41st IMMS

May 4-7, 2025

Fiera di Primiero - Italy

41st Informal Meeting on Mass Spectrometry

4th – 7th May 2025

Auditorium of Fiera di Primiero (TN) Italy

ORGANIZING COMMITTEE:

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Gianluca Bartolucci, Sara Crotti, and Pietro Traldi

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László Drahos and Károly Vékey

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41st IMMS Program

Sunday, May 4th

17:00-18:00 Registration
18:00-19:30 Informal get together party

Monday, May 5th

09:00-09:30 *Opening Ceremony*

Session 1: PHOTOIONIZATION, ENERGY TRANSFER, AND MOLECULAR DISSOCIATION DYNAMICS
Chairpersons: Luisa Mattoli and Piero Traldi

OP01	09:30-09:50	**Károly Vékey** THE SUN: PHOTOIONIZATION AND COLLISIONAL ENERGY TRANSFER
OP02	09:50-10:10	**Emilie Bertrand** ENERGY CALIBRATION OF A QUADRUPOLE ION TRAP BY RESONANT ACTIVATION
OP03	10:10-10:30	**Anestis Alexandridis** EVALUATION OF LIGAND INFLUENCE ON GOLD(I)-ALKYNE COMPLEXES USING (THRESHOLD) COLLISION INDUCED DISSOCIATION
OP04	10:30-10:50	**Peter C. Burgers** ANALYZING MELTING PHENOMENA USING ENTHALPY/ENTROPY DIAGRAMS: WHAT CAN WE LEARN?

10:50-11:20 *Coffee break and Poster Exhibition*

Session 2: ADVANCES IN MASS SPECTROMETRY: PEPTOID ANALYSIS, MULTI-OMICS, AND ION MOBILITY TECHNIQUES
Chairperson: Károly Vékey

OP05	11:20-11:40	**Pascal Gerbaux** MASS SPECTROMETRY OF PEPTOIDS AS PEPTIDOMIMETIC COMPOUNDS: FROM SEQUENCE TO ARTIFICIAL ENZYME DESIGN
OP06	11:40-12:00	**Gabriele A. Zingale** BEYOND LINEARITY: ***SPECTRASAGE*** REVEALS BRANCHED PEPTIDE PRODUCTS OF 20S PROTEASOME ACTIVITY ON MONO-UBIQUITYLATED TAU PROTEOFORMS
OP07	12:00-12:20	**Lei Ye** HOST-GUEST CHEMISTRY OF THE CYCLOPARAPHENYLENE DICATION (CPP^{2+})
OP08	12:20-12:40	**Viktória Goldschmidt Gőz** MULTIPASS COLLISIONAL CROSS SECTION MEAUREMENT STRATEGIES BY CYCLIC ION MOBILITY SPECTROMETRY
	12:40-14:30	*Lunch time*

Session 3: OMICS AND MASS SPECTROMETRY IN DISEASE DIAGNOSIS AND THERAPEUTIC DEVELOPMENT
Chairperson: László Drahos

OP09	14:30-14:50	**Andrea Armirotti** TOWARDS SAFE TREATMENTS FOR CYSTIC FIBROSIS: THE ROLE OF OMICS SCIENCES
OP10	14:50-15:10	**Summra Ahmed** MICROBIAL SIDEROPHORE INTERCALATED IN LIPOCALIN: NATIVE MASS SPECTROMETRY AND MOLECULAR MODELING
OP11	15:10-15:30	**Marta Sousa Silva** DECODING BIOTHREATS: FT-ICR-MS METABOLOMICS FOR THE IDENTIFICATION OF ***BACILLUS CEREUS***
OP12	15:30-15:50	**Samuele Scurati** THE FUTURE OF HIGH-THROUGHPUT OMICS: ZENOTOF 7600 SYSTEM FOR LIPIDOMICS AND METABOLOMICS

OP13 15:50-16:10 **Vladimir Havlicek**

MICROBIAL AND MAMMALIAN METABOLITES IN THE MASS SPECTROMETRIC DIAGNOSIS OF INFECTIOUS DISEASES

16:10-16:40 Coffee break and Posters Exhibition

Session 4: ADVANCEMENTS IN MASS SPECTROMETRY FOR DRUG ANALYSIS, MYCOTOXIN DETECTION, AND OLIGONUCLEOTIDE CHARACTERIZATION
Chairperson: Peter C. Burgers

OP14 16:40-17:00 **Rutuja H. Patil**

MASS SPECTROMETRY REVEALS VIRAL AND ZINC-DEPENDENT REGULATION OF FUNGAL MYCOTOXINS

OP15 17:00-17:20 **Antonio Triolo**

CHARACTERIZATION OF ANTISENSE OLIGONUCLEOTIDES BY ION PAIR HPLC-HIGH RESOLUTION MS: METHOD DEVELOPMENT USING DESIGN OF EXPERIMENTS

OP16 17:20:17:40 **Eleonora Cecchin**

ANALYTICAL AND CLINICAL VALIDATION OF A LC-MS/MS METHOD FOR THE QUANTIFICATION OF IMATINIB AND NORIMATINIB IN DRIED BLOOD SPOT COLLECTED USING VOLUMETRIC DEVICES

OP17 17:40-18:00 **Giovanna Nevola**

EXTRACTIVE-LIQUID SAMPLING ELECTRON IONIZATION-MASS SPECTROMETRY (E-LEI-MS): DRUG SCREENING AND DIRECT ANALYSIS IN PHARMACEUTICAL AND FORENSIC APPLICATION

19:30 Social Dinner

QR code to reach the social dinner restaurant:

Tuesday, May 6th

Session 5: EXPLORING PROTEIN STRUCTURE AND DYNAMICS: INSIGHTS FROM MASS SPECTROMETRY AND TANDEM TECHNIQUES
Chairperson: Thomas Drewello

OP18	09:00-09:20	**László Drahos** HOW MUCH INFORMATION DO TANDEM MASS SPECTRA CONTAIN?
OP19	09:20-09:40	**Ceyda Seren Ceyhan** REGULATION OF KERATIN 8 PHOSPHORYLATION DURING CELL DIVISION
OP20	09:40-10:00	**Sandra Hybsier** THE SYNERGY BETWEEN PEA TECHNOLOGY AND MASS SPECTROMETRY
OP21	10:00-10:20	**Émilie Hirschler** CROSS-LINKING MASS SPECTROMETRY OF THE ANTIMICROBIAL PEPTIDES MAGAININ 2 AND PGLA REVEALS HETERODIMERIZATION IN MICELLAR MEDIUM
OP22	10:20-10:40	**Alice Vetrano** PROTEIN SECONDARY STRUCTURE THROUGH SHORT-RANGE CROSS-LINKS
	10:40-11:00	*Coffee break and Posters exhibition*

Session 6: MASS SPECTROMETRY APPROACHES IN PROTEOMICS, CHEMICAL NETWORKS, AND FIELD DEPLOYABLE ANALYSIS
Chairperson: Marek Šebela

OP23	11:00-11:20	**Giuseppe Grasso** THE HUMAN 20S PROTEASOME CLEAVES BETA-AMYLOID (Aβ1-40) RELEASING PEPTIDES THAT ACT AS INHIBITORS OF ITS CHYMOTRYPSIN-LIKE ACTIVITY
OP24	11:20-11:40	**Béla Paizs** CHEMICAL ANNOTATION PROPAGATION FOR MOLECULAR NETWORKS

OP25 11:40-12:00 **Miroslav Polášek**
SELECTED ION FLOW TUBE MASS SPECTROMETRY OF VOLATILE METAL CARBONYLS

OP26 12:00-12:20 **Enrico Davoli**
FIELD DEPLOYABLE ION TRAP MS FOR DIRECT AND SPME MS&MS/MS ANALYSIS: BEYOND THE LABORATORY

12:20-16:00 *Lunch time and walking on the mountains*

16:00-16:30 *Coffee break and Posters exhibition*

Session 7: PROTEOLYSIS, METABOLISM, AND BIOTECHNOLOGY: NEW FRONTIERS IN PROTEOMIC RESEARCH AND INDUSTRIAL APPLICATIONS
Chairperson: Vladimir Havlicek

OP27 16:30-16:50 **Diego Sbardella**

MINING OF N- AND C- TERMINI INTRODUCES THE VITREOUS HUMOR DEGRADOME AND HIGHLIGHTS ALTERED PROTEOLYTIC PATTERNS OF STRUCTURAL AND NON STRUCTURAL COMPONENTS IN RHEGMATOGENOUS RETINAL DETACHMENT

OP28 16:50-17:10 **Marek Šebela**
MASS SPECTROMETRIC EVALUATION OF PROTEOLYTIC ACTIVITY IN WINE VINEGARS

OP29 17:10-17:30 **Nicola Cimino**

AUTOMATED FRACTIONATION OF LOW VOLUME PLASMA SAMPLES FOR LC-MS MULTI-OMICS

OP30 17:30-17:50 **Javier F. Montero-Bullón**
STATIONARY METABOLIC FLUX ANALYSIS OF THE BIOTECHNOLOGICAL CHASSIS ***ASHBYA GOSSYPII*** FOR RIBOFLAVIN PRODUCTION ON A NON-CONVENTIONAL XYLOSE SUBSTRATE

Wendesday, May 7th

Session 8: INNOVATIVE ANALYTICAL TECHNIQUES IN FOOD AUTHENTICATION, CONTAMINANT DETECTION, AND ENVIRONMENTAL MONITORING
Chairperson: Pascal Gerbaux

OP31	09:00-09:20	**Silvia Pianezze** LATEST APPLICATIONS OF THE LC-CO-IRMS FOR FOOD AND DIETARY SUPPLEMENTS AUTHENTICATION
OP32	09:20-09:40	**Emanuele Ceccon** LATEST TRENDS IN PFAS TESTING AND REGULATIONS LANDSCAPE: FROM PPB TO PPQ IN WATER, SERUM AND BEYOND
OP33	09:40-10:00	**Achu Kuriakose** ELUCIDATING CONTAMINANT SORPTION DYNAMICS USING SPME-LEI-QqQ MASS SPECTROMETRY
OP34	10:00-10:20	**Giorgio Zanoni** INSIGHT ON LUGANA FLAVOR WITH A NEW LC-MS METHOD FOR THE DETECTION OF POLYFUNCTIONAL THIOLS
	10:20-10:50	*Coffee break*

Session 9: MASS SPECTROMETRY IN AGRIFOOD AND NATURAL PRODUCTS
Chairperson: Carlos Cordeiro

OP35	11:50-11:10	**Franco Biasioli** AN INFORMAL VIEW ON THE PATH OF DIRECT INJECTION MASS SPECTROMETRY FOR RAPID VOLATILE COMPOUND MONITORING FROM IMMS29 TO IMMS41: AGRIFOOD APPLICATIONS, GLOBAL QUALITY AND SUSTAINABLE INNOVATION
OP36	11:10-11:30	**Annarita Panighel** USE OF ORTHOGONAL MS-APPROACHES IN THE STUDY OF AROMA COMPOSITION OF NATIVE SICILIAN GRAPES AND WINES

OP37	11:30-11:50	**Katarzyna Pawlak** COMPREHENSIVE QUALITATIVE AND QUANTITATIVE ANALYSIS OF SAPONINS IN SAPONARIA OFFICINALIS
OP38	11:50-12:10	**Tiziana Nardin** HIGH-RESOLUTION MASS SPECTROMETRY (HRMS) TO INVESTIGATE THE ALKALOID PROFILE OF MILK
	12:10-12:30	*Award for the Best young oral presentation*
	12:30-13:00	*Final remarks and presentation of the 42th IMMS*

Book of Abstracts QR code:

Posters session:

Authors and titles

P1. **A. Márton; Gy. Ferenc; É. Hunyadi-Gulyás; Z. Darula, V. Goldschmidt-Gőz, G. Schlosser, and Z. Kupihár**
STRUCTURAL CHARACTERIZATION OF OLIGONUCLEOTIDES WITH DOUBLE HELIX FORMING ABILITIES USING MULTIPLE METHODS.

P2. **V. Pashynska, M. Kosevich, O. Boryak, I. Voloshin, V. Karachevtsev, and P. Kuzema**
MASS SPECTROMETRIC CHARACTERIZATION OF NANOCOMPOSITES OF ANTICANCER DRUGS WITH MoS2 AS POTENTIAL MULTIFUNCTIONAL THERAPEUTIC NANOPLATFORMS.

P3. **M.Kosevich, V. Shelkovsky, O. Boryak, V. Zobnina, P. Kuzema, and V. Karachevtsev**
DIVERSITY OF FEATURES OF NANOCOMPOSITES OF TRANSITION METAL DICHALCOGENIDES WITH ORGANIC COMPOUNDS AS REVEALED BY LASER DESORPTION/IONIZATION MASS SPECTROMETRY.

P4. **F. Villanelli, L. Calamai, E. Maseroli, S. Marchiani, M.E. Ragosta, G. Danza, M. Cirillo, and L. Vignozzi**
A NEW METHOD FOR KETONE BODIES DETERMINATION IN SERUM AND SEMINAL FLUID BY GC-MS.

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P6. **R. Kamguem; Q. Duez; T. Robert; P. Gerbaux, and J. De Winter**
PHOTOSENSITIVE MACROCYCLIC PEPTOIDS FUNCTIONALIZED WITHAZOBENZENES.

P7. **E. Piplart, A. Serez, G. Henrard, J. De Winter, M. Frère, J. Cornil, and P. Gerbaux**
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P12. **D. Papp, K. N. Enyedi, and G. Schlosser**
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P13. **F. Errante, M. Pallecchi, M. Menicatti, L. Giovannelli, A. M. Papini, P. Rovero, and G. Bartolucci**
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P43. **A. Pap, M. Tangos, E. Hunyadi-Gulyas, E. Klement, A. Pettko-Szandtner, N. Hamdani, and Z. Darula**
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A MULTI-DISEASE STATISTICAL ANALYSIS OF FREE FATTY ACID SIGNATURES ASSESSED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY.

Abstracts,

ORAL COMUNICATIONS

OP1

THE SUN: PHOTOIONIZATION AND COLLISIONAL ENERGY TRANSFER

N. Solem[1,2], C. Alcaraz[1,2], R. Thissen[1,2], K. Nagy[3], Á. Révész[3], L. Drahos[3] and K. Vékey[3]

[1]CNRS, Institut de Chimie Physique, Université Paris-Saclay, UMR 8000, Orsay 91405, France
[2]Synchrotron SOLEIL, L'Orme des Merisiers, 91192 Saint Aubin, Gif-sur-Yvette, France
[3]HUN-REN Research Centre for Natural Sciences, Magyar Tudósok krt. 2, 1117 Budapest, Hungary

SOLEIL (the Sun) is a large-scale synchrotron facility located in France, providing intense photon beams across a broad spectral range, from IR to X-Rays, depending on the beam line that is used. The DESIRS beamline provides with VUV light (5 to 35 eV), ideal for single photon ionization and dissociation of small molecules. Connected to the DESIRS beamline, the CERISES (Cherry) instrument is a guided beam, tandem mass spectrometer specifically dedicated to the study of ion molecule collisions and reactions. In the first part of this presentation, we will introduce general aspects of the SOLEIL synchrotron and details the CERISES mass spectrometer, highlighting its capabilities and emphasizing the unique opportunities it presents for conducting fundamental research using advanced experimental techniques.

In the second part of the lecture, we will show some of our most surprising results. Most MS/MS studies rely on CID, and it is widely used. Excitation and energy exchange (from the kinetic energy into the internal energy of the fast ion) is a most important feature of the collision (and of MS/MS). We know that the degree of excitation increases with the collision energy, but we know relatively little what happens in a single collision. The maximum energy transfer is limited (to the center of mass collision energy); but the literature disagrees how much is really exchanged. Some studies indicate a few %, some others close to 100 %. We shall show our results obtained using buthylbenzene molecular cation produced by one photo ionization, and fragmented by single collision with Xe, at well-defined collision energies.

OP2

ENERGY CALIBRATION OF A QUADRUPOLE ION TRAP BY RESONANT ACTIVATION

E. Bertrand, F. Kalem, F. Rosu, V. Gabelica

University of Geneva, Switzerland.

In mass spectrometry, working with a fragile system (metabolites, lipids, amino acids, nucleic acids, organic pollutants, etc...) is a challenge. In tandem mass spectrometry (MS/MS), the nature of the observed fragments and their intensity depend on the ion internal energy distribution. Under appropriate conditions, the internal energy distribution of a group of ions can be defined by an effective temperature (T_{eff}): the temperature to which ion should be physically heated to give the same spectrum as the experimental one. A method to calibrate T_{eff} in a quadrupole ion trap by resonant activation is to perform dissociation kinetics on a group of thermometer molecules with known Arrhenius parameters. The calibration method used in this work is inspired by the one described before for a ThermoFinnigan LCQ [1].

In this work, the selected thermometers molecules are Bradykinin $(M+H)^+$, $(M+2H)^{2+}$ and $(M+3H)^{3+}$, Leucine Enkephalin $(M+H)^+$, Ubiquitin $(M+11H)^{11+}$ and *holo*-Myoglobin $(M+9H)^{9+}$. Their Arrhenius parameters had been already estimated (**Table 1).**

Table 1. Published Arrhenius parameters of the thermometer ions used.

Thermometer molecules	Charge	Log A	E_a (eV)	Ref.
Bradykinin	$[M+H]^+$	12.12	1.27	[2]
	$[M+2H]^{2+}$	7.63	0.82	[2]
	$[M+3H]^{3+}$	9.3	0.79	[2]
Leucine enkephalin	$[M+H]^+$	12.55	1.28	[3]
Ubiquitin	$[M+11H]^{11+}$	16.7	1.55	[4]
Holo-Myoglobin	$[M+9H]^{9+}$	8	0.9	[5]

The calibration is performed in a Bruker amaZon quadrupole ion trap. As shown with the dissociation kinetics obtained for Leucine enkephalin, the precursor ion was isolated and fragmented at different resonant activation amplitudes and activation times, and the logarithm of the survival yield is plotted as a function of the activation amplitude (**Figure 1.a.**). The dissociation kinetics fit a pseudo-first order kinetics, and the dissociation rate constants were determined by linear regression. The effective temperature at each activation amplitude could be calculated using the modified Arrhenius activation (**Figure 1.b.**). The same data processing is performed for the other thermometer molecules and leads to the complete calibration of the amaZon quadrupole ion trap. The T_{eff}(K) = intercept + slope x Amp(V) calibration curve is used in order to determine the Arrhenius parameters for G-quadruplex $(G_nT_4G_4)_2$ [6] and for duplexes with varying GC content [7].

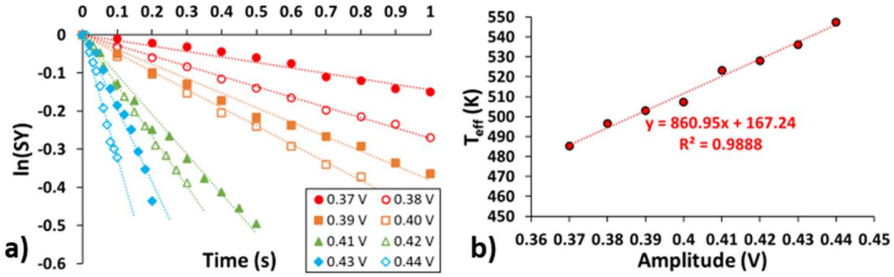

Figure 1. a. Dissociation kinetics of the protonated Leucine Enkephalin as a function of the resonant activation amplitude. **b.** Effective temperature as a function of the resonant activation amplitude.

We found that the dissociation kinetics obtained are highly sensitive to the fragmentation width (frag. width), which is the experimental parameter influencing the frequency profile of the secular frequency of the studied ion. For the same thermometer molecules, depending on the frag. width value, the activation waveform will be composed of one, three or more frequencies. For example, as shown in Figure 2, in the case of the Leucine Enkephalin, with a frag. width of 5.7 m/z there is only one frequency peak and with a frag. width of 5.8 m/z, there are three frequencies peaks. When there is only one frequency peak, the activation is harsher and centered on a specific isotopic peak. It means that the obtained dissociation rate contants and T_{eff} are higher. In the case of activation with three frequencies, the activation resonating with the entire isotopic distribution and its amplitude is diluted, resulting in softer activation, but the isotopic distribution is more homogeneously activated. To define comparable conditions among calibration and calibrated molecules, the fragmentation width resulting in an applied waveform of three frequencies, always centered at the same secular frequency determined by q_z.

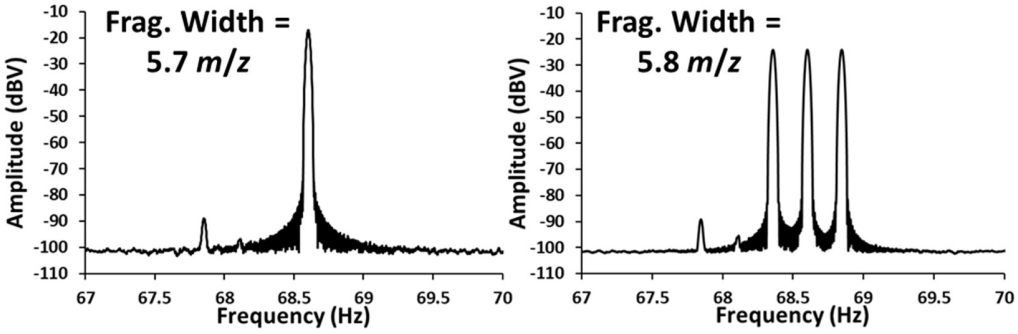

Figure 2. Amplitude of the frequency applied at the end cap electrodes obtained in the case of Leucine Enkephaline with the Frag. Width set at 5.7 m/z or 5.8 m/z.

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OP3

EVALUATION OF LIGAND INFLUENCE ON GOLD(I)-ALKYNE COMPLEXES USING (THRESHOLD) COLLISION INDUCED DISSOCIATION

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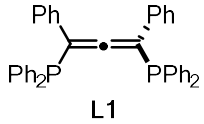
Modern chemistry is increasingly dependent on catalysis in order to access synthetically important scaffolds. In the case of catalysis using transition metals, the properties of the metal center are highly influenced by the metal's environment, and more specifically by the nature of the ligands. Axial ligand chirality is one of the cornerstones of enantioselective catalysis, with biaryl-type diphosphine ligands being one of the most explored classes [1]. In sharp contrast, the axial chirality of allene-containing ligands such as **L1** (Figure 1) remains severely underexplored.



L1

Figure 1. 1,3-Bis(diphenylphosphine)allene ligand.

In order to probe the potential of ligand **L1** in gold(I)-catalyzed reactions, the dinuclear complex **Au1** and its BH_3-protected mononuclear analogue **Au2** (Figure 2) were synthesized, with **Au1** providing promising results in preliminary investigations [2].

Figure 2. The studied 1,3-bis(diphenylphosphine)allene gold(I) complexes.

To explore the electronic effects of the ligand on the metal center, threshold collision-induced dissociation (TCID) of an adduct of the complex at low gas pressure is a reliable approach. While this technique commonly employs custom-made instruments, our group has shown that commercially available instruments with modifications can also provide comparable results [3]. In addition to TCID, collision-induced dissociation (CID) in higher gas pressure can also provide data on the electronic nature of the ligand, thus allowing the measurement of critical energies for complexes of very different sizes [4]. These experiments were performed in a triple quadrupole mass spectrometer with modifications allowing for ion-molecule reactions prior to mass selection (Figure 3).

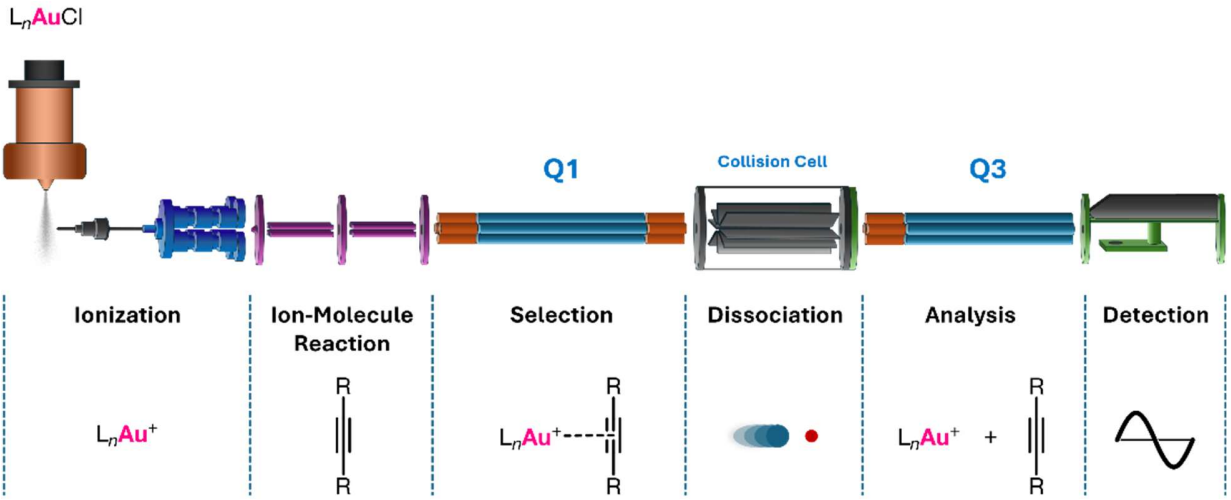


Figure 3. Triple quadrupole mass spectrometer modified for ion-molecule reactivity.

A series of catalytically relevant gold(I) complexes including 1,3-bis(diphenylphosphine)allene gold(I) complexes were ionized, then coordinated with an alkyne ligand in the gas phase. The resulting adduct was selected, then subjected to CID.

In order to estimate the critical energies of the gold-alkyne adducts, RRKM simulations from the *MassKinetics* software [5] were used. The comparison with the results obtained from the two dissociation techniques (threshold or high-pressure) will be examined in detail [6].

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OP4

ANALYZING MELTING PHENOMENA USING ENTHALPY/ENTROPY DIAGRAMS: WHAT CAN WE LEARN?

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The question has recently been raised that although melting may be well known, is it also well understood? [1] Not entirely, it appears, despite the fact that today many accurate [2] thermochemical data are available from different sources, for example from the Yaws compilations [3]. We have gathered available data, notably melting temperatures (*Tm*), enthalpies (*ΔHfusion*) and entropies (*ΔSfusion*) for the melting processes of inorganic and organic compounds which will be discussed in this presentation; we have also compiled available data for the melting (denaturation) processes of DNA/DNA duplexes on which we also report.

For inorganic compounds we will discuss the interesting case of the melting of the alkali halides where the melting temperatures of the lithium halides are remarkably lower than those of the others. In an enthalpy/entropy diagram, where *ΔHfusion* is plotted against *ΔSfusion* , the lithium halides are shifted towards much lower values leading to significant lower melting temperatures. For LiCl, see figure 1, for which *Tm* is depressed by about 200 K, the lowering of *ΔHfusion* (which would lead to a depression in *Tm* of 300 K) is offset by a concomitant lowering of *ΔSfusion* (which would lead to an increase of 100 K).

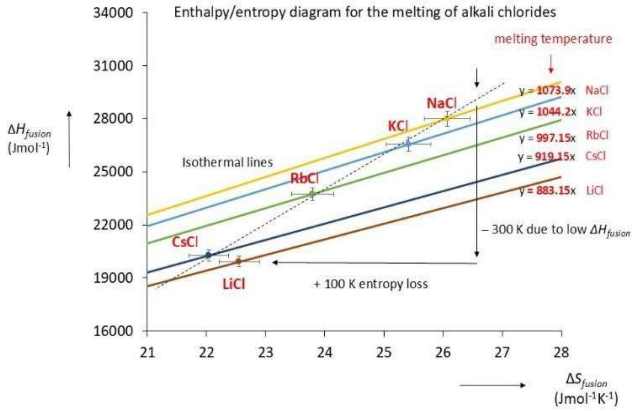

Figure 1. Enthalpy/entropy diagram for the melting of alkali chlorides. $Tm = \Delta Hfusion/\Delta Sfusion$ ($\Delta G = 0$). The data point for LiCl is shifted considerably towards lower *ΔHfusion* and lower *ΔSfusion*.

An elegant rationalization for this melting point depression is provided by considering the melting of the alkali halides as a random walk (or Brownian motion) of spherical particles, where the entropic force ($= 2 \bullet k \bullet Tm/r$, where k is Boltzmann's constant) balances the Coulombic force ($= 1/(D \bullet r^2)$), leading to $Tm = 1/(2 \bullet k \bullet D \bullet r)$, where D is the dielectric constant of the melt [4].

Since D is high for the lithium halides, Tm will be low. This also neatly rationalizes the very low Tm values of the thallium(I) halides which have large dielectric constants. We can find no other theory that can rationalize these effects.

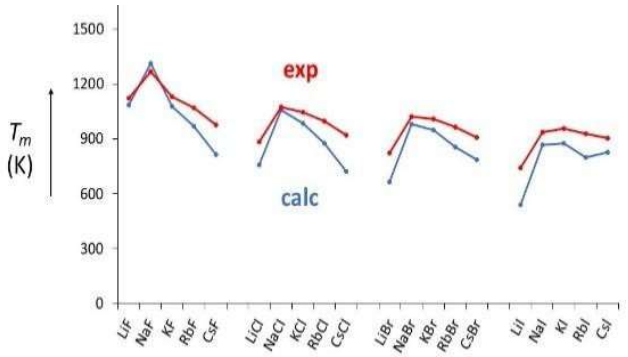

Figure 2. Experimental and calculated melting temperatures of the alkali halides from $Tm = 1/(2\bullet k\bullet D\bullet r)$, derived using the concept of entropic force. The dielectric constant D at melt can be equated to $LBE/\Delta H_{fusion}$ where LBE is the lattice binding enthalpy [4]. Although this simple formula leads to an, on average, lower Tm than the experimental values, it does reproduce the low melting points of the lithium halides.

For organic compounds we will discuss the odd-even effect frequently observed for aliphatic chains where the melting points (but not the boiling points) show a zigzag pattern rather than a monotonic trend as a function of the number of carbon atoms.

A third example concerns the melting of DNA duplexes [5] from which it follows that enthalpy/entropy compensations are highly relevant in nature's systems [6]. (For these denaturation processes, $\Delta G \neq 0$.) For example, a duplex of a 35 base-pair DNA forms in an aqueous solution from its constituent single strands with an exothermicity of -1213 $kJ\bullet mol^{-1}$, but is also associated with a dramatic entropy change of -3376 $J\bullet mol^{-1}\bullet K^{-1}$ [7].

