and myopathy. For proteomics analysis, the samples were first depleted of 14 high abundance proteins. The low abundant proteins were then denatured, reduced, and carbamidomethylated. Following this, tryptic digestion was performed using trypsin in a 1:25 enzyme to protein ratio. For N-glycomics, intact proteins were treated with PNGase F to release N-glycans, which were subsequently purified through ethanol precipitation. The glycans were then permethylated using iodomethane to increase hydrophobicity and improve ionization. Liquid chromatography with tandem mass spectrometry (LC-MS) analysis for both sets of samples was performed using an Ultimate 3000 nano-UHPLC coupled to an Orbitrap Fusion Lumos mass spectrometer.

The preliminary results revealed significant disruptions in the complement cascade pathways in both Pompe disease and myopathy, furthermore, cardiomyopathy was upregulated in both conditions. Notably, decreased cell polarization was identified exclusively in myopathy. Additionally, a significant change in sialylation was observed in myopathy compared to control, while fucosylation was altered in pompe. Future work for this project will focus on the analysis of glycopeptides and the integration of data from all three omics approaches to develop a more comprehensive understanding of these diseases.

YSL 2: Are Conventional Efficiency Measurements Reliable in Chiral Chromatography?

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Efficiency-flow profiles such as the van Deemter provide valuable insights for optimizing separations, yet the shape of the measured peak is often overlooked. Chiral separations are regularly asymmetric, so reliance on the assumption that peaks are Gaussian overestimates the calculated efficiency. The most accurate method of determining peak efficiency is from the statistical moment analysis of the raw chromatogram. However, this approach suffers from several problems, such as baseline noise and peak overlap, that render it impractical in many cases. Sophisticated peak models that incorporate higher-order moments have been used to account for the peak shape of highly asymmetrical or unresolved peaks. However, broader adoption may be needed. By examining the discrepancies in efficiency-flow profiles derived from conventional methods and moment analysis of peak models, we underscore the critical need to consider peak shape in chiral separations.

KL-03: Capillary Zone Electrophoresis for Quantitative Analysis of Intact Proteins in Pharmaceutical and Biomedical Samples

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Proteins are important biomacromolecules that play crucial roles in all living organisms. Many proteins, especially monoclonal antibodies, have also become important biopharmaceuticals, which represent a rapidly growing broad class of therapeutic agents for the advanced treatment of life-threatening diseases. Capillary electrophoresis (CE) emerged as a mature and robust separation technique increasingly popular in peptidomic and proteomic analysis. CE is recognized as a valued separation technique for its high separation efficiency, low sample consumption, good economic and ecological aspects, and complementarity to traditional LC techniques. It offers attractive features for miniaturization, sample preparation automation, and integration with the separation step. In CE analysis of proteins, UV spectrophotometry and mass spectrometry (MS) are the most frequently used detection techniques. CE-UV/MS offers fast and highly efficient protein separations on multiple levels of complexity, including intact protein analysis. Besides multiple advantages, CE has also several drawbacks, including protein adsorption on the fused-silica capillary wall and the relatively low concentration sensitivity due to the low injected sample volume (nanoliter scale). In this work, we present applications of CE-UV/MS methods for quantitative analysis of small intact proteins (molecular masses <20 kDa) in pharmaceutical matrices and biological fluids (human serum, plasma, urine, and saliva) and show the advantages and limitations of these methods.

Acknowledgements
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KL-04: Cell Separations in Sepsis and Cancer

Dimitri Pappas

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Microfluidic and 3D printed technologies continue to find new, relevant applications in disease diagnosis. To that end, we present recent advances in cell separations based on microfluid dynamics as applied to sepsis and cancer. We demonstrate that the length scales of these microsystems can be exploited for both label-free cell separations and affinity separations. Our group has pioneered affinity methods for isolating activated leukocytes in sepsis patients. Our device requires minimal operator intervention and utilizes CD64, CD69, and CD25 as the biomarker targets for detecting sepsis in liquid biopsies. We assessed the effectiveness of our 3D printed multi-zone cell separation device by testing it on clinical samples obtained from both septic patients (n=35) and healthy volunteers (n=8) and validated its performance accordingly. Our results showed a statistically significant difference in cell capture between septic and healthy samples (with p values of 0.0001 for CD64, CD69, and CD25), suggesting that 3D printed multi-zone cell capture is a reliable method for distinguishing sepsis. In parallel efforts, we developed and validated a 3D printed affinity separation device for cancer cell detection in liquid biopsies with minimal operator intervention using CD71 (transferrin receptor) as the affinity target. Cancer cell isolation was demonstrated with concentration as low as 30 cells per mL. Samples with initial cancer cell concentrations as low as 0.05% were separated with a capture purity of 94-98% and an enrichment factor of 98X-1900X for COG-LL-332 cells. Clinical plasma samples of breast and prostate cancer patients were tested, showing our 3D printed CD71 affinity device could isolate cancer cells from these liquid biopsies. The low detection concentration, high enrichment factor, simple fabrication and conjugation, and applicability to multiple cancer types makes this a promising approach for liquid biopsies and sepsis detection.

L2: Improve Chromatographic Performance Towards Metal-Sensitive Analytes using UPLC

Jorge Smith

Mass Spectrometry Specialist for Waters Corporation

Liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) has become the default platform for proteomics, metabolomics, and lipidomics due to its specificity, sensitivity, and sample compatibility. However, interactions between transition metals in LC systems and analytes containing phosphate groups result in poor chromatographic performance or even analyte loss. Using systems, such as ACQUITY Premier UPLC, where the metal surfaces had been treated with a hybrid inorganic/organic surface material to form an effective surface barrier mitigated these undesired interactions. When employed for the analysis of the TCA Cycle, Phosphorylated and Carboxylate Lipids or tryptic digests of Alpha and Beta Casein, along with synthetic "PhosphoMix" standards, the use of such a system showed significant improvements in chromatographic peak shape and analyte response together with superior spectral quality and sequence coverage. The hybrid surface system thus offered

significant advantages for the analysis of this type of analytes when compared to conventional LC/MS. In this presentation we will demonstrate the application of this LC technology combined with accurate mass MS for the analysis of plasma proteins and lipids as well as TCA cycle analytes.

L3: Applications of Micro Free Flow Electrophoresis

Michael Bowser

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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. Analyte streams are defected laterally according to their electrophoretic mobility giving rise to individual paths through the separation chamber. FFE has recently been miniaturized into a microfluidic format (μFFE), requiring less sample and reagents, a simplified flow profile, and better heat dissipation.

The continuous nature of μFFE separations suggests a number of novel analytical applications. For example, we have demonstrated how introducing a buffer gradient into the μFFE device can be used to efficiently optimize a range of separation conditions in as little as five minutes. A gradient can be introduced at the sample channel to titrate a fluorescently labeled aptamer with increasing concentrations of its protein target. Due to the continuous nature of gradient μFFE, complete coverage of the binding curve is possible in as little as five minutes. μFFE also offers the potential for impressive microscale purification. We have demonstrated how incorporating μFFE selections simplifies isolation of high affinity aptamers while increasing the size of the selection pool. μFFE is an ideal second stage for two dimensional separations. We have shown how directly coupling continuous μFFE separations with nano liquid chromatography or capillary electrophoresis allows us to produce high peak capacity 2D separations in <5 minutes. More recently we have begun integrating online μFFE into continuous flow affinity assays that will allow real time monitoring of peptides involved in cellular communication.

L4: High Sensitivity Analysis of Biosignatures using Capillary Electrophoresis and Laser-induced Fluorescence

Laura Casto-Boggess

The University of North Carolina at Charlotte, Charlotte, NC

The search for extraterrestrial life on icy moons requires high-sensitivity detection of biosignatures with amino acid detection limit requirements down to low nanomolar and picomolar concentrations in low volume samples. Existing approaches for in-situ trace analysis of organic amines and amino acids for space exploration leverage highly sensitive laser-induced fluorescence detection on a capillary electrophoresis separation channel. Fluorescent amine-reactive probes, including 3-carboxy-6,8-difluoro-7-hydroxycoumarin succinimidyl ester (Pacific blue) and 5-carboxyfluorescein succinimidyl ester, are used to target primary amine-containing species. Optimization of labeling and detection of

fluorescently tagged amino acids will be discussed. Modifications to the detection modality using frequency-encoding and convolution strategies for enhanced detection will also be presented.

YSL 3: LC-MS/MS of Permethylated *O*-Glycans, Free Oligosaccharides, and Glycosphingolipid Glycans Using Mesoporous Graphitized Carbon Column

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Glycosylation, a prevalent post-translational modification, plays a critical role in various biological processes and diseases. Despite significant research on *N*-linked glycans, *O*-linked glycans and other small glycan structures, including glycosphingolipid (GSL) glycans and free oligosaccharides, remain underexplored due to their structural complexity and low abundance. To achieve efficient isomeric separation of these small glycans, we employed the Mesoporous Graphitized Carbon (MGC) column, an efficient alternative to nano Porous Graphitized Carbon columns, which has been recently demonstrated to show efficient isomeric separation of *N*-glycans and *N*-and-*O*-glycopeptides. To extend the application of the MGC column for the isomeric separation of *O*-glycans, free oligosaccharides, and GSL glycans, we optimized the chromatographic conditions using standard samples, leading to improved retention and isomeric separation. Initial trials using a 50% Acetonitrile/Isopropanol (ACN/IPA) mixture as mobile phase B showed poor retention and incomplete separation of the glycans. Switching to 100% ACN with 0.1% formic acid improved retention and partial isomeric separation. Further optimization with 80% ACN enhanced these results, but full separation of some isomers was still not achieved. Finally, using 100% methanol with 0.1% formic acid significantly improved both retention and isomeric separation for all tested glycans. To validate the MGC column's effectiveness for isomeric separation of small glycans from biological samples, human milk-derived glycan samples were analyzed. *O*-glycans, free oligosaccharides, and GSL glycans were released from human milk, and permethylated, before been analyzed. The result showed successful isomeric separation, including separation of several small glycans, highlighting the method's capability across different glycan types. This study highlights the MGC column's robustness and reproducibility, suggesting its applicability for advanced glycomics, especially in biological samples where small glycans play significant roles in disease progressions.

This work was also supported by grants from the National Institutes of Health, NIH (1R01GM112490, 1R01GM130091, and 1U01CA225753, The Robert A. Welch Foundation under Grant No. D-0005 and The CH foundation.

YSL 4: Daidzein to Equol: Cyclodextrin-Based Chiral Separations of Key Metabolites

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Interest in daidzein, a plant hormone with a structure similar to human estrogen, has been on the rise as a preventative measure and treatment for various ailments. Daidzein, an achiral molecule, naturally occurs in many legumes and can be readily consumed by humans. Once consumed, if a person has the necessary bacterial enzymes, daidzein goes through three key reduction steps to become equol which has even more biological activity. All three reduction metabolites have at least one chiral center. The use of a single cyclodextrin-based chiral stationary phase provides the best enantiomeric separation of all daidzein metabolites and is discussed in detail.

YSL 5: Moving Towards a Proteomics Blood-Based Diagnosis of Alzheimer's Disease Using Human Brain Tissue, CSF, and Plasma Samples by Leveraging a nanoLC-FAIMS-MS/MS Approach

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Alzheimer's Disease (AD) is the most common form of dementia with cases and costs of caregiving projected to increase dramatically in the future. This study aims at identifying key proteins to create a sensitive and accurate AD screening/diagnostic tool. For this proteomics study, a total of 95 samples were used with 39 human brain tissue samples from the University of California San Diego (UCSD) Alzheimer's Disease Research Center (ADRC), 16 CSF samples purchased from Golden West Biologicals Inc., and 40 plasma samples from Washington University at St. Louis (WUSTL) Knight Alzheimer's Disease Research Center (KADRC). The sample prep included a beads beating protocol for the brain tissue and a depletion step for plasma. The samples were then denatured, reduced, alkylated and digested by trypsin followed by a C18 cleanup step. Samples were then analyzed using an Ultimate 3000 nano UHPLC coupled to an Orbitrap Fusion Lumos mass spectrometer with FAIMS-Pro interface with three compensation voltages (CV) of -40, -55, and -70. The column used was an Acclaim™ Pepmap™ C18 column (0.075 mm x 500 mm, 3 µm). The LC gradient was 141 minutes long with a flow rate of 0.300 µL/min. The raw files were then processed using Proteome Discoverer for identification and quantitation with proteins of "High" or "Medium" confidence kept for further processing. Data cleaning, statistical analysis and visualization were carried out using R. Results show 4491 proteins quantified in brain tissue with 949 statistically significant (429 up, 520 down), 930 proteins quantified in CSF with 218 statistically significant (189 up, 29, down, and 504 proteins quantified in plasma with 158 statistically significant (98 up, 60 down). Three proteins were found to be statistically significant in all three samples (IGHA2, HSPG2, and DKK3) with 15 additional proteins being statistically significant and in common between brain and plasma samples.

YSL 6: Unveiling Glycan Profiles to Study Breast Cancer Brain Metastasis

Joy Solomon[1], Sherifdeen Onigbinde[1], Wenjing Peng[1], Parvin Mirzaei[1], Akhila Reddy[1], Byeong Gwan Cho[1], Mona Goli[1], Moyinoluwa Adeniyi[1], Judith Nwaiwu[1], Mojibola Fowowe[1], Oluwatosin Daramola[1], Waziha Purba[1], Yehia Mechref[1]

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Breast Cancer, aside from its high incidence, has shown to have a high metastatic possibility, forming secondary tumor sites in organs such as bones, lungs, liver, brain. Brain metastatic breast cancer has gained attention because of its increased incidence rate and its low survival rate. Despite the increasing incidence of brain metastasis from breast cancer, the underlying mechanisms remain poorly understood. Altered glycosylation is known to play a role in various diseases, including cancer metastasis. Most of the existing research focuses on gene and protein expressions. Hence, there is a need to investigate glycan moiety alterations during the breast cancer brain metastasis process to understand the roles glycans play in this biological process. We investigated the *N* and *O*-glycans released from five breast cancer cell lines including MDA-MB-231, MDA-MB-231BR, MDA-MB-361, HTB131, HTB22 and from one brain cancer cell line, CRL-1620 utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS). The *N*-glycomics experiment was initiated with extraction of the proteins. Then the glycans were released using PNGase-F enzyme. The released glycans were separated from the proteins, reduced, and permethylated. For the *O*-glycomics, after protein extraction, the *O*-glycans were subjected to a chemoenzymatic release. The released *O*-glycans were then subjected to solid-phase permethylation. The resulting permethylated glycans were resuspended in an aqueous solution containing 20% acetonitrile and 0.1% formic acid before undergoing LC-MS analysis. Significance in glycan changes was assessed through the Mann-Whitney U test, calculating p-values at a 95% confidence level. An increase of *N*-glycan $HexNAc_4Hex_5DeoxyHex_1NeuAc_1$, $HexNAc_5Hex_6DeoxyHex_1NeuAc_3$, and $HexNAc_6Hex_7DeoxyHex_1NeuAc_3$ were observed in 231BR, suggesting that they may play important roles during the breast cancer brain metastasis process. The expressions of *O*-glycan $HexNAc_1Hex_1NeuAc_1$, $HexNAc_1Hex_1NeuAc_2$, and $HexNAc_2Hex_3$ were significantly altered across all other cell lines in comparison to 231BR, suggesting that they may play a role in the metastatic process.

