MSB 2024

40th International Symposium on Microscale Separations and Bioanalysis

May 19–22, 2024, Brno, Czech Republic

Program and Abstract Book

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Final Program and Abstract Book

www.msb2024.org

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CONTENT

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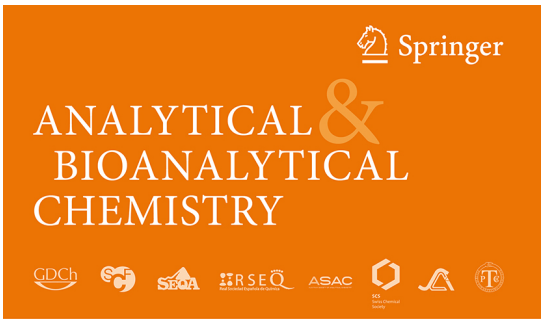
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EXHIBITORS

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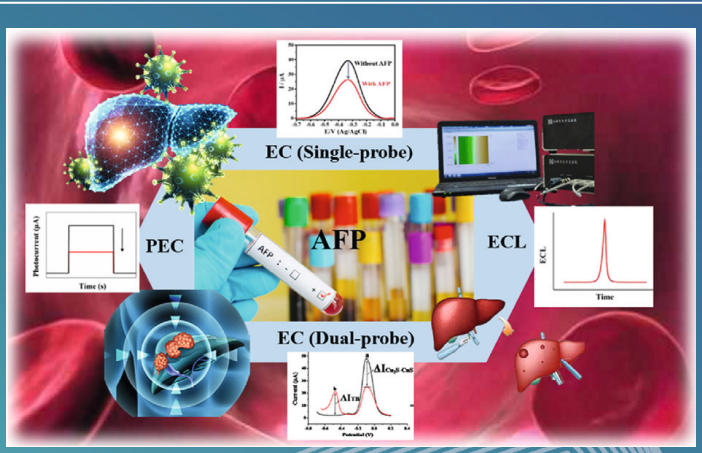

Featured Article

Electrochemical immuno-biosensors for the detection of the tumor marker alpha-fetoprotein: A review

Chen-Wei Shan, Zhencheng Chen, Guo-Cheng Han, Xiao-Zhen Feng, Heinz-Bernhard Kraatz

(*Published in Article 125638 in issue 271*)

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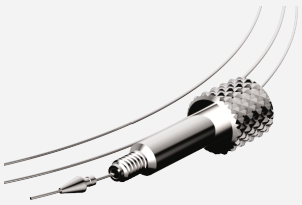

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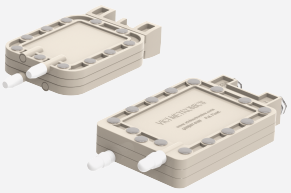

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WELCOME FROM THE MSB 2024 CHAIR

On behalf of the organizing committee of the 40th International Symposium on Microscale Separations and Bioanalysis – MSB 2024, it is my great pleasure to welcome you to Brno, Czech Republic.

MSB began as the HPCE symposium in 1989, focused tightly on the emerging technology – High Performance Capillary Electrophoresis – that ultimately sequenced the human genome and enabled a revolution in molecular biology. The conference has evolved into an annual interactive forum for the discussion of research on the frontiers of microscale separation science and bioanalysis. That was accompanied by a format change that has made this meeting more dynamic and inclusive, having a double-blind abstract review process that ensures the best science is presented. In keeping with this theme, MSB 2024 encompasses a range of microscale separations research, from fundamental technology development to high-impact applications in the fields of health, medicine, and the environment. The meeting begins with a series of short courses, which lead to an opening plenary session. In the following days, the scientific program will be held in two parallel sessions. We include oral presentations from young scientists and poster sessions to ensure that no research topic is left wanting for conversation.

In addition to our invited plenary and keynote presenters, we welcome our 44 oral presentations selected via the double-blind review process. Our goal is to set the stage for the future of microscale science. 1/3 of the allocated presentation time is reserved for questions, leaving ample opportunity for discussion of each presentation before advancing to the next talk.

At the start of the symposium, I would like to thank you for your contribution and participation and acknowledge our sponsors for their generous support. Without delegates and sponsors, there would be no symposium. I warmly invite all of you to join me in creating a stimulating microclimate for a special and rewarding MSB 2024 event.

Frantisek Foret
MSB 2024 chair
Institute of Analytical Chemistry
of the Czech Academy of Sciences

www.msb2024.org

COMMITTEES

MSB 2024 chair

Frantisek FORET, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic

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MSB 2024 IN HONOR OF BARRY KARGER

Barry L. Karger, PhD, is the Director Emeritus of the Barnett Institute for Chemical and Biological Analysis, and James L. Waters Emeritus Chair and Distinguished Professor Emeritus at Northeastern University. Throughout his career, Dr. Karger has made major advances in understanding the fundamentals of chromatography and capillary electrophoresis techniques and to their application, particularly in the fields of protein and DNA analysis.

Dr. Karger founded the Barnett Institute of Chemical and Biological Analysis in 1973, which has been a significant contributor to developing analytical methods for the biological sciences and the instrumentation and biotechnology industries. The Barnett Institute has produced over 500 PhDs, postdocs, and staff, many of whom have gone on to have distinguished careers in academia and industry.

Dr. Karger began his research career in gas chromatography. He was at the forefront of high-performance liquid chromatography and was among the first to develop reversed-phase liquid chromatography (RPLC). He introduced a means of classifying mobile phases in RPLC, leading to a better understanding of selectivity, and he was the first to show direct enantiomeric separations using liquid chromatography using chiral chelate additives. In 1973, Karger co-authored the textbook, An Introduction to Separation Science, which was used to train over two generations of analytical chemists in chromatographic fundamentals.

Dr. Karger played a significant role in the Human Genome Project using capillary electrophoresis. He was the first to show that polymer separation media could be blown out of the capillary column and reloaded with fresh polymer with no change in the separation performance of DNA fragments, allowing automated DNA sequencing. His linear polyacrylamide polymer matrix was used to sequence 40% of the first human genome sequence.

Dr. Karger is also known for his research on the analysis of peptides and proteins, especially the native-denatured behavior of proteins in RPLC. Focusing his work on the coupling of mass spectrometry with liquid chromatography and capillary electrophoresis facilitated the analysis of low-volume clinical samples. It led to the identification of important cancer and blood biomarkers. His research also expanded to include detailed characterization of protein biopharmaceuticals — applying powerful analytical techniques routinely used today in the biopharmaceutical field.

Dr. Karger won the Steven dal Nogare Memorial Award for Chromatography (1975), the American Chemical Society (ACS) Award in Chromatography (1982), the M.S. Tswett Medal in Chromatography (1986), the ACS Award in Analytical Chemistry (1990), the A.J.P. Martin Medal (1991), the EAS Symposium Award for Outstanding Achievements in the Field of Separations

Science (1997), the ACS Award in Separations Science and Technology (1998), and the Halász Medal of the Hungarian Chemical Society for Chromatography and Separation Science (2002). He has also received the CaSSS Award (2003), the Michael Widmer Award (2004, Switzerland), the Torbern Bergman Medal (2008, Sweden), the Csaba Horváth Medal (2008), the Golay Medal, (2009), the Csaba Horváth Lectureship (2010, Hungary), the J. Heyrovsky Honorary Medal (2010, Czech Republic), the Arnold O. Beckman Medal (2014), and the Lifetime Achievement in Chromatography Award by LCGC (2022).

SYMPOSIUM HISTORY

Originally established as the International Symposium on High-Performance Capillary Electrophoresis (HPCE), the first event was held April 10-12, 1989, at the Park Plaza Hotel in Boston, MA. Professor Barry Karger from Northeastern University founded the meeting which featured presentations discussing the principles of separations in capillaries under high electric fields, including instrumentation development and applications, particularly in biotechnology.

The HPCE symposium was introduced when capillary electrophoresis (CE) branched off from the HPLC community, giving the technology the necessary focus at a time when CE instrumentation was first being commercialized. The Scientific Advisory Board (SAB) drove the symposium series under its diligent chairman Barry Karger until 2000, followed by Frantisek Svec. The series was organized worldwide by Prof. Karger until 2000, after which by CASSS in the USA and by separate bodies in Europe and Asia.

At HPCE 2004 in Salzburg, the SAB changed the symposium name to MicroScale Bioseparations (MSB), since the attendees' interests expanded into the related techniques of micro- and nano-HPLC, microfluidic separations, and Lab-on-a-Chip applications, while the fascination with CE slowly decreased. The stylized logo was created at the same time and captured the acronym MSB in a DNA helix motif given the prominent role that electrically-driven microseparations have played in DNA sequencing and the early completion of the Human Genome Project.

At MSB 2012 in Geneva, Switzerland, Beckman-Coulter established the prestigious Arnold O. Beckman Medal and Award for Outstanding Scientific Achievements in The Field of Electrodriven Separations Techniques which has become an essential element of the MSB series.

After the MSB 2012 symposium, the SAB changed. This was not done just by including new members but also by introducing new key concepts by which future meetings of the series will be organized. The symposium aims to create a confidential ambiance with significant room for discussion and with over seventy percent of the program built from contributed abstracts using a blind review process. The board also changed its name to Strategic Program Committee (SPC). To further broaden the scope of the series to a wider range of scientists, the SPC approved the

acronym MSB to refer to Microscale Separations and Bioanalysis. The new official conference name was used for the first time at MSB 2016 in Niagara-on-the-Lake, Canada.

In January 2018, the SPC took the bold step of creating an official, incorporated society to ensure the longevity of the MSB symposium series. Previous members of the SPC now form the Board of Directors of the Society for Microscale Separations and Bioanalysis (SMSB).

PREVIOUS HPCE AND MSB MEETINGS

Year	*Location*	*Chair(s)*
1989	Boston	Barry Karger
1990	San Francisco	Barry Karger
1991	San Diego	James Jorgenson
1992	Amsterdam	Frans Everaerts
1993	Orlando	Barry Karger
1994	San Diego	Shigeru Terabe
1995	Würzburg	Heinz Engelhardt
1996	Orlando	Barry Karger
1997	Anaheim	William Hancock
1997	Kyoto	Shigeru Terabe
1998	Orlando	Barry Karger, Salvatore Fanali
1999	Palm Springs	Edward Yeung
2000	Saarbrücken	Heinz Engelhardt
2001	Boston	Barry Karger, William Hancock
2002	Stockholm	Douglas Westerlund
2003	San Diego	Aran Paulus, Andras Guttman
2004	Salzburg	Wolfgang Lindner
2005	New Orleans	Michael Ramsey
2005	Kobe	Yoshinobu Baba, Koji Otsuka
2006	Amsterdam	Gerard Rozing
2007	Vancouver	Robert Kennedy
2008	Berlin	Andreas Manz
2009	Boston	Jonathan Sweedler
2009	Dalian	Hanfa Zou
2010	Prague	Frantisek Foret
2011	San Diego	Annelise Barron
2012	Geneva	Franka Kalman, Gerard Rozing, Jean-Luc Veuthey
2012	Shanghai	Rong Zeng
2013	Charlottesville	Jeff Chapman, James Landers

2014	Pécs	Ferenc Kilár, Attila Felinger, András Guttman
2015	Shanghai	Fukui Zhang, Pengyuan Yang, Norman Dovichi, Amy Guo
2016	Niagara-on-the-Lake	Philip Britz-McKibbin, Karen Waldron, Sergey Krylov
2017	Noordwijkerhout	Govert Somsen, Rawi Ramautar
2018	Rio de Janeiro	Marina Tavares, Emanuel Carrilho
2019	Corvallis	Vincent Remcho, Karen Waldron
2020	Saint-Malo (Virtual)	Myriam Taverna, Serge Rudaz
2021	Boston (Virtual)	Alexander Ivanov, Kimberly Hamad-Schifferli, Jarrod Marto
2022	Liège	Marianne Fillet, Heidi Ottevaere
2023	Tallahassee	Michael Roper, Rebecca Pompano, James Edwards
2024	Brno	Frantisek Foret

SCIEX MICROSCALE SEPARATIONS INNOVATIONS MEDAL AND AWARD

The SCIEX Microscale Separations Innovations Medal and Award (previously the Arnold O. Beckman Award) is an annual award given to an individual for remarkable career achievements, with particular consideration being given to the development of new methods, techniques and high-impact applications in the field of electro driven separations. The award is supported by SCIEX, a key driver in capillary electrophoresis technology, and comprises a medal, a diploma, and a monetary prize. The award is focused on a capstone achievement in the preceding 12 months but also recognizes that this key achievement is often built on a foundation of prior efforts.

The 2024 SCIEX Microscale Separations Innovations Medal and Award will be presented to:

Hervé Cottet

Prof. Hervé Cottet is a full professor at the Biomolecule Institute (IBMM) in Montpellier, France. His research work concentrates on the interface between separation sciences, analytical chemistry, polymers, and pharmaceutical sciences, with expertise in Capillary Electrophoresis and Taylor Dispersion Analysis. He is both interested in the fundamentals and practical (or industrial) applications of CE and TDA. Recent advances include the development of polyelectrolyte multilayer capillary coatings, the limitation of solute adsorption, the optimization of separation efficiency in CE, the study of biomolecular interactions, the monitoring of biomolecule aggregation, the characterization of vaccine formulations, and the development of various TDA applications. He has co-authored more than 150 scientific articles (h-index=40) dealing with CE and/or TDA. He has (co-)supervised more than 20 PhD students.

Previous Award Winners

2023	Christian Neusüß
2022	James Landers
2021	Peter Willis
2020	Detlev Belder
2019	Aaron Wheeler
2018	Amy Herr
2017	Shigeru Terabe
2016	Bohuslav Gaš
2015	Gyula Vigh
2014	Barry Karger
2013	Stellan Hjertén
2012	Pier Giorgio Righetti

MEDAL OF JAROSLAV JANÁK

The Medal of Jaroslav Janák for contributions to the development of analytical sciences was established by the Institute of Analytical Chemistry of the Czech Academy of Sciences. Named after the inventor of the gas chromatograph (patented in 1952), the founder of the institute (1956), and its long-term director, the medal is awarded to scientists who have significantly contributed to the development of separation sciences.

In 2024, the Medal of Jaroslav Janák goes to:

Milos V. Novotny

Milos V. Novotny, a native of Brno (Czech Republic), is a Distinguished Professor Emeritus and Adjunct Professor of Medicine at Indiana University. He has resided with his family in Bloomington (Indiana) for more than 50 years.

Milos Novotny was raised and educated in Brno, studying at Masaryk University, where he received the degrees of Magister and RNDr (Biochemistry) and Dr.Sc. (Chemical Sciences). Before emigrating to Sweden in January 1968, and later to the U.S.A., he was for 3 years on the research staff of the Institute of Analytical Chemistry of the Czechoslovak Academy of Sciences in Brno. While working at the Royal Karolinska Institute in Stockholm, he accepted the Robert A. Welch Postdoctoral Fellowship at the University of Houston (Texas) during 1969-1971. In August 1971, he was appointed to the faculty of Indiana University (IU) where he rose through the ranks to a full professor, James H. Rudy Professor, Lilly Chemistry Alumni Chair, and Distinguished Professor.

At IU Milos Novotny established a strong and internationally recognized research group playing a major role in developing modern chromatographic and electrophoretic analytical techniques. He was a pioneer in the preparation of glass capillary GC columns and coupling of capillary GC with mass spectrometry; later on, in capillary LC, capillary SFC, and in the early developments in capillary electrophoresis. As a member of the NASA Viking 1975 Science Team, Novotny designed the miniaturized GC column to search for organic molecules on the Planet Mars. In the search for new LC microcolumns, miniaturized detectors, and capillary LC-MS methodologies, Milos Novotny educated numerous students and research associates to become future leaders in academia and top industrial positions. He received several teaching awards at IU.

For his research accomplishments, Dr. Novotny has been recognized by more than 40 different awards, medals, and other distinctions on three different continents. Among the most prestigious

are 4 awards from the American Chemical Society: Chromatography (1986); Chemical Instrumentation (1988); Separation Science and Technology (1992); and Analytical Chemistry (2005); also Ralph N. Adams Award in Bioanalytical Chemistry (2008). He was named the R&D Magazine Scientist of the Year in 1994. His international awards include Marcel J.E. Golay Award and the A.J.P. Martin Gold Medal (U.K.). Milos Novotny received honorary doctorates from Uppsala University (Sweden) and Charles University in Prague. He was elected to two foreign academies: the Royal Society for Sciences (Sweden) and the Learned Society of the Czech Republic. His research interests remain wide-ranging, from separation science to structural analysis of biological molecules, proteomics, glycoscience to chemical communication in mammals. He and his coworkers have been credited with structural identification of the first mammalian pheromones. He has co-authored over 540 articles, reviews, and patents, which are widely cited (h-index 102).

While maintaining extensive international scientific collaborations, Dr. Novotny has particularly intensified contacts in his homeland after the Velvet Revolution in 1989. He has collaborated with scientists at Charles University, Masaryk University, the University of Pardubice, and the University of South Bohemia. The Czech Academy of Sciences honored him with the J. E. Purkyne Medal and the Jaroslav Heyrovsky Medal for advancing chemical sciences. After retiring from Indiana University in 2011, Dr. Novotny held a part-time research position at the Masaryk Memorial Cancer Institute in Brno.

András Guttman

Professor András Guttman is heading the Horváth Csaba Memorial Laboratory of Bioseparation Sciences at the University of Debrecen (Hungary) and the Translational Glycomics Research Group at the University of Pannonia (Veszprem, Hungary). Professor Guttman graduated from the University of Veszprem (Hungary) in chemical engineering, where he also received his first doctoral degree. His work is focused on capillary electrophoresis, CE-MS-based glycomics, and glycoproteomics analysis of molecules of biomedical and biopharmaceutical interests.

Professor Guttman had previous academic appointments at Northeastern University (Boston, MA) and the University of Innsbruck (Austria) holding the Marie Curie Chair of the European Commission. His former industrial affiliations included Novartis (La Jolla, CA), Genetic BioSystems (San Diego, CA), and Beckman Instruments (Brea, CA), where he developed high-resolution capillary electrophoresis and microfluidics-based bioanalytical methods.

Professor Guttman has published nearly 400 scientific papers, and 38 book chapters, authored one and edited four textbooks, and holds more than 40 patents. He is a member of the Hungarian Academy of Sciences and was the past president of the American Chemical Society Hungary

Chapter. Professor Guttman is the Editor in Chief for Current Molecular Medicine and serves on the editorial boards of a dozen international scientific journals. He is on the Board of the Society of Hungarian Academicians in America and is the associate director of CASSS.

Professor Guttman is in the top 0.5% of scientists in the world and has been recognized by numerous awards, including the Analytical Chemistry Award of the Hungarian Chemical Society in 2000, the CASSS CE Pharm Award, the Arany Janos Medal of the Hungarian Academy of Sciences, and the Pro Scientia award of University of Pannonia in 2013. In 2014 he received the Dennis Gabor Award of the Novofer Foundation. In 2017 Professor Guttman received the Dal Nogare Award of the Delaware Valley Chromatography Forum and the Grand Prize of the Swedish Chamber of Commerce. He was a Fulbright Scholar of the US Department of State in 2012 and named a distinguished Brain Pool Fellow of the National Research Foundation of Korea in 2021. Professor Guttman also received the 2024 Jedlik Anyos Award of the Hungarian Intellectual Property Office and the Cross of Merit of the Hungarian Republic.

Previous Medal Winners

2023	Bohuslav Gaš
2022	Martin Gilar
2022	František Švec
2018	Ludmila Křivánková
2016	Petr Boček
2015	Pavel Jandera
2014	Jaroslav Janák

MSB 2024 YOUNG SCIENTISTS AWARD

The MSB 2024 Young Scientists Award is intended to give talented young scientists extra encouragement. It will be presented to a young researcher whose outstanding work sets an example for other scientists. All presenters who are graduate students or postdocs at the end date of the meeting are eligible for consideration (proof of status will be required). An international jury of scientists will judge the qualified presentations and choose the winners. The prize consists of a certificate, a cash amount (sponsored by Wiley – Electrophoresis and MDPI - Micromachines), and a book voucher (sponsored by Springer - Analytical and Bioanalytical Chemistry). The winners will be announced and awarded at the Closing Ceremony on Wednesday.

MSB 2024 Young Scientists Award Nomination:

Antonín Bednařík, Brno, Czech Republic
Constantin Blöchl, Leiden, The Netherlands
Zahia Bouchelaghem, Orléans, France
Christoph Gstöttner, Leiden, The Netherlands
Mathilde Jégo, Orsay, France
Ignaas S.M. Jimidar, Brussels, Belgium
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Egzontina Shabani, Pardubice, Czech Republic
Denisa Smolková, Brno, Czech Republic
Ruben Szabó, Debrecen, Hungary
Dora Szerenyi, Veszprem, Hungary
Rebeka Török, Veszprem, Hungary
François-Xavier Vidal, Lyon, France
Tobias Waldmann, Aalen, Germany
Delaram Zohouri, Orsay, France

MSB 2024 BEST POSTER AWARD

All posters presented at MSB 2024 will be considered for an MSB 2024 Best Poster Award. An international panel of scientists will review the posters. Posters will be up during the entire symposium. Presenters of a poster with an even number should be at their poster during the poster session on Monday; presenters of posters with an odd number should be at their poster during the poster session on Tuesday. The poster prize consists of a certificate, and a cash amount (sponsored by the Royal Society of Chemistry – Analyst and Analytical Methods). Poster prize winners will be presented and awarded at the Closing Ceremony on Wednesday.

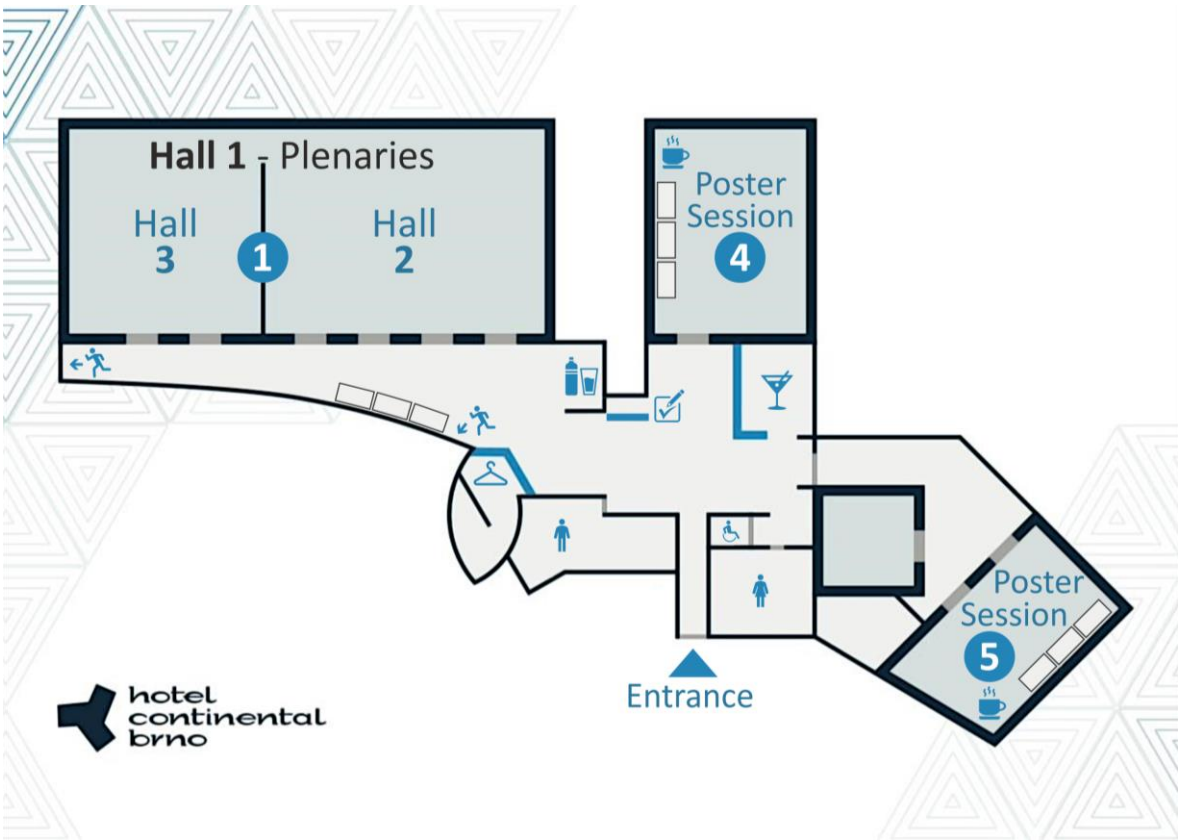
CONFERENCE VENUE

Congress Center of the Hotel Continental****
Kounicova 6, Brno, Czech Republic
www.continentalbrno.cz/en

All symposium meeting rooms are located on the first floor.

- The opening and closing ceremonies and plenary lectures will be in room 1.
- The oral parallel sessions are in rooms 2 and 3.
- Poster sessions, exhibitor table tops, and coffee breaks are in rooms 4 and 5.
- The symposium registration desk is located in the lobby.

Lunches will be served in the Hotel Restaurant on the ground floor.



GENERAL INFORMATION

Badge

Each registered participant must wear the official symposium name badge to gain admittance to the meeting, symposium rooms, and social gatherings. Badge sharing is not permitted.

Registration and Information Desk

The symposium registration desk is located in the lobby of the Congress Center.

Opening hours:
Sunday 12:00 – 20:00
Monday 8:30 – 17:00
Tuesday 8:30 – 16:00
Wednesday 8:30 – 16:00

Exhibitors

Visit the exhibitors' table tops in the lobby and rooms 4 and 5. Take the time to thank them for their generous support of the symposium by letting them share their latest services and products with you.

SOCIAL PROGRAM

MSB recognizes that good science is all about strong interaction between people. Therefore, next to a comprehensive scientific program with active discussion, MSB 2024 will provide ample opportunities to build up and strengthen social networks. All social events are included in the registration and are open to each delegate. So, join us and take the occasion to meet up with old friends and make new friendships.

Welcome Reception

Sunday, May 19, 2024, 18:20-20:00 - Congress Center of the Hotel Continental, Kounicova 6, Brno

Refreshments and wine just after the Opening Plenary Session.

Tour of the Mendel Museum: "Discover the story of the founder of genetics – Gregor Johann Mendel"

Tuesday, May 21, 2024, 16:00-18:45 - Mendel Museum, Mendlovo náměstí 1a, Brno

The exhibition displays original objects belonging to the "Father of Genetics", his school reports, books, or teaching aids. The crowning piece of the display is Mendel's seminal work, Versuche über Pflanzenhybriden (Experiments with Plant Hybrids).

Banquet

Tuesday, May 21, 2024, 19:00-21:00 - Augustinian Abbey, Mendlovo náměstí 1, Brno

Our banquet dinner in the Garden of Eden of the Monastery courtyard with the tour of the Mendel Museum allows you to see the places where the era of Modern Genetics started.

Farewell Reception – sponsored by VICI AG International

Wednesday, May 22, 2024, 16:00-17:00 - Congress Center of the Hotel Continental, Kounicova 6, Brno

A last drink before returning home.

All symposium participants and accompanying persons are kindly welcome to all social events.

SHORT COURSES

MSB 2024 offers three stimulating and highly informative short courses for symposium participants. The short courses are lectured by recognized experts in the field and run in parallel on Sunday, May 19, 2024, from 13:00 to 16:00. The course fee is 50 euros.

1. Ion Mobility Spectrometry

presented by Tim Causon, BOKU - University of Natural Resources and Life Sciences, Vienna, Austria

Course Description:
Ion mobility (IM) complements existing analytical methods as it involves millisecond-timescale separations of gas phase ions according to their structure and not only their mass. Combined with mass spectrometry, IM-MS is now a powerful technology that can be used in diverse analytical applications. This short course will introduce the basics of IM theory, separation principles, and the derivation of collision cross section (CCS) from IM-MS measurements. Important instrumental aspects of IM spectrometry and IM-MS technologies will be introduced, and examples from diverse application fields (e.g., food, metabolomics, environmental analysis) will be shown to highlight how ion mobility can be applied successfully in analytical methods.

Instructor:
Assoc. Prof. Tim Causon is an Associate Professor at the Institute of Analytical Chemistry, University of Natural Resources and Life Sciences, Vienna (Austria). His main areas of research are liquid chromatography, molecular mass spectrometry, and ion mobility-mass spectrometry (IM-MS) addressing diverse analytical method development questions and fundamental studies of ionization and properties of small molecular ions. Current research topics include applications of these analytical techniques for optimization of both upstream (e.g., microbial cell factories) and downstream (e.g., purification) ends of the bioprocessing continuum, fundamental investigations of gas-phase isomers of small molecule systems, and addressing the urgent need for harmonization of IM-MS measurement and reporting standards. Starting in 2024, he will be the coordinator of the MSCA Doctoral Network "MobiliTraIN" (Ion Mobility Mass Spectrometry Training Network).

2. Mass Spectrometry Imaging

presented by Jan Preisler - Antonin Bednarik, Masaryk University, Brno, Czech Republic

Course Description:
This course comprehensively explains mass spectrometry imaging (MSI) principles and techniques. The most common ionization techniques involved in MSI, namely matrix-assisted laser desorption/ionization (MALDI), desorption electrospray (DESI), secondary ion mass spectrometry (SIMS) and laser ablation inductively-coupled plasma (LA ICP), are reviewed. The MALDI technique

is emphasized, as it presents the most widespread MSI platform. Sample preparation methods are discussed in detail, including tissue sectioning and storage, washing protocols, matrix application, and on-tissue reactions. Current instrumentation, a common data format (imzML) structure, software tools for data processing and visualization, selected applications, and the latest achievements in MSI are presented.

Instructors:
Prof. Jan Preisler is a professor of analytical chemistry at Masaryk University in Brno, Czech Republic. He received his Ph.D. with Ed Yeung at Iowa State University, Ames, and spent four years in the group of Barry Karger at Barnett Institute, Boston. His research interests include the development of instrumentation and methods for bioanalytical chemistry, MS imaging, time-of-flight mass spectrometers, characterization and analytical applications of nanoparticles, single-particle analysis, and development of new sample introduction techniques for inductively coupled plasma mass spectrometry. He pioneered using kHz lasers to increase the throughput of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in imaging applications and to detect parallel column separations.

Antonin Bednarik, Ph.D. is a postdoc researcher at Masaryk University in Brno, Czech Republic. His research activities include the development of MALDI MS and MSI instrumentation, analysis of volatile organic compounds, the development of novel MSI techniques, and applications of MSI in the analysis of nanoparticles and lipidomics. After receiving his Ph.D., he spent one year as a postdoc in the group of Klaus Dreisewerd in Münster, where he developed on-tissue Paternò Büchi derivatization protocol for MALDI MSI of lipid double bond positional isomers. He continues studying on-tissue derivatization reactions for MSI of lipid isomers and their potential in clinical applications.

3. Introduction to Miniaturization and Microfluidics

presented by Petr Kuban - Jakub Novotny - Tomas Vaclavek - Jana Krivankova, Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

Course Description:
The main objective of this short course is to provide the participants with an overview of the basic concepts of miniaturization and microfluidic technology. We will discuss the fundamentals of microfabrication techniques involved in manufacturing microfluidic devices as well as the main benefits of utilizing such devices in analytical chemistry. In a series of lectures, the participants will gain knowledge and skills on two primary techniques for fabricating microfluidic devices in glass and polymeric materials. The methods will include glass chip fabrication techniques, such as photolithography, metal deposition, and etching. The information regarding the fabrication of plastic- and polymer-based systems, such as PDMS chips, will encompass casting and other soft lithography techniques. Eventually, there will be a comprehensive lecture on 3D printing, showing 3D printing techniques, such as FDM and SLA, discussing the advantages of various printing materials, economic and sustainability aspects, etc., and practical examples of a 3D printed casing for microfluidic devices. The participants will also gain knowledge of other computer-controlled

methods associated with fabricating microfluidic devices, such as CNC micromachining. Last but not least the control of microfluidic devices with the use of an Arduino microcontroller, basic programming using the IDE, and examples of the use of Arduino in analytical instruments will be shown. Additionally, specific applications like single-cell analysis and droplet microfluidics, and more will be discussed as a part of this course.