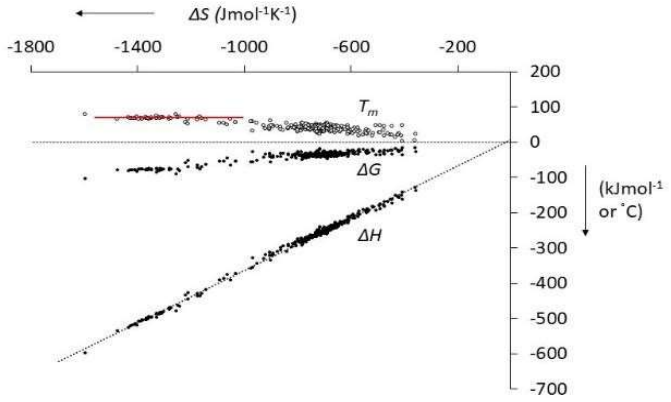

Figure 3. Enthalpy/entropy diagram fc melting of DNA duplexes. $\Delta G = \Delta H - T\Delta S$. be seen that very large differences in ΔH a underly the relatively small differences in n points (red line) and Gibbs free energies means that for mismatches the weakening duplex bonding is compensated by an incre its dynamics as has been pointed out by Wi et al.[6]

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OP5

MASS SPECTROMETRY OF PEPTOIDS AS PEPTIDOMIMETIC COMPOUNDS: FROM SEQUENCE TO ARTIFICIAL ENZYME DESIGN

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The relationship between structure and activity has captivated scientists for the past decades. By taking inspiration from the folding of proteins and enzymes, chemists currently seek to design sequence-defined macromolecular chains that can undergo intramolecular cross- linking reactions and collapse into bioinspired nanoparticles (see Fig. 1) [1]. Over the years, non-natural compounds able to dynamically fold and unfold into well-defined secondary structures called 'foldamers' have aroused interest in many fields, e.g. in catalysis.

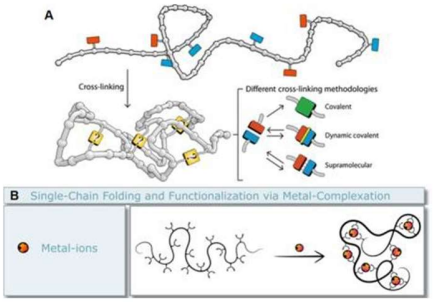

Fig. 1. (A) Collapse of a sequence-defined macromolecule via different cross-linkings. (B) metal ion induced folding of a polymer chain containing ligand moieties. Metal cations are responsible for both the collapse and catalytic activity. Adapted from [1].

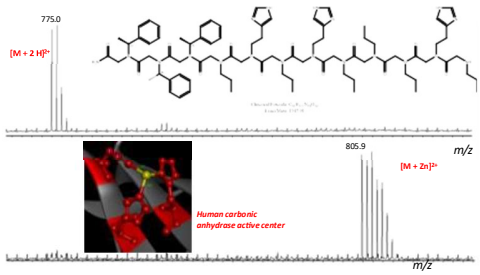

Fig. 2. Artificial Carbonic anhydrase active center : 3-imidazole peptoid. Mass spectrometry demonstration of the Zn^{2+} ion complexation : Electrospray analysis of the peptoid without (up) and with (bottom) the addition of a Zn^{2+} salt (Zn:peptoid = 1:1)

Peptoids are poly-N-substituted glycines belonging to the peptidomimetic polymer family (see Fig. 2) [2]. The combination of the side chains that are appended to the backbone nitrogen atoms in peptoids plays a key role for stabilizing the secondary structures, *i.e.*, helices, threaded-loop or ribbons, both in solution and in the solid state [2].

In associating organic synthesis with structural characterization, we are now conducting research on artificial (metallo)enzymes.

These biomimetic compounds are synthetic organic (macro)molecules designed to mimic enzymatic functions toward large-scale applications. Our focus is to develop sequence-defined peptoid-based enzyme mimics. Peptoids are typically synthesized using the submonomer method [2]. This method involves the stepwise construction of the polymer chain, allowing for the creation of tailor-made, sequence-defined structures with functional groups at selected positions. Peptoid-based catalysts are unique due to improved catalytic performance through chemogenetic optimization. This involves parallel optimization of both the direct metal surroundings (first coordination sphere) and the polymer scaffold (second coordination sphere), which are crucial for achieving (regio)(stereo)selectivity. To design the initial peptoid sequences, we draw inspiration from 3D

structures of metallo-proteins archived in the Protein Data Bank (PDB). Using a reductionist approach, we are creating short, heterogeneous peptoids that are intended to mimic the active sites of these proteins. Our initial research candidate is the Carbonic Anhydrase (CA) family of metalloenzymes (see Fig. 2) [3], present in many organisms and which catalyse the hydration and dehydration of CO_2. CA enzymes feature a Zn^{2+} ion at the center of their active site, coordinated to one water molecule and three histidine residues, essential for CO_2 conversion.

In recent years, by taking huge advantages from the unique combination between organic synthesis and mass spectrometry, we turned mass spectrometry into an inescapable analytical method to tackle the structure complexity of tailor-made original peptoids, i.e., from the primary sequence confirmation to the 3D structure establishment, mostly by using collision- induced dissociation and ion mobility experiments, together with theoretical chemistry. In a bottom-up approach, from sequence to 3D structure, our strategy is to prepare tailor-made model peptoids to answer specific questions, such as (i) how to sequence de novo peptoid by tandem mass spectrometry, (ii) are solution phase secondary structures, typically the helical structures, conserved upon ionization/desolvation, (iii) are ion mobility experiments together with Molecular Dynamics simulations able to describe the gas phase structures of peptoids and to tackle subtle differences between conformers, (iv) are the gas phase complex ions generated upon electrospray ionization of peptoid/metal salt solution mimicking the 3D structures, including the coordination sphere of the metal ion (Zn^{2+}, Ni^{2+}…) of the metalloenzyme mimics and (v) can we take advantage of the online coupling between flow chemistry and mass spectrometry to detect transient intermediates of the catalytic reactions?

In the present communication, by using selected examples [4-5], the efficiency of mass spectrometry experiments for the in-depth structural characterization - ***from the primary to the ternary structures*** - of original bio-inspired peptoids will be demonstrated.

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OP6

BEYOND LINEARITY: *SpectraSage* REVEALS BRANCHED PEPTIDE PRODUCTS OF 20S PROTEASOME ACTIVITY ON MONO-UBIQUITYLATED TAU PROTEOFORMS

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We here introduce SpectraSage, a novel software tool specifically developed by us for the Mass Spectrometry (MS) identification at MS1 level of peptides generated during enzymological studies in vitro (substrate:enzyme). SpectraSage finds particular application for the analysis of complex proteolytic events, as in the case of digestion of branched substrates by proteolytic systems with unknown catalytic specificities. It excels in identifying long, branched peptides that are challenging to detect using conventional MS (*i.e.* MS/MS) approaches, overcoming limitations in identifying non-tryptic cross-linked peptides. This software significantly enhances the capabilities of MS analysis, providing a valuable tool for researchers investigating protein degradation mechanisms and other biological processes involving protein-protein interactions *in vitro*.

To demonstrate the power of SpectraSage, we applied it to investigate the degradation of mono-ubiquitylated (mono-Ub) tau protein (MAPT gene) by the 20S proteasome. The Ubiquitin Proteasome System (UPS) is crucial for cellular protein homeostasis. While traditionally believed to require poly-ubiquitin chains for substrate recognition, recent studies suggest that the uncapped 20S proteasome can degrade mono-Ub substrates independently. Tau protein is essential for neuronal function, and its misfolding and aggregation are implicated in neurodegenerative diseases. This test study investigates the degradation of synthetic and semi-synthetic mono-Ub tau proteoforms covering the 4RD domain of the protein by the 20S proteasome. By means of SpectraSage, we show that the 20S proteasome can efficiently degrade mono-Ub tau proteins in vitro, generating unique branched peptide fragments. Notably, we observed that the position of the ubiquitin moiety significantly influenced the degradation pattern and the resulting peptide fragments.

By employing SpectraSage to analyze these complex degradation products, we gained crucial insights into the interplay between ubiquitylation and proteasomal degradation of tau protein, highlighting the importance of site-specific ubiquitylation in modulating protein fate. This study demonstrates the significant advancements SpectraSage brings to the field of mass spectrometry, enabling deeper understanding of complex biological processes and paving the way for future discoveries in proteomics and related fields.

OP7

HOST-GUEST CHEMISTRY OF THE CYCLOPARAPHENYLENE DICATION (CPP^{2+})

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Cycloparaphenylenes ([n]CPPs) are ring molecules ("nanohoops") composed of para-linked benzene rings. "[n]" refers to the number of benzene units. The [n]CPPs with n= 5-12 are available in macroscopic quantities. One amazing feature is their facile oxidation into fairly stable molecular dications. The CPP^{2+} dications possess (4N+2) π electrons and show a unique "in-plane aromaticity". Their stability results in part from strain release upon oxidation. A second unique feature relates to their host-guest chemistry. Since the CPP ring features a convex outside and a concave inside, it provides both elements of shape complementary for noncovalent π π-binding. Thus, the CPP molecule may act both as host as well as guest molecule. In this way a large enough CPP ring may act as host for fullerenes or smaller CPP rings.

Here, we focus on the combination of these unusual properties and investigate the formation of dicationic $CPP^{2+}\supset$guest complexes. Employing ESI we generated the complexes $[n]CPP^{2+}\supset C_m$ with n=10, 11, and 12 and m= 60 and 70 (fullerenes) and selected organic ligand-bearing fullerene derivatives as guests. Moreover, ring-in-ring complexes of the type $[n]CPP^{2+}\supset$[m]CPP with n > m were found. The fragmentation behaviour and relative stabilities are studied in energy-resolved CID experiments. As a consequence of their bigger π–system, the $[n]CPP^{2+}\supset$fullerene complexes are more stable than the ring-in-ring complexes. The dicationic complexes show two distinct dissociations, i.e. the dissociation into CPP^{2+} and the neutral guest, and/or dissociation with electron transfer, yielding $CPP^{+\bullet}$ and the guest radical cation. For all complexes, the electron transfer is highly exothermic and there is clear indication that the host-guest distance within the complex is a decisive criterion influencing the electron transfer.

OP8

MULTIPASS COLLISIONAL CROSS SECTION MEAUREMENT STRATEGIES BY CYCLIC ION MOBILITY SPECTROMETRY

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Ion mobility spectrometry (IMS) is a widely used analytical technique for determining the collisional cross section (CCS), a physical parameter essential for identifying and characterizing the structure of various molecules, such as components in complex biological samples. Drift tube ion mobility spectrometry (DTIMS) offers the fastest and most straightforward method for CCS determination, as CCS values can be directly calculated from DTIMS measurements. However, DTIMS has limited ion mobility resolution due to the short ion drift path. In contrast, cyclic IMS (cIMS) instruments utilize traveling wave ion mobility (TWIM) technology within a cyclic ion mobility cell, providing enhanced mobility resolution. In cIM-MS instruments, CCS values are typically obtained from single-pass measurements after calibration. Achieving high ion mobility resolution through multipass separation in the cyclic ion mobility cell requires more complex calibration strategies.

In this work, we compared the multipass CCS measurements methods [1-4] available for cyclic ion mobility mass spectrometers [5]. The first method was the so called “same pass method”, in which calibrants are analyzed in the same pass number as the analytes. This strategy was published firstly in the literature [1]. The “average ion velocity method” [2] applies an average ion velocity, calculated from the path length traveled by the ions in the cyclic cell and from the drift time (t_{corr}) corrected with the dead time (t_0). The third strategy was the “projected single-pass method” [3], in which the calibrants were measured in one-pass only but the corrected drift times (t_{corr}) were used for CCS calibration. But this, the t_{corr} multipass drift times of the analytes were divided by the number of passes to obtain a virtual „corrected single-pass drift time” for each analytes. Finally, the last calibration strategy was the “perturbed periodic drift time method” which is considered the most accurate [4]. Here, the physical changes within the cyclic ion mobility cell during multipass measurements were also taken into account and corrected mathematically. In this calibration method, various separation times were measured for the calibrants and the analytes. By considering the differing velocities of ions under perturbed and unperturbed fields, the so-called “perturbed periodic drift time” was determined based on the separation times, and the number of passes, which were then used for calibration and CCS determination.

Our aim was to compare these mulipass calibration strategies to identify possible major differences and to acquire experimental experience of their precision. Our experiments demonstrated that CCS valuses, determined by DTIMS and by single-pass cyclic TWIMS were identical within 1% , therefore single-pass cIM data were used as references to probe the quality of multipass calibration strategies.

When comparing the results to single-pass cIM data as a reference, the "perturbed periodic drift time method" published recently by Xia et al, showed the smallest difference, where drift times, measured over multiple passes and scaled to a single pass are used to determine CCS. Additionally, we performed experiments with three different calibration sets and 30 test molecules (10 from each compound class). These results demonstrated that not only the multipass CCS measurement strategy, but the quality of the calibration set is a significant factor as well during exact CCS measurements.

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OP9

TOWARDS SAFE TREATMENTS FOR CYSTIC FIBROSIS: THE ROLE OF OMICS SCIENCES

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Cystic Fibrosis (CF) is the most common genetic disease, with around 100000 affected people worldwide. It is caused by mutations of CFTR gene, which encodes for a very important anion transporter, highly expressed in human epithelial cells. Over the last years, new modulator drugs have greatly improved life quality and expectancy for CF patients, with the triple drug combination (ETI: Elexacaftor, Tezacaftor, Ivacaftor) representing a powerful therapeutic option. Over the last years, we have worked quite extensively to characterize the action of ETI and to dissect possible side effects by using omics sciences. We previously demonstrated [1] that bronchial epithelial cells (BE), from both CF and non-CF individuals, show accumulation of saturated sphingolipids when treated with ETI. We recently discovered that this drug interacts with an important human enzyme, Δ-4 sphingolipid desaturase (DEGS), which converts dihydroceramides (dHCer) into ceramides [2,3]. Given the role of this enzyme in the central and peripheral nervous systems [4], we aim at investigating potential long-term side effects of the use of ETI during neurodevelopment, by conducting a long-term exposure study in mice. To evaluate the effect of ETI during pregnancy and breast feeding, we will expose CD-1 female mice to ETI through food. After three weeks, the females will be allowed to mate and, for the whole duration of pregnancy and breastfeeding, they will be exposed to ETI. We will quantify ETI levels in mum's and pup's blood and tissues and we will measure, if any, the accumulation of dHCer. The neurological abilities of newborn mice and their general wellbeing will also be assessed through behavioral tests. Morphological and functional assessment of PNS and CNS will also be carried out. LC-MS will play a major role in this project, enabling both targeted, highly sensitive studies and untargeted surveys of the changes associated with ETI exposure, particularly in the brain.

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OP10

MICROBIAL SIDEROPHORE INTERCALATED IN LIPOCALIN: NATIVE MASS SPECTROMETRY AND MOLECULAR MODELING

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The struggle for nutrients is an important element in the progression of an infection. Microbes produce siderophores to chelate iron while the host's immune cells counteract by producing lipocalins [1]. Enterobactin (Ent) is a catechol siderophore isolated from most *Enterobacteriaceae* [2,3]. Nutritional immunity to pathogens is partially controlled by lipocalin proteins (Lcn1/Lcn2) secreted by some immune cells during microbial invasion [4].

The intercalation of the siderophore with the lipocalin protein was investigated using analytical and computational tools. The non-covalent complexes Lcn1-Ent/Lcn2-Ent were characterized by electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS) (Synapt G2S*i*, Waters) using home-made gold-plated ESI emitters. The spectra of a 20 µM concentration of Lcn1 and Lcn2 in 150 mM ammonium acetate were recorded in a 1:1 stoichiometric ratio with desferri-Ent. The stability of the Lcn1-Ent complex was tested in the pH range of 5.4–9, Ent concentration of 0.2-200 µM, and with ion block temperatures of 50 to 120 °C. The interaction was modeled using SwissDock, KVFinder, LigPlot, and Chimera tools.

The Lcn1-Ent complex was found to be pH and concentration-sensitive. The optimal conditions were pH 6.4–7.4, 20 µM (1:1) with no thermal effect. Native MS data performed at pH 7.4 and equimolar concentration (20 µM) of Ent and Lcn1/Lcn2 indicated partial intercalation of Lcn1-Ent and complete incorporation of Lcn2-Ent (Fig. 1). Molecular modeling identified critical amino acids (Lys114, Asp25, and Leu33) for Lcn1-Ent and (Lys125 and Lys134) for Lcn2-Ent. Identifying proteins involved in innate immunity and studying binary and ternary complexes of metallophores may pave the way for discovering new therapeutic strategies.

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OP11

DECODING BIOTHREATS: FT-ICR-MS METABOLOMICS FOR THE IDENTIFICATION OF *Bacillus cereus*

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"*In a bioterrorism scenario, can we afford to misidentify a microbial threat?*" Genome sequencing is currently the gold standard for microbial identification. However, it may fail to distinguish closely related species with overlapping genetic features. This is the case of *Bacillus cereus* and *Bacillus cytotoxicus*, two members of the *Bacillus cereus* group with distinct pathogenic profiles [1]. Moreover, these bacteria primarily exist as highly resilient spores in environmental samples, further complicating their identification using traditional methods. *Bacillus cereus* is a well-known opportunistic pathogen, commonly associated with foodborne illnesses and localized infections. Nevertheless, certain strains can carry virulence factors that make them highly toxigenic. In contrast, *Bacillus cytotoxicus* is a thermotolerant species with a narrower ecological niche but considered more virulent due to its potent cytotoxins. Despite these differences, genomic data alone often fails to clearly differentiate the two species, making difficult their classification and risk assessment. This distinction becomes particularly relevant in the context of biowarfare. The *Bacillus cereus* group includes strains that possess genetic elements similar to *Bacillus anthracis*, the causative agent of anthrax. Some *B. cereus* strains can acquire virulence plasmids, making them capable of causing anthrax-like infections. Misidentification or delayed differentiation of these resilient bacterial spores could have severe consequences in the event of a biological attack or an accident, where rapid and accurate detection is critical for an effective response. Here we show that FT-ICR-MS-based untargeted metabolomics is an effective method for an accurate identification of *Bacillus cereus* and *Bacillus cytotoxicus*, ultimately contributing to improved microbial forensics and biosurveillance strategies.

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OP12

THE FUTURE OF HIGH-THROUGHPUT OMICS: Zeno TOF 7600 SYSTEM FOR LIPIDOMICS AND METABOLOMICS

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Untargeted lipid analysis presents a unique set of challenges, primarily due to the significant overlap of isobaric compounds across different lipid classes. Additionally, the relatively low number of diagnostic fragment ions generated by collision-induced dissociation (CID) makes accurate compound identification more difficult, increasing the risk of false positives when using data analysis software. To address these challenges, a new approach was developed that combines electron-activated dissociation (EAD) with the ZenoTOF 7600 system. This method not only enhances lipid structural characterization but also reduces the likelihood of false positives. By integrating data from both DDA and DIA (SWATH) experiments, the overall coverage of untargeted lipidomics analysis was significantly improved. In practice, samples are analyzed using both DDA and SWATH in positive and negative ion modes, employing both CID and EAD fragmentation techniques, which are then processed with MS-DIAL software for lipid species identification.

Beyond lipidomics, the ZenoTOF 7600 system also excels in untargeted metabolomics experiments. DDA is a widely used analytical technique in metabolomics, enabling the detection and potential quantification of all metabolites within a sample. The untargeted workflow integrates a TOF MS scan to detect all precursor ions within a specified mass range, followed by MS/MS scans that analyze those precursors meeting predefined criteria. Furthermore, the Zeno trap in the ZenoTOF 7600 system significantly boosts MS/MS sensitivity for both DDA and DIA analyses, improving spectral quality and enabling more confident spectral matching, ultimately increasing metabolite coverage.

Moreover, the ability to integrate the ZenoTOF 7600 system with the Acoustic Ejection Mass Spectrometry (Echo MS) system opens a new frontier in high-throughput analysis and non- invasive, contactless sampling.

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OP13

MICROBIAL AND MAMMALIAN METABOLITES IN THE MASS SPECTROMETRIC DIAGNOSIS OF INFECTIOUS DISEASES

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Microorganisms can produce metallophores, low molecular weight metal chelators, which regulate the levels of these critically important nutrients in their environment. These substances are produced both in the absence of metal ions and at high concentrations that are toxic to microorganisms. Metallophores are synthesized not only by microbes (especially bacteria, mycobacteria and filamentous fungi), but also by plants, parasitic protozoa and arthropods, and mammals. In the event of an incipient infection, a battle for metal nutrients is unleashed between the infected host and the microbial pathogen. The basic tug-of-war is mainly for iron, zinc or copper. The better-equipped opponent, i.e., the one with richer (in number) and more efficient (in recognition) molecular tools to assemble these essential nutrients, wins the battle. Thus, metallophores are not mere fitness factors of microorganisms, but important factors of virulence in general. The mammalian host defends itself against ongoing infection, for example, by secreting lipocalins - proteins that bind microbial siderophores or by elevated concentration of toxic metals.

Evolutionarily engineered pathogenic microorganisms can chemically modify their siderophores, increasing their molecular diameter so that they cannot form a complex with lipocalin, but retaining their metal-binding abilities. These are called "stealth" metallophores and their function is identical to that known from technologies used in warfare. The most successful pathogens then send mock targets or other intelligent strategies to improve their own defences against the host immune system. In this talk, we will show that microbial metallophores are early and specific markers of infectious diseases that are released into the environment during the intense cell division phase of a pathogen. The presence of metallophores in an infected host distinguishes colonization from the invasive course of infection, represents a benefit that diagnostic approaches used to date do not provide.

Using two patient examples from two prospective and retrospective study, we will demonstrate the advantages of the new "infectious metallomics" method over standard techniques (molecular techniques based on DNA sequencing, serological and microscopic techniques, culture). We will also show the importance of microbial toxins and quorum sensing molecules in diagnosis and other mechanisms by which the host defends itself against bacterial or fungal infection.

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OP14

MASS SPECTROMETRY REVEALS VIRAL AND ZINC-DEPENDENT REGULATION OF FUNGAL MYCOTOXINS

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Aspergillus fumigatus is a major fungal pathogen affecting millions of individuals annually. Its virulence is closely linked to secondary metabolites such as gliotoxin (Gtx) and other alkaloids, whose production is influenced by environmental factors like zinc availability [1]. Gtx features a redox-active disulfide bridge, enabling dynamic regulation of its bioactivity. Additionally, infection with Aspergillus fumigatus polymycovirus 1 (AfuPmV-1) has been shown to impair stress resistance, but its effect on fungal metabolism remains largely unexplored. This study demonstrates that mycovirus infection is a novel stress factor that modulates Gtx and other alkaloid secretion using gene expression analysis and metabolomics.

Three isogenic lines of Af293, one naturally virus-infected (VI) with AfuPmV-1, one virus-free (VF) chemically cured of AfuPmV-1, and one re-infected (VR) with AfuPmV-1, were grown in M9 medium in the presence and absence of zinc. Fungal metabolites: Gtx, bismethyl-gliotoxin (BmGtx) and fumiquinazolines were quantified by liquid chromatography-12T FTICR mass spectrometry, and gene expression levels of Gtx biosynthetic (*gli*) genes were quantified by RT-qPCR over time.

MS metabolomics revealed *A. fumigatus* responds to AfuPmV-1 infection via Gtx and BmGtx secretion. All strains produced Gtx, bmGtx, and Gtx E, while Gtx G (four-sulfur variant) was exclusively detected in virus-infected strains (VI, VR) under Zn-depleted conditions (Figure 1). Similarly, upregulated expression of *gli*- genes correlated with relative AfuPmV-1 RNA levels in the VI strain. In zinc-rich conditions, neither Gtx nor BmGtx was detectable in the VF strain throughout the 96-h cultivation period. Conversely, both infected (VI+VR) strains showed kinetics of the appearance of Gtx resembling a Gaussian curve, reaching peak concentrations at 48 h (Figure 1).
Several fumiquinazoline alkaloids were also identified and detected at higher concentrations in VI *A. fumigatus* than the VF, highlighting a broader impact of mycovirus infection on fungal secondary metabolism.

The results display how zinc availability and mycovirus infection drive metabolic shifts in A. fumigatus, revealing dynamic regulation of secondary metabolites and offering new potential therapeutic targets.

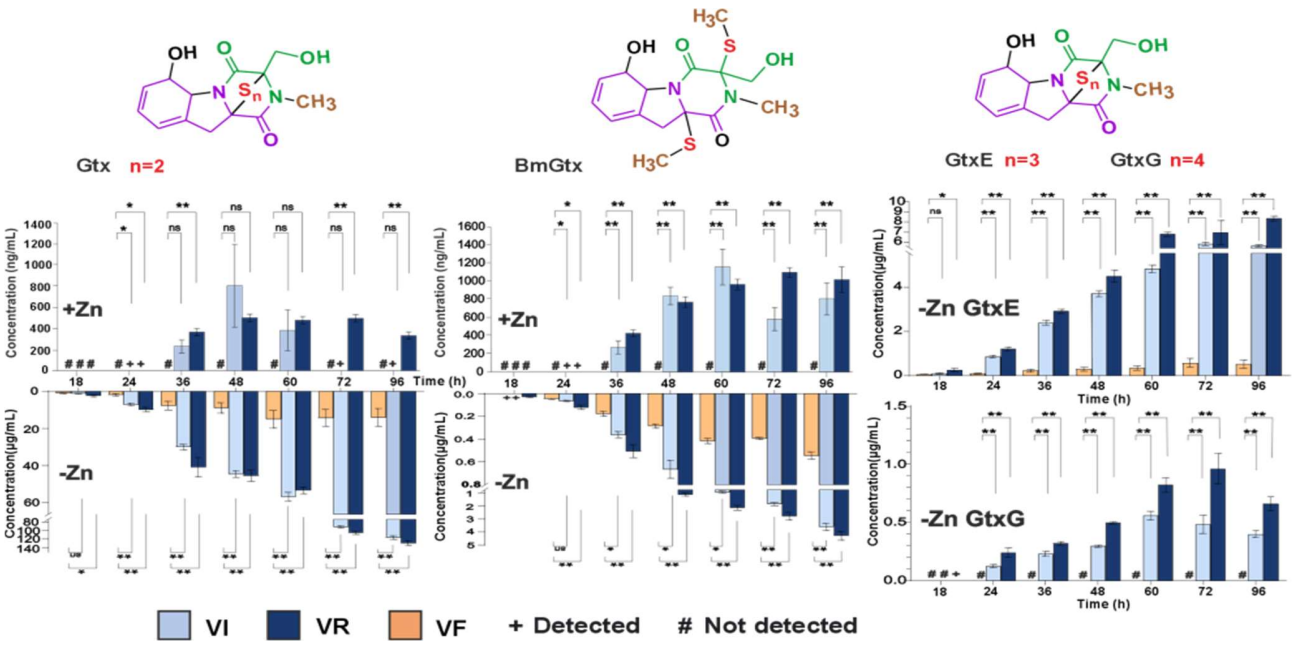

Figure 1. Polymycovirus modulates biosynthesis of Gtx analogues. Secretion profiles of (left) Gtx, (middle) BmGtx, (right) GtxE and GtxG recorded in zinc-regulated experiments in the culture supernatant. Data are presented as means ± standard error of the mean. Asterisks indicate statistically significant differences (one-way ANOVA with Tukey's multiple comparisons test; *p ≤ 0.05 and **p ≤ 0.01). VF, virus-free; VI, virus-infected; VR, virus- reinfected.

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OP15

CHARACTERIZATION OF ANTISENSE OLIGONUCLEOTIDES BY ION PAIR HPLC – HIGH RESOLUTION MS: METHOD DEVELOPMENT USING DESIGN OF EXPERIMENTS

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Since their introduction in 1998 with the approval of Fomiversen for the treatment of CMV retinitis, Antisense Oligonucleotides (ASO) have been finding growing use in the therapy of genetic, oncologic, inflammatory and viral diseases [1]. Chemically, they are short sequences of single-stranded RNA/DNA nucleotides, prepared by solid-phase synthesis (SPS) and modified to improve stability, pharmacokinetic properties and binding affinity; they have base sequences complementary to a defined mRNA sequence, to which they bind with high specificity inhibiting the expression of genes involved in the targeted disease.

Their molecular mass normally ranges from 6 to 18 kDa, somewhat midway between small molecules and most protein therapeutics; given the structural similarity to their biological counterparts, and at the same time their synthetic origin, from a Regulatory point of view ASO cannot be considered as biotherapeutics, nor as small-molecule drugs. Consequently, application of the existing guidelines, i.e. those defining manufacturing process, characterization, specifications and analytical control is not always possible or appropriate [2]. In the effort to fill the gap, several position papers and, very recently, a draft guideline from European Medicines Agency have been issued [3].

As for any new drug under development, identification and quantification of the product-related impurities are key analytical issues, strictly connected not only to quality, but even more to safety for patients (the occurring impurities do not have to alter the drug pharmaco-toxicological profile) and efficacy (the total amount of impurities determines the assay potency of the drug substance).
Indeed, the characterization and analysis of ASO drug substances, drug products and their impurities pose specific challenges:

- the preparation by SPS involves tens of iterative steps, generally without purification; this produces a very complex impurity profile; consequently, the impurities are usually considered collectively in different classes (n-1, n+1, phosphate, abasic etc.), rather than individual impurities as with small drug molecules;
- impurities are closely related to each other and to the full-length oligonucleotide: they have very similar physico-chemical properties, molecular mass and same UV absorption maxima (260 nm);
- due to the presence of an acidic phosphorotioate (or phosphate) group for each residue, ASO have a net negative charge and are very polar: hence, they are difficult to separate chromatographically.

Altogether, difficulty in separation and complex impurity profiles render unavoidable that many impurities are unseparated from the main product and from each other [4] [5], requiring the use of orthogonal separation techniques (e.g. Anion Exchange HPLC and Ion Pair Reverse Phase HPLC) and/or

orthogonal detection methods (e.g. HPLC-UV/MS), with complex method setup and data processing procedures.

