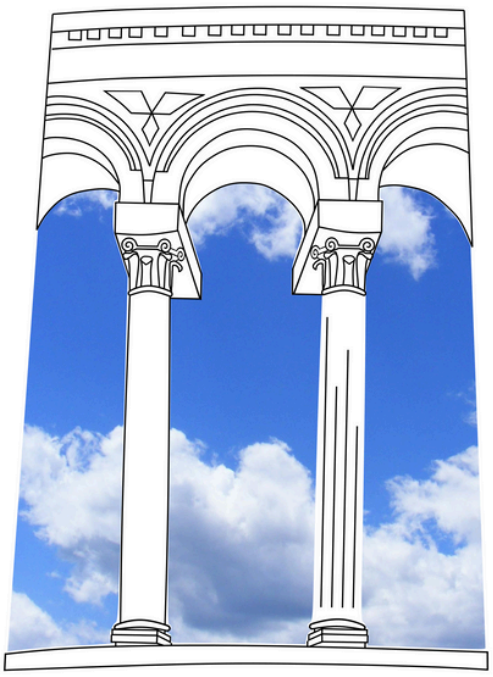

# 12th MS J-day

Pisa

22-23rd May 2025

1st International Edition

# BOOK OF ABSTRACTS

Società Chimica Italiana
Divisione di Spettrometria di Massa

Società Chimica Italiana
Gruppo Giovani

Cultural Heritage | Environment | Life Science

MS Technology | Food Science | Plant Science

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| | 15:00 | Giulia Caroti<br>*University of Pisa* | The chemistry of mixed media paints: the key role of the paint microstructure in the co-oxidation of proteins and lipids |
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# Scientific Program

## May 22nd

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# Scientific Program

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# Picture this: Mass Spectrometry Imaging to unveil the multiomic layers of the tumor immune environment

**Greta Bindi**[1], Claudia Fumagalli[1], Natalia Shelly Porto[1], Nicole Monza[1], Glenda Santos de Oliveira[1], Vanna Denti[1], Lisa Pagani[1], Clizia Chinello[1], Eleonora Bossi[1], Simone Serrao[1], Vasco Coelho[2], Manon Van Der Ploeg[3], Ieva Palubeckaite[4], Manfred Wuhrer[4], Giorgio Cazzaniga[5], Daniela Besozzi[2], Vincenzo L'Imperio[5], Fabio Pagni[5], Giuseppe Paglia[1], Fulvio Magni[1] and Andrew Smith[1]

[1]*Proteomics and Metabolomics Unit, Department of Medicine and Surgery, University of Milan-Bicocca, Vedano al Lambro, Italy*
[2]*Department of Informatics, Systems and Communication (DISCo), University of Milan-Bicocca, Milan, Italy*
[3]*Pathology Unit, Leiden University Medical Center, Leiden, Netherlands*
[4]*Center for Proteomics and Metabolomics (CPM), Leiden University Medical Center, Leiden, Netherlands*
[5]*Department of Medicine and Surgery, Pathology Unit, University of Milan-Bicocca, IRCCS Fondazione San Gerardo dei Tintori, Monza, Italy*

The cellular and molecular composition of the microenvironment of solid tumors is known to influence disease progression, prognosis, and response to treatment[1], and the advent of spatial multiomics approaches has greatly contributed to advancing this rapidly growing field[2]. Spatial multiomics enables the correlation of complementary layers of information on a single biological sample; when integrated, these multiomics levels collectively generate a molecular snapshot of the tumor microenvironment (TME). In this context, Matrix Assisted Laser Desorption Ionization-Mass Spectrometry Imaging (MALDI-MSI) is a spatial omic approach that represents a turning point in TME research, as it enables the specific detection of metabolites and lipids, N-glycans, proteins, or even mass-tagged antibodies, all while maintaining the spatial coordinates of analytes and in a non-destructive manner. In this study, we demonstrate the feasibility of mapping the spatial lipidome, N-glycome, proteome and target antigens on single tissue sections of immunogenic tumors through MALDI-MSI. Furthermore, we describe how this workflow enabled the distinction of diverse molecular signatures of intratumoral and distal lymphocytes, highlighting the necessity of spatial multiomics in TME research. Lastly, we present the development of multicellular 3D models to replicate tumor-immune cell interactions, demonstrating how this workflow can be extended to study the TME's response to different stimuli and treatments in a controlled and reproducible experimental setting. Through this integrated workflow, we highlight the importance of spatial multiomics in deepening our understanding of the TME and its implications for improving therapeutic strategy and personalized oncology.

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2. Wan-Chen Hsieh, Bugi Ratno Budiarto, Yi-Fu Wang, et al.; J. of Biomed. Sci.