Instructors:
Assoc. Prof. Petr Kuban obtained his doctorate from the Department of Analytical Chemistry, Stockholm University, Sweden and spent three years as a post-doc at the Department of Chemistry and Biochemistry, Texas Tech University, USA. He is the Head of the Department of Bioanalytical Instrumentation at the Institute of Analytical Chemistry of the Czech Academy of Sciences in Brno, Czech Republic. His main research interests include micro-column separation techniques such as capillary electrophoresis and HPLC, flow injection analysis, sample pretreatment and preconcentration, miniaturization, microfluidics, and developing novel analytical instrumentation for clinical diagnostics.

Jakub Novotny, Ph.D. aimed his doctoral studies at the Institute of Analytical Chemistry in Brno, Czech Republic, towards the applications of microfluidics in biochemistry, with a specific emphasis on microfabrication methods. This involved the development of microfluidic devices using CNC fabrication methods (e.g. micro-milling), 3D printing, and photolithography. During his postdoctoral research in Lund, Sweden, he explored the techniques related to acoustofluidics (study of the interaction of soundwaves with fluids and dispersed particles at the microscale). His current work continues to revolve around acoustofluidics and the development of acousto/microfluidic devices.

Tomas Vaclavek, Ph.D. has received his Ph.D. in Biochemistry from Masaryk University, developing various microfluidic systems focused on single-cell manipulation, non-optical particle detection, and intracellular compound isolation with subsequent analysis by mass spectrometry. His collaborations provided valuable experience with the miniaturization of biosensing systems or developing microfluidic interfaces for 2D liquid separations. Currently, he develops micromachined nanospray interfaces as ion sources for highly sensitive bioanalyses.

Jana Krivankova, Ph.D. finished her doctoral studies at the Brno University of Technology. Since 2016, she explored research related to fabrication techniques, focused on developing and optimizing droplet-based microfluidic devices. Her current research deals with the application of photon-conversion nanoparticles and automation on microfluidic chips to detect clinically relevant protein markers.

SCIENTIFIC PROGRAM

MSB 2024 Young Scientists Award Nomination

May 19, 2024 (Sunday)

12:00		Registration desk opens
13:00–16:00		SHORT COURSES, registration required – Rooms 2, 3, 4
		SC1 – Ion Mobility Spectrometry
		SC2 – Mass Spectrometry Imaging
		SC3 – Introduction to Miniaturization and Microfluidics
16:30–16:40		Opening Ceremony – Room 1 *Chair: Frantisek Foret, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic*
16:40–17:00		Interview of Barry Karger with the MSB 2024 chair
		Plenary Lectures 1 – Room 1 *Chair: Frantisek Foret, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic*
17:00–17:40	PL1	Microdroplet Arrays: The Next Generation of Multi-Well Plates for High Throughput Analysis Petra Dittrich, ETH Zurich, Basel, Switzerland
17:40–18:20	PL2	Applications of DNA Capillary Electrophoresis in Molecular Cancer Diagnostics: Above and Beyond Sanger Sequencing Marek Minarik, Elphogene & Charles University, Prague, Czech Republic
18:20–20:00		Welcome Reception

May 20, 2024 (Monday)

	Session 1 - Microfluidics I - Room 2 *Chair: Jan Petr, Palacký University Olomouc, Olomouc, Czech Republic*	
09:00-09:30	KN1	Novel Electrokinetic Approaches for Particle Separation Applied to Organelles and Microplastics Alexandra Ros, Arizona State University, Tempe, AZ, USA
09:30-09:50	O1	On-Chip Depletion-Zone Isotachophoresis of Exosomes: A Solution to Overcome the Purity Limitations of Current Techniques Andrea Capuano, EXIT071, Leiden, The Netherlands
09:50-10:10	O2	Frontal Analysis Continuous Capillary Electrophoresis: An Approach to Predict Plasma Proteins and Polymeric Nanoparticles Interactions Mathilde Jégo, University Paris Saclay, Orsay, France
10:10-10:30	O3	Chip Electrophoresis of Fluorescently Labelled Virus Particles Victor U. Weiss, TU Wien, Vienna, Austria
	Session 2 - Glycomics - Room 3 *Chair: Gabor Jarvas, University of Pannonia, Veszprem, Hungary*	
09:00-09:30	KN2	Comprehensive Characterization of Mammalian Brain N-Glycome: Isomer-Sensitive Nano-LC-MS/MS Analysis and its Application to Alzheimer's Disease Models Hyun Joo An, Chungnam National University, Daejeon, Korea
09:30-09:50	O4	SSSMuG: Same Sample Sequential Multi-Glycomics Edward S.X. Moh, Macquarie University, Sydney, Australia
09:50-10:10	O5	Comprehensive O-Glycan Analysis by Porous Graphitized Carbon Nano-Liquid Chromatography-Mass Spectrometry Tao Zhang, Leiden University Medical Center, Leiden, The Netherlands
10:10-10:30	O6	BODIPY-based Fluorescent Labeling Tag for Oligosaccharide and N-Linked Glycan Analysis by High-Performance Liquid Chromatography with Fluorescence Detection Denisa Smolková, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic
10:30-11:00	Coffee Break	

	Session 3 - Advances in Microscale CE and LC Separations I - Room 2 *Chair: Rawi Ramautar, Leiden University, Leiden, The Netherlands*	
11:00-11:30	KN3	Multidimensional Assessment of Polymer Nanoparticles Govert W. Somsen, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
11:30-11:50	O7	Highly Sensitive Two-dimensional Profiling of N-linked Glycans by Hydrophilic Interaction Liquid Chromatography and Dual Stacking Capillary Gel Electrophoresis Takayuki Kawai, Kyushu University, Fukuoka, Japan
11:50-12:10	O8	Going Big: Non-Denaturing HRMS- Hyphenated Separations Unleash the Analysis of Complex Proteoform Mixtures Over 100 kDa Andrea Gargano, University of Amsterdam, Amsterdam, The Netherlands
12:10-12:30	O9	Endotoxin Quantification by the Chemical Instrumental HPLC-Kdo-DMB Assay Anika Hoffmann, HES-SO Valais-Wallis, Sion, Switzerland
12:30-12:50	O10	A Novel Selective Comprehensive Two-Dimensional Online nanoLC-CZE-MS Platform for Proteoform Characterization Tobias Waldmann, Aalen University, Aalen, Germany
	Session 4 - New Trends in MS and IMS - Room 3 *Chair: Tim Causon, BOKU, Vienna, Austria*	
11:00-11:30	KN4	Imaging of Individual Nanoparticles by Mass Spectrometry Jan Preisler, Masaryk University, Brno, Czech Republic
11:30-11:50	O11	Taylor-Aris Dispersion Assisted Mass Spectrometry for the Direct Injection Analysis of Proteins with High Matrix Content Ruben Szabó, University of Debrecen, Debrecen, Hungary
11:50-12:10	O12	Rapid Distinction and Assignment of Positional Isomers of New Psychoactive Drugs in Mixtures by Trapped Ion Mobility Mass Spectrometry Hany A. Majeed, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
12:10-12:30	O13	Metal Ionization in Gas Phase Mass Spectrometry (MIG MS): A New Tool for Analysis of Volatile Organic Compounds Antonín Bednařík, Masaryk University, Brno, Czech Republic
12:30-12:50	O14	The Potential of Stainless Steel Needles to Integrate Microextraction and Mass Spectrometry Jaime Millán-Santiago, University of Cordoba, Cordoba, Spain
12:50-14:30	Lunch - Hotel Restaurant	

14:30–15:30	Poster Session 1 – Rooms 4 and 5	
	Session 5 – Microfluidics II – Room 2 *Chair: Jakub Novotný, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic*	
15:30–16:00	KN5	When Microfluidics Meets Magnetic Nanomaterials: Recent Approaches in Bioanalysis Zuzana Bílková, University of Pardubice, Pardubice, Czech Republic
16:00–16:20	O15	Nanogels for Multifunctional Biomolecular Electrophoresis Assays Lisa Holland, West Virginia University, Morgantown, WV, USA
16:20–16:40	O16	Dry and Wet Assembly Approaches for Arranging Ordered Particle Monolayers and Arrays Ignaas S.M. Jimidar, Vrije Universiteit Brussel, Brussels, Belgium
16:40–17:00	O17	Investigation of the Effect of Induced Macromolecular Crowding on Hyaluronidase Catalytic Activity and Interactions using Capillary Electrophoresis and Microscale Thermophoresis Zahia Bouchelaghem, University of Orleans. Orleans, France
17:00–17:20	O18	Novel Approaches for the Analysis of Organoids and Organ-on-Chip Samples using Liquid Chromatography and Mass Spectrometry Steven Ray Haakon Wilson, University of Oslo, Oslo, Norway
	Session 6 – Biomarkers – Room 3 *Chair: Pavel Kubáň, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic*	
15:30–16:00	KN6	The Study of Sputome: A Need for Sputum as a Rich Source of Protein Biomarkers for the Non-Invasive Diagnosis of Infectious and Chronic Diseases Norberto A. Guzman, Princeton Biochemicals, Princeton, NJ, USA
16:00–16:20	O19	Monitoring the Effectiveness of Chemotherapy Treatments Utilizing Artificial Intelligence-based N-Glycome Analysis Rebeka Török, University of Pannonia, Veszprem, Hungary
16:20–16:40	O20	N-Glycosylation Analysis of Homogenized Oral Squamous Cell Carcinoma Soft Tissue Samples by CE-LIF Eniko Gebri, University of Debrecen, Debrecen, Hungary
16:40–17:00	O21	Ionic Liquids Assisted Micellar Electrokinetic Chromatography of Urine Catecholamine Metabolites for the Investigation of Neuroblastoma Ilona Olędzka, Medical University of Gdańsk, Gdańsk, Poland
17:00–17:20	O22	Novel Magnetic Solid-Phase Microextraction Approach with Ionic Liquids and a Surfactant as Coating Materials for Pretreatment of Biological Samples Alina Plenis, Medical University of Gdańsk, Gdańsk, Poland

May 21, 2024 (Tuesday)

	SCIEX Session - Room 1 *Chair: Vincent T. Remcho, Oregon State University, Corvallis, OR, USA*	
09:00-09:10	SCIEX Medal & Award	
09:10-09:50	PL3	Capillary Electrophoresis and Taylor Dispersion Analysis: Recent Advances and Present Challenges in Health Applications Hervé Cottet, University of Montpellier, Montpellier, France
09:50-10:20	Coffee Break	
	Session 7 - Microscale Separations for Omics Sciences - Room 2 *Chair: Andrea Gargano, University of Amsterdam, Amsterdam, The Netherlands*	
10:20-10:50	KN7	Native N-Glycome of Single Mammalian Cells and ng-Level Blood Isolates Deciphered using Label-Free Capillary Electrophoresis-Mass Spectrometry Alexander R. Ivanov, Northeastern University, Boston, MA, USA
10:50-11:10	O23	Deciphering the Phosphorylation Barcode of G Protein-Coupled Receptors (GPCRs) using CZE-TDMS Kevin Jooß, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
11:10-11:30	O24	Intramolecular Disulfide and Charge Variant Separation and Characterization of Various Antibody Subunits with CE-MS/MS Jasmin Schairer, Aalen university, Aalen, Germany
11:30-11:50	O25	Toward 1000-fold Sensitivity Improvement of Capillary Electrophoresis coupled with Laser-Induced Fluorescence Detection for Aminopyrene Trisulfonic Acid Fluorophore Chenchen Liu, Kyushu University, Fukuoka, Japan
11:50-12:10	O26	Charge to Move Forward in Volume-Restricted Metabolomics Rawi Ramautar, Leiden University, Leiden, The Netherlands
12:10-12:30	O27	Online Electrokinetic Sample Cleanup and Evaluation Method for APTS Labeled N-Glycan Separation by Capillary Electrophoresis Gabor Jarvas, University of Pannonia, Veszprem, Hungary
	Session 8 - Bioanalysis/Cellular Analysis - Room 3 *Chair: Lucie Korecká, University of Pardubice, Pardubice, Czech Republic*	
10:20-10:50	KN8	Bioanalytical Approaches for Monitoring Cellular Communication Michael Roper, Florida State University, Tallahassee, FL, USA
10:50-11:10	O28	Single Islet Metabolomics using Capillary LC-MS James Edwards, Saint Louis University, St. Louis, MO, USA
11:10-11:30	O29	Kinase/Small Inhibitor Interaction Evaluated Directly in Cell Lysates and Whole Cells: A Combined Capillary Electrophoresis and Microscale Thermophoresis Study Reine Nehmé, University of Orleans, Orleans, France

11:30–11:50	O30	Digging into the Multifaceted Variability of Antibody Molecules: Fc-Proteoform Profiling Illuminates Autoimmune Responses in Rheumatoid Arthritis Constantin Blöchl, Leiden University Medical Center, Leiden, The Netherlands
11:50–12:10	O31	Isotachophoresis for Electrokinetic Preconcentration of Extracellular Vesicles by Capillary Electrophoresis Delaram Zohouri, University Paris Saclay, Orsay, France
12:10–12:30	O32	Enrichment and Identification of Ceramide Synthase 2 in Subcellular Components: Novel Insights from Porcine Pancreatic Tissue Egzontina Shabani, University of Pardubice, Pardubice, Czech Republic
12:30–14:00	Lunch – Hotel Restaurant	
14:00–15:00	Poster Session 2 - Rooms 4 and 5	
16:00–18:45	Tour of the Mendel Museum	
19:00–21:00	Banquet – Augustinian Abbey (Garden of Eden of the Monastery courtyard)	

May 22, 2024 (Wednesday)

	Session 9 - Biosensors - Room 2 *Chair: Steven Ray Haakon Wilson, University of Oslo, Oslo, Norway*	
09:00-09:30	KN9	Glycosylation as a Tool for Biomarker Discovery in Cancer using Exosomes Jan Tkac, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia
09:30-09:50	O33	Artificial Intelligence-Aided Massively Parallel Spectroscopy for Bioaffinity Assays and Droplet Microfluidics Antonín Hlaváček, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic
09:50-10:10	O34	3D Made Electrochemical Sensors Agata Michalska, University of Warsaw, Warszawa, Poland
10:10-10:30	O35	From Separation to Treatment: Development of a Microbead-Based Extracorporeal CTC Capture Platform Dora Szerenyi, University of Pannonia, Veszprem, Hungary
	Session 10 - Pharma and Biopharma Applications - Room 3 *Chair: Kevin Jooß, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*	
09:00-09:30	KN10	AQbD, ICH Q14 – Should I Bother and What Does This Mean for My CE Method Development? Cari E. Sänger – van de Griend, Kantisto BV, Baarn, The Netherlands
09:30-09:50	O36	Towards Immunoglobulomics - IgG, IgA and IgM Fc Profiling by Light Chain Affinity Capturing by Nano-LC-MS Christoph Gstöttner, Leiden University Medical Center, Leiden, The Netherlands
09:50-10:10	O37	Ultra-Miniaturized Weak Affinity Chromatography Coupled with Mass Spectrometry (nano-WAC-MS) as a Powerful Screening Strategy of Native Membrane Proteins in Fragment Based Drug Discovery: Adenosine Receptor as a Case-Study François-Xavier Vidal, Claude Bernard University Lyon 1, Lyon, France
10:10-10:30	O38	Unravelling Functional Changes in Antibody Proteoforms using Affinity CE-MS Elena Domínguez-Vega, Leiden University Medical Center, Leiden, The Netherlands
10:30-11:00	Coffee Break	
	Session 11 - Advances in Microscale CE and LC Separations II - Room 2 *Chair: Victor U. Weiss, Vienna University of Technology, Vienna, Austria*	
11:00-11:30	KN11	Biopharmaceuticals by Capillary Electrophoresis: Mass Spectrometry, Affinity, Isoelectric Focusing, Process Analysis Hermann Wätzig, University of Braunschweig, Braunschweig, Germany
11:30-11:50	O39	Behavior of Weak Electrolytes in the Diffuse Layer of the Double Layer Bohuslav Gas, Charles University, Prague, Czech Republic
11:50-12:10	O40	Refractive Index Detector Based on a Young Interferometer for Electroseparation Methods Ruchi Gupta, University of Birmingham, Birmingham, United Kingdom

12:10–12:30	O41	Characterization of Nanoparticles in Mixtures by Capillary Electrophoresis and Taylor Dispersion Analysis Hyphenated to ICP-MS Jan Petr, Palacky University Olomouc, Olomouc, Czech Republic
	Session 12 – Point-of-Care Devices – Room 3 *Chair: Elena Dominguez Vega, Leiden University Medical Center, Leiden, The Netherlands*	
11:00–11:30	KN12	Exhaled Breath, Saliva, Sweat: New, Emerging, Alternative Samples in Non-Invasive Medical Diagnostics Petr Kubáň, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic
11:30–11:50	O42	Fully Autonomous Processing and Analysis of Dried Blood Spots Collected by Volumetric Absorptive Microsampling Pavel Kubáň, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic
11:50–12:10	O43	Electrochemical Immunoassay-based Sensors Towards Point-of-Care Diagnostics: Recent Progress and Challenges in Multiple Biomarkers Detection Lucie Korecká, University of Pardubice, Pardubice, Czech Republic
12:10–12:30	O44	Microfluidic Capillary Electrophoresis for In-line Dual-stage Enrichment and Unattended Sampling: From Instrumental Conception to Bioanalytical Applications Thanh Duc Mai, University Paris Saclay, Orsay, France
12:30–14:00	Lunch – Hotel Restaurant	
	Plenary Lectures 2 – Room 1 *Chair: Frantisek Foret, Institute of Analytical Chemistry of the CAS, Brno, Czech Republic*	
14:00–14:10	Jaroslav Janák Medal & Award	
14:10–14:50	PL4	Comprehensive N-Glycan Profiling in Cancer Research: Past, Present and Future Milos V. Novotny, Indiana University, Bloomington, IN, USA
14:50–15:30	PL5	Analysis of Proteins and Peptides by Native and SDS Capillary Agarose Gel Electrophoresis Online Coupled to Electrospray Ionization Mass Spectrometry Andras Guttman, University of Debrecen, Debrecen, Hungary
15:30–17:00	Young Scientists and Best Poster Awards	
	Announcement of MSB 2025	
	Closing Remarks, Farewell Drink	

POSTERS

Poster Instructions

- Posters should be up Monday-Wednesday during the entire symposium.
- Posters should preferably be mounted on Sunday afternoon May 19, but not later than Monday morning May 20 before 9:00.
- Poster presentations are assigned a number that will also be attached to the poster board; authors should mount their posters only on their assigned boards.
- The preferred poster size is A0 (portrait). Nevertheless, the maximum poster size is 100 x 120 cm vertical (portrait) orientation. The poster does not necessarily have to fill the entire board area.
- Poster mounting material will be available.
- Two poster sessions are scheduled: Monday, May 20, 14:30-15:30, and Tuesday, May 21, 14:00-15:00.
- Presenters of a poster with an even number should be at their poster during the poster session on Monday; presenters of posters with an odd number should be at their poster during the poster session on Tuesday.
- Poster should be taken down on Wednesday, May 22 between 13:30 and 14:00.
- Any posters left after the symposium closing session will be removed by the organizers and recycled.

List of Poster Presentations

- in the alphabetical order of the presenting author

P1 Noncovalent Labeling of Proteins in Sodium Dodecyl Sulfate Capillary Gel Electrophoresis
Felicia Auer, Andras Guttman

P2 Electrophoretic Separation of DNA Fragments in Deuterated Water
Jan Badin, Ivona Voráčová, Petr Táborský, Marcus Gassmann, František Foret

P3 Deterministic Lateral Displacement for Separation in Microscale: Particle and Microbial Cell Analysis
Violina B. Barbosa, Laura Cerqueira, João M. Miranda, Nuno F. Azevedo

P4 Identification of Mephedrone Synthesis Reagents using CEMs
Iwona Biel, Katarzyna Czyżowska, Paulina Kraus, Paweł Mateusz Nowak, Michał Woźniakiewicz

P5 Analysis of N-linked Glycans by CE/LIF Using Various Glycoproteomic Protocols
Janette Bobalova, Denisa Smolkova, Dana Strouhalova, Richard Cmelik, Jana Lavicka

P6 Tools That Improves Concentration Sensitivity in Capillary Electrophoresis-Frontal Analysis for Affinity Studies
Taťána Bržezická, Lenka Kohútová, Hana Mlčochová, Tereza Zapletalová, Zdeněk Glatz

P7 Separation Conditions for Oligonucleotides by CE-MS
Maria Butnariu, Dušan Koval

P8 Microfluidic Capillary Electrophoresis – Mass Spectrometry for Rapid Charge-Variant and Glycoform Assessment of Monoclonal Antibody Biosimilar Candidates
Ruben Cageling, Sara Carillo, Anja Boumeester, Karin Lubbers-Geuijen, Jonathan Bones, Kevin Jooß, Govert W. Somsen

P9 Unlocking New Perspectives: Fluorinated Sugars and Their Enhanced Lectin Binding Abilities
Jakub Červený, Martin Kurfiřt, Pavla Bojarová, Jindřich Karban

P10 Study of Selected Analytes in in Vitro Fertilization Culture Medium by Capillary Electrophoresis
Petra Crhonková, Taťána Bržezická, Lenka Kohútová, Zdeněk Glatz

P11 Multi-Material 3D-Printing Fabrication of Microfluidic Devices
Reverson F. Quero, Fernando Henrique M. Costa, Mathias Stahl Kavai, Dosil P. de Jesus, José A. F. da Silva

P12 Determination of Unbound Fraction of Selected Antiepileptic Drug Using Ultrafiltration and LC-MS method
Viktoria Ďurčová, Marta Pelcová, Zdeněk Glatz, Jan Juřica

P13 Sorbentless Dried Blood Spot Sampling for Automated DBS Analysis
Miloš Dvořák, Sylvie Profousová, Pavel Kubáň

P14 Investigation of the Effect of Maternal Obesity and Gestational Diabetes on the N-Glycosylation of Human Immunoglobulins
Anna Farkas, András Guttman, Oksana Matsyura, Lesya Besh, Sándor G. Vári

P15 Decoding the Human Seminal Plasma Metabolome: Assessment of the Performance of Different Sample Preparation Strategies
Luz Alonso-Dasques, María Morán-Garrido, Laura Mayo-Martínez, Érica A Souza-Silva, Ameer Y. Taha, Coral Barbas, Víctor González-Ruiz

P16 Investigating Collagen-Protein Interactions Using Affinity Capillary Electrophoresis: Method Development and Use of Correction Factors
Sophie Hartung, Christin Scheller, Hermann Wätzig

P17 Automated Sample Preparation of Human Tissue Specimens to Search for N-Glycan-based Biomarkers
Eniko Gebri, Kinga Hogyor, Adrienne Szabo, Gabor Jarvas, Zuzana Demianova, Andras Guttman

P18 Liquid Biopsy Testing – Isolation Method for Targeted Nucleic Acid Biomarkers
Helena Hrušková, Roman Řemínek, František Foret

P19 New Tools for Peptide Retention Time Predictions in Proteomics
Kateřina Hrůzová, Martina Nechvátalová, Jan Valášek, Jiří Urban

P20 Combining Three-Dimensional Printed Miniaturized Microextraction Device and Elemental Extractant for Detection of Toxic Metal Ions
Shivangi Singh, Emmanuvel Arputharaj, Yu-Hui Huang, You-Rong Wu, Yeou-Lih Huang

P21 Microfluidic Automation of Library Preparation for Nanopore Sequencing
Jacob F. Hess, Julian Rüdiger, Tobias Hutzenlaub

P22 Microfluidic Automation of Sample Preparation Techniques for Proteomics
Jan-Niklas Klatt, Michelle Hinrichs, Tobias Hutzenlaub

P23 Online Coupling of Size-Exclusion Protein Separation with Monolithic Enzymatic Reactor
Anna Kosmáková, Aryna Paulenka, Jiří Urban

P24 The Evaluation of Galectin-1 – Glycopeptide Interactions by Affinity Monolith Chromatography
Maria Butnariu, Dušan Koval

P25 Microfluidic Chip for Cell Lysis: Towards Single-Cell Immunochemistry in Microdroplets
Jana Krivankova, Julie Weisova, Antonin Hlavacek

P26 How Can Electrochemistry Help with Drug Testing?
Anna Kubíčková, Lucie Pražáková, Jan Fischer

P27 Humic Acid Modified Paper as an Affordable Cation Exchanger Sorbent to Isolate Basic Drugs from Saliva Samples
Carlos Calero-Cañuelo, Rafael Lucena, Soledad Cárdenas

P28 Sample Preparation for Proteomic Analysis - Greenness Evaluation
Katarína Maráková, Radovan Tomašovský, Marina Opetová, Kevin A. Schug

P29 A Simplified Protocol for Intact Exosome Separation using Low-Pressure Size-Exclusion Chromatography
Ondrej Moravek, Zuzana Vankova, Malena Mandzi, Robert Jirasko, Zuzana Kozovska, Rudolf Kupcik, Zuzana Bilkova, Michal Holcapek

P30 From Model to Practice: Developing an Enrichment and Recovery System to Facilitate Rapid Pathogen Detection
Patrick Raphael Muschak, Zehua Liu, Sonja Berensmeier, Sebastian Patrick Schwaminger

P31 Acoustophoretic Focusing of Microparticles in Glass Microfluidic Device
Jakub Novotny, Lucie Brezinova, Anna Tycova

P32

P33 Glycoform Equivalence Assessment of Biotherapeutics with N-and O-Glycosylation Sites by Sequential Intact Mass Spectrometry
Myung Jin Oh, Hyun Joo An

P34 First Data on Alpelisib Concentrations in Plasma Determined by HPLC-FLD Method
Eva Krejčířová, Marta Pelcová, Zdeněk Glatz, Jan Juřica

P35 Therapeutic Drug Monitoring of Colistin Supported by Lipidomics in Critically Ill Patients

Juraj Piestansky, Ivana Gerhardtova, Ivana Cizmarova, Marian Koval, Andrej Kovac

P36 Novel Approaches using Fluorescence Spectroscopy for Smoke Taint Determination
Erin Kalbaugh, Vincent T. Remcho

P37 Novel Microsampling Approach Based on Solid-Phase Microextraction for Monitoring the Level of Tryptophan and Its Metabolites in Human Serum and Urine Samples
Anna Roszkowska, Ilona Olędzka, Piotr Kowalski, Natalia Miękus-Purwin, Kamila Langowska, Tomasz Bączek

P38 Determination of Organic Acids in Infants Faeces Using a CE-C4D In-house Built Instrument
Marcelina Rusin, Joanna Pluta, Aneta Woźniakiewicz, Justyna Dobrowolska-Iwanek, Michał Woźniakiewicz

P39 Application of the DLLME/GC-MS Method of the Identification of Aromatic Amines Derived from Azo Dyes for Forensic Analysis of Fibers
Anna Sałdan, Michał Woźniakiewicz, Paweł Kościelniak

P40 Identification of Aspergillus Species using CE in Capillary with Roughened Part and MALDI TOF MS
Jiří Šalplachta, Anna Kubesová, Pavel Karásek, Filip Růžička, Michal Roth

P41 Enzyme Kinetic Studies in Droplet Microfluidic Device with Fluorescence Detection
Michal Sedlák, Lukáš Jordán, Marta Pelcová, Zdeněk Glatz

P42 Rhodamine B-based Labeling for Oligosaccharide and Glycan Analysis by CE/LIF
Jozef Sestak, Filip Dusa, Denisa Smolkova, Richard Cmelik, Andras Guttman, Jana Lavicka

P43 Synthesis of Bifunctional Non-Covalent Molecularly Imprinted Polymers (MIPs) for Selective Extraction of Catecholamines and their Metabolites
Antons Podjava, Artūrs Šilaks, Laura Bernāte, Jorens Kviesis, Valda Valkovska

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ABSTRACTS

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KN – keynote presentations

O – oral presentations

P – posters

PL1

Microdroplet Arrays: The Next Generation of Multi-Well Plates for High Throughput Analysis

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Summary

Droplet microfluidics has emerged as a powerful method for high throughput applications, e.g. for screening reaction conditions, synthesizing particles, or single-cell analysis and other bioanalytical applications. We employ droplet-based methods on an open platform, which is conceptionally similar to a multi-well plate, however, has a massively increased density of wells and hence, throughput. Thousands of aqueous nL-droplets deposited on a custom-made glass slide that have a defined hydrophilic-hydrophobic surface pattern. The droplets are covered by fluorinated oil and remain stable for several days. Compounds and fluids can be added by further spotting runs at any time. Moreover, our automated deposition system allows for the creation of chemical gradients along the surface and therefore, fine-tuned concentration-depending screening applications. In addition, we can employ optical microscopy for droplet assessment as well as MALDI-MS imaging for analysis of droplet composition. In this presentation, the use and versatility of the method for various applications will be discussed. After validation of the platform, we first confirmed the use of the platform for protein analysis and determination of posttranslational modifications. Next, we adapted the platform for cell analysis. For example, the biosynthesis of an enzyme could be monitored by both fluorogenic assay as well as label-free by mass spectrometry. Further recent advancements of the platform include the analysis of supernatant by droplet splitting, the production of chemical gradients and the use of hydrogels for embedding cells. In addition, a conceptionally similar approach for analysis of cell-secreted compounds will be presented.

PL2

Applications of DNA Capillary Electrophoresis in Molecular Cancer Diagnostics: Above and Beyond Sanger Sequencing

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Summary

In the late 1990s the Sanger sequencing of the tumor DNA became a dominant application for capillary electrophoresis, especially after the importance of acquired somatic DNA mutations for cancer initiation and progression was revealed by the work of Bert Vogelstein's group at Johns Hopkins University [1]. However, a decay from the tremendous expansion of the technology started when other non-CE sequencing approaches (termed next-generation sequencing (NGS) or massive-parallel sequencing (MPS)) arrived in the late 2000s [2].

Nowadays, despite to the slow retreat from the DNA sequencing arena, DNA separation by CE still has significant utility in molecular cancer diagnosis. The dominating applications are based on PCR fragment analysis, where capillary CE as well as Chip-CE platforms are widely used. Perhaps the most routine application (sometimes accepted as a golden standard) is the CE detection of microsatellite instability (MSI) with the purpose of (i) revealing hereditary cancer predisposition caused by inherited infidelity of the DNA repair system (Lynch syndrome) and (ii) assessing the tumor mutator phenotype (tumor mutation burden), a major biomarker for prediction of immunotherapy response [3]. In addition to MSI, there is a family of CE-based DNA mutation detection techniques. Using precise temperature settings during the run, they rely on subtle differences in electromigration properties to resolve wildtype (non-mutated) and tumor (mutated) DNA fragments [4].

The last area of CE usage in cancer diagnostics is related to modern sequencing. From the early days, most NGS technologies relied on DNA library preparation workflows that use CE for profiling of the library before putting it onto the sequencer. Although not a separation per se, CE-based NGS library quality control is essential for obtaining quality data and limiting wasteful sequencing runs.

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PL3

Capillary Electrophoresis and Taylor Dispersion Analysis: Recent Advances and Present Challenges in Health Applications

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Summary

In this presentation, the complementarity of Capillary Electrophoresis (CE) and Taylor dispersion analysis (TDA) will be exemplified for the characterization of biopharmaceutical samples, including mRNA loaded lipid nanoparticles (LNP) and vaccine formulations [1-3]. CE and TDA can be implemented on the same equipment, and share similar advantages (low injected volumes, automation of the analysis, and the absence of sample filtration). Combining CE and TDA allows determining both the charge and the size of the analytes, which are generally considered as critical quality attribute in the pharmaceutical industry. Taylor dispersion analysis (TDA) is a promising technique for the determination of diffusion coefficients and hydrodynamic radii of a myriad of nanoscale objects, including ultra-small nanoparticles (below 5 nm). The principle of this method is based on the band broadening of a solute plug injected in a miniaturized Poiseuille flow (50 µm i.d. capillaries). It allows determining the hydrodynamic radius of virtually any mixture of solutes, on a range of size ranking between 0.1 and 300 nm. TDA is insensitive to the presence of dusts (contrary to scattering techniques), and leads to a fair size distribution of the sample generally based on the weight-average of the constituents. With straightforward implementation, the absence of calibration, no filtration of the sample, TDA is a method of choice for the size-characterization of solutes in health applications.