In the effort of studying a general and possibly simplified procedure for the analysis of therapeutic oligonucleotides and impurities, we developed an Ion Pair Reverse Phase HPLC – High Resolution MS method using Fomiversen (cited above) and Tofersen (used for the therapy of SLA) as model compounds, with a Thermo Vanquish UHPLC chromatograph interfaced to a Thermo Q Exactive Plus mass spectrometer.

In the preliminary experiments, an OFAT (One Factor At a Time) approach was used to optimize mass parameters and determine which ion-pairing agent was the most appropriate for chromatography. In this first phase, the research focused on the intensity of the height of the MS signal and the chromatographic peak shape, and the following optimized values, valid for both model compounds, were selected: type of ion-coupling agent, N,N-diisopropylethylamine; Sheath Gas, 60 units; Auxiliary Gas Temperature, 400 °C; S-lens, 90 units.

Subsequently, optimization continued through Design of Experiments (DoE), utilizing Response Surface Methodology (RSM) with Box-Behnken matrix [6].

The considered factors in the RSM included the concentrations of N,N-diisopropylethylamine and hexafluoroisopropanol in the mobile phase, as well as the elution gradient slope. The evaluated responses included height and width of the main peak (from the UV trace), as well as critical resolutions between selected impurities (from their extracted MS chromatograms). The hypothesized models were found to be valid and significant with good prediction quality, and through RSM it was possible to define a multidimensional space (sweet spot) where the requirements for the responses considered were met, performing their simultaneous optimization.

In conclusion, two distinct IP RP HPLC-MS methods were developed for Fomiversen and Tofersen, based on common mobile phase components and MS parameters, and on DoE application for the chromatographic parameters and UV signal optimization. In addition, 9 out of 11 detected Tofersen impurities above 0.1% were identified, whereas 3 of 4 Fomiversen impurities were identified.

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OP16

ANALYTICAL AND CLINICAL VALIDATION OF A LC-MS/MS METHOD FOR THE QUANTIFICATION OF IMATINIB AND NORIMATINIB IN DRIED BLOOD SPOT COLLECTED USING VOLUMETRIC DEVICES

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Imatinib mesylate, a potent BCR-ABL1 and KIT tyrosine kinases inhibitor, is the standard of care for treating chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST) [1]. Its pharmacokinetics and plasma exposure (minimum steady-state plasma concentration, C_{trough}) vary significantly, affecting both efficacy and toxicity. A target C_{trough} of 1000 ng/mL for CML patients and 1100 ng/mL for GIST patients has been established [2]. In this context, therapeutic drug monitoring (TDM) is a proven, feasible tool for optimizing dosing by accounting for individual pharmacokinetic differences [3-6]. The use of dried blood spot (DBS) sample (also known as microsampling) significantly enhances TDM feasibility in clinical practice [7,8].

This study aims to develop and validate an LC-MS/MS method for quantifying imatinib and its active metabolite, norimatinib, in volumetric DBS samples collected using Capitainer B and HemaXis DB10 devices [9,10]. Notably, this is the first assay to employ these devices while simultaneously quantifying both imatinib and norimatinib.

The chromatographic system consisted of a Shimadzu Nexera XR (Tokyo, Japan) and separation was achieved using an XTerra MS C18 column. Detection occurred with a SCIEX 4000QTrap tandem mass spectrometer using electrospray ionization in positive ion mode.

Analytical and clinical validation was conducted following the latest International Council for Harmonisation (ICH) M10 guideline on bioanalytical method validation [11] and the recommendations by Capiau et al. [12] for DBS specific parameters. Two volumetric collection devices, HemaXis DB10 and Capitainer B, were evaluated for their analytical and clinical performance.

The assay was linear over the range 240-6000 ng/mL for imatinib and 48-1200 ng/mL for its metabolita, with accuracy (89 %-107 %) and precision (≤6 % CV) consistently meeting validation standards across a hematocrit range of 22-55 % for both devices. Recovery ranged from 84 % to 92 %, and no significant matrix effects were observed, confirming the efficacy of the optimized extraction process and method's reliability. Stability tests confirmed analyte preservation under various conditions, including autosampler maintenance, desiccator conditions (20 °C, ≤35 % humidity), and simulated home sampling, ensuring long-term accuracy and precision. Clinical validation eas conducted on 52 paired

DBS-plasma samples and conversion factors were determined for each analyte, enabling the conversion of DBS measurements to plasma concentrations. Statistical evaluations, including Bland-Altman and Passing-Bablok regression analyses, demonstrated a strong correlation between the estimated and measured plasma concentrations for both devices. The conversion method was further tested on an additional set of 25 to 31 samples, with 80 to 97 % of the sample falling with ±20 % difference.

This study proved that DBS sampling offers a practical alternative to plasma-based TDM. The validated LC-MS/MS method ensures accurate quantification of imatinib and norimatinib, while both HemaXis DB10 and Capitainer B devices offer reliable options for clinical implementation.

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OP17

EXTRACTIVE-LIQUID SAMPLING ELECTRON IONIZATION-MASS SPECTROMETRY (E-LEI-MS): DRUG SCREENING AND DIRECT ANALYSIS IN PHARMACEUTICAL AND FORENSIC APPLICATION

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Extractive-Liquid Sampling Electron Ionization-Mass Spectrometry (E-LEI-MS) is a recently developed real-time analytical approach suitable for the direct analysis of many compounds in different matrices [1]. The main features of the E-LEI-MS system are the absence of sample pre-treatment and the combination of ambient sampling with the high identification power of electron ionization (EI). The E-LEI-MS setup (**Figure 1**) includes a solvent-release mechanism for extracting the analytes from the sample surface and a capillary system for transferring the solution to the EI source by the suction force of the MS high vacuum. The vaporizing process takes place in the vaporization microchannel using the high temperature of the transfer line, where the analytes reach the gas phase before entering in the EI source.

The E-LEI-MS capability to provide real-time data was evaluated for identifying active pharmaceutical ingredients (APIs) and excipients in twenty industrial drugs. The twenty samples chosen are representative of sixteen APIs belonging to different therapeutic classes in six different pharmaceutical forms. Furthermore, two supplement tablets were purchased from a legal website as suspected of counterfeiting. Analyses of pharmaceuticals have been performed using the E-LEI-MS system coupled with an Agilent 7010 GC/QqQ-MS. Source and Vaporization Micro-Channel (VMC) temperatures were optimized according to the molecular weight, boiling point, and degradation temperature of the analytes. The extraction solvent was selected based on the pharmaceutical form of the sample. Diclofenac pharmaceutical forms, tadalafil and supplementary tablets were also tested using the E-LEI-MS system coupled with an Agilent 7250 GC/Q-ToF-MS to obtain HR results

Nine out of sixteen APIs and four excipients were unambiguously identified using the NIST library and predicted spectra library. Six samples were unsuitable for the E-LEI-MS analysis due to the chemical-physical characteristics of the API or to the special coating of some tablets (e.g. gastro-resistant material).

One of the tablets sold as supplement resulted in containing sildenafil, known as Viagra.

The E-LEI-Q-Tof-MS system was also involved in the analysis of twenty benzodiazepines (BDZs) in solutions and in fortified cocktails. The BDZs were analyzed using source and transfer line temperatures of 350 °C and 400 °C, respectively. ACN was used as the extraction solvent. Six BDZ solutions, selected for their common use, were used to fortify the cocktails at concentrations of 20 mg/L and 100 mg/L. The fortified cocktails were analyzed on a glass surface as 20 mL dried spots to simulate the forensic scenario of BDZs being used as rape drugs, where glass represents a crime scene sample.

Nineteen BDZs analyzed as standard solutions were correctly identified by libraries. The six BDZs contained in the fortified cocktails at a concentration of 100 mg/L were identified by the NIST library. The BZDs in the fortified cocktails at a concentration of 20 mg/L were identified using a library created by collecting the accurate mass EI spectra acquired during the standard solutions analysis.

The results confirmed the E-LEI-MS capability in real-time targeted and untargeted analysis, providing results in less than five minutes. The pharmaceutical forms analysis and the accurate identification of BDZs demonstrated the potential of this analytical approach as a valuable screening technique in applications requiring the rapid acquisition of qualitative data as quality control tests, and detection of counterfeits and adulterations.

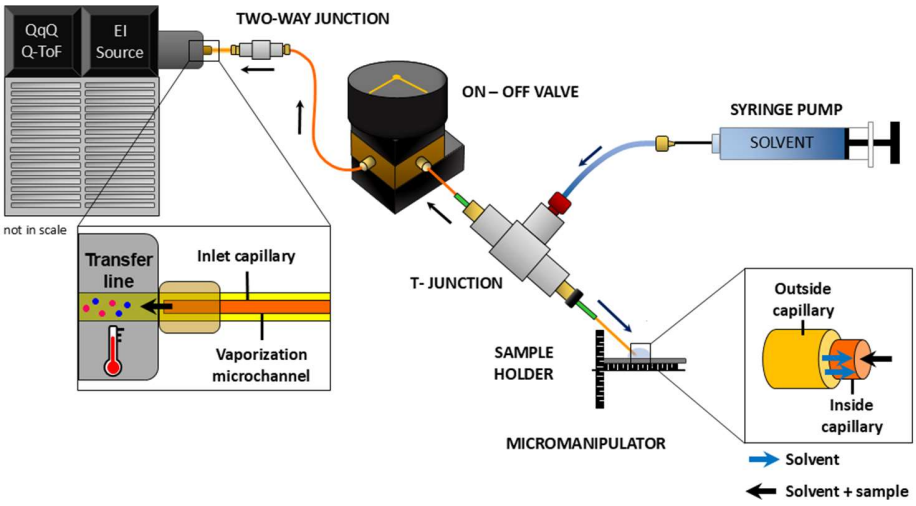

Figure 1. Scheme of the E-LEI-MS configuration.

Acknowledgement

This work has been funded by the European Union - NextGen-erationEU within the framework of PNRR Mission 4 - Component 2 - Investment 1.1 under the Italian Ministry of University and Research (MUR) program PRIN 2022" – grant number 202224R9NL - CUP: H53D23003760001. The authors are grateful to Agilent Technologies for providing the MS instrumentation and Laboratorio di Tossicologia, A.S.T. AV1, 61122 Pesaro, Italy for providing and preparing the BDZ standards and samples.

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OP18

HOW MUCH INFORMATION DO TANDEM MASS SPECTRA CONTAIN?

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Selecting the optimal collision energy is critical in tandem mass spectrometry (MS/MS), as it significantly influences spectral quality. However, determining the optimal collision energy often requires tedious evaluation of all tandem mass spectra during this optimization process. In this study, we developed a novel metric to quantify the information content of tandem mass spectra without the need for structure elucidation.

Using Shannon entropy, a concept from information theory, we introduced an information-content metric integrating peak distribution analysis and noise-reduction techniques. Our approach was validated using tandem MS spectra from a complex proteomic sample containing over 3,000 HeLa peptides. The results demonstrated strong correlations between our metric and peptide identification scores, confirming its effectiveness in identifying optimal collision energy settings. This method provides a robust tool for optimizing experimental conditions in MS/MS analyses without requiring prior structural information about the analytes.

OP19

REGULATION OF KERATIN 8 PHOSPHORYLATION DURING CELL DIVISION

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Keratins are cytoskeletal proteins classified as Type I and Type II intermediate filaments. These filamentous structures provide mechanical strength and stability to the cells, but it is not known well how these robust structures are regulated and function during dynamic processes, such as cell division. To better understand the biochemistry of cell division and the regulation of keratins, I focused on keratin 8 in cancer cell division. Keratin 8 is a good candidate with a maintained expression during tumorigenesis and has diverse expression patterns in different cell types. During cell division, these filamentous structures split into non-filamentous clusters with mechanisms that are not well understood. To uncover the phosphorylation-dependent disassembly, I analyzed the cell cycle-dependent phosphoproteome of keratin 8 in different cell lines and investigated its commonly regulated phosphorylation sites. I took label-free and dimethyl labeling-based [1] quantitative approaches combined with TiO_2 phosphopeptide enrichment methodology (Figure 1., Figure 2.). I applied DDA and DIA-based mass spectrometry methods and identified and quantified commonly regulated or cell-type specific phosphorylation sites of keratin 8 during cell division. I focused on a common phosphorylation site on keratin 8 across different cells and performed a targeted analysis with PRM-based mass spectrometry to increase the specificity. To take an orthogonal approach, the role of keratin 8 phosphorylation was investigated by high-resolution imaging. Our study brought a better understanding of the regulation and functioning of keratins during cell division.

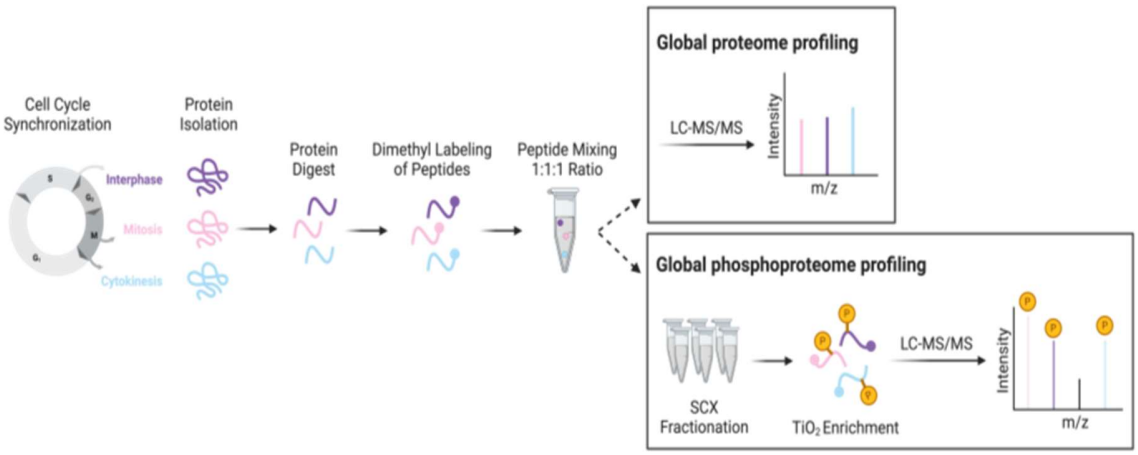

Figure 1. Experimental workflow of cell cycle-dependent and quantitative proteome and phosphoproteome profiling.

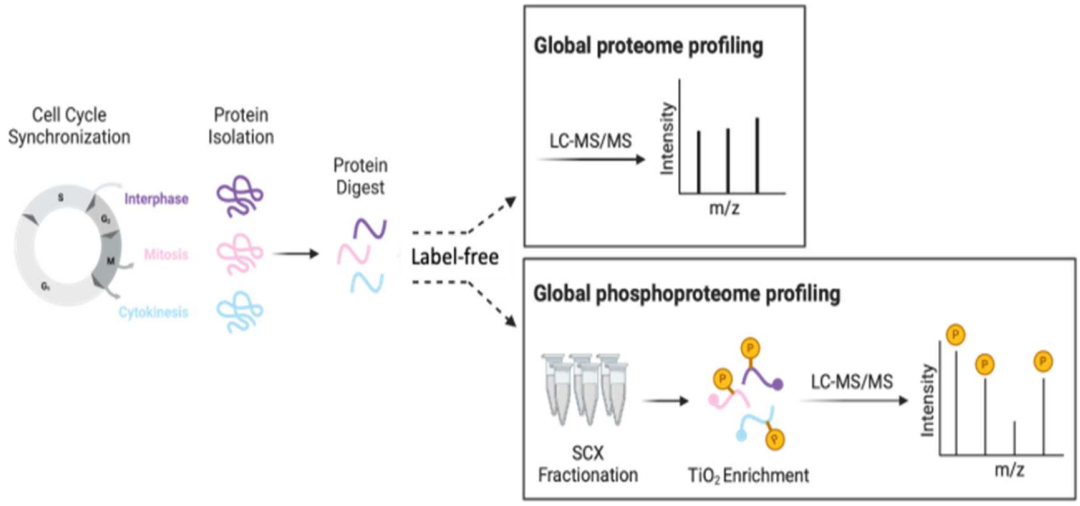

Figure 2. Experimental workflow of cell cycle-dependent and label-free proteome and phosphoproteome profiling.

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OP20

THE SYNERGY BETWEEN PEA TECHNOLOGY AND MASS SPECTROMETRY

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As the functional regulators of phenotype, proteins are a primary focus of translational and clinical research. Technologies for protein expression profiling are generally classified as being targeted or untargeted. Targeted assays analyze a pre-selected set of proteins and generally rely on affinity-based reagents (e.g., antibodies) to bind specific proteins. Untargeted assays, on the other hand, measure proteins in a theoretically global manner without the use of affinity reagents.
Two commonly used proteomic technologies for targeted and untargeted analyses are PEA and "bottom-up" LC-MS, respectively.

PEA and MS are complementary technologies, which have been successfully used together to increase proteome coverage, validate biomarkers, and perform multiomic research.

OP21

CROSS-LINKING MASS SPECTROMETRY OF THE ANTIMICROBIAL PEPTIDES MAGAININ 2 AND PGLA REVEALS HETERODIMERIZATION IN MICELLAR MEDIUM

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This work focuses on the structural characterization of two antimicrobial peptides (PGLa and magainin 2) in a membrane-mimetic environment. PGLa and magainin 2 are known to be unstructured in aqueous buffer, but when in presence of membrane mimetics, they form amphipathic alpha helixes [1]. Both peptides exhibit antimicrobial properties on their own but when added together in a 1/1 cocktail, a synergism of one order of magnitude is observed [2]. While the synergistic mechanism of action is not well understood, it was suggested that it could take its origin in the formation of a heterodimer (or peptide complexes) [3]. In a context of growing antimicrobial resistance and major threats to public health, antimicrobial peptides are of great interest as they are considered less prone to antimicrobial resistance [4]. We therefore proposed to use cross-linking (XL) coupled to mass spectrometry (MS) to help decipher the synergism behavior of PGLa and magainin 2.

XL experiments were carried out in HEPES buffer in presence of dodecylmaltoside (DDM) detergent micelles or lipidic vesicles (POPE:POPG 3:1 and DMPE:DMPG 3:1). Disuccinimidyl suberate (DSS) or disuccinimidyl glutarate (DSG) were used as cross-linkers. The XL reaction was followed by MALDI-MS and cross-linked peptides were analyzed before- or after- enzymatic digestion with trypsin and pepsin by nano-liquid chromatography-MS/MS (easy-nLC1000 coupled to a QExactivePlus Orbitrap). Linkage sites identification was achieved using pLink2.

The live monitoring of XL-MS carried out in DDM micelles by MALDI-MS showed the formation of intramolecular cross-linked monomers, but also provided direct evidence of a specific PGLa/magainin 2 heterodimer (Figure 1). No other oligomeric states were detected. Interestingly, such heterodimer is only observed under membrane mimetic environment when proper folding occurred (verified by circular dichroism experiments). Monitoring the reaction using MALDI-MS facilitated a rapid optimization of conditions to achieve the best balance between stabilizing complex formation and avoiding unspecific aggregation. It also revealed some biases likely to be introduced insidiously during XL-MS experiments.

Performing the XL in POPE:POPG liposomes appeared much more difficult in terms of XL efficiency. Indeed, even at high cross-linker/peptide ratio, the heterodimeric species was barely detected, despite still being the only multimeric species identified.

Modifying the lipid composition to smaller lipids (DMPE and DMPG) revealed a different behavior. As a matter of fact, the results observed were similar to what was visible in DDM micelles

(Figure 1). These results suggest that interactions between the peptides might occur differently in both lipid vesicles media. MALDI-MS highlighted the need to adjust the XL workflow compared to its use in large-scale protein-protein interaction mapping in order to avoid technical biases arising from the rapid nature of the XL reaction. The optimized conditions could be used in more complex peptidomic studies realized with intact bacteria or to help characterize membrane protein complexes.

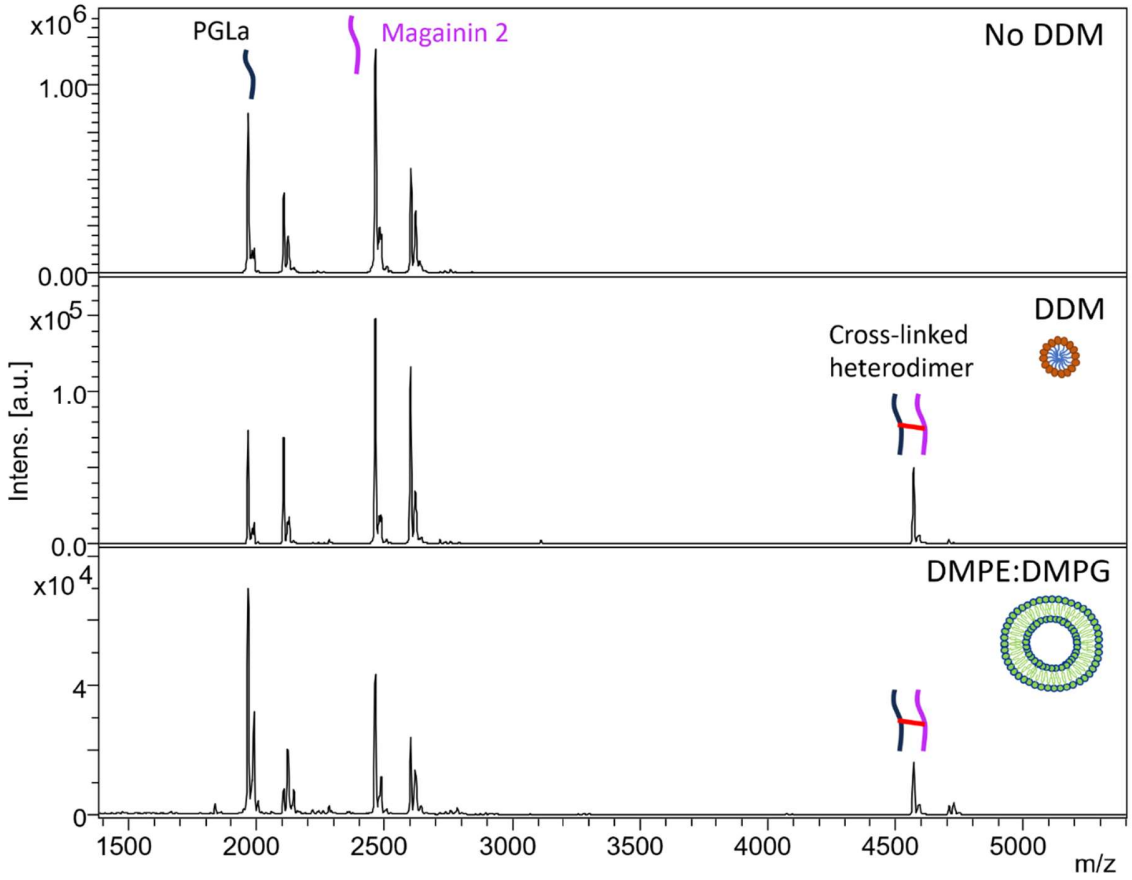

Figure 1. MALDI mass spectra of PGLa and Magainin 2 cross-linked with DSS without DDM (top); in presence of DDM (middle) and in presence of DMPE:DMPG liposomes (down)

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OP22

PROTEIN SECONDARY STRUCTURE THROUGH SHORT-RANGE CROSS-LINKS

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Cross-linking mass spectrometry (XL-MS) has become an essential tool in structural biology, as it provides valuable information on protein conformations and interactions. While long-range cross-links are widely used to define tertiary and quaternary structures, short-range cross-links (those spanning fewer than 20 residues) have often been overlooked and considered of little structural significance.
In this study, we systematically analyzed these short-range cross-links to explore their relationship with the secondary structure of proteins. We introduce X-SPAN, a software designed to integrate publicly available XL-MS datasets from system-wide experiments with AlphaFold-predicted protein structures for entire organismal proteomes. Specifically, we analyzed 13 publicly available system-wide XL-MS datasets, comprising a total of 669910 cross-links from 4 organisms. By applying our filtering criteria, selecting only unique intra-protein cross-links with a maximum spacing of 20 amino acids in the primary sequence, we identified 79871 cross-links, of which 47013 were found in continuous motifs (α-helices, β-strands coils and mixed elements).

Our analysis unveils distinct cross-linking patterns that depend on the spatial constraints of secondary structure elements, with α-helices, β-strands, and coils exhibiting characteristic behaviors. We demonstrate that α-helices display a periodic cross-linking pattern consistent with their helical pitch, whereas coils and β-strands show nearly monotonic distributions.
Additionally, our analysis highlights context-dependent cross-linking grammar, where the local amino acid environment modulates cross-linking efficiency.

By comparing experimental cross-linking data with AlphaFold-predicted structures, we find that cross-link distributions are strongly influenced by the predicted local confidence score (pLDDT). This work provides a novel framework for benchmarking AlphaFold's local accuracy.

Beyond these structural insights, our study demonstrates the potential of short-range cross-links as quality control metrics for XL-MS experiments to distinguish between native distributions from potential artifacts.

We anticipate that our short-range cross-link database will serve as a valuable resource for studying local secondary structure switches and their potential roles in protein function and allosteric regulation.

OP23

THE HUMAN 20S PROTEASOME CLEAVES BETA-AMYLOID (Aβ1-40) RELEASING PEPTIDES THAT ACT AS INHIBITORS OF ITS CHYMOTRYPSIN-LIKE ACTIVITY

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In neurodegenerative processes, accumulation of amyloidogenic peptides and proteins, such as β-amyloid (e.g., $A\beta_{1-40}$, $A\beta_{1-42}$), typically overwhelms the degradative capacity of pathways deputed to their physiological clearance, such as the Ubiquitin Proteasome System (UPS).

The UPS is the main intracellular proteolytic pathway and the major surveyor of protein homeostasis (proteostasis). The 26S proteasome holoenzyme, which is a multi-enzymatic complex, is the proteolytic core of the UPS and carries out the degradation of almost all cellular proteins including amyloidogenic peptides and proteins. There is compelling evidence that the activity of proteasome complexes progressively decreases during neurodegeneration progression thereby favoring amyloid deposition and amyloid plaques formation. However, the mechanisms driving this proteolytic impairment are unknown yet. In the case of Alzheimer's disease, different studies have highlighted that proteasome particles are inhibited by the soluble oligomers of Aβ amyloidogenic peptides [1]. Conversely, whether the proteasome cleaves and/or is inhibited by $A\beta_{1-40}$, $A\beta_{1-42}$ monomers is still debated.

In this study, employing synthetic Aβ sequences (10 μM) of pathogenic interest (i.e., full-length $A\beta_{1-40}$, $A\beta_{1-18}$, $A\beta_{1-24}$, $A\beta_{18-40}$, $A\beta_{12-36}$, etc.) fed to 10 nM human 20S proteasome (affinity purified) we first sought to characterize the digestion fragments by top down mass spectrometry (MS) approaches, according to well-known standard procedures [2]. Thereafter, we assayed whether monomeric peptides behave as h20S inhibitors by validated fluorimetric assays in vitro (peptides conc. range 100 nM – 10 μM). MS spectra, which were analyzed by SpectraSage, a software recently developed by us and successfully applied to the release of branched fragments [3], clarified that, among the individual peptides tested, full-length $A\beta_{1-40}$ is the only 20S substrate, and that different fragments spanning over the 15-35 residues of Aβ are generated during the catalytic cycle. Interestingly, the informatics analysis retrieved evidence that the 20S shows a net catalytic preferences for valine and glycine residues in P1 and P1' position.

Conversely, biochemical studies clearly suggested that, regardless of their identity, peptides harboring the 18-24 stretch of Aβ1-40 inhibit the h20S chymotrypsin-like activity, which is the most relevant one for biological processes, although with different pattern and modalities which are being investigated further (Aβ18-40 > Aβ12-36 > Aβ1-24).

Interestingly, since several fragments released by the h20S digestion of Aβ1-40 correspond to peptides showing a strong inhibitory potency, this study introduces the working hypothesis that the catalytic activity of the h20S proteasome on Aβ1-40 promotes its progressive inhibition.

This process, which has been referred as “suicide” for enzymes others than the h20S, to the best of our knowledge, was never reported for proteasome complexes. If future studies will confirm this scenario, these findings may contribute to explain why boosting proteasome activity towards amyloid peptides by delivery of pharmacological proteasome activators was found to introduce paradoxical proteotoxic effects.

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OP24

CHEMICAL ANNOTATION PROPAGATION FOR MOLECULAR NETWORKS

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In molecular networking (MN, Watrous et al., PNAS, 2012) highly similar MS/MS spectra are connected in a graph and it is assumed that the corresponding molecules are structurally also similar. Some of the MS/MS spectra (nodes) in MN can usually be annotated by matching to spectral libraries and nodes adjacent to these are attempted via annotation propagation. Recently we have introduced the Universal Fragmentation Model (UFM, DOI 10.26434/chemrxiv-2024-fmf67) to accurately predict fragmentation chemistry for general metabolites including rearrangement pathways and corresponding fragment structures. Here we combine UFM with MN and demonstrate automated, fragmentation chemistry based annotation propagation.

UFM predicts fragmentation pathways by considering both gas-phase ion chemistry and quantum chemical information. In case of ionization by protonation UFM explores all plausible protonation sites along with dissociations and rearrangements by hydride transfer, ring formation and opening reactions. In MN mode the UFM software performs fragment assignment and validation for the annotated MS/MS spectrum using the annotation provided and stores fragment structures and pathways using a modified version of the Morgan algorithm. Then the node-adjacent MS/MS spectrum is compared to the annotated MS/MS to identify fragments which differ in a chemically meaningful way based on plausible mass differences and the stored fragment structures. The Morgan coding of the original fragment is then altered accordingly generating typically a limited number of modified fragment structures. These are then re-assembled using pathways computed for the annotated molecule giving reverse-engineered parent structures for the node-adjacent MS/MS spectrum. Typically, a limited number of these are produced for which UFM is rerun for validation against the node-adjacent MS/MS spectrum and the highest-scoring candidate structure is accepted as propagation.

This presentation will demonstrate successful structure propagation for fentanyl (Vincenti, Front. Chem. 2020), floxacin and synthetic cannabinoids (Magny, JASMS, 2024) MNs using our combined MN&UFM strategy.