# Advancing Dye Identification in Historical Textiles using Mass Spectrometry-Based Molecular Databases

**Diego Tamburini**[1]

[1]*The British Museum, London (UK)*

The identification of colouring materials used for textile dyeing is challenging, due to the outstanding variety of natural sources that have been used by humans throughout history and across the globe. The invention of synthetic dyes further complicates the challenge, adding more chemical formulations to the dyers' palette. As a result, comprehensive molecular databases are key to provide accurate identifications. However, reliable reference materials need to be sourced and analysed to build such databases, often requiring substantial research. Considering the variety of molecular classes in the dye world, mass spectrometry emerges as the state-of-the-art technique in this research field. This presentation will showcase the potential of mass spectrometry techniques, especially high-pressure liquid chromatography coupled to electron spray ionisation and quadrupole time-of-flight (HPLC-ESI-QToF) for dye analysis. A selection of case studies from the British Museum's collection will highlight the challenges of archaeological and historical textile analysis in a museum context.

Cultural Heritage

# Understanding the Curing Process of Pre-Polymerized Oils: A Molecular Approach to Historical Painting Techniques.

**Alessandro Arrigo**[1], Emanuele Arusa[1], Giulia Caroti[1], Celia Duce[1], Ilaria Bonaduce[1]

*1 Department of Chemistry and Industrial Chemistry, Università di Pisa, Pisa, Italy*

Pre-polymerized oils are treated drying oils that have been widely used as painting mediums since the fifteenth and early sixteenth centuries. Their presence in paintings alongside raw siccative oils implies that artists made conscious choices about when and how to use them, as their physico-chemical properties differ in viscosity, adhesivity, and drying time.[1]
Despite the curing of raw linseed oils has been studied and characterized in detail[2,3], much less is known about the curing of pre-polymerized oils which depends on the type of treatment.
The aim of this work is to study the curing process of pre-polymerized oil paints combined with carbon black, which has an antioxidant effect and inhibits the radical chain propagation[4]. The paints were exposed to different ageing conditions in order to gain a better understanding of historical painting techniques and the stability of paint layers over time.
In this study three types of commercial pre-polymerized linseed oils were used. An heat-bodied oil (boiled in an inert atmosphere), a thickened oil (exposed to sunlight under air flow), and a catalyzed oil (heated in presence of lead oxide).
Those oils were firstly analysed by thermogravimetric analysis (TGA) under an air flow at a constant temperature of 80°C, revealing distinct oxidation and oxidative degradation behaviours, as well as different curing kinetics. Volatile species released from the model paint layers as a result of oxidative degradation were analysed over time using SPME-GC/MS. The results from TGA and SPME-GC/MS proved to be consistent, allowing us to confirm the different curing kinetics and to identify oxidative degradation products with their relative abundance, which varied significantly among the samples. Ongoing experiments will focus on obtaining information about the thermal stability of the different organic fractions of the model paints through EGA-MS analysis. Moreover HPLC-MS will be employed to characterize the glyceride composition.
The combination of the obtained results obtained with those from the upcoming analyses is expected to improve the understanding of the curing process in different types of treated oils, thus providing new information on the molecular characteristics of the formed paint films.

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Cultural Heritage

# Investigating Silk Ageing through Thermoanalytical Techniques

**Cecilia Campi**[1], Ilaria Bonaduce[1], Ilaria Degano[1]

*1 Department of Chemistry and Industrial Chemistry, Università di Pisa, Pisa, Italy*

Silk has long been considered one of the most valued textiles, used for over 5,000 years, thanks not only to its natural luster but also to its remarkable mechanical and thermal properties [1]. However, silk is delicate and prone to degradation and dirt accumulation over time. Furthermore, conventional wet cleaning methods present challenges in both cleaning and preserving silk-based heritage artifacts [2].

This work is part of the MOXY project, which aims to develop a novel, non-contact cleaning method based on the generation of a cold plasma of Atomic Oxygen (AO) applicable to delicate objects. AO is a highly reactive species capable of oxidizing carbon-based contaminants and removing them without mechanical contact, thereby minimizing damage to the underlying surface. Before evaluating the effects on silk of this innovative technology, it is essential to study its aging and oxidation and establish a clear understanding of the degradation pathways affecting this fragile material in different conditions. This level of detail on silk degradation, particularly at the molecular level, remains, in fact, incomplete in the literature. A thermoanalytical approach is at the core of this research, using analytical pyrolysis - EGA-MS and multi shot Py-GC/MS - to assess the thermal stability of silk proteins and obtain molecular-level information. In order to provide insights into the degradation pattern of silk, the reference materials were subject to different environmental conditions, leveraging the effects of UV-Vis light, elevated temperature, and relative humidity. Historical samples have been investigated with the same approach. The specific impact of dyes and mordants was also assessed by including in the experimental set-up silk samples dyed with carminic acid (using alum as a mordant) and Rhodamine B (a direct dye).