Through various examples of applications, the advantages and limits of TDA and CE will be presented. For both techniques, one of the main limitations comes from solute adsorption. In the case of CE, we recently deciphered and quantified the impacts of electroosmotic heterogeneity and solute adsorption on peak broadening for intact protein separations in polyelectrolyte multilayer (SMIL) coated capillaries [4]. This could be realized by the systematic experimental determination of the curve representing the plate height H versus the solute velocity u. In optimized conditions, very efficient and repeatable protein separations could be obtained using SMIL coatings. In the case of TDA, the impact of solute adsorption can be greatly limited experimentally by using a “plug-in-front” methodology.

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PL4

Comprehensive N-Glycan Profiling in Cancer Research: Past, Present and Future

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Summary

The intimate knowledge of aberrant glycosylation associated with human cancers was sparse until the first years of this century, when new analytical methodologies based on capillary LC, CE and the electrospray and MALDI mass spectrometry started to contribute to glycomic and glycoproteomic capabilities. The N-glycans immediately became the preferred target of glycomic studies because they were methodologically easier, but most importantly, their structural diversity and some known biosynthetic pathways have made N-glycans the perceived "zip codes" of the respective glycoproteins and their cellular environments. The first glycomic studies reported in the literature by different groups were often mutually inconsistent for either methodological reasons or differences in sample source and treatment. The methodologies matured within the next decade and more meaningful sets of clinical samples became available. Microderivatization and sample preconcentration techniques have been emphasized by our research group as the adjuncts to mass spectrometry and CE-LIF detection as demonstrated in examples of the studies pertaining to prostate cancer, pancreatic cancer, different types of breast cancer, and the profiles obtained from sera and tissues of ovarian cancer patients. As the result of gradual methodological improvements, some 60-90 N-glycans can be routinely monitored in different sample types and then statistically correlated with cancer conditions. However, with the more comprehensive sample isolation and simple group separation approaches, over 220 N-glycans can be identified, including the structural details associated with the isomerism of fucosyl and sialyl residues.

Some seemingly important glycans have been structurally elucidated within these profiles: paucimannosidic glycans; hyperfucosylated structures; multiply-branded glycans with three different sialyl linkages; and glycans with sulfation. The importance of the sample repositories, clinical information and medical collaborations cannot be overstated. To advance further the potential of glycomics for the sake of early diagnosis and prognostic evaluations will necessitate simplified measurements and enhanced sample throughput.

PL5

Analysis of Proteins and Peptides by Native and SDS Capillary Agarose Gel Electrophoresis Online Coupled to Electrospray Ionization Mass Spectrometry

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Summary

A novel coaxial sheath flow reactor interface (CSFRI) is introduced for CE-ESI-MS coupling, especially beneficial in CGE of protein and peptides, both in native- and SDS-gel electrophoresis separation modes. The major benefit of using the CSFRI approach is the continuous closed circuit-based transport of the separated analytes from the coaxial setting via the flow reactor tube to the ESI source, robustly stabilizing the electrospray process [1]. This arrangement also offers the option to conduct post-column reactions in the flow reactor section, e.g., to capture non-MS-friendly background electrolyte components, such as sodium dodecyl sulfate in SDS-CGE. In addition, this novel interface design allows safe and efficient decoupling of the electric circuit from the mass spectrometer, i.e., no current flow from the CE into the MS and vice versa, enabling stable electrospray formation independent of the capillary electrophoresis part of the system. Most importantly, the CSFRI connection does not require any microfabrication and specially modified (i.e., etched, sharpened, etc.) capillaries; a conventional blunt edge, rugged fused silica capillary with 30-50 µm i.d. and 365 µm o.d. can be simply and safely attached and detached to and from any commercial ESI sources originally developed and optimized for the actual mass spectrometer used with no modification requirements. In SDS capillary agarose gel electrophoresis mode, addition of γ-cyclodextrin to the sheath liquid efficiently removed the SDS content of the sample and the background electrolyte in the flow reactor section by inclusion complexation, while maintaining good separation efficiency and decreasing ion suppression. Optimization of the agarose based sieving matrix will be presented in detail [2] along with examples of the analysis of peptides and proteins in SDS-CGE-MS mode using the coaxial sheath flow reactor interface.

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KN1

Novel Electrokinetic Approaches for Particle Separation Applied to Organelles and Microplastics

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Summary

Electrokinetic phenomena are essential elements in separation sciences, yet novel approaches are required for addressing cutting-edge analytical challenges. Among the traditional combination of electrophoretic and electroosmotic phenomena employed in the microenvironment, dielectrophoresis (DEP) has been explored either as a unique particle migration tool or in combination with other electrokinetic techniques. DEP can be induced in microfluidic devices by designing constrictions and arrays of such leading to inhomogeneous electric fields via the application of potential differences along a microchannel. We have designed microfluidic devices that allow DEP-induced separation of organelles via a unique periodic driving mechanism. We have particularly focused on mitochondria and the underlying optimization of their separation in small and large size fractions which are actively steered into opposing directions. Our latest work focused on the optimization of this ratchet-migration mechanism in the continuous separation mode with numerical methods, which are in excellent agreement with experimental observations.

Furthermore, we scout the capabilities of dielectrophoretic separation of micro- and nanoplastics (MNPs), for which implications in human health and disease are alarming. While evidence has been found that MNPs accumulate in organs, we postulate that they circulate in the blood stream in sub-µm dimensions. However, techniques to analyze and enumerate their abundance and composition in this size range are lacking. With DEP-based approaches we have started to characterize micro- and nanoplastics as they interact with blood constituents. To allow these studies, we developed protocols to generate realistic micro- and nanoplastic particles by cryo-grinding and studied their dielectrophoretic characteristics in alternating current electric fields. These studies will inform the design of microfluidic separation devices exploiting dielectrophoresis to characterize MNPs in human body fluids.

KN2

Comprehensive Characterization of Mammalian Brain N-Glycome: Isomer-Sensitive Nano-LC-MS/MS Analysis and its Application to Alzheimer's Disease Models

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Summary

In the mammalian brain, over 70% of the proteome is known to be glycosylated, and a large fraction of this glycosylation is N-glycome. N-glycosylation plays an important role in neurobiology, including in the development of the nervous system and the regulation of neurotransmitter receptors. Particularly, aberrant glycosylation has garnered substantial interest due to its association with many brain diseases and disorders, including Alzheimer's disease. To understand the diverse functions of N-glycome at the molecular level, a comprehensive characterization is highly required. However, the heterogeneity of glycans in the biosynthetic process leads to the production of various isomers and hundreds of N-glycans, making it difficult to obtain in-depth structural insights due to this structural complexity. To overcome these challenges, we developed an analytical method using porous graphitized carbon nano-LC-MS/MS, an isomer-sensitive and reproducible analytical platform. The unique isomer selectivity of porous graphitic carbon underscores the importance of analyzing isomeric structures in glycomics, facilitating the clear identification of complex glycan structures that include bisected and hybrid-type glycans with novel features. Simultaneously, the use of MS/MS has defined the structure of brain-specific N-glycans including sulfated LacNAc, sialylated HexNAc, sialylated LacdiNAc, non-sulfated HNK-1, HNK-1, and phosphorylated mannose. As a next step, we applied this analytical platform to actual disease mouse model, specifically focusing on Alzheimer's disease. Subsequent statistical analyses based on our established database revealed differences in N-glycome composition across five key regions. Presently, further investigations are underway to understand the role of the specific molecular targets that have undergone changes.

Acknowledgement

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KN3

Multidimensional Assessment of Polymer Nanoparticles

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Summary

Polymer nanoparticles (NPs) are increasingly utilized for multiple purposes. For instance, polymer NPs are key constituents of advanced paints and coatings, and are used as sophisticated carriers for new therapeutic agents. NP dimensions such as size, chemical content, and charge strongly determine the overall quality and end-product properties. These characteristics need to be assessed analytically, which can be quite challenging.

We developed a comprehensive online two-dimensional liquid chromatography system that is capable of determining both NP size and encapsulated cargo using one integrated method [1]. Hydrodynamic chromatography (HDC) was used in the first dimension to separate the intact NPs and to determine the particle-size distribution. Fractions from the first dimension were taken comprehensively and the NPS were disassembled online, releasing their payload. Reversed-phase liquid chromatography (RPLC) was used as a second dimension separation to analyze the quantity, quality and stability of the cargo molecules as function of NP-size distribution.

For the determination of the surface charge of NPs, we employed capillary electrophoretic (CE) principles. Experimentally acquired electrophoretic mobilities (EMs) of NPs, were transformed into NP zeta potentials and, subsequently, into surface-charge densities (SCDs) using appropriate theoretical models. We developed a suitable CE method using well-defined polystyrene NPs as benchmarking compounds, taking the effect of NP size and size distribution on obtained EMs into account. We used CE for the assessment of SCDs of several new industrial copolymer NPs varying in acidic monomer content. CE results revealed that the CSD of some NPs was not only determined by chemical composition, but also by the (previously unknown) presence of adsorptive species such as surfactants.

Acknowledgement

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KN4

Imaging of Individual Nanoparticles by Mass Spectrometry

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Summary

The detection of single microscopic entities has always been a challenging subject. Laser-assisted mass spectrometry methods can offer chemical information as well as imaging capabilities. Here, we demonstrate the mass spectrometry imaging of individual metal nanoparticles using two ionization techniques: laser ablation inductively coupled plasma (LA ICP) [1] and subatmospheric pressure laser desorption/ionization (LDI) [2,3]. The principles allowing the detection of single nanoparticles are revealed, and the detection efficiency is discussed. The potential applications of the methods are demonstrated in detecting nanoparticles on biological tissues and imaging viable cells on 3D aggregates of human colorectal carcinoma cells.

Acknowledgement

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KN5

When Microfluidics Meets Magnetic Nanomaterials: Recent Approaches in Bioanalysis

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Summary

In bioanalysis, the advantages of microfluidics and superparamagnetic materials have been demonstrated many times in practice. It is therefore not surprising that attempts have been made to combine these two systems and multiply these advantages. In a short time, the magnetic particles have been adopted as the intelligent carriers in microfluid devices. The versatility of magnetic particles allows them to be easily integrated into the microchannels or microchambers of microfluidic devices. Appropriately adjusted magnetic field enables static or dynamic self-assembly of magnetic particles. Their parameters are the basis for the magnetic field modeling, so the equilibrium or motion of the particles, their torque, orientation, and angular velocity can be easily controlled. Thanks to these flexible properties, we have several options how to manipulate with magnetic particles. It is worth mentioning at least magnetic fluidized bed or magnetic bead chains, magnetic droplets and magnetophoresis. As a result, the highly efficient systems finding wide application have been achieved. Most of such microfluidic devices were developed for searching of clinically important targets, for specific capturing and isolation of cells or biomolecules, for controlled catalysis, and finally for the synthesis, control and sensing of magnetic nanoparticles. One of the most interesting magnetically active nanomaterials are TiO_2 nanotubes decorated by Fe_3O_4 nanoparticles (TiO_2NTs@Fe_3O_4NPs) [1] whose unique properties and versatile applicability will be commented [2,3].

Acknowledgement

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KN6

The Study of Sputome: A Need for Sputum as a Rich Source of Protein Biomarkers for the Non-Invasive Diagnosis of Infectious and Chronic Diseases

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Summary

Many ailments can be diagnosed while they are asymptomatic, meaning that the patient has no signs or symptoms of a progressing disease. If caught early in their formation, these maladies can be effectively treated, leading to successful chances for curative therapies as it halts the disease from advancing, therefore improving the quality of life, and long-term survival for the patient. However, further improvements in cutting edge precision technologies for early disease detection that are also simple to use, rapid, and affordable are not only necessary, but vital for well-being of people and the future of global public health.

The hallmark of non-invasive approaches has been liquid biopsies based on genomic biomarkers. As such, biological fluids permit any measurable molecular indicator or signature to provide significant information on wellness and disease. Among the bodily secretions used for non-invasive diagnostics is sputum, a complex viscous hydrogel meshwork, that has gained growing recognition as a rich biological source of biomarkers of airway infections, pulmonary diseases, and serves as a determinant to reveal other illnesses.

Respiratory tract diseases are a major problem, and on the rise, due to climate change affecting the health of many individuals around the world, in addition to putting stress on healthcare facilities and services. I therefore highlight the need to use expectorated or induced sputum specimens as a routine sample of valuable protein biomarkers for the diagnosis of these chronic maladies, to predict inflammation and disease progression, as well as to monitor the effectiveness of treatments. I also discuss the need for fast and reliable point-of-care methods for the detection and quantification of crucial protein biomarkers in sputum samples, and some of the limitations and challenges faced when dealing with very complex matrices to identify and characterize their constituents.

KN7

Native N-Glycome of Single Mammalian Cells and ng-Level Blood Isolates Deciphered Using Label-Free Capillary Electrophoresis-Mass Spectrometry

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Summary

Aberrant glycosylation represents an attractive source of potential biomarkers for the diagnostic, prognostic, and treatment monitoring of various human diseases, including autoimmune, genetic, oncological, and neurodegenerative pathologies. Contrary to the rapidly growing field of single-cell proteomics, single-cell glycomics (SCG) demonstrated lagging progress. Deciphering cell-to-cell glycome variations and developing glycomic techniques for sub-microliter volumes of minimally invasive liquid biopsies (e.g., blood, circulating rare cells, extracellular microvesicle isolates) can be crucial in designing advanced diagnostic and therapeutic approaches. In this work, we developed an integrated platform coupling online in-capillary sample processing with high-sensitivity label-free capillary electrophoresis-mass spectrometry (CE-MS) for N-glycan profiling of single mammalian cells and ng-level amounts of blood-derived protein, extracellular vesicle, and total plasma isolates. Native N-glycans were enzymatically released from the cell surface prior to their inline CE-MS analysis in the described proof-of-concept experiments. Direct and unbiased characterization and quantification of single-cell surface N-glycomes were demonstrated for HeLa and U87 cells, with the detection of up to 100 N-glycans per single cell. Specific N-glycosylation patterns were demonstrated for HeLa and U87 single cells, based on a thorough differential analysis of qualitative and quantitative SCG profiles. Significant differences in fractional distributions and abundances of the N-glycans detected in HeLa and U87 single cells were observed, reflecting unique molecular features for each cell type. Interestingly, N-glycome alterations were observed at the single cell level when HeLa and U87 cells were stimulated with lipopolysaccharide (LPS), which manifested the change in the phenotypic cell state reflected on the cell surface. Overall, we demonstrated in the presented here proof-of-concept study that our developed SCG workflow could effectively and accurately characterize the single-cell N-glycome of different mammalian cell lines and detect N-glycome alterations at the single-cell level.

The developed workflow was also applied to the profiling of ng-level amounts of blood-derived isolates of protein (e.g., total IgG, total IgM), extracellular vesicles, and total plasma, resulting in over 170, 220, and 370 quantitated N-glycans, respectively. These numbers of identified N-glycans corresponding to sub-0.5 ng-levels of serum proteins and nL/pL-levels of plasma isolates, largely exceed the profiling results reported in other N-glycan profiling studies of similar complexity blood-derived isolates.

KN8

Bioanalytical Approaches for Monitoring Cellular Communication

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Summary

Islets of Langerhans are the endocrine portion of the pancreas and are composed of several cell types that release peptide hormones into the bloodstream for regulating glucose levels. Proper control of blood glucose is dependent on the amounts and dynamics of hormones released from these cells. Defects in the secretion of these hormones are associated with a number of metabolic diseases, including diabetes and the metabolic syndrome. Because the dynamic profiles of hormone secretion are essential to proper glucose control, examining secretion from single or small groups of islets are essential, necessitating analytical tools with high sensitivity.

In this talk, a number of analytical strategies our group has developed which enable monitoring secretion of hormones and small molecules released from islets with high time resolution will be discussed. Microfluidic systems are an ideal platform to interrogate islets as they reduce dilution of the secreted components and can be used to deliver complex glucose profiles like those observed in vivo. We have developed a number of analytical approaches that use microfluidic systems to measure hormone and small molecule secretions from single or small groups of islets of Langerhans. Initially, microfluidic electrophoretic immunoassays were used to measure insulin secretion from single islets. While highly sensitive, they were difficult to use due to the shallow channels required to limit Joule heating. As such, a homogeneous fluorescence anisotropy competitive immunoassay for insulin was developed which allowed for larger channels to be used [1]. To increase the throughput of the assay, a fluorescence anisotropy imaging system was employed for measurement of insulin secretion from 12 groups of islets in parallel with minimal fluidic inputs [2]. To automate the system further microfluidic valves and other fluidic elements were implemented [3]. Finally, antibody-free assays using LC- and SPE-MS/MS providing multi-analyte monitoring of hormone secretion will be described [4]. These systems offer the potential for combining the benefits of microfluidics with high information content detection.

Acknowledgement

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KN9

Glycosylation as a Tool for Biomarker Discovery in Cancer using Exosomes

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Summary

Analysis of glycans (complex carbohydrates attached to protein or lipids) is a hot scientific discipline, especially for disease diagnostics including diagnostics of various types of cancer. The main reason behind that statement is the fact that 70% of all intracellular proteins and 80% of membrane proteins are glycosylated. Thus, glycoproteins are new types of biomolecules that can be used as disease biomarkers.

The lecture will provide an introduction showing why glycans and exosomes are so promising as cancer biomarkers. For example, glycans are information-rich molecules involved in many physiological and pathological processes. Part of the lecture will be devoted to showing how new biomarkers are validated and what are the hot trends in the diagnosis of various types of cancer.

The final part of the lecture will be devoted to describing what exosomes are, and how they can be isolated and used in cancer diagnostics with a focus on glycan analysis.

Acknowledgement

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KN10

AQbD, ICH Q14 – Should I Bother and What Does This Mean for My CE Method Development?

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Summary

In this presentation, a short, practical, and pragmatic overview is given on the impact of the new ICHQ14 guideline and Analytical Quality by Design (AQbD) on method development and more specifically on CE methods. The high-level guidelines are translated into a practical method development process flow, that can be applied on new methods as well as on adapting existing methods or platform methods. The discussion will focus on the need or not for AQbD outside the pharmaceutical industry and what or what not it can contribute to analytical science, as well as demonstrate that AQbD is a lot more than Design of Experiments (DoE).

KN11

Biopharmaceuticals by Capillary Electrophoresis: Mass Spectrometry, Affinity, Isoelectric Focusing, Process Analysis

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Summary

In order to contribute to the scientific research on the SARS-CoV-2, we developed two CIEF imaging methods to characterize the quality and stability of messenger ribonucleic acid (mRNA) vaccines, particularly mRNA encapsulated in lipid nanoparticles (LNPs). A variety of stressed and lipid composition altered samples were measured. Results were supported by data from an encapsulation assay and particle sizing. A method using 9 M urea as an additive showed two broad and jagged peaks, with the peak shape providing detailed information. The summed peak area of both peaks showed RSDs ranging from 2 to 8% when measured in triplicate, and appears to depend on the size of the LNPs. In the second method, a combination of 5.5 M urea and 2 M N-ethylurea was used. This method is characterized by high reproducibility of the pI value (< 0.5%). The reproducible peak area (RSD of 2-7%) correlates linearly with the mRNA content. This is also true for the first method. Stress is evident from the change in pI and peak area. In addition, experiments were performed with the addition of a fluorescent dye, which greatly increased the sensitivity of the methods. Both methods can be used to characterize LNP stability, e.g., in studying different storage times at different temperatures and freeze-thaw cycles, as well as the ability of the methods to distinguish lipid compositions and measure batch-to-batch variability [1].

Collagen is a very important and highly abundant structural protein but hard to analyse due to its insolubility. In order to investigate collagen in a liquid environment and to maintain its biological function, we milled and suspended collagen in a phosphate buffer pH 7.4, 12.5 mM using a dual zentrifuge (ZentriMix 380R) to obtain particles with a size below 5 µm. Using these small collagen particles, affinity CE (ACE) was performed to study binding properties of Human Serum Albumin, Human Fibronectin and Collagenase Type I from Clostridium histolyticum.

Antibody self-interaction including aggregation has been correlated to hydrophobic patches as well as the electrostatic potential distribution on a protein surface. These approaches rely on 3-D structures which may not always be available but can be predicted with an increasing precision from the sequence.

A capillary zone electrophoresis (CZE) method was developed for the monitoring of the mAb concentration during cell culture processes. CZE method development rules are outlined, particularly discussing various capillary coatings, such as a neutral covalent polyvinyl alcohol (PVA) coating, a dynamic successive multiple ionic-

polymer (SMIL) coating, and dynamic coatings using BGE additives such as triethanolamine (T-EthA) and triethylamine (TEA). The dynamic T-EthA coating resulted in most stable electro-osmotic flows (EOFs) and most efficient peak shapes [2]. A general update on method development and validation in CE can be found in [3].

CE-MS has been around for a long time and has recently evolved into a mature technique [4]. We demonstrate excellent structural information with the MauriceFlex system for the Waters mAb, the NIST mAb and matuzumab. This new system did not show a single failure for technical reasons during two months of use.

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KN12

Exhaled Breath, Saliva, Sweat: New, Emerging, Alternative Samples in Non-Invasive Medical Diagnostics

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Summary

The analysis of biological fluids plays a vital role in clinical diagnosis. Among available options, non-invasive samples are gaining significant traction due to their ease of collection, lower costs, and improved patient comfort. Our research group has dedicated the past decade to developing methods for acquiring these samples, including exhaled breath condensate (EBC), saliva, and sweat. This lecture will explore the use of non-invasive samples in medical diagnostics. We will begin by providing an overview of various non-invasive samples, their collection techniques, and their relevance in clinical settings.

The section on EBC will delve into the instrumentation developed in our laboratory, ranging from simple portable collectors for single exhalations to advanced devices with active cooling for collecting larger volumes of EBC. We will discuss selected applications, such as analyzing EBC for inorganic and organic molecules to diagnose pulmonary diseases, gastroesophageal reflux disease (GERD), and other potential areas.

The section on saliva will focus on its relevance in specific diagnostic cases. We will compare collection methods and showcase its use in diagnosing GERD and Barrett's esophagus. Finally, we will discuss sweat sampling and analysis for cystic fibrosis diagnosis. Recent advancements like skin-wipe and skin-wash techniques using simple cotton swabs or 3D-printed devices will also be presented.

The lecture will conclude by outlining sample preconcentration and pretreatment methods for analyzing trace amounts of various compounds in non-invasive samples. This will highlight the diverse approaches used with microcolumn separation techniques in clinical diagnostics.

Acknowledgement

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O1

On-Chip Depletion-Zone Isotachophoresis of Exosomes: A Solution to Overcome the Purity Limitations of Current Techniques

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Summary

This paper introduces a novel microfluidics-based technology capable of implementing on-chip isotachophoresis using one single electrolyte for the concentration and separation of essential sources of biomarkers, such as exosomes. Exosomes are extracellular vesicles produced by every cell that transport nucleic acids, proteins, lipids, and metabolites. They play a crucial role in cell-cell communication in a disease condition and normal metabolic processes in healthy individuals [1]. By leveraging the depletion-zone isotachophoresis (dzITP) principle [2], we have employed a method that replaces the trailing electrolyte with an ion-depleted zone, creating a barrier that anions cannot cross. Negatively charged analytes trapped between the ion-depleted zone and the leading electrolyte are, therefore, concentrated, depending on their electrophoretic mobility, in bands that occupy different positions in the microfluidic channel, thus mirroring the capabilities of classical isotachophoresis. The practical application of this technology is demonstrated through the concentration and separation of exosomes derived from cell cultures and blood plasma, implemented with silicon/glass-based microchips. Interestingly, although the exosome samples used in this study are pre-purified by density- and size-based techniques (e.g., tangential flow filtration and ultracentrifugation), the dzITP-based chip is capable of further separating extracellular vesicles from contaminants such as proteins or residues of cleaved extracellular vesicles. Thus, we demonstrate that dzITP can potentially obtain purer samples than currently routinely used techniques. The method described allows for on-chip isotachophoresis by simply preparing the solution of exosomes in a buffer that already contains the leading electrolyte. No other solution is required to operate the microchip. In this study, the buffer conditions are fine-tuned (e.g., ionic strength) to achieve the highest concentration of exosomes in the isolated band of interest. Following the assessment of the bandwidth of the exosomes, around a million-fold concentration is achieved in the device, in line with comparable devices described in the literature [3].

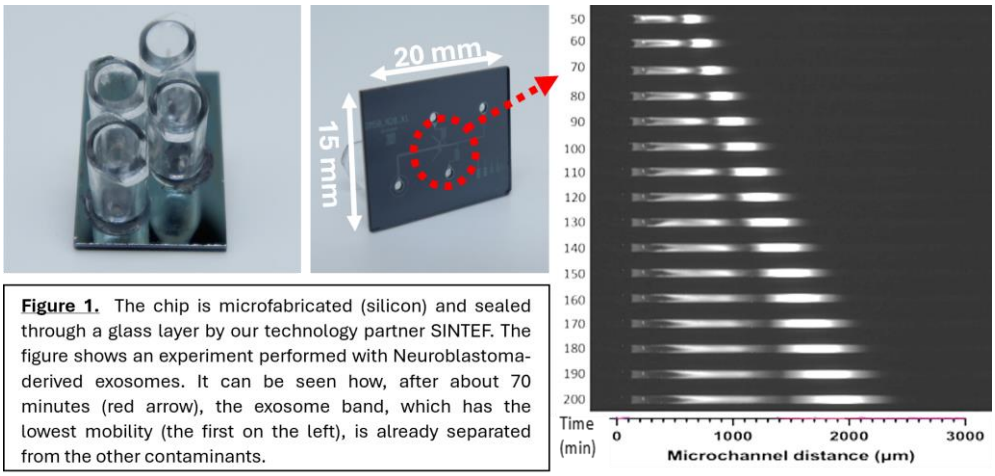

Figure 1. The chip is microfabricated (silicon) and sealed through a glass layer by our technology partner SINTEF. The figure shows an experiment performed with Neuroblastoma-derived exosomes. It can be seen how, after about 70 minutes (red arrow), the exosome band, which has the lowest mobility (the first on the left), is already separated from the other contaminants.

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O2

Frontal Analysis Continuous Capillary Electrophoresis: An Approach to Predict Plasma Proteins and Polymeric Nanoparticles Interactions

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Summary

Polymeric-based nanoparticles (PNPs) have gained attention over the past decades for their use either as nanocarriers to vehicle drugs or as therapeutic agents [1]. However, once administered into the bloodstream, a dynamic interplay occurs between their surface and plasma proteins. Consequently, studying NP-protein interactions is of great interest to predict their in vivo fate and biodistribution, especially towards abundant plasma proteins, such as human serum albumin (HSA). A few capillary electrophoresis (CE)-based methods have been reported to estimate NP-plasma protein binding parameters [2]. Among them, frontal analysis continuous capillary electrophoresis (FACCE) is a methodology of choice, providing plateau heights proportional to the free ligand concentration. Based on the size and surface charge of our PNPs, their electrophoretic mobility did not allow for efficient electrokinetic injection during the FACCE process, so measurement of HSA was rather required. Although FACCE has been used once to measure dendrimer-HSA interactions [3], this was done on highly charged dendrimers able to be electrokinetically introduced. To date, no FACCE quantitating the protein counterpart has been reported. Therefore, our work aims at evaluating the affinity between PDMAC-based NPs [4] and HSA, by selectively injecting HSA into the capillary. Since the FACCE principle relies on mobility differences between the free and the bound ligand, our PNPs were fully characterized. Considering that HSA is a protein prone to adsorb to the silica capillary, extensive optimization of the method was needed under physiological conditions, including silica modification. High intermediate precision was obtained (RSD ~ 1.5%) as well as a linear calibration ($R^2 > 0.99$) between plateau heights and HSA concentrations. Moreover, after HSA-PNPs mixture incubation, free HSA was detected with high repeatability (RSD < 2%). This fast technique allows henceforth the construction of adsorption isotherms in less than 30 min, giving access to the intrinsic binding constant and stoichiometry of the HSA-PNPs interactions.

Acknowledgement

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O3

Chip Electrophoresis of Fluorescently Labelled Virus Particles

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Summary

Capillary zone electrophoresis (CZE) on a commercially available chip electrophoretic platform has already been demonstrated in a series of papers (e.g. [1, 2]). Focusing on fluorescently labelled particles of a human rhinovirus (common cold virus), it was possible to detect native, infectious virus particles and to separate virions from a co-purified contaminant, which was likewise modified via the applied fluorophore. Subsequent measurements followed the binding of virus particles to recombinant receptor molecules and receptor-decorated liposomes [3, 4]. Furthermore, the application of molecular beacons - small oligonucleotide probes showing a closed hairpin conformation in the absence of a complementary sequence and hence spatial proximity of a fluorophore and quencher - was possible, to target the release of the viral RNA genome via an increase in fluorescence [5].

On the basis of these experiments, we turned to the analysis of virus-like particles (VLPS) based on SARS-CoV2, the source of the recent COVID-19 pandemic. VLPs resemble native virions but no longer include the genomic material of the parent virus inside their core, hence, they are no longer infectious. We were able to demonstrate that also for this bionanoparticle analyte labelling via a fluorophore was possible. Resulting particles were still recognized by antibodies binding the VLP surface despite capsid modification via the applied dye. Nanoparticle tracking analysis enabled us to assess particle loss during the labelling process and subsequent removal steps of excess dye prior to CZE analysis. In overall, we found also CZE analysis of fluorescently labeled SARS-CoV2 VLPs possible via the chosen commercially available chip electrophoretic setup. Furthermore, we believe that CZE enables to target the question of VLP stability upon storage – an important parameter e.g. for VLP based vaccine development.

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O4

SSSMuG: Same Sample Sequential Multi-Glycomics

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Summary

The mammalian glycome is structurally complex and diverse, composed of many glycan classes such as N- and O-linked glycans, glycosaminoglycans (GAGs), glycosphingolipids (GSLs) and other distinct glycan features such as polysialic acids (PolySia), sulfation and proteoglycan attachment stubs. Various methods are used to analyze these different components of the glycome, but they require pre-fractionated/partitioned samples to target each glycan class individually. To address this need for a knowledge of the relationship between the different glycan components of a biological system, we have developed a sequential release workflow for analysis of multiple conjugated glycan classes (PolySia, GAGs, GSL glycans, N-glycans O-glycans) from the same tissue lysate, termed SSSMuG – Same Sample Sequential Multi-Glycomics. With this sequential glycan release approach, five glycan classes were characterized (or four glycan classes plus proteomics) using enzymatic or chemical release from a single sample immobilized on a polyvinylidene difluoride membrane. The various released glycan classes were then analyzed by a variety of HPLC and/or MS techniques, such as C18 for DMB-labelled sialic acid quantitation, ZIC-HILIC for 2-AB labelled GAG disaccharide separation, and PGC-LC-MS for reduced GSL, N- and O-glycans. Compared to single glycan class release approaches, SSSMuG was able to identify more glycans and more proteins with higher intensity analytical peaks and provides a better comparative normalization of the different glycan classes of the complex glycome. Applying this to a brain lysate sample, we were able to obtain an in-depth glycomics analysis of the mouse brain. To this end, the SSSMuG technology workflow will be a foundation for a paradigm shift in the field, transforming glyco-analytics and facilitating the push towards multi-glycomics and systems glycobiology.