OP25

SELECTED ION FLOW TUBE MASS SPECTROMETRY OF VOLATILE METAL CARBONYLS

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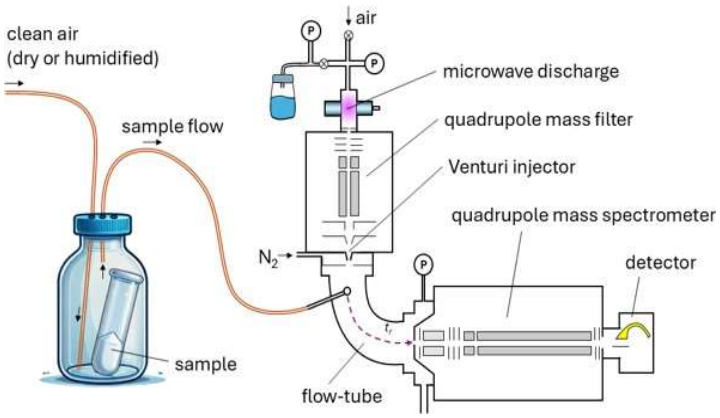
Volatile metal carbonyls can be formed during various anthropogenic activities, especially by the reaction of CO and dispersed metal under specific temperature and pressure conditions. They were convincingly detected in industrial H_2 or N_2 gases containing CO as an impurity [1], in automobile exhaust [2], and urban air [3]. The presence of volatile $Mo(CO)_6$ and $W(CO)_6$ in the landfill gas [4] and $Mo(CO)_6$, $W(CO)_6$, and $Ni(CO)_4$ in the sewage gas produced by the fermentation of sewage sludge at municipal water treatment plants has also been reported [5]. Conversely, $Fe(CO)_5$ has not been detected in the environment because it showed low stability under a CO atmosphere in the presence of water vapor, and it also slowly decomposes when exposed to sunlight. The limited stability to attack by oxygen from ambient air and moisture applies to varying degrees to all volatile monomeric metal carbonyls [1], making the potential toxicity concerns and the need for continuous monitoring relevant only to some specific work areas.

Various analytical methods have been developed for metal carbonyl measurements, including chemiluminescence, mass spectrometry (MS), IR spectrometry, AAS, GC-EDC, and GC-ICP-MS. We aimed to investigate the feasibility of selected ion flow tube mass spectrometry (SIFT-MS) for the detection and quantification of $Fe(CO)_5$, $Mo(CO)_6$, and $W(CO)_6$. This paper will present and discuss the results of a pilot study of SIFT-MS-related ion-molecule chemistry of these metal carbonyls.

The SIFT-MS [6] is a direct mass spectrometry technique that applies precisely controlled soft chemical ionization to analyze volatile compounds in air with typical LOD at the ppt level. A general scheme of the SIFT-MS apparatus is shown in Figure 1.



The reagent ions were generated from a mixture of laboratory air and water vapor in a microwave plasma ion source. The H_3O^+, NO^+, $O_2^{+\cdot}$, OH^-, $O_2^{-\cdot}$, $O^{-\cdot}$, and NO_2^- reagent ions were used in this study. Individual reagent ion species were mass-selected by a quadrupole mass filter and injected into the flow of carrier gas (N_2) at a pressure of 0.4 mbar via a Venturi orifice. The sample vapors mixed with dry or moist air were admitted into theflow tube heated up to 120°C, where their molecules reacted with the selected

reagent ions during a defined reaction time (4 ms). The product ions formed in these reactions were analyzed by the second quadrupole mass filter. With the H_3O^+ reactant ion, protonated molecules MH^+ were observed for all three metal carbonyls. Low abundances (typically less than 1% of the base peak) of $(M-CO)H^+$ fragment ions were also observed. Hydrated fragment ions $(M-CO+H_2O)H^+$ were only observed when moist air was used, as shown in Figure 2.

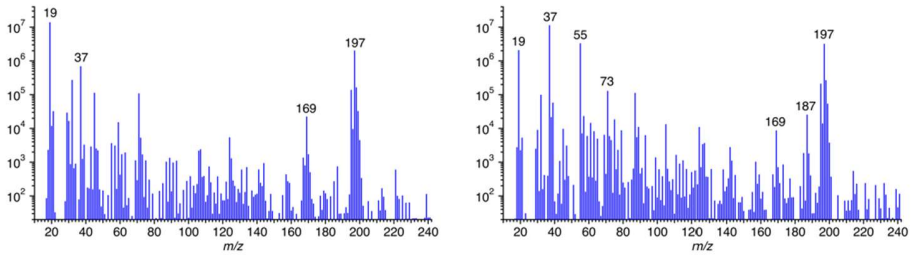


Figure 2. SIFT-MS of $Fe(CO)_5$ with H_3O^+ in dry (left panel) and humidified (right panel) air.

With NO^+ and $O_2^{+\cdot}$ reagent ions, molecular cation radicals $M^{+\cdot}$ were observed together with low-abundant ions $(M-CO)^{+\cdot}$ for all three metal carbonyls. In the case of $Fe(CO)_5$, abundant peaks corresponding to $(M+4)^{+\cdot}$ ions were present in the spectra. These ions were tentatively assigned as adducts of $(M-CO)^{+\cdot}$ and O_2 molecules present as trace impurities in the nitrogen carrier gas flow. In addition to these products, abundant mono- and dihydrated FeO_2^+ ions were observed in moist air. With negative reactant ions, the ion-molecule chemistry is much more complex, so only a few examples are given in the following. Reactions of OH^-, O_2^-, O^-, and NO_2^- with $Fe(CO)_5$ form FeO_2^-, FeO_3^-, and FeO_4^- product ions. The ions, tentatively assigned as $(M+OH-CO_2)^-$, were observed with OH^- reagent. $Mo(CO)_6$ and $W(CO)_6$ reactions with O_2^-, O^- yielded ions XO_n^-, where X is a metal atom and n = 3, 4, and 5. Ions $(M+NO_2-nCO)^-$ (n = 1, 2) were observed in $Mo(CO)_6$ reacting with NO_2^-, while $W(CO)_6$ showed no such products. The ions $(M+OH-nCO)^-$ and $(M+O_2-nCO)^-$ (n = 2, 3) were observed in reactions of OH^-, O_2^- with $Mo(CO)_6$ and $W(CO)_6$. Quantum chemistry calculations were performed on peculiar product ions to investigate their structure and stability using DFT (Gaussian 16, B3LYP functional, Def2TZVPP basis set). This study provided experimental data on the kinetics of reactions involving SIFT-MS reagent ions and volatile metal carbonyls, aiding the analysis of effluents from metal volatilization devices used in environmental studies.

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OP26

FIELD DEPLOYABLE ION TRAP MS FOR DIRECT AND SPME MS&MS/MS ANALYSIS: BEYOND THE LABORATORY

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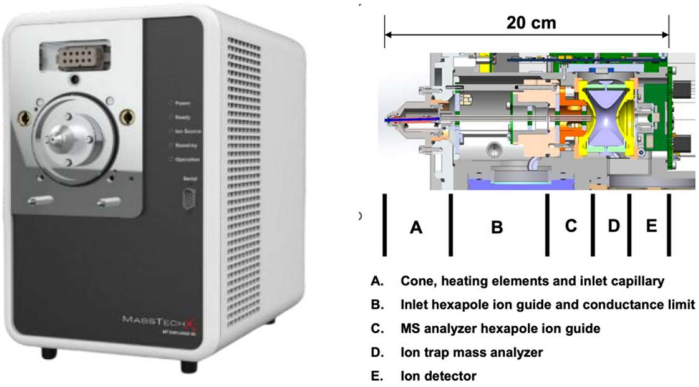
SRA Instruments SpA

The development of portable analytical tools, including mass spectrometers, has rapidly advanced. Small instruments are available on the market for analyses in different fields. However, for portability, some characteristics of mass spectrometry (MS), such as resolution, sensitivity, or the ability to operate in MS/MS mode, are often compromised. An innovative field-deployable MS [1], with an Atmospheric Pressure Interface has been modified to desorb Solid-Phase Microextraction (SPME) fibers [2]. The instrument is fully integrated into a chassis, without external pumps or gases, weights approximately 17 kg and has better than unit resolution over a 2000 Da range. This study demonstrates the SPME approach's sensitivity and repeatability using this compact mass spectrometer shoved in Figure 1, both in MS and MS/MS modes, enhancing specificity for direct analysis.



1. MTE30 mass spectrometer and schematics.

Table 1. Main specs for the MTE30 EXPLORER

Mass analyzer type:	3D quadrupole ion trap
Mass range:	35 up to 2,000 Da
Mass resolution:	better than 0.5 Da
Mass accuracy:	better than 0.25 Da
Mass scan rate:	up to 4,000 Da/sec
Throughput rate:	up to 5 spectra/sec
MS/MS and MS^n capability:	available
Ion modes:	positive and negative
Source of buffer gas:	metal hydride container (no compressed gas cylinder used)

All MS experiments were carried out on a MT Explorer 30 (MTE30, MassTech Inc., Columbia, MD, USA) coupled to a modified Direct Sampling Atmospheric Pressure (DSAP) source equipped with APCI and secondary ESI/ESI modules. The data were acquired in MS and MS/MS modes using proprietary MODAS software. The qualitative data analysis was performed using ChromExplorer 1.5.1 software. The results were validated with an Agilent 5975 bench top GC/MS, using different procedures, depending on sample type

The desorption unit accepts the SPME fiber in an injector-like unit, the Liquid Direct Sampling Atmospheric Pressure (LDSAP), equipped with a glass liner, and can be heated up to 250 °C. Using headspace solid-phase microextraction (HS-SPME) or direct environmental air sampling, we demonstrate trace level detection with good repeatability.

Here we report the results for the analyses of tobacco products, both as HS (headspace) and direct air analysis. In the first case, e-liquids are analysed, identifying both the presence of nicotine and that of new nicotine alkaloids derivatives on the market, such as metatine (6-methyl nicotine). In the second case, the presence of ETS (Environmental Tobacco Smoke) is detected by analysing nicotine in the air at levels of a few parts per billion (ppb).

An application of HS-SPME data is also presented to monitor release of histamine in fish and fish products, due to bacterial decarboxylation of histidine. Histamine is toxic and is regulated in fish products, with different levels depending on countries.

Quantitative or semi-quantitative analyses can be performed by using isotopically labelled internal standards or external calibration, depending on sample type.

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OP27

MINING OF N- AND C- TERMINI INTRODUCES THE VITREOUS HUMOR DEGRADOME AND HIGHLIGHTS ALTERED PROTEOLYTIC PATTERNS OF STRUCTURAL AND NON STRUCTURAL COMPONENTS IN RHEGMATOGENOUS RETINAL DETACHMENT

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Vitreous humor (VH) is a fluid of the posterior chamber of the eye that provides mechanical and nutritional support to the retina. Since the retina is considered "a window to the brain", growing attention is being paid to VH composition to figure out patho-physiological processes of the nervous system and to identify biomarkers of neurodegeneration onset and progression.

Starting from this perspective, we here introduce a pilot study designed to investigate and compare the N-terminome (and C-terminome), and, in parallel, the total proteome of VH fluids isolated from subjects who underwent eye surgery for rhegmatogenous retinal detachment (RRD, n=8), which is a severe condition characterized by the detachment of the neurosensory retina from the Retinal Pigment Epithelium (RPE), and pucker (enrolled as controls, n=8).

To this aim, we optimized a protocol based on trypsin-digestion after labeling of free N-termini in solution by TMT pro Zero.

N-termini and C-termini were then searched using MSFragger/FragPipe software following a validated strategy centered on semi-tryptic peptide searches. The repertoire of N- and C-termini identified and quantified (>1000, FDR≤0.01 by target-decoy validation) constitute the first library of proteolytic events and proteolytic sites of human VH, covering structural and non-structural elements of this fluids. This result has fostered the ongoing development of targeted approaches for boosting the coverage of N-terminome (e.g., TAILS) in order to broaden our current knowledge on the VH degradome and to figure out relevant proteolytic processes of retina layers.

Although sample size is limited, comparison between the N- and C-termini landscapes and the perturbations of total (tryptic) proteome highlighted robust alterations of the repertoire of cleaved proteins between RRD and control subjects. Strengthened by immunoblotting studies, datasets envisage that RRD is characterized by unbalanced proteolysis of structural and non-structural components involved in the regulation of immune processes, proteolytic control and, in particular, angiogenesis,

which may promote the morpho-functional alterations that are thought to predispose to RRD complications and irreversible blindness.

OP28

MASS SPECTROMETRIC EVALUATION OF PROTEOLYTIC ACTIVITY IN WINE VINEGARS

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Plant organisms represent a rich source of proteolytic enzymes. Aspartic endopeptidases (e.g., cardosin, cyprosin, and phytepsin), serine endopeptidases (e.g., cucumisin), and cysteine endopeptidases (e.g., actinidin, bromelain, ficin, and papain) are well known in the life sciences. Some of these enzymes are used as reagents in experimental biochemistry, particularly for protein digestion, as well as in food processing. Bromelain, ficin, papain, and actinidin are efficient meat tenderizers. Additionally, plant proteases from asparagus, broccoli, ginger, and leek juices, and various fruit vinegars have been shown to be useful for meat tenderization. Cardosins and cyprosins are effective milk coagulants, serving as plant-based rennet substitutes in cheese production.

Fresh grapevine juice, as well as fermented juice or bottled wine, contains a cysteine protease [1]. This enzyme (CYSP) exhibits a high sequence similarity to the RD21A peptidase from Arabidopsis, oryzain alpha from rice, and many other plant cysteine endopeptidases. In this study, we focused on CYSP activity in wine and vinegars with potential applications in meat tenderization and milk coagulation. The experimental set of wine vinegars included commercial samples (white wine vinegars and balsamic vinegar) and a red wine vinegar prepared by fermenting grapevine juice in the laboratory.

Azocasein, sodium caseinate, gelatin from bovine skin, a group of protein standards (hen lysozyme, horse cytochrome c, myoglobin, and yeast alcohol dehydrogenase 1), and minced beef were used to study the activity and cleavage specificity of the proteases in the vinegars. Enzyme activity was assayed by digesting azocasein or visualized using gelatin in polyacrylamide gels after native electrophoresis. This approach confirmed the presence of CYSP in the negatively stained bands through peptide sequencing. Digestion of beef meat and sodium caseinate with wine and vinegars, followed by peptide purification and MALDI-TOF/TOF tandem mass spectrometry, allowed for the identification of the digested meat proteins and casein isoforms, as well as the determination of cleavage sites. It was shown that the most frequent C-terminal amino acids in the peptides were F, Y, H, E, L, and R. This cleavage pattern is similar not only to CYSP but also to the cleavage preference of pepsin and other aspartic proteases.

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OP29

AUTOMATED FRACTIONATION OF LOW-VOLUME PLASMA SAMPLES FOR LC-MS MULTI-OMICS

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Agilent Technologies, Inc

LC-MS multi-omics analyses dramatically benefit from automated sample preparation, which reduces variability introduced during manual handling of multiple sample fractions. This technical overview demonstrates a comprehensive, automated method for the fractionation of polar metabolites, lipids, and proteins from single 20 µL plasma samples. The method comprises multiple configurations, which enables the user to selectively collect any combination of sample fractions, from metabolites alone to metabolites, lipids, and proteins. Automated sample fractionation is achieved using an Agilent Bravo Metabolomics Sample Prep Platform with an Agilent Captiva EMR–Lipid plate, which separates polar metabolite and lipid fractions. Using the Agilent AssayMAP Bravo Protein Sample Prep Platform, the optionally collected protein precipitate is further prepared with standard methods. Performance of method configurations for metabolite, lipid, and protein fractionation was assessed with LC-MS using the Agilent 1290 Inifinity II Bio LC System or Agilent 1290 Infinity II LC System with an Agilent 6495 triple quadrupole LC-MS (LC-QqQ) or Agilent Revident quadrupole time-of-flight (LC-Q-TOF). The assessment detected broad compound coverage with highly reproducible recovery, making this configurable and automated sample preparation platform an integral component of an Agilent end-to-end LC-MS multi-omics workflow.

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OP30

STATIONARY METABOLIC FLUX ANALYSIS OF THE BIOTECHNOLOGICAL CHASSIS *ASHBYA GOSSYPII* FOR RIBOFLAVIN PRODUCTION ON A NON-CONVENTIONAL XYLOSE SUBSTRATE

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Traditional genetic engineering relies on stepwise modifications targeting specific enzymatic reactions while overlooking whole-cell metabolic effects on productivity. Engineered biotechnological microorganism cultures on non-conventional carbon substrates— increasingly relevant due to the current interest in bioresidue-based feedstocks—often face such challenges[1]. Our research group relies on a collection of engineered *Ashbya gossypii* - an industrial riboflavin producer, including xylose-assimilating strains for lignocellulosic residue utilization[2], which exhibit systemic metabolic constraints. Specifically, in xylose cultures as the sole carbon source, growth unexpectedly increases, while riboflavin production declines compared to glucose-fed counterparts. This study integrates genome-scale metabolic simulations and metabolic flux analysis (MFA) to better understand the metabolic performance of these xylose-based cultures, with a focus on riboflavin productivity.

The recombinant in-house strain, overexpressing the cryptic XDH-XR pathway for xylose assimilation, was assayed in glucose and xylose media and further analyzed by fluxomics. To this end, an updated version of the genome-scale metabolic model iRL766[3] was established to evaluate optimal metabolic states and detect essential fluxes in riboflavin production. Culture in minimal media with 1,2-^{13}C-xylose supplementation, alongside extracellular metabolite monitoring, enabled proteinogenic amino acid labeling and steady-state metabolic flux reconstruction. Comparative analysis of actual metabolic fluxes under xylose versus glucose feeding[4] and steady-state *in silico* simulations provided insight into systemic effects influencing riboflavin biosynthesis.

In silico metabolic modeling predicted an ideal flux map outlining enzymatic differences in xylose-fed adaptation. However, the model suggests a fully functional metabolic network upon xylose input that should yield canonical growth and riboflavin tites. This theoretical model, based on non-oxidative pentose phosphate pathway (PPP) carbon processing, renders glycolysis obsolete and suggest NADPH fueling via isocitrate dehydrogenase. Yet, real flux measurements via ^{13}C-MFA confirmed deviations from the *in silico* model, identifying key metabolic burdens limiting riboflavin production and explaining upregulated biomass accumulation relative to glucose-fed strains. A priori, xylose feeding leads to an NADPH deficit due to the truncated PPP lacking its oxidative phase, which normally provides ~60% of the required NADPH. The overexpressed XDH-XR pathway further disrupts balance by consuming NADPH and generating NADH. Real flux data showed limited isocitrate dehydrogenase activity and instead a backflush through gluconeogenesis toward the oxidative PPP phase to compensate NADPH supply. Nevertheless, actual fluxes exceed *in silico* requirements for both growth and riboflavin

synthesis to match glucose-fed cultures. Most likely, futile cycles draining carbon as CO_2 and overcompensating NADPH levels—aligned with increased biomass—impair riboflavin biosynthesis. The excess NADH generated by XDH-XR is theoretically accommodated through a diminished TCA cycle. However, actual fluxes show increased TCA cycle activity in xylose-fed cultures, along with ethanol production, indicating that real adaptation cannot rely on TCA regulation alone. NADH oxidation in mitochondria would require NADH shuttles, but these remain untraceable within our model's carbon transitions. These findings suggestfurther effort and engineering strategies to optimize the NADPH/NADH balance.

Apart from increased TCA cycle activity, reduced glycolysis fluxes observed in xylose metabolism reinforces the idea of deficient catabolite repression in glucose absence. Increased gluconeogenic flux, also linked to oxidative PPP recirculation, could be equally affected. Regulatory interventions, such as controlled growth modulation via catabolite repression mechanisms, could shift metabolism toward riboflavin production rather than growth, enhancing titers.

Despite ribose phosphate immediate availability from xylose uptake and its role as a precursor in riboflavin biosynthesis, *in silico* models do not suggest improved riboflavin channeling but rather similar performance. In fact, experimental fluxes indicate reduced riboflavin partitioning in favor of growth, potentially due to inadequate enzymatic activation, insufficient catalytic efficiency, competition with PPP fluxes, or precursor limitations like GMP depletion driven by excessive growth.

This study highlights the importance of systems biology in deciphering metabolic constraints in engineered strains utilizing non-conventional substrates. Fluxomics defines theoretical productivity limits while revealing real cellular constraints, including cofactor imbalances (e.g., excess NADPH), unintended flux rerouting and regulatory bottlenecks. While enzymatic-level bottlenecks remain elusive, this approach effectively identifies heavily burdened pathways, guiding metabolic engineering refinements. Ultimately, fluxomics bridges the gap between theoretical models and whole-cell bioproduction, supporting rational and effective engineering strategies for industrial biotechnology.

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OP31

LATEST APPLICATIONS OF THE LC-CO-IRMS FOR FOOD AND DIETARY SUPPLEMENTS AUTHENTICATION

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The LC-co-IRMS represents an innovative technique based on the oxidation in acid conditions of all the carbon-based compounds of a sample mixture, previously separated from each other through an appropriate analytical column. Since its introduction in the market in 2004, the LC- co-IRMS has been used to analyse various matrices [1].
Nevertheless, the potential of this techniques is still far from being fully exploited. In this work, we presented some of the latest LC-co-IRMS applications that our group developed for traceability purposes.

In a recent study, the LC-co-IRMS was applied to check for the fraudulent addition of exogenous sugars to Italian authentic wine must. A database of about 100 samples from 16 different Italian regions was considered to set reference values for the carbon isotopic ratio ($\delta^{13}C$) of glucose and fructose in this matrix [2].

Besides sugars, organic acids have also been considered. The addition of biosynthetic citric acid obtained though the fermentation of cheap starting materials like cane sugar by the fungus *Aspergillus Niger* was detected in matrices such as tomato sauce, lemon and orange juice.
Finally, dietary supplements and drugs have also been studied. Levodopa is an amino acid prescribed for Parkinson disease. Natural levodopa can be extracted from plants like the *Mucuna pruriens*, but cheaper analogues can be chemically synthesised or biochemically obtained from the fermentation of sugars by various fungi [3]. The LC-co-IRMS led to the characterisation of the different levodopa sources, pointing out the possibility to detect fraudulent additions of the biochemical active principle to products declared as natural.

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OP32

LATEST TRENDS IN PFAS TESTING AND REGULATIONS LANDSCAPE: FROM PPB TO PPQ IN WATER, SERUM AND BEYOND

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Restek S.r.L.

Among many PFAS destruction technologies for remediation project, formation of byproducts is often ignored and quantitation of ultrashort-chain PFAS can play a critical role in understanding the missing mass Fluorine balance study. As already stated by the European Journal TFA is going to play a critical role.

Majority of detected PFAS is highly dominated in the form of ultrashort-chain and short chain PFAS, yet, testing of those compounds is untouched. In addition, emerging PFAS compounds are even more susceptible to forming ultrashort-chain and short chain PFAS byproducts.

We will go through the different technologies needed to stick at all regulations

Accurate quantitation of ultrashort-chain and short chain PFAS in various water matrices (tap water, bottled water, wastewater) and serum samples will be presented. Critical tips and tricks in ultrashort-chain PFAS analysis will be discussed as well.

OP33

ELUCIDATING CONTAMINANT SORPTION DYNAMICS USING SPME-LEI-QqQ MASS SPECTROMETRY

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Water quality in the European Union is increasingly threatened by the presence of emerging pollutants (EPs) and microplastics (MPs) in freshwater systems. MPs, ranging from 100 nm to <5 mm[1], are ubiquitous environmental contaminants that adsorb hazardous organic pollutants, including Polycyclic Aromatic Hydrocarbons (PAHs), Per- and Polyfluoroalkyl Substances (PFASs), and pesticides, posing significant risks to ecosystems and human health[2], [3], [4]. This study introduces an innovative analytical approach combining Solid Phase Microextraction (SPME) with Microfluidic Open Interface (MOI) and Liquid Electron Ionization Mass Spectrometry (LEI-MS) to investigate pollutant adsorption onto MPs. LEI-MS offers reduced matrix effects, allowing external calibration for a direct-MS technique and providing an effective tool for continuous monitoring of organic contaminants in aquatic environments[5], [6].

Experimental procedures were conducted in a 500 mL glass bowl, containing water fortified with PAHs (50 ppb), pesticides (80 ppb) and microplastic pellets (200 pellets of Low Density Poly Ethylene (LDPE) and 254 pellets of Polypropylene (PP)), with their mass recorded prior to the experiment. Adsorption dynamics were assessed by collecting 1 mL aliquots from the solution at specific time intervals. After had determining the equilibration time in the kinetic experiment, the thermodynamic experiment was performed in a scaled down version of the kinetic experiment. After reaching the equilibrium at 30 hours, 8 aliquots of 1 mL were taken and extracted with Hydrophilic-Lipophilic Balance/Polyacrylonitrile (HLB/PAN) and C_{18}/PAN SPME fibers. The MOI enables direct coupling of SPME with LEI-MS. The MOI-LEI-MS system facilitates rapid analysis of liquid samples by integrating direct sample introduction into the ionization source. MOI operates on the principle of flow-isolated desorption, allowing the insertion of the SPME fiber into a chamber, where analytes are desorbed and transferred to the ionization region. LEI generates gas-phase analytes before they reach the electron ionization source, ensuring efficient ionization.

The research focuses on the kinetic and thermodynamic aspects of pollutant adsorption on PP and LDPE MPs. Kinetic analysis of PAH adsorption (4-n-nonylphenol, naphthalene, anthracene, pyrene) revealed that PAHs followed pseudo-second-order kinetics. Similarly, the adsorption of pesticides (metalaxyl, chlorpyrifos, alachlor, chlorfenvinphos, dichlorvos, atrazine) on LDPE MPs adhered to pseudo-second-order kinetics. Thermodynamic analysis, including equilibrium adsorption capacity (Qe) calculations, demonstrated higher adsorption of chlorpyrifos (6.0 µg/g), anthracene (3.6 µg/g), pyrene (3.6 µg/g) and chlorfenvinphos (3.2 µg/g) compared to other compounds, while naphthalene (2.7 µg/g),

4-n-nonylphenol (2.2 µg/g) , alachlor (1.2 µg/g), metalaxyl (1.0 µg/g), dichlorvos (1.0 µg/g), and atrazine (<1.0 µg/g) exhibited lower adsorption. These findings provide critical insights in MP-EP interactions, enhancing understanding of contaminant transport and persistence in freshwater ecosystems. This study informs microplastic pollution dynamics and supports strategies to mitigate emerging chemical threats to water quality.

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OP34

INSIGHT ON LUGANA FLAVOR WITH A NEW LC-MS METHOD FOR THE DETECTION OF POLYFUNCTIONAL THIOLS

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The analysis of important aroma compounds, such as polyfunctional thiols, requires a reliable and straightforward method. Despite advances in odorant detection, each method currently involves lengthy and complex sample preparation. In this paper, we present a novel high-throughput method that includes derivatization with ebselen, SPE purification, and LC-MS analysis for the quantification of 15 thiols with enological relevance, as well as its comparison with a recently developed QuEChERS-based method. Furthermore, the analysis of 43 Lugana wines was conducted to gain insight into the thiolic composition of this typical Italian wine characterized by tropical flavors and to demonstrate the efficacy of this fast, reliable, and environmentally friendly method.

OP35

AN INFORMAL VIEW ON THE PATH OF DIRECT INJECTION MASS SPECTROMETRY FOR RAPID VOLATILE COMPOUND MONITORING FROM IMMS29 TO IMMS41: AGRIFOOD APPLICATIONS, GLOBAL QUALITY AND SUSTAINABLE INNOVATION

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Years ago, at the 29th Informal Meeting on Mass Spectrometry, we presented advancements in the application of Direct Injection Mass Spectrometry (DIMS), specifically Proton Transfer Reaction Mass Spectrometry (PTR-MS), for monitoring volatile organic compounds (VOCs) in agrifood applications. At that time, quadrupole mass analyzers dominated the field, while time-of-flight (ToF) analyzers were still emerging. Many challenges identified then have since been addressed, but others remain, particularly due to the difficulty in untangling complex volatile mixtures released by agrifood products and processes without separation.

Since that conference, we developed an automated facility based on PTR-ToF-MS for the rapid, high-sensitivity profiling of VOCs, which we have applied to various agrifood-related questions. Despite its limitations, our approach offers unparalleled efficiency in terms of sensitivity, time resolution, and throughput, enabling experiments that would otherwise be impossible. Together with a general overview of the potential of the analytical approach in terms of global quality, traceability and massive screening, we will discuss two paradigmatic examples: fermentation monitoring and nose-space analysis.

Fermentation, also known as "ancient biotechnology," is experiencing a resurgence due to its green and sustainable potential in addressing modern food production challenges, such as label cleaning, new products development, valorisation of alternative proteins and energy-efficient food processing. The term fermentation summarizes a family of microbial-based bioprocesses that provides the basis of more than 5000 foods on a global scale. In these products, VOCs are associated with product quality but also associate with microbial metabolites and, so the possibility of monitoring the phenomena online is particularly interesting. PTR-ToF-MS demonstrates versatility in monitoring key fermentation pathways, studying starter cultures and microbiomes. Our approach is based on the original coupling of a headspace autosampler with PTR-TOF-MS and original data handling and data mining approaches. This allows for simultaneous incubation and continuous VOC sampling from over 100 samples, each containing a substrate inoculated with suitable microorganisms, whereas the PTR-TOF-MS guarantees VOC quantitiation and tentative identification at high time resolution. We have applied this method to monitor VOCs released during fermentation of the principal categories of foods and food-related microbes, including yeasts (for bread, beer, wine, sourdough, and dairy fermentations) and lactic acid bacteria (for

yoghurt, kefir, wine and bread). This technique provides an ideal tool for screening strains, assessing different interactions, and optimizing process conditions. It can support strain selection, develop solutions inspired by nature and support sustainable industrial process, also investigating the biotransformation of flavor compounds by microorganisms.

Understanding the complex relationship between aroma release and perception requires advanced analytical techniques that monitor both chemical stimuli and sensory responses. We combined PTR-ToF-MS nose-space analysis with dynamic sensory analysis to investigate aroma release and perception in real-time. This approach provides detailed information on the concentration and dynamics of specific volatiles. At the same time, dynamic sensory analysis captures consumer perception, offering insights into how these compounds are experienced. We applied this approach to both simplified food models and more complex food combinations that better reflect real-world situations. Our results show that it is possible to track flavor compound release during food consumption and demonstrate that the relationship between flavor perception and VOCs concentrations is not always straightforward. For example, an increase in VOC concentration in the nose-space may be associated with a decrease in flavor perception. This finding highlights the complexity of aroma perception, suggesting that factors beyond instrumental analysis—such as individual sensory responses or environmental conditions—play a significant role. These techniques have applications in fields like food science, fragrance development, and consumer product testing, where understanding both the release and perception of aromas is crucial for improving product quality and consumer satisfaction.