The changes in thermoanalytical properties of aged silk were assessed and depended not only on the environmental conditions (thermal and photo-ageing showed different outcomes) but also on the dyes and mordants used. Interestingly, some combinations exhibited a protective effect, significantly slowing down the aging process of silk.

**References**

1. C. Holland et al. Advanced healthcare materials, 8, (2019), pp1800465.
2. F. Vilaplana et al. Analytical and bioanalytical chemistry, 407 (2015), pp 1433-1449.

Cultural Heritage

# The chemistry of mixed media paints: the key role of the paint microstructure in the co-oxidation of proteins and lipids

**Giulia Caroti**[1], Rafaella Georgiou[1], Celia Duce[1], Ilaria Bonaduce[1]

*1 Department of Chemistry and Industrial Chemistry, Università di Pisa, Pisa, Italy*

Lipids and proteins both undergo oxidation during time. In a paint, the reactions that take place during lipid oxidation are various[1] and can be seen as a competition between two different phenomena: oxidative degradation - leading to the formation of polar moieties - and cross-linking - leading to the formation of the polymeric network.[2] Protein oxidation occurs through three different phenomena: fragmentation of the polypeptidic chain, cross linking and oxidation of amino acid side chains.[3] When lipids and proteins are in contact, radical species formed due oxidation can react with each other, leading to the formation of co-oxidised species.[4]

This work is part of a research regarding the combined use of both lipids and proteins in the same paint layers by the Old Masters in the 15th century. Depending on the painting technique, oil and proteinaceous binders can be mixed in different ways resulting in paint layers with different microstructure and thus different interfaces at which lipids and proteins can interact. Two different painting techniques have been investigated: tempera grassa - an emulsion of oil in water, in which proteins and pigment particles are dispersed - and protein coated pigment - an oil paint in which the pigment particles are coated with a thin layer of proteins. A tempera grassa is characterized by multiple interfaces: pigment/protein, pigment/oil, oil/protein, oil/air, protein/air. In a protein coated pigment, the oil is in direct contact with oxygen, but the protein layer mostly prevents the interaction of the oil with the pigment particles.

The aim of this work was to investigate the different chemistry of oils and proteins in tempera grassa and protein coated pigment, from the fresh paints to the formation of solid paint layers. A multi-analytical approach was implemented. The presence of peroxides and hydroperoxides was monitored with Differential Scanning Calorimetry (DSC) and the mass change due to oxidation was investigated with ThermoGravimetrical Analysis (TGA) under air flow at constant temperature. Data were compared with those obtained by monitoring the molecular composition of the volatile products of oxidative degradation of model paint layers using Solid Phase Micro Extraction - Gas Chromatography - Mass Spectrometry (SPME-GC-MS). Finally, the thermal stability of the different organic fraction, from the most volatile to the tightly cross-linked polymeric network, was investigated with Evolved Gas Analysis - Mass Spectrometry (EGA-MS).

This study reveals that reaction pathways are highly influenced by the microstructure of the paint layer, leading to the formation of paint films with distinct properties. Furthermore, proteins were found to exhibit an antioxidant effect against lipid oxidation in both paint formulations.

**References**

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4. Schaich, K.M.J.L.o.p., Co-oxidation of proteins by oxidizing lipids. 2008. 2: p. 183-274.

# Impact of Atomic Oxygen and Laser Surface Cleaning Techniques on Cultural Heritage Materials: A Mass Spectrometry Investigation

**Elena Ducoli**[1], Kirill Shumikhin[1,2,3], Silvia Pizzimenti[1], Alessia Andreotti[1], Antonella Manaretti[1], Celia Duce[1], Anton Nikiforov[4], Tomas Markevicius[4], Klaas Jan van den Berg[3], Ilaria Bonaduce[1]

[1]*Università di Pisa, Italy*
[2]*Sapienza Università di Roma, Italy*
[3]*Universiteit von Amsterdam, The Netherlands*
[4]*University of Ghent, Belgium*