Tuesday, October 1, 2024

PL-02: Importance of D-Amino Acids in Biological Systems: Detection and Analysis

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Chiral small metabolic molecules can be important in regulating a variety of biological functions and provide information about the presence and progression of disease. Precise analysis of less prevalent D-amino acids as free entities or as constituents of peptides can be difficult when analyzing complex physiological matrices. Because enantiomers and epimers have the same exact mass, stereoselective separations are essential. However, sensitive detection also is necessary because trace levels of these analytes are involved. Multidimensional separations often provide the best avenue for accurate and quantitative results. Faster and "greener" chiral analyses are becoming more routine and will be discussed as well as their use in studies of various pathologies[1-4].

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KL-05: Specific Interactions in Liquid Phase Separations

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In this study, we focus on the theoretical understanding for π interactions due to the separation of aromatic halogens, H/D isotopes, sugar chains and glycoproteins. Additionally, we focus on the molecularly imprinted polymers (MIPs) using the weak intermolecular interactions.

We report an experimental evaluation for Halogen-π (X-π) interaction by liquid chromatography (LC) using carbon-material coated columns providing strong π interactions in normal phase mode. We suppose the existence of bimodal interactions, π-π and X-π interaction, between halogenated benzenes and aromatic materials. Then, we successfully demonstrated the effective separations of di- or tri-bromo benzene isomers, several halogenated benzenes, and 11 brominated benzene analogues by optimization of the mobile phase conditions with a C_{70}-coated column [1]. Additionally, we studied kinetic and geometric H/D isotope effects based on OH-π and/or CH/D-π interactions in liquid chromatography. Finally, we demonstrated the effective H/D isotopologue separations by utilizing the trade inquiry between kinetic and geometric isotope effects, complementary [2]. Furthermore, 5-boronopicolinic acid (BPA), which is one of boronic acid derivatives containing a pyridine moiety show a lower pK_a, is used to prepare silica-gel based columns with poly(ethylene glycol) (PEG) and poly(ethylene imine) (PEI) conjugated BPA. The optimized column, BPA-PEI1800-PEG600-SiO_2 packed column, was suitable for the online LC concentration of the glycoprotein (HRP), even at low concentrations, which are below the detection limit in the typical LC analysis [3].

As another application of the weak intermolecular interactions, we designed the MIPs for the halogenated aromatic compounds. Firstly, we succeeded in the effective chromatographic separation of diiodobenzene isomers on the uniformly sized MIPs prepared by multi-step swelling and polymerization. The MIPs selectively recognized halogenated thyroid hormones via halogen bonding and could be applied to screening endocrine disruptors [4]. Next, we revealed the unique molecular recognition ability using the MIP with a flexible crosslinker. This study found the presence of the suitable length of the spacer in a crosslinker during the MIP preparation for the accurate molecular recognition. Furthermore, according to the thermodynamic approaches, the MIP also indicated the induced-fit type molecular recognition, briefly memorizing the stable molecular recognition sites at polymerization temperature [5].

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KL-06: 3D Printed Microfluidic Devices Integrating Solid-Phase Extraction and Electrophoretic Separation

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3D printing provides a powerful, iteratively optimizable approach for the creation of improved devices for miniaturized chemical analysis. We have developed novel 3D printers that enable the

formation of integrated microfluidic systems that offer a broad range of separation functions for the analysis of biomarkers related to human health. Porous polymer monoliths formed in these microfluidic devices can be combined with miniaturized pumps and valves to improve sample preparation processes, including immunoaffinity and reversed-phase extraction. Automated preconcentration and fluorescence labeling on these monoliths can then provide small volumes of enriched sample for rapid microchip electrophoresis. We have developed 3D printed microdevices for high-performance electrophoretic separation of disease-linked biomarkers [1]. We have further integrated solid-phase extraction and fluorescence labeling with microchip electrophoresis in these 3D printed systems [2]. We are working to combine immunoaffinity extraction, solid-phase extraction, and microchip electrophoresis in a single device for automated, rapid determination of proteins and peptides connected to risk of preterm birth. 3D printed microfluidics have excellent potential to advance chemical analysis by reducing sample and reagent volumes, offering rapid separations, improving limits of detection, and automating processes.

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KL-07: Promise of MitoQuinone in TBI Therapeutics: Lessons from Clinical TBI Trajectories

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Mild traumatic brain injury (mTBI) or concussion accounts for the bulk of all head injuries and represents a major health concern. To date, there is no FDA-approved drug for TBI in general and rmTBI in particular. Among the TBI secondary injury pathophysiological events, oxidative stress and mitochondrial bioenergetics dysfunction have emerged as major pathomechanistic instigators. Mitochondrial dysfunction is evident acutely and can extend to chronic time points post-injury observed in mild TBI. Recently, Mitoquinone (MitoQ), a synthetic mitochondria-targeted antioxidant drug, is a viable contender in different TBI modalities. In this work, different experimental mouse models of TBI investigated the neurotherapeutic roles of (MitoQ), at acute (3 days), subacute (7 days), and chronic (30 days) time points which were evident at the molecular, neural, and behavioral levels providing a new dimension for Neurotherapy. This work was evaluated on both the proteomics and glycomics levels. Data from this work extended our knowledge of the need for novel methods to assess markers of brain insult and therapy which will be discussed in the realm of the outcomes of the MitoQ study.

L5: Bruker Daltonics – 4D Proteomics Update De-risking Discovery

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The challenges of modern proteomics are addressed by platforms that are both highly sensitive and have extremely fast scan speeds. Bruker's timsTOF line of systems addresses these needs simultaneously in all their platforms. The timsTOF Ultra 2 excels at workflows that have minimum sample availability at the low pico gram level yet maintaining the capability to span the dynamic range up to 800ng. This is made possible with the field upgradable CSI Ultra 2 configuration as well as the ion charge control. Here we show the acute selectivity of the new timsTOF Ultra 2 applicable to the high and low input workflows.

L6: Investigation of Chiral Separation of α-Hydroxy Acid Biomarkers via SPP-Teicoplanin

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α-Hydroxy acid determination has a wide variety of applications in the food industry, dermatology, and most importantly in medical diagnosis. The enantiomeric separation of α-hydroxy acids is crucial since specific enantiomers of these compounds can be used as biomarkers for the diagnosis and prognosis of cancer, brain diseases, kidney diseases, diabetes, etc. [1]. There were limited examples of the separation of α-hydroxy acid enantiomers using liquid chromatography in the literature. Moreover, the methods were lengthy and had low selectivity at room temperature. This work presents fast and sensitive HPLC methods for the enantioseparation of 11 α-hydroxy acids using a superficially porous particle–based teicoplanin chiral stationary phase (TeicoShell) [2,3]. Specific mobile phases containing ammonium formate and potassium dihydrogen phosphate were used to be compatible with MS and UV detection methods, respectively. It was observed that mobile phase acidity and ionic strength significantly affected enantioresolution and enantioselectivity [3]. Interestingly, higher ionic strength resulted in increased retention and enantioresolution. The effect of organic modifiers was investigated as well. Using acetonitrile as the organic modifier usually resulted in greater enantioresolution compared to methanol. However, sometimes using acetonitrile with high ammonium formate concentrations led to lengthy retention times which could be avoided by using methanol as the organic modifier. Additionally, determination of enantiomeric content of single enantiomer commercial standards showed presence of enantiomeric impurity in all the standards which emphasizes the importance of developing chiral separation methods [3].

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YSL 7: Characterizing Insect-Mediated Microbial Degradation of Insecticides Through LC Methodologies

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The mechanism elucidating the resistance of some insects to pesticides is a relatively recently explained phenomenon. Similarly, the ability of insects to detoxify their plant diet is not fully understood. As part of an interdisciplinary project to explore the symbiotic relationship of insects and their digestive bacteria, analytical methods for detection and quantification of pesticides and plant toxins were developed. For the pesticide racemic malathion, the ability of bacterial lines to degrade the pesticide chirally can result in either enhanced or reduced toxicity, as the two enantiomers have different toxicity levels. In terms of plant toxins, including chlorogenic acid, oxalic acid, and rutin, insect gut bacteria have co-evolved with the insect over time, providing the ability to degrade otherwise toxic allelochemicals. The methods developed to characterize this varied class of insecticides, and their degradation (or lack thereof) by hundreds of bacterial lines, are discussed.

YSL 8: Identifying Dysregulated Lipids in Human Brain of Alzheimer's Disease and their Effects on Biological Pathways

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Introduction: Alzheimer's disease (AD) is the most common neurological disorder and the leading cause of dementia, severely affecting cognitive functions such as memory, speech, and physical coordination. It ranks as the seventh leading cause of death in the United States, with cases expected to triple in the next 30 years. Current AD diagnoses primarily depend on clinical symptoms, but alterations in lipid rafts—critical for cell signaling—may also play a role in the disease's progression.

Methods: In this study, 18 brain tissue samples from AD patients and 18 from healthy controls were analyzed. The samples were lysed, and their protein concentrations were determined. Lipids were extracted using the absolute methanol extraction method. LIPIDOMIX® and GM1-d3 Internal Standard were spiked into the samples, and the mixture was analyzed on an LC-MS/MS platform.

Preliminary Data: A total of 598 lipid ions were identified and quantified, with 123 showing significant differences between AD and control samples ($p < 0.05$; $q < 0.05$ upon Welch t-test with Benjamini-Hochberg correction). Of these, 117 were upregulated, and 6 were downregulated. The study focused on lipid subclass levels; 20 out of 44 quantified lipid subclasses were upregulated in AD samples. Notably, upregulated lipids included phosphatidylglycerol ($q < 0.00002$), phosphatidylserine

(q < 0.00002), lysophosphatidic acid (q < 0.01), GD2 (q < 0.04), and GD1a with fold changes of 19.3, 12.7, 8.1, 4.5, and 4.0 respectively. Additionally, hexosylceramide and ganglioside GM3 were significantly upregulated in female AD patients compared to males. System biology analysis suggested that dysregulated fatty acids may activate glycerophospholipid biosynthesis, contributing to the upregulation of phospholipids. Dysregulation of sphingomyelin, sphingosine-1-phosphate, and ganglioside GD1a is associated with sphingolipidoses, indicating a potential link to AD pathology.

Novel Aspect: This study identifies distinct lipid signatures in AD brains, enhancing our understanding of lipid- related pathophysiology in Alzheimer's disease.

YSL 9: LC-MS/MS-Based Metabolic Profiling: Investigating Serum and CSF in TBI Patients

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Metabolomics is a powerful tool in systems biology, crucial for unraveling the complex biochemical alterations associated with Traumatic Brain Injury (TBI). TBI, caused by external head impact, initiates a cascade of molecular events that significantly alter the brain's metabolic profile. Through metabolomic studies, we can identify distinct metabolic signatures at various stages of TBI, offering valuable insights into the underlying pathophysiological processes. This knowledge not only deepens our understanding of the molecular mechanisms involved but also paves the way for advancements in diagnostics and targeted therapies.

In our study, we focused on analyzing metabolites in the serum and cerebrospinal fluid (CSF) of TBI patients to uncover the detailed molecular mechanisms underlying the injury. To extract polar metabolites, we used 100 µL of CSF or serum samples, mixed with 200 µL of Dichloromethane/Methanol (DCM/MeOH) (1:2 v/v) and 75 µL of DCM along with cold water. After centrifugation at 5000 rpm for 15 minutes, the supernatant containing the polar metabolites was collected, dried, and then resuspended for Liquid Chromatography-Mass Spectrometry (LC- MS/MS) analysis.

Our analysis examined 38 samples (19 CSF and 19 serum) from TBI patients, collected on days 1, 3, and 5 post-injury. Using Compound Discoverer for metabolite identification, we detected 797 metabolites in CSF and 1113 in serum. Significant changes were noted: 39 metabolites in CSF between days 1 and 3, 38 between days 1 and 5, and 11 between days 3 and 5. In serum, 131 significant metabolites were observed between days 1 and 3, 186 between days 1 and 5, and 68 between days 3 and 5. Pathway analysis with IPA highlighted distinct enriched pathways on different days, revealing significant alterations in pathways related to tRNA charging, lipid metabolism, and neurotransmitter

release. This highlights the ever-evolving landscape of metabolic transformations that unfold in the wake of traumatic brain injury.

Wednesday, October 2, 2024

PL-03: Ion Separations in Hollow Tubes: Mirage or Oasis?