The main limitation of DIMS is the limited specificity of the analysis, which often cannot resolve all ambiguities in compound identification, requiring confirmation from more established methods. We will shortly discuss how ongoing technological advancements, and in particular the implementation of Ion Mobility Spectrometry in the field, aim to improve analytical precision while maintaining non-invasiveness, sensitivity, and speed. The compliance of PTR-MS with the principles of green analytical chemistry and the potential of this approach to support future sustainable innovation in agro-food are highlighted.

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OP36

USE OF ORTHOGONAL MS-APPROACHES IN THE STUDY OF AROMA COMPOSITION OF NATIVE SICILIAN GRAPES AND WINES

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The aromatic complexity of wines is deeply influenced by the volatile compounds in native grape varieties and the winemaking practices used.
This study investigates the aromas of base wines and sparkling wines produced by using some Sicilian autochthonous white and red grape varieties (Catarratto, Frappato, Nero d'Avola, and Zibibbo) and glycosidic aroma precursors in grapes using orthogonal techniques of Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). Namely, GC-MS was used to identify and quantify free volatile compounds in wines and LC-HRMS for characterization of intact structures of glycosidic aroma precursors in grapes which were correlated to GC-MS data [1,2].

The findings revealed distinct differences in the aromatic profiles of the grape varieties, providing valuable insights into how sparkling wine production practices influence the aroma characteristics of the wines.

To elucidate the effects of refermentation, the fermentative volatile profiles of base wines were compared to corresponding sparkling wines. Yeast metabolism during the refermentation induces rearrangement in the composition of volatile esters, increasing the complexity and uniqueness of the sparkling wines [3,4,5].

Understanding these changes offers valuable insights into the sensory attributes of these Sicilian sparkling wines and guides winemaking practices aimed at enhancing their flavor profiles.

Acknowledgments
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OP37

COMPREHENSIVE QUALITATIVE AND QUANTITATIVE ANALYSIS OF SAPONINS IN SAPONARIA OFFICINALIS

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Plant-derived biosurfactants, particularly saponins, exhibit unique properties due to their glycosidic linkages, which connect triterpene or steroid aglycones with one, two, or three sugar groups (glycones) [1]. The type and quantity of saponins produced by a plant vary based on species, organ, developmental stage, and environmental conditions, with some plants synthesizing up to 100 distinct saponins that differ in both aglycone and glycone composition [2]. These variations significantly influence the surface activity and biological properties of plant extracts, creating challenges for their standardization as biotechnological raw materials.

Key insights into saponin biosynthesis have been derived from studies on genome overexpression of aglycone cyclization reactions, which are specific to certain saponin classes but lack the resolution to differentiate between subclasses [3]. As a result, current data do not establish direct correlations between specific saponin types and their biological functions. Structural characterization of saponins is most effectively achieved using Nuclear Magnetic Resonance (NMR) spectroscopy [2]; however, purification and enrichment processes are time-consuming and risk degradation or structural modifications. An alternative approach involves liquid chromatography (LC) coupled with mass spectrometry, for identifying isolated saponins [3]. However, the determination of saponins is often limited by the scarcity of high-purity reference standards. Consequently, saponins are frequently characterized through the analysis of monosaccharides and/or aglycones obtained via chemical degradation.

The work aimed to develop a strategy for tracking changes in the content of glycosides synthesized by *Sapponaria officinalis* L. in extracts obtained using methods with industrial potential. The final method enabled tracking of changes in the structure of saponins influenced by the growing conditions of plants extraction and storage method of saponins.

The developed method, which includes the purification of soapwort extracts, reversed-phase chromatographic separation, and parent ion scanning, enabled the detection of over 200 glycoside species containing 11 distinct triterpenoid aglycones. In the final step, a multiple-reaction monitoring method was established to investigate the impact of extraction and purification conditions on saponin composition. The study revealed that acidification, alkalization, and elevated extraction temperatures enhance the overall extraction efficiency of glycosides. However, these conditions also contribute to increased de-esterification of oligosaccharide moieties containing pentoses and deoxyhexoses.

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OP38

HIGH-RESOLUTION MASS SPECTROMETRY (HRMS) TO INVESTIGATE THE ALKALOID PROFILE OF MILK

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The transfer of alks into dairy products raises important questions regarding food safety and potential health implications for consumers. Infact, investigation of alk focus on toxic alks, such as pyrrolizidines, tropanes, or quinolizidines, whose presence in milk is mainly derived from contaminated animal feed products [1,2].

In this study, 48 daily milk samples were collected, over three consecutive days, from 16 lactating cows grazing on two alpine pastures in Northeast Italy, with 8 cows per pasture. Simultaneously, alpine herbs selected by the cows during grazing were gathered using the hand-plucking method. Additionally, 12 bulk milk samples were taken from a herd of 110 cows.

Both the herbage and milk samples were analysed using liquid chromatography coupled with high-resolution mass spectrometry, leading to the identification of 41 alkaloids through pure standards and 116 putatively identified compounds. The study found a transfer rate of 0.4% for pyrrolizidine alkaloids, 2.7% for indole alkaloids, and 12% for steroidal alkaloids from the ingested herbs to the milk. A partial least squares discriminant analysis (PLS-DA) based on alkaloid profiles correctly classified 67% of milk samples according to the cows' grazing location.

Although the transfer of alkaloids was minimal, which is reassuring from a health perspective, the findings highlight the potential of alkaloids as natural markers for the traceability of mountain dairy products.

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Abstracts,
POSTER SESSION

P01

STRUCTURAL CHARACTERIZATION OF OLIGONUCLEOTIDES WITH DOUBLE HELIX FORMING ABILITIES USING MULTIPLE METHODS

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Although oligonucleotides are known biomolecules for a long time and some of their properties have been extensively investigated (melting temperature, CD, MS) , only very few studies appeared around the investigation of their double, triple and quadruple helix forming abilities by ion mobility mass spectrometry. [1-4]

In our work we aimed to do a comparative ion mobility mass spectrometry study of oligonucleotides having double helix forming abilities. For this study oligonucleotides having rather different hybridization properties were synthesized including linear-scrambled sequences (a), linear-double-stranded (b), linear partially- and fully-hairpin-forming (c and d), cyclic scrambled sequences (e) and cyclic double stranded (f) structures. (**Figure 1.**) These oligonucleotides of variable length and thermodynamic properties were analyzed and compared by multiple techniques. Their sequence and structure were confirmed by HPLC-UV and ESI-MS/MS. Their thermal stability (melting temperature) was measured by a programmable Peltier-heating-cooling cuvette containing UV-photometer, while further optical properties were investigated by CD spectroscopy. Other structural properties, including shape (Collision Cross Section) and double helix forming abilities were analyzed by cyclic ion mobility mass spectrometry beside the HPLC-UV investigation of their chromatographic retention.

The results show that all the analytical methods support and well complement each other.

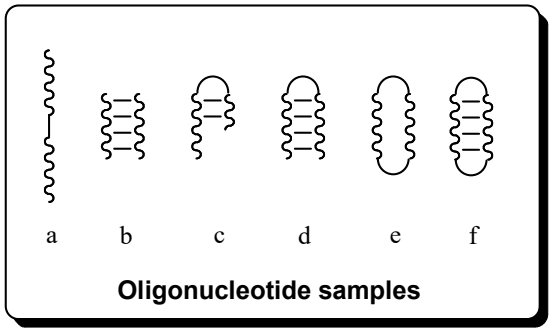

Figure 1. Schematic structure of the analyzed oligonucleotides

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P02

MASS SPECTROMETRIC CHARACTERIZATION OF NANOCOMPOSITES OF ANTICANCER DRUGS WITH MoS_2 AS POTENTIAL MULTIFUNCTIONAL THERAPEUTIC NANOPLATFORMS

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Two-dimensional transition metal dichalcogenides, including MoS_2, are known as promising materials for the development of multifunctional nanoplatforms for biomedical applications. The unique physicochemical properties of MoS_2 2D nanosheets provide the possibility of this nanomaterial utilization as a photothermal transduction agent for photothermal therapy (PTT) of cancers [1]. At the same time, MoS_2-based nanostructures are considered as potential biocompatible nanocarriers for drug delivery that is crucial for some anticancer drugs to protect them from rapid clearance in the bloodstream and to accumulate in the targeted organs [2]. A combination of chemotherapy with PTT provided by MoS_2 multifunctional nanocarriers can result in synergetic positive effects in some cancers treatment. To control the production technology of MoS_2-based nanoplatforms loaded by anticancer agents and to confirm the drugs` stability, the (MoS_2+drug) composites may be examined by matrix-free laser desorption/ionization (LDI) mass spectrometry (MS). This method has already demonstrated efficiency in the study of MoS_2 based nanostructures [2, 3].

In the current study we applied LDI MS for a comparative study of nanoparticles obtained from ultrasound-treated aqueous dispersions of MoS_2 with selected anticancer drugs, namely doxorubicin (DOX), 6-thiopurine (TP), and 2-thioadenine (TA). In our previous research [3], it was shown that LDI negative ion mode is optimal for observation of the characteristic spectral clusters of Mo-containing ions („fingerprints") originating from nanocomposite, while the characteristic peaks of the anticancer drugs are better recorded in the positive ion mode. Analysis of the mass spectra of samples containing DOX and MoS_2 prepared by different ultrasound treatment regimes (with different times of sonication) enabled us to select the optimal conditions for the (MoS_2+DOX) nanocomposite production. The MS data confirmed DOX stabilty in the nanocomposite, that is important for its therapeutic activity preservation, while changes in the MoS_2 characteristic clusters intensity distribution testified to the active exfoliating role of DOX in ultrasound technology of MoS_2 nanocarriers preparation. In contrast to the situation with DOX, certain features of the LDI mass spectra of (MoS_2+TP) and (MoS_2+TA) nanocomposites indicated the presence of critical interactions between MoS_2 nanocarriers and sulfur-containing anticancer drugs and the effect of MoS_2 on the structure of these anticancer drugs withing the composite. Namely, the catalytic activity of MoS_2 stimulated the covalent dimerization reaction of thioderivatives by forming S–

S bridges, while the adsorption of TP and TA on MoS_2 nanosheets changed the pattern of the Mo-containing clusters sputtered under LDI conditions.

The effects observed during LDI MS characterization of MoS_2 nanocomposites with the studied anticancer drugs demonstrate that chemical structure of an anticancer agent and peculiarities of its interaction with 2D nanosheets influence significantly on the behavior of the drug within the nanocomposite and on potential MoS_2-based multifunctional nanoplatforms applicability for the drug delivery purposes combined with PTT.

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P03

DIVERSITY OF FEATURES OF NANOCOMPOSITES OF TRANSITION METAL DICHALCOGENIDES WITH ORGANIC COMPOUNDS AS REVEALED BY LASER DESORPTION/IONIZATION MASS SPECTROMETRY

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In modern nanotechnology, combining of inorganic materials with organic compounds is aimed at production of nanomaterials for the most diverse applications [1]. Desorption mass spectrometric techniques, laser desorption/ionization (LDI) in particular, can provide direct analytical data on the composition of such nanomaterials and, at the same time, give some indirect information on physical and chemical processes occurring in the composite. In this study we tried to highlight such information for composite 2D nanomaterials obtained by sonication of aqueous mixtures of transition metal dichalcogenides (TMD) MoS_2, $MoSe_2$, WS_2 with a number of organic compounds of different classes: polyethyleneglycol polymer PEG-600, cationic dye methylene blue (MB), cysteine (Cys) amino acid, and thiol thioglycerol (TG). In the case of physical interactions within thus formed nanocomposites the mass spectra will be a mere superposition of mass spectra of their components, while in the case of chemical interactions the transformation products may be detected by the corresponding changes in mass values.

The first case is illustrated by the example of (PEG-600 + TMD) nanocomposites. Positive ion LDI mass spectra of the nanocomposites of the polymer with all three TMD consisted of two bell-shaped sets of cationized oligomers $[HO\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}H]\bullet K^+$, $[H\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}H]\bullet K^+$ which practically coincided with the mass spectra characteristic of pure PEG-600. Negative ion LDI mass spectra contained sets of clusters sputtered from TMD with appropriate isotopic distributions caused by the polyisotopic nature of the atoms in the cluster: $Mo_xS_yO_z^-$, $Mo_xSe_yO_z^-$, $W_xS_yO_z^-$ (oxygen may appear due to oxidation). In this case physical adsorption of PEG at the TMD nanosheets surface caused "shielding effect" reflected in decrease of abundances of the sputtered clusters as compared with the spectra of pure TMD. Pegylation of TMD enhances their dispersability in water and thus bioavailability for biomedical applications of TMD-based 2-D nanomaterials [2].

Organic cationic dye MB exhibits other type of effect. In the positive ion LDI mass spectra of (MB + TMD) nanocomposites the threshold of appearance of the MB organic cation was observed at the laser power values about 30% lower than those required for sputtering of other organic compounds from the TMD carriers. The effect was explained by the existence of the "performed" MB cations at the nanosheets surface; plane heterocyclic cations were electrostatically bound with the partially negatively charged TMD surface. Such a structure may be referred to as a "polysalt" of a row of organic cations

coordinated with the sets of sulfur atoms at the surface. The adsorption of MB in the form of single cations ("monomers") was confirmed by recording of intact cations of MB in the LDI mass spectra, since we have shown earlier [3] that in the case of aggregation of MB its reduction product appears under desorption mass spectrometric conditions due to redox interactions in the aggregates. In the negative ion mode the distribution of TMD-related cluster sets was somewhat affected by MB adsorption. Mass spectrometry-derived data confirming the stability of (MB + TMD) nanocomposite are of interest for applications of this nanomaterial in combined cancer therapy, since TMD are tested for photothermal therapy while MB is applied in photodynamic therapy.

In the LDI mass spectra of redox-active sulfur-containing amino acid cysteine composite with MoS_2, redox transformation of cysteine promoted by catalytic activity of MoS_2 was observed. The product of cysteine oxidation which resulted in formation of its covalent dimer bound by –S-S- disulfide bridge - (2Cys – 2H), cystine, was recorded in the protonated, cationized, and deprotonated forms. This is an example of chemical interactions within nanocomposites.

The strongest chemical interactions were observed for the systems incorporating thiol TG: (MoS_2 + TG), (WS_2 + TG). Bare atomic Mo^+ or W^+ ions were recorded in the positive ion LDI mass spectra of these composites, while in the mass spectra of pure TMD molybdenum or tungsten were observed in the composition of their negatively charged clusters with sulfur and/or oxygen. The explanation for this effect was proposed, supported by the presence of S_n^- (n=1-3) and $[TG + S]^-$ ions. The thiol group of TG interacted with sulfur atoms at the edges of TMD nanosheets via formation of disulfide bond followed by cleavage and removal of sulfur from the nanosheet. Molybdenum atoms deprived of their sulfur ligands were easily liberated under LDI. At the same time, some share of TG molecules were adsorbed at the surface of the nanosheets shielding them from laser impacts, which resulted in the suppression of sputtering of $Mo_xS_yO_z^-$ or $W_xS_yO_z^-$ clusters containing more than one Mo or W atom.

The four described cases illustrate how the diversity of properties of nanocomposites is reflected in their LDI mass spectra. The described approach can be further applied to evaluate certain properties of nanocomposites of TMD with organic compounds.

Experimental details: 2D nanosheets of TMD MoS_2, $MoSe_2$, WS_2 were prepared by sonication of their crystalline powders in aqueous medium without any additives or with addition of organics either as exfoliating agents or components of the nanocomposites. LDI mass spectra of solid dried nanocomposites were obtained using Autoflex II mass spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser (337 nm).

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P04

A NEW METHOD FOR KETONE BODIES DETERMINATION IN SERUM AND SEMINAL FLUID BY GC-MS

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Ketone bodies play crucial roles in body metabolism including serving as energy fuels for extrahepatic tissues like brain, heart, or skeletal muscle, ketone bodies play pivotal roles as signaling mediators, drivers of protein post-translational modification (PTM), and modulators of inflammation and oxidative stress (1).

In sperm metabolism Ketone bodies could serve as an alternative energy source for sperm, especially in conditions of glucose deprivation (e.g., fasting, ketogenic diets, diabetes). High ketone levels in semen may indicate a shift in energy metabolism, which could impact sperm motility, viability, and overall quality. The study of ketone bodies (3-Hydroxybutiric Acid, 3-HBA and Acetoacetic Acid, AA) in semen could help understand the impact of metabolic diseases on male reproductive health and provide insights into how different diets or metabolic states influence spermatogenesis and sperm function.

In addition, it is well known for other molecules, that no correlations were recorded between seminal fluid and serum concentrations, and information related to ketone bodies in both matrices are not available.

In our knowledge there are no published methods for ketone bodies determination in seminal fluid and no commercial kits are available for this matrix. The proposed method was optimized to quantify 3-HBA and AA in both serum and seminal fluid. While 3-HBA is stable at every pH, and can be easily measured after esterification, AA determination by GC-MS is troublesome as this molecule is not volatile as such and undergoes spontaneous and rapid de-carboxylation in acidic environment required for its esterification (2). In addition, seminal fluid contains a huge number of metabolites such as biogenic amines which easily reacts with the carbonyl group of AA, especially during the dry out phase following deproteinization step. This problem was overcome oximating the keto group of AA with hydroxylamine (HY) in the dry out phase, before silylation with *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). No difficulties were encountered for 3-HBA silylation in the presence of HY.

The derivatized extracts were analyzed by GC-MS with a HP5-MS chromatographic column (0.18 mm x 0.18 μm x 20 m) with a Markes Benchtof MS spectrometer coupled to an Agilent 7890 GC.

The method showed good sensitivity and a good linearity in a range that includes the values reported in literature for other matrices (3).

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P05

DESIGN OF A PEPTOID-BASED ARTIFICIAL CATALYST INSPIRED BY CARBONIC ANHYDRASE FOR CO_2 CAPTURE

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The capture and conversion of CO_2 are critical challenges in mitigating the impact of anthropogenic emissions on climate change. In living organisms, carbonic anhydrase, a metalloenzyme containing a zinc ion at its active site, efficiently catalyzes the reversible hydration of CO_2 into bicarbonate [1]. However, its application at a large-scale is hindered by stability and cost limitations.

In order to develop an efficient alternative, an artificial carbonic anhydrase is being designed using peptoids. These peptide-like oligomers possess an N-substituted glycine backbone that offers enhanced stability and structural tunability to mimic the enzyme active site [2].

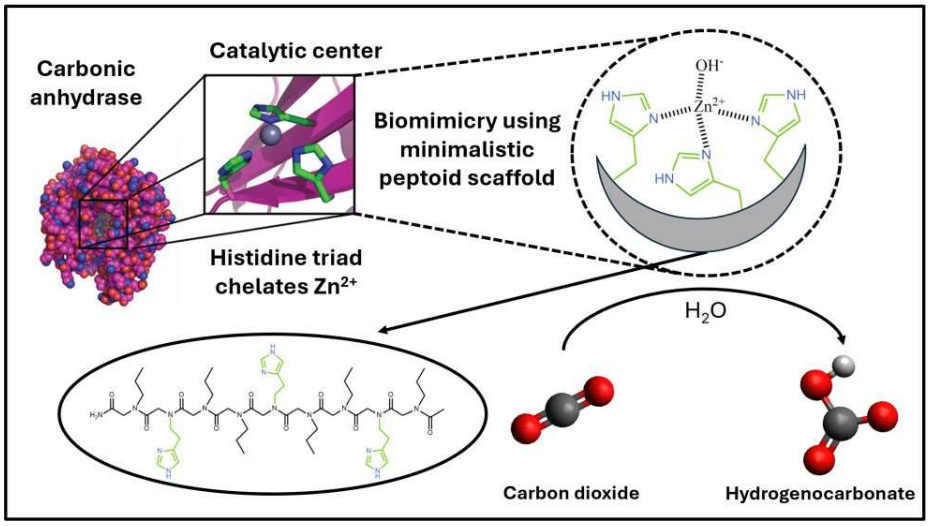

Figure 1. Minimalist design of a peptoid-based artificial enzyme inspired by carbonic anhydrase.

In the present communication, we report on the investigation by mass spectrometry of the complexation of various metal ions (Zn^{2+}, Cu^{2+}, Ni^{2+}), known as modulators of the enzymatic activity [3], with tailor-made peptoid sequences bearing from zero to three pending imidazole metal ligands. The designed original peptoids have been prepared using an optimized solid-phase protocol based on the method developed by Zuckermann et al.,[2] and have been fully characterized by LC-MS and LC-MSMS experiments. Electrospray ionization of peptoid/metal salt solutions revealed the formation of

different peptoid ions, depending on the nature of the metal ion and the sequence of the peptoids. Notably, we found that at least two imidazole ligands are required for the detection of potentially catalytically active $[M+Zn]^{2+}$ ions. To gain deeper insights into the conformations of these complexes in the gas phase, we conducted ion mobility spectrometry (IMS) experiments using a Waters Synapt G2-Si mass spectrometer. The experimental collision cross sections of all detected singly and doubly charged peptoid ions were measured and compared with theoretical CCS values derived from the 3D atomistic structures generated via molecular dynamics (MD) simulations. This structural analysis provides crucial information on the stability and conformational changes of $[M+Met]^{2+}$ complexes in the gas phase, offering key insights for optimizing metal coordination and enhancing catalytic efficiency.

By combining synthetic chemistry, ion mobility spectrometry, and molecular modelling, our approach provides a framework for a rational design of efficient artificial enzymes for CO_2 capture.

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P06

PHOTOSENSITIVE MACROCYCLIC PEPTOIDS FUNCTIONALIZED WITH AZOBENZENES

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Azobenzenes are a particularly interesting class of molecular photoswitches due to their ability to switch reversibly between their *trans* and *cis* configurations under the influence of light. This property is exploited in many fields, including energy storage, smart materials and pharmacology. By dynamically modifying their molecular interactions and physico-chemical properties, azobenzenes enable innovative applications such as light-activatable sensors, molecular actuators and controlled-release drugs [1], [2].

On the other hand, peptoids are an emerging family of peptidomimetics due to their ease of synthesis, their ability to fold into well-defined secondary structures and their great chemical structural diversity. Thanks to their cellular permeability and proteolytic resistance, peptoids have been widely studied in biomedical applications, notably drug delivery [3], [4]. In addition, recent studies in the field of solar energy storage showed that incorporating pending azobenzene residue(s) onto a linear peptoid backbone improves the storage properties [5]. However, linear peptoids are flexible, which reduces their ability to adopt the specific conformations required. The cyclization of peptoids is an elegant strategy to reduce their conformational flexibility, thereby optimizing their applications in pharmacology, biotechnology or materials science [3], [4].

In this context, the integration of azobenzenes onto cyclic peptoids is promizing for the design of photoreactive materials and bio-inspired molecules. Their enhanced stability and structural modularity provide an ideal platform for harnessing azobenzene properties while introducing functional diversity. This project aims to investigate how grafting an azobenzene residue onto a cyclic peptoid plateform may influence the peptoid conformation and the azobenzene photochemical properties, including cis-isomer half-life, absorption spectrum, and photostationary state composition.

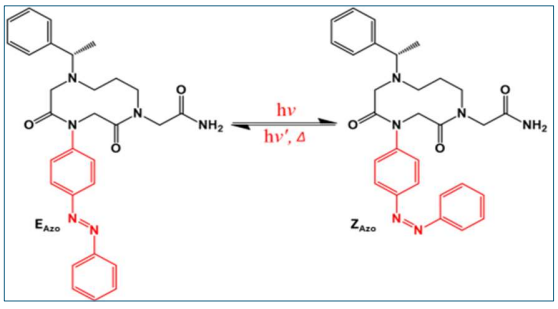

Figure 1. Photoisomerization of a cyclic peptoid-grafted azobenzene.

Liquid chromatography and ion mobility separation associated to mass spectrometry (LC-MS and IMS-MS) are used to thoroughly investigate the conformational dynamics of these unique hybrid systems.

As a starting point, three-unit linear and cyclic peptoids, containing one azobenzene-functionalized monomer, were successfully synthesized and characterized. The cyclic peptoid was obtained by microwave-assisted cyclization of the linear precursor involving an intramolecular nucleophilic substitution.

As shown in Figure 2, the LC-MS chromatogram of the linear peptoid displays two signals, corresponding to the presence of both azobenzene photoisomers. Compared to the linear precursor, when analyzing the cyclic products, numerous signals were detected. These peaks are attributed to the formation of stereoisomers upon cyclization. The signal at 13.07 min is tentatively attributed to a linear peptoid produced upon an HCl elimination for the chloropropyl side chain. Additional studies were conducted using IMS-MS to confirm these structure assignments.

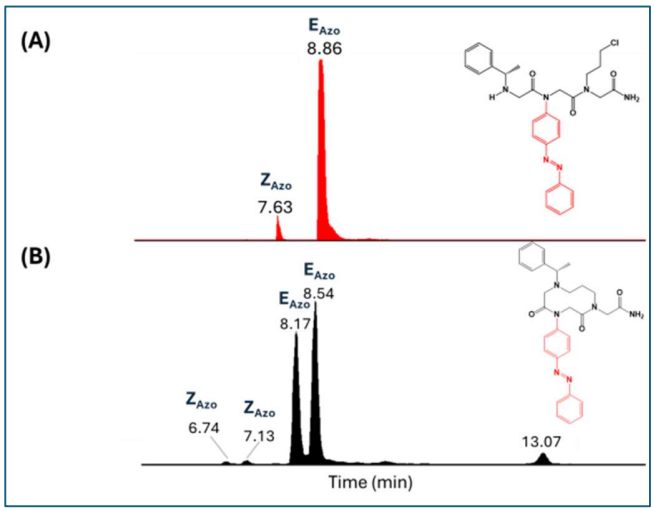

Figure 2. LC-MS analysis of the (A) linear peptoid; and the (B) cyclic counterpart.

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P07

SELF-ASSEMBLED MONOLAYERS BASED ON AZOBENZENE DERIVATIVES AS MOST SYSTEMS

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Azobenzene (AZO) derivatives exhibit a reversible photoisomerization between ground-state trans (E) and metastable cis (Z) forms[1]. Pristine AZO and many of its derivatives absorb in the UV range to promote E-to-Z isomerization while the reverse isomerization is generally triggered by visible light for releasing the energy. Densely packed self-assembled monolayers (SAM) of azobenzene derivatives anchored on rigid metal substrates exhibit a high yield of photoisomerization (>96 %) due to cooperative switching effects[1]. For grafting on metallic surfaces such as gold or silver, the azobenzene core is typically end-substituted by a thiol group to generate a covalent Au/Ag-S bond upon chemisorption[2]. Interestingly, switches can also be grafted on glass substrates using silanes as anchoring unit[3]. Glass is a strongly appealing substrate for our project due to its transparency, further allowing for spectroscopic investigations, and its high commercial availability. In the present study, the strategy for anchoring azobenzene derivatives onto glass surfaces is to perform a click reaction between azobenzenes with an alkyne group and grafted silanes end-substituted by an azide group.

In the present communication, we will present our preliminary results related to the preparation of azobenzenes and fluorine-substituted azobenzenes chromophores. The determination of the MOST properties of the isolated chromophores are performed before the SAM preparation to assess the impact of alkyne groups on the chromophore photoswitching properties and also to further evaluate the role of the intermolecular interactions within the close-packed assemblies on the MOST properties. Thus, we evaluated the absorption properties by UV-Vis spectroscopy and the half-life time by mass spectrometry. LC-MS (Liquid Chromatography-Mass Spectrometry) is here originally used to separate the isomers and to measure the proportion of both as a function of time to obtain the azobenzene half-life time.

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P08

PROFILING OF THE PERIPHERAL BLOOD MONONUCLEAR CELLS PROTEOME BY SHOT-GUN PROTEOMICS IDENTIFIES ALTERATIONS OF IMMUNE SYSTEM COMPONENTS, PROTEOLYTIC BALANCE, AUTOPHAGY AND MITOCHONDRIAL METABOLISM IN GLAUCOMA SUBJECTS

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Glaucoma is a chronic optic neuropathy and the second cause of irreversible blindness worldwide. Although the pathogenesis of the disease is not fully understood, death of retinal ganglion cells and degeneration of the optic nerve is likely promoted by a combination of local and systemic factors. Growing attention has been paid to non-intra-ocular pressure risk factors, including mechanisms of inflammation and neuro-inflammation.

In this study, we set up a shot gun proteomics study to characterize by Mass Spectrometry (MS) the global proteome perturbations of PBMC of patients with primary open-angle glaucoma (POAG) compared to non-glaucomatous controls. Data were acquired by Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA), both under library free searches (DIA-NN Software) and Gas Phase Fractionation (GPF) refined DIA library.

The approach identified >4,500 proteins and a total of 435 differentially expressed proteins between POAG and control subjects. Clustering and rationalization of proteomic datasets and immunodetection of selected proteins by Western blotting, highlighted significant alterations of immune system compartments (i.e., complement factors, regulators of immune functions and lymphocytes activation) and pathways serving key roles for immune system such as proteolysis (e.g., matrix metalloproteinases and their inhibitors), autophagy (i.e., beclin-1 and LC3B), cell proliferation (Bcl2), mitochondrial (i.e., sirtuin) and energetic/redox metabolism (i.e., NADK).

Based on these findings, this proteomic study fosters the perspective that circulating immune cells suffer from heterogeneous alterations of central pathways for cell metabolism and homeostasis which are worth being deepened to elucidate their possible contribution to the mechanisms of neuroinflammation and glaucoma pathogenesis.