The surface cleaning is a crucial procedure for cultural heritage conservation. Usually, it involves the use of solvents (wet cleaning) or physical removal of dirt from the surface (dry cleaning). However, these methods have their limitations in terms of efficacy, safety and sustainability. Search for new approaches lead to the development of non-contact methods, which present significant advantages when working with solvent-sensitive and mechanically unstable heritage object surfaces. Such as the use of laser systems, that are versatile and allow to adjust parameters like laser source and pulse duration, even if this method has limitations too, specifically when applied to particularly sensitive surfaces.
In this regard recently, an alternative for cleaning the surface of works of art was developed based on the use of atomic oxygen (AO) in atmospheric pressure plasma. It's cleaning mechanism is based on the oxidation of the contaminants by atomic oxygen cold plasma. Given its mechanism of action, this technique is a promising non-contact technique particularly useful for cleaning carbon-based contaminants from porous, water-sensitive and fragile surfaces. The pioneering use of AO cleaning for cultural heritage conservation showed promise before[1]. However, before applying it to artworks, it is necessary to systematically study the chemical effects of AO on the substrate materials to evaluate the cleaning mechanism and carry out a systematic comparison to other established techniques. To this aim, this study systematically investigates the impact from AO cleaning and compares it to cleaning carried out with two types of lasers: Er:YAG[2], suitable for varnish thinning, and Nd:YAG[3], commonly used by conservators for soot removal. Experiments were performed on model oil paint layers (naturally aged for two years), consisting of ultramarine blue pigment and two different types of binders: linseed oil, used as oil binder since antiquity, and safflower oil, frequently encountered in modern paint formulations. Treated and untreated samples of identical composition were analyzed using a combination of analytical techniques such as SEM and optical microscopy to compare the surface morphology of the layers, ATR-FTIR and EGA-MS to assess the molecular changes undergone by the binder, and SPME-GC-MS[4] to monitor the evolution of VOCs released from paint layers over time.
In conclusion, the results show that AO cleaning is less invasive (ATR-FTIR, EGA-MS) compared to laser and, unlike the latter, it does not seem to cause long-term damage (SPME-GC-MS).

**References**

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Cultural Heritage

# The transformation of egg proteins in aged painting layers through UV-induced processes: a proteomic perspective

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Understanding the aging process of pictorial binders is crucial for artwork conservation and for identifying the chemical transformations that affect their stability over time. Among historical binders, egg tempera was widely used due to its durability; however, protein degradation can compromise the integrity of the painting layer, particularly when interacting with inorganic pigments [1]. This study simulated the natural aging of egg-based paint by subjecting non-pigmented egg white, as well as egg white mixed with calcium carbonate (white pigment) or hematite (red pigment), to UV-Vis irradiation at 50 °C for up to 864 hours.

ATR-FTIR spectroscopy highlighted structural protein modifications, with shifts in the Amide I and II bands suggesting increased intermolecular β-sheet formation as an indicator of protein aggregation [2]. Proteomic analysis using three different enzymes (Trypsin, AspN, and GluC) confirmed these findings. Matrix assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-ToF-MS) and reversed phase liquid chromatography-electrospray ionization-mass spectrometry (RPLC-ESI-MS/MS) revealed a significant decline in peptide signal-to-noise ratios with aging, demonstrating the increasing difficulty in extracting and digesting aggregated proteins. These findings highlight the extensive structural alterations occurring over time.

Additionally, the study detected several aspecific and semi-specific peptides, mainly derived from the most degradation-prone regions of ovalbumin, supporting the notion that protein breakdown occurs alongside aggregation [3]. Importantly, prolonged aging conditions led to previously unreported non-enzymatic post-translational modifications (PTMs) in aged proteinaceous binders, including methylation, formylation, and diglycyl modifications. These changes likely contribute to the destabilization of the pictorial layer, offering new insights into the complex chemical evolution of egg tempera paintings.

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# Nitrofuran metabolite residues in honey: the challenge with small molecules

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Nitrofurans, once widely used to prevent bacterial infections in bee colonies, are antimicrobial agents not authorised for use in food-producing animals in the European Union due to their potential health risks [1]. Nitrofurans are rapidly metabolised, hence in food controls the detection of parent drugs would be ineffective [2]. Their metabolites are highly stable and can persist in honey posing a public health concern.

The European Commission requests to each Member State to include in their National Monitoring Control Plans the detection of the following five metabolites: 1-aminohydantoin (AHD), 3-amino-5-methyl-morpholino-2-oxazolidinone (AMOZ), 3-amino-2-oxazolidinone (AOZ), 3,5-dinitrosalicylic acid hydrazide (DNSH) and semicarbazide (SEM). For these analytes a Reference Point of Action (RPA) was set at 0.5 µg/kg with the UE Regulation 1871/2019 in different matrices. Therefore, the laboratories involved in Official Control in Food Safety have to use detection method with a Detection Capability (CCβ) lower than the RPA value.

In this context, our laboratory had to face the challenge of upgrading the analytical method for five nitrofuran metabolites by the development and validation of a LC-MS/MS screening procedure on a highly efficient system. The final procedure involved a single liquid-liquid extraction step, after hydrolysis and derivatization of these small molecules with 2-nitrobenzaldeide to the corresponding 2-nitrobenzaldeide imine-type derivatives [3]. Detection of the analytes was performed by LC-MS/MS on a Kinetex Biphenyl (50 x 2.1 mm, 1.7 µm) column in HPLC Agilent 1290 Infinity coupled to a SCIEX QTRAP 7500 MS equipped with a H-ESI in positive and negative polarity and operating in MRM mode. The validation was performed according to the EU Regulation 808/2021 criteria, considering the following parameters: specificity, β Error and ruggedness.