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Jorgenson and Lukacs [1] introduced capillary zone electrophoresis (CZE) in 1981, a technique that is widely used today with many manufacturers offering commercial instrumentation. So, the title question will seem to be moot. In the wake of CZE exhibiting hitherto unequaled separation efficiencies, more than one manufacturer offered instruments for the separation and determination of small ions shortly thereafter. None successfully competed with ion exchange chromatography for ion separations as introduced in its suppressed conductometric form by Small et al. in 1975 [2] and commercialized as *Ion Chromatography* in 1977. What is far less cited is the work of Jorgenson and Guthrie in 1983 [3] in which they showed chromatographic separations just as impressive as CZE. The tubes were smaller, however; absorbance detection was impractical. Jorgenson prophetically remarked that OTLC will succeed only if suitable detectors are available. In this decade Liu and his students have shown amazing separations (1000 peaks an hour) in 2 μm dia. capillaries, fluor tagging analytes and using LIF detection or using a one-of-a-kind MS system [4]. I admittedly hold the biased view that such detectors defeat the purpose of miniaturizing the rest of the separation system. We have pursued with many pauses and bursts, ion chromatography in open tubes since 1997 [5]; more often than not, the goal seemed to be a mirage. This is an account of the journey; others have given their own account of the tortuous path [6].

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KL-08: Octadecyl Monolith for the Reversed-Phase Capillary Electrochromatography of Pre-column Derivatized Mono- and Oligosaccharides with Three Different Ultraviolet Absorbing Tags

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In this talk, an in house developed octadecyl monolithic (ODM) column has been exploited in the reversed-phase capillary electrochromatography (RP-CEC) of precolumn derivatized mono- and oligosaccharides with three different tagging agents, namely 1-naphthylamine (1-NA), 2-aminoanthracene (2-AA) and 3-amino-2,7-naphthalenedisulfonic acid (ANDSA). These three derivatizing agents, which differed in their charges, nonpolar characters and optical absorption properties, led to different RP-CEC elution patterns and UV detection signals. In fact, the limit of detection of the derivatized sugars were 50 µM for the ANDSA- and 1-NA-sugar derivatives and 35 µM for the 2-AA-sugar derivatives due to the presence of three fused aromatic rings in 2-AA versus 2 fused rings in the 1-NA and ANDSA tags. Furthermore, while the longer ANDSA-oligosaccharides eluted later than the shorter ones and the ANDSA-monosaccharides, 1-NA- and 2-AA-sugar derivatives necessitated the presence of borate ions at alkaline pH in the mobile phase to form in situ charged derivatives to facilitate their separation by RP-CEC, and the elution order was the reversal of that observed with the ANDSA-sugar derivatives; that is the mono- eluted later than the larger size oligosaccharides. In addition, plots of log t_R vs. number of glucose residues (n_{Glc}) for derivatized glucose and maltooligosaccharides yielded straight lines with slopes representing log h where h is the retention time modulus (i.e., ratio of retention time of two neighboring derivatives differing in one glucosyl residue). In the case of 1-NA and 2-AA derivatives, h was smaller than unity while it was greater than unity in the case of ANDSA-sugar derivatives because the elution occurred in the order of decreasing size of the homologous sugar derivatives in the former than in the later derivatives. The prepared ODM column was stable for more than a month of continuous use, a fact that allowed a good repeatability for intraday and interday analyses.

KL-09: Targeted and Untargeted Analysis of Psilocybin Mushrooms using LC-MS

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Psilocybin mushrooms have drawn recent attention due to their potential for the treatment of major depressive disorders and anxiety. A desire exists to continue the investigation of psilocybin treatments for mental health problems. Prior clinical studies have relied exclusively on the use of synthetic psilocybin. In order to provide a reliable dose of psilocybin and psilocin from a natural product "magic" mushrooms, reliable methodologies are needed to understand their composition. A liquid chromatography – triple quadrupole – mass spectrometry method was developed to study and establish the potency of different psilocybin mushrooms [1]. Mushrooms were milled using a Fritsch Pulverisette 11 blade mill; 50 mg of mushroom was extracted twice using 5 mL of methanol acidified with 0.5% acetic acid. A Shimadzu LCMS-8040 was used in reversed phase mode (5 cm Supelco Biphenyl phase). Confirmed also with interlaboratory testing at Millipore Sigma, a series of different psilocybin mushroom strains were established to contain between 0.9 – 1.2% of psilocybin and psilocin by weight. To further investigate the compositional differences among psilocybin mushrooms (and compared to regular mushrooms), an untargeted analysis was also performed using liquid chromatography – quadrupole – time-of-flight – mass spectrometry (Shimadzu LCMS-9030). Psilocybin mushrooms could be well differentiated from regular mushrooms based on the reversed phase analysis performed. Some chemical compound classes could be identified that clearly differentiate the different mushroom types. However, additional work is ongoing to elucidate the presence of other tryptamine alkaloids of interest, especially their potentially different levels of abundance in different psilocybin mushroom strains.

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L7: Considerations and Capabilities of Ion Chromatography

Tom Cardwell

Shimadzu Scientific Instruments

Ion chromatography (IC) maintains many fundamental ideas used in LC, but for ions rather than molecules. A few hardware considerations are pivotal to understand for the success of IC separations. Presented will be the current hardware strategies used across the market, including the use of detectors beyond conductivity. Detector and sample introduction technologies have afforded industries other than drinking water to incorporate IC in their processes. Presented will be aspects of IC used for oil and gas, fuel additives, batteries, and pharmaceutical drugs.

L8: Reducing Hepatitis C Diagnostic Disparities with a Point of Care Assay for HCV Antigen detection using a Handheld Microfluidic Device with Sequence Oscillatory Flow Controls

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Viral hepatitis continues to be a significant global health issue, with chronic hepatitis B (HBV) and hepatitis C (HCV) responsible for approximately 1 million deaths each year, primarily due to complications such as liver cancer and cirrhosis. Annually, more than 1.5 million individuals contract HCV, with vulnerable populations, including American Indians and Alaska Natives (AI/AN), being disproportionately affected. Although direct-acting antivirals (DAAs) have proven highly effective, the timely and accurate diagnosis of HCV remains a major challenge, particularly in settings with limited resources. The current two-step HCV testing approach is both costly and time-intensive, often resulting in patient loss before appropriate care is administered. Point-of-care (POC) HCV antigen (Ag) testing presents a viable alternative, offering the potential for early detection, even during the acute phase of infection. However, there is currently no FDA-approved POC HCV Ag test that meets the required sensitivity and specificity for detecting low viral loads. To address this gap, we developed a handheld and fully automated smartphone-based POC HCV Ag assay using the catalytic property of platinum nanoparticles (PtNPs), advanced deep learning image processing, and miniature microfluidic devices with sequential oscillatory flow controls. The overall accuracy of the developed system in determining the presence of HCV was 94.94% (95% CI, 87.54% to 98.60%). Accuracies were 94.74% (95% CI, 82.25% to 99.36%) for samples collected from general populations (n=38) and 95.12% (95% CI, 83.47% to 99.40%) for samples collected from AI/AN populations (n=41). This user-friendly, cost-effective, and portable HCV detection device paves the way for reducing HCV-related health disparities by improving accessibility for underserved community members, particularly within the AI/AN populations, ultimately enhancing equity in HCV-related care.

YSL 10: LC-MS/MS-Based Proteomic Profiling of Small Extracellular Vesicle Alterations in Severe Traumatic Brain Injury

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Effective therapies for traumatic brain injury (TBI) rely on identifying reliable diagnostic markers that indicate the development and progression of the injury. Small extracellular vesicles (sEVs), secreted by most cells, can cross the blood-brain barrier (BBB) and carry important biomolecules, such as proteins. Consequently, sEVs are a promising resource for understanding the pathophysiology of TBI. In this study, we utilized liquid chromatography-tandem mass spectrometry (LC-MS/MS) to perform a comprehensive proteomic analysis of sEVs derived from the serum and cerebrospinal fluid (CSF) of patients with severe traumatic brain injury (sTBI). Our goal was to investigate the time-dependent proteomic changes associated with sTBI and identify potential biomarkers for injury progression. A two-tailed Student's t-test revealed 164 differentially expressed proteins in serum-sEVs and 114 in CSF-sEVs when comparing control groups with sTBI groups at 1-, 3-, and 5-days post-injury. Notably, we observed significant increases ($p < 0.05$) in astrocyte-expressed glial fibrillary acidic protein (GFAP) and neuronal ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in both serum- and CSF-sEVs at these time points. In healthy subjects, GFAP levels were higher in serum sEVs than in CSF sEVs. However, from days 1 to 5 post-injury, CSF sEVs showed a significant increase compared to serum sEVs ($p < 0.05$). We also detected elevated levels of C-reactive protein (CRP) in CSF sEVs, a sensitive biomarker for predicting poor clinical outcomes in TBI and reflecting responses to trauma and chronic inflammation. These differentially expressed proteins may help distinguish between injured and healthy individuals. Further investigation of these proteins will shed light on the biological mechanisms and molecular pathways involved in TBI over time.

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YSL 11: An Innovative Method for Analyzing IgG Glycosylation Significance in Traumatic Brain Injury

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Traumatic brain injury (TBI) is a critical public health issue, affecting millions globally and particularly impacting young adults. Dysregulated glycosylation patterns, a feature in both TBI and neurodegenerative diseases, play a significant role in immune responses following brain injury. Given the pivotal role of IgG in the immune response post-TBI, a comprehensive investigation into the glycosylation of IgG becomes essential. This study introduces a novel approach to analyzing IgG glycosylation in serum samples from TBI patients. By utilizing an on- membrane digestion method, IgG-specific *N*-glycans were released using the GlycINATOR enzyme, while *N*-glycans from other glycoproteins were liberated with PNGase F. High-resolution LC-MS/MS analysis identified 19 IgG *N*-glycans and 84 *N*-glycans from other glycoproteins, with significant changes observed across different time points post-TBI. The findings indicated a significant increase in sialylation in both IgG and other glycoprotein-derived glycans in TBI patients when compared to controls. In contrast, fucosylation levels were elevated in IgG but reduced in other glycoproteins. Specifically, eight IgG *N*-glycans exhibited significant changes on Day 1, nine on Day 3, and eleven on Day 5 post-injury. Among the *N*-glycans from other glycoproteins, fourteen showed significant changes on Day 1, thirty on Day 3, and 27 on Day 5. Interestingly, four glycans (3500, 4501, 4502, and 5512) exhibited significant changes across both IgG-specific and other glycoprotein *N*-glycans, although with varying trends over time. Additionally, the study explored isomeric IgG glycans, noting changes in their expression post- TBI. Future work will investigate fucosylation or afucosylation on core GlcNAc of IgG and validate these findings through PRM analysis of purified IgG from TBI samples. The insights gained from this research could pave the way for developing quantitative ELISA assays, offering clinically valuable diagnostic and prognostic tools for better TBI management and personalized treatment approaches.

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KL-10: Vibrating Sharp-edge Spray Ionization: A New Paradigm for Coupling Condensed-phase Separations with Mass Spectrometry

Stephen J. Valentine, Peng Li, Olanrewaju Awoyemi, Sandra Majuta, Anthony DeBastiani, Madison Pursell, Daud Sharif

West Virginia University, Morgantown, WV

For the past ~35 years, electrospray ionization (ESI) has been the mainstay ionization source for liquid chromatography (LC) – mass spectrometry (MS) analyses. Despite its tremendous sensitivity, ESI has largely remained unchanged in form and practice since its inception. Recently, a new ionization source named vibrating sharp-edge spray ionization (VSSI) has emerged as a competitive ion source methodology for LC-MS analyses due to its exquisite sensitivity; 5 to 100 fold enhancements over state-of-the-art ESI have been demonstrated for various molecular systems. Additionally, VSSI offers opportunities to increase the breadth and depth of 'omics analyses. This can be achieved through coupling novel source features to VSSI such as atmospheric pressure chemical ionization (APCI) and in-droplet hydrogen/deuterium exchange (HDX). The current status of VSSI as an alternative source for condensed phase separations as well as developments on the horizon will be covered in this presentation.

L9: LC Hardware Considerations for Better Biomolecule Separations

Joe Lopez

Shimadzu Scientific Instruments

The chemical and physical properties of biomolecules are more important and variable than for small molecules in regards to analytical separations. Aspects such as adsorption to metal surfaces, physical orientation, and net charge on biomolecules are new considerations for those trained in small molecule separations. These properties can also be dynamic depending on analytical conditions, leading to inconsistency in biomolecule separations and quantitation. Presented will be aspects to consider, recommendations and the latest offerings for more robust biomolecule separations.

L10: The Power of PASEF in Lipidomics Research

Beixi Wang

Applications Scientist, Small Molecule and Metabolomics, Bruker Daltonics

Lipids play a crucial role in biological systems as they are essential for cell membrane structure, energy storage, and acting as signaling molecules. Imbalances in the lipidome are associated with a variety of disease mechanisms, including cardiovascular and metabolic diseases. Identifying alterations in lipid metabolism is crucial in characterizing disease states, developing diagnostics and prognostic indicators. One of the major challenges in lipidomics analysis is differentiating isobaric and isomeric lipids. Enter the world of Trapped Ion Mobility Spectrometry (TIMS), which can separate lipid ions based on size and shape prior to mass analysis. This additional dimension of separation provides cleaner, in-depth MS2 coverage as well as a measured CCS value that can be used for more confident lipid annotations. Here we apply LC-TIMS-MS for comprehensive lipidome coverage, quantitative analysis, unknown identification, and ultra-high sensitivity workflows down to the single cell.

L11: Advances in Multidimensional Mass Spectrometry for Resolving Complex Biological and Environmental Sample Mixtures

Touradj Solouki

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Understanding the intricate composition of biological and environmental samples at the molecular level is imperative for advancements in health, ecology, and chemistry. However, the complex nature of sample mixtures often presents significant challenges for conventional analytical techniques, especially in separating and identifying individual components. This presentation will feature examples of ultrahigh-resolution and multidimensional mass spectrometry instrumentation, along with data analysis approaches that illustrate improved precision and the ability to analyze highly complex samples. Specifically, multidimensional data for characterization of unprocessed biological samples as well as environmental samples such as crude oil that contains thousands of molecules, will be presented. These cutting-edge analytical methods are crucial for overcoming the limitations of traditional separation techniques and pushing forward innovation in complex mixture characterization.