O5

Comprehensive O-Glycan Analysis by Porous Graphitized Carbon Nano-Liquid Chromatography-Mass Spectrometry

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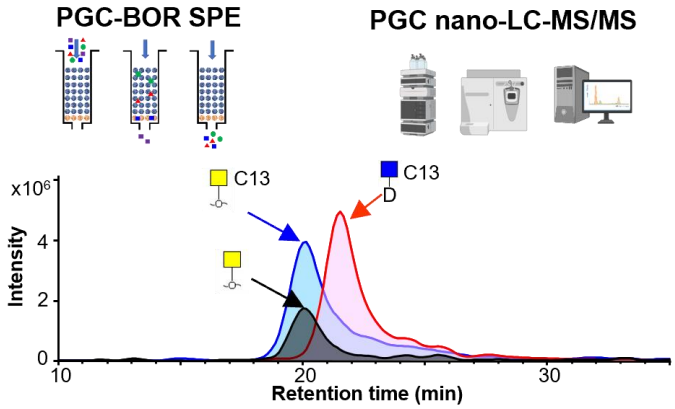
Summary

The diverse and unpredictable structures of *O*-GalNAc-type protein glycosylation present a challenge for its structural and functional characterization in a biological system. Porous graphitized carbon (PGC) liquid chromatography (LC) coupled to mass spectrometry (MS) has become one of the most powerful methods for the global analysis of glycans in complex biological samples, mainly due to the extensive chromatographic separation of (isomeric) glycan structures and the information delivered by collision induced fragmentation in negative mode MS for structural elucidation [1,2]. However, current PGC-based methodologies fail to detect the smaller glycan species consisting of one or two monosaccharides, such as the Tn (single GalNAc) antigen, which are broadly implicated in cancer biology. This limitation is caused by the loss of small saccharides during sample preparation and LC. Here, we upgraded the conventional PGC nano-LC-MS/MS-based strategy for *O*-glycan analysis, enabling the detection of truncated *O*-glycan species and improving isomer separation. This was achieved by the implementation of 2.7 µm PGC particles in both the trap- and analytical LC columns, which provided an enhanced binding capacity and isomer separation for *O*-glycans. Furthermore, a novel mixed-mode PGC-boronic acid-solid phase extraction during sample preparation was established to purify a broad range of glycans in an unbiased manner, including the previously missed mono- and disaccharides. Taken together, the optimized PGC nano-LC-MS/MS platform presents as a powerful component of the toolbox for comprehensive *O*-glycan characterization and revealing biological function of *O*-glycosylation in immune responses and cancer research.



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O6

BODIPY-based Fluorescent Labeling Tag for Oligosaccharide and N-Linked Glycan Analysis by High-Performance Liquid Chromatography with Fluorescence Detection

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Summary

Glycosylation analysis is still challenging, not only because of the extreme structure complexity and conjugation diversity of glycans but also because of instrumental aspects such as the sensitivity limits of analyses. Therefore, glycan analysis by chromatographic methods is very often combined with fluorescence detection in addition to MS. The majority of fluorescent labeling employed before LC separation is based on 2-aminobenzamide, which has several disadvantages such as low labeling yield, poor fluorescence properties, and MS ionization efficiency. Therefore, even after several decades of development of new labels, there is still a need for new labeling tags with improved characteristics.

We present the application of a newly synthesized fluorescent label designed for oligosaccharide and glycan analysis by high-performance liquid chromatography with fluorescence detection (HPLC/FLD). The novel hydrazide derivative of dipyrromethene boron difluoride (BODIPY) was synthesized from 2,4-dimethylpyrrole, methyl succinyl chloride, and boron trifluoride etherate followed by a reaction with hydrazine. The synthesized label was characterized by several analytical methods including NMR, UV/Vis and fluorescence spectroscopy, and mass spectrometry. The labeling reaction via hydrazone formation chemistry was optimized by labeling of maltooligosaccharide standards. The analysis of maltohexaose labeled by BODIPY-hydrazide followed by HPLC/FLD analysis provided the limit of detection in the low tens of femtomole. The presented method based on fluorescence detection is at least 30 times more sensitive than the standard approach employing labeling by 2-aminobenzamide. In addition, the labeling method by BODIPY-hydrazide was used for N-linked glycan profiling of several glycoproteins (ribonuclease B, immunoglobulin G) by RP-HPLC/FLD as well as HILIC/FLD analysis.

O7

Highly Sensitive Two-dimensional Profiling of N-linked Glycans by Hydrophilic Interaction Liquid Chromatography and Dual Stacking Capillary Gel Electrophoresis

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Summary

Glycosylation is one of most important post-translational modifications of proteins. Since biological samples often contain diverse glycans, highly sensitive, quantitative, and comprehensive profiling methods are required. Here, we newly developed a two dimensional (2D) separation system, which couples hydrophilic interaction liquid chromatography (HILIC) and capillary gel electrophoresis (CGE) via large-volume dual preconcentration by isotachophoresis and stacking (LDIS) [1]. Glycans labeled with 8-aminopyrene-1,3,6-trisulfonic acid were firstly separated into around 100 fractions by HILIC, which were then preconcentrated and separated by LDIS-CGE, and finally detected with laser-induced fluorescence. As a result, limit of detection was estimated to be 12 pM (60 amol, S/N = 3) and good linearity in the calibration curve (R^2 > 0.999) was realized in the 2D analysis of maltoheptaose.

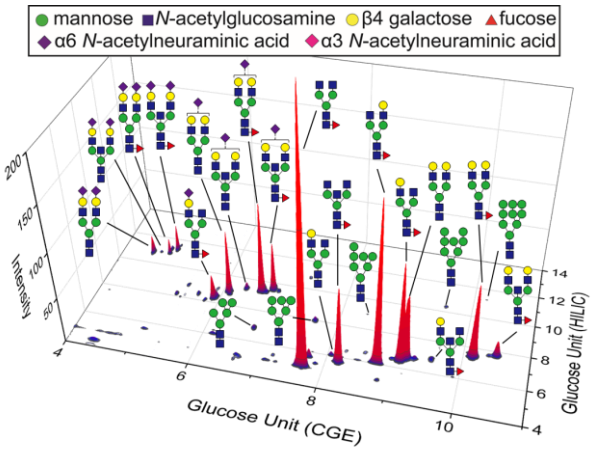

Figure 1. HILIC/CGE 2D profile of IgG N-glycans.

Finally, 2D profiling of N-linked glycans obtained from standard glycoproteins and cell lysates were demonstrated. As shown in Figure 1, high resolution 2D profile of IgG N-glycans was successfully obtained by the data alignment using triple internal standards [2]. N-glycans were well distributed on the HILIC/CGE 2D plane based on the glycan size, number of sialic acids, linkage type, and so on. Specific minor glycans were also identified from HeLa cell lysates.

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O8

Going Big: Non-Denaturing HRMS- Hyphenated Separations Unleash the Analysis of Complex Proteoform Mixtures Over 100 kDa

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Summary

In the last decade, significant progress has been made in native mass spectrometry (MS), enabling the characterization of large proteins and protein complexes in application areas such as biopharmaceutical and structural biology studies. Yet, to date, many studies that apply native MS use purified samples and direct infusion with nanospray sources, reducing the application potential of this technique.

Hyphenating native separations to native MS allows the measurement of complex samples, resolving proteoforms according to specific mechanisms (and therefore aiding identification) and increasing the dynamic range of the measurement. However, the approaches to perform native-MS hyphenated separations are currently geared towards analyzing biotechnological protein products available in relatively large amounts and need to be more sensitive for biological studies. In our research, we aimed to extend the application of native separation methods to study intact proteins and complexes in microscale format to allow for the analysis of biological samples. The separations were developed at nanoflows, facilitating desolvation during electrospray ionization and increasing MS detection sensitivity.

In this presentation, we will discuss our results obtained using non-denaturing capillary zone electrophoresis (in collaboration with Aalen University), nanoflow size exclusion chromatography, and nanoflow ion-exchange chromatography, and their hyphenation to MS. Focus of the talk will be in particular the use of nanoflow cation exchange chromatography. Results from the analysis of reference proteins between 10 and 150 kDa, a model cell lysate, and serum immunoglobulin G by a salt-mediated pH gradient with volatile additives will be discussed. Proteins presented non-denatured mass spectra, and low detection limits were achieved (0.22 pmol of monoclonal antibodies). Excellent chromatographic separations were obtained, including the resolution of different proteoforms for large proteins (over 140 kDa). The proposed native hyphenated separations setup shows great potential for analyzing diverse proteins in native top-down proteomics and provides unprecedented opportunities for clinical applications.

O9

Endotoxin Quantification by the Chemical Instrumental HPLC-Kdo-DMB Assay

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Summary

Bacteria are ubiquitous in the environment, leading to the constant risk of endotoxin contamination in the pharmaceutical and the biotechnology industries. Endotoxins are molecules integrated into the outer membrane of Gram-negative bacteria and released constantly in large quantities. They are strong immunostimulants, even quantities as low as pg mL^{-1} present in the human bloodstream, trigger severe reactions such as fever, sepsis, or potentially fatal organ failure. Consequently, strict quality controls of these contaminants are requested by health authorities.

Endotoxin testing employs biological assays such as the rabbit pyrogen test, monocyte activation test, or the Limulus Amoebocyte Lysate (LAL) assay, considered inhere as the gold standard. While highly sensitive, all these assays have significant drawbacks. They are all susceptible to strong sample matrix interferences, leading to a phenomenon known as “low endotoxin recovery” which endangers patient health. Moreover, the LAL assay is validated with endotoxin recovery values of 50 – 200%, and a measurement accuracy of 25% leading to large experimental errors.

Recognizing these limitations which come from the enzymatic nature of these assays, a chemical assay presents a viable solution. Our HPLC-Kdo-DMB assay [1] uses the rare sugar acid Kdo, present in each endotoxin molecule as endotoxin marker. Kdo is released quantitively by mild acidic hydrolysis. Sensitive detection is obtained by Kdo derivatization with the fluorophore DMB. Matrix effects are minimized by the separation of Kdo-DMB by RP-(U)HPLC from potential interfering matrix compounds. This chemical endotoxin quantification approach significantly reduces the likelihood of “low endotoxin recovery”. Its current limit of quantification is at 30 EU mL^{-1}, with ongoing research efforts to reduce it to 0.25 EU mL^{-1}, a health authority requirement for pharmaceutical applications.

The novel assay has been employed to monitor endotoxin release in bioreactor cultivations [2], assess the efficacy of downstream process filtrations, and analyze high protein load matrixes.

Acknowledgement

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O10

A Novel Selective Comprehensive Two-Dimensional Online nanoLC-CZE-MS Platform for Proteoform Characterization

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Summary

CE-MS has been used to separate proteoforms on intact protein levels, however, selectivity and sensitivity are often not sufficient for complex biological samples. We therefore developed a two-dimensional (2D) heart-cut nanoLC-CZE-MS platform and have shown that this allows the pre-separation of intact proteins from a complex matrix and a 280-fold increased sensitivity compared to a one-dimensional CZE-MS approach. Furthermore, with this 2D approach, a higher proteoform selectivity can be gained [1]. While the transfer of a peak from the first to the second dimension is efficient using our two-dimensional heart-cut approach, the characterization of multiple chromatographic peaks or partly separated proteoforms is time consuming, requires high sample amounts, and might lead to incomplete proteoform characterization. Therefore, we expanded the nanoLC-CZE-MS platform to perform selective comprehensive nanoLC-CZE-MS. There, the RPLC column is connected to a storage capillary by a 10-port valve to decouple the storage capillary from the first dimension while performing the second dimension analysis. This allows the storage of a fraction from the first dimension for subsequent 2D analysis. The volume of the stored fraction depends on the volume of the storage capillary and can be easily adjusted. The storage capillary is also connected to an 8-port nanoliter valve with four 20 nL internal loops. Hence, 20 nL fractions can be transferred from the storage capillary to the second dimension. Here, we discuss different coupling approaches for RPLC-CZE-MS and show the essential parameters of our selective comprehensive two-dimensional nanoLC-CZE-MS platform. In addition, we show initial results from the analysis of a cell lysate using our selective comprehensive two-dimensional online nanoLC-CZE-MS platform, demonstrating its highly selective and sensitive analysis of proteoforms.

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O11

Taylor-Aris Dispersion Assisted Mass Spectrometry for the Direct Injection Analysis of Proteins with High Matrix Content

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Summary

Electrospray ionization mass spectrometry (ESI-MS) is a predominant tool in the analysis of proteins; however, protein samples often contain ESI-MS incompatible components. This is the case for protein pharmaceuticals such as monoclonal antibodies (mAbs), which are stabilized with non-volatile salts, buffer components and often detergents as well. In case of native protein analysis, the use of phosphate-buffered saline (PBS) solution is a prevalent option for mimicking the native conditions. Although direct infusion is the fastest and most straightforward method for introducing samples to the MS, due to the interferences of the aforementioned MS incompatible components the results often provide low-quality spectra or no information at all.

Taylor-Aris dispersion occurs in case of sample plugs moving slowly through a small inner diameter capillary, causing symmetrical band broadening due to the radial diffusion of analytes across the pressure-driven parabolic velocity profile [1]. This dispersion is more substantial in case of high molecular weight components with low diffusion coefficients, resulting in relatively wide peaks for proteins and narrow peaks in case of low molecular weight matrix components. Consequently, a matrix-free zone forms in the front and rear portions of the sample plug, where ESI-MS measurements can provide clean spectra of the protein, without the need for any conventional separation technique.

We have recently demonstrated the use of Taylor-Aris Dispersion Assisted Mass Spectrometry (TADA-MS) for the simple, fast and low-cost analysis of large molecules in MS incompatible matrices, such as mAbs in their original formulation or native protein complexes in PBS solution [2].

Acknowledgement

The discussions with Prof. Attila Felinger and Dr. Krisztian Horvath about TA dispersion, and with Prof. Gyöngyi Gyémánt about native proteins were highly appreciated. Financial support was provided by the National Research, Development and Innovation Office, Hungary (K142134) and the New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-23-3-I-DE-115).

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O12

Rapid Distinction and Assignment of Positional Isomers of New Psychoactive Drugs in Mixtures by Trapped Ion Mobility Mass Spectrometry

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Summary

New psychoactive substances (NPS) are structural derivatives of conventional illicit drugs, designed to circumvent the law. Some NPS are positional isomers of the original drug and pose a challenge to identify confidently in forensic casework, often requiring laborious and time-consuming approaches. Here we present a new trapped ion mobility mass spectrometry (TIMS-MS) method for the fast and highly selective analysis of NPS isomers found in real cases. Solutions of (mixtures of) cathinones were directly infused into a TIMS-time-of-flight mass spectrometer via electrospray ionization (positive mode) requiring no or only little sample preparation. The study focused on cathinones, a popular class of NPS comprising a large number of positional isomers. The suitability of the new method for the highly accurate identification of cathinone isomers in challenging mixtures will be demonstrated.

Applying TIMS-MS, each individual cathinone exhibited a bimodal mobility distribution due to the presence of protomers, leading to a complex profile of convoluted peaks when isomer mixtures were analyzed. However, addition of a neutral crown ether to the sample prior to TIMS analysis, resulted in a single peak for each cathinone isomer, which greatly reduced the complexity of the obtained mobilograms and facilitated the analysis of mixtures. Recorded mass spectra and extracted-ion-mobilograms were processed with an in-house developed script. Based on TIMS-MS data acquired for pure standards, a model was created that allowed accurate deconvolution of mobilograms of isomer mixtures. This way, the NPS composition of unknown samples could be established, revealing the relative contribution of individual isomers to the total MS signal. Relative amounts down to 10% could assigned reliably. The developed workflow was successfully used for the unambiguous identification of NPS isomers in confiscated forensic case samples in less than 5 min per sample.

O13

Metal Ionization in Gas Phase Mass Spectrometry (MIG MS): A New Tool for Analysis of Volatile Organic Compounds

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Summary

In the current work, we proposed the ions of coinage metals belonging to 11 group – Cu^+, Ag^+ and Au^+ as chemical ionization agents for mass spectrometry (MS) analysis of volatile organic compounds (VOCs). Among these ions, especially Au^+ attracts considerable interest due to its rather high reactivity associated with relativistic effects and ability to activate C-H and C-C bonds.

The metal ions were generated by laser ablation of a metal nanolayer with the UV laser (355 nm) in a commercial dual sub-atmospheric pressure MALDI/ESI interface attached on ultra-high resolving power mass spectrometer with orbital trap. VOCs belonging to different chemical classes (alkenes, alcohols, aldehydes, ketones, aromatic compounds, carboxylic acids, ethers, and organosulfur compounds) were infused via the ESI capillary and quickly formed ion-molecular complexes in the ion source with general formula $[M+VOC+H_2O]^+$ and $[M+2VOC]^+$ [1]. Utilization of Ag^+ ions yielded also intense signal of $[M+VOC]^+$. Au^+ ions being the most reactive among the studied metal ions interacted with VOCs in a way that was more intricate and often resulted in formation of side products through hydride abstraction, loss of water or cleavage of carbon chain. These side reactions complicated the spectra, but on the other hand, they also allowed detecting saturated hydrocarbons, which did not produce any signals with Ag^+ and Cu^+. The detection limits of the selected compounds in the gas were in the range of 0.1 – 1.4 nmol/L [2].

The developed technique brings novel utilization of the dual MALDI/ESI interface for studying gas-phase chemistry of metals and expands the portfolio of currently available methods for the analysis of VOCs.

Acknowledgement

We gratefully acknowledge the financial support of the Czech Science Foundation (projects 19-20927Y, 21-12262S and 24-10924S).

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O14

The Potential of Stainless Steel Needles to Integrate Microextraction and Mass Spectrometry

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Summary

Sustainability and affordability are relevant features of novel microextraction devices. On the one hand, the use of sorbent materials derived from renewable or natural sources is relevant to minimize the environmental impact of the sample preparation step and, thus, of the whole analytical procedure. In addition, considering the affordability of the elements as a criterion in the design of these units helps to improve their transferability, given their low cost and ease of implementation in any laboratory, regardless of its economic resources. The fact that these elements are available on the market is an added value as it leads to a higher reproducibility of the extraction devices.

In this context, we have investigated the potential of stainless-steel needles (SS-Ns) as sorbent hosts. Their easy combination with syringes facilitates the construction of a very versatile microextraction device, since it can be easily adapted to the sample volume to be analyzed simply by selecting the appropriate volume of the syringe. Disposable plastic syringes can also be used to avoid cross-contamination between samples. The conductivity of stainless steel allows it to be used as an electrospray emitter in ambient mass spectrometry. This last combination simplifies the whole analytical procedure, as the on-line elution of the retained analytes is efficiently transferred to the mass spectrometer inlet.

This communication describes the applications developed by our research group using SS-Ns where the sorbent phase is either placed as a thin film coating the inner wall of the needle or placed in the holder. The determination of drugs in biological fluids has been selected as the analytical problem. The different sorbents used are sustainable (e. g., cotton, polydopamine), and the interface with the mass spectrometer was made up of commercial elements [1,2].

Acknowledgement

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O15

Nanogels for Multifunctional Biomolecular Electrophoresis Assays

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Summary

Significance. Capillary electrophoresis is a powerful separation technique for volume-limited samples in biomedical and pharmaceutical research. An advanced method is developed for native enzyme analyses providing insight into protein structure and function.

Introduction. Protein-based assays are difficult to achieve with capillary electrophoresis under native conditions because of the interaction of proteins with the separation channel. By miniaturizing the enzymatic reactions are integrated in real-time with separation-based assays. Biocompatible gels advance nanoscale reactions by enabling new separation modalities critical to evaluating and harnessing enzyme activity. Self-assembled phospholipid nanogels are thermally reversible media that maintain the biological function of complex biomolecules and provide a means to create and embed multifunctional assays in capillary separations. In this presentation nanogel technologies are described to create nanoliter reactions zones to interrogate biomolecules in seconds.

Experimental Approach. To date, as many as 7 sequential processing steps have been performed in series in microscale channels. This is possible because the viscosity of nanogel is thermally dependent and thermally reversible. 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 temperatures using an automated capillary electrophoresis instrument. Once the nanogel is loaded into the capillary, the fluids are then locked in place by raising the temperature to gel the material. 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. In this way, a series of discreate reaction zones is created and integrated with a separation step. Enzyme reactors of this low volume are mixed electrophoretically and then the substrate and products, or products, are separated, detected, and quantified.

Results and Discussion. This approach is automated and reduces the time for enzymatic conversion from hours to seconds. 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 it transforms standard electrophoresis methods into sophisticated multifunctional separations that are programmed, erased, and repeatedly run.

Conclusions. An approach for nanoliter enzyme reactions under native conditions is demonstrated that leverages the low sample volume requirements of capillary electrophoresis. The technology outlined in this presentation provides new strategies to either leverage or evaluate enzyme specificity and activity.

O16

Dry and Wet Assembly Approaches for Arranging Ordered Particle Monolayers and Arrays

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Summary

Our group focuses on developing versatile assembly methods, primarily dry approaches, of colloidal particles into ordered monolayers and arrays in an open format, potentially for Lab-on-a-Chip analytical systems [1,2]. To address the assembly of ordered arrays, we proposed a novel, more automatable, and completely dry assembly method to attain a non-closely packed array of silica, polystyrene, or PMMA, microspheres within 10 s [3]. As shown in Fig. 1a, the agglomerated microspheres are offered to an electrostatic cell, which is fluidized by applying an electric field. Subsequently, the particles are attracted to a perforated silicon device by applying a vacuum force and a brushing step to remove excess particles. From Figs. 1b-c, compared to most existing methods in literature, this system's merit is that any desired geometrical particle array can be assembled on a large scale. Furthermore, we demonstrate that these arrays can be transferred to other soft elastomeric surfaces, paving the way for the potential fabrication of hierarchical materials or the desired ordered packing structures for High-Performance Liquid Chromatography (HPLC) columns using a layer-by-layer strategy in a bottom-up approach [2]. Another approach that we recently reported involves the manual rubbing of dry powders (silica and PMMA) on fluorocarbon-patterned substrates using a PDMS stamp to rapidly (≈ 20 s) obtain ordered arrays of hexagonal closely packed (HCP) crystals of powder particles with diameters ranging between 500 nm to 10 µm (cf. Fig. 1d) [4]. Our findings elucidate that the triboelectric charging and contact mechanics force are critical contributors to attain tunable HCP crystal patterns on a wafer-scale (as shown in Figs. 1e-f) [4]. We envision these assembled arrays as a promising open microfluidic platform for performing bioassays and other biosensing applications.

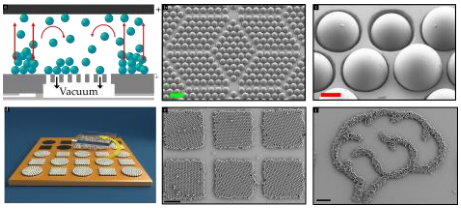

Figure 1. (a) Dry powder is offered to an electrostatic cell, which is levitated by applying an electric field E≥1.5 MVm-1 and subsequently captured on a perforated silicon device using a vacuum force. (b-c) Any geometrical array can be assembled. (d) Schematic illustration of the manual rubbing technique to assemble dry powder into closely packed crystal (HCP) structures on substrates using a PDMS (rubber) stamp. (e-f) SEM images of patterned HCP crystal structures comprising PMMA microspheres. Scale bar: green = 20 µm; red = 2 µm; black = 100 µm.

Acknowledgement

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O17

Investigation of the Effect of Induced Macromolecular Crowding on Hyaluronidase Catalytic Activity and Interactions Using Capillary Electrophoresis and Microscale Thermophoresis

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Summary

The extracellular matrix (ECM) is dominated in-vivo by macromolecular crowding and resultant excluded volume effects [1]. It is composed of a large quantity of various macromolecules which fill the interstitial space within cells forming a hydrated gel [2]. ECM is a highly dynamic structure. It is constantly regenerated, remodeled and degraded to maintain tissue homeostasis, through the action of metalloenzymes such as collagenase, hyaluronidase (Hyal) and elastase [3]. The present investigation is part of a large multidisciplinary project, X-Crowd, aiming to scrutinize the kinetics of these enzymes in a realistic picture. For this purpose, crowded environments mimicking the ECM in-vitro are used. The crowding environment was simulated using dextran at two different molecular weights (40 and 476 kDa), respecting so the ratio between enzyme and crowder size.

We first intended to study the activity of Hyal, a glycosidase responsible for the degradation of hyaluronic acid (HA), a large polysaccharide responsible for skin hydration and cartilage lubrication. Capillary electrophoresis (CE), thanks to its miniaturized dimensions, was advantageously used to monitor the enzymatic reaction, after optimizing the injection step, taking into account the media viscosity and complexity. To better understand the effect of dextran on the catalytic activity of Hyal, a small substrate, decasaccharide (10-mers), was first used. Results were compared to those obtained with the high molecular weight natural substrate, HA. Moreover, the interaction between Hyal and the Dextran was characterized using microscale thermophoresis (MST), a biophysical miniaturized technique based on fluorescence detection. Hyal was thus labeled with ATTO-647 and studied in the presence of dextran at different buffer and pH conditions. CE combined to MST allowed to disentangle the impact of crowding on Hyal kinetics and confirmed the importance of considering it for the evaluation of compounds.

Acknowledgement

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O18

Novel Approaches for the Analysis of Organoids and Organ-on-Chip Samples Using Liquid Chromatography and Mass Spectrometry

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Summary

Organoids and organ-on-chip (OoC) systems are emerging as alternatives to animal experiments in disease modeling and drug studies. However, samples for these organ models can be both limited in size and complex. We are currently developing analytical approaches for analyzing such samples, with a focus on automated and miniaturized sample preparation coupled on-line to liquid chromatography and mass spectrometry [1]. Two approaches will be focused upon: electromembrane extraction (EME), and automated filtration/filter backflush (AFFL) based systems. We find that EME is promising for chip-format sampling of liver organoids [2], allowing for fully automated drug metabolism studies. The AFFL system has been applied for sterol biomarker discovery studies of non-alcoholic fatty liver disease (NAFLD) [3] and is currently being developed for monitoring drug metabolism in both organoids and OoCs. We find that both of these approaches are suited for efficient sample clean-up of limited samples, and are focusing on method validation following FDA-guidelines. Taken together, efficient and miniaturized sample preparation and separation systems allow for LC-MS-based analysis of organoids and OoCs.

Acknowledgement

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O19

Monitoring the Effectiveness of Chemotherapy Treatments Utilizing Artificial Intelligence-based N-Glycome Analysis

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Summary

A unique methodology is presented to investigate the impact of chemotherapy on individuals with lung cancer through the examination of serum N-glycome, coupled with data analysis employing artificial intelligence-based machine learning techniques. The research encompassed serum specimens from 33 lung cancer patients undergoing chemotherapy, emphasizing 21 specific asparagine-linked glycan structures both before and after treatment. Enzymatic release, fluorophore labeling, and capillary electrophoresis with laser-induced fluorescent detection (CE-LIF) were utilized for the analysis of N-linked glycan structures. Employing Quadratic Discriminant Analysis (QDA) classifier data processing techniques revealed a correlation between structural modifications in the targeted N-glycans attributable to chemotherapy. This integrated bioanalytical-artificial intelligence approach represents a novel contribution to the field, holding the potential for accurate and expeditious assessment of treatment outcomes.

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O20

N-Glycosylation Analysis of Homogenized Oral Squamous Cell Carcinoma Soft Tissue Samples by CE-LIF

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Summary

Oral squamous cell carcinoma (OSCC) is an aggressive disease with a glycoproteomically unmapped progression and a low, five-year survival rate. Besides the most commonly known risk factors of alcohol consumption, tobacco, poor oral hygiene, HPV infection, long-term immunosuppressant therapies may also increase the risk and change the therapeutic response of secondary malignancies. Alterations of protein N-glycosylation have a pivotal role in tumorigenesis and metastasis formation. Thus, our study aimed to identify novel glycobiomarkers for more precise prognosis suggesting more efficient therapeutic alternatives for oral cancers. Oral mucosal soft tissue samples were obtained by incisional biopsy from five patients with OSCC, both from the malignant and the opposite healthy gingival sides, as well as from seven age-sex matched healthy controls with the appropriate Ethical Permissions and Informed Patient Consents (DE RKEB/IKEB: 6152-2022). The collected tissues were properly homogenized, followed by N-glycan profiling of endoglycosidase released and fluorophore-labeled carbohydrates using capillary electrophoresis coupled with ultra-sensitive laser-induced fluorescent detection. Significant ($p<0.05$) differences have been identified between the malignant tissue samples of OSCC patients and the healthy controls, indeed between the healthy and the positive control oral mucosal samples, while there were no differences between the N-glycan profiles of the malignant tumor and the positive control samples. We can conclude that the automated sample preparation in conjunction with high-resolution CE-LIF-based glyocoanalytical method reported in this presentation proved to be an efficient and sensitive workflow for glycobiomarker-based molecular diagnostics of oral malignant lesions.

O21

Ionic Liquids Assisted Micellar Electrokinetic Chromatography of Urine Catecholamine Metabolites for the Investigation of Neuroblastoma

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Summary

Neuroblastoma (NBL) is a malignant tumor originating from neural tube cells and is the most common cancer diagnosed in infants. Nearly 50% of its cases occur in children under 2 years of age. NBL is characterized by the increased production of catecholamines (CAs) and their metabolites therefore the quantification of CAs is important for the diagnosis especially the measurement of urinary VMA, HVA and also metoxycatecholamines such as metanephrine (M) and normetanephrine (NM). Capillary electrophoresis (CE) was used in this study due to its number of advantages such as high resolution, speed and low consumption of reagents. However, due to poorer detection limits and lower repeatability, compared to chromatographic techniques, the addition of ionic liquids (ILs) to the separation buffer was investigated to solve these problems. Among the 12 tested imidazolium-based ILs with various substituents in position 1 and various anions, 1-hexyl-3-methylimidazolium chloride turned out to be the most optimal. Literature data confirmed that coating the capillary wall with a cationic layer can increase its surface stability, thereby improving the repeatability of the separation process [1,2]. In this study, micellar electrokinetic chromatography (MEKC) with separation buffer composed of 5 mM sodium tetraborate, 150 mM boric acid, and 50 mM SDS and MeOH (15%, v/v) (apparent pH 7.27) was employed [3]. The isolation of analytes of interest from urine samples was performed by using solid-phase extraction with hydrophilic–lipophilic-balanced columns and methanol as eluent. The obtained results demonstrated that HVA and VMA are easily extracted at pH of 5.5, while a sample pH of 9.0 facilitated the extraction of M and NM. The validation data confirmed the method's linearity ($R2 > 0.996$) for all analytes within the range of 0.25–10 µg/mL. The applicability of the optimized SPE-MEKC-UV method was confirmed by employing it to quantify clinically relevant CAs in real urine samples from pediatric neuroblastoma patients.

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O22

Novel Magnetic Solid-Phase Microextraction Approach with Ionic Liquids and a Surfactant as Coating Materials for Pretreatment of Biological Samples

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Summary

Sample pretreatment based on magnetic nanoparticles (MNPs) can be considered an interesting alternative compared to LLE and SPE because of high specific surface area and the possibility of their separation from the matrix using external magnetic field. It allows to miniaturize the sample preparation procedure and reduce consumption of harmful organic solvents. However, the functionalization of magnetic sorbents by delivering the functional groups on their surface is required for obtaining high extraction efficiency. In recent years, the increased utilization of ionic liquids (ILs) and surfactants in the method development has been observed due to their structural diversity, unique and eco-friendly properties [1,2]. On the other hand, there are no papers reporting the use of these compounds as coating materials of MNPs for drug extraction from biological fluids.

In the study, ten different ILs and a double-chained surfactant in the functionalization of Fe3O4 MNPs were tested. The influence of the cation, anion, length and amount of alkyl substituents in these structures in respect to the extraction results of the prepared MNPs was evaluated. They were also used in combination with silica as a coating material. The most effective MNPs were characterized by FT-IR, XRD, TG and TEM techniques [3], and applied for the isolation of epirubicin from biological fluids before liquid chromatography with fluorescence detection (LC-FL) [4]. The developed method was optimized and validated according to the FDA and ICH criteria. Linearity was confirmed in the range of 1-1000 and 1-10000 ng/mL for plasma and urine samples, respectively. The extraction efficency was ≥ 80 %. Finally, the MNP-LC-FL method was successfully applied for epirubicin quantification in real clinical samples collected from a pediatric cancer patient. Summarising, a novel magnetic solid-phase microextraction approach based on a double chained surfactant for pretreatment of biological samples was developed as an interesting alternative to others in pharmaceutical and clinical investigations.