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# Amino acid profiling of barattiere (Cucumis melo) by hydrophilic interaction liquid chromatography and high-resolution MS

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Southern Italy, particularly Apulia, is home to numerous traditional landraces of immature melons (Cucumis melo), which are cherished by local populations as an alternative to the more commonly consumed cucumber (C. sativus). Among these, the barattiere stands out as a rare heirloom Apulian variety, valued for its sweeter, juicier pulp, and superior digestibility compared to cucumber. This improved digestibility is attributed also to the absence of metabolites like cucurbitacins, which are known for their potential adverse effects on the gastrointestinal tract [1-3]. Since barattiere fruits are typically consumed in an immature, unripen state, the chemical changes occurring throughout their maturation remain largely unexplored. Previous metabolomic studies on other plant families, such as Rosaceae, have demonstrated that amino acids (AAs), some of which are linked to sour flavours, can vary significantly with maturation [4]. To bridge this knowledge gap, a quantitative profiling of AAs in barattiere fruits was conducted by hydrophilic interaction liquid chromatography coupled with high-resolution mass spectrometry through an electrospray source (HILIC-ESI-HRMS). AAs were extracted from barattiere samples via a straightforward procedure using acidified water [5], and the resulting extracts were directly analysed without prior derivatization. HILIC enabled the separation of all proteinogenic AAs within 20 minutes, while also detecting non-proteinogenic AAs characteristic of Cucurbitaceae plants. AAs were quantified in whole fruit as well as in specific anatomical parts, namely the epicarp/mesocarp and the placenta, revealing significant differences in concentration across these tissues. The HILIC-ESI-HRMS method proved to be highly efficient for the rapid profiling of AAs in barattiere fruits, offering promising opportunities for further research into the metabolic profiles of local plant landraces. This study paves the way for a deeper understanding of their nutraceutical potential, promoting the valorization of traditional cultivars.

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Food Science

# Direct Detection of Oregano Adulteration with AP-MALDI-MS

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Food fraud is a growing concern in the global supply chain, with dried oregano identified as one of the most vulnerable herbs to adulteration [1]. Rapid and reliable analytical techniques are essential to ensure food authenticity and protect consumers [2]. In this study, it is reported an innovative application of atmospheric pressure matrix-assisted laser desorption ionization mass spectrometry (AP-MALDI-MS) for the detection of adulteration in dried oregano leaves, enhancing quality control measures [3]. A dataset comprising 44 samples, including authentic oregano, common adulterants, and intentionally adulterated samples, was analyzed using positive and negative ion modes. The spectral data were subjected to multivariate statistical analysis, employing partial least squares discriminant analysis (PLS-DA) and random forest (RF) classifiers to distinguish between authentic and adulterated samples. The results demonstrated that the RF classifier, built up with negative ion mode data, achieved the highest performance, with an overall accuracy of 87.0% on the test set. Key discriminant metabolites were identified, including flavonoids and their glycosylated derivatives [4], which were further characterized through collision-induced dissociation and database matching. The study highlights the potential of AP-MALDI-MS as a cost-effective and rapid screening tool for food authenticity assessment, reducing the need for labor-intensive sample preparation while maintaining high sensitivity and specificity [5]. This approach offers a promising solution for regulatory agencies and food industry stakeholders to combat fraudulent practices in the spice trade [6].

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# UHPLC-Q-Orbitrap-HRMS combined with chemometrics as a tool for authenticating the origin and cultivation of Italian Lupinus albus L.

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Lupin seeds are the edible portion of lupin plants, included in the Fabaceae family. They have recently garnered global attention as a sustainable alternative to other legumes. Their resilience to environmental stress and climate adaptability, combined with significant nutritional benefits, make them an attractive crop [1]. Lupins are rich in proteins (>40%) and fibers (>28%), claiming a low-fat content (<6%); they also constitute a potential source of minerals and vitamins. Additionally, they offer bioactive phytochemicals, such as polyphenols, phytosterols and triterpenes, providing antioxidant and anti-inflammatory properties. However, they also contain antinutritional elements, notably quinolizidine alkaloids. The chemical composition of lupins can be influenced by several factors such as species or variety, geographical origin, farming practices, environmental stress, harvesting and processing methods [2,3]. In this study, Lupinus albus L. samples from four southern-central Italian regions were subjected to an untargeted metabolomics approach using ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS). The aim was to discriminate between samples based on their geographical origin and assess the metabolic variances resulting from distinct agricultural practices (conventional vs. organic farming). Unsupervised Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed to explore natural clustering tendency among the samples. Additionally, a supervised Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) revealed robust clustering and predictive capability, identifying over 25 putative biomarkers across various compound classes, including alkaloids, amino acids, glycerophospholipids, and fatty acids. The results highlighted the impact of the origin and farming techniques on the metabolomic composition and distinctiveness of lupin samples; this could be crucial for cultivation optimization, traceability ensurance and quality assessment of lupins, aiming to increase consumers attention and preferences, given their potential nutritional properties.

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Food Science

# Bioactive extracts from asparagus by-product

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The circular economy is a growing strategy gaining attention worldwide, especially in agriculture and food production. Asparagus (Asparagus officinalis L.) by-products, including lower stems, and discarded spears, are rich in bioactive compounds with potential applications in the food, pharmaceutical, and cosmetic industries. These by-products contain high levels of polyphenols which exhibit antioxidant properties. This work aimed to valorize waste products deriving from the production chain of Asparagus officinalis, which are usually discarded during harvesting and processing, and transform them into useful and sustainable resources. A chemical characterization of the bioactive compounds of the edible part and waste products of asparagus was carried out by UHPLC Q-Orbitrap HRMS. The evaluation of the total polyphenol content (TPC) and the antioxidant activity was carried out by spectrophotometric assays. To evaluate the impact of the cooking process on the polyphenol content, an in vitro gastrointestinal digestion (INFOGEST Protocol) was performed on the edible part after cooking. The results obtained confirmed that, although the edible fraction has a higher phenolic content, the residual biomass also has a significant phytochemical composition. These results highlight the importance of valorizing residual biomasses in the nutraceutical and pharmaceutical fields, opening up prospects for the development of functional ingredients and innovative formulations. The valorization of asparagus by-products represents a promising opportunity for sustainable innovation in the food and health industries.

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Food Science

# Antioxidant activity of anthocyanins from red cabbage by an AAPH-incubating method using liquid chromatography coupled with high resolution tandem mass spectrometry

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TRecent research highlights the potential of natural antioxidants as alternatives to synthetic antioxidants in the food industry [1]. Among them, anthocyanins, a class of water-soluble flavonoids responsible for the red, purple, and blue colors in fruits and vegetables, stand out for their ability to prevent oxidative stress by neutralizing reactive oxygen species (ROS) [2]. Red cabbage appears as a valuable source for its higher anthocyanin content [2]. When assessing antioxidant activity of complex matrices, traditional assays (DPPH, FRAP, ORAC) do not distinguish the contribution of individual compounds nor account for differences in reaction kinetics. For these reasons, the method proposed in this study to evaluate antioxidant activity involves the use of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), a radical initiator able to release peroxyl radicals (ROO•) under controlled conditions (37°C and pH 7.4), using HPLC-HRMS/MS [3][4]. Extraction of anthocyanins from red cabbage was performed using ultrasound assisted extraction (UAE). Thirteen anthocyanins were identified and quantified with HPLC-HRMS/MS using cyanidin chloride as external standard. The base structure of anthocyanins was cyanidin 3-diglucoside 5-glucoside with the glucoside residues nonacylated, monoacylated and diacylated with sinapic, ferulic and p-coumaric acid as main phenolic acids. The reaction with AAPH revealed that anthocyanins conjugated with sinapic acid displayed the highest antioxidant activity. Notably, cyanidin 3-(disinapoyl)-diglucoside 5-glucoside showed the fastest reaction rate (1.05E-02 μM/min). Interestingly, the nonacylated form cyanidin 3-diglucoside 5- glucoside also demonstrated strong antioxidant reactivity. Validation was performed using coulometric array detector. The analysis showed that cyanidin 3-(disinapoyl)-diglucoside 5-glucoside had the lowest half-wave potential (525 mV), indicating superior electron-donating ability. This approach effectively identifies anthocyanins with antioxidant properties, assesses their radical-scavenging activity, and relates their activity to chemical structures. These findings enhance understanding of anthocyanin reactivity, revealing their capacity to neutralize peroxyl radicals and their potential as natural alternatives to synthetic additives.

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Food Science

# Evaluation of the phytochemical profile of walnut fresh fruit extract (Juglans Regia) and its in vitro and in vivo biological activity

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In this study [1], a maceration of triturated walnut fresh fruit was performed, and the phytochemical profile of the dried extract was determined by HPLC-ESI-Q-TOF-MS. The characterization of the compounds was conducted using accurate mass data, ion source fragmentation, fragmentation pattern, bibliographic research and comparison with analytical standards, leading to the identification of 38 compounds. The main phenolic acids and flavonoids were quantified; the extract was rich in gallic acid and its derivatives, caffeoylquinic acids and coumaric acid derivatives.

In vitro assays were performed to determine the total phenolic content and the antioxidant and enzyme inhibition activities. The results of CUPRAC (206.93 mg TE/g) and FRAP (139.56 mg TE/g) assays highlighted the high reducing ability of the extract.

Then, its anti-proliferative effect was determined in vitro on human cells, specifically DU145 prostate carcinoma and PNT1A normal prostate cell lines, by real-time and label-free impedance-based cell proliferation assay. Based on the IC50, the extract exhibited a time- and dose-dependent anti-proliferative activity against prostate cancer cells.

Finally, in vivo studies were conducted on adult male Wistar rats with testosterone-induced benign prostatic hyperplasia (BPH). BPH is a non-malignant ageing-associated urological disorder. The limitations of the current therapies, e.g. adverse side effects and poor treatment response, push the development of novel approaches for its treatment [2]. The administration of the extract reversed the testosterone-induced biochemical and histomorphology changes in the rats with testosterone-induced BPH.

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# TTXs in molluscs and blue crabs from Adriatic Sea: do these species represent a risk for human consumption?

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INTRODUCTION: Tetrodotoxins (TTXs) are neurotoxins produced by bacteria from Pseudomonas, Vibrio, and Alteromonas genera which accumulate in host organisms through the food chain. TTXs are primarily found in pufferfish (Tetraodontidae) but also occurring in other species. [1,2,3]. EFSA reports that shellfish meat with concentrations below 44 µg eq. TTX/kg is safe for human consumption, but further data are required, especially for gastropods [4]. In this work, TTXs have been monitored in molluscs (bivalves, various gastropods species and echinoderms) and blue crabs (Callinectes sapidus) from the Adriatic Sea. Blue crab is a non-autochthonous species that became invasive in the Mediterranean Sea. Its feeding habits, based on bivalves and gastropods, can lead to TTXs biomagnification phenomena, also promoting the toxin spread in the local marine ecosystems. This study aims to assess levels, time-space patterns, origin and circulation of TTXs in the ecosystem.
MATERIALS AND METHODS: In the frame of research project RC IZSUM 02/2022, 137 samples of marine organisms have been collected in 2023 (66 Mytilus galloprovincialis, 58 various gastropods species, 3 C. sapidus and 10 Paracentrotus lividus) and 121 in 2024 (55 M. galloprovincialis, 27 various gastropods species, 26 C. sapidus and 13 P. lividus). Mussels and echinoderms were sampled in the Conero Riviera (Marche region, North-Central Adriatic Sea), gastropods throughout the whole North-Central Adriatic Sea and blue crabs from the northern areas of the Adriatic Sea. The TTXs extraction and chromatographic separation have been performed following the EURLMBSOP [5], with some modifications and using a HPLC-MS/MS system (HILIC column-QqQ-ESI+) for the instrumental determination.
RESULTS AND DISCUSSION: The majority of the samples analysed had TTXs levels below the EFSA threshold value. In 2023, TTXs traces were found in 19 of the 66 mussels (29%) analysed, with levels ranging from LOD (3.0 µg/kg) to 39.0 µg/kg with a mean of 16.0 µg/kg. In 2024, TTXs were measured in 11 samples out of 55 (20%), ranging between LOD and 119.0 µg/kg with a mean of 41.1 µg/kg. These results confirmed that the Conero Riviera is an area significantly affected by TTXs contamination, as already reported by Bacchiocchi et al. [1]. Moreover, traces of TTXs at LOD levels were detected only in one sample of echinoderms collected in 2023. All gastropods and blue crabs analysed had TTXs levels < LOD, suggesting the absence of contamination and then no risk for human consumption. In conclusion, the circulation of TTX in the marine local ecosystem suggests that further studies are necessary to better understand the phenomenon and related exposure risks.

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Food Science

# Evaluation of metabolic profile and anti-glycative properties of Diospyros kaki after simulated in vitro digestion

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Advanced glycation end products (AGEs), which are generated in the non-enzymatic reaction between reducing sugars and the amino groups of lysine or arginine side chain in proteins, are involved in various diseases, such as diabetes, cardiovascular, and neurodegenerative diseases. Glucose, fructose, and dicarbonyl compounds such as MGO (methylglyoxal) and GO (glyoxal) are mainly involved in the formation of endogenous AGEs [1]. Several studies showed that plant extracts counteract the formation of AGEs in vitro for the presence of different phenolic compounds [2]. However, it is well known that vitro studies are preliminary to in vivo invesitgations, also because plant extracts' metaobolites generally are poor bioaccessible [3]. Therefore, the aim of this study is to investigate the antiglycative properties of the edible extract of Diospyros kaki and to monitor the change in phenolics profile following simulated in vitro digestion. In particular, model systems consisting of a glycation agent (MGO, glucose, fructose) and a protein (bovine serum albumin-BSA) were set up under physiological conditions (37 °C, pH 7.4) to evaluate the antiglycative properties at different stages of the glycation process. In addition, the MGO and GO trapping ability of the extracts has been investigated [4]. Then, the main secondary metabolites present in the methanolic extract of Diospyors kaki leaves were identified by RP-HPLC-DAD-ESI-MSn. Finally,the extract was submitted to a simulated in vitro digestion process (consisting of oral, gastric, and intestinal phases, and using different enzymes and electrolyte mixtures for each phase) to study its bioaccessibility [5]. Consequently, the different phases were analyzed by RP-HPLC-DAD-ESI-MSn to compare the phenolic profile with the undigested one. The results showed that the extract effectively inhibited the formation of AGEs at the intermediate and final stages of the glycation reaction, mainly in presence of fructose (about 90%). In addition, it possesed great trapping properties towards MGO and GO (about 90%). According to literature data, these properties could be attributed to the identified secondary metabolites, such as kaempferol-3-O-glucoside, quercetin-3-O-galactoside, and other kaempferol and quercetin derivatives. However, during the in vitro simulated digestion process, a reduction of concentrations of various secondary metabolites was observed, partly due to a dilution effect and partly due to the low stability of these compounds during the process. However, the digested extract maintained low antiglycative properties.

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# Rapid sonochemical synthesis of molecularly imprinted polymers (MIPs) coupled with high-resolution mass spectrometry for the selective extraction of phytoprostanes in plants

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Phytoprostanes (PhytoPs) are non-enzymatic products of lipid peroxidation derived from α-linolenic acid. While PhytoPs are not essential for the metabolic activity of living cells, they are considered components of the oxidative damage detection systems and act as excellent biomarkers for oxidative degradation in plant-based foods. Among these PhytoPs, in the literature is reported a limited number of analytical methods resulting from homemade chemical synthesis few of them are available in commerce. Various methodologies are employed to qualitatively and quantitatively detect PPs in foods. Recent advancements in the identification of PhytoPs primarily employing UHPLC-MS/MS, allowing for rapid and precise measurement [2]. Additionally, methods integrating biosensors and high-resolution mass spectrometry are emerging, promising to further refine quantification in complex matrices like food and biological samples. The extraction of PhytoPs can be limited by factors such as the complexity of the matrix, the stability of the compounds, and the efficiency of the extraction method. In recent years, researchers introduced molecularly imprinted polymers (MIPs) extraction for target compounds or for class selective extraction [3]. In this work, an alternative strategy for fast MIPs synthesis was proposed and apply to the PhytoPs compounds, in commercial food samples analysed by UHPLC-MS/MS, as targeted method. A Orbitrap IQ-X tribrid mass spectrometry was employed to confirm the results using the high resolution and mass accuracy, allowing for precise identification of PhytoPs. MIPs were synthesized via a low-cost and rapid (5 min) sonochemical free-radical polymerization, using 4-cyclopentene-1,3-dione as a dummy template. To this aim, we tested methacrylic acid (MAA) and methacrylamide (MMA) as monomers, using ethylene glycol dimethacrylate as the cross-linker and 2,2 azobisisobutyronitrile as the initiator. MAA MIP based in dispersion solid phase extraction (dSPE) yielded the best result than the other. The evaluation of the performance of MAA-MIP-dSPE were performed with 5 of isoprostanes standard (8R-Isoprostane (8-ISOR), 8S isoprostane (8-ISOS), 8-keto-isoprostane (8 KETO), 11β- isoprostane (11β) and 5IPF2A-VI) and phytoprostane A1, obtaining satisfactory recovery and good reproducibility. Proposed MIP was tested on food samples proving the selective extraction of PhytoP forms by their putative identification.

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Food Science

# A Fused Approach Using Ion Mobility Spectrometry and Spectroscopy for Honey Authentication

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Food fraud is a growing global concern, driven by globalization and impacting economies, consumer health, and market trust. The honey industry is particularly vulnerable to fraudulent practices, such as mislabeling of botanical and geographical origins. However, conventional target-based analyses lack established chemical markers for authenticity verification. To address this challenge, there is a pressing need for fast, reliable, and cost-effective analytical methods to ensure honey authenticity and support regulatory frameworks.

In this study, over 130 honey samples from various botanical origins (primarily acacia, citruses, and multi-flower) and geographical regions were analyzed using Head-Space Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS) alongside UV-Vis and Raman spectroscopy. HS-GC-IMS, a highly sensitive and selective technique, enables the detection and identification of volatile and semi-volatile organic compounds in complex mixtures, reaching detection limits in the mid-pptv (parts per trillion by volume) range without requiring sample enrichment [3]. Notably, all three techniques (HS-GC-IMS, UV-Vis, and Raman) offer the advantage of minimal sample preparation, eliminating the need for organic solvents or hazardous reactants.

Since these techniques provide complementary insights, in this study an exploratory analysis employed mid-level and high-level data fusion to assess variations based on botanical and geographical origin. Ultimately, this study lays the foundation for a robust honey authentication method aligned with the principles of Green Analytical Chemistry (GAC) [4].

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Food Science

# A Fused Approach Using Ion Mobility Spectrometry and Spectroscopy for Honey Authentication

**ZINGARO???**

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Life Science

# Brain proteomics provides new insights into the