L12: Slow Your Flow for Improved Top-down Analysis of Proteins

William Russell

The University of Texas Medical Branch at Galveston, Galveston, TX

Liquid chromatography-mass spectrometry (LC-MS) intact mass analysis and LC-MS/MS peptide mapping are key technologies used in assays for developing and characterizing biologics and protein reagents. Analysis can be confounded by protein modifications and truncations, which increase the difficulty of precise proteoform characterization owing to inherent limitations in peptide and intact protein analyses. Top-down MS (TDMS) directly addresses this ambiguity through fragmentation of targeted proteoforms. We optimized our existing flow-programmed online buffer exchange (OBE) chromatographic approach for high-throughput TDMS applications, resulting in improved ESI sensitivity and increases in TDMS sampling time. OBE is essentially shortened size exclusion chromatography (SEC), a nonadsorptive chromatographic mode, which provides excellent sample recovery, direct compatibility with MS, and negligible protein–protein separation. This HPLC-based approach streamlines sample injection and buffer exchange, while providing several minutes of MS/MS acquisition time at micro-flow ESI sensitivity. One key feature of our study is the simple and effective use of flow-programmed (fp) denaturing OBE to provide several minutes of MS/MS acquisition time for a given protein analyte with improvements in sensitivity due to microliter/minute flow rates. A second key feature of this study is the use of a new software platform called TDAcquire X, an innovative data analysis technique to generate high-quality TDMS results for sequence and modification elucidation. This technique involves forming composite proteoform spectral match (cPrSM) results by aggregating

the fragment data from different scan types across multiple injections that constitute an experiment. Data "depth" is leveraged using a probability-based approach to remove low-quality fragment ions which are less consistently detected. This strategy yields high-quality fragment data which we demonstrate can be used to determine highly significant fragments to precisely reveal PTM occupancy for characterizing positional isomers.

YSL 12: Glycoproteomics Profiling of Biofluids in Severe Traumatic Brain Injury Patients

Moyinoluwa Adeniyi[1], Mojibola Fowowe[1], Joy Solomon[1], Sherifdeen Onigbinde[1], Waziha Purba[1], Md Mostofa Al Amin Bhuiyan[1], Judith Nwaiwu[1], Cristian D. Gutierrez Reyes[1], Ava Puccio[2], Firas H. Kobaissy[3], Stefania Mondello[4], Yehia Mechref[1]

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Protein glycosylation is critical in various physiological and pathological processes, including neurological development and diseases. Traumatic Brain Injury (TBI) is a highly heterogeneous condition that poses a significant global health challenge, particularly affecting the younger adult population, with millions of cases reported annually. This underscores the need for advanced biomarker discovery to enhance diagnosis, prognosis, and therapeutic strategies. Cerebrospinal Fluid (CSF), being in direct contact with the brain and spinal cord, serves as an ideal medium for investigating the biochemical and molecular alterations following TBI. Also, several proteins have been reported to cross the blood-CSF barrier. In this study, a Liquid Chromatography Mass Spectrometry (LC-MS/MS) glycoproteomics approach was utilized to analyze *N*-glycoproteomics changes in CSF and serum samples collected from severe TBI (sTBI) patients and healthy controls. The samples were obtained at three critical time points post-injury: day 1, day 3, and day 5, representing key stages in the disease progression. A total of 270 *N*-glycopeptides were identified in the CSF samples. Pairwise comparisons between TBI cohorts and controls revealed 94, 95, and 41 differentially expressed glycopeptides on day 1, day 3, and day 5, respectively. Notably, more significant differences were observed between day 1 and day 3 versus controls, compared to day 5. Among the differentially expressed N-glycopeptides, 24 exhibited changes across the different time points. These include common serum proteins known to cross the blood-CSF barrier and those involved in inflammatory and immune responses. These alterations in glycoproteins may reflect the immune system's response to sTBI. Our findings hold the potential to provide crucial insights into the progressive *N*-glycoproteome changes in the biofluids of sTBI patients, contributing to a deeper understanding of TBI pathophysiology and offering potential avenues for biomarker development.

Acknowledgement:

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YSL 13: Heart Cut 2D-LC in a Single Chromatograph for a Difficult Isomeric Separation

Siddharth Jaya Sajeevan J, Troy T. Handlovic, M. Farooq Wahab, Daniel W. Armstrong

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A two-dimensional methodology, achieved by reconfiguring a single chromatograph, was adapted for separations that are challenging with a single enantioselective column. Allethrin, a pyrethroid with three chiral centers resulting in eight stereoisomers (four diastereomers), presents one of the most challenging chiral separations known in the literature. A shape-selective porous graphitic carbon (PGC) stationary phase was used in the first dimension to achieve diastereomeric separation. A six-port 2-position rotary valve was used to heart-cut the four diastereomeric peaks and send them to the second dimension. An immobilized-cellulose tris(3,5- dichlorophenylcarbamate) column was used in the second dimension to attain enantiomeric separation with chromatographic resolutions >2.7 for all enantiomeric pairs. Also, the best chiral separation of allethrin was found to be on the (2-hydroxypropyl)-β-cyclodextrin column, which resolved seven of the eight isomers in a single dimension. The developed 2D method was used to verify the enantiomeric purity of a formulation of allethrin in a commercial pesticide.

YSL 14: Quantitation of Intact Proteins in Biological Fluids Using tITP-CZE-MS with Off-Line Microelution SPE Sample Pretreatment

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Protein analysis in biological samples is one of the key areas of biomedical research. To miniaturize the entire analytical process and lower its environmental impact, attention is currently being paid to the development of greener approaches aimed at the targeted quantitation of intact proteins. In this field, capillary electrophoresis is becoming more popular and meets the criteria for greener techniques [1,2]. When combined with mass spectrometry, it can compete with established chromatographic techniques in terms of performance and meets the requirements to become a routine part of practice [3,4]. However, when it comes to the analysis of biological matrices, its reliable application requires a comprehensive optimization of the separation and detection conditions in addition to the implementation of effective preconcentration techniques and pretreatment procedures [5].

In this work, we focused on the development of an on-line hyphenated capillary zone electrophoresis-mass spectrometry method (CZE-MS) employing off-line microelution solid-phase extraction (μSPE) as a sample pretreatment step for the quantitation of intact proteins with molecular masses <20 kDa in biological fluids (human serum, plasma, urine, and saliva). Various preconcentration techniques can be used to enhance the sensitivity of the CZE-MS method [6,7]. 19- to 127-fold increase in signal intensity was achieved by employing transient isotachophoresis (tITP) as an in-capillary preconcentration method. Off-line μSPE with various eluate treatment procedures was evaluated to ensure the compatibility of the sample pretreatment method with the selected in-capillary preconcentration, separation, and detection process. Achieved extraction recoveries of spiked proteins were in the range of 76-100% for urine, 12-54% for serum, 21-106% for plasma, and 25-98% for saliva when the eluate was evaporated and reconstituted in the solution of the leading electrolyte to achieve the tITP process [8]. The optimum method was validated across different biological matrices, offering good linearity, accuracy, and precision, and making it suitable for proteomic studies in different biological samples.

Acknowledgements

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YSL 15: LC-MS/MS Characterization of the *N*-glycosylation of Spike protein S1 Derived from 11 variants of SARS-CoV-2

Cristian D Gutierrez Reyes[1]; Sherifdeen Onigbinde[1]; Andrew I. Bennett[1]; Akeem Sanni[1]; Peilin Jiang[1]; Oluwatosin E Daramola[1]; Mojgan Atashi[1]; Vishal Sandilya[1]; Mojibola O Fowowe[1]; Yehia Mechref[1]

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After the SARS-CoV-2 outbreak in late 2019, there was an 11-month stretch of relative evolutionary stability of the virus. However, since late 2020, SARS-CoV-2 has undergone several mutations quickly to produce 'variants of concern' with observed differences in their transmissibility and infectivity. The highly glycosylated S protein plays a vital role in host cell invasion, making it the principal target for vaccine development. Different mutations on the S protein of the SARS-CoV-2 variants may cause distinct glycosylation patterns, thus reducing the efficacy of current vaccines against variants of concern. In this study, we will provide a comprehensive characterization of *N*-glycosylation in eleven variants of the SARS-CoV-2 S1 subunit protein. Initially, the S1 proteins from the SARS-CoV-2 variants alpha, beta, gamma, delta, epsilon, eta, iota, kappa, lambda, mu, and omicron were denatured. The denatured proteins were subjected to bottom-up glycoproteomics analysis using trypsin and the combinations trypsin-Glu C, and trypsin-chymotrypsin. The *N*-glycopeptides identification and quantitation were performed by combining the software Proteome Discoverer (Thermo Scientific) and Byonic (Protein Metrics). The *N*-glycoproteomics profiles of the eleven variants were compared and investigated. The native SARS-CoV-2 S1 protein has thirteen reported *N*-glycosylation sites located in the positions N17, N61, N74, N122, N149, N165, N234, N282, N331, N343, N603, N616, and N657. Additionally, a comparison of *N*-glycosite variant differences comparing the macro and micro-heterogeneity will be provided. Furthermore, *N*-glycosylation modifications on functional domains and regions across the variants are being examined.

Thursday, October 3, 2024

KL-11: Advancing Proteomics: Efficient Mass Spectrometry Techniques for Analyzing Protein Interactions and Modifications

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Deciphering systems-level interactomes and post-translational modification (PTM) networks requires cutting-edge proteomics methodologies. A key obstacle is the precise identification and quantification of protein-protein interactions and PTMs on a large scale. The complexity of interactome networks demands specialized pull-down techniques or system-wide experiments to clearly pinpoint protein complexes and their modifications. PTMs are typically low in abundance and exhibit a wide range of structures, necessitating novel strategies for accurate detection in extensive studies.

Chemistry-based methods, paired with advanced mass spectrometry, have become increasingly effective in mapping interactomes and enriching low-abundance modifications. Enhancing chemical structures with specific features can improve their detection and quantification in mass spectrometry. The selective cleavage of chemical bonds in the gas phase within a mass spectrometer is particularly useful, allowing modified peptides to be identified by their unique signature masses in the spectra.

This presentation will cover innovative techniques, including affinity purification, spacer chain-restricted crosslinking, and advanced mass spectrometry. We will also introduce cutting-edge approaches for profiling lipid PTMs and assessing proteolytic activities within cellular systems on a large scale. These advancements will provide scientists with powerful tools to thoroughly investigate complex biological systems.

KL-12: Coupling 3D-Printed Microfluidic Devices with Mass Spectrometry for Advanced Liquid Chromatography Separation

Peng Li

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Mass spectrometry is a powerful detection method for liquid chromatography (LC) separations. Recently, the rapid development of 3D printing technology has enabled the direct fabrication of microfluidic channels using consumer-grade 3D printers, offering significant potential to customize and enhance LC-MS experiments. However, stereolithography-based (SLA) 3D-printed devices face challenges with chemical leaching, which can introduce contaminant molecules that may appear as isobaric ions and/or significantly suppress the ionization of target analytes during MS analysis. To address this issue, we have developed a simple surface modification strategy to isolate chemical leachates from the channel solution, thereby eliminating contaminant peaks in MS analysis and enabling the direct coupling of 3D-printed devices with MS detection. Based on this work, we demonstrated the first 3D-printed serpentine SEC column compatible with spray-based MS analysis. We believe this advancement paves the way for creating LC columns with custom geometries and integrating additional functional units for various LC-MS applications.

KL-13: Current State of Erythropoietin (EPO) Analysis in Doping Control and the Potential of Liquid Chromatography/Capillary Electrophoresis Mass Spectrometry as a Better Alternative

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The current analytical protocol for erythropoietin (EPO) used by the World Anti Doping Agency (WADA) can be solely based on results obtained by Western bolt based on sarcosyl polyacrylamide

gel electrophoresis (SAR-PAGE) separations. Although alternative methods such as isoelectric focusing (IEF) – PAGE and different blotting procedures are recommended by WADA's EPO technical document, they are not always performed in practice because they are not mandatory. The currently used Western blot for EPO detects all EPO receptor agonists, and lacks the specificity for the types of molecules detected, especially for recombinant EPOs. False positives can be reported in cases of high endogenous EPOs, and interpretations made by experts can be subjective. Because the primary structure of endogenous EPO is the same as the recombinant ones, the main difference lies in the structures of glycans attached to the proteins. Capillary electrophoresis (CE) and liquid chromatography (LC) coupled with high resolution tandem mass spectrometry (MS) are complementary methods, and can offer clear molecular evidence between the glycans of endogenous and recombinant EPOs. CE-MS and LC-MS methods, and the results obtained will be presented. The results showed the acetylation of sialic acids, N-acetylneuraminic (Neu5Ac)/N-glycolylneuraminic acid (Neu5Gc) variation, and elongation of the glycan chains with LacNAc repeat. CE/LC-MS methods can be better alternatives to the current SAR-PAGE Western blot to avoid false positive and false negative results.

L13: Advance your LC Experience with SFC

A. Paige Wicker

Shimadzu Scientific Instruments

LC and LCMS have become a prominent separation technique in laboratories, even causing original GC methods to be transferred to LC due to varying advantages. Modern instrument advancements have allowed Super-Critical Fluid Chromatography (SFC) to move out of the R&D realm and into analytical workflows. SFC can offer additional opportunities within an LC lab through a broader separation field than reversed-phase (RP) LC, increased throughput, complimentary selectivity to RP-LC, enhanced chiral resolution and increased MS sensitivity. Presented will be a description of SFC hardware, method variables, and application parallels between LC and SFC. Flexibility in detectors, fraction collectors, and scale supports the varying examples that will be shown for pharmaceutical compounds, pesticides, and separations typically performed by normal phase LC.

L14: Digital Restoration of Chromatographic Data for Signal-to-Noise Enhancement

M. Farooq Wahab

University of Texas at Arlington, Arlington, TX

Inverse problems in signal processing focus on determining the underlying cause or input from an observed output. For instance, noise and instrumental distortion may affect a recorded chromatogram. This talk will explore whether it is possible to reconstruct an estimated trace of the "true" chromatogram, which is free from detection artifacts and noise-corrupted chromatograms. The first section will explore

new advanced denoising techniques that leverage mathematical optimization. Conventional digital filters embedded in current chromatographic instruments tend to broaden chromatographic peaks while reducing noise. New peak-width preserving techniques that effectively suppress noise will be introduced. The second section will address the enhancement of digital resolution in denoised chromatograms using Fourier analysis, regularization methods, and iterative techniques.