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O23

Deciphering the Phosphorylation Barcode of G Protein-Coupled Receptors (GPCRs) using CZE-TDMS

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Summary

G protein-coupled receptors (GPCRs) are a critical class of biomolecule that are regulated in time and space by post-translational modifications. An area of long-standing interest is mapping modification states (proteoforms) of GPCRs to specific stimuli or disease states, however, characterizing proteoforms of GPCRs is inherently challenging due to their hydrophobicity and low copy number. Even with recent advancements in membrane mimetics and mass spectrometry instrumentation, current workflows for GPCR characterization require large amounts of relatively clean and homogenous protein, which limits their application to endogenous samples. Here, we describe a capillary electrophoresis top-down mass spectrometry (CE-TDMS) workflow to characterize proteoforms of both intact and partially cleaved GPCRs. By this approach, we are able to resolve multiple proteoforms of both beta-2-adrenergic receptor and metabotropic glutamate receptor 2 without the need for prior desalting while using very small volumes of samples (<40 nL injections). In addition, for smaller phosphorylated protein analytes (4.2 kDa), CE resolves phosphorylated isomers, which significantly improves the fragmentation coverage that can be accomplished in comparison to conventional reverse phase-liquid chromatography (RP-LC). Localization of these phosphorylation hot spots was also achieved. Overall, this study demonstrates that CE-TDMS is successful for mass limited samples in complex matrices, making it an ideal candidate to pursue in-depth GPCR proteoform characterization.

O24

Intramolecular Disulfide and Charge Variant Separation and Characterization of Various Antibody Subunits with CE-MS/MS

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Summary

The analysis of antibodies is an ever-evolving field of research. Antibodies can vary, for example, in their glycosylation or amino acid sequence based on the host cell, the purification process, and the storage conditions. The characterization on subunit level enables a detailed mass spectrometric characterization of posttranslational modifications (PTMs) of monoclonal antibodies (mAbs). For the subunit analysis, various mAbs were enzymatically digested and further reduced. However, the implemented reduction often leaves the intramolecular disulfide bridges intact, sometimes without realizing it. Here, we present a capillary electrophoretic (CE) method based on a neutral-coated capillary for the separation of Immunoglobulin G-degrading enzyme of *Streptococcus pyogenes* (IdeS) digested and reduced mAb subunits followed by mass spectrometry (MS) and MS/MS. Our CE approach enables the separation of (i) different subunit moieties, (ii) various reduction states, and (iii) positional isomers of these partly-reduced subunit moieties. The location of the remaining disulfide bridges can be determined by middle-down Electron transfer higher energy collisional dissociation (EThcD) experiments. Applying the presented CE-MS/MS method, reduction parameters such as the use of chaotropic salts were studied. For the antibodies investigated, urea significantly improved the subunit reduction compared to the approach without a chaotropic salt. Using urea in the reduction process, subunit proteoforms like lysin or glycosylation variants could be separated and identified as well as several other low abundant charge and size variants. The presented CE-MS method is a powerful tool for the variant characterization of mAbs on the subunit level. It not only enables understanding of disulfide bridge reduction processes in antibodies, but also the characterization of variants.

Acknowledgement

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O25

Toward 1000-fold Sensitivity Improvement of Capillary Electrophoresis coupled with Laser-Induced Fluorescence Detection for Aminopyrene Trisulfonic Acid Fluorophore

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Summary

Glycan analysis holds significant importance in various fields such as medicine, biotechnology, nanotechnology, bioenergy, and materials science. One widely used method for glycan analysis involves labeling glycans with aminopyrene trisulfonic acid [1] (APTS) and analyzing them using capillary electrophoresis (CE) coupled with laser-induced fluorescence (LIF). However, the sensitivity of CE-LIF faces challenges due to the growing demand for detecting trace amounts of glycans and conducting single-cell or subcellular glycan analysis.

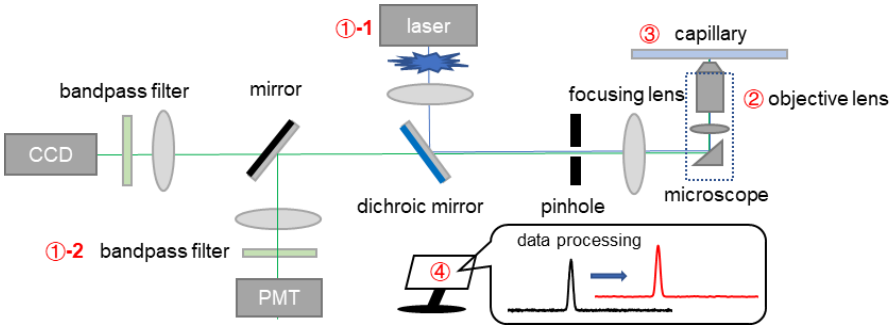

Figure 1. Schematic setup of LIF system.

In our research, we focus on optimizing a LIF system setup to enhance the sensitivity for detecting APTS-labeled glycans (Fig. 1). An inverted microscope (Olympus, IX73) was employed for detection. A 450 nm laser and a 530±55 nm bandpass filter were customized for APTS. The detection window of the capillary was etched to approximately 10 µm thickness. In terms of data processing, we employed 3 times binomial smoothing to reduce background noise fluctuations [2]. The signal-to-noise ratio (SNR) was calculated for sensitivity evaluation [3]. A 1000-fold enhancement in SNR was achieved with our CE-LIF system compared to commercial CE system. We are continuing to work on improving sensitivity and plan to apply our CE-LIF system to glycan analysis.

Acknowledgement

The authors have declared no conflict of interest.

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O26

Charge to Move Forward in Volume-Restricted Metabolomics

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Summary

In metabolomics, advanced analytical workflows are needed to study biochemical changes in small-volume biological samples, notably for samples originating from 3D microfluidic cell culture models, individual zebrafish larvae and neonatal clinical blood sampling. Recent work from our lab revealed that capillary zone electrophoresis-mass spectrometry (CE-MS), regardless of utilizing a sheath-liquid or sheathless interface, is a strong analytical tool for probing polar and charged metabolites in biological samples with a good reproducibility. Moreover, in a simulated metabolomics study, CE-MS was able to find the right set of differential metabolites between controls and cases. These studies clearly indicate the value of CE-MS for biomarker discovery and comparative metabolomics studies.

Given our ambition to address volume-restricted biomedical questions with metabolomics, we report in this presentation on the development of new CE-MS-based analytical workflows for the highly efficient and sensitive analysis of polar (endogenous) metabolites in neonatal plasma and individual zebrafish larvae. As only nanoliters of samples are consumed by a single CE-MS analysis, multiple injections/assays can be performed on the same valuable volume-limited sample allowing for technical replicates and/or probing different classes of ionogenic metabolites. We show how these new CE-MS-based workflows can be employed in a reliable way for the quantitative analysis of creatinine, and many more endogenous compounds, in neonatal plasma samples using a starting amount of less than 5 microliter, whereas gold standard clinical chemistry approaches require often a minimum of 100 microliter for only creatinine determination. Hence, the proposed CE-MS-based workflow will contribute to minimizing both the amount and frequency of blood collecting required for diagnostic purposes in a neonatal setting.

We also demonstrate the utility of a new CE-MS workflow for the profiling of metabolites in extracts from individual zebrafish larvae and pools of small numbers of larvae. More than 70 endogenous metabolites could be observed in a pool of 12 zebrafish larvae, whereas 29 endogenous metabolites were detected in an extract from only 1 zebrafish larva. So far, zebrafish has proven to be a very effective model for stress research, in particular for studies on the effects of cortisol, with a clear role of the glucocorticoid receptor during stress. However, the role of the mineralocorticoid receptor (MR) on mediating the effects of cortisol is less known. By using wild-type (WT) and ubiquitous MR-knockout (MRKO) zebrafish larvae exposed to exogenous cortisol treatment, our CE-MS-based metabolomics workflow revealed the implication of metabolic pathways solely activated via MR. Taken together, CE-MS has the potential to identify novel pathways and mechanisms of action in zebrafish larvae and is a viable analytical approach for volume-restricted metabolomics.

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O27

Online Electrokinetic Sample Cleanup and Evaluation Method for APTS Labeled N-Glycan Separation by Capillary Electrophoresis

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Summary

Capillary electrophoresis (CE) is one of the most frequently used liquid phase separation techniques for the analysis of complex carbohydrates. Since glycans in most of the cases lack chromophore or fluorophore groups, their CE analysis usually requires tagging by a charged fluorophore. To speed up the derivatization reaction, a large excess of the labeling reagent is typically used, therefore, a purification step is necessary prior to CE analysis using the industry standard low pH gel-buffer system. In addition to representing an extra sample preparation step with the associated labor and cost, the purification process also holds the risk of losing some of the sample components. In this presentation we demonstrate an online electrokinetic sample cleanup process with electroosmotic flow (EOF) assisted separation in a bare fused silica capillary using alkaline pH background electrolyte and normal polarity of separation voltage [1]. 8-Aminopyrene-1,3,6-trisulfonic acid (APTS) labeled maltooligosaccharides were analyzed first to understand the complex effect of the downstream EOF and the counter current electromigration of the sample components including the labeling dye. The use of 150 mM caproic acid - 253 mM Tris (pH 8.1) running buffer facilitated the entrance of the sample components of interest into the separation capillary, while the excess labeling reagent was excluded, therefore, did not interfere with the detection. The alkaline caproic acid - Tris running buffer was then applied to the N-glycome analysis of human serum samples, showing excellent separation performance, and more importantly, without the need of the extra sample purification step. Next to the radically new separation method, the required novel data evaluation method will be introduced as well [2].

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O28

Single Islet Metabolomics using Capillary LC-MS

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Summary

Dysfunction in insulin secretion profiles from islets of Langerhans is a hallmark of type II diabetes. Insulin secretion is metabolically driven from various sources including glucose, glutamine, fatty acids, and lactate. Interestingly, when individual islets, comprising about 500 cells are stimulated using glucose, their secretion profiles fall into one of three categories. To date, the metabolic changes that are associated with these different profiles are not known. This is due to metabolite analysis from islets being available only from batches of islets. Using chemical tagging and capillary based LC-MS, we are able to analyze over 80 metabolites from a single islet.

Derivatization and analysis: First, 3-(diethylamino) propionyl chloride tag was synthesized by reacting 3-(diethylamino) propanoic acid and 1-chloro-N,N,2-trimethyl-1-propenylamine (Ghosez's reagent) in dimethylformamide. Islet lysate was reacted with 3-(diethylamino)propionyl chloride with pyridine for 30 minutes. The first step reaction was quenched with N,N-Diethylenediamine followed by addition of HATU/HOBt and allowed to react for 2 hours.

Sample injection and Separation: Individual islets were injected using a bomb injection method to load the entirety of the sample. Analytes are separated with 5mM ammonium carbonate buffer (pH= 9)/acetonitrile on RPLC capillary 50µm x 23 cm column packed with Kinetex Evo C18 2.6µm particles reverse phase column (RP). nESI analysis on a Q-Exactive mass spectrometer operating at resolution = 70k.

The primary/secondary amines and alcohols was derivatized to amides and esters from the acyl chloride tag (1st step) and is stable under neutral condition Ghosez's reagent offers. Organic acids and other carboxylic acids were derivatized to amide using the diamine tag (2nd). The reaction after second step has an average reaction efficiency of 98% and average % RSD of 7. 80 specific metabolites were targeted for analysis and all 80 were detected in single islets as well as from populations down to 25 cells. Islet secretions were also analyzed for metabolite changes, as insulin is co-secreted with a number of neurotransmitters and biogenic amines. Islets were subjected to low glucose, stimulatory glucose for 20 minutes and stimulatory glucose for 40 minutes. Our results show substantial metabolic differences in energy metabolism and amino acid profiles between these groups.

Our dual stage derivatization increases sensitivity and S/N of analytes to allow the analysis of 8o metabolites from individual islets down to single cell levels.

Acknowledgement

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O29

Kinase/Small Inhibitor interaction Evaluated Directly in Cell Lysates and in Whole Cells: A Combined Capillary Electrophoresis and Microscale Thermophoresis Study

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Summary

Cell life is orchestrated by molecular interactions that controls signaling pathways. Studying these interactions remains a challenge if one wishes to work under conditions close to in vivo. In this project, we have combined capillary electrophoresis (CE) and microscale thermophoresis (MST) to characterize protein/ligand interaction in terms of affinity and thermodynamics [1,2]. Our ambition was to succeed in these miniaturized assays by working directly with cellular lysate or even in whole cells, without radioactivity, immobilization steps, or purification steps.

For this purpose, we report and validate for the first time the use of the red fusion miRFP670 protein as a fluorophore for MST opening the way to original bioanalysis applications. Indeed, in contrast to the commonly used Green fluorescent protein (GFP) protein, miRFP670 allows to conduct MST analyses an optical window (650-900 nm) where background autofluorescence of cells is low [3]. We chose LIM kinases (LIMKs) as enzymes of interest because, despite their established implication in numerous pathologies [4], no inhibitor has passed the clinical trial stage, undoubtedly due to a lack of holistic understanding of these therapeutic targets. We successfully overexpressed miRFP670-LIMKs in HEK293 cells as confirmed by western blot analyses. Maintaining the catalytic activity of LIMK in the cellular lysate towards its substrate, cofilin, even after its tagging with miRFP670, was controlled using CE. A poly(diallyldimethylammonium) or PDADMAC-coating of the silica capillaries was mandatory for the developed CE method to be reliable. Cell lysates were then analyzed by MST, again without any purification step. K_d, ΔH, ΔS and ΔG values for LIMK interactions with various small inhibitors were successfully obtained. Very recently, we went further by conducting miRFP670-LIMK / inhibitor affinity assays directly within whole cells. This remarkable approach provides, in a straightforward manner, insights into the cellular permeability of a potential inhibitor.

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O30

Digging into the Multifaceted Variability of Antibody Molecules: Fc-Proteoform Profiling Illuminates Autoimmune Responses in Rheumatoid Arthritis

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Summary

Autoantibodies and their post-translational modifications (PTMs) are insightful biomarkers of autoimmune diseases informing clinical decisions. However, current methodologies are mainly focused on IgG1 glycosylation, which represents only a subset of the IgG proteome. Quite the contrary, sequence variations, multiple glycosylation sites, and additional PTMs cumulate in structural and functional complexity of the immunologically decisive Fc-domain. To grasp this complexity, including low abundant autoantibody subpopulations, comprehensive yet sensitive methodologies are urgently needed and will allow to understand the role of (auto)antibody-proteoforms and fully unravel their biomarker potential.

Here, we present a holistic analytical approach based on isolation and analysis of the intact Fc-subunit to decipher (auto)antibody Fc-proteoforms and demonstrate its capabilities for the prototypic autoimmune disease rheumatoid arthritis (RA). To this end, Fc-subunits were obtained from anti-citrullinated protein antibodies (ACPA) via antigen-specific immunocapturing and total IgG via Fc-specific capturing. Analytical characterization of Fc-subunits required the development of a nanoscale reversed-phase HPLC approach coupled to mass spectrometry via dopant-enriched nanoESI that allowed separation of IgG allotypes and subclasses, while providing the necessary sensitivity to assess antigen-specific antibodies. Characterization of paired plasma and synovial fluid samples of RA patients revealed a clear molecular distinction of ACPA compared to total IgG besides plasma- and synovial fluid-dependent differences. Prominent changes in glycosylation included high fucosylation in ACPA and low galactosylation in synovial fluid-derived ACPA. Monitoring of hitherto neglected IgG features such as allotype ratios, C-terminal truncations, and doubly-glycosylated Fc-subunits, extended the current view of (auto)antibody complexity. Integration of this wealth of Fc-proteoforms showed a separation of patients that differed in disease activity and led to the identification of disease-associated proteoforms. Taken together, the developed methodology provided comprehensive allotype- and subclass-specific Fc-proteoform profiles surpassing state-of-the-art peptide approaches and uncovered disease-associated Fc-proteoforms of biomarker potential, thus calling for implementation of such methodologies in autoimmunity and beyond.

O31

Isotachophoresis for Electrokinetic Preconcentration of Extracellular Vesicles by Capillary Electrophoresis

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Summary

Extracellular vesicles (EVs) are membrane-enclosed nanoparticles (30-1000nm) secreted by cells that exhibit an intrinsic heterogeneity in size and molecular composition (Intravesicular or membrane) and can be found in body fluids. They have gained high interest in recent years as disease/prognostic biomarkers or biotherapeutic agents and drug nanocarriers [1]. The heterogeneity and complexity of EVs along with limitations in current techniques for their isolation and characterization have hindered their full exploitation [2]. Thus, emerging approaches that bring information on their size, charge and morphology are needed. Capillary zone electrophoresis (CZE) using laser-induced fluorescent (LIF) or UV with or without large volume sample stacking for on-line preconcentration have been recently proposed for EVs separation/characterization [3,4]. However, insufficient detection sensitivity regardless of the detection modes is still the main limitation of CE. Here, we report the development of an in-line electrokinetic preconcentration method based on capillary isotachophoresis (ITP) under extremely high ionic strength (IS) to increase the detection sensitivity of CZE-UV of intact EVs. Isolated EVs are composed of a wide range of vesicles exhibiting different electrophoretic mobilities, while being very slow anions. In addition, they are prone to lysis or aggregation. Because of this, the development of ITP to preconcentrate EVs is very challenging. We focused on selecting the appropriate BGE allowing low UV background signal and current generation, decreased EOF, high stacking effect and high compatibility with the subsequent ITP method. Then, a special attention was given to the electrolyte composition, co-ions and EVs sample matrix. Our developed ITP-UV method demonstrated a detection limit down to 8.3× 108 EV/mL, allowing an enrichment of 25 folds, compared to CZE-UV. The best enrichment factor obtained so far with the few attempts reported for electrokinetic preconcentration of EVs is only 10 folds [4]. ITP-LIF could also be used to provide more specific profiling of EVs.

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O32

Enrichment and Identification of Ceramide Synthase 2 in Subcellular Components: Novel Insights from Porcine Pancreatic Tissue

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Summary

Ceramide Synthase 2 (CerS2), a pivotal enzyme within the sphingolipid metabolic pathway, plays a critical role in various cellular processes. Although CerS2 is widely expressed in several mammalian tissues, such as the kidney, liver, lung, and intestine, its concentration in pancreatic tissue remains relatively low, setting challenges to its isolation and identification. Here, we present a novel, robust, and easy methodology for enriching and localizing CerS2 within pancreatic tissues.

Our approach involves the utilization of an automated homogenization technique coupled with a special buffer composition adjusted for pancreatic tissue homogenization. Subsequently, low and high-speed centrifugation steps were utilized to enrich nuclear, mitochondrial, and membrane proteins, assisting the identification of CerS2 within subcellular fractions. Immunodetection following 1D electrophoresis confirmed the presence of CerS2 in these fractions. Furthermore, CerS2 identification was performed by MALDI-MS analysis after protein separation by 1D and 2D electrophoresis, followed in gel digestion. This comprehensive approach provides valuable insights into the isolation, subcellular localization, and molecular characteristics of CerS2 within pancreatic tissue.

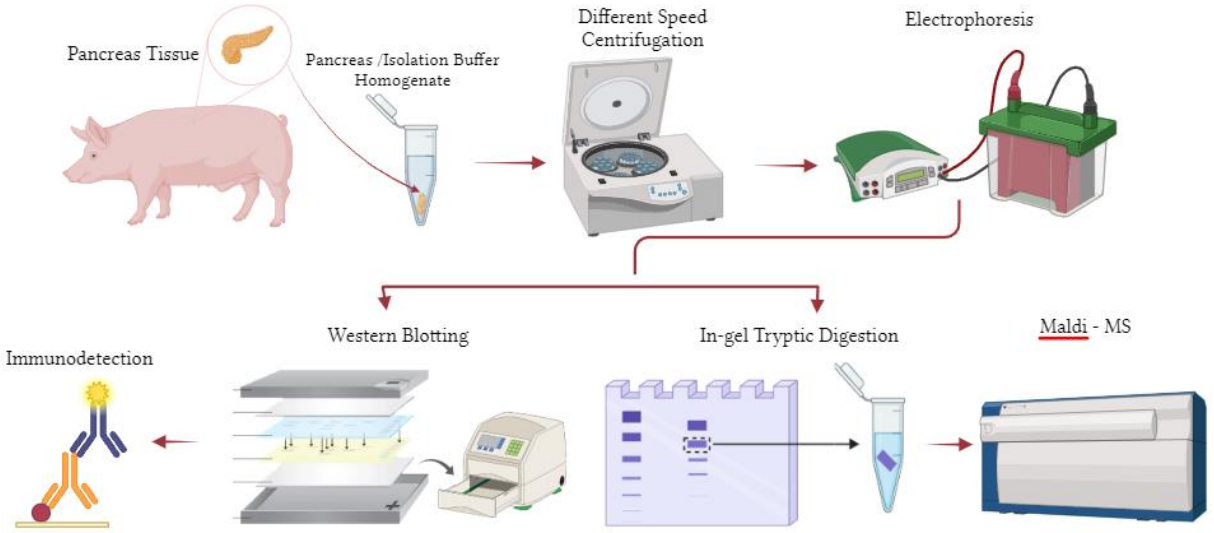

Fig 1. Graphical abstract

Acknowledgement

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O33

Artificial Intelligence-Aided Massively Parallel Spectroscopy for Bioaffinity Assays and Droplet Microfluidics

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Summary

Single-molecule heterogeneous immunochemical assays with photon-upconversion labels (UCNPs) appeared as an ultrasensitive advancement of immunochemical methods possessing femtomolar detection limits [1]. However, the method relies on separation on solid surfaces, which complicates its adaptation for microfluidic systems. Here, we discuss massively parallel spectroscopy (MPS) as a new tool for single-molecule bioaffinity assays in a free dispersion without a need for any separation [2]. Compared to cross-correlation spectroscopy – a method, that detects single-molecules by confocal detectors, MPS operates in 1000 times larger detection volumes. This provides opportunities for faster analysis and low detection limits. To investigate the capabilities of MPS in bioaffinity assays, bioconjugated UCNPs with excitation in the near-infrared region (976 nm) are prepared as a model. The UCNPs are doped with either Tm^{3+} or Er^{3+} providing virtually background-free emission at 450 and 802 nm or 554 and 660 nm, respectively. These UCNPs are conjugated to biotinylated bovine serum albumin (Tm^{3+} doped) or streptavidin (Er^{3+} doped). The MPS data are processed by a specialized convolutional neural network, and the limit of detection (1.6 fmol L^{-1}) and linearity range (4.8 fmol L^{-1} to 40 pmol L^{-1}) for the bioconjugated UCNPs are estimated [2,3]. MPS is then used to observe the bioaffinity clustering of bioconjugated UCNPs. This observation is correlated with native electrophoresis and bioaffinity assays on microtiter plates. A competitive MPS bioaffinity assay for biotin with a limit of detection of 6.6 nmol L^{-1} is developed. MPS in complex biological matrices (cell culture medium) is performed without apparently increasing background. Compatibility with polydimethylsiloxane microfluidics is demonstrated by recording MPS from microdroplets containing UCNPs, which pass through a detection area in the channel of the microfluidic chip.

Acknowledgement

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O34

3D Made Electrochemical Sensors

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Summary

Making electrochemical, potentially disposable, ion-sensors requires application of electronically conducting substrates, e.g. screen printed electrodes. The latter however, is not always optimal choice. The aim of this work was to investigate possibility of making electrochemical sensors using 3D printing or drawing.

Electrochemical sensors require the presence of electronically conducting track, that has to be typically isolated from solution and at some part modified by a receptor layer. A common alternative for glassy carbon supports are screen printed electrodes. The latter were proven successful in many applications, however, they are not best suited for longer (hours) time scale sensor contact with sample due to spontaneous hydrolysis of materials used to printing. This process is leading to change of the properties of the conducting layer, formation of ions which is a problem in case of ion-selective sensors.

The alternative approach can benefit from 3D printing of the conducting track. The hot melt approach used allows application of polymer rich in the presence of a conducting material, e.g. carbon to prepare conducting track. At the same process electrically non-conducting polymer to prepare insulation (from solution) can be used. The problems related to spontaneous changes of support material are mitigated, an additional advantage is lack of constrains with respect to geometry of the sensor. The unique advantage of the proposed approach is, among others, possibility of using properties of the polymer applied to prepare the 3D support to help to assure excellent adhesion of the receptor layer to the conducting substrate, to minimize variation of recorded signals. The sensors obtained using this approach are characterized with high stability of performance in time, analytical parameters well comparable with those of classical sensors, as shown on example of potassium sensors [1]. This can be an attractive alternative for making disposable sensors even at low resources condition.

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O35

From Separation to Treatment: Development of a Microbead-based Extracorporeal CTC Capture Platform

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Summary

Tumor derived circulating cells (CTCs) play a key role in the forming of metastases, which process is majorly responsible for the death of cancer victims. Invasive diagnostic methods, like surgical manipulation, radiotherapy or systemic cancer treatments can cause the dissemination of CTCs and even promote the survival of malignant cells. To prevent further progression, metastatic cancer could be stopped from developing by eliminating circulating cells from the bloodstream. Based on existing extracorporeal treatments, we developed a platform technology, which is capable to specifically capture EpCAM-positive tumor cells by immobilizing anti-EpCAM (CD326) covalently onto the surface of chemically altered glass microbeads. A laboratory scale system has been designed and used for the investigation of the capture efficiency of the proposed technology by utilizing HTC116 cell spiked model media. Surface pretreatment was characterized by goniometry while the capture performance was monitored by flow cytometry and fluorescent microscopy. The demonstrated 30,000 circulating tumor cells per gram bead specific capture ability corresponds to the capture capacity of more than 15 million cells in case of an average volume hemoperfusion cartridge during a two-hour treatment. This capture efficiency and throughput allow the therapeutic utilization of the proposed technology and may be used for the mitigation of metastatic cancer death. After further developments, the presented platform technology could be an additional tool to existing treatments.

O36

Towards Immunoglobulomics - IgG, IgA and IgM Fc Profiling by Light Chain Affinity Capturing by Nano-LC-MS

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Summary

The constant domain (Fc) of antibodies is responsible for the binding to effector cells and thereby triggering downstream immune responses. However, antibodies exhibit post-translational modifications (PTMs) with glycosylation as one of the most important PTM which can modulate these immune responses. Until now endogenous antibodies and their PTMs are characterized using bottom-up approaches resulting in a loss of information on the combination of multiple PTMs. Due to new developments in mass spectrometry, intact or middle-up protein analysis are increasingly applied for glycoform characterization, since it provides comprehensive structural information compared to bottom-up analysis. In middle-up analysis, antibodies are cleaved via specific proteases into two subunits, Fc and variable domain (Fab). So far, only IgG has been studied using middle-up approaches. Therefore, endogenous IgGs are captured by FcXL beads which bind to the Fc of IgG, followed by a hinge-region cleavage by IdeS and elution of the Fc subunits under acidic conditions. In this project, we have developed a middle-up analysis platform for sequential Fc profiling of IgG, IgA and IgM. To capture different antibody isotypes a light chain affinity capturing, using a mixture of kappa and lambda light chain beads, was established. After capturing, the Fc/2 subunits of IgG, IgM and IgA1 were sequentially released by specific proteases directly providing the Fc portions and eliminating any elution step. The Fc/2 subunits of each isotype were individually analyzed by nano-RPLC-MS. Comparing the light chain capturing and traditional FcXL approach no bias for IgG Fc/2 profiles could be observed. For IgM and IgA Fc/2 subunits very complex glycosylation profiles containing multiple N-glycosylation sites were detected which were annotated by integrating the middle-up and site-specific bottom-up information. Next to glycosylation, other modifications such a C-terminal tyrosine truncation were observed. In addition to Fc/2 subunits, the corresponding joining chains from IgA and IgM were detected and different glycoforms could be annotated. The proposed method was applied to characterize three independent donors resulting in different profiles, therefore illustrating the potential of the approach to study antibody Fc/2 changes.

O37

Ultra-Miniaturized Weak Affinity Chromatography Coupled with Mass Spectrometry (nano-WAC-MS) as a Powerful Screening Strategy of Native Membrane Proteins in Fragment Based Drug Discovery: Adenosine Receptor as a Case-Study

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Summary

Fragment-based drug discovery (FBDD) is a structure-guided approach in which the first screening step (against a target protein) aims to identify small binding molecules ("hits") with potential for subsequent evolution into high-affinity ligands ("drug-like" compounds). Due to their inherent small size, these binding molecules (called fragments) have a weak affinity (µM to mM range) for the target. Although several biophysical techniques are available for fragment screening of soluble proteins, none are suitable for native membrane proteins such as G protein-coupled receptors (GPCRs), which are important targets for therapeutic agents. The key criteria for membrane protein fragment screening are low protein consumption (very difficult to produce proteins), unbiased conformational states and speed (low stability proteins).

Here we propose a strategy that combines the preparation of nanodisc-embedded GPCRs and the ultra-miniaturisation of weak affinity chromatography coupled with mass spectrometry.

The preparation of home-made affinity nanocolumns (less than 1 µL in volume) is proposed according to a "generic process" (i.e. applicable to any membrane protein): (i) in-situ synthesis (75 µm i.d. capillary) of a new highly hydrophilic monolith to minimize non-specific interactions [1], (ii) in-situ grafting of streptavidin (resulting in the so-called generic columns), (iii) in-situ capture of the biotinylated nanodiscs containing the receptor. The full characterization of affinity nanocolumns by frontal affinity chromatography is presented in terms of total target density (approximately 100 pmol/µL, i.e. less than 1 µg per column, an unrivalled protein consumption), active target density (approximately 92 pmol/µL) and low non-specific binding. The coupling of the affinity nanocolumns (flow rate 200 nL/min) to a nanospray mass spectrometer was then optimized by addition of a make-up flow (600 nL/min), resulting in a robust coupling.

Finally, the results of a fragment screening against the adenosine receptor AA2AR (50 fragments/injection) are presented and the relative advantages of different types of "control columns" are discussed.

Acknowledgement

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O38

Unravelling Functional Changes in Antibody Proteoforms Using Affinity CE-MS

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Summary

Antibodies recruit immune responses via interaction with different Fcγ receptors (FcRs). These interactions are strongly influenced by structural features including glycosylation. Unfortunately, common approaches, such as SPR provide an overall affinity response for all different glycoforms and assessment of their individual binding require tedious production or enrichment of specific forms. In particular, assessment of the influence of Fab glycosylation in binding represents a huge challenge due to the complexity of generating homogeneous forms.

In our lab, we have exploited the capabilities of mobility-shift affinity CE-MS to study the binding of antibodies and FcRs in a proteoform-resolved fashion. To this end, the FcR receptors were added to the background electrolyte whereas the mixture of antibody glycoforms were injected in the CE. We will show that the approach is able to determine the relative binding of different glycoforms based on the shifts on their mobility. For FcγRIIIa the obtained affinity profiles were benchmarked towards affinity LC using the same constructs providing similar results. Due to low amounts of receptor required, the developed affinity CE-MS platform was ideal for testing a large variety of FcRs, namely FcγRIIa, FcγRIIb, FcRn and FcγRIIIa and including different allotypic variants. As anticipated, Fc glycosylation was key for the binding. Hemiglycosylated antibodies showed strong decrease in the binding towards the FcγRs while non-Fc glycosylated forms showed near no binding. Fc-glycoforms behaved differently between receptors with clear differences for afucosylated and high mannose variants. Interestingly, different receptor allotypes also revealed some glycan-sensitive differences. Furthermore, we explored the potential of the approach to investigate the influence of Fab glycosylation in FcR binding. Our results showed an altered binding for the Fab-glycosylated variants. As Fab glycans are far from the binding site, the change in binding properties most likely correlates to a change in protein conformation in the interaction surface.

O39

Behavior of Weak Electrolytes in the Diffuse Layer of the Double Layer

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Summary

The structure of the double layer on the boundary between solid and liquid phases is described by various models. We have proposed a mathematical model of diffusion and electromigration in the diffuse layer of the double layer considering the high deviation from electroneutrality. The liquid phase is allowed to be a solution of weak multivalent electrolytes of any valence and any complexity. The mathematical model joins together the Poisson equation, the continuity equation for electric charge, the mass continuity equations, and the modified G-function [1].

In the present paper we have additionally implemented the Navier-Stokes equation into the model to describe the electroosmotic flow (EOF) acting inevitably in electromigration separation methods. The model is solved by the numerical simulation software COMSOL and allows to calculate (i) the velocity profiles of liquid, (ii) volume charge density, (iii) electric potential, (iv) deviation from electroneutrality and (v) concentration profiles of all ionic forms of all electrolytes in the diffuse part of the double layer.

The model can depict, e.g., a pH drop in the solution of a multivalent weak electrolyte, here the phosphate buffer, in the diffuse layer in the vicinity of a silica wall, see Fig.1.