P09

CHEMICAL PROTEOMIC INVESTIGATION OF FATTY ACID PHOTODECARBOXYLASE DEACTIVATION

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Fatty acid photodecarboxylase (FAP) has recently been identified in the microalgae Chlorella variabilis NC64A.[1] FAP is a photoenzyme belonging to the glucose-methanol-choline (GMC) class. It is involved in lipid metabolism and it catalyses the decarboxylation of fatty acids to alkanes in response to light exposure.[1] The FAP is a photoenzyme with great potential for technological applications in green chemistry, as hydrocarbon synthesis plays a crucial role in fuel and solvent production.[2]

Beisson et al. investigated the elementary steps responsible for the catalytic activity of FAP.[1,2] According to their hypothesis, the illumination of FAP triggers an electron transfer from the fatty acid to the cofactor flavin adenine dinucleotide (FAD). This generates a semi- quinone FAD radical (FAD•-) and a carboxyl radical. The latter undergoes a rapid decarboxylation, forming an alkyl radical (R•). The final alkane may be generated through a hydrogen atom transfer from a cysteine or asparagine residue present in FAP active site.

The problem arises when the FAP is not bound to the substrate. Under this condition, light illumination triggers a rapid and irreversible FAP deactivation. This makes economically unprofitable the industrial application of FAP. FAP deactivation has never been investigated at a molecular level, but understanding this process could unlock the potential for real-world FAP applications.

Holmann et al. hypothesised that FAP deactivation may involve covalent modification of amino acid residues near the active site (Figure 1).[3] We aim to investigate this hypothesis by leveraging mass spectrometry-based chemical proteomics. High-resolution tandem mass spectrometry could reveal both the specific amino acid residues undergoing photoinduced covalent modifications and the precise nature of these modifications. These chemical modifications will be incorporated into molecular dynamics simulations of FAP to elucidate their mechanistic role at the molecular level.

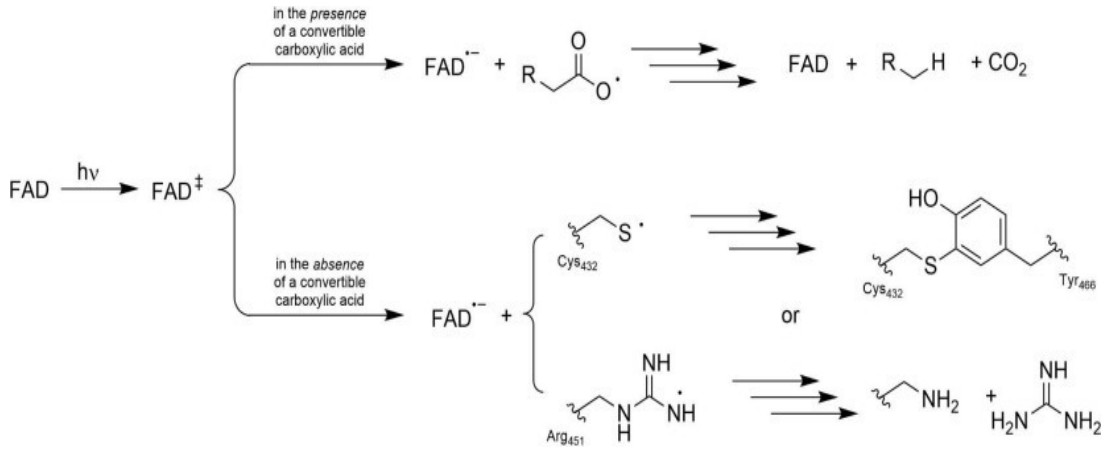

Figure 1. Proposed mechanisms of FAP catalytic activity in presence and absence of substrate. Adapted from ChemBioChem 2021, 22, 2420–2423.[3]

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P10

APPLICATION OF ALKALI METAL ADDUCT ION FORMATION IN CYCLIC ION MOBILITY MASS SPECTROMETRIC ANALYSIS OF CYCLODEXTRIN ISOMERS

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Cyclodextrins (CDs) are cyclic oligomers consisting of α-D-glucopyranose monomer units, having widespread applications across various fields. Several regioisomers are formed upon substitution of CDs. These isomers have different complexing properties; therefore, their identification and quantification are essential [1]. Ion mobility separates gas-phase ions based on their size, charge, and shape as they are guided through an inert buffer gas by an electric field. The cyclic ion mobility−mass spectrometer (cIM-MS) is an advanced version of traditional linear instruments, featuring a traveling wave ion mobility cell with a unique cyclic geometry. The main advantage of cIM-MS is that the path length can be adjusted and optimized for specific separation challenges [2].

In this work, cyclic ion mobility separation of two cyclodextrin regioisomers was achieved along with their identification by tandem mass spectrometry (MS/MS). Two constitutional isomers were investigated: Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (2,6- DIMEB) and Heptakis(2,3-di-*O*-methyl)-β-cyclodextrin (2,3-DIMEB). In an ion mobility measurement, it is not always evident which ion species of the isomeric analytes can be separated. In addition, detection of the components as single gas phase species (as single peaks) is also important to an unequivocal identification of the isomers. In many cases, reference materials are not available, hence, the ion mobility-resolved isomers can only be identified based on their MS/MS spectra. Different adduct ions can yield fragment ions with different structures via collisional induced dissociation (CID), therefore, various adduct types were tested in this work: $[M + H]^+$, $[M+ NH4]^+$, $[M + Li]^+$, $[M + Na]^+$, $[M + K]^+$, $[M + Rb]^+$, $[M + Cs]^+$, $[M – H]^-$, and $[M + Fo]^-$ ions of the two CD isomers were probed during the cIM-MS/MS method development.

All of the tested adduct ions of the isomers could be separated by the cIM except the $[M + H]^+$, $[M – H]^-$ and $[M + Fo]^-$ ions. A 10-pass cIM experiment demonstrated, that the peaks of the protonated molecules, $[M + H]^+$, consist of several protomers with different gas phase configuration. CID of the protonated ions yielded cleavages at the glycosidic bonds only, - between the glucoside units - which did not provide information about the location of the methyl-groups on the glycosidic units. The fragmentation pattern of the $[M + NH4]^+$ ion was similar to the $[M + H]^+$ ion, due to the loss of ammonia, similarly to the formate-adduct ion which dissociated after the loss of formic acid resulting an MS/MS spectrum identical to the $[M – H]^-$. However, all of the alkali metal adduct ions could be separated by the cIM and their peaks were also uniform showing a single conformer only. Our results

demonstrated that the Li- and Na-adduct ions of the CD isomers are applicable for the isomer distinction from both an ion mobility and a mass spectrometric point of view. During the CID process, the K-, Rb-, and Cs-adduct ions lost their alkali cation only, therefore the dissociation of the CD did not happen. On the contrary, the Li- and Na-adducts yielded structure specific fragment ions via crossring cleavages beside the glycosidic cleavages. Comparing the MS/MS spectra of the $[M + H]^+$, $[M + Li]^+$ and $[M + Na]^+$ it was also observed that the $[M + Li]^+$ ion showed properties of both the $[M + H]^+$ and $[M + Na]^+$ ions. The MS/MS spectrum of the $[M + Li]^+$ exhibited characteristic structure specific fragment ions just as the MS/MS spectrum of $[M + Na]^+$ but with slightly lower intensities.

Our results demonstrate the effectiveness of cyclic ion mobility to separate isomeric cyclodextrin molecules. Combination of cIM with MS/MS fragmentation verified the exact structure of the constitutional isomers. It was observed that the careful selection of the adduct type is essential for the successful analysis.

Acknowledgement

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P11

LIPID ALTERATIONS IN PHYSIOLOGICAL AND SERTOLI CELL-ONLY SYNDROME HUMAN TESTICULAR TISSUE SECTIONS AND SEMINAL PLASMA SAMPLES

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Azoospermia, the absence of sperms in semen, affects around 15% of infertile males. Sertoli cell-only syndrome (SCOS) is the most common pathological lesion in the background of non-obstructive azoospermia and is characterized by the complete absence of germinal epithelium, Sertoli cells are exclusively present in the seminiferous tubules. Studies have shown a correlation between successful spermatogenesis and male fertility with lipid composition of spermatozoa, semen, seminal plasma or testis. The aim of this research was to discover the correlation between Johnsen scoring system and phospholipid expressions in testicular cryosections of SCOS patients. MALDI imaging mass spectrometry is used to determine spatial distributions of molecular species, such as phospholipids. PC, PE, and SM are the most abundant phospholipids in mammalian cells and testis. Sphingomyelins, structural components of plasma membranes, are crucial for spermatogenesis and sperm functions. Plasmalogens, are unique PCs in testis with strong antioxidative properties.

In this study, human testicular phospholipid species were investigated by MALDI IMS. Spatial expression levels of the investigated lipid species are significantly altered in the normal and the SCOS individuals. Tissue cryosections of 12 non-obstructive azoospermic patients with Johnsen scores 2 to 10 were investigated by MALDI IMS.

Azoospermia, defined as the absence of sperms in the semen occurs in ca. 15% of infertile male population. Previously, several analytical techniques have been used for lipidomic investigations of spermatozoa, semen, seminal plasma and testicular samples. These studies showed a significant correlation between successful spermatogenesis and male fertility with lipid compositions. MALDI IMS is a powerful method to determine the spatial distributions of molecular species, such as phospholipids. In this study, SM, PCs and plasmalogens were determined directly from non-obstructive azoospermic human testicular cryosections by MALDI TOF/TOF IMS. Our findings showed a strong relationship between the Johnsen scoring system and the expression levels of phospholipids. PC, PE and SM are the most abundant phospholipids in mammalian cells, as well as in human testis.

In mammalian cells the glycerophophatidylcholines mainly occur in their diacyl form, additionally, alkenyl-acyl and alkyl-acyl species are also present. Our results showed down regulation of monounsaturated and polyunsaturated diacyl-PC species (16:0/18:2, 16:0/18:1, 18:1/18:1 and 18:0/20:5)

in all SCOS testicular tissue samples, while the concentration of PC (16:0/20:4), PC (16:0/16:0) and PC (16:0/16:1) over represented in azoospermic conditions.

Sphingomyelins are structural components of plasma membranes with a crucial importance in apoptosis, cell ageing, development, spermatogenesis, sperm functions. In physiological conditions of spermatogenesis, the concentration of SM (16:0) is significantly higher than in SCOS conditions, in contrast the expression levels of some PC species. PCs are potential choline donors in SM biosynthesis, accordingly, the results may indicate the irregularity of SM synthetic pathway in SCOS conditions (Figure 1.). Previous studies have also shown similar effect of SM/PC ratio during spermatozoa epididymal maturation [1].

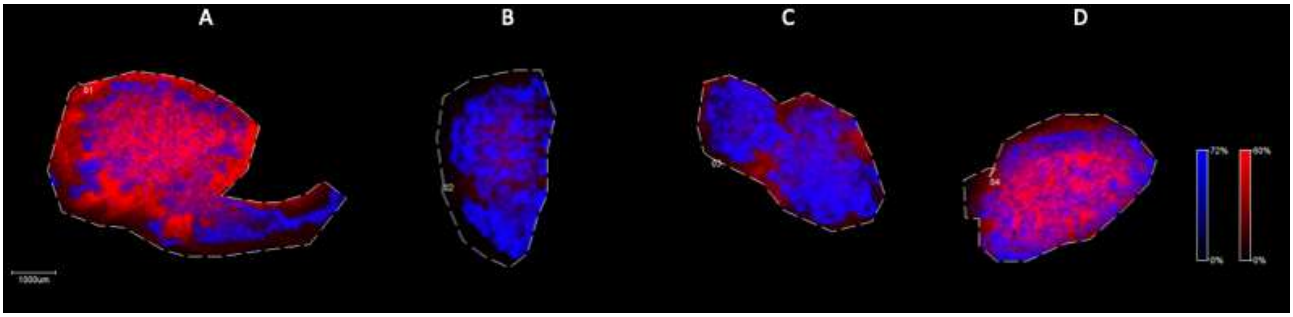

Figure 1. Merged ion images of SM (16:0) (m/z 703, red) and PC (16:0/20:4) (m/z 782, blue) in testicular tissue samples by MALDI IMS in positive mode with using of CHCA as matrix. (A) Johnsen score 8-10, (B) Johnsen score 2, (C) Johnsen score 2, (D) Johnsen score 6-8. Scale bar: 1000 μm.

Plasmalogen are unique PCs in testis with strong antioxidative character, because the alkenyl ether bond is highly sensitive for reactive oxygen species (ROS). Their significant down regulation shows increased oxidative nature and higher ROS concentration in SCOS testicular samples.

Our preliminary MALDI IMS data collected from human testicular tissue cryosections and seminal plasma samples showed a relationship between Johnsen scoring system and the local distribution of phospholipid species.

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P12

CYCLOPEPTIDE DEAMIDATION STUDIED BY CYCLIC ION MOBILITY MASS SPECTROMETRY

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The non-enzymatic deamidation of asparagine amino acids in proteins under physiological conditions is a key post-translational modification, which has been associated with various diseases, as well as cellular aging. During deamidation, a mixture of *L*- and *D-iso*aspartyl and aspartyl derivatives is formed through a succinimide intermediate. This process is particularly favourable for peptides and proteins containing an NGR (Asn-Gly-Arg) sequence motif, from which *iso*DGR and DGR derivatives can form, typically in a 3:1 ratio, strongly affecting the biological activity of the molecules. The monitoring of this process and the structural verification of the modified residues is difficult by mass spectrometry, because there is only a 1 Da mass difference between the asparagine and (iso)aspartyl derivatives, and these peptide isomers and diastereomers can be challenging to be distinguished under standard experimental conditions. Ion mobility spectrometry, especially the high-resolution cyclic ion mobility spectrometry provides an alternative approach to address this issue.

In our experiments, we focused on the deamidation of a small bioactive cyclopeptide and the structural analysis of the products by cyclic ion mobility mass spectrometry. To study the conversion of the NGR motif-containing cyclo[KNGRE]-NH_2 peptide, we applied a multi-cycle ion mobility separation combined with UPLC-MS analysis. The main advantage of the Waters Select Series Cyclic IMS mass spectrometer used in the measurements is that, in addition to high-resolution and high-mass accuracy, the increased path length used for ion mobility separation allows even components with the same molecular mass to be separated from each other. By synthesizing one of the expected derivatives, the cyclo[KDGRE]-NH_2 peptide, as a reference compound, we were able to separate and identify all the resulting products, including D/*iso*D derivatives and the deamidated C-terminal derivative. During our work, we studied the deamidation process in detail – affecting both the asparagine residue and the *C*- terminal amide group – to explore the effect of solvent composition and temperature on this spontaneous conversion.

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P13

APPLICATION OF 2D-CHROMATOGRAPHY FOR THE DETECTION OF COSMETIC PEPTIDES IN SKIN HOMOGENATES

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Peptides are biologically active molecules of great interest due to their ability to interact with various biological systems. Many peptides have been developed for cosmetic applications, particularly to enhance skin firmness and reduce wrinkles by modulating collagen turnover. However, unlike the pharmaceutical sector, the cosmetic market lacks strict regulations, and no specific guidelines currently exist for assessing the dermal stability of cosmetic peptides. Given their topical administration and the limited data on peptide resistance to skin proteases, we developed an HPLC-MS/MS method to evaluate peptide stability in human skin homogenates [1]. We further refined this approach by incorporating 2D chromatography and introducing, for the first time, a novel internal standard for peptide quantification: a scrambled peptide sequence derived from the original analytes [2]. This combination significantly improved analytical performance and sensitivity, making this methodology the most accurate and reliable tool available for assessing peptide stability against dermal proteases in complex matrices such as human skin homogenates.

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P14

HIGH-RESOLUTION MASS SPECTROMETRY CHARACTERIZATION OF ANTHOCYANIN COMPOSITION IN BLUE AND RED BERRIES

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Anthocyanins are a major group of natural pigments that characterize the color of purple, blue and red berry fruits, such as blueberry, raspberry, and grape. In plants, they serve as free radical scavengers lowering the oxidative stress due to yellow and green light alongside and attracting seed dispersals and pollinators with their vivid color spectrum. For humans, anthocyanins are highly beneficial due to their use as pigments and nutraceuticals (e.g., antioxidants) in the food and pharmaceutical industries [1,2,3].
In this study, the structures of anthocyanins in blueberry, raspberry, and red grape were characterized by using ultra-high-performance liquid chromatography coupled to Quadrupole Time-of-Flight (UHPLC/QTOF) MS and performing MS/MS fragmentation experiments. Two grapevine PIWI cultivars tolerant to main fungal diseases (Cabernet Cortis and Cabernet Volos), two blueberry cultivars (Titan *Vaccinium virgatum* and Biloxi *V. corymbosum*), and two raspberry cultivars (Amira and experimental cross Li28, both *Rubus idaeus*) were studied. Berries were crushed using liquid nitrogen, the power was extracted using methanol, and extracts were analysed by positive ionization mode. Anthocyanin profiles of these resistant grape varieties are characterized by the presence of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv) 3-*O*-monoglucoside and 3,5-*O*-diglucoside derivatives, and of their acetyl and *p*-coumaroyl glucoside derivatives. A total of 21 structures of anthocyanins were identified in both grape varieties.
In blueberry, monoglucoside and pentoside derivatives of Cy, Dp, Pt, Pn, Mv, and Cy succinyl-monoglucoside were found. Biloxi berry also had several acetyl- and *p*-coumaroyl-glucoside derivatives absent in Titan. Raspberry samples resulted quantitatively and qualitatively poorer in anthocyanins and characterized by sophoroside and glucorutinoside derivatives just of the simpler aglycones, such as pelargonidin (Pg) and Cy [3,4].
The findings of the study implement the HR-MS/MS dataset useful for the characterization of natural anthocyanins and increase the knowledge of these fruit varieties as sources of nutraceuticals and natural colorants.

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P15

LABEL-FREE prmPASEF PROTEOMICS DATA ANALYSIS WORKFLOW SELECTION – BENCHMARKING OF AI-BASED AND DATA-DRIVEN APPROACHES

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As the prevalence of deep global proteomic analyses widens, the need for accurate and reliable label-free targeted proteomics becomes more pressing. Although methods used in conjunction with stable isotope-labeled (SIL) peptides already exist, little attention is given to the more accessible label free approaches and their data analysis. Today, ample confidence in the PRM analysis can be achieved even without SIL peptides due to modern advances in the MS systems such as the dimensionality expansion of the PASEF technology, or the availability of additional data from AI platforms such as Koina. Thus, the aim of this work was to develop and benchmark selected data analysis approaches for label-free prmPASEF with the data-driven and AI systems in mind.

Benchmarking dataset was prepared using a constant human proteome background (30 proteins measured) and a spike-in of three yeast proteins (ENO1, HXK2 and ADH1) varying in their concentrations. After in-solution digestion, a label-free prmPASEF analysis was performed using the Dionex UltiMate 3000 RSLC coupled to a Bruker timsTOF Pro2 system. Results were imported into Skyline-daily v.24.1.1.398 and manually checked for integrity. Separate transition areas were then exported into the R environment v.4.4.3 for subsequent analysis.

Calibration curve was plotted for each of the spiked-in proteins and an LOD and LOQ were calculated using the blank sample approach. Moreover, the limit of linearity was determined by piecewise linear approximation. Only the samples with spiked-in proteins which fit in the linear range were used for further analysis. Assuming the ratio of signals between peptide fragments is constant, data-driven and AI-based approaches were proposed. As such, missing data imputation (MDI) strategies were investigated where no MDI, k-nearest neighbors, data-driven and AI based methods were compared. Data consolidation and statistical testing methods (i.e., mathematical summing, best peak selection or AI scaling with a univariate statistical testing; p-value integration or multivariate testing) have also been developed and benchmarked.

In conclusion, this study shows that a data analysis workflow selection is a crucial part of the proteomic analysis pipeline. Each of the methods proposed can be characterized by several advantages and disadvantages, with varying complexity and performance.

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P16

INFLUENCE OF THE NORMALIZATION EFFECT ON THE RELATIONSHIP BETWEEN SAMPLE SIZE, EXTRACT CONCENTRATION AND PICK AREA: A NOT-TARGETED RELATIVE GC MS/MS STUDY OF BY2 SUSEPENSION CELLS METABOLOME

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In this study we examined the effect of sample size and extract concentration (dilution) on the final result of non-targeted GC/MS metabolomic study of BY2 suspension cells performed using various normalization approaches.

To address the above issues, we decided to experimentally check the influence of frequently used, user-available, and user-friendly normalization techniques on the effectiveness of analysis of plant samples varied in size. We were particularly interested in evaluating which data normalization technique would best reflect the defined differences in concentrations of the particular compounds introduced by varied sample sizes and extract dilutions. The artificially introduced difference in metabolite concentrations enables us to analyze the impact of sample preparation and, among others, the normalization technique on the final result of GC/MS data. According to our hypothesis, the final results will be massively influenced by the applied normalization approach, and, most importantly, this influence will be different for particular groups of compounds, i.e., carbohydrates, lipids, or organic acids.

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P17

TAYLOR-ARIS DISPERSION ASSISTED MASS SPECTROMETRY (TADA-MS): DIRECT INJECTION ANALYSIS OF PROTEINS WITH HIGH MATRIX CONTENT

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Electrospray ionization mass spectrometry (ESI-MS) is an important technique in protein analysis. Although direct infusion is the fastest and most straightforward sample introduction method, numerous matrix components can cause significant interferences resulting in low-quality spectra or no information at all.

Taylor-Aris dispersion occurs when a sample plug travels slowly through a small inner diameter capillary, leading to symmetrical band broadening due to the radial diffusion of analytes across the pressure-driven parabolic velocity profile [1]. The extent of dispersion is greater in case of high molecular weight components with low diffusion coefficients, resulting in broader peaks for proteins and narrow peaks in case of low molecular weight matrix components. Consequently, a matrix-free zone containing only the protein forms in the front and rear portions of the sample plug, allowing ESI-MS measurements to provide clean spectra, without the need for conventional separation techniques [2].

Taylor-Aris Dispersion Assisted Mass Spectrometry can be practical in the analysis of native proteins and protein complexes, as it provides a fast, partial buffer exchange, therefore samples can be present in any matrix (eg. PBS) and transferred to ammonium acetate just seconds before ionization [3].

The applicability of this method was first demonstrated in a CE-MS system, as the standard dimensions of commercial CE instruments are just in the optimal region for Taylor dispersion. However, a syringe pump or LC system can be just as easily employed [4].

Establishing the optimal operating conditions for these analyses can be challenging, as Taylor-Aris dispersion and sample ionization are influenced by numerous factors. We found that the expected taylograms under different operating conditions can be well described mathematically. This enabled the in-silico investigation into the effects of operating conditions for Taylor-Aris Dispersion Assisted Mass Spectrometry [5].

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P18

HIGH-PERFORMANCE ANALYTICAL METHOD FOR PRECISE EVALUATION OF MEMBRANE TREATMENT PERFOMANCE

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Water scarcity and drought, increasingly frequent and widespread due to climate change, pose a significant global challenge. Addressing acute water shortages requires urgent and sustainable solutions, with water reuse playing a crucial role. This project explores the treatment and reuse of various water sources through membrane separation processes. To ensure water safety for both drinking and irrigation, a fast UHPLC-MS/MS (Waters Aquity H-class ULPC coupled Xevo TQS micro) method was developed for the simultaneous quantification of target microcontaminants, including four pesticides (atrazine, imidacloprid, metolachlor, and tebuconazole) and their five metabolites (desethyl atrazine, desisopropyl atrazine, desnitro imidacloprid, metolachlor ESA, and metolachlor OA) following membrane treatment. The average limits of quantitation were below 1 µg/L without sample enrichment. For trace quantification (ng/L -low µg/L) of the target analytes, a solid-phase extraction (SPE) and microscale solid phase extraction (MSPE) method was developed and optimized using Oasis HLB cartridges (Waters) and HLB solid phases (Chromservice), respectively, achieving average recoveries above 70 % for all target compounds, except metolachlor and desnitro imidacloprid. The developed LC-MS/MS method was applied in pilot membrane studies to quantify pesticides in different matrices and assess membrane removal efficiencies, providing precise evaluation of membrane performance.

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P19

MALDI MSI analysis of changes in kidneys' lipids after morphine administration

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The kidneys play a vital role in maintaining homeostasis in the organism. Their primary function is connected with blood filtration and getting rid of waste, such as toxins and drug metabolites. They are essential for regulating blood pressure via neuronal and physical mechanisms and maintaining fluid and electrolyte balance.

Opioids are used to case the physical pain as well as to create the feeling of euphoria. They influence kidneys as active substances that influence the central nervous system and as xenobiotics that have to be safely excreted from the body. One of the most important functions of the kidneys is a urine output mechanism, which may be affected by opioids. By many hormonal and neuronal mechanisms within the periphery, kidneys and central nervous system may cause changes in urine and sodium deletion. Opioids also have an influence on sodium reabsorption in renal tubules and on the response of the kidney to acute injury.

In our study, we decided to have a closer look at the molecular changes caused by morphine administration in the kidneys' tissue. MALDI mass spectrometry imaging is a technique that allows the comparison of control and experimental tissues and finds changes in localization or quantity of all the substances that are able to ionize under certain circumstances (e.g., positive or negative ion mode and MALDI matrix). Such analysis is very useful, especially when we are looking for changes in lipids.

In our experiment, male rats were administered morphine subcutaneously in a dose of 10 mg/kg once a day for 10 days. The five morphine and five control rat kidneys were cut using cryostat in 12µm thickness and then put on InteliSlide. In our research, we used DHB as a matrix. SunCollect® system – wet-interface technique – was used for its deposition. MALDI- MSI analysis was performed in the positive mode using an Ultraflex Mass Spectrometer. The analysis of changes after morphine administration was made for the medulla and cortex. PLS analysis shows the most important lipid peaks differentiating morphine from control samples: m/z 796.4 and 824.4 for medulla and m/z 616.2, 741.4, 796.4, 820.6 for kidney's cortex.

Our research shows the impact of morphine administration on the lipids profile in the kidney's medulla and cortex, which could be responsible for its toxicity. Such analysis may, in the future, help find a way to prevent this effect, especially, when we consider using morphine as a pain cessation agent.

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P20

PROTEOMIC DIFFERENCES IN RAT'S SPINAL CORD UNDER THE INFLUENCE OF MORPHINE: A TIME-DEPENDENT INVESTIGATIONS.

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Drug addiction is a serious global problem. In particular, the abuse of morphine and other opioids (such as heroin, fentanyl, desomorphin) has been rising dynamically in recent years [1]. Research aimed at uncovering the molecular mechanisms underlying addiction has primarily focused on the central nervous system (CNS), which undeniably plays a key role in modifying human behavior. However, the spinal cord, as a major signal-conducting structure between the CNS and peripheral tissues, should not be overlooked—especially given its high density of opioid receptors localized on neurons within the dorsal horn [2].

Moreover, our preliminary, unpublished data indicate that ethanol exposure during adolescence significantly impacts the effectiveness of pain-relieving drugs in adulthood. This delayed interaction effect could be explained by long-term or even permanent changes in the levels of certain proteins within the CNS or spinal cord proteomes. The observed proteomic differences in the spinal cord between naive and morphine-treated animals may provide valuable insights for predicting physiological responses to chronic pain treatment, assessing the safety of therapeutic strategies, and designing novel treatments for opioid addicted that mitigate peripheral effects of opioid withdrawal.

In our experiments, to comply with the 3R rule in animal research, we utilized spare tissues from lipidomic profiling of the rat CNS. The rats were treated with morphine hydrochloride solution at a standard dose of 10 mg/kg i.p. once daily for 5, 10, and 15 days. After tissue isolation samples were stored in -80C until homogenized in 4% SDS to extract membrane-associated proteins. Before precipitation, protein concentrations were equalized, followed by reduction and alkylation. Peptide mixtures were generated via overnight trypsin digestion. For analysis, a data-dependent acquisition (DDA) spectral library was created using peptide mixtures from all samples, separated on a Gemini C18 (1 mm × 100 mm) column at pH 8.0. All DDA and data-independent acquisition (DIA) analyses were performed using an Ultimate 3000 chromatograph coupled to the Exploris 240 mass spectrometer. Additionally, we compared the correlation of results between the DIA spectral library-based approach and the direct-DIA method.

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P21

LC-MS/MS-BASED APPROACH FOR CONFIRMING THE AUTHENTICITY OF SELECTED PERFUMES

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Perfumes are widely available and commonly used cosmetics. Products from well-known and reputable brands enjoy high popularity; however, due to the presence of valuable ingredients in their formulations, they are often expensive. Consequently, recent years have seen a significant rise in the availability of counterfeit perfumes. These imitations differ from the originals in terms of composition, quality, and longevity. The lack of rigorous quality control in counterfeit products causes potential health risks to consumers, who may be exposed to toxic and prohibited substances. Moreover, the expansion of counterfeit perfumes results in financial losses for legitimate manufacturers. Therefore, the development of analytical techniques for the rapid and reliable identification of counterfeits has become essential [1,2]. This study aimed to establish a simple and a cost-effective methodology for verifying the authenticity of perfumes using LC-MS/MS methodology. The examined samples included original products from well-known brands as well as their lower-cost substitutes.

Methodology of sample preparation was as simple as possible: each perfume sample was diluted 100-fold in a 4 % ACN+ 0.1 % HCOOH aqueous solution. Analyses were performed using Ultimate 3000 LC connected *on-line* to AmaZon SL MS equipped with nanoFlow ESI ion source. Separation was carried out using system consisting of two Acclaim PepMap C18 columns: trap cartridge (5mm x 1mm) and 75 μm ID x 150 mm capillary column. Injection volume was 1 μl. The mobile phase consisted of solvents: A = H_2O+0.1 % HCOOH and B = ACN + 0.1 % HCOOH, with a gradient: 10-90 % B over 45 min, a flow rate of 300 nl/min, scan range 100-600 m/z. To assess repeatability, at least four replicates were performed.

Considering results: at each comparison level, it was possible to clearly differentiate between original product and its counterfeit: EIC (level 1), MS spectra (level 2), fragmentation spectra (level 3). Proposed methodology can be enhanced by shortening analysis times or design simple algorithms helpful for comparison. This approach serves as a viable alternative to GC-MS, which has been commonly used to date, as it allows for the analysis of thermolabile compounds while covering a broader mass range.

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P22

PLS ANALYSIS IN THE INTERPRETATION OF MASS SPECTROMETRY IMAGING DATA. ANALYSIS OF KIDNEY TISSUE AFTER MORPHINE ADMINISTRATION

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PLS is a widely used technique in the spectrometric field owing its success to properly handle a large number of correlated variables [1]. PLS is based on Principal Component Analysis (PCA), but it is a discriminatory technique, where and at the beginning, we have to define the number of classes. This class assignment - in our case the data from morphine and control tissues - "guides" the search for new directions. In this method, the more new directions (Latent variables—lv.) we include in the model, the better results we will have, as a Y variance increases. Nevertheless, we have to be careful to overfit the model since it will be taking more and more "noise" into consideration. To find the proper lv value for the model a Cross-Validation (CV) approach is used. Thus, the minimum CV error indicates the highest lv that we could consider and does not overfit the model, which may lead to lowering its prediction ability.