L15: A Custom-made Autosampler for Capillary Electrophoresis

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Aim and Background: The present study was aimed at optimising a custom-made autosampler for capillary electrophoresis (CE). Different research groups have explored the potential of developing bespoke CE devices for a number of reasons: i. science democratisation; ii. affordability; iii. in-situ analyses [1]. The proposed strategies demonstrated a range of performance and operational characteristics. However, the majority of the approaches were based on a single injection, lacking the automation of multiple analyses. The primary result was a higher operator activity, which in turn increases the potential for errors. Consequently, the present study was focused on developing an automated CE sampler for processing multiple samples, limiting the error sources.

Method: The buffer was composed by histidine 15 mM, acetic acid 50 mM, 18-crown-6 4 µM [2]. The separation occurred in a fused silica capillary (I.D. 25 µm, E.L. 42.5 cm) at 20 kV. Samples were injected by pressure (0.6 psi x 3590 ms). A Capacitively-Coupled Contactless Conductivity Detector was used for the detection [excitation frequency 320 kHz, amplitude 240 Vpp (peak-to-peak)].

Results: The developed autosampler allowed the injection from six different vials. Custom-made polymethylmethacrylate pieces were conceived for assembling four commercial items: needle, pre-puncher, and two electric motors. The instrument was tested for detecting ammonium, potassium, calcium, sodium, magnesium, and barium (ISTD), and showed a repeatability of injection better than 2.4%. The optimized device was tested for analyzing three mineral waters. After a proper dilution (10 or 20 folds), samples were injected without extraction procedure, showing accuracies within ±10%.

Conclusions: The developed device represents a valid alternative to commercial systems. In addition, its reduced cost could result in an effective instrumental approach in low resource contexts for a wide variety of applications, including pharmaceutical analysis, environment pollutants, and food safety.

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ABSTRACTS: Poster Presentations

P-01: Explore the Antileukemic Activity of the Synthetic Retinoid ST1926 in HTLV-1 Positive and Negative Malignant T Cells through LC-MS/MS Proteomics

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In this study, we investigated the potential of the synthetic retinoid ST1926 as a therapy for leukemia, particularly Adult T-cell leukemia/lymphoma (ATL) associated with the human T-cell lymphotropic virus type 1 (HTLV-1). Using advanced liquid chromatography-mass spectrometry techniques, we aimed to understand how ST1926 affects protein expression in both HTLV-1 positive and negative malignant T cells. Our research provides valuable insights into the mechanisms behind the antitumor efficacy of ST1926 and its potential for innovative treatments against HTLV-1-associated T-cell malignancies.

In this regard, we treated the HTLV-1 positive malignant T-cell line (HuT102) and the HTLV-1-negative leukemic cell line (Molt-4) with 1 μM ST1926 at different time points (2, 12, and 24 h). After treatment, we collected total cellular proteins and analyzed them using LC-MS/MS techniques. The data analysis revealed distinct proteomics profiles induced by ST1926 in the treated cells compared to control samples. Our findings show significantly differentially expressed proteins across groups, potentially influencing crucial cellular processes such as cell proliferation, apoptosis, cell death, invasion, metastasis, and metabolism. Targeting these proteins with ST1926 therapy may lead to personalized treatments for T-cell malignancies. We complemented our investigation with gene ontology and ingenuity pathway analysis to deepen our analysis. Understanding the molecular alterations in these cells is crucial for developing targeted therapeutic strategies against T-cell malignancies commonly associated with HTLV-1 infection. This study reveals the distinctive influence of ST1926 on protein expression in both HTLV-1 positive and negative malignant T cells through precise LC-MS/MS analysis.

P-02: Exploring Tianeptine: Chiral Separation, Enantiomeric Determination and Biological Effects

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Tianeptine, a tricyclic antidepressant, has gained significant attention due to its unique pharmacological profile and therapeutic efficacy in treating major depressive disorders. Unlike traditional antidepressants that primarily target serotonin reuptake inhibition, tianeptine enhances serotonin uptake, particularly in the hippocampus and the cortex [1-2]. This atypical mechanism of action, combined with its efficacy in improving mood, cognition, and neuroplasticity, makes tianeptine a subject of ongoing research and clinical interest. The importance of chiral separation of tianeptine could provide deeper insights into the drug's pharmacodynamics and pharmacokinetics. This is particularly relevant given that the enantiomers of tianeptine may interact differently with biological targets, leading to variations in their therapeutic and adverse effects [1-2]. In this study simple and fast methods for enantiomeric separation of tianeptine were reported for the first time. Tianeptine enantiomers were obtained using preparative liquid chromatography, and vibrational circular dichroism was used to determine the absolute configuration of each enantiomer. Additionally, the enantioselective binding of tianeptine to various proteins was investigated.

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P-03: LC-MS/MS Characterization of the *N*-glycosylation of Spike protein S1 Derived from 11 variants of SARS-CoV-2

Cristian D Gutierrez Reyes[1]; Sherifdeen Onigbinde[1]; Andrew I. Bennett[1]; Akeem Sanni[1]; Peilin Jiang[1]; Oluwatosin E Daramola[1]; Mojgan Atashi[1]; Vishal Sandilya[1]; Mojibola O Fowowe[1]; Yehia Mechref[1]

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After the SARS-CoV-2 outbreak in late 2019, there was an 11-month stretch of relative evolutionary stability of the virus. However, since late 2020, SARS-CoV-2 has undergone several mutations quickly to produce ‘variants of concern’ with observed differences in their transmissibility and infectivity. The highly glycosylated S protein plays a vital role in host cell invasion, making it the principal target for vaccine development. Different mutations on the S protein of the SARS-CoV-2 variants may cause distinct glycosylation patterns, thus reducing the efficacy of current vaccines against variants of concern. In this study, we will provide a comprehensive characterization of *N*-glycosylation in eleven variants of the SARS-CoV-2 S1 subunit protein. Initially, the S1 proteins from the SARS-CoV-2 variants alpha, beta, gamma, delta, epsilon, eta, iota, kappa, lambda, mu, and omicron were denatured. The denatured proteins were subjected to bottom-up glycoproteomics analysis using trypsin and the combinations trypsin-Glu C, and trypsin-chymotrypsin. The *N*-glycopeptides identification and quantitation were performed by combining the software Proteome Discoverer (Thermo Scientific) and Byonic (Protein Metrics). The *N*-glycoproteomics profiles of the eleven variants were compared and

investigated. The native SARS-CoV-2 S1 protein has thirteen reported *N*-glycosylation sites located in the positions N17, N61, N74, N122, N149, N165, N234, N282, N331, N343, N603, N616, and N657. Additionally, a comparison of *N*-glycosite variant differences comparing the macro and micro-heterogeneity will be provided. Furthermore, *N*-glycosylation modifications on functional domains and regions across the variants are being examined.

P-04: Exploring the Impact of Fasting on Biological Pathways through LC-MS/MS of Profiling Serum Proteome

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Introduction: The influence of fasting on cellular processes has emerged as a groundbreaking area in biomedical research. Fasting induces amino acid utilization for glucose generation and activates autophagy, crucial for energy conservation and maintaining cellular health during nutrient scarcity. In this study, we employed LC/MS-MS to investigate the impact of fasting on biological pathways.

Methods: In this study, we examined the serum proteome changes of 28 human subjects before and after a period of fasting. For low-abundance protein collection, we depleted serum samples with the Agilent Human 14 Multiple Affinity Removal Column. Protein concentration was measured using Thermo Scientific's Micro BCA™ Protein Assay Kit. An Acclaim PepMap C18 capillary column (75 µm x 15 cm, 2 µm, 100 Å) in the Ultimate 3000 nanoUHPLC coupled with QE-HF mass spectrometer for LC-MS/MS analysis.

Preliminary Data: In the proteomics study, 463 proteins were identified and quantified using Proteome Discoverer software in both pre-fasting and post-fasting serum samples. Principal Component Analysis (PCA) plot revealed distinct patterns between the two groups, highlighting the impact of fasting on the serum proteome. Statistical analysis with the Mann-Whitney U-test and Benjamini-Hochman correction identified 138 significant proteins (p-value < 0.05), with 48 upregulated and 90 downregulated. The altered expression of these proteins was visualized using a heatmap, providing a clear representation of their differential regulation. Ingenuity Pathway Analysis (IPA) revealed some functional implications of these protein changes. Proteins associated with neurological diseases (THBS1, CTNNB1, FLT4) were found to activate sensory system development, while inflammatory responses featured proteins such as THBS1, ADAMTS13, APCS, C4A, C4B, CTNNB1, and PGLYRP2. Additionally, proteins like NCAM1, LBP, IGFBP7, CTNNB1, and FCGR2A activated pathways leading to the synthesis and metabolism of fatty acids.

Novel Aspect: Differential protein expression resulting from fasting reveals intricate molecular mechanisms influencing key metabolic processes, vascular system development, and inflammatory responses.

P-05: Are Conventional Efficiency Measurements Reliable in Chiral Chromatography?

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Efficiency-flow profiles such as the van Deemter provide valuable insights for optimizing separations, yet the shape of the measured peak is often overlooked. Chiral separations are regularly asymmetric, so reliance on the assumption that peaks are Gaussian overestimates the calculated efficiency. The most accurate method of determining peak efficiency is from the statistical moment analysis of the raw chromatogram. However, this approach suffers from several problems, such as baseline noise and peak overlap, that render it impractical in many cases. Sophisticated peak models that incorporate higher-order moments have been used to account for the peak shape of highly asymmetrical or unresolved peaks. However, broader adoption may be needed. By examining the discrepancies in efficiency-flow profiles derived from conventional methods and moment analysis of peak models, we underscore the critical need to consider peak shape in chiral separations.

P-06: Moving Towards a Proteomics Blood-Based Diagnosis of Alzheimer's Disease Using Human Brain Tissue, CSF, and Plasma Samples by Leveraging a nanoLC-FAIMS-MS/MS Approach

Andrew I. Bennett[1], Cristian D. Gutierrez-Reyes[1], Vishal Sandilya[1], Sherifdeen Onigbinde[1], Mojgan Atashi[1], Wenjing Peng[1], Yehia Mechref[1]

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Alzheimer’s Disease (AD) is the most common form of dementia with cases and costs of caregiving projected to increase dramatically in the future. This study aims at identifying key proteins to create a sensitive and accurate AD screening/diagnostic tool. For this proteomics study, a total of 95 samples were used with 39 human brain tissue samples from the University of California San Diego (UCSD) Alzheimer’s Disease Research Center (ADRC), 16 CSF samples purchased from Golden West Biologicals Inc., and 40 plasma samples from Washington University at St. Louis (WUSTL) Knight Alzheimer’s Disease Research Center (KADRC). The sample prep included a beads beating protocol for the brain tissue and a depletion step for plasma. The samples were then denatured, reduced, alkylated and digested by trypsin followed by a C18 cleanup step. Samples were then analyzed using an Ultimate 3000 nano UHPLC coupled to an Orbitrap Fusion Lumos mass spectrometer with FAIMS-Pro interface with three compensation voltages (CV) of -40, -55, and -70. The column used was an Acclaim™ Pepmap™ C18 column (0.075 mm x 500 mm, 3 µm). The LC gradient was 141 minutes long with a flow rate of 0.300 µL/min. The raw files were then processed using Proteome Discoverer for identification and quantitation with proteins of “High” or “Medium” confidence kept for further processing. Data cleaning, statistical analysis and visualization were carried out using R. Results show 4491 proteins quantified in brain tissue with 949 statistically significant (429 up, 520 down), 930 proteins

quantified in CSF with 218 statistically significant (189 up, 29, down, and 504 proteins quantified in plasma with 158 statistically significant (98 up, 60 down). Three proteins were found to be statistically significant in all three samples (IGHA2, HSPG2, and DKK3) with 15 additional proteins being statistically significant and in common between brain and plasma samples.

P-07: Glycoproteomics Profiling of Biofluids in Severe Traumatic Brain Injury Patients

Moyinoluwa Adeniyi[1], Mojibola Fowowe[1], Joy Solomon[1], Sherifdeen Onigbinde[1], Waziha Purba[1], Md Mostofa Al Amin Bhuiyan[1], Judith Nwaiwu[1], Cristian D. Gutierrez Reyes[1], Ava Puccio[2], Firas H. Kobaissy[3], Stefania Mondello[4], Yehia Mechref[1]

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Protein glycosylation is critical in various physiological and pathological processes, including neurological development and diseases. Traumatic Brain Injury (TBI) is a highly heterogeneous condition that poses a significant global health challenge, particularly affecting the younger adult population, with millions of cases reported annually. This underscores the need for advanced biomarker discovery to enhance diagnosis, prognosis, and therapeutic strategies. Cerebrospinal Fluid (CSF), being in direct contact with the brain and spinal cord, serves as an ideal medium for investigating the biochemical and molecular alterations following TBI. Also, several proteins have been reported to cross the blood-CSF barrier. In this study, a Liquid Chromatography Mass Spectrometry (LC-MS/MS) glycoproteomics approach was utilized to analyze *N*-glycoproteomics changes in CSF and serum samples collected from severe TBI (sTBI) patients and healthy controls. The samples were obtained at three critical time points post-injury: day 1, day 3, and day 5, representing key stages in the disease progression. A total of 270 *N*-glycopeptides were identified in the CSF samples. Pairwise comparisons between TBI cohorts and controls revealed 94, 95, and 41 differentially expressed glycopeptides on day 1, day 3, and day 5, respectively. Notably, more significant differences were observed between day 1 and day 3 versus controls, compared to day 5. Among the differentially expressed N-glycopeptides, 24 exhibited changes across the different time points. These include common serum proteins known to cross the blood-CSF barrier and those involved in inflammatory and immune responses. These alterations in glycoproteins may reflect the immune system's response to sTBI. Our findings hold the potential to provide crucial insights into the progressive *N*-glycoproteome changes in the biofluids of sTBI patients, contributing to a deeper understanding of TBI pathophysiology and offering potential avenues for biomarker development.

Acknowledgement:

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P-08: Daidzein to Equol: Cyclodextrin-Based Chiral Separations of Key Metabolites

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Interest in daidzein, a plant hormone with a structure similar to human estrogen, has been on the rise as a preventative measure and treatment for various ailments. Daidzein, an achiral molecule, naturally occurs in many legumes and can be readily consumed by humans. Once consumed, if a person has the necessary bacterial enzymes, daidzein goes through three key reduction steps to become equol which has even more biological activity. All three reduction metabolites have at least one chiral center. The use of a single cyclodextrin-based chiral stationary phase provides the best enantiomeric separation of all daidzein metabolites and is discussed in detail.