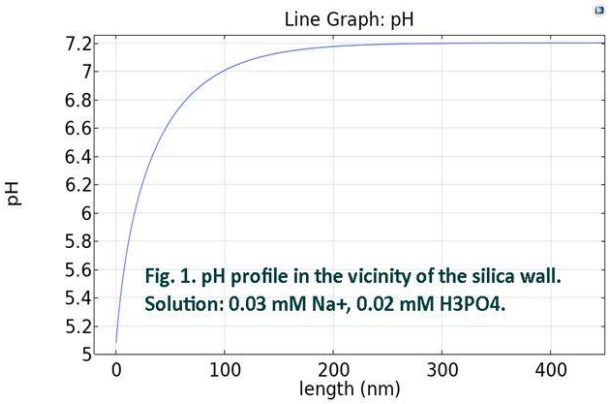

Fig. 1. pH profile in the vicinity of the silica wall. Solution: 0.03 mM Na+, 0.02 mM H3PO4.

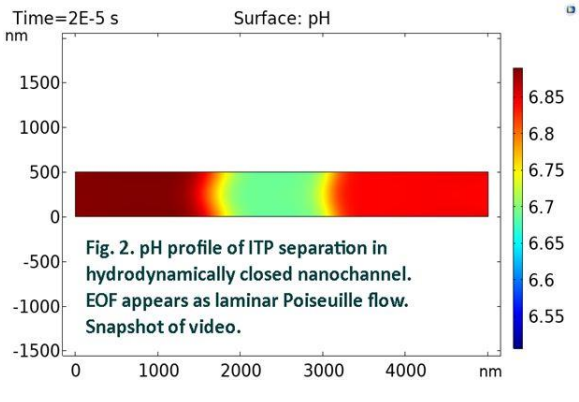

Fig. 2. pH profile of ITP separation in hydrodynamically closed nanochannel. EOF appears as laminar Poiseuille flow. Snapshot of video.

When a tangential electric field is applied, as it is in channels of electromigration methods, we can calculate spatial profiles of all significant parameters of electromigration, such as, e.g., pH profiles, as demonstrated in Fig. 2.

Due to the electrical double layer the composition of electrolyte solutions near surfaces is different when compared with the rest of the solution. Practical examples and video sequences are presented to demonstrate behavior of the diffuse layer. The presented model is applicable in complex biological systems for the calculation of all quantities of solutions in the vicinity of cell membranes or tissues.

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O40

Refractive Index Detector Based on a Young Interferometer for Electroseparation Methods

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Summary

Detectors based on absorption [1], fluorescence [2], and refractive index (RI) [3] have been reported for electroseparation methods. RI detectors are universal but are less sensitive than absorption and fluorescence detectors. The detection limit of RI detectors is ≈10^{-6} M for proteins, corresponding to a RI resolution of ≈10^{-5} refractive index units (RIU).

In this presentation, I will describe a RI detector based on an optofluidic Young interferometer (YI) with two wedge-shaped light beams that passed through the depth of sample and reference microfluidic channels where electroseparations occurred. The two beams then overlapped with each other to produce interference fringes that contained information on the RI difference between each point in the sample and reference microchannels. The RI difference is related to the concentration of analyte bands/peaks. Our YI provided whole channel visualisation of electroseparation methods with a spatial resolution of 295 µm along the length of microchannels.

The RI resolution of our YI was 2.04 × 10^{-6} RIU per mm of the optical pathlength and can be tailored by changing the depth of microchannels. By using a Fresnel biprism with a cylindrical lens to generate two virtual slits, we obtained high quality, high intensity interference fringes without diffraction effects from the channel edges, which in turn allowed short camera acquisition times. Further, we developed a fast Fourier transform algorithm for real-time analysis of interference fringes, and obtained a temporal resolution of 2 s. Finally, we applied our YI to study electrophoretic transport [4] and electrophoresis combined immunoassays of exemplar proteins, and pre-concentration of oligonucleotides by isotachophoresis.

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O41

Characterization of Nanoparticles in Mixtures by Capillary Electrophoresis and Taylor Dispersion Analysis Hyphenated to ICP-MS

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Summary

In this contribution, a novel methodology will be introduced for advanced characterization of the behavior of nanoparticles (NPs) in their mixtures in aqueous high-ionic strength conditions. Our approach utilizes Taylor dispersion analysis (TDA) connected to inductively coupled plasma mass spectrometry (ICP-MS) to probe metal-derived nanoparticles. The coupling of TDA and ICP-MS relies on our in-house developed interface connecting a capillary electrophoresis instrument with ICP-MS [1]. With this methodology, we are able to distinguish between magnetic Fe_3O_4-based, Au and Ag NPs. We can accurately determine their hydrodynamic size, and elemental composition in their mixtures and different pH (2.5, 4.5, 7.5, and 9.5) [2]. Moreover, the application of an electric field for a short period (e.g., 45 s) before TDA allows for the separation of ions from NPs. This approach was successfully applied, for instance, to distinguish between silver ions and Ag NPs at physiological pH. This distinction is of significant importance, especially in toxicity studies involving Ag-based products. Moreover, the setup can also be used to target protein-modified ultrasmall fluorescence NPs with core size of 1.7 nm in phosphate-based media, where traditional characterization techniques often fail. The future perspectives in the view of studies of interparticle interactions and self-assembling processes will be also mentioned.

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O42

Fully Autonomous Processing and Analysis of Dried Blood Spots Collected by Volumetric Absorptive Microsampling

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Summary

Dried blood spot (DBS) processing is usually based on sub-punching a small DBS section and might have a direct consequence on quantitative DBS analyses [1]. Simple, flexible, and user-friendly volumetric absorptive microsampling (VAMS) devices were, therefore, introduced for accurate DBS collection recently. These devices transfer an exact blood volume onto a sampling sorbent, use the whole DBS for subsequent processing/analysis, and have proven to be suitable for remote patient-centric DBS collection and quantitative clinical analysis [2].

DBS processing typically requires transformation from a dry to a liquid sample, however, commercial instruments for DBS processing are not compatible with the new VAMS devices. All clinical assays of DBSs collected by VAMS are, thus, based on manual or semi-automated processing and analyses. They are tedious, labour- and time-intensive, prone to processing errors, and there is an urgent quest for the development of simple and cheap solutions for unmanned pretreatment and analyses of DBSs collected by VAMS.

One such solution was recently developed in our laboratory. A single, off-the-shelf capillary electrophoresis (CE) instrument was employed for executing all tasks of the analytical protocol and the actual contribution will summarize fundamentals and the most recent applications of the novel concept for the autonomous quantitative CE analyses of DBSs collected by VAMS. The proposed concept enabled high-throughput analyses (several hundred DBS samples per day) and its suitability was exemplified by the determination of endogenous clinical markers (e.g. organic ions and amino acids) in DBS samples. This concept represents a progressive analytical tool for personalized healthcare, screening populations at risk, and can be also useful in critical (e.g. pandemic) situations. Besides, it can be easily extended to the determination of a wide range of analytes in various dried biological materials and might, thus, play an important role in clinical, toxicological, and forensic analyses in the future.

Acknowledgement

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O43

Electrochemical Immunoassay-based Sensors Towards Point-Of-Care Diagnostics: Recent Progress and Challenges in Multiple Biomarkers Detection

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Summary

Specific biomarkers detected in body fluids and tissues are crucial elements for disease diagnosis, prediction of its progression, and evaluation of personalised treatment effectiveness. Although the detection of a single specific biomarker is sufficient, the simultaneous detection of multiple disease-associated biomarkers (cancer biomarkers, inflammatory proteins, etc.) significantly improves the diagnostic power of biomarkers in terms of specificity and sensitivity from the Receiver operating characteristic curve (ROC) [1].

Electrochemical biosensors meet the criteria of Point-of-Care (POCT) devices and represent the alternative to a commonly used instrumentally and time-consuming laborious diagnostic methods (ELISA, immunohistochemistry, PCR). Biosensors are a group of cheap, portable, sensitive, quick-responsive and accurate devices enabling complete automation. Among electrochemical biosensors, immunosensors, which are based on the principle of highly specific affinity interactions between antigens and two corresponding antibodies identical with ELISA method, are therefore of interest in clinical diagnosis. Recently, the most common enzyme-based immunosensors have been replaced by nanomaterials-based sensors to overcome the shortcomings of enzymes. From electroactive nanoparticles, gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), quantum dots (QDs), carbon nanotubes (CNTs), graphene, and carbon quantum dots (CQDs) are the most commonly used. Moreover, these nanomaterials enable the simultaneous detection of multiple biomarkers since they provide separate, non-interfering and mutually unaffected electrochemical signals.

Here, we present two examples of the development of electrochemical magneto-immunoassay-based sensors for simultaneous stripping voltammetric detection of ovarian cancer biomarkers, namely proteins HE4, CA125, AFP [2,3], and pro-inflammatory biomarkers PTX3 and CALR, as potential predictive markers of preterm labour. Silica nanoparticles (SiNPs) combined with electroactive QDs (CdTe, PbS) were used for site-directed labelling of detection antibodies and providing non-interfering signals. Disposable screen-printed carbon electrodes were used for measurements, enabling the droplet analysis. Cut-off limits gained for detected biomarkers meet the criteria for clinical importance.

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O44

Microfluidic Capillary Electrophoresis for In-line Dual-stage Enrichment and Unattended Sampling: From Instrumental Conception to Bioanalytical Applications

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Summary

In bioanalysis with capillary electrophoresis (CE), we often encounter the challenges of insufficient detection sensitivity (e.g. for detection of biomarkers in biological matrices) and real-time monitoring of target molecules in biological fluids. To address these challenges, we report herein the development of new microfluidic CE setups for i) in-line dual enrichment stages to boost the detection performance in CE and ii) automatic sampling and monitoring of the analyte concentrations from a continuous micro-flow. With the dual-enrichment stages, two completely different preconcentration approaches can be realized in the same capillary, without any loss of pretreated samples. In the first stage, a dynamic magneto-extraction of target analytes on circulating magnetic beads is implemented within the capillary. Then, electrokinetic preconcentration of eluted analytes via large volume sample stacking is carried out to focus them into a nano band, prior to CE separation of enriched analytes. For this purpose, the movement of magnetic beads and analyte's flow inside the capillary was precisely controlled with different push-pull pressure / vacuum controllers and valves that are conventionally employed for microfluidics. Magnetic fields were generated via different magnetic tweezers set along the capillary to allow capture and release of magnetic beads containing enriched analytes. The developed enrichment principle and its associated instrument were demonstrated for CE separation of target double-stranded DNA, with enrichment factors of up to 125. Different ways to integrate the microfluidic CE system with a novel lab-on-valve setup to allow automatic sampling from a continuous flow and analyte separation without any manual intervention are under investigation.

P1

Noncovalent Labeling of Proteins in Sodium Dodecyl Sulfate Capillary Gel Electrophoresis

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Summary

Sodium dodecyl sulfate (SDS) capillary gel electrophoresis stands as a commonly employed technique in molecular biology laboratories and the biopharmaceutical sector for size-based protein separation. In recent times, there has been a notable surge in the development of multicapillary electrophoresis systems aimed at enhancing throughput [1,2]. However, almost all of these systems support fluorescent detection only, necessitating labeling of the sample proteins with a fluorophore [3]. To overcome the laborious pre-separation derivatization process, we have developed an innovative in-column labeling strategy using a non-covalent labeling dye that is incorporated as a key ingredient in the gel-buffer system. This approach obviates the need for any additional pre- or post-column derivatization steps. Furthermore, a comprehensive theoretical treatment has been conducted to elucidate the underlying principles of this novel online labeling process, including the impact on the electroosmotic flow. Evaluation of the influence of ligand concentration on both effective and apparent electrophoretic mobilities, peak area, and resolution was undertaken in alignment with theoretical considerations. Conversely, concerning the resolution of a biopharmaceutical protein test mixture comprising intact and subunit fragments of a therapeutic monoclonal antibody, including the non-glycosylated heavy chain, the ligand concentration was optimized in the background electrolyte.

Acknowledgement

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P2

Electrophoretic Separation of DNA Fragments in Deuterated Water

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Summary

Separation of DNA fragments according to their length is crucial for sequencing and other analyses. This is often performed via electrophoresis with fluorescence detection using various staining dyes. The intensity of the fluorescence signal can be affected by the physicochemical properties of the solvent (background electrolyte in case of electrophoresis), such as the temperature, pH, polarity, and other properties of the solvent. Compared to regular water, deuterated water provides lower fluorescence quenching and lower absorbance of the excitation radiation, which should result in a higher fluorescence signal [1]. This effect has been observed for various fluorophores. Deuterated water also affects the CE separation. It can decrease electroosmotic flow, thus improving the electrophoresis separation in electrolytes containing sieving matrices. This work aims to study the influence of heavy water on separation and detection limits for DNA electrophoresis on a chip and in a capillary.

Selected DNA dyes were first characterized using a spectrofluorometer. Fluorescence intensities of the stained DNA in normal and deuterated water were compared to choose the most affected dyes for further electrophoretic experiments. The Agilent 2100 Bioanalyzer (red excitation) and 5200 Fragment Analyzer (blue excitation) were used for separations. The separation poly(n,n-dimethyl acrylamide) gels containing background electrolytes were prepared for both instruments in light and heavy water. For comparison, the gels from commercial kits were dried using vacuum and lyophilization, redissolved in normal and deuterated water, and used to separate DNA ladders labeled with selected fluorescent dyes on both instruments. An undocumented increase in the fluorescence signal of selected DNA-dye complexes was observed in several cases. Some experiments also confirm previously published [2] observations about longer separation times and higher resolution in heavy water-based solutions.

Acknowledgement

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P3

Deterministic Lateral Displacement for Separation in Microscale: Particle and Microbial Cell Analysis

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Summary

Microbial contamination in industry presents a public health risk and cause product deterioration resulting in economic losses. Standard analysis relies on microbial separation from background matrix and identification by culture-dependent or -independent methods. However, these procedures are laborious, time- and reagent-consuming. Conversely, microfluidics allows manipulation of small liquid volumes at microscale, thus allowing microorganisms separation at high throughput. Here, we evaluated separation in microfluidic channel using particles and cells.

The proposed microchannel relies on deterministic lateral displacement (DLD) method allowing size-based separation [1] with 3.36µm critical diameter (Dc) [2]. Theoretically, particles above Dc are displaced laterally, while particles below Dc are non-displaced. The microchannel was designed with AutoCAD® and fabricated in polydimethylsiloxane (PDMS) using soft-lithography. DLD array configuration was confirmed with scanning electron microscopy. Then, the microchannel was used for the separation of fluorescence polystyrene microparticles (D=2µm; D=6µm) and bacterial (*Escherichia coli*) and yeast (*Saccharomyces cerevisiae*) cells suspended in sodium dodecyl sulfate (SDS) supplemented with dextran (40,000 g/mol) at 0.2 µL/min (Q_{inlet}=0.001m/s; Re = 0.11, laminar flow). Density of dispersant (ρ=1.047g/cm3) matched density of microparticles (ρ=1.05g/cm3) and demonstrated constant viscosity (η=0.008 Pa.s) at varying shear stress, confirming Newtonian fluid behaviour. Finally, trajectories of microparticles and cells were visualized through streak line photography, followed by quantification of displaced and non-displaced modes to evaluate separation efficiency. Qualitative data revealed that 2 µm particles and *E. coli* cells were non-displaced, while 6 µm were displaced. The *S. cerevisiae* showed displaced and non-displaced trajectories. Moreover, quantitative analysis confirmed that 88.24% of 2 µm particles were non-displaced, while 96.55% of 6 µm particles were displaced. Similarly, 89.29% of *E. coli* were non-displaced, while 88.89% of *S. cerevisiae* were displaced. Concluding that particle and cell trajectories were consistent with the theoretical Dc (3.36µm), which demonstrates the possible applicability of this strategy to separate yeast from bacteria.

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P4

Identification of Mephedrone Synthesis Reagents using CEMs

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Summary

One of the most commonly used synthetic cathinone is 4-methylmethcathinone (mephedrone, MEP) [1]. There are several synthesis pathways for mephedrone but the production of MEP from 4-methylpropiophenone (MPP) is most commonly used because this primary precursor is commercially available over the Internet, and the synthesis does not require complex and professional laboratory equipment. Interestingly, mephedrone could theoretically be used in the synthesis of pseudoephedrine (PEP) or ephedrine (EP), as it was speculated [2].

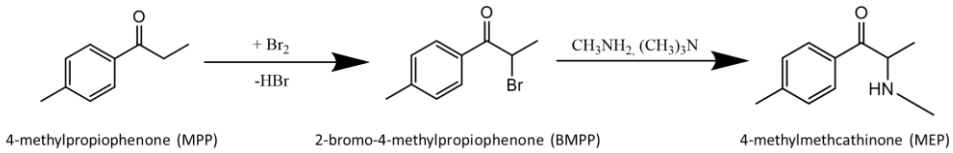

Fig. 1. Synthesis of mephedrone from commercially available substances.

This work aimed to develop an approach for the identification of these substances by combining two methodologies - CE-DAD and CE-C^4D. The analytes can be classified based on their chemical structure, with some being neutral (MPP and BMPP), while others have the potential to ionize in a solution (methylamine - MA, trimethylamine - TMA, EP, PEP, and MEP) or absorb radiation in the UV range (MPP, BMPP, MEP, EP, and PEP). To accommodate these differences, two distinct methods have been proposed. These include a portable capillary electrophoresis system with C^4D detection and a commercial, bench-top capillary electrophoresis system. The results demonstrated successful methods optimization, with peak resolution for EP, PEP, and MEP achieved under specific background electrolyte conditions (CE-C4D: BGE M; CE-DAD: BGE U). The method's repeatability was evaluated, and it showed satisfactory intra-day and inter-day precision for MEP, EP, and PEP for both methods combined into the efficient qualitative protocol for mephedone profiling.

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P5

Analysis of N-linked Glycans by CE/LIF Using Various Glycoproteomic Protocols

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Summary

Glycosylation occurs in the production of many protein-based biologics and can have a major impact on their biological, clinical and pharmacological properties. Analysis and characterization of glycans is an increasingly important area for both the medical and biopharmaceutical industries as it provides indispensable information, thus understanding glycan profiles helps in the development of new therapeutic agents [1].

Here, we present several modified glycoproteomic protocols that differ, for example, in the purification step, the enzyme used, and the digestion procedure with the aim of efficient and sensitive analysis of *N*-linked glycans. Ribonuclease B of different concentrations was chosen as a model glycoprotein.The most successful protocol was based on DTT reduction during thermal denaturation. In addition, two reducing agents (DTT and TCEP) were compared, but no significant results were observed.

Derivatization of the released glycans was performed using Cascade Blue hydrazide (8-(2-hydrazino-2-oxoethoxy)pyrene-1,3,6-trisulfonic acid trisodium salt), as a more reactive analogue of 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt followed by CE/LIF analysis [2].

Acknowledgement

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P6

Tools That Improves Concentration Sensitivity in Capillary Electrophoresis-Frontal Analysis for Affinity Studies

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Summary

Various pharmacokinetic processes such as absorption, distribution, metabolism and elimination are significantly affected by binding between drug and plasma proteins since these proteins, especially human serum albumine (HSA), act as reservoirs and also mediate drug transport. The characterization of that interaction is therefore crucial in drug development. Understanding the mechanism of interaction involves the determination of key parameters such as the binding constant (K_b), the stoichiometry of the interaction and also identifying the binding sites for drugs on plasma proteins. One of the methodes for determining K_b and number of binding sites is capillary electrophoresis-frontal analysis (CE-FA). CE-FA belongs to more robust affinity modes of capillary electrophoresis (CE). CE is generally a well-known techique for its high resolution, speed of analysis and low reagent consumption but it often faces a challange as is low concentration sensitivity due to narrow capillary and ultraviolet-visible (UV-VIS) detection. The focus of these studies was to improve the concentration sensitivity of CE-FA affinity interaction experiments using three different approaches. One of them is combination of CE-FA with mass spectrometry detection for evaluation binding between HSA and antidiabetics. This set-up provided more sensitive experiments with almost three times lower limits of detection than with the usual UV-VIS detection. Another two approaches focused on HSA-salicylic acid as a model system. One utilized a contactless conductivity detector, which provided sixfold lower limits of detection. The next combined UV-VIS detection with on-line preconcentration technique, which improved sensitivity by seventeenfold compared to conventional method. These three different tools thus offer improved concentration sensitivity and versatility for more efficient drug-protein affinity studies.

P7

Separation Conditions for Oligonucleotides by CE-MS

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Summary

Capillary electrophoresis (CE) is a valuable tool in the analysis and characterization of (bio)molecules and their interaction [1,2]. CE is particularly useful for the analysis of polar and charged molecules, which makes it a complementary technique to other methods, such as RP-HPLC. As an inherently miniaturized technique, its advantage is the very low consumption of sample, which is in the order of nanoliter volumes for a solution of analyte in the range of micromolar concentrations per one analysis. CE is very compatible with a mass spectrometer because it allows for highly sensitive and information-rich detection. The online coupling of CE and MS is established through an appropriate ESI-based interface, such as a nanoflow sheath-liquid interface [3,4].

The analysis of two different length mixtures of standard oligonucleotides was conducted by CE-MS. The oligonucleotides were separated as anions in acidic BGEs and analyzed by a TOF-based MS in positive mode. Volatile ammonium acetate and formate background electrolytes (BGE) were in focus for the suitability with the MS. Different pH values were tested to achieve the best results. The highest impact on separation was observed in the 2 – 3.5 pH region thanks to partial deprotonation of nucleobases due to their different pK_as. Furthermore, small amounts of EDTA in the buffer proved to be helpful for the improvement of the peak shape; a sheath liquid containing a higher amount of formic acid helped with the ionisation of longer-sequence oligonucleotides; and minimising the amount of ammonia present in the system significantly decreased adduct formation. In the end, a good separation was achieved, proving the good compatibility between the CE separation and the MS detection.

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P8

Microfluidic Capillary Electrophoresis - Mass Spectrometry for Rapid Charge-Variant and Glycoform Assessment of Monoclonal Antibody Biosimilar Candidates

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Summary

During the early development of a monoclonal antibody (mAb) biosimilar, multiple cell lines are set up to increase the likelihood of identifying a cell line possessing the desired properties. During this process, mAbs produced from each candidate cell line must undergo screening based on critical quality attributes (CQA), including glycosylation profile and charge heterogeneity [1]. Typically, various methodologies such as released N-glycan analysis, cation exchange chromatography (CEX) or capillary electrophoresis (CE) with UV detection are employed to obtain CQA information. However, the sample preparation of the released N-glycan method is laborious [2], and both the CEX-UV and CE-UV methods only yield the relative quantification of charge variants [3]. Therefore, there is a need for a single method that can address these challenges, enabling the rapid and comprehensive analysis of generated mAb samples.

To address this need, we developed a microfluidic capillary electrophoresis - mass spectrometry (MCE-MS) method for the rapid screening of mAb biosimilar candidates. Initially, we evaluated the background electrolyte and found that the addition of dimethyl sulfoxide enhanced the MS signal by more than 60-fold. The method performance was assessed by mimicking an early-stage cell line selection using five biosimilar candidates alongside their originator mAb. Well-separated charge variants were obtained for all samples. Based on the deconvoluted mass spectra, both the basic and acidic charge variants could be identified, in addition to the glycosylation profiles of each sample. Based on this information, a similarity score was implemented to select the best clones for further process development.

The robustness of the MCE-MS method was further highlighted through a transferability study conducted at two separate laboratories. To conclude, the MCE-MS method demonstrated here has the potential to become an indispensable tool for early-stage clone screening and process development of biosimilars.

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P9

Unlocking New Perspectives: Fluorinated Sugars and Their Enhanced Lectin Binding Abilities

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Summary

The interaction between carbohydrates and lectins is crucial in a variety of biological processes [1]. Modifying carbohydrates, such as through fluorination, has emerged as a promising strategy to enhance their binding affinity to lectins, improve metabolic stability, and create effective probes for 19F NMR analysis. Although previous studies have demonstrated that fluorination significantly influences the binding profiles to biomolecules, the specific effects of monofluorination on the affinity of monovalent carbohydrate ligands have been ambiguous, often resulting in decreased binding [2]. This study details the synthesis and binding analysis of monofluorinated derivatives of N,N'-diacetylchitobiose (GlcNAcβ4GlcNAc) and LacdiNAc (GalNAcβ4GlcNAc) with wheat germ agglutinin (WGA), a plant lectin known for its role in glycan recognition and crop protection. Our aim was to synthesize methyl β-glycosides of N,N'-diacetylchitobiose and LacdiNAc, applying monofluorination systematically at all potential hydroxyl positions to assess their binding affinity to WGA. We aimed to determine how fluorination at specific sites influences the binding profile, using enzyme-linked lectin assays (ELLA), isothermal titration calorimetry (ITC), and advanced NMR techniques to decipher the molecular basis for observed changes in affinity to WGA. This approach aimed to investigate the potential of fluorinated glycomimetics as high-affinity lectin ligands and as 19F NMR-active probes. Significant results were obtained, indicating that fluorination in particular hydroxyl positions can dramatically change the binding affinity of carbohydrate ligands to WGA. Remarkably, 6'-fluorination of chitobioside led to a ten-fold increase in binding affinity to WGA, an enhancement of unprecedented magnitude attributed to strengthened CH/π interactions following deoxyfluorination. Furthermore, the study provided the first characterization of the binding profile of LacdiNAc to WGA, showing that its 4'-fluoro derivative binds more strongly than the unmodified compound. These discoveries highlight the critical role of conformational preorganization in lectin binding and open new avenues for the development of glycomimetic drugs and diagnostic tools.

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P10

Study of Selected Analytes in in Vitro Fertilization Culture Medium by Capillary Electrophoresis

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Summary

The infertility rate of couples in reproductive age is 15 % and this number is still rising [1]. Due to this fact, assisted reproduction technologies are increasingly in demand, particularly *in vitro* fertilization. Embryos that have reached the blastocyst stage after *in vitro* cultivation are then transferred into the uterus, with multiple embryos usually being transferred at the same time. However, this leads to multiple pregnancies, which are risky for the mother and the fetus as well. On the other hand, singleton pregnancies are much safer and therefore the main aim is to transfer only single embryo with the highest potential to develop into a healthy fetus [2].

Clinical studies focus on the non-invasive assessment of this potential based on culture media analysis, as its composition reflects embryo's metabolism and development. Analysed components are mainly pyruvate (Pyr), lactate (Lac) and amino acids. These analytes are metabolised differently during embryo's development and based on their concentrations it is possible to assess whether the embryo has developed into high-quality blastocyst [3]. However, it is important to distinguish if these changes in culture media composition are due to the embryo metabolism or media aging and possible degradation of analytes.

The aim of this study is to monitor the stabilities of Pyr, Lac and amino acids in culture medium under different storage conditions. The chosen method was capillary electrophoresis, that can be performed in several operating modes and also combined with many types of detections. Pyr and Lac were determined specifically with capillary zone electrophoresis mode using contactless conductivity detection and amino acids with micellar electrokinetic capillary chromatography mode using laser-induced fluorescence detection.

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P11

Multi-Material 3D-Printing Fabrication of Microfluidic Devices

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Summary

In the context of the fabrication of microfluidic devices, additive manufacturing is very promising, as it enables the generation of objects with high structural complexity. As expected, the use of 3D printing has accelerated in recent years, due to improvements in printers, the development of new materials, and the introduction of new printing methodologies. Many groups have demonstrated the capabilities of 3D printing for the production of microfluidic devices, including the comparison of different printing techniques. Although microfluidic devices can be successfully created in a single material substrate, the use of multi-material printing allows the inclusion of features to the devices, promoting more functionality to them. In this work we describe an alternative approach to prepare multi-material objects and microdevices by DLP 3D printing by changing the resins in a single vat.

The proposed instrumentation can be easily incorporated in commercial printers. The structure of our printer is based on the Bene5 mono msla kit from Nova3D. Assemble of resin vat, peristaltic pumps, resin reservoirs, inclination system, Z-motor, and ultraviolet illumination system were developed in our lab, including open-source solutions for hardware and firmware. Basically, we developed an inclination vat system to automatically replace the substrate resins. This procedure can be added to the G code for automation, or alternatively can be conducted manually by using a touch screen. We have developed our own resin formulations to include PEDOT:PSS or copper nanoparticles (CuNP) to prepare conductive tracks, dyes to prepare fluorescent or colorful materials, and porogenic agents to prepare porous resins.

The proposed multi-material 3D printing system is based on the replacement of resins in a single vat, which was designed to optimize the resin exchange without cross-contamination and resin loss. So, we developed a 30 degrees vat inclination system to help the replacement of resins and cleaning the object and vat, to remove any residuals of the former printed layers. A perfect adhesion was achieved between layers of different materials. By using this protocol, we were able to print objects up to four materials, which include microfluidic devices containing embedded electrodes for contactless conductivity detection (C^4D) and microchannels as narrow as 42 µm.

The proposed multi-material 3D printing instrument could be successfully applied on the preparation of complex microfluidic devices containing diverse features. We are particularly interested on the incorporation of conductive tracks for the integration of electrochemical sensors, fluid control and voltage application aiming electrophoretic separations.

Acknowledgement

FAPESP, CAPES, CNPq.

P12

Determination of Unbound Fraction of Selected Antiepileptic Drug Using Ultrafiltration and LC-MS method

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Summary

Total plasma concentrations are commonly used for therapeutic drug monitoring of antiepileptic drugs (AED). On the other hand, drug activity and at least some of adverse effects depend on the unbound fraction of a drug in the bloodstream. The determination of free (unbound) concentrations by ultrafiltration is expected to reflect the effective drug concentration. Further, saliva is considered as a plasma ultrafiltrate, thus referring to free drug concentration. Therefore, the aim was to develop a method for the measurement of total and unbound AED concentrations in plasma and correlate them to saliva AED concentration. As a model drug, lamotrigine (LTG) was chosen since it is one of the most widely used AED and is moderately bound to albumin (~55 %).

The samples of saliva and plasma were obtained from the patients treated at the Neurological clinic of the St. Anne´s Hospital, Brno upon subscription of Informed Consent. The study was approved by the Ethics Committee of St. Anne´s Hospital in Brno (Approval No. 3V/2023). The plasma samples for the determination of unbound LTG concentrations were processed by ultrafiltration and liquid-liquid extraction by ethyl acetate. The same extraction step was performed for plasma and saliva samples too. The extraction efficiency of LTG and its internal standard (LTG-13C15N4) was about 90 % for all matrices.

The developed LC-MS method was performed on the Kinetex C 18 Polar column (3x100 mm, 2.6 µm), which was maintained at 35 °C during the whole acquisition period. The mobile phase consisted of solution A (0.1 % formic acid in aqua) and solution B (acetonitrile containing 0.1 % formic acid). Multistep gradient was set in the range from 5 % to 90 % B within 10 minutes. Following elution from the LC column, ESI-qTOF analysis using the maXis impact Bruker instrument enabled identification and quantification of the drug. Analytical data were processed using Compass DataAnalysis 4.1 and Compass QuantAnalysis 2.1 software (both supplied by Bruker). The detector response was linear over the concentration range 0.5-20 µg/ml for saliva samples. Calibration range for ultrafiltrates was from 0.5 to10 µg/ml. The method for saliva matrix was fully validated according to European Medicines Agency guidelines. For other matrices, only linearity, intra-day precision, and accuracy were assessed. All patient samples were processed within a day of their collection, and thus no analyte stability data were acquired for plasma and ultrafiltrate.

All LTG plasma concentrations were within the recommended therapeutical range (3-15 µg/ml). Pilot data show that the concentrations measured in plasma ultrafiltrate and saliva were in close proximity, even though total plasma concentrations are much higher and wildly differ between individual samples. More data must be available to determine the correlation between saliva and plasma concentrations and saliva and ultrafiltrate concentrations.

Acknowledgement

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P13

Sorbentless Dried Blood Spot Sampling for Automated DBS Analysis

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Summary

At present, the majority of dried blood spot (DBS) samples are collected on cellulose-based sampling cards. Recently, novel sampling materials and approaches were developed as attractive alternatives for blood collection. The rationale for their development was the detrimental effect of blood hematocrit on quantitative DBS analysis, the reduced elution recoveries of analytes, and the limited suitability for patient-centric blood collection of standard DBS cards. Moreover, porous sampling sorbents contain random production-borne species that can be released during sample preparation and contaminate the resulting eluates [1-4].