During our study, we were looking for the best parameters to indicate certain m/z values that are important for discrimination between morphine and the control group (tissue: kidney, cortex). For all the samples, we tested two m/z ranges: more general 400 – 1800 m/z and narrower, 600 – 1000 m/z focused on lipids. For the kidney cortex, the narrower range of m/z was chosen (600 – 1000 m/z) since it offered a model with higher PLS Y variance. LV=3 model allowed faster separation for two classes reaching a variance of 85.85 % and a model error of around 0.3. It was better in comparison with the broader range (400 – 1800 m/z) where the next lv=4 was indicated as the best. For a certain lv we may indicate the m/z values that discriminate between two classes.

Additionally, PLS analysis allows the order of the data based on their similarity. It means that we may take the spectra characteristic for cortex, medulla and inner layer and test different possibilities for their arrangement. For example, in the first step, we may assume that the spectra characteristic for the cortex is between the medulla and the inner layer, then we may assume that the spectra for the inner layer are between the cortex and the medulla, or the spectra for the medulla are between the cortex and the inner layer. For each permutation we are able to create a model and look for the one with the highest PLS Y variance. Both for control and morphine tissues, the situation when we have inner layer between the cortex and medulla creates the best model with lv=3. For control tissues, we have a variance of 93.9 % and a model error of around 0.048, and for morphine tissues, we have PLS variance of 96.23 % and model error of around 0.034. Values common for control and morphine tissues are cortex 772, 844, medulla 782, 804 and 832. Our data demonstrate the utility of PLS analysis in MALDI MSI measurements.

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P23

ACORN FLOUR EXTRACTS: STUDY OF NUTRITIONAL AND CHEMICAL PROFILE AND BIOAVAILABILITY OF POLYPHENOLS THROUGH CACO-2 CELLS

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In the last decade, the revalorization of alternative foods is continuously increasing to promote the circular economy and to find alternative ingredients to be incorporated in the human diet. In this context, acorn, especially as flour, represents an interesting candidate thanks to its numerous properties. First of all, it has a great nutritional value, as it is rich in unsaturated fatty acids, macro and microelements, vitamins and antioxidants [1,2]. Moreover, acorn flour is gluten-free so it can be used by people suffering from celiac disease [3-5].

The aim of this work was to study the nutritional and chemical composition of acorn flour, especially in terms of polyphenolic content. In addition, the bioavailability of polyphenols of acorn flour extracts was studied through Caco-2 cells.

The nutritional analysis was carried out following the procedures of AOAC [6]; the quantitative analysis of 36 polyphenols was determined through HPLC-MS/MS [7]; a MTT assay determined the viability of Caco-2 cells exposed to acorn flour extracts.

The nutritional analysis confirmed the previous results found in literature. In fact, acorn flour revealed a great nutritional profile, mainly for the mineral content (mostly potassium and calcium) and the fatty acids composition (44.92% of oleic acid, 36.51% of linoleic acid and 14.09% of palmitic acid). Results from the analysis of 36 phenolic compounds revealed gallic and ellagic acids as the main polyphenols in acorn flour with a concentration of 135.88 mg·kg- 1 and 539.56 mg·kg-1, respectively. Detection was performed in the dynamic-multiple reaction monitoring (dynamic-MRM) mode, and the dynamic-MRM peak areas were integrated for quantification. The most abundant product ion was used for quantitation, and the others for qualification. Finally, acorn aqueous extracts were prepared from acorn flour at four different temperatures (20, 60, 80 and 100 °C) to study the bioavailability of polyphenols using Caco-2 cells in trans-well plates for 2, 3 and 4h. Previously viability on Caco-2 cells of aqueous acorn extracts were tested through MTT assay. Results demonstrated that bioavailability was higher after 2 hours of exposure for all acorn extracts (Figure 1) and that the extract at 60 °C had the highest bioavailability value, mainly due to gallic and ellagic acids; on the other hand, the extract at 20 °C had the highest bioavailability of hyperoside, while in the extract at 100°C, 4- hydroxybenzoic acid resulted to be the most bioavailable (Figure 2).

In conclusion, this study demonstrated the high value of acorn flour thanks to its nutritional properties and bioactive compounds; moreover, the high bioavailability of gallic and ellagic acids in the extract at 60 °C after 2 hours of exposure makes the acorn flour interesting also as possible nutraceutical.

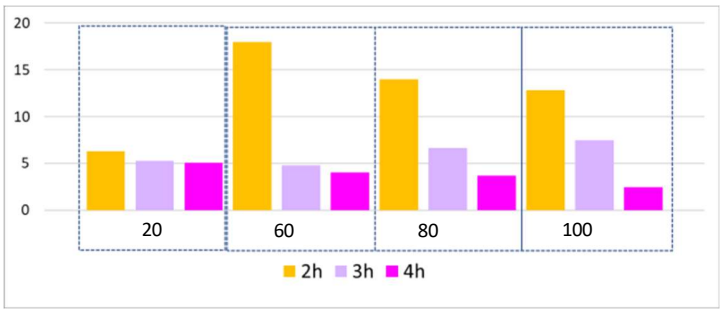

Figure 1. Bioavailability Polyphenols (%) after 2-3-4 hours of treatment.

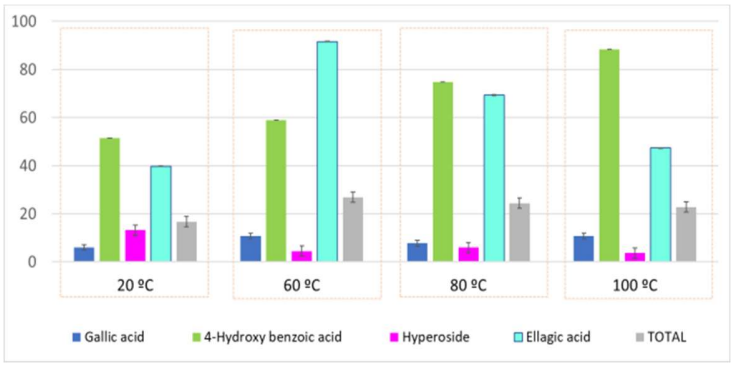

Figure 2. Bioavailability Polyphenols (%) in extracts at 20-60-80-100 °C.

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P24

HUMAN CORNEA CULTURE: AN UNTARGETED METABOLOMICS APPROACH TO STUDY MEDIUM CHANGES BY UHPLC-QTOF

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Human cornea culture is one of the main adopted approaches to preserve and storage human donor corneas from the harvesting to transplantation. During this process, corneal tissue is maintained at certain temperature (28−37 °C) into an organ culture media to a maximum of 4 weeks with the aim to mimic the original environmental conditions of the eye [1]. As yet, researchers have studied the impact of storage media on the biochemical properties of cornea during human cornea culture by directly manipulating and destroy corneal tissues. The present research proposes, for the first time, the study of medium changes during human cornea culture without handling and damaging the donor cornea still suitable for transplantation. Therefore, the objective of this work was to develop an untargeted UHPLC-QTOF analytical method coupled to a bioinformatics approach for studying the medium changes during human cornea culture which can be useful for discovering biomarkers capable of predicting tissue health status.

The metabolomics investigation has been performed in TISSUE-C (AL.CHI.MI.A. SRL, a medium intended for corneal storage at 31 °C) with and without a donor cornea preserved for 20 days. Data were acquired in SCAN mode and in Auto MS/MS mode (data depedent acquisition, DDA) and data matrices were obtained by MS-DIAL and processed and elaborated by Excel and MetaboAnalyst.
A total of 173 metabolites belonging to diverse chemical classes, for instance, amino acids, dipeptides, lipids, vitamins, sugars, and carboxylic acids, were found in medium conserved with or without cornea. This preliminary study suggested some differences in metabolites abudance produced by conservation time but the main significant results were achieved when the cornea were introduced in the system, especially after 10 and 20 days. The metabolic pathway analysis (MetPA) resulted in 36 differentially expressed pathways (Figure 1) belonging to amino acids routes, vitamins pathways (especially B vitamins), energetic routes, lipid metabolism, oxidative pathways, etc [2]. Once again these data confirmed the marked impact of the investigated tissue in the medium constituents during conservation time and it is hoped that the current approach can be exploited in future works by incresing the number of samples for discovering biomarkers related to the health status of corneal tissue without handling and destroying it.

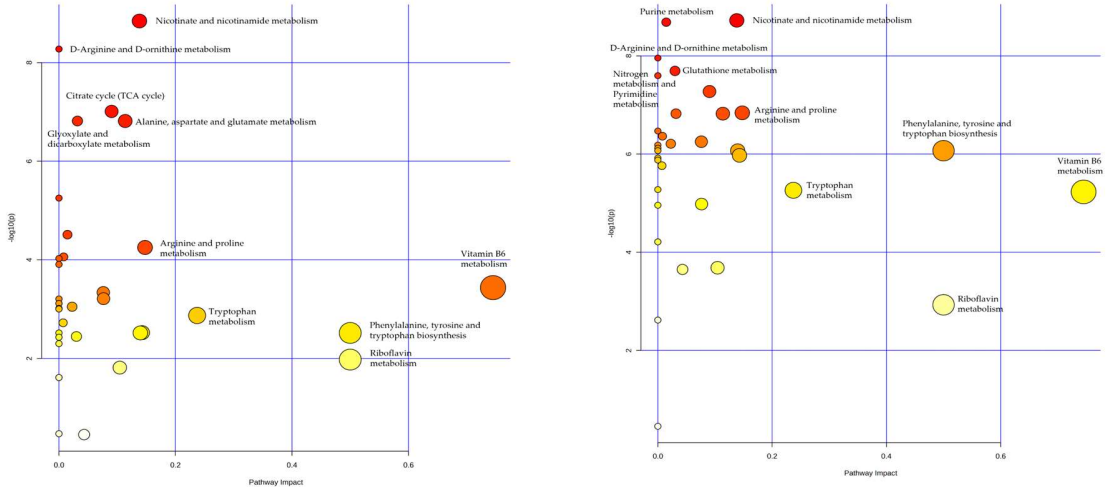

Figure 1. Overview of metabolic pathway analysis performed on annotated metabolites at 10 days (on the left) and 20 days (on the right) of conservation times, comparing medium with and without cornea.

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P25

CHEMOMETRIC CLASSIFICATION OF TEA LEAVES USING CHEMICAL INGREDIENT PROFILING

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Tea (Camellia sinensis) is one of the world's most cherished beverages, celebrated for its diverse processing methods that generate unique chemical fingerprints. Variations in oxidation, fermentation, and other post-harvest treatments result in distinct profiles of bioactive compounds. This chemical diversity not only influences flavor and aroma but also underpins the potential health benefits attributed to different tea types. Considering these factors, establishing rapid and reliable analytical methods for tea classification is of considerable interest to both industry and research communities [1].

In this study, we applied liquid chromatography–mass spectrometry (LC-MS) combined with principal component analysis (PCA) to systematically differentiate tea leaves based on their chemical ingredient profiles. Our analysis focused on quantifying key phenolic compounds: green teas were found to be enriched in non-oxidized catechins (e.g., epigallocatechin gallate), black teas showed higher levels of oxidation-derived compounds such as theaflavins, and pu-erh teas exhibited markers indicative of fermentation processes. The chemometric approach allowed us to reduce complex datasets into discernible clusters corresponding to each tea type, demonstrating the efficacy of this method for rapid product differentiation and quality control [2].

Beyond classification, our findings offer insights into the influence of regional cultivation practices and specific processing protocols on tea quality. The integration of analytical techniques provides a robust platform for authentication and quality assurance in the tea industry. Looking ahead, future research will focus on incorporating additional analytical modalities, such as metabolomics and proteomics, to further correlate chemical profiles with sensory attributes and health benefits, thereby enhancing product development and consumer confidence [3].

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P26

QUANTITATIVE ANALYSIS OF LACTOYLGLUTATHIONE: A NEW 2D-HPLC-MS/MS APPROACH WITHOUT ION-PAIR AGENTS

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Lysine lactylation (KLa) is a recently identified post-translational modification (PTM) with significant implications in cancer. KLa occurs on both histone and non-histone proteins and may involve enzymatic or non-enzymatic mechanisms, utilizing Lactyl-CoA and Lacotylglutathione (LGSH) as donor of the lactyl moiety, respectively [1-2]. The specific regulatory enzymes that mediate enzymatic lactylation remain still unclear, although acetyl-synthetase (ACSS2), acetyl-transferases (p300), and deacetylases (HDAC1-3) have also been proposed as potential mediators for histone lactylation [3-5]. The glyoxalase 1 (GLO1) pathway involved in LGSH generation is instead engaged in the non-enzymatic lactylation strategy. Interestingly, the lactylation of histone proteins provides additional evidence, linking tumor metabolic reprogramming to epigenetic regulation and transcriptional changes. In prostate cancer (PCa), lactate fuels mitochondrial metabolism and promotes epigenetic changes, primarily through histone acetylation [6]. However, the role of histone lactylation in PCa remains largely unexplored. Published method for the determination of LGSH use an high performance liquid chromatography coupled to tandem mass spectrometry (HLPC-MS/MS) system with the support of an ion coupling agent [7] which have a positive effect on the retention and chromatographic separation of polar and water-soluble analytes such as LGSH, but strongly negatively affect the detection sensitivity in mass spectrometry. The aim of this work was to develop a high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) method for the determination of LGSH in PCa cells extracts, without the support of the ion-pair agents. In order to analyze the minimal variations in concentration of LGSH, we used a two-dimensional (2D) HPLC system coupled to a LTQ-Orbitrap, equipped with an ESI source. The development of the method involved both the optimization of the chromatographic system and the MS/MS acquisition. The use of 2D chromatography allowed to eliminate most of the polar molecules, during the loading step of injected sample, and to minimize the ion suppression effect on the analyte detection. MS/MS studies were conducted to select the best fragmentation conditions (collision energy and activation time). Furthermore, the selected product ions were analyzed and characterized in high resolution mass spectrometry (HRMS at 30k FWHM) in order

to guarantee the selectivity of the method. LGSH was quantified in sample extracts of lactate-treated and untreated PCa cells. The obtained results show that lactate exposure in PCa cells significantly increased LGSH levels.

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P27

TARGETED MS-BASED QUANTITATIVE ASSAY FOR ASSESSMENT OF MELANOMA PROGRESSION

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This project investigates the role melanoma cell derived exosomes (MTEX) play in modifying the tumor microenvironment (TME), promoting melanoma progression, and predicting response to immunotherapy. Melanoma cells use MTEX to communicate with and alter functions of other cells in the TME. MTEX captured from patients' plasma can serve as a liquid tumor biopsy. When the proteome of MTEX was studied by mass spectrometry in data-dependent mode (DDA), several highly overexpressed proteins were identified which subdivided melanoma patients (n=15) into those with progressive disease (PD) and those with no evidence of disease (NED). Among them, ALIX had the greatest discriminating power and emerged as a potential biomarker of melanoma progression after oncological therapy. To confirm this, ALIX and the associated overexpressed proteins were evaluated as potential biomarkers of melanoma progression in an independent larger cohort (n=37) of patients by targeted mass spectrometry-based proteomics.

Exosomes (sEV) were isolated from plasma and separated into three groups: unseparated, MTEX and Non-MTEX. Filter Aided Sample Preparation (FASP) method was used for sample preparation. A system comprising of a nano-liquid chromatograph UltiMate 3000 coupled with a high-resolution quadrupole-Orbitrap mass spectrometer was utilized for development of a parallel reaction monitoring-based targeted assay. The list of peptides representing the proteins to be quantified was based on the previously obtained DDA data. The open source Skyline software was employed to generate an inclusion list.

The hypothesis was tested that ALIX protein carried by MTEX serves as a biomarker of disease progression and response to therapy. The patients were divided into PD and NED groups based on clinical evaluation at the time of blood draw. The targeted proteins abundance was compared in both groups. ALIX expression levels did not discriminate the two groups in the Principal Component Analysis, and ALIX abundance in MTEX varied broadly among patients. In contrast, the abundance of four other proteins, CD81, contactin-1 (CNTN1), moesin (MSN) and ubiquitin-ribosomal protein eL40 fusion

protein (UBA52) in MTEX differentiated patients with PD from those with NED. Further bioinformatics analysis is in progress to examine the poor discriminating ability of ALIX relative to that of the four other MTEX proteins. The data normalization as well as clinical criteria used for assignments of patients as PD vs NED are under scrutiny.

The newly developed targeted proteomics assay was successfully used to identify a group of 4 proteins in MTEX that differentiated melanoma patients with PD from those with NED, suggesting that the assay has a potential to study plasma exosomes in search for cancer biomarkers.

Acknowledgments

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P28

T CELL-DERIVED SMALL EXTRACELLULAR VESICLES FROM PLASMA OF MELANOMA PATIENTS AND HEALTHY DONORS – PROTEOMIC STUDY

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Small extracellular vesicles (sEV) released by T cells play important role in immune regulation. The thorough analysis of proteome of T cell-derived small extracellular vesicles in human plasma involves separating them from a diverse combination of circulating vesicles using anti-CD3 antibody immune capture. Here, we compared proteomic profiles of T cell-derived CD3(+) sEV from the plasma of melanoma patients (MPs) and healthy donors (HDs). The reason for investigating CD3(+) sEV in the plasma of melanoma patients is supported by existing preliminary data and the developing perspective that these sEVs, reflecting the protein content of T cells, could act as a "liquid T cell biopsy"[1].

Blood samples were obtained from 10 HDs and 10 MPs (IRB approval #04-001). The thawed and pre-cleared plasma was processed by ultrafiltration, followed by size exclusion chromatography (SEC). Vesicles recovered in fraction #4 were characterized as sEVs according to the ISEV criteria [2[. The T cell-derived sEV (CD3(+)sEV) were separated from the non-T cell-derived sEV (CD3(−)sEV) by immune capture with anti-CD3 mAbs. The separated sEV fractions were prepared for proteomic analysis using a modified FASP protocol [3]. LC-MS/MS analysis of tryptic peptides were performed with the use of the Dionex UltiMate 3000 RSLC nanoLC System in conjunction with the Q Exactive Plus Orbitrap MS.

294 proteins were found in CD3(+) sEV, with 226 also present in T cells, suggesting that the protein composition of CD3(+) sEV closely mirrors that of T lymphocytes. In the analysis of pathways enrichment, proteins found in CD3(+) sEV were linked to vesicle localization and the immune system. The differentiation of the MP and HD cohorts was attributed to the presence of 66 proteins found in CD3(+) sEVs. The DEPs (differentially expressed proteins) were related to functions associated with cancer. Additionally, 41 DEPs that were increased in MPs were linked to signalling pathways that rely on Rho GTPases, cytokines, and MAPK family kinases. As the BRAF status was known in 5 MP donors,

we were able to search for a possible link between the level of these proteins and the BRAF status in sEV of MPs. Although the data are preliminary, the abundance of ITGB3 and YWHAB, two sEV proteins associated with BRAF-related pathways, correlated with the BRAF mutation status in two MPs.

The proteomic profiles of CD3(+) sEV were distinct in MPs from those in HDs and recapitulated the proteome of tumor-responding T cells, potentially serving as a "liquid T cell biopsy".

Acknowledgments
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P29

PROTEOMIC ANALYSIS ON SHORT MONOLITHIC COLUMNS

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We present an application of short monolithic columns based on polyhedral oligomeric silsesquioxane methacrylate and stearyl methacrylate [1] or zwitterionic sulfobetaine [2] monomers for the gradient separation of peptides. The HPLC analyses of peptides originated from tryptic digests of bovine serum albumin and ovalbumin were performed on a portable chromatographic system assembled in our laboratory and connected to ESI-MS instrument.
The monolithic capillary columns (0.32 × 50 mm) were prepared in fused silica capillaries when the polymerization mixture was optimized to achieve high selectivity, efficiency, and favorable permeability. Then, the prepared columns were applied for the fast and effective gradient separation of peptides and peptide mixtures under RPLC or HILIC conditions. The number of detected peptides was comparable on both types of monolithic columns.

Acknowledgments
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P30

QUECHERS EXTRACTION AND A STRAIGHTFORWARD CLEAN-UP PROCEDURE FOR THE QUANTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN RIPENED CHEESE USING GC-MS/MS.

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Polycyclic Aromatic Hydrocarbons (PAHs) are chemicals made up of hydrogen and carbon atoms arranged in two or more fused aromatic rings. Over a hundred different PAH compounds exist, typically in complex mixtures. These compounds are known environmental carcinogens and can originate from both human activities and food processing. For non- smokers, the primary route of PAH exposure is through food consumption. Due to their lipophilic nature, PAHs can easily accumulate in fatty food matrices such as milk, meat, edible oils, fish, and cheese. This study aimed to develop an effective and straightforward sample preparation procedure for the determination of PAHs in ripened cheese samples.

For this study, 60 samples of non-smoked hard cheese were collected from the market. The optimized protocol for PAH quantification consisted of three steps: 1) a rapid QuEChERS extraction using cyclohexane as the solvent, 2) a quick and efficient mechanical clean-up with Silica Gel (70-200 mesh ASTM), and 3) concentration under a gentle stream of nitrogen. PAH analysis was performed using an Agilent Intuvo 9000 GC system coupled with an Agilent 7000 Series Triple Quadrupole MS, employing a HP-5MS Ultra Inert capillary column (30 m × 0.32 mm id × 0.25 μm film thickness). Chromatograms were acquired in selected-ion monitoring (SIM) mode, and target compounds were identified based on their ions and retention times. Eighteen different PAHs were considered for method development: naphthalene (NaP), 2- methylnaphthalene (MeNaP2), 1-methylnaphthalene (MeNaP1), acenaphthene (Ace), acenaphthylene (Acp), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (B[a]a), chrysene (Chr), benzo[b]fluoranthene (B[b]f), benzo[k]fluoranthene (B[k]f), benzo[a]pyrene (B[a]p), indeno[1,2,3-c,d]pyrene (I[cd]p), dibenzo[a,h]-anthracene (D[ah]a), and benzo[g,h,i]perylene (B[ghi]P).

Once validated for linearity, repeatability, reproducibility, recovery, limit of detection (LOD), and limit of quantification (LOQ), the method was applied to analyze the entire set of collected samples. In three cheese samples were identified and quantified the lower molecular weight PAHs: NaP, MeNaP2 and MeNaP1. However, the degree of contamination in these samples was very low, with the total concentration not higher than 30 μg/kg.

The experiments conducted in this study demonstrated that the proposed method effectively clean-up the sample while achieving acceptable recoveries, repeatability, and reproducibility for PAH quantification in cheese. Therefore, this method could be successfully applied to different food matrices with high fat content.

P31

IDENTIFICATION OF BACTERIA BY COMBINATION OF PREPARATIVE ISOELECTRIC FOCUSING AND MALDI-TOF MS

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Identification of microorganisms from a complicated matrix is required for diagnostic purposes in medicine, environmental studies and food industry. Many different conventional techniques, such as polymerase chain reaction, DNA-typing, specific antibodies, are used for characterization of variety of microorganisms. In recent years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a fast and reliable technique for identification of various microorganisms including pathogenic ones. Nevertheless, sample preparation, including pre-separation and pre-concentration techniques, is still a crucial step in the analysis of microbial samples.

The objective of this study is to demonstrate the ability of method combining preparative isoelectric focusing (IEF) in a cellulose-based separation medium with MALDI-TOF MS analysis to identify microorganisms. Three opportunistic human pathogens, two bacteria (*Micrococcus luteus* and *Dietzia* sp.) and one yeast (*Rhodotorula mucilaginosa*), were first separated and concentrated by preparative IEF. Zones of the separation medium with microbial cells were then collected with a spatula, the fractions were simply processed and the microorganisms were finally analyzed by MALDI-TOF MS.

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P32

MINIATURIZED CHROMATOGRAPHIC SYSTEM FOR PROTEOMIC ANALYSIS

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We introduce the miniaturized chromatographic system assembled and evaluated in our laboratory. It consists of a high-pressure syringe pump and a pair of low-pressure syringe pumps ensuring rapid and reproducible analyses; a high-pressure injector for accurate sample injection; a capillary column ensuring fast and efficient gradient separation and an CCD spectrometer-based optical detector [1,2]. Figure 1 shows the suggested system connected to ESI-MS instrument. The gradient separations of tryptic digests of proteins were achieved in time less than 6 minutes utilizing short monolithic capillary column. The proposed system provides comparable results to the benchtop analytical HPLC system if the same capillary column is used. Chromatographic system needs only less than 500 µl of mobile phase per analysis and regeneration of the column.

Figure 1. Miniaturized chromatographic system connected to ESI-MS instrument.

Acknowledgments
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P33

THE EXPRESSION OF E50K OPTINEURIN DOES NOT INDUCE EFFECTS ON UPS, BUT ALTERS NF-KB SIGNALING THROUGH TRAF2 UBIQUITYLATION

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The E50K optineurin (OPTN) mutation, in which the glutamic acid is replaced by a lysine residue, causes retinal degeneration and it is linked to familiar and juvenile cases of normal tension open-angle glaucoma and amyotrophic lateral sclerosis (ALS).

Herein, we sought to investigate how the E50K-OPTN affects global proteasome activity and Ub-signaling in a SH-SY5Y cell strain.

To this aim, cultivated cells were transfected with OPTN-GFP, E50K OPTN-GFP, or GFP vectors over 48h before cell harvesting (n=3).

Proteasome activity was measured by the kinetics of digestion of LLVY-amc in crude cell extracts and by native gel electrophoresis.

Ub signaling was investigated by monitoring the abundance of proteins ubiquitylated with K48 and K63 linkages by Western blotting and by diGLY proteomics (DIA, library-free search using DIA-NN).

The rate of LLVY-amc digestion and the uncapped/capped particles ratio (by native-gel) was comparable across all experimental conditions.

A slight drop of K48 and K63-labelled Ub-proteins was observed in E50K transfected cells as compared to the others experimental conditions, which were all comparable.

The diGLY approach showed that, as compared to control cells, overexpression of OPTN-E50K, OPTN-wt or GFP, did not affect the number of Ub-peptides identified and quantified (>6000 Ub-remnants, FDR<0.01). However, among 120 Ub-peptides exclusive of OPTN-E50K cells, the Ub-Lys27 of TRAF2 (TNF Receptor-associated factor 2) was identified.

This study suggests that, at least under the conditions tested, E50K has not a dominant effect on proteasome activity, but can alter the signaling of NF-kB through modulating TRAF2 ubiquitylation.

P34

SINGLET OXYGEN AS A PROBE FOR PROTEIN STRUCTURE ELUCIDATION

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Covalent labeling techniques combined with mass spectrometry (MS) provide valuable insights into protein structure, conformational dynamics, and the identification of regions involved in interactions. These footprinting approaches rely on labeling solvent-exposed protein sites with suitable chemical probes.

Singlet oxygen ($^1O_2$) is a reactive species that can interact with a variety of biomolecules, including proteins, making it an ideal candidate probe for stable, covalent protein labeling in MS-based approaches. In this work, we generate singlet oxygen under mild conditions using a photodynamic approach with phthalocyanine and a red laser. The resulting singlet oxygen reacts with solvent-accessible amino acid residues, particularly tyrosine, tryptophan, histidine, methionine, and cysteine. Under specific conditions, oxidation of phenylalanine was also detected. The technique demonstrated versatility, not being limited to a narrow range of conditions, although pH and buffer composition were found to influence the yield and types of reaction products. Our findings, tested on short peptides and proteins, demonstrate that singlet oxygen can be effectively utilized as a labeling probe in structural MS studies, providing a valuable addition to current technologies detecting solvent-accessible surface area of proteins.

P35

COMPARATIVE ANALYSIS OF CAROTENOID AND FAT-SOLUBLE VITAMIN CONTENT IN DAIRY PRODUCTS: A COMPARISON BETWEEN ALPINE AND INDUSTRIAL PRODUCTS

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Carotenoids and fat-soluble vitamins are essential bioactive compounds for humans, playing a key role in various processes necessary for maintaining health [1], except for vitamin D, which can be synthesized through UV exposure. Because dairy products are major sources of these micronutrients, especially the carotenoids and fat-soluble vitamins, the diet of cows whether they are grazers, is a key determinant of their levels [2]. Such compounds may represent valuable markers in mountain product traceability.

Simultaneous extraction and quantification of carotenoids and fat-soluble vitamins are particularly difficult owing to their differing polarity and solubility. It provided an extraction and analytical method for simultaneous analysis of these compounds in cheeses, butter, and creams. Subsequently, various products derived from mountain pastures (N=26) were compared with industrially produced ones (N=9).

The developed method enabled the quantification of lutein, β-carotene, retinol, α-tocopherol, β/γ-tocopherol, δ-tocopherol, vitamin K1, and vitamins D2 and D3 using high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) with an APCI source. The analysis revealed that mountain cheeses contained higher levels of α-tocopherol, β/γ-tocopherol, retinol, lutein, vitamin K1, and vitamin D2 (KW test). In butter samples, alpine products also showed higher concentrations of lutein, β-carotene, vitamins D2 and D3, and δ-tocopherol.

Overall, creams did not show significant differences except for lutein content; however, in general, the concentrations tended to be higher in mountain products.

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P36

A NEW APPROACH FOR STRUCTURAL BIOLOGY: CROSS-LINKER TARGETING AROMATIC AND CYSTEINE RESIDUES IN PROTEINS

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Chemical cross-linking coupled with mass spectrometry has become a rapidly evolving field of structural biology. Its ability to capture protein dynamics, protein-protein interactions and interaction networks combined with the advantages of mass spectrometry make it a versatile technique. Despite being widely used, most of the cross-linking experiments target amino acids containing primary amino groups due to their high reactivity, solvent accessibility and frequent occurrence. Cross-linking chemistry targeting other amino acids faces challenges, such as low reactivity of these residues, unwanted side-products, low abundance of targeted residues. As of today, there is no cross-linker connecting aromatic amino acids due to their intrinsic low reactivity. In this study, we present a new cross-linker based on the Togni reagents that can be utilized in two distinct ways. Both methods lead to generation of fluoroalkyl radical species that modify and connect the aromatic residues as well as cysteines. This new cross- linker can be activated by ascorbic acid in an FFAP (Fast FluoroAlkylation of Proteins) fashion developed in our laboratory or it can be activated by reaction with thiol groups of cysteine residues. To test the ability of the new reagent to cross-link aromatic residues, we modified the apo form of myoglobin to demonstrate the FFAP way of activation. RhoA protein was chosen to demonstrate the activation of the cross-linker through reaction with cysteine residues. Bottom-up approach was utilized to locate the cross-linked residues. The obtained results show that the presented cross-linker connects aromatic residues as well as cysteines in protein sequences and thus provides new structural information in the chemical cross- linking field.