P-09: Serum Proteome Profiling of Diabetic Patients Treated with Novel Antidiabetics Show Improved Cognitive Function, Cardiovascular Function and Reduce Inflammatory Responses

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Introduction: In the realm of chronic metabolic disorders, type 2 diabetes has been associated with cognitive impairment, particularly in elderly patients. This study investigates the cognitive effects of different antidiabetic treatments in patients with type 2 diabetes, focusing on metformin monotherapy versus combination of metformin with novel antidiabetic such as dipeptidyl peptidase-4 inhibitors (DPP-4i) and sodium-glucose transport protein 2 inhibitors (SGLT2i). The differential expression of proteins was investigated to unravel potential insights into the cognitive effects of the combined treatment approach.

Method: Serum samples were collected from 160 participants, comprising control subjects, patients treated with metformin alone, and those receiving combination therapy. Tryptic digested peptides were analyzed using LC-MS/MS, resulting in the identification of 741 proteins, with several exhibiting significant differential expressions between treatment groups.

Preliminary data: Significant differences were observed in the expression of 83 proteins when comparing the control group to the metformin monotherapy group, and 79 proteins in the control versus combination therapy group. Notably, only 18 proteins displayed significant differences between the metformin monotherapy and combination therapy groups. Proteins such as multimerin-1 and ryanodine receptor 1 were significant across all comparisons. Ingenuity pathway analysis revealed that proteins associated with neurological diseases and inflammatory responses were differentially expressed depending on the treatment regimen. Specifically, proteins implicated in neurological diseases, including GFAP, VWF, GPX3, F11, GRIN2C, SNCA, and KGN1, were upregulated, while FN1, DBH, and CNTN1 were downregulated. Additionally, inflammatory response-related proteins such as CD44, F11, ECM1, KNG1, and SNCA were upregulated, whereas CPB2 and PGLYRP2 were downregulated. The data suggests that combination therapy may exert a more pronounced effect on pathways related to cognitive function, cardiovascular health, and inflammation compared to metformin monotherapy. These findings have important implications for understanding the potential therapeutic benefits of combination therapy in managing cognitive impairment in patients with type 2 diabetes.

P-10: Evaluation of LC-MS Mobile Phase Additives for Separation of Peptide Epimers/Isomers

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Peptides are crucial to the proper functioning of various physiological processes in biological systems. While peptides are commonly thought to consist of L-amino acids as their building blocks, the study of epimerization/isomerization of peptides has been increasingly crucial to investigation of various human diseases and novel therapeutics. Separation and analysis of peptide isomers can be challenging since they differ only by the inversion of chirality of stereogenic centers or through other isomerization mechanisms like the conversion of L-aspartic acid to L/D-isoaspartric acid. Most commonly, liquid chromatography coupled to mass spectrometry (LC-MS) is used for analysis of peptides. Typical methods for separation of peptides by LC-MS include the use of acidic mobile phase additives to improve chromatographic peak shapes and increase ionization efficiency. In general, various mobile phase additives have been explored for separation and analysis of peptides. However, the effect of additives on separation of peptide stereoisomers has not been thoroughly reviewed and will be discussed herein.

P-11: A Multi-Omics Investigation Uncovering the Biological Mechanisms Underlying Pompe Disease and Muscle Myopathy

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Pompe disease, also known as glycogen storage disease II (GSD-II), is a rare genetic disorder resulting from mutations in the gene responsible for producing the enzyme acid-alpha glucosidase (GAA). GAA is crucial for breaking down glycogen into glucose. Mutations in GAA gene can reduce the amount of or completely eliminate the enzyme, leading to muscle deterioration. In contrast, myopathies encompass a broad category of muscle disorders, primarily affecting muscle structure, metabolism, or ion-channel function. Due to the overlapping symptoms between Pompe disease and myopathies, misdiagnosis or delays in diagnosis are common.

In this study, blood serum samples from 26 control, 14 Pompe, and 30 myopathy patients were analyzed to uncover the biological mechanisms underlying Pompe and myopathy. For proteomics analysis, the samples were first depleted of 14 high abundance proteins. The low abundant proteins were then denatured, reduced, and carbamidomethylated. Following this, tryptic digestion was performed using trypsin in a 1:25 enzyme to protein ratio. For N-glycomics, intact proteins were treated with PNGase F to release N-glycans, which were subsequently purified through ethanol precipitation. The glycans were then permethylated using iodomethane to increase hydrophobicity and improve ionization. Liquid chromatography with tandem mass spectrometry (LC-MS) analysis for both sets of samples was performed using an Ultimate 3000 nano-UHPLC coupled to an Orbitrap Fusion Lumos mass spectrometer.

The preliminary results revealed significant disruptions in the complement cascade pathways in both Pompe disease and myopathy, furthermore, cardiomyopathy was upregulated in both conditions. Notably, decreased cell polarization was identified exclusively in myopathy. Additionally, a significant change in sialylation was observed in myopathy compared to control, while fucosylation was altered in pompe. Future work for this project will focus on the analysis of glycopeptides and the integration of data from all three omics approaches to develop a more comprehensive understanding of these diseases.

P-12: An Innovative Method for Analyzing IgG Glycosylation Significance in Traumatic Brain Injury

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Traumatic brain injury (TBI) is a critical public health issue, affecting millions globally and particularly impacting young adults. Dysregulated glycosylation patterns, a feature in both TBI and neurodegenerative diseases, play a significant role in immune responses following brain injury. Given the pivotal role of IgG in the immune response post-TBI, a comprehensive investigation into the glycosylation of IgG becomes essential. This study introduces a novel approach to analyzing IgG glycosylation in serum samples from TBI patients. By utilizing an on- membrane digestion method, IgG-

specific *N*-glycans were released using the GlycINATOR enzyme, while *N*-glycans from other glycoproteins were liberated with PNGase F. High-resolution LC-MS/MS analysis identified 19 IgG *N*-glycans and 84 *N*-glycans from other glycoproteins, with significant changes observed across different time points post-TBI. The findings indicated a significant increase in sialylation in both IgG and other glycoprotein-derived glycans in TBI patients when compared to controls. In contrast, fucosylation levels were elevated in IgG but reduced in other glycoproteins. Specifically, eight IgG *N*-glycans exhibited significant changes on Day 1, nine on Day 3, and eleven on Day 5 post-injury. Among the *N*-glycans from other glycoproteins, fourteen showed significant changes on Day 1, thirty on Day 3, and 27 on Day 5. Interestingly, four glycans (3500, 4501, 4502, and 5512) exhibited significant changes across both IgG-specific and other glycoprotein *N*-glycans, although with varying trends over time. Additionally, the study explored isomeric IgG glycans, noting changes in their expression post- TBI. Future work will investigate fucosylation or afucosylation on core GlcNAc of IgG and validate these findings through PRM analysis of purified IgG from TBI samples. The insights gained from this research could pave the way for developing quantitative ELISA assays, offering clinically valuable diagnostic and prognostic tools for better TBI management and personalized treatment approaches.

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P-13: Chiral Separation of Synthesized Dihydropyridine Derivatives: A Chromatographic Approach for Potential Calcium-channel Blockers

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Calcium channel blockers (CCBs) are essential in cardiovascular disease treatment, particularly hypertension and angina. Their ability to inhibit calcium ion influx into the heart and smooth muscle cells makes them vital for controlling blood pressure and preventing heart-related disorders. However, the therapeutic performance and safety of CCBs are heavily influenced by the chirality of their molecules. Different enantiomers often exhibit distinctive pharmacological effects. The effective separation of enantiomers is fundamental to chiral drug development due to the distinct pharmacological activities of different enantiomers. This study focuses on the chiral separation of newly synthesized dihydropyridine derivatives, which are being explored as potential next-generation CCBs. Through a comprehensive chromatographic approach, employing both high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC), the performance of various chiral stationary phases (CSPs) in separating these complex molecules was evaluated.

P-14: LC-MS/MS of Permethylated *O*-Glycans, Free Oligosaccharides, and Glycosphingolipid Glycans Using Mesoporous Graphitized Carbon Column

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Glycosylation, a prevalent post-translational modification, plays a critical role in various biological processes and diseases. Despite significant research on *N*-linked glycans, *O*-linked glycans and other small glycan structures, including glycosphingolipid (GSL) glycans and free oligosaccharides, remain underexplored due to their structural complexity and low abundance. To achieve efficient isomeric separation of these small glycans, we employed the Mesoporous Graphitized Carbon (MGC) column, an efficient alternative to nano Porous Graphitized Carbon columns, which has been recently demonstrated to show efficient isomeric separation of *N*-glycans and *N*-and-*O*-glycopeptides. To extend the application of the MGC column for the isomeric separation of *O*-glycans, free oligosaccharides, and GSL glycans, we optimized the chromatographic conditions using standard samples, leading to improved retention and isomeric separation. Initial trials using a 50% Acetonitrile/Isopropanol (ACN/IPA) mixture as mobile phase B showed poor retention and incomplete separation of the glycans. Switching to 100% ACN with 0.1% formic acid improved retention and partial isomeric separation. Further optimization with 80% ACN enhanced these results, but full separation of some isomers was still not achieved. Finally, using 100% methanol with 0.1% formic acid significantly improved both retention and isomeric separation for all tested glycans. To validate the MGC column's effectiveness for isomeric separation of small glycans from biological samples, human milk-derived glycan samples were analyzed. *O*-glycans, free oligosaccharides, and GSL glycans were released from human milk, and permethylated, before been analyzed. The result showed successful isomeric separation, including separation of several small glycans, highlighting the method's capability across different glycan types. This study highlights the MGC column's robustness and reproducibility, suggesting its applicability for advanced glycomics, especially in biological samples where small glycans play significant roles in disease progressions.

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P-15: Development of Simple and Rapid tITP-CZE-UV method for Salivary Lysozyme determination

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Biomarkers have a fundamental importance in medicine, as they offer new possibilities for simple screening, diagnosis, and monitoring of diseases, as well as prediction of treatment. Saliva is a suitable source of biomarkers, mainly because it is easy to collect, and the sampling is noninvasive. One potential biomarker in saliva is lysozyme. Its level in saliva is closely related to several diseases, which makes it an interesting biomarker candidate.

This work aims to develop a CZE-UV method for quantitatively analyzing salivary lysozyme using simple UV detection. Analyses were performed by CZE using transient isotachophoresis (tITP) as a pre-concentration step. The CZE was carried out in an uncoated fused silica capillary (75 µm I.D. × 52 cm). The samples were hydrodynamically injected by applying 50 mbar for 100 s. The cationic regime of the separation was used, and a separation voltage of +15 kV was applied. UV detection was provided by a photodiode array detector.

In this work, we optimized the parameters of the tITP-CZE-UV method, including injection mode and length, BGE composition, saliva treatment, and final composition of the sample to prevent non/specific adsorption onto the insert surface. Validation was performed using an artificial saliva matrix and an internal standard. The results showed excellent linearity over two orders of magnitude (r^2=0.9997), good intra-day precision (1.23-4.09%) and accuracy (-0.56-5.36%).

The optimized tITP-CE-UV method is sufficiently robust and selective to determine the lysozyme in the salivary matrix without extensive sample treatment. The LLOQ of lysozyme in water and artificial saliva is 1 µg/mL, which is sufficient for the intended application, as the concentration of salivary lysozyme in healthy people ranges from 10 to 70 µg/mL [1].

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P-16: LC-MS/MS-Based Proteomic Profiling of Small Extracellular Vesicle Alterations in Severe Traumatic Brain Injury

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Effective therapies for traumatic brain injury (TBI) rely on identifying reliable diagnostic markers that indicate the development and progression of the injury. Small extracellular vesicles (sEVs), secreted by most cells, can cross the blood-brain barrier (BBB) and carry important biomolecules, such as proteins. Consequently, sEVs are a promising resource for understanding the pathophysiology of TBI. In this study, we utilized liquid chromatography-tandem mass spectrometry (LC-MS/MS) to perform a comprehensive proteomic analysis of sEVs derived from the serum and cerebrospinal fluid (CSF) of patients with severe traumatic brain injury (sTBI). Our goal was to investigate the time-dependent proteomic changes associated with sTBI and identify potential biomarkers for injury progression. A two-tailed Student's t-test revealed 164 differentially expressed proteins in serum-sEVs and 114 in CSF-sEVs when comparing control groups with sTBI groups at 1-, 3-, and 5-days post-injury. Notably, we observed significant increases ($p < 0.05$) in astrocyte-expressed glial fibrillary acidic protein (GFAP) and neuronal ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in both serum- and CSF-sEVs at these time points. In healthy subjects, GFAP levels were higher in serum sEVs than in CSF sEVs. However, from days 1 to 5 post-injury, CSF sEVs showed a significant increase compared to serum sEVs ($p < 0.05$). We also detected elevated levels of C-reactive protein (CRP) in CSF sEVs, a sensitive biomarker for predicting poor clinical outcomes in TBI and reflecting responses to trauma and chronic inflammation. These differentially expressed proteins may help distinguish between injured and healthy individuals. Further investigation of these proteins will shed light on the biological mechanisms and molecular pathways involved in TBI over time.

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P-17: Quantitation of Intact Proteins in Biological Fluids Using tITP-CZE-MS with Off-Line Microelution SPE Sample Pretreatment

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Protein analysis in biological samples is one of the key areas of biomedical research. To miniaturize the entire analytical process and lower its environmental impact, attention is currently being paid to the development of greener approaches aimed at the targeted quantitation of intact proteins. In this field, capillary electrophoresis is becoming more popular and meets the criteria for greener techniques [1,2]. When combined with mass spectrometry, it can compete with established chromatographic techniques in terms of performance and meets the requirements to become a routine part of practice [3,4]. However, when it comes to the analysis of biological matrices, its reliable application requires a comprehensive optimization of the separation and detection conditions in addition to the implementation of effective preconcentration techniques and pretreatment procedures [5].

In this work, we focused on the development of an on-line hyphenated capillary zone electrophoresis-mass spectrometry method (CZE-MS) employing off-line microelution solid-phase extraction (μSPE) as a sample pretreatment step for the quantitation of intact proteins with molecular masses <20 kDa in biological fluids (human serum, plasma, urine, and saliva). Various preconcentration techniques can be used to enhance the sensitivity of the CZE-MS method [6,7]. 19- to 127-fold increase in signal intensity was achieved by employing transient isotachophoresis (tITP) as an in-capillary preconcentration method. Off-line μSPE with various eluate treatment procedures was evaluated to ensure the compatibility of the sample pretreatment method with the selected in-capillary preconcentration, separation, and detection process. Achieved extraction recoveries of spiked proteins were in the range of 76-100% for urine, 12-54% for serum, 21-106% for plasma, and 25-98% for saliva when the eluate was evaporated and reconstituted in the solution of the leading electrolyte to achieve the tITP process [8]. The optimum method was validated across different biological matrices, offering good linearity, accuracy, and precision, and making it suitable for proteomic studies in different biological samples.

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P-18: Identifying Dysregulated Lipids in Human Brain of Alzheimer's Disease and their Effects on Biological Pathways

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Introduction: Alzheimer's disease (AD) is the most common neurological disorder and the leading cause of dementia, severely affecting cognitive functions such as memory, speech, and physical coordination. It ranks as the seventh leading cause of death in the United States, with cases expected to triple in the next 30 years. Current AD diagnoses primarily depend on clinical symptoms, but alterations in lipid rafts—critical for cell signaling—may also play a role in the disease's progression.

Methods: In this study, 18 brain tissue samples from AD patients and 18 from healthy controls were analyzed. The samples were lysed, and their protein concentrations were determined. Lipids were extracted using the absolute methanol extraction method. LIPIDOMIX® and GM1-d3 Internal Standard were spiked into the samples, and the mixture was analyzed on an LC-MS/MS platform.

Preliminary Data: A total of 598 lipid ions were identified and quantified, with 123 showing significant differences between AD and control samples ($p < 0.05$; $q < 0.05$ upon Welch t-test with Benjamini-Hochberg correction). Of these, 117 were upregulated, and 6 were downregulated. The study focused on lipid subclass levels; 20 out of 44 quantified lipid subclasses were upregulated in AD samples. Notably, upregulated lipids included phosphatidylglycerol ($q < 0.00002$), phosphatidylserine ($q < 0.00002$), lysophosphatidic acid ($q < 0.01$), GD2 ($q < 0.04$), and GD1a with fold changes of 19.3, 12.7, 8.1, 4.5, and 4.0 respectively. Additionally, hexosylceramide and ganglioside GM3 were significantly upregulated in female AD patients compared to males. System biology analysis suggested that dysregulated fatty acids may activate glycerophospholipid biosynthesis, contributing to the upregulation of phospholipids. Dysregulation of sphingomyelin, sphingosine-1-phosphate, and ganglioside GD1a is associated with sphingolipidoses, indicating a potential link to AD pathology.

Novel Aspect: This study identifies distinct lipid signatures in AD brains, enhancing our understanding of lipid- related pathophysiology in Alzheimer's disease.

P-19: N-Glycan Alterations in Kidney of Rats Chronically Exposed to Glyphosate-Based Herbicide

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The extensive use of Glyphosate-based herbicides (GBHs) has raised concerns regarding possible adverse effects on human health and the environment. Glyphosate has been associated with various toxicological outcomes, including carcinogenicity, endocrine disruption, and neurotoxicity. While significant research has focused on the broader toxicological impacts of glyphosate, there remains a critical gap in our understanding of how glyphosate affects specific biochemical pathways, particularly glycosylation, in vital organs such as the kidney. The comprehensive study of glycan structures are essential in understanding the complex carbohydrate modifications that regulate cellular functions, signaling pathways, and disease processes. Following exposure, glyphosates are primarily excreted through feaces and urine, making the kidney a sensitive target of its toxic effects. Given the kidney's central roles in detoxification, metabolism, and homeostasis, understanding the impact of glyphosate on glycosylation pathways in this organ is of paramount importance. In this study, rat kidney tissues were homogenized and lysed. The extracted proteins underwent denaturation and PNGase F digestion, followed by *N*-glycan purification step to eliminate the proteins. The released glycans underwent reduction and permethylation. The permethylated *N*-glycans were dried and resuspended in a solution of 20% acetonitrile (ACN) and 0.1% formic acid for LC-MS/MS analysis. This analysis utilized an Ultimate 3000 LC system interfaced with an Orbitrap Fusion Lumos Mass Spectrometer. Chromatographic separation was performed on an Acclaim Pepmap™ C18 column. Data processing was conducted using Xcalibur Freestyle, quantitation using Skyline software while analysis employed IBM SPSS, Origin, for interpretation. We identified 120 *N*-glycans in the kidneys after exposure to GBH, covering different glycan types such as high mannose, fucosylated, sialylated and sialofucosylated. Brain structures such as $HexNAc_5Hex_3Deoxy_0NeuAc_0$ were identified which could indicate the possible link between the kidney and the brain. Fucosylated and Sialofucosylated N-glycans were the most prevalent. This study emphasizes the potential of glycomics in enhancing therapeutic strategies.

P-20: Heart Cut 2D-LC in a Single Chromatograph for a Difficult Isomeric Separation

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A two-dimensional methodology, achieved by reconfiguring a single chromatograph, was adapted for separations that are challenging with a single enantioselective column. Allethrin, a pyrethroid with three chiral centers resulting in eight stereoisomers (four diastereomers), presents one of the most challenging chiral separations known in the literature. A shape-selective porous graphitic carbon (PGC) stationary phase was used in the first dimension to achieve diastereomeric separation. A six-port 2-position rotary valve was used to heart-cut the four diastereomeric peaks and send them to the second dimension. An immobilized-cellulose tris(3,5- dichlorophenylcarbamate) column was used in the second dimension to attain enantiomeric separation with chromatographic resolutions >2.7 for all enantiomeric pairs. Also, the best chiral separation of allethrin was found to be on the (2-hydroxypropyl)-β-cyclodextrin column, which resolved seven of the eight isomers in a single dimension. The developed 2D method was used to verify the enantiomeric purity of a formulation of allethrin in a commercial pesticide.

P-21: Investigating the Effects of Alteration in Lipid Profile on Biological Pathways in Diabetic Patients Treated with Different Drugs

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Introduction: This study explores the neuroprotective effects of metformin, SGLT2 inhibitors, and DPP-4 inhibitors in Type 2 Diabetes Mellitus (T2DM). It investigates the combination of DPP-4i and SGLT2i with metformin seeks to investigate changes in the lipid profile and assess whether these innovative antidiabetic drugs can improve cognitive function in individuals with T2DM.

Methods: In this study, serum samples from 161 Type 2 Diabetes Mellitus (T2DM) patients undergoing treatment with metformin, DPP4i, and SGLT2i, along with a follow-up after six months, were analyzed. The study focused on nonpolar lipids, extracting them and employing reverse-phase chromatography separation. The Acquity UPLC BEH 130 Å (2.1 x 100 mm) column on a Vanquish UHPLC system coupled with a Quadrupole Exactive HF was used for chromatographic separation. Lipidomics LC-MS/MS data were analyzed using Lipid search software, with lipid quantitation based on area abundance. Statistical analysis involved Mann Whitney-U followed by the Benjamini-Hochberg correction to identify significant lipids in different cohorts.

Preliminary Data: A total of 451 lipids were identified and quantified in the study using Lipid search. Patients with type 2 diabetes were grouped into those treated with metformin alone or with additional DPP-4i/SGLT2i. The Principal Component Analysis (PCA) showed distinct clustering among control, treatment, and follow-up cohorts. Ingenuity Pathway Analysis (IPA) indicated that differentially expressed lipids in metformin alone and metformin with novel antidiabetics-treated groups activated pathways related to neurological diseases, inflammatory responses, and others. Upregulated lipids, such as anandamide and 2-arachidonoylglycerol, linked to neurological diseases and inflammation,

while downregulated lipids included ceramides and sphingolipids. IPA highlighted pathways in neurological diseases and inflammatory responses.

Novel Aspect: Lipidomic changes in T2DM patients treated with metformin and novel antidiabetics may contribute to the modulation of cognitive function, inflammatory responses, and cardiovascular function.

P-22: LC-PRM-MS/MS Reveals Significant Metabolomic Alterations in Parkinson's Disease Frontal Lobe Tissue

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Parkinson's disease (PD) is characterized by the specific degeneration of dopaminergic neurons in the substantia nigra. This decline in dopamine levels has been observed in the frontal lobes of PD patients, contributing to the cognitive impairment associated with the disease. While various omics studies have shed light on the molecular mechanisms underlying different aspects of PD pathologies, limited research has explored metabolomic changes in the frontal lobe of PD patients. Therefore, this study identified metabolites relevant to PD pathogenesis, utilizing a highly sensitive liquid chromatography-parallel reaction monitoring-mass spectrometry (LC-PRM-MS) method. Polar metabolites were extracted from 100 µL of brain tissue lysate. A 200 µL mixture of dichloromethane/methanol (DCM/MeOH, 1:2 v/v) was introduced to the samples, followed by 30 seconds of vortexing. After a 60-minute incubation period at room temperature, 75 µL of DCM and cold water were added, and samples were centrifugated at 5000 rpm for 15 minutes. The metabolites (upper layer) were carefully collected, dried, and resuspended for LC-PRM-MS analysis with Ultimate 3000 interfaced to Q Exactive HF MS, Thermo Scientific. The raw data were analyzed with Freestyle and quantified using Skyline MS. Comprehensive analyses were conducted utilizing SPSS 29.0, OriginPro 2.0, and Genesis 1.8.1. Untargeted LC-MS analyses on six pooled control samples and six PD samples was conducted, Compound Discoverer identified 560 metabolites with 385 showing statistically significant differences between the pooled samples ($p < 0.05$). Subsequent PRM analysis validated 254 metabolites while further statistical analysis showed 118 significantly different metabolites in PD compared to controls. 3D Unsupervised Principal Component Analysis (PCA) with a confidence level of 95% revealed distinct clustering and separation between the two sample groups. Furthermore, IPA shows the inhibition of nucleotide catabolism and that of the nuclear/membrane-initiated estrogen.

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P-23: The Effect of Chiral Changes on Antibodies, Detection, and Sample Preparation: A Study of Epimeric Beta Amyloids

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The discovery of D-amino acids has opened new avenues for disease analysis, yet there remains a significant gap in our understanding and the availability of tools to identify, extract, and detect these chiral changes. This study investigates the impact of chiral modifications on antibody-antigen interactions, specifically focusing on epimeric and isomeric beta-amyloid (Aβ) peptides. These modifications may influence the deposition of Aβ plaques and contribute to neurodegeneration in Alzheimer's disease (AD) patients, and potentially altering the binding affinity of therapeutic antibodies.

This study uses immunoprecipitation to assess the binding affinities of four antibodies against 18 different epimeric and/or isomeric Aβ peptides. Tandem mass spectrometry was utilized as a detection method, though it yielded highly variable results when analyzing these chiral peptides. To further explore these variations, we investigated the ionization and fragmentation patterns of epimeric peptides in a mass spectrometer using selected reaction monitoring, selected ion mode, and product ion scans. The results revealed significant differences in the ionizability and fragmentation behavior between epimeric and isomeric peptides. To gain insight into the effects of chiral changes on secondary structures such as beta sheets and random coils, we employed circular dichroism and dynamic light scattering techniques. These studies highlighted variations in the folding kinetics and structural changes associated with epimerization.

Overall, this work provides a comprehensive examination of the influence of chiral changes on the behavior of beta-amyloid peptides, offering new insights into how these modifications affect detection methods like mass spectrometry. Additionally, it underscores the implications for sample preparation techniques such as immunoprecipitation, which are crucial for the design of antibodies used in immunotherapies.

P-24: *In vivo* Stable Labeling (GlyProSILC) of Mitochondria Glycans and Proteins

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Mitochondria are vital organelles in eukaryotic cells and a central hub in several cellular processes such as energy biosynthesis, Ca^{2+} homeostasis, metabolic regulation, cell proliferation, and death. Mitochondria proteomics research has dramatically increased with the aid of advanced technology.

Mitochondria glycoproteomics research is a growing research area. Thus, to facilitate mitochondria glycoproteomics studies, we labeled mitochondria proteins and glycans *in vitro* using stable isotopes amino acids (^{13}C and ^{15}N). To achieve our aim, 231 and 231BR cancer cell lines were cultured in normal media and media containing stable isotopes (heavy media). The normal media contained L- [$^{12}C_6$, $^{14}N_4$]-arginine (Arg0) and L- [$^{12}C_6$, $^{14}N_2$]-lysine (Lys0) amino acids, while the heavy media contained L- [$^{13}C_6$]-arginine (Arg6), L- [$^{13}C_6$, $^{15}N_2$]-lysine (Lys8) and L-[$^{15}N_1$]-glutamine. Media were changed daily, and cells were cultured to the fourth generation to ensure complete labeling. Cells were harvested after the fourth generation and mitochondria were enriched using differential centrifugation. Enriched mitochondria were lysed, and proteins were extracted. Extracted mitochondria protein samples were utilized for proteomics, glycomics, and glycoproteomics analysis. All samples were analyzed on the Dionex 3000 UltiMate nano-LC system coupled with an Orbitrap Fusion Lumos Tribrid Mass Spectrometer (Thermo Scientific). All proteomics data were processed using Proteome Discoverer 2.5. At a 5% false discovery rate (FDR), Using a mitochondria-specific database downloaded from Mitocarta 3.0, 577 and 544 proteins were identified in the light 231BR and 231 mitochondria respectively; 511 and 528 proteins were identified in the heavy-labeled 231BR and 231 mitochondria respectively. Glycan structures such as $HexNAc_3Hex_6DeoxyHex_1NeuAc_0$, $HexNAc_4Hex_5DeoxyHex_2NeuAc_0$, $HexNAc_4Hex_5DeoxyHex_3NeuAc_0$, $HexNAc_4Hex_6DeoxyHex_2NeuAc_0$ were identified in both 231BR and 231 cells but not detected in their respective mitochondria samples. Together both protein and glycan motifs were labeled with stable isotope amino acids. Therefore, labeling proteins and glycans moieties would facilitate mitochondria glycoproteomic studies.

P-25: Characterizing Insect-Mediated Microbial Degradation of Insecticides Through LC Methodologies

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The mechanism elucidating the resistance of some insects to pesticides is a relatively recently explained phenomenon. Similarly, the ability of insects to detoxify their plant diet is not fully understood. As part of an interdisciplinary project to explore the symbiotic relationship of insects and their digestive bacteria, analytical methods for detection and quantification of pesticides and plant toxins were developed. For the pesticide racemic malathion, the ability of bacterial lines to degrade the pesticide chirally can result in either enhanced or reduced toxicity, as the two enantiomers have different toxicity levels. In terms of plant toxins, including chlorogenic acid, oxalic acid, and rutin, insect gut bacteria have co-evolved with the insect over time, providing the ability to degrade otherwise toxic allelochemicals. The methods developed to characterize this varied class of insecticides, and their degradation (or lack thereof) by hundreds of bacterial lines, are discussed.

P-26: LC-MS/MS-Based Approach for Examining Disease-Related *N*-Glycopeptides in Small Extracellular Vesicles: A Case Study on Traumatic Brain Injury

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Small extracellular vesicles (sEVs) play a key role in intracellular communication and hold promise as reservoirs for disease biomarkers. Profiling protein post-translational modifications (PTMs) within sEVs is particularly valuable for early diagnosis, as PTMs, like glycosylation, are essential to cellular functions. However, investigating glycosylation in sEVs presents several challenges. In this study, we introduce a specialized workflow for *N*-glycopeptide analysis in sEVs, using traumatic brain injury (TBI) as a case study, to identify potential disease biomarkers while addressing the challenges specific to sEVs.

sEVs were isolated from cerebrospinal fluid (CSF) and serum samples of patients with severe traumatic brain injury (sTBI) at 1-, 3-, and 5-days post-injury. Proteins were extracted using 5% sodium deoxycholate (SDC) with bead beating at 4,000 rpm for 30 seconds, followed by trypsin digestion. The digested proteins were then analyzed using an Acclaim PepMap 100 C18 column on a 3000 Ultimate nano-LC system coupled with an Orbitrap Fusion Lumos Mass Spectrometer (Thermo Sci.). *N*-glycopeptides were identified using Byonic software, with subsequent statistical analysis performed via the student's t-test, ANOVA, and the Benjamini-Hochberg procedure for false discovery rate (FDR) control.

The unbiased *N*-glycoproteomic analysis of sEVs from cerebrospinal fluid (CSF) and serum revealed 155 and 162 *N*-glycopeptides, respectively, with a 1% FDR. Prior to conducting the *N*-glycopeptide analysis, a proteomic purity assessment confirmed the presence of exosome marker proteins (CD9, CD63, and CD81) in the isolated sEVs. Further characterization using Transmission Electron Microscopy (TEM) and nano-Flow Cytometry (nFCM) will validate the vesicles' morphology, size distribution, and concentration. This study underscores the potential of these identified *N*-glycopeptides as robust biomarkers for severe traumatic brain injury detection, with implications for the development of rapid diagnostic tests.

P-27: Unveiling Glycan Profiles to Study Breast Cancer Brain Metastasis

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Breast Cancer, aside from its high incidence, has shown to have a high metastatic possibility, forming secondary tumor sites in organs such as bones, lungs, liver, brain. Brain metastatic breast cancer has gained attention because of its increased incidence rate and its low survival rate. Despite the increasing incidence of brain metastasis from breast cancer, the underlying mechanisms remain poorly understood. Altered glycosylation is known to play a role in various diseases, including cancer metastasis. Most of the existing research focuses on gene and protein expressions. Hence, there is a need to investigate glycan moiety alterations during the breast cancer brain metastasis process to understand the roles glycans play in this biological process. We investigated the *N* and *O*-glycans released from five breast cancer cell lines including MDA-MB-231, MDA-MB-231BR, MDA-MB-361, HTB131, HTB22 and from one brain cancer cell line, CRL-1620 utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS). The *N*-glycomics experiment was initiated with extraction of the proteins. Then the glycans were released using PNGase-F enzyme. The released glycans were separated from the proteins, reduced, and permethylated. For the *O*-glycomics, after protein extraction, the *O*-glycans were subjected to a chemoenzymatic release. The released *O*-glycans were then subjected to solid-phase permethylation. The resulting permethylated glycans were resuspended in an aqueous solution containing 20% acetonitrile and 0.1% formic acid before undergoing LC-MS analysis. Significance in glycan changes was assessed through the Mann-Whitney U test, calculating p-values at a 95% confidence level. An increase of *N*-glycan $HexNAc_4Hex_5DeoxyHex_1NeuAc_1$, $HexNAc_5Hex_6DeoxyHex_1NeuAc_3$, and $HexNAc_6Hex_7DeoxyHex_1NeuAc_3$ were observed in 231BR, suggesting that they may play important roles during the breast cancer brain metastasis process. The expressions of *O*-glycan $HexNAc_1Hex_1NeuAc_1$, $HexNAc_1Hex_1NeuAc_2$, and $HexNAc_2Hex_3$ were significantly altered across all other cell lines in comparison to 231BR, suggesting that they may play a role in the metastatic process.

P-28: Models, Metrics, and Methods for Greening Analytical and Preparative Chromatography

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Research in green chemistry has moved away from mandated waste treatment and environmental remediation plans and towards strategies to prevent pollution at its source. Chromatographic separations, especially those in the liquid phase, are prolific pollution generators and therefore research into greening them is needed. Prior to greening an analytical technique, an understanding of the current models of green chemistry needed including the red/green/blue (RGB) model of green chemistry, perceived states of green chemistry, and life cycle style of thinking [1]. From there, modern metrics will be discussed that readily “quantify” a method’s greenness allowing for method-to-method sustainability comparisons [2]. Finally, examples of simple and effective methods to green any chromatographic separation are displayed including changing the column hardware/packing, substituting mobile phase components, and optimizing instrumental parameters. All methods are exemplified with real world examples at the analytical and preparative scale.

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P-29: LC-MS/MS-Based Metabolic Profiling: Investigating Serum and CSF in TBI Patients

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Metabolomics is a powerful tool in systems biology, crucial for unraveling the complex biochemical alterations associated with Traumatic Brain Injury (TBI). TBI, caused by external head impact, initiates a cascade of molecular events that significantly alter the brain's metabolic profile. Through metabolomic studies, we can identify distinct metabolic signatures at various stages of TBI, offering valuable insights into the underlying pathophysiological processes. This knowledge not only deepens our understanding of the molecular mechanisms involved but also paves the way for advancements in diagnostics and targeted therapies.

In our study, we focused on analyzing metabolites in the serum and cerebrospinal fluid (CSF) of TBI patients to uncover the detailed molecular mechanisms underlying the injury. To extract polar metabolites, we used 100 µL of CSF or serum samples, mixed with 200 µL of Dichloromethane/Methanol (DCM/MeOH) (1:2 v/v) and 75 µL of DCM along with cold water. After centrifugation at 5000 rpm for 15 minutes, the supernatant containing the polar metabolites was collected, dried, and then resuspended for Liquid Chromatography-Mass Spectrometry (LC- MS/MS) analysis.

Our analysis examined 38 samples (19 CSF and 19 serum) from TBI patients, collected on days 1, 3, and 5 post-injury. Using Compound Discoverer for metabolite identification, we detected 797 metabolites in CSF and 1113 in serum. Significant changes were noted: 39 metabolites in CSF between days 1 and 3, 38 between days 1 and 5, and 11 between days 3 and 5. In serum, 131 significant metabolites were observed between days 1 and 3, 186 between days 1 and 5, and 68 between days 3 and 5. Pathway analysis with IPA highlighted distinct enriched pathways on different days, revealing significant alterations in pathways related to tRNA charging, lipid metabolism, and neurotransmitter release. This highlights the ever-evolving landscape of metabolic transformations that unfold in the wake of traumatic brain injury.

P-30: Glycome Profiling of Small Extracellular Vesicle N-glycans as Disease Biomarkers: A Traumatic Brain Injury Case Study

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Small extracellular vesicles (sEVs) are crucial for intercellular communication and are involved in various physiological and pathological processes. Analyzing glycomic profiles helps reveal the role of glycans in vesicles, which is crucial for understanding disease-related changes. *N*-glycans in sEVs have been underexplored due to research challenges. This study introduces a glycomics workflow to analyze these *N*-glycans, using severe traumatic brain injury (sTBI) as a case study. In this study, serum and cerebrospinal fluid (CSF) samples were collected from control and sTBI patients on days 1, 3, and 5 post-injuries. sEVs were isolated using a specialized kit, and their proteins extracted using sodium deoxycholate (SDC) lysis and bead beating. The proteins were then transferred to a membrane filter, digested with PNGase F, and the resulting *N*-glycans were recovered, reduced, permethylated, and analyzed via LC-MS/MS. This study outlines a workflow for analyzing the glycomics of sEVs using just 200 µL of serum or CSF. The yield of sEVs depends on the type of biofluid and isolation method. Given the low abundance of sEVs in CSF, which is closely linked to the CNS and valuable for studying neurotrauma and neurodegenerative diseases, the study employs a commercial extraction method and a technique to minimize sample loss during *N*-glycan preparation. The study compares glycan changes in sTBI patients at different time points (Day 1, 3 and 5) with controls. Initial findings indicate a progressive decrease in sialylation in CSF-EVs as sTBI advances, with similar trends observed in serum-EVs after day 3. No significant changes were found in other glycan structures. Further analysis will reveal glycomic changes in sTBI, validated against standard preparation techniques. This research aims to improve sEV glycomics and help discover disease biomarkers.

P-31: Understanding Protein Accumulation in Cleome Seeds Using NanoLC-MS/MS-Based Proteomics

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Cleome contains species spanning a developmental progression from C3 to C4 photosynthesis. *Cleome gynandra* and *Cleome hassleriana* are two species within the Cleome genus known for their diverse medicinal and nutritional properties. The proteomic analysis of Cleome *gynandra* and Cleome *hassleriana* offers a comprehensive overview of the protein traits of these species. The study leverages

nano liquid chromatography-mass spectrometry (nanoLC-MS) to identify and analyze the common and unique proteins in these species respectively. The abundance ratio was used to compare proteins between Cleome *gynandra* and Cleome *hassleriana*, indicating relative intensities or quantities in different samples. The analysis revealed several proteins involved in biological and biochemical pathways, including defense-related proteins, inhibitors, and calmodulin, which are involved in signal transduction pathways. This highlights the involvement of these proteins in various physiological processes, from cellular signaling to stress responses. Overall, the integrated analyses provide a holistic view of the metabolic and proteomic landscapes of Cleome *gynandra* and Cleome *hassleriana*, enriching our understanding of their biochemical mechanisms and genetic responses. This study offers valuable insights into the metabolic states and pathways of these species, with broader implications for agricultural and genetic research, particularly in understanding plant biochemistry and developing therapeutics.

P-32: Proteomic Insights into Fusarium *Oxysporum* Adaptation and Host Interaction: A Study of Cotton Root Influence on Pathogen Protein Expression.

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Fusarium wilt of cotton is an important and widespread disease caused by the fungus *Fusarium Oxysporum* f.sp.*vasinfectum* (Fov1). The disease is transmitted by several mechanisms, including spores and mycelium in the soil. Initially, the fungus infects the roots and spreads all over the plants via the vascular system resulting in wilting and death of the plant. This study investigates the impact of cotton upland (G. *hirsutum L.*) root presence on the protein profile of F*. oxysporum* to better understand the pathogen's adaptive responses and interactions with its host. Using advanced proteomic technique nano liquid chromatography-mass spectrometry (nano LC-MS), we analyzed the protein expression profiles of F*. oxysporum* in the presence and absence of cotton roots.
Our results revealed substantial differences in the protein profiles of F*. oxysporum* when exposed to cotton roots. Notably, a total of number of 719 proteins were identified from Fov1, and 746 from Fov1 when cocultured with cotton roots. 34 proteins were uniquely identified when cocultured with roots, associated with stress responses, defense mechanisms against the host's immune response, and pathogen virulence. Conversely, seven proteins were expressed only in Fov1. primarily linked to basic metabolic processes and cell growth.

This study offers significant insights into the molecular mechanisms governing the interactions between F*. oxysporum* and cotton and identifies potential targets for innovative disease management strategies. Additional research is needed to investigate the functional roles of these identified proteins and explore their potential applications in boosting crop resistance and controlling fungal diseases.

P-33: Exploring Metabolic Diversity: A Non-Targeted Metabolomics Study of Cleome *gynandra* and Cleome *hassleriana*

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Cleome gynandra and *Cleome hassleriana* are two species within the genus cleome that exhibit significant medicinal and ecological potential in addition to its application in food. The research aimed to provide valuable insights into the metabolic profiles and potential applications of these two species using ultra-performance liquid chromatography-mass spectrometry analysis. UPLC-MS for quantitative and qualitative metabolomics studies to characterize and compare the metabolomic signatures of both species. This research highlights the value of metabolomics in revealing the functional and chemical diversity within the cleome genus and lays the groundwork for future investigations into their therapeutic and economic potential.

The comparative analysis between the two species unveiled significant differences in their metabolic pathways, reflecting their ecological functions and evolutionary adaptations. These differences provide valuable insights into how each species manages biotic and abiotic stresses and suggest potential areas for applied research. Notably, cleome gynandra was found to harbor a unique array of bioactive compounds that align with its ethnomedicinal uses, suggesting its potential for novel therapeutic applications. Conversely, cleome hassleriana displayed a distinct metabolic profile that may contribute to its adaptation to varying environmental conditions, highlighting its possible role in defense mechanism and antifungal and antimicrobial.

This nontargeted metabolomic profiling of cleome gynandra and cleome hassleriana offers valuable insights into the complex biochemical landscapes of plants, revealing both known and novel compounds with potential applications in medicine, agriculture, and industry. Future studies could explore the functional roles of identified metabolites and assess their practical applications in health and environmental management.