This contribution focuses on the development of a new DBS sampling concept eliminating the three major drawbacks of contemporary DBS collection: blood hematocrit effects, DBS eluate contamination, and retention of target analytes by sampling sorbents. A small drop of blood with an exact volume (e.g. 5 µL) was collected in a specific container (e.g. non-porous plastic CE vial) with no sorbent. The blood was dried directly inside the container, which was fully compatible with commercial CE autosamplers for direct DBS elution and analysis. Several parameters (including DBS collection repeatability, analyte stability in DBS, and DBS eluate contamination) were examined and compared for DBSs collected by the proposed sorbentless concept and various commercial DBS sampling sorbents, demonstrating the superior performance of the former in several aspects. The commercial sorbents exhibited considerable contamination by inorganic and organic ions (e.g. Ca2+, Na+, Mg2+, chloride, sulfate, formate, and acetate), whereas the contamination was eliminated for the sorbentless concept. The stability of selected model analytes (except for lactate) was considerably better in the sorbentless DBS samples, possibly due to the increased microbial degradation of the blood matrix within standard cellulose-based sorbents. Various strategies suitable for sorbentless DBS sampling were suggested offering simple, convenient, economical, and repeatable patient-centric blood collection free from hematocrit effects and contamination. The actual concept is suitable for blood self-sampling as well as for sampling in medical centers, requires mL volumes of capillary blood only, and enables autonomous DBS processing, which makes it highly attractive for future analyses in clinical, therapeutic, and forensic science. Further research will also address the capability of the sorbentless DBS sampling for analytes retained by standard sampling sorbents.

Acknowledgement

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P14

Investigation of the Effect of Maternal Obesity and Gestational Diabetes on the N-Glycosylation of Human Immunoglobulins

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Summary

Obesity during pregnancy increases the risk of developing gestational diabetes mellitus (GDM) causing various negative consequences for both mother and infant. Hence, there is a crucial need to identify early biomarkers with sufficient sensitivity that can also be applied to women not categorized in the high-risk group. Maternally derived IgG passing through the placenta plays a significant role in shaping the immune system of newborns, while in breast milk, it is responsible for mucosal immunity after birth. IgA plays a crucial role in alleviating inflammatory processes, potentially mitigating the adverse effects of maternal obesity and GDM.

N-glycosylation influences many properties of proteins including function and structural features. A distinctive N-glycosylation motif specific to a protein, cell, tissue, or species is formed through the highly regulated activity of multiple enzymes. These glycosylation-controlling enzymes are extremely sensitive to physiological or pathological changes in cell function, possibly making the unique glycan pattern a valuable indicator of the current state of the body.

We analyzed IgG N-glycosylation in serum samples from mothers, children, and maternal milk samples as well as the IgA N-glycome of serum collected from mothers to explore the potential of these complex sugars as biomarkers for low-grade inflammatory conditions, particularly in the context of obesity and GDM. While IgG N-glycosylation is not a proper biomarker to predict GDM, these sugar structures reflect possible inflammation occurring in maternal obesity. On the other hand, the IgA N-linked carbohydrate profile may be a potential biomarker for the development of GDM. The obtained N-glycosylation patterns of milk IgG, on the other hand, indicated that the profiles were influenced by both maternal obesity and GDM. Differences were also observed between IgG N-glycosylation of maternal and child origin, suggesting a selective transport process of these molecules.

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P15

Decoding the Human Seminal Plasma Metabolome: Assessment of the Performance of Different Sample Preparation Strategies

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Summary

The steady decline in the number of births for the past five decades has continued not only due to the changes in sociocultural factors, but also to increasing fertility issues [1]. With more than half of the fertility problems in couples arising from the male partner, traditional semen analysis still fails to diagnose the etiology and mechanisms of such conditions [2]. Therefore, new omics approaches are emerging as an alternative to retrieve more information from seminal liquid and, thus, to better understand and diagnose male sub- and infertility [3].

In the present work, we first compared different sample preparation techniques enabling the analysis of oxylipins in human seminal plasma. These compounds were chosen based on their relevance as signaling molecules. Samples were analyzed using a targeted metabolomics strategy for 88 molecules. Different protein precipitation treatments optionally followed by solid phase extraction (SPE) yielded similar but distinctive profiles. Each protocol was characterized on the base of their alpha diversity, which measures the richness of metabolites within each sample, and beta diversity, which quantifies the differences between two groups based on their composition. Information was represented in a low-dimensionality space (principal coordinates analysis, PCoA) based on Bray-Curtis distances. Our results confirmed the presence of a panel of other oxylipins in addition to the expected prostaglandins. Finally, SPE and solid phase microextraction (SPME) using HLB sorbent were compared aiming at a broader metabolomic seminal plasma analysis. SPE yielded a slightly more diverse profile of compounds for untargeted analyses, but at the expenses of a complex and time-consuming protocol compared to protein precipitation.

In all, our results show that the oxylipin profile obtained from seminal liquid remains quite unaffected by variations to the sample preparation protocol such as deproteinization solvent, with SPE providing a characteristic coverage when untargeted analyses are performed. In this direction, comparison of SPE versus SPME revealed a better removal of interferents for the latter in the mid- to long retention time region, with SPE providing a better coverage over the more polar region.

Acknowledgement

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P16

Investigating Collagen-Protein Interactions Using Affinity Capillary Electrophoresis: Method Development and Use of Correction Factors

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Summary

The project aims to investigate the binding properties of proteins with a collagen product designed for wound healing in order to determine its biocompatibility. In mobility shift affinity capillary electrophoresis, the presence of a ligand can alter the analyte's effective electrophoretic mobility. By fitting the data to a model function using non-linear regression, the strength of the interaction can be estimated. The project presents several challenges, including the non-solubility of collagen at neutral pH, the increasing current in runs with high collagen concentrations, and the evaluation and use of various mobility correction factors.

To mimic the natural conditions of the human body, a phosphate buffer was chosen as the BGE. Due to the poor solubility of collagen in neutral mediums, a procedure was established to make parts of the collagen product suspendable, which was achieved by grinding and freeze-drying. Next, a CE method was developed using coated capillaries to reduce protein adhesion and constant power as the separation mode to compensate for an increase in current. Recent investigations have shown that the EOF in the LPA-coated capillary is not negligible as previously assumed. Therefore, an EOF marker had to be established in the method.

To ensure reliable mobility values for analytes, it is necessary to apply correction factors that counteract viscosity and dielectric changes during evaluation. As only small amounts of collagen material are available, the method by Allmendinger et al. [1] was adapted to determine viscosity using an Agilent 7100 capillary electrophoresis instrument. In addition, sodium salicylate was used as a non-interacting mobility marker to correct for dielectric changes, following the method of Britz-McKibbin et al. [2]. Subsequently, mobility shift affinity capillary electrophoresis was performed using collagen as ligand at various concentrations and epidermal growth factor as the analyte. The corrective actions were then compared and evaluated.

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P17

Automated Sample Preparation of Human Tissue Specimens to Search for N-Glycan-based Biomarkers

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Summary

Oral squamous cell carcinoma (OSCC) accounts for the largest proportion of oral malignancies, making it the sixth most common cancer. Because of the sensitivity of the mucosa, the risk of developing secondary tumours is higher. Current knowledge suggests that OSCC is caused by genetic alterations. Environmental factors such as smoking, alcohol and areca nut consumption play a role in its development [1,2]. In most cases, malignant tumours are diagnosed only at a late stage. Biomarkers are analysed for risk assessment, screening, diagnosis and prognosis. The cancer biomarkers are mainly analysed as proteins and nucleic acids from tissue biopsies, blood, saliva, and so on. Saliva, as a biomarker source, contains various proteins, DNA and RNA molecules, but is limited by the sensitivity of detection. However, developments in the field of cancer biomarkers are needed to achieve higher sensitivity and specificity. The search for new biomarkers is essential for the early diagnosis and treatment of patients to avoid the development of complications [3].

In this work, we analyzed the *N*-glycan profile of oral cancer tissue samples. During the process of sample preparation, the tissues were homogenized in a magnetic bead homogenizer and the resulting supernatants were subject for sample further preparation. The automated homogenization results were compared with the results of manual potter homogenization. The obtained *N*-glycans were separated by capillary electrophoresis with laser-induced fluorescence detection. The glycan profiles of the tumorous samples were compared with the results of the non-tumorous control group to find significant differences between the glycan profiles of the two groups.

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P18

Liquid Biopsy Testing – Isolation Method for Targeted Nucleic Acid Biomarkers

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Summary

Liquid biopsy is a relatively new scientific technology, where cancer can be detected by analyzing the free circulating tumor DNA fragments from biological fluids like blood plasma. These short DNA fragments can provide significant information about the occurrence and the stage of the disease. In addition, such a diagnostic tool is low invasive, requiring only venous blood sampling [1,2].

However, liquid biopsy imposes special demands on the blood sample treatment. The presence of larger DNA fragments and other compounds can significantly reduce the quality of the obtained diagnostic data. For this reason, the development of new techniques for sample pretreatment is crucial [2].

In the presented work, we developed preparative gel electrophoresis combined with an electrodialysis device (PGE-ED). The aim was the isolation of short DNA fragments from the original sample and great emphasis was placed on the reduction of the presence of fragments larger than 1000 base pairs and salts. This purification should result in higher specificity and sensitivity of the subsequent analysis of tumor DNA fragments.

The design of the device enables the parallel separation of multiple samples and is fully compatible with automation robots. For the fabrication, 3D printing was used, providing fast and low-cost manufacturing of the product. The principle of the separation is based on electromigration techniques. These methods enable the efficient partition of DNA fragments in an external electric field. The fractionation is mediated by the sieving effect of the porous separation media. At the end of the separation process, the purified fraction can be collected from the device, and DNA analyses performed.

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P19

New Tools for Peptide Retention Time Predictions in Proteomics

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Summary

Retention time in MS-based proteomics has the potential to be used as a quality evaluation metric for peptide identification. Since peptide retention time represents its complex behaviour between mobile and stationary phases, a sufficient model can greatly enhance targeted and untargeted proteomics. Peptide retention time prediction algorithms have been studied over the last five decades to find the ideal descriptor for retention time predictions.

We developed deep learning models for peptide retention time prediction in different gradient elution conditions in RPLC mode. We combined peptide sequences and gradient slope into an input of a neural network, and we predicted retention time and retention width. Our dataset was made of peptides obtained from a tryptic digest of α-casein, β-casein, BSA and HeLa cell lines. These peptides were analysed in LC-MS under different gradient elution conditions.

Models with different amino acid encodings and neural network architectures were evaluated. We optimised hyperparameters for each neural network to reach the highest accuracy and to overcome overfitting. Embedding layer with backpropagation and bidirectional long-short term memory units recurrent neural network architecture outperformed other architectures.

The pre-trained model was then used on a dataset of peptides from the proteomics identifications database to evaluate the generalisation ability. The developed model was able to both correctly predict the elution behaviour of peptides from their sequence only and distinguish the retention behaviour of non-modified and modified peptides.

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P20

Combining Three-Dimensional Printed Miniaturized Microextraction Device and Elemental Extractant for Detection of Toxic Metal Ions

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Summary

Development of sensitive and selective analytical tools that can enrich the targeted chemical for analysis is necessary for detecting and identifying toxic materials within a complex matrix. We developed a straightforward procedure for using an inductively coupled plasma mass spectrometer in conjunction with a microextraction platform device to enrich and detect different toxic metal ions from various sample matrices. Using lysozyme, a structurally directing compact globular protein, and carboxymethylcellulose as a core template, we created a well-defined carbo-gel nanosphere extractant using hydrothermal carbonization. The extractant was placed inside a repackable, multiple-use 3D-printed miniaturized microextraction device. The fabricated extractant's surface chemistry and structural significance were investigated using infrared spectroscopy, electron microscopy and spectroscopy technologies. The analytical performance showed good precision, appropriated detection limits and linear dynamic ranges with acceptable correlation coefficients for the analytes of interest. The developed approach can sustain more than six regeneration cycles without deteriorating the analytical performance, proving its applicability and sustainability in the real and spiked biological samples. Compared to other solid-phase extraction approaches, our device by significantly reduced the amount of sample and sorbents needed for each analysis and the improved extraction kinetics significantly reduced needed extraction times. The multiplex approach of both extractant and device development can significantly improve selective analytical sensing of toxic or emerging contaminants of concern in biomedical solutions.

P21

Microfluidic Automation of Library Preparation for Nanopore Sequencing

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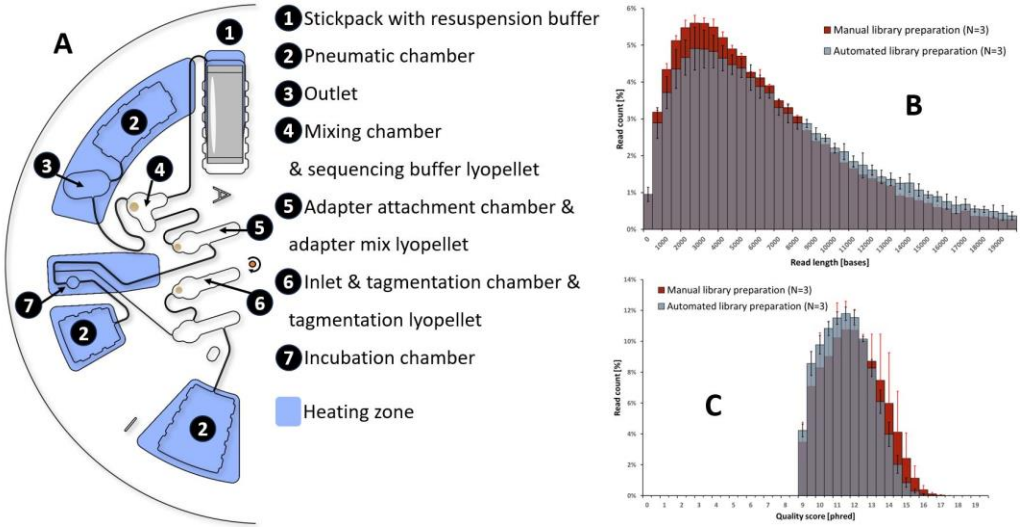
Summary

Due to its error proneness, high costs and contamination risk, library preparation for sequencing is a bottleneck when establishing the complete workflow starting from the material to be analyzed up to bioinformatics. Established robotic automation solutions capable of tackling these challenges mainly target high-throughput scenarios and require high investment costs. In contrast, microfluidic solutions can facilitate automation with low investment costs and the potential to reduce reagent consumption [1]. We present an automated library preparation for nanopore sequencing on a centrifugal microfluidic LabDisk (Fig. A). All necessary reagents are pre-stored thus the required user interaction is minimized to only two pipetting steps: DNA sample input and library output. Using lambda DNA as an input sample, the distribution of read lengths and the quality score of the reads of the libraries prepared by the LabDisk and of the manually prepared libraries were comparable (Fig. B&C). With extracted DNA of Staphylococcus aureus (genome size 2.81 Mb) prepared by the LabDisk, the sequencing time until reaching 95% genome coverage was 90 minutes. In future we will combine the presented library preparation approach with microfluidic DNA extraction workflows [2] on one single LabDisk. We will apply this work to detect a panel of mutation and methylation markers for therapy monitoring of colorectal carcinoma.



Acknowledgement

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P22

Microfluidic Automation of Sample Preparation Techniques for Proteomics

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Summary

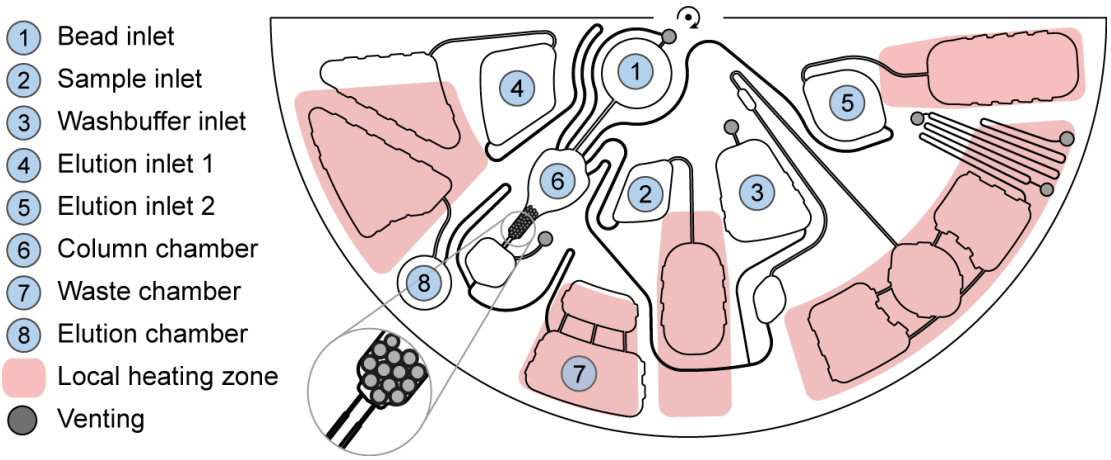

Figure 1: Schematics of the microfluidic LabDisk for solid phase extraction. Fluid propulsion is realised by a combination of centrifugal force and thermopneumatic pumping by local heating zones under the LabDisk.

The performance of the sample preparation significantly affects the downstream analysis via LC-MS/MS for bottom-up proteomics and is considered to be a major bottleneck with regard to reproducibility [1]. This is due to the numerous steps that are necessary to generate highly pure peptide samples from complex samples such as cell lysates or formalin-fixed paraffin-embedded (FFPE) tissue. Essential elements of the sample preparation process, such as homogenization of crude samples, on-bead digestion and enrichment/purification of peptides often rely on bead handling. Bead based processes, including solid phase extraction (SPE), can efficiently be implemented using centrifugal microfluidics. Efficient separation of liquid and solid phases by the artificially created gravity field and the reduction of reaction volumes enable less adsorptive losses and higher reproducibility compared to manual workflows. We present automated SPE on the centrifugal LabDisk platform demonstrated by peptide desalting of tryptic HEK-293 digestions. The microfluidic approach featured more precise results for peptide quantification (median CV of 9.3% versus median CV of 12.6% for manually desalted samples). Future work aims to transfer the microfluidic SPE concept to additional workflow steps such as on-bead (SP3) digestion and phosphor peptide enrichment. Integration of this concept with centrifugal microfluidic bead-based FFPE tissue homogenization promises ultrasonic free, highly reproducible sample preparation for FFPE tissue.

Acknowledgement

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P23

Online Coupling of Size-Exclusion Protein Separation with Monolithic Enzymatic Reactor

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Summary

Proteins, one of the main communication entities in any cell, are an increasingly frequent subject of interest for many scientists. Since they contain a lot of information, their comprehensive analysis is necessary. Two-dimensional liquid chromatography is an increasingly used method in proteomics analysis.

Two contradictory approaches usually characterize proteins. In a more common bottom-up approach, the proteins are first digested into peptides that undergo chromatographic separation and mass spectrometry detection. Obtained information allows the reassembling of the protein structure and revealing its function. In contrast, in the top-down protocol, the whole intact protein is introduced in the mass spectrometer, providing information about individual proteoforms related to sequence variations or post-translational modifications.

This work aims to couple size-exclusion separation of proteins followed by online proteolysis on a capillary-based monolithic reactor with the immobilized enzyme. The reversed-phase gradient elution of emerging peptides is used to characterize the online coupling.

The coupling of size-exclusion separation with the monolithic reactor has been thoroughly optimized. The sample concentration, mobile phase composition, and flow rate were tuned to describe their effect on the proteolytic activity of the enzymatic reactor. The effect of the immobilization buffer composition was also tested. The online coupling will be utilized to develop a new concept of comprehensive two-dimensional liquid chromatography combining top-down and bottom-up analysis of proteomics samples.

Acknowledgement

Financial support by the Czech Science Foundation project 23-07581S is gratefully acknowledged.

P24

The Evaluation of Galectin-1 – Glycopeptide Interactions by Affinity Monolith Chromatography

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Summary

Weak affinity chromatography on monolithic support is a powerful technique for quantification of the specific interactions between immobilized ligand and its target. This approach is convenient for weak-to-moderate binding strengths with typical dissociation constant of the complex falling to the range of mM to µM. Weak affinity chromatography can be performed in frontal mode (FAC) and it proved to be a very powerful tool, yet the main disadvantage of FAC is being time-consuming. The staircase frontal affinity analysis is a modification of the FAC and implies the consecutive percolation of ligand solutions on the affinity stationary phase until the breakthrough curve is observed, forming, in the end, a staircase pattern [1]. The poly(GMS-*co*-EDMA) acrylate-based monolithic columns were prepared from the 75 µm ID transparent fused silica capillaries on which grafting of streptavidin was carried out through reductive amination. Then, the biotinylated target protein was attached to the monolithic column functionalised with streptavidin [2,3]. In our case, a protein of interest was galectin-1 stemming from a group of galectins, which have affinity for beta-galactosides. A set of specifically designed glycopeptides was evaluated as ligands for galectin-1, which was grafted on the monolithic column through the streptavidin-biotin linkage. The length of glycopeptides was 8 amino acids with 0-2 saccharide moieties, glucose or thiolactose, attached to the side-chain by an appropriate linker. The measurements were conducted in staircase frontal analysis on the monolithic columns using a capillary electrophoresis system with UV detection. The dissociation constant *Kd* values of the ligands were then calculated from a double reciprocal plotting showing the amount of ligand captured on the monolithic column vs. the ligand concentration [2,3]. The values obtained varied from 10 µM for the compound with the highest affinity, to 100 µM for the standard, thiodigalactoside.

Acknowledgement

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P25

Microfluidic Chip for Cell Lysis: Towards Single-Cell Immunochemistry in Microdroplets

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Summary

Based on previous experimental results [1,2], we present a droplet-based microfluidic platform for cell lysis, which integrates a) passive cell encapsulation, b) mixing the microdroplet content, and c) the cell lysis (Fig. 1A). Microdroplets are produced continuously by pumping fluids (Fluorinert oil and assay buffer containing detergent Triton-X) from external pressure-driven reservoirs. Hela cells in assay buffer stained with Propidium iodine are passively encapsulated into the droplets (Fig. 1B). Further, the droplet content is mixed and the cells are lysed in the incubation part of the chip (Fig. 1C). The lysed cells are indicated by red fluorescence (Fig. 1D). Single-cell lysates will be mixed with photon-upconversion nanoparticles forming immunochemical complexes, and will be analyzed by massively parallel spectroscopy without separating, washing, and other common steps of immunochemical assays [2].

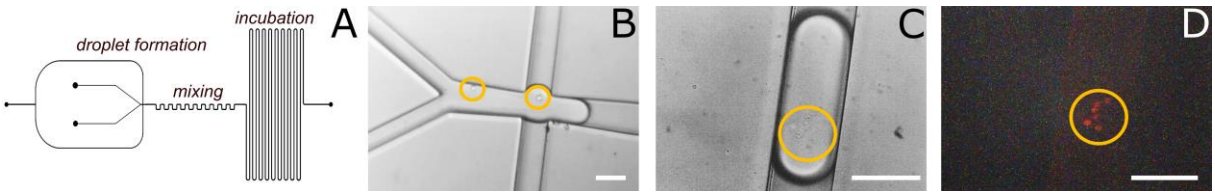

Figure 1. Microfluidic chip for cell lysis. Scheme of the chip (A). Micrographs of cell encapsulation in bright-field mode (B, C), and lysed cells in epifluorescence mode (D). Scale bar: 100 µm.

Acknowledgement

We acknowledge financial support from the Czech Science Foundation (21-03156S) and Institutional support RVO: 68081715.

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P26

How Can Electrochemistry Help with Drug Testing?

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Summary

Drug stability studies are an essential part of any new drug development process, providing information on changes in the stability of a drug over time. Oxidative degradation is one of the fundamental mechanisms used for the forced degradation of drugs in stress tests. According to the World Health Organization (WHO), oxidation is accomplished by electron transfer using a 0.1-3% hydrogen peroxide solution on the drug or active pharmaceutical ingredient (API) at both ambient and elevated temperatures for hours to days [1,2].

In this work, a groundbreaking electrochemical method was employed instead [3]. The potential of this approach was investigated on the API, abacavir sulfate, sample by utilizing two distinct electrode materials: platinum and boron-doped diamond (BDD) [4]. Abacavir samples underwent controlled oxidation, followed by meticulous analysis via chromatography coupled with mass spectrometry. The resulting degradation products were scrutinized, with comparisons drawn against conventional chemical oxidation using 3% hydrogen peroxide. Additionally, the impact of pH on degradation kinetics and product formation was systematically explored.

Remarkably, both electrochemical and traditional oxidation methods yielded identical degradation products, identified through mass spectrometry. These findings were consistent across experiments conducted on large-surface platinum electrodes and BDD disc electrodes. Notably, the electrochemical process achieved rapid degradation of abacavir—crucial for pharmaceutical stability assessments—in mere minutes, contrasting starkly with the protracted hours required for hydrogen peroxide oxidation.

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P27

Humic Acid Modified Paper as an Affordable Cation Exchanger Sorbent to Isolate Basic Drugs from Saliva Samples

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Summary

The impact of the sample preparation step has led to the use of natural materials as precursors for the synthesis of sorptive phases to reduce the environmental impact [1,2]. In this work, two natural materials such as cellulose and humic acids (HA) have been combined to prepare a flat sorptive phase. The preparation procedure was performed using the dip-coating technique, which stands out for its simplicity and low reagent consumption. In fact, the synthesis consisted of dipping the paper in HA aqueous solution followed by simple thermal curing. The final phase has been characterized by infrared spectroscopy, ultraviolet-visible diffuse reflection spectroscopy, and scanning electron microscopy. Cocaine, codeine, methadone, and methamphetamine have been selected as target compounds to study the performance of this method, followed by its analysis by direct infusion mass spectrometry. Variables related to the extraction (such as pH, ionic strength, number of dips, and extraction time) have been studied deeply. The large number of acid groups of the humic acid provides the phase with a mixed-mode cationic exchange capacity, which has been evaluated and satisfactorily demonstrated. The limits of detections were established at 1.5 $mg \cdot L^{-1}$ for codeine and 0.3 $mg \cdot L^{-1}$ for the rest of the analytes. Moreover, the intra/inter-day precision and accuracy have been studied at 3 concentration levels. Finally, the method has been applied to saliva samples from patients undergoing treatment with codeine and was able to quantify 96 % of the samples satisfactorily.

Acknowledgement

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P28

Sample Preparation for Proteomic Analysis - Greenness Evaluation

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Summary

Proteins are essential molecules for all living organisms and play a role also as important biopharmaceuticals. The complex structures of these molecules often need sophisticated sample preparation and analytical methods for their thorough characterization. Capillary electrophoresis (CE) has emerged as an eco-friendly technique in proteomic analysis, offering rapid, high-resolution separations with minimal sample and reagent use, aligning with environmental sustainability goals [1].

Proteomic analysis can be conducted at various levels, from intact proteins to peptides and released glycans. The top-down approach, analyzing proteins in their native or denatured forms, and the middle-up approach, which involves partial enzymatic digestion, are gaining attraction, especially in the research of monoclonal antibodies. While Green Analytical Chemistry (GAC) [2] encourages reducing sample preparation, achieving direct analysis in proteomic applications remains challenging. Traditional sample preparation methods can be labor-intensive, time-consuming, and produce hazardous waste. To align with the principles of Green Sample Preparation (GSP), researchers are exploring greener techniques. This includes using safer solvents, but also preferring automation and miniaturization of the analytical process to reduce environmental impact. Our study assesses various sample preparation methods for proteomic analysis using AGREEprep software and evaluates the greenness of selected analytical methods with AGREE software [3].

The results highlight the trend toward more sustainable practices in proteomic research, emphasizing miniaturization of analytical devices and integrating on-line and in-line sample preparation approaches. This shift towards eco-friendly techniques not only addresses environmental concerns but also enhances efficiency and throughput in proteomic analysis.

Acknowledgement

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P29

A Simplified Protocol for Intact Exosome Separation using Low-Pressure Size-Exclusion Chromatography

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Summary

Exosomes, small extracellular vesicles, are formed through the inward budding of the endosomal membrane (endocytosis), leading to the accumulation of intraluminal vesicles within multivesicular bodies, which subsequently fuse with the plasma membrane, releasing exosomes into the extracellular space. They are an essential component of cellular and tissue processes, such as intercellular communication, immune response, programmed cell death, inflammation, and the transport of morphogens. Because of their versatile role, exosomes also play a decisive role in various pathological conditions, such as cancer or neurodegenerative diseases. These nano-sized vesicles carry a diverse cargo of biomolecules, including nucleic acids, proteins, lipids, and metabolites. Studies have shown that the composition of exosomes often reflects the physiological or pathological state of cells, tissues, organs, and organisms. This characteristic makes exosomes, especially oncosomes, promising structures to investigate the pathogenesis of disease and allow early and accurate disease diagnosis. Despite their potential, the widespread use of exosomes is hampered by the limited possibility of isolating exosomes quantitatively and in sufficient quality.

Consequently, efforts have been made to implement a robust exosome isolation method that offers a high yield of intact exosomes without contaminating proteins, lipoproteins, or microvesicles. This study shows a specific configuration with a low-pressure size-exclusion chromatography, which disposes of several advantages, such as a high capacity, robustness, providing a pure fraction of intact exosomes with minimal contamination. What makes this technique unique? Optimized conditions enabled us to separate exosomes into fractions varying in exosome size. We established a protocol for the isolation and fractionation of exosomes. Methods such as DLS, NTA, Dot-Blot, ELISA, and MS were applied to control the separation process.

Acknowledgement

This work was supported by the ERC Advanced grant No. 101095860 sponsored by the European Research Council, and by the SGS_2024_002 project of the University of Pardubice.

P30

From Model to Practice: Developing an Enrichment and Recovery System to Facilitate Rapid Pathogen Detection

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Summary

Identifying microorganisms quickly and accurately is essential for safe drinking water, a persistent global challenge. Traditional culturing methods, capable of detecting one colony-forming unit (CFU) per 100 mL, remain the gold standard despite taking up to 10 days. Biosensors offer a quicker, on-site alternative without needing a sterile lab or specialised staff. However, they are typically limited to small samples and thus require a pre-concentration step, for which filtration is often used. It can suffer from low recovery rates, reducing the total limit of detection [1].

To improve upon this, we focused on optimising the filter holder design. We used COMSOL Multiphysics to compute the flow over and through ceramic membranes (Ø 47 mm, 0.14 µm cut-off). Our goal was to increase the shear rate, ensure its uniform distribution over the membrane, and minimise dead volume while staying within the filter's 4 bar pressure limit and processing 100 mL of sample within 10 minutes. We modelled the fluid flow inside the filter module using Brinkman's Law and laminar flow conditions. We considered diffusion (Fick's Law) and convection to model ion transport through the system.

We validated the accuracy of our model experimentally by comparing in- and outflow and pressure and by evaluating a 1 M NaCl[PM1] tracer peak against our simulations. We 3D-printed promising designs and tested them with 100 mL water samples doped with Escherichia coli (down to <1 CFU/mL). We analysed the recovery rate after back-flushing via fluorescence staining, plating, and microscopy. It reached up to 97.6% (±14.6) with a concentration factor of 20 in less than 10 minutes, making it a practicable option for utilisation with biosensors at the point-of-care. Future work will be focused on increasing the concentration factor while integrating the current laboratory-benchtop design into an autonomous, parallel, and fully portable enrichment system.

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P31

Acoustophoretic Focusing of Microparticles in Glass Microfluidic Device

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Summary

Acoustofluidics describes the interaction of sound waves with fluids and dispersed particles. Resonating sound waves form, so-called, acoustic standing waves (ASWs), inducing perturbations in the fluid that can manifest as a radiation force and, in turn, cause a migration phenomenon known as acoustophoresis. In microfluidic devices made from rigid materials, the microchannel itself acts as the resonator, and the shape of the ASW is dictated by the ratio of the wavelength to the width of the channel.

Here, we present a glass microfluidic device for acoustophoretic focusing of particles suspended in liquid media. The glass device comprises a Y-shaped microchannel junction with two inlets and one outlet. A piezoceramic transducer, serving as the acoustic emitter, is securely attached to the glass surface near the microchannel to prevent any attenuation of the acoustic energy. The wavelength of the acoustic signal is set to double the width of the channel, generating a single-node ASW in the center of the channel. The dispersed particles migrate along the pressure gradient generated by the standing wave and accumulate at the node. We propose that the instant temporary accumulation of particles could benefit microscopic or spectral methods, such as surface-enhanced Raman spectroscopy (SERS), by concentrating them at a designated location in the channel, such as the focal point, thereby enhancing the output of these methods.

For our glass device, we have also designed a 3D-printed latch-on case providing quick-release fluidic connections and a sturdy mount compatible with standard microscope stage frames, without hindrances to any functionality of the device.

Acknowledgement

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P33

Glycoform Equivalence Assessment of Biotherapeutics with N-and O-Glycosylation Sites by Sequential Intact Mass Spectrometry

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Summary

Glycosylation is a crucial attribute of biotherapeutics with significant impacts on quality, safety, and efficacy. Therefore, ensuring consistent glycosylation requires a systematic review of biotherapeutics, including diverse glycan structures (microheterogeneity) and variable occupancy of individual sites (macro-heterogeneity), from drug design to upstream and downstream bioprocessing. Various methods have been employed for glyco-characterization at different levels-glycan, glycopeptide, and intact protein. In particular, intact protein analysis is considered a facile and rapid glycoform monitoring approach used throughout the product development lifecycle to determine suitable glycosylation lead candidates and reproducible product quality. However, intact glycoform characterization of diverse and complex biotherapeutics with multiple N- and O-glycosylation sites can be very challenging. To address this, a robust analytical platform that enables rapid and accurate characterization of biotherapeutics with highly complex multiple glycosylation using two-step intact glycoform mass spectrometry has been developed. We used darbepoetin alfa, a second-generation EPO bearing multiple N- and O-glycosylation sites, as a model biotherapeutics to obtain integrated information on glycan heterogeneity and site occupancy through step-by-step MS of intact protein and enzyme-treated protein. In addition, we performed a comparative assessment of the heterogeneity from different products, confirming that our new method can efficiently evaluate glycosylation equivalence. This new strategy provides rapid and accurate information on the degree of glycosylation of a therapeutic glycoprotein with multiple glycosylation, which can be used to assess glycosylation similarity between batches and between biosimilar and reference during development and production.

Acknowledgement

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P34

First Data on Alpelisib Concentrations in Plasma Determined by HPLC-FLD Method

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Summary

In 2022, the FDA approved alpelisib (ALP) also for the treatment of patients with noncancerous PIK3CA-related overgrowth spectrum (PROS), including PIK3CA associated venous malformations [1], which offers a novel therapeutic approach to current PROS management strategies. The most common adverse reactions were reported hyperglycemia, diarrhoea, or stomatitis [1]. There is no antidote for an overdose of alpelisib, so patients are treated symptomatically. However, ALP exposure shows considerable inter-individual variability and, along with adverse effects, induces the necessity for therapeutic drug monitoring (TDM).

Analytical methods for ALP determination comprise LC separation hyphenated to MS/MS detection or alternatively more affordable fluorescence detection [2]. In this study, ALP analysis was performed on an Agilent 1200 Series liquid chromatograph combined with a fluorescence detector. The excitation and emission wavelengths were set to 311 nm and 384 nm, respectively. A Kinetex Core-Shell C18 column (100 × 3 mm, 2.6 µm) heated to 40 °C was used for separation. The mobile phase consisted of ammonium acetate (10 mM, pH 6.6) and acetonitrile at 65:35 (v/v) in isocratic mode. The flow rate was 0.5 mL/min, and the total time of a single run was 8 min. Analytical data were processed using Agilent OpenLAB CDS software. After optimizing the separation conditions, a suitable liquid-liquid extraction procedure was examined. The excellent extraction yield was obtained with a mixture of dichloromethane-isopropanol, 9:1 (v/v). The internal standard (IS) glibenclamide was added before extraction. The method was validated in terms of linearity, precision, accuracy, and stability according to the European Medicines Agency guideline. The limit of detection for ALP was determined to be 1.1 ng/mL (the limit was based on the signal-to-noise ratio of 3), and the limit of quantification was 30 ng/mL. The samples of plasma were obtained from the patients treated at the Children's Oncology Clinic of the University Hospital Brno upon subscription of Informed Consent by their parents or legal guardians. The study was approved by the Ethics Committee of the University Hospital Brno (Approval No.01-130923/EK).

The developed HPLC-FLD method was applied to determine ALP in patient plasma samples. Quantification was based on the peak area ratio of the analyte to the IS, and the obtained concentrations were within the chosen calibration range (30–1000 ng/mL). The method is sensitive enough for the TDM of ALP in human plasma. The upcoming study will target its clinical utilization.

Acknowledgement

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P35

Therapeutic Drug Monitoring of Colistin Supported by Lipidomics in Critically Ill Patients

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Summary

Polymyxins (including colistin) are naturally occurring cyclic lipopeptides with a narrow antibacterial spectrum, mainly against Gram-negative pathogens which are now used as the last therapeutic option for infections caused by Pseudomonas aeruginosa, Actinetobacter baumanii, and Klebsiella pneumoniae [1]. These antibiotics (ATBs) entered the clinic in the late 1950s, but their use declined in the 1970s due to potential nephrotoxicity and neurotoxicity. However, since the 2000s clinicians have increasingly had to use polymyxins as one of the few therapeutic options for treatment of resistant Gram-negative bacteria [2]. Therapeutic drug monitoring (TDM) of such ATBs can be an effective tool to set optimal therapy management of critically ill patients which includes optimization of the dosage regimens, minimization of the unwanted side reactions, and also preventing bacteria resistance. Capillary electrophoresis (CE) is a promising alternative method for the analysis of drugs and their metabolites in biological fluids. It offers several advantages in TDM, i.e., relatively fast analysis time, simple instrumentation, environmental friendliness, better resolution, high separation efficiency allowing multicomponent analysis, and low cost of analysis compared to HPLC. Recent trends in clinical practice are oriented towards the personalized medicine. Such approach typically demands the fundamental understanding of the disease, identification of drug targets for therapy and also discovery of relevant biomarkers of diseases or for monitoring the effectiveness of drug treatment leading to the clinical follow-up in medical therapy. These goals can be fulfilled with the use of “omics” disciplines, e.g., lipidomics. Here, we present a comprehensive therapy optimizing strategy based on implementation of TDM of colistin performed by advanced capillary zone electrophoresis-tandem mass spectrometry (CZE-MS/MS) approach combined/supported with a complex lipidomics approach.

Acknowledgement

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P36

Novel Approaches using Fluorescence Spectroscopy for Smoke Taint Determination

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Summary

Smoke taint, a wine fault characterized by a smoky flavor and aroma, has led to significant agricultural and economic loss. Conventional smoke taint analysis uses both gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) to quantify levels of smoke taint markers. Although these techniques offer high sensitivity, these instruments are often stationary, expensive, and require expertise. Here, we introduce an alternative technique for smoke taint determination using fluorescence spectroscopy that is simple, inexpensive, and rapid. Two fluorescent probes (1 and 2), each consisting of a fluorophore bound to a thiol recognition moiety, were synthesized, and used to detect several thiophenol smoke taint markers in grapes and wine. At neutral pH, the electron-withdrawing dinitrobenzene sulfonyl recognition moiety selectively reacts with thiophenol smoke taint markers to yield a fluorescent product. The signal generated from this reaction can be measured and used to determine smoke taint risk. As proof of concept, thiophenol smoke taint markers were extracted from spiked grape juice and wine using solid phase extraction (SPE). The SPE eluate was then mixed with a fluorescent probe in phosphate buffer (pH 7.3). Results using probe 1indicate that this method is rapid (10 min), selective, and sensitive (~37 ppb). Preliminary results using probe 2 suggest even higher sensitivity. We are actively pursuing the incorporation of these fluorescent probes in microfluidic architectures for field use in vineyards and wineries. This approach offers a promising alternative to conventional methods for smoke taint determination.

Acknowledgement

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P37

Novel Microsampling Approach Based on Solid-Phase Microextraction for Monitoring the Level of Tryptophan and Its Metabolites in Human Serum and Urine Samples

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Summary

Solid phase microextraction (SPME) is minimally invasive sample preparation technique that facilitates isolation of low molecular weight compounds directly from biological matrix [1]. Quantitative analysis can be performed since SPME can be coupled to various separation and detection methods, such as liquid chromatography tandem mass spectrometry (LC-MS/MS) [2]. In this study, SPME utilizing biocompatible probes along with LC-MS/MS analysis is proposed as a novel strategy to monitor the levels of tryptophan (Trp) and its metabolites in complex biological samples as potential predictors of neurodegenerative diseases resulting from asymptomatic microembolism to the central nervous system. Trp and its metabolites formed in the serotonin pathway (e.g., serotonin, 5-hydroxyindoleacetic acid, melatonin, and N-acetylserotonin) and in the kynurenine pathway (e.g., kynurenin, kynurenic acid, xanthurenic acid) are considered as good predictive biomarkers of the central nervous system and cardiologic diseases [3]. Optimization of the SPME method concerned the selection of extraction phase (coating), desorption mixture, and sample pH, among others. SPME utilizing DVB coating showed the best performance towards simultaneous extraction of 14 compounds (8 analytes and 6 deuterated internal standards) from urine and serum samples. In optimized conditions, the SPME-LC-MS/MS method enabled the analysis of even trace amounts of studied compounds in 8.5 min. The developed methodology was applied in the monitoring of Trp and its metabolites in real human urine and serum samples obtained from patients with paroxysmal atrial fibrillation. Assessment of the relationship between the level of Trp and its selected metabolites in both biological matrices was used for the evaluation of changes in those systems in patients with the episodes of paroxysmal atrial fibrillation. The proposed SPME-based methodology may facilitate in future more in-depth analysis of the role of serotonin and kynurenin pathways in the development of neurological disorders.

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P38

Determination of Organic Acids in Infants Faeces Using a CE-C4D In-house Built Instrument

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Summary

Short-chain fatty acids (SCFAs) are aliphatic carboxylic acids, such as formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, and 2-methyl-butanoic acids. SCFAs are produced during colonic bacterial fermentation of complex carbohydrates from the diet that remain undigested in earlier sections of the gastrointestinal tract and they can serve as substrates for subsequent biochemical transformations, leading to the production of other organic acids like lactic acid or succinic acid. Short-chain fatty acids demonstrate several important functions, e.g. immunoregulatory, neuroprotective, hepatoprotective, and even anticancerogenic activity [1,2]. Numerous studies have proven that the changes in the bacterial consortium constituting the intestinal flora have an impact on short and long-term health disorders. In the literature on the subject, several studies indicated a relationship between the early development of the gut microbiota and the risk of developing asthma and allergic diseases in the later stages of life [3]. This research presents the development and validation of a capillary electrophoresis method with a contactless capacitively coupled conductivity detector (CE-C^4D) for the analysis of organic acids in infant faeces. The study focused on optimizing the background electrolyte, mainly dynamic capillary coating with polybrene, and utilizing cyclodextrins to achieve efficient separation. The final BGE consisted of 10 mM MES/His (pH 5.75) + 1% β-CD, and the separation of nine organic acids was performed in a dynamic polybrene-coated capillary with a total length of 60 cm (a voltage: +12 kV at outlet). The validation study was performed, and the method was found suitable for the purpose. The developed method was used in a pilot study to investigate the concentration of organic acids in real faecal samples collected from children to evaluate its feasibility.

Acknowledgement

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P39

Application of the DLLME/GC-MS Method of the Identification of Aromatic Amines Derived from Azo Dyes for Forensic Analysis of Fibers

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Summary

In forensic investigation, disclosed microfibers can play a crucial role, as they can link a suspect with a victim or a crime scene. One of the most widely used types of dyes in the textile industry is azo dyes. Therefore, there is a high probability that a fiber dyed with azo colorants will be secured as evidence.

Due to the reductive cleavage, azo dyes can break down into aromatic amines, some of which are known to be harmful or carcinogenic. Therefore, specific azo colorant use is limited, and the relevant standard [1] describes methods of controlling their presence in textiles. However, the described procedures require a large amount of the sample (1 g of fabric), making it unpractical or even impossible to use in forensic analysis where the sample size is often limited to single fibers of mass ca. a few micrograms.

The study aimed to redesign the method for the identification of aromatic amines derived from azo dyes by reducing the sample preparation process scale and the fiber sample size for identification of dyes and discrimination of fibers.

The developed procedure includes the extraction of dyes, reductive cleavage of azo groups, and the extraction of obtained amines by Dispersive Liquid-Liquid Microextraction (DLLME) followed by the GC-MS analysis.

The redesign of the standard method to the microscale reduces the volume of reagents and cost of analysis and, more importantly, enables its application for forensic fiber analysis.

To initially evaluate the procedure in a real-world scenario, it was employed to analyze polyester fiber samples secured from clothing. The analysis of 1 cm long threads (approximately 0,5 mg) confirmed that it was possible to identify amines based on the NIST database and retention times with good repeatability which makes the first step to set up the whole forensic discrimination procedure.

Acknowledgement

The research has been supported by a grant from the Faculty of Chemistry under the Strategic Program Excellence Initiative at Jagiellonian University.

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P40

Identification of Aspergillus Species using CE in Capillary with Roughened Part and MALDI-TOF MS

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Summary

Aspergillus species are the most common and widely distributed fungi. They produce airborne spores which can cause an infection in immunocompromised patients. It was also found that some chronic wounds can be infected with *Aspergillus* spp. Since the infections caused by *Aspergillus* spp. are associated with high mortality if the disease is not treated, reliable identification of an etiological agent is crucial for successful therapy. Currently, diagnosis of *Aspergillus* infections relies on combination of radiological data, microbiological methods, and histopathologic examination. However, these diagnostic methods are not specific and sensitive enough and are time-consuming.

The aim of this study was to develop a method for identification of *Aspergillus* spp. in clinical samples based on a combination of capillary electrophoresis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *Aspergillus* spp. conidia were on-line concentrated after their dynamic adhesion onto the roughened part of fused silica capillary (prepared by etching of the inner capillary surface with supercritical water) and subsequently off-line identified by MALDI-TOF MS. Conidia of four *Aspergillus* spp., *A. niger*, *A. fumigatus*, *A. flavus*, and *A. parasiticus*, were selected for this work.

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P41

Enzyme Kinetic Studies in Droplet Microfluidic Device with Fluorescence Detection

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Summary

Enzyme kinetics is important for a wide range of disciplines. As many enzymes play their role in human diseases, they are potential therapeutical drug targets. On the other hand, biotechnology field is other emerging area utilizing kinetic data in process optimization. However today multiple well plate format offers high degree of automation it is still characterized by significant consumption of all reagents.

Droplet microfluidics (DM) enables the translation of chemical and biological assays to scales and rates unachievable in conventional laboratory workflows. Since the droplets are isolated and protected by the oil (water-in-oil concept), they can be individually manipulated and virtually regarded as separate microreactors. Compared to the conventional technologies, microfluidics offers several advantages such as reduced reagent consumption, shorter analyses times, and rapid production of data in given time.

Working in minuscule volumes presents new possibilities in sample manipulation as well as challenges. The "hearth" of the DM components is typically polydimethylsiloxane chip with integrated droplet generator, incubation channel and other functional elements, connected to precise syringe pumps. Many different detection techniques were reported in combination with DM [1]. Laser induced fluorescence is one of them being often employed.

In this study we monitored enzyme reaction of β-galactosidase using synthetic fluorogenic substrate. First, chip fabrication process, inner surface modification as well as water-oil phase ratio and flow rates were optimized as all these parameters have significant impact on uniform-sized droplets generation and repeatability. The optimized setup allowed on-line substrate concentration variation by setting corresponding flow rates of individual reaction components. Finally, enzyme activity of β-galactosidase, which is an important enzyme used in biotechnology and bioanalysis was measured with its fluorogenic substrate in setup which allowed to measure the reaction rate simply by focusing on different distances in the incubation channel, corresponding to different reaction times.

Acknowledgement

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P42

Rhodamine B-based Labeling for Oligosaccharide and Glycan Analysis by CE/LIF

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Summary

Fluorescent labeling represents a key step in oligosaccharide and glycan analysis by capillary electrophoresis with laser-induced fluorescence detection. In this work, we present a synthesis of the rhodamine B-based fluorescent tag modified by a hydrazide functionality for saccharide derivatization via hydrazone formation chemistry. The labeling conditions were optimized to obtain a high reaction yield. The labeled oligosaccharides and glycans were separated in polybrene-coated capillaries and the separation conditions (BGE compositions, separation, voltage, capillary length, etc.) were optimized. The labeled products were detected with a 532-nm laser and 550-nm longpass or 560/10-nm bandpass optical filters. The optimized labeling and separation conditions were applied for CE/LIF analysis of glycans released from model glycoproteins such as ribonuclease B and immunoglobulin G.

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P43

Synthesis of Bifunctional Non-Covalent Molecularly Imprinted Polymers (MIPs) for Selective Extraction of Catecholamines and their Metabolites

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Summary

Catecholamines (CAs) are important hormones and neurotransmitters present in bodily fluids. Abnormal concentrations of CAs are linked to cardiovascular and neurodegenerative diseases as well as adrenogenic tumors (e.g. neuroblastoma, pheochromocytoma). Thus, simultaneous determination of both CAs and their metabolites in urine or plasma helps reliably diagnose these ailments [1]. However, matrix complexity and low analyte concentrations in biological fluids require complicated sample preparation. Solid-phase extraction (SPE) using molecularly-imprinted polymers (MIPs) is a promising sample purification technique with high throughput and selectivity [2]. In our previous study, we showed that the CAs and their metabolites can be selectively isolated from plasma with a semi-covalent dual-mode MIP [3]. The synthesis procedure had practical drawbacks which prompted us to switch to a non-covalent strategy where the anions of CAs and metanephrines are interacting with the sorbent by anion-exchange rather than commonly used cation exchange interactions. This substantially reduced MIP synthesis time, prevented binding site degradation and improved solvent flow-through characteristics to employ the MIPs in a cartridge format, making them compatible with aqueous mobile phases. MIPs with varying cross-linker/monomer ratios were synthesized in different solvent systems to test changes in sorption selectivity. Two MIPs made in ACN-DMFA-MeOH system demonstrated moderate recovery for CAs (59-77%) and high recovery for HVA and VMA (85-112%). These MIPs are selective towards CAs (IF 3.5-5.2) but demonstrated lower IF values for MN, NMN, HVA and VMA (1.1-1.4). MIPs synthesized in other solvent systems showed increased degradation of CAs during SPE, resulting in low recoveries (possibly due to poor solvent throughput). These results show that two MIP candidates are viable for further optimization as well as experiments with spiked biological samples.

Acknowledgement

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P44

Navigating the Complex Landscape of Glycoproteomics: Challenges in Large-Scale Data Analysis

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Summary

Glycoproteomics is crucial for understanding the complex network of glycosylation processes that influence cellular functions and the pathology of diseases. The study of glycoproteins in complex biological samples is fraught with analytical challenges that impede the precise identification and quantification of glycoforms. Among these challenges, false positive identifications stand out as a critical issue where the peptide component is accurately identified, but the glycan is incorrectly assigned due to factors such as overalkylation, adduct formation, and the inherent mass similarity of monosaccharide combinations.

To address this critical challenge, our strategy employs the verification of glycoform MS2 identifications through retention time (RT) alignment, taking advantage of the sialylation-induced RT shifts. Measurements of blood serum samples were conducted using a high-resolution LC-MS Orbitrap Fusion, which provides the necessary analytical precision for accurate glycoform identification. By drawing a connection between sialylation levels and RT, we enhance our ability to accurately identify glycoforms, thereby reducing the chances of false positive identifications. This analytical approach permits the investigation of glycoforms within designated RT intervals, enabling the identification of glycoforms that have previously gone undetected in complex biological samples like blood serum.

Our methodology highlights the complexity of glycoproteomic analysis and introduces a robust framework for overcoming some of the most pressing analytical challenges.

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P45

Micromachined Nanospray Interfaces for Fast and Sensitive Bioanalyses

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Summary

In this poster we present a nanospray interface for capillary electrophoresis (CE) – mass spectrometry (MS) analyses of biomolecules. This interface utilizes a micromachined electrospray device based on silicon technology and the liquid junction concept for CE-MS coupling.

UV-lithography, reactive-ion etching and deep reactive-ion etching techniques were used to pattern the nanospray device on both sides, creating an emitter on the front side and a star-shaped structure on the back side of the 10 x 10 mm silicon die. The emitter was 15 µm tall and its internal diameter (ID) was 7 µm, providing for low flow rates (below 100 $nL.min^{-1}$) and thus high ionization efficiency. The star-shaped structure was 150 µm deep and 600 µm wide and it basically acted like a funnel collecting the CE effluent and sheath liquid into a narrower 150 µm long/100 µm ID inner channel. This inner channel stretched into 100 µm long and 7 µm ID emitter channel, completing the fluidic path from the back to the front side of the silicon die.

The nanospray device was operated in front of the mass spectrometer entrance while inserted in a custom-designed nanospray module. This miniaturized nanospray module integrated a three-port liquid junction ferrule together with a conductive insert for direct application of voltage on the nanospray device. Its applicability was demonstrated by measuring mass spectra of Pierce™ Positive Ion Calibration Solution and bovine cytochrome C using flow rate of approximately 85 $nL.min^{-1}$.

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P46

Design of Experiments-Based Optimization of Microflow LC-MS Method Applicable in Proteomics Analysis

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Summary

Due to the reduced radial dilution of analytes, liquid chromatography-mass spectrometry (LC-MS) utilizing narrow columns with an internal diameter of 50 – 75 µm is almost exclusively used in ultra-trace proteomics analysis of limited sample amounts. Micro-flow LC-MS employing columns with an inner diameter of 1 mm is a viable alternative in cases where the sample amount is not limited. It offers robust qualitative and quantitative performance characteristics, simplicity of implementation, and an extensive range of high-quality micro-flow columns. Additionally, micro-column LC analysis is also easily applicable in routine clinical applications.

In this work, we have utilized the design of experiments protocol to optimize the micro-flow LC-MS method and to explore the main factors controlling its sensitivity in bottom-up proteomics analysis.

We divided the optimization process into several consecutive steps planned by Box-Behnken designs. The experimental conditions providing the highest response in each stage have been selected in a subsequent step. Signal intensity and number of identified peptides were all used as optimization criteria.

In liquid chromatography, we tested the effect of mobile phase flow rate, column temperature, and gradient time. In electrospray coupling, we optimized the position of the capillary, its temperature, voltage, and the flow of sheath, auxiliary, and sweep gases. Finally, we focused on optimizing the ion injection time, automatic gain control target, and Orbitrap analyzer resolution utilizing both full MS and MS/MS scans. We also compared the influence of dual ionization source for electrospray and MALDI and conventional electrospray ionization source.

During the optimization process, the signal intensity and number of identified peptides increased four times and two and a half times, respectively. After optimization with a semi-complex proteomic sample, we analyzed the real-life sample and compared the obtained results with the literature findings.

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P47

Top-down Analysis of Snake Venoms with CZE-MS

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Summary

Venoms contain numerous biologically active compounds, primarily peptides and proteins, which have the potential to cause lethal effects [1]. When combined with capillary zone electrophoresis, the top-down mass spectrometric technique becomes highly effective in studying the structural and dynamic characteristics of intact proteins. This approach can be applied to complex protein molecules such as snake venom [2]. In this work we demonstrated the favourable analytical performance of capillary zone electrophoresis coupled with mass spectrometry (CZE-MS) for the intact protein analysis of similar venom samples. Using 1 M formic acid (pH=1.9) as BGE, minimal adsorption and narrow peaks shapes - thus good separation efficiencies - were obtained for the protein components of the venom samples. The precision of migration times and peak areas were 1.9-2.8 RSD% and 0.8-7.2 RSD%, respectively and the theoretical plate numbers were 32000-238000 for peaks having signal-to-noise ratio (S/N) larger than 50.

More than 250 different neuropeptides (7-10 kDa) were detected in the venoms obtained from snakes of 9 different subspecies (belonging either to Naja or Dendroaspis species). The protein contents of the venoms of the same subspecies collected from different geographical regions are similar and differ only in a few (less than 10%) components. However, the venoms collected from different organism (within the same species) exhibit very different protein patterns.

Notably, our findings revealed distinct protein patterns among venoms from different subspecies, emphasizing the unique fingerprinting potential of venom in distinguishing snake populations.

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Analysis of Biothiols in Non-Invasive Sample Matrix with the Use of Gold-Based Nanostructures

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Summary

In biomedical research, there is a long lasting way to search for new biomarkers of various diseases. Biothiols, whose levels and differences are related to many pathological states in the organism, including, but not limitied to Alzheimer's disease, cancer or cardiovascular disease [1,2] are such biomarker examples. While there are methods for routine analysis of biothiols in human plasma and urine samples, determination of these analytes in non-invasive samples, such as saliva or exhaled breath condensate (EBC) is still a topic of ongoing research.

In this work, we have focused on the analysis of EBC. A challenging aspect of the EBC analysis is the rather low concentrations of the analytes, which are considerably lower than in plasma or urine samples. We first describe an in-house built device that allows collection of sufficient amount of EBC (several mililiters, at -20 °C). A selective preconcentration by using gold nanoparticles and utilization of gold-thiol interaction, is then employed as a first step. The gold nanoparticles could be used as they are, or, they can be grafted onto a magnetic nanoparticle core, which enables magnetic separation.

After adsorption of biothiols on the gold surface, they are desorbed by addition of an excess of thiol-desorption agent and analysed by capillary electrophoresis with laser-induced fluorescence detection [3]. There are multiple compounds that could be used for biothiol desorption, such as dithiothreitol, 2-mercaptoethanol or thioglycolic acid. In the final step, we derivatized the desorbed thiols from the nanoparticles by a fluorescent label – eosin-5'-maleimide (EMA). The whole procedure is utilized for extraction of biothiols from large volumes of EBC, in order to determine the low nanomolar levels of biothiols in EBC as well as comparing the thiol content to the spiked EBC samples.

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P49

Simple Separation Device for Fast Sample Desalination

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Summary

The successful analyses of the complex samples depend not only on sensitive and selective separation and detection methods but also, in many cases, mainly on sample collection and its pretreatment. The sample pretreatment includes purification, concentration, and simplification of complex, primarily biological, samples. The biological matrices' complexity impacts the method's sensitivity and reproducibility.

Electromigration methods are often used for separation and pretreatment of biological samples [1]. They are usually limited by the injected volume of the sample, generally up to hundreds of µL, while several mL of the sample are available. In the case of epitachophoresis, several mL of the sample can be injected in a single run [2]. However, a high amount of inorganic salts, mainly NaCl, causes the prolongation of separation time significantly [3]. Therefore, a simple device that in short time removes the inorganic salts and exchanges the buffer simplifies sample preparation increases the volume of the sample to be injected, and, last but not least, saves time.

The new concept of purification technique based on the electromigration methods in discontinuous electrolyte systems is presented. It enables the purification and desalination of large samples from small charged molecules, sample simplification, and buffer exchange. It can also be used to separate two substances of different sizes quickly. The presented device is easy to fabricate and set, with low operation costs. The device is simple to use and works very efficiently. The device design enables accessible sample collection and processing of large samples. The presented application examples show the desalting of a DNA ladder dissolved in six millilitres of 0.9% NaCl or blood plasma sample finished in 15 to 35 minutes, depending on salt content and used electric power. Qubit confirmed 90% of the DNA recovery. Subsequent capillary electrophoresis analysis confirmed no loss of DNA fragments.

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P50

The New Format of Stop-Flow Thermophoretic Measurement in the Narrow-Bore Transparent Capillary

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Summary

The microscale thermophoresis technique (MST) usually involves the use of disposable glass capillaries. However, the total capacity of these capillaries is much larger than the amount of sample used directly in the thermophoretic measurement. An alternative approach that reduces the usage of sample, reagents, and waste is to use narrow-bore capillaries, typically used in the capillary electrophoresis technique (CE), along with injecting a smaller sample segment. In this work, a new approach using a UV-Vis transparent and flexible capillary is presented. Such improvement makes the analytical process much easier to handle than traditional non-transparent silica capillaries and allows for thermophoretic analysis with a smaller sample volume. The sample is delivered to the detection window using pressure-induced flow or electrophoretically, while simultaneously performing electrophoretic separation. The primary focus is on the Transparent Capillary Stop-Flow MicroScale Thermophoresis (TCSF-MST) approach, which has been demonstrated to be effective in systems utilizing microscale thermophoresis for three distinct purposes: (i) monitoring the progress of the fluorescent derivatization reaction; (ii) determining the critical micellization concentration of a surfactant; and (iii) quantitative analysis of fluorophores based on the internal standard calibration method. The results indicate that this new TCSF-MST method could be an interesting extension of the traditional MST and is consistent with the principles of "green" and "white" analytical chemistry.

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Capillary Electrophoresis Analysis of Brain Tissue N-Glycosylation

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Summary

High sensitivity diagnostic methods are in great demand for early detection and treatment decisions of different illnesses. Biomarkers are important biomolecules to diagnose different diseases and their various clinical parameters. Altered protein glycosylation was already reported on this subject in the 1960s. Since then, glycobiomarkers have been extensively investigated in diagnostic research covering ailments such as cancers, inflammatory processes, autoimmune diseases, and other conditions including Alzheimer's disease. The expanding knowledge about the role of N-glycosylation in various syndromes suggests that glycobiomarkers can indicate the presence of certain diseases, track their progression, assess the risk of their development, and monitor the effects of drug treatments in therapeutic and diagnostic approaches.

In glycomics research, next to high-performance liquid chromatography and mass spectrometric methods, capillary electrophoresis-based (CE) separation techniques are also frequently applied. Its greatest advantage lies in requiring a small sample quantity, high separation efficiencies and short separation times. In this work the asparagine linked glycan profiles were investigated from swine brain samples without and with formalin treatment. Sample preparation optimization focused on automated tissue homogenization, delipidation, denaturation, enzymatic release of N-glycans, fluorescent labeling by aminopyrenetrisulfonate, and analysis by capillary electrophoresis with laser-induced fluorescent detection. The GUcal software was used to identifify the separated glycan peaks in the obtained electropherograms. The migration time and peak area reproducibility of the protocol were 0.17 and 3.67% RSD, respectively. The developed workflow will be applied for Alzheimer's disease research using the 2500 brain sample depository of our university.

P52

Analysis of NIST mAb Reference Material by Parallel CE-SDS

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Summary

Monoclonal antibody drug products are complex biomolecules that can contain undesirable species such as antibody fragments and non-glycosylated species leading to size heterogeneity. Characterization of size heterogeneity and impurities is necessary to determine the purity of a mAb drug product or if a given candidate or purification is suitable for downstream applications.

Agilent has developed a 12-channel, parallel Capillary Electrophoresis-SDS (CE-SDS) instrument and reagents. To demonstrate the capabilities of the ProteoAnalyzer, the NIST mAb was characterized under both reduced and non-reduced conditions and the results were compared to the NIST data sheet.

P53

Enhanced Fluorescent Detection of Oxaliplatin via BSA-Copper Nanoclusters: A Targeted Approach for Cancer Drug Monitoring

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Summary

A novel fluorescence sensing approach has been proposed for the precise determination of the anticancer drug oxaliplatin (OXA-Pt). This method entails synthesizing blue-emitting copper nanoclusters (CuNCs) functionalized with bovine serum albumin (BSA) acting as stabilizing agent. Upon excitation at 330 nm, the resultant probe exhibits emission at 460 nm. Notably, the fluorescence response of BSA@CuNCs substantially increases upon incubation with OXA-Pt due to multiple binding sites between the drug and the fluorescent probe. These binding interactions encompass hydrogen bonding, hydrophobic interaction, and the high affinity between the SH groups (cysteine residues of BSA) and platinum (in OXA-Pt). Consequently, this interaction induces aggregation-induced emission of BSA@CuNCs. The probe demonstrates a broad response range from 0.05 to 140.0 µM, along with a low detection limit of 16.0 nM, determined based on a signal-to-noise ratio of 3. Furthermore, the probe effectively detects OXA-Pt in human serum samples, yielding acceptable results. This study represents a significant advancement in the development of a straightforward and efficient sensor for monitoring platinum-containing anticancer drugs during chemotherapy.