Acknowledgments
This work was supported by the Czech Science Foundation (grant number 22-27695S and 25-18181S) and by the Ministry of Education, Youth and Sports of the Czech Republic grant for Talking Microbes – understanding microbial interactions within One Health framework (grant no. CZ.02.01.01/00/22_008/0004597).

P37

DIELECTRIC BARRIER DISCHARGE IONISATION MASS SPECTROMETRY (DBDI-MS): ION CHEMISTRY AND SENSITIVITIES FOR SELECTED VOCS

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Dielectric Barrier Discharge Ionization (DBDI) is a highly sensitive method for real-time mass spectrometric analysis of vaporised and gaseous samples [1]. In DBDI, a high voltage applied across electrodes separated by a dielectric material creates a non-thermal plasma, forming analyte ions. Understanding the ion chemistry involved is crucial, despite commercial use often relying on numerical data processing. Our research examines ionisation mechanisms in the presence of air constituents like water vapour, providing insights into product ion formation. This knowledge is essential for accurate mass spectral interpretation and responsible DBDI application.

Figure 1 shows the experimental arrangement used for this study. Gaseous samples containing variable concentrations of VOC vapours were introduced from a Nalophan bag into the DBDI source through a PEEK capillary at a known flow rate. The concentrations of the analytes in the bag were constantly monitored by SIFT-MS.

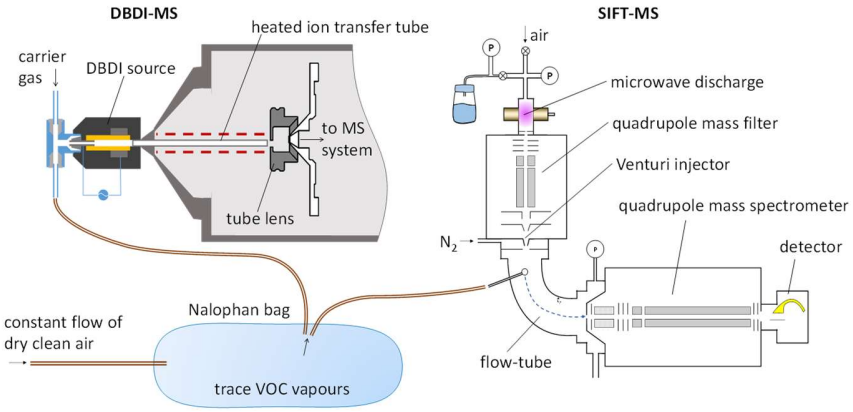

Figure 1. Parallel DBDI-MS and SIFT-MS experimental setup

Mass spectra were obtained for several ion transfer tube temperatures and different tube lens voltages. A heated ion transfer tube, to which the DBDI source is connected, enables the efficient transportation of ions generated in the ion source to the mass analyser, transitioning from near ambient pressure to vacuum. The temperature of the ion transfer tube modifies ion collisions with carrier gas molecules inside the tube, breaking up adducts or weakly bound clusters. The tube lens voltage influences the collisional energy in a low-pressure region. Increasing the tube lens voltage leads to reducing the intensity of higher mass product ions, such as the protonated hydrates $MH^+(H_2O)$ and the proton-bound dimer M_2H^+, while increasing the fraction of fragment ions (see Figure 2).

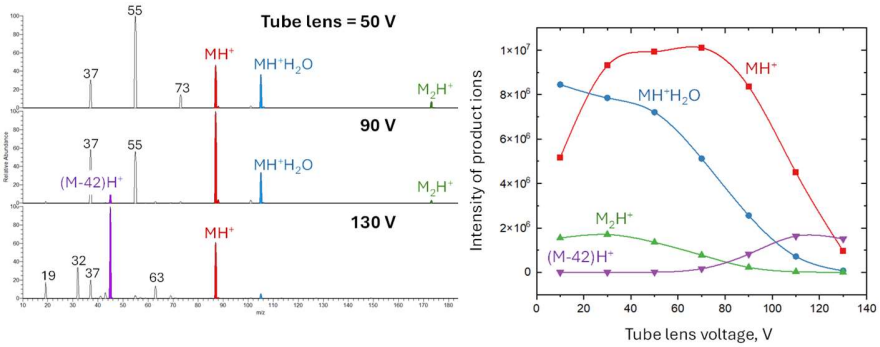

Figure 2. DBDI-MS spectra for 2-pentanone for 300°C ion transfer tube temperatures and three different tube lens voltages (left part), and the dependence of the main product ion signals on tube lens voltages.

The sensitivity of DBDI-MS was determined for various compounds, demonstrating variability and a tendency to increase with the analyte molecule size. The comparative results are shown in Figure 3 as plots of the DBDI-MS total product ion signals as functions of SIFT-MS measured absolute concentrations (in parts per billion by volume, ppbv).

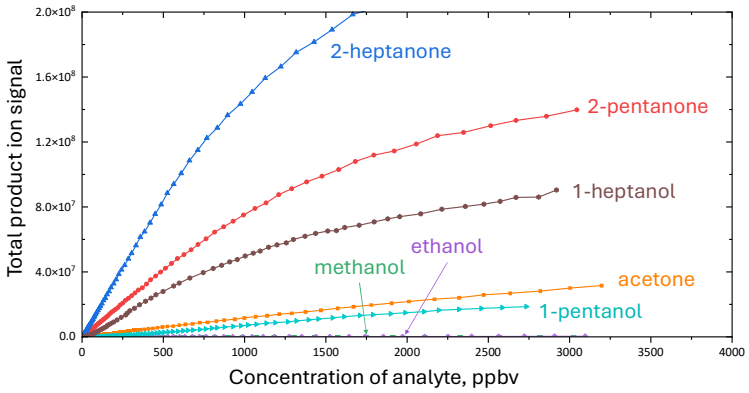

Figure 3. Dependences of DBDI-MS total product ion signal on the analyte concentrations.

In summary, the sensitivity of DBDI-MS varies significantly with the size of the analyte molecule. It is also influenced by the temperature of the ion transfer tube and the voltage applied to the tube lens. Understanding these factors is essential for accurate quantitation of trace amounts of VOC vapours.

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P38

POTENTIAL OF MALDI-TOF MS FOR DIFFERENTIATION BETWEEN MRSA AND MSSA STRAINS

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Staphylococcus aureus, commonly known as the golden staphylococcus, is a Gram-positive, spherical bacterium that frequently colonizes human skin and mucous membranes of approximately 30% of people. While it often exists harmlessly, it can become a versatile pathogen capable of causing a broad spectrum of infections, ranging from minor skin conditions to serious, potentially fatal diseases. *Staphylococcus aureus* represents the leading cause of nosocomial infections globally, possessing an exceptional ability to acquire resistance to commonly prescribed antibiotics. The prevalence of resistant strains has increased dramatically in recent years, with methicillin-resistant *Staphylococcus aureus* (MRSA) strains constituting a particularly serious worldwide health concern. The rapid and accurate differentiation between MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) strains is crucial for implementing appropriate and timely treatment protocols. The study explored the efficacy of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in distinguishing between methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains of Staphylococcus aureus. The present study investigated various sample preparation approaches to optimize MALDI-TOF MS for MRSA/MSSA differentiation:

- Intact cells
- Cell lysates
- "Washed pellets" (residue remaining after lysis)

Additionally, bacteria were cultivated with specific concentrations of various antibiotics to enhance differentiation capabilities.

The results obtained from this investigation are promising, suggesting that MALDI-TOF MS, when optimized with appropriate sample preparation techniques and analytical approaches, could effectively differentiate between MRSA and MSSA strains. This capability has significant implications for clinical microbiology laboratories, potentially enabling more rapid diagnosis and improved patient outcomes through targeted antimicrobial therapy.

Acknowledgments
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P39

EMPLOYING BALLISTIC GRADIENTS, VACUUM JACKETED COLUMNS AND BENCHTOP MULTI REFLECTING TIME-OF-FLIGHT (MRT) TO INCREASE LIPIDOMIC THROUGHPUT WHILST MAINTAINING HIGHLY CONFIDENT IDENTIFICATIONS

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Increasing patient cohort sizes is essential for identifying new disease biomarkers and enhancing omics studies. However, with thousands of samples, throughput becomes a limiting factor. Reducing separation time can compromise peak capacity and feature detection. Vacuum jacketed columns (VJC) significantly increase peak capacity and narrow peak widths, allowing faster chromatography without information loss or maintaining chromatography duration with increased peak capacity. Vacuum jacketed columns were applied to analyze the lipidome of healthy controls and cancer patients. Lipids from serum samples of healthy controls and patients with bladder, colon or kidney cancer were extracted using IPA and spiked with EquiSPLASH™ as an internal control. Lipids were separated on a 1mm diameter Phenyl-Hexyl column (either 100 or 50mm length), with corresponding VJC format columns also used. A standard 10-minute gradient was used with all columns before scaling down to sub-1 minute gradients. The eluate was directed towards a benchtop multi reflecting time-of-flight (MRT) mass spectrometer operating at 100Hz scan speed in data-independent mode. Data were analysed using both in-house and third-party informatic tools. Datasets were aligned, peak picked, and normalized using Lipostar informatics, and lipid identifications were obtained using the LipidMAPS™ database. Benchmarking with a conventional 2.1mm column and 10-minute gradient identified several biomarkers related to cell proliferation and signaling pathways, showing differences in relative abundances between healthy controls and cancer groups. Scaling down to 1mm and shorter columns maintained gradient time and identified a similar number of features, though increased flow rate and reduced gradient time decreased peak capacity and feature detection. However, major biomarkers were still observed, and samples were separable by abundance. Multivariate data analysis, via unsupervised principal component analysis (PCA), generated similar profiles regardless of the chromatographic method. Finally, using a 1mm vacuum jacketed column with a 10-minute gradient increased peak capacity and identified more features. The high scan speed of the benchtop multi reflecting time-of-flight (MRT) instrument allowed profiling of sub-1 second peaks without compromising mass accuracy or resolution. The use of VJCs with reduced gradient time in combination with MRT increased throughput by 30% while maintaining performance.

P40

ON THE NATURE OF REACTIVE MANGANESE-OXYGEN COMPLEXES IN THE CATALYTIC OXIDATIVE COUPLING OF ARYLLITHIUMS: A COMBINED MASS SPECTROMETRIC AND THEORETICAL APPROACH

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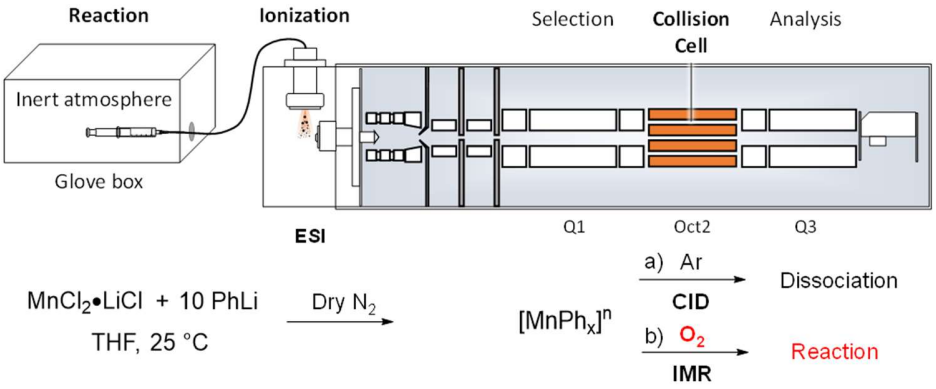
The oxidative coupling of organolithium by ligand-free $MnCl_2$ under air [1] has attracted much attention due to the demand for sustainable catalysts using abundant and non-toxic first row transition metal complexes and oxygen as green oxidant.[2] This coupling is a useful method to synthesize homo or heterocoupled products (biaryls, dialkynes or arylalkynes) and understanding its mechanism could provide essential information for the further development of catalysts and synthetic applications.

Electrospray ionization coupled to tandem mass spectrometry has proved to be a highly effective approach for identifying reaction intermediates.[3]

Therefore, we study the interaction of oxygen with manganese complexes that allowed C-C and C-O coupling reactions to occur using a triple quadrupole specially modified for ion-molecule reactions (IMR) coupled with electrospray ionization.

As the expected complexes are extremely sensitive to water and oxygen, a glove box was coupled to the spectrometer. After selecting the ions of interest with the first quadrupole (Q1), they are transferred inside the collision cell. Collision-induced dissociation (CID) can be achieved by introducing argon, or an ion molecule reaction (IMR) can be performed by introducing dioxygen. Product ions are analyzed by the second quadrupole (Q3) to provide MSMS spectra (Scheme 1).



Scheme 1. Instrumental setup allowing formation of $[MnPh_x]^n$ complexes in inert atmosphere and their subsequent analysis in a modified triple-quadruple instrument. a) collision on argon in the collision cell will lead

to collision induced dissociation (CID); b) collision on dioxygen in the collision cell will lead to ion molecule reaction (IMR)

In addition, solution phase studies using stoichiometric or sub-stoichiometric quantities of isotopically enriched $^{18}O_2$ released from an endoperoxide have been performed to corroborate the findings from the gas phase. Based on these results, a theoretical study was conducted to shine some light on the gas phase and solution processes. Taking into account the specificities of the condensed phase, DFT calculations have revealed a number of potential mechanistic scenarios for this oxidative C-C coupling reaction.[4]

Acknowledgments
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P41

COMBINED GC-MS AND GC-IRMS TO AUTHENTICATE HIGH VALUE NATURAL PRODUCTS

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GC-MS is a well-established technique that has been used in analytical laboratories for many decades, while GC-IRMS (Fig.1) is a more recent method widely applied in the field of food traceability. In this work, we present the application of these two techniques in combination to assess the authenticity of certain high-value food products. These methods were used to analyze a range of commercial samples with the aim of distinguishing between natural and synthetic origins. The approach involved the extraction and analysis of volatile organic compounds alongside the measurement of stable isotope ratios. VOCs, which are responsible for the characteristic aroma of a product, are often the main target of adulteration due to their sensory and economic relevance. By first establishing reference isotopic values from verified natural sources, it was then possible to assess the authenticity of various products and detect potential adulteration with synthetic components. The integrated use of GC-MS and GC-IRMS thus provided a robust analytical framework for verifying product origin and integrity.

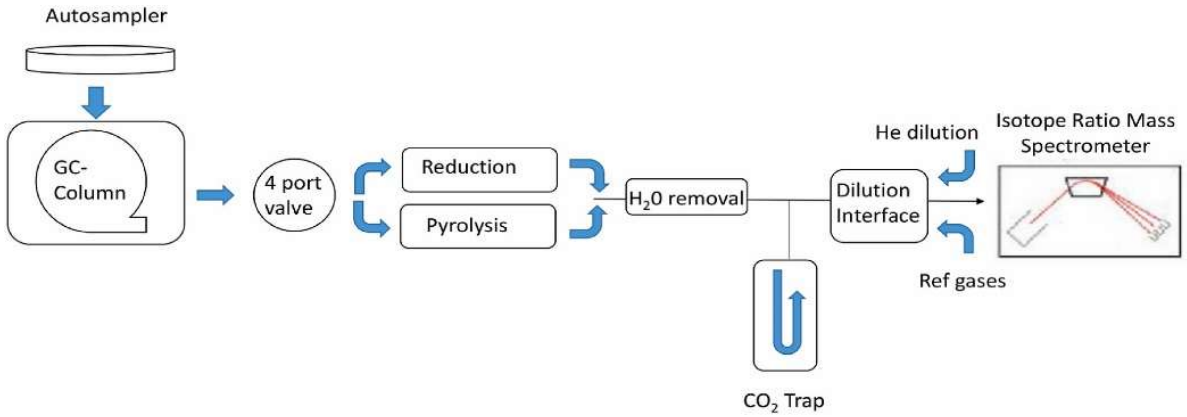

Fig 1. GC-IRMS scheme [1]

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P42

SOLUTIONS AND CHALLENGES IN FOCUSING TRAP AND SPECTRAL DECONVOLUTION FOR CHARACTERIZATION OF ODOUR ACTIVE VOCs IN HOT MIX-ASPHALT

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Volatile chemicals are emitted from a variety of anthropic emissions and, in recent years, the attention toward Volatile Organic Compounds (VOCs) has increased versus the odorous-active compounds, that can strongly limit the use of the territory. The ISO 16000-6:2021 classified Semi(S)VOCs (after n-hexadecane on a 5% phenyl 95% PolyDiMethylSiloxane (PDMS) Gas Chromatographic (GC) capillary column), VOCs (eluting between and including *n*-hexane and *n*-hexadecane), and Very(V)VOCs (eluting before *n*-hexane). The characterization of odor impact from asphalt mixture plants is challenging; odors can be released from different plant areas, resulting in a complex source localization and a difficult estimation and measure of odors. Already in the European Collaborative Action (2013) Report no. 29, it was stated that, especially for VVOCs, European harmonized test methods are not yet available [1]. Different sampling and analytical strategies have been developed for the quantification of VOCs in air usually performed on passivated canisters. Typically, this step is performed by refocusing the sample on micro traps filled with light adsorbents kept at -150 °C [2]. Since VVOCs are not fully retained on traps filled with solid sorbents maintained at temperatures ranging from +5 to -15 °C, cryogenic cooling is often mandatory to obtain quantitative recoveries during the sampling step. Although dehydrating agents have been used to make possible the collection of large volumes of air at any relative humidity, these devices are not free from artifacts. Selective losses of VOCs have been reported with magnesium perchlorate and potassium carbonate sorbents whereas sample contamination and selective removal of VVOCs have been observed with Nafion tubes

This work shows the measurement of trace VVOCs, VOCs, and SVOCs in air sampled collect from channeled emissions of asphalt mixture plants by Nalophan® gas bags, including formaldehyde, hydrocarbons, volatile sulfur compounds (VSCs) and hydrogen sulfide, by Thermal Desorption - GC/Mass Spectrometry (Markes Unity-xr and Agilent 8890 GC coupled with Agilent 5977C MSD, SRA Instruments) equipped with an automated unique, three-stage preconcentration and water-management mechanism that operates entirely without liquid cryogen thanks to a dedicated focusing traps (Kori and H_2S Trap, Markes International Ltd). Success of this application has greatly benefited from

computational workflow, applied to the complex raw mass spectrometry data to obtain qualitative and quantitative information of the matrix through deconvolution, which has proven to be mandatory in certain chromatographic zones due to the existence of co-elution in unresolved complex mixtures (UCMs) [3]. Deconvolution reconstructed a pure mass spectrum for each component, permitting the identification of co-eluted VOCs by both matching the Electronic Ionization (EI) mass spectra and the Linear Temperature-Programmed Retention Index (LTPRI) with reference values provided by spectral library.

The use of a 60 meters capillary column, with 100% PDMS column stationary phase permitted the use of "standard non-polar" Retention Indices (RI) library, containing the largest number of records.

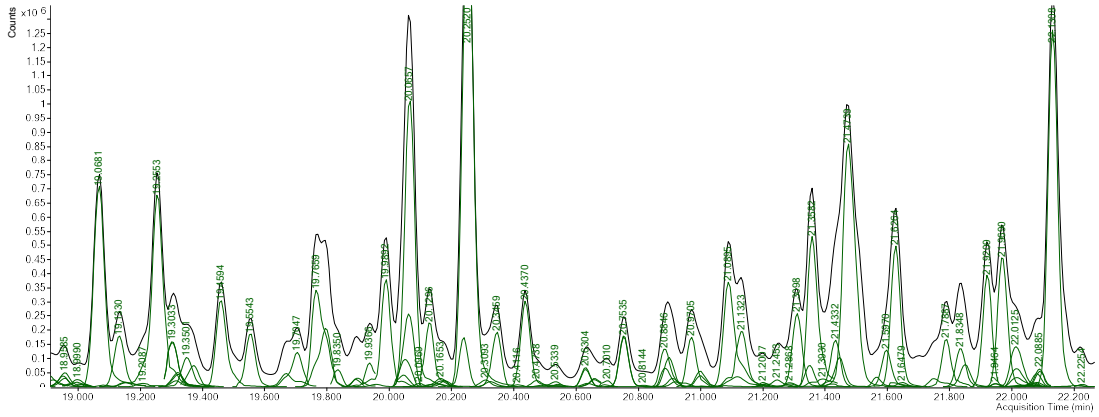

Figure 1. Example of a part of deconvolution results.

The described approach permitted a semi-untargeted screening of VOCs in complex matrix by single quadrupole mass spectrometer, with multiple outcomes. Several oxygenated VOCs were found with relatively low abundances under the UCMs produced by co-elution of much more abundant alkanes in the range between heptane and dodecane.

Samples collected in typical asphalt production conditions produced between 2000 and 4500 deconvolution results, with 300 to 500 library hits considering a minimum match factor of 70/100. VVOCs resulted chromatographically resolved, while early eluting peaks (hydrogen sulfide, formaldehyde, acetaldehyde) were qualified by standard injection and Single Ion Monitoring (SIM) acquisition, since EI spectra of such light molecules is characterized by an insufficient number of peaks to achieve a robust deconvolution under the predominant early elution of carbon dioxide.

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EXPLORING THE HUMAN HEART PHOSPHOPROTEOME USING ION MOBILITY MASS SPECTROMETRY (IM-MS)

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In LC-MS analysis of complex protein samples, reducing sample complexity is crucial, and IM-MS is becoming a popular approach which may help to achieve this goal. A key advantage is its on-line gas-phase fractionation, which might eliminate the need for off-line methods like high-pH reversed-phase fractionation. High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) is one such IM-MS technique, using high- and low-electric fields and additional low DC voltage to selectively filter ions from the ion source to the mass spectrometer.

In our study, LC-MS measurements were performed on an Orbitrap Fusion Lumos mass spectrometer coupled with a Thermo Ultimate 3000 HPLC, with or without FAIMS, to investigate whether FAIMS can increase phosphoproteome coverage in heart biopsy samples. Furthermore, we compared the quantification results of TMT-labeled samples, using MS2-based quantification in combination with FAIMS or the conventional SPS-MS3 method without FAIMS. Non-FAIMS data were collected using 60- or 120-minute gradients, while FAIMS data were collected either applying 2CVs with a 60-min gradient, or applying 3CVs in combination with a 120-min gradient. The 120-minute non-FAIMS measurement yielded the highest number of phosphopeptide identifications. FAIMS with 2CVs (60-minute runs) improved the results by ~10% compared to the 60-minute non-FAIMS runs, but still performed worse than the 120-minute non-FAIMS measurement, whereas FAIMS results using 3CVs with a 120-min gradient lagged behind the other approaches. Overall, longer gradient times without FAIMS performed best in phosphopeptide analysis. If analysis time is a critical factor, the second-best approach was the FAIMS run with 2CVs (60-minute gradient), which yielded 20% fewer identifications compared to the 120-minute non-FAIMS run.

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THE LIQUID ELECTRON IONIZATION (LEI) INTERFACE AS NOVEL STRATEGY FOR TARGETED AND UNTARGETED ANALYSIS IN A FORENSIC APPLICATION

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Carbofuran (CF) is a carbamate insecticide, nematicide, and acaricide largely used for its broad spectrum of activity. Although CF is no longer available in the EU market, a case of intentional animal poisoning using CF happened in the Czech Republic, but the origin of this pesticide remains unknown. In this study, 13 CF formulations from countries where it is still available have been characterized using a novel non-targeted analyses (NTA) workflow. The characteristic compounds found in CF formulations were then searched in poisoned animal samples to determine the provenience of the formulation used. After that, a quantitative method was validated to establish CF concentration in animal samples.

The analyses were conducted using the liquid electron ionization (LEI) interface coupled with a high-resolution mass spectrometer, the quadrupole time-of-flight (Q-TOF). The LEI interface allows coupling liquid chromatography (LC) with electron ionization (EI), as shown in **Figure 1**, extending the EI searchable library spectra to compounds not amenable to GC [1]. In addition, while EI spectra commonly lack measurable molecular ions, the QTOF's low-energy EI capabilities allow the acquisition of high-informative spectra under standard EI conditions and softer fragmentation at lower energies (15 and 9 eV), which yields molecular ions for most compounds.

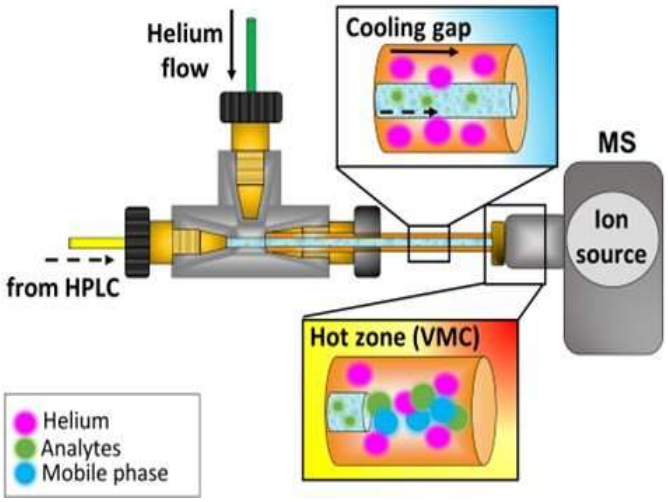

Figure 1. Scheme of LC-LEI-QTOF/MSMS system.

The 13 CF formulation, in granular and liquid form, and three 3 real samples from animals (one liver and two gastric contents) were provided by Charles University of Prague. Regarding the CF formulation, the extraction of compounds from granules was performed by immersing the granules in ultrapure water and sonicating them for 10 minutes. The extracts were then filtered using syringe filters. While liquid formulations were diluted in methanol (MeOH) and filtered. For real samples, one liver from a fox, and the gastric content of an eagle and a red kite, were extracted using a modified Accelerated Solvent Extractor (ASE). Reverse phase liquid chromatography (RPLC) separation was performed using a Kinetex column (XB-C18 150x2.1, 1.7 μm) with a gradient elution (5% B hold for 1 min; 5-100% B in 6 min; 100% B hold for 15 min) using ultrapure water + 0.1% formic acid (A) and ACN + 0.1% formic acid (B). The analyses were performed in Scan mode (mass range m/z 85-600) at 70 eV, and low energies (9 and 15 eV), as well as in MS/MS mode.

The NTA workflow developed in this work includes different steps: (1) deconvolution of co-eluted compounds; (2) comparison of deconvoluted spectra with the NIST library; (3) molecular ion determination; (4) MS/MS analyses using the suspected molecular ion as precursor ion; (5) molecular structure determination comparing the HR-MS/MS fragmentation with ChemSpider database.

The system allowed for the identification of 7 compounds with high confidence levels, 1 or 2a of the Schymanski scale [2]. The library did not recognize 4 compounds, but their structures were proposed with high scores by the Molecular Structure Correlator (MSC) software, and the satisfactory accurate masses, allow their identification with a confidence level of 2b. For 3 compounds, the molecular formulas were proposed with good accuracy (below 5 PPM), and only for one compound, it was not possible to establish the possible formula, perhaps due to its low concentration. Regarding the quantitative analysis, the method demonstrated excellent performance. As expected, negligible ME was confirmed also without the use of an internal standard, confirming that the LC-LEI-QTOF-MS/MS system is a good alternative for qualitative and quantitative analysis of volatile and low volatile fractions of complex samples. As a result, in two of the three samples, CF was detected and quantified. The concentrations found in CF-positive samples resulted well above the LD50 values [3], confirming the suspected intentional poisoning.

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A MULTI-DISEASE STATISTICAL ANALYSIS OF FREE FATTY ACID SIGNATURES ASSESSED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Fatty acids are classified based on chain length into short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), and long-chain fatty acids (LCFAs), which are essential for various physiological processes, including gut health. The gut microbiota plays a significant role in SCFA production, and its imbalance (dysbiosis) is often linked to alterations in fatty acid profiles. On the other hand, both MCFAs and are generally encountered in the diet, especially from milk and dairy products. Overall, these metabolites exert various essential function for host health, such as the regulation of energy metabolism, gene expression, ion channels and pump activities, membrane trafficking, and the modulation of immune processes.

In this study, through a dedicated gas chromatography-mass spectrometry protocol, we evaluated the serum of SCFAs, MCFAs a LCFAs abundances in 54 patients with adenomatous polyps, 55 colorectal cancer patients, 54 patients affected by celiac disease, 67 patients affected by Sars-Cov 2, 100 amiotrophic lateral sclerosis patietns and 100 healthy controls, in order to explore potential disease clusters based on fatty acid distribution.

Multinomial logistic regression effectively distinguished healthy individuals, celiac disease patients, and those affected by COVID-19 and ALS, but faced challenges in differentiating adenoma from colorectal cancer patients. Fisher's linear discriminant analysis (LDA) performed poorly, with an accuracy deficit in classifying ALS patients, who were misidentified as healthy. Additionally, LDA struggled with distinguishing between adenoma and colon cancer patients. Quadratic discriminant analysis (QDA) showed an improved ability to classify healthy controls and achieved better overall classification accuracy compared to LDA. However, QDA faced difficulties in differentiating adenoma, colon cancer, and cardiovascular disease patients, which were not confused in other models. Despite the varying performance of the models, one consistent finding was the difficulty in distinguishing adenoma from colorectal cancer. This similarity was attributed to the fact that adenoma is considered a precursor to colon cancer, and patients with adenomas exhibit fatty acid levels closely resembling those of colon cancer patients. Overall, the results suggest that while fatty acid profiling can differentiate several disease states, adenoma and colon cancer share substantial overlap in their fatty acid signatures, reflecting the

pathological continuum between the two conditions.

This study highlights the potential of fatty acid profiling as a diagnostic tool, while also illustrating the challenges in distinguishing conditions with overlapping metabolic features. Further research into the role of gut microbiota and fatty acid metabolism in these diseases may offer valuable insights for early diagnosis and targeted interventions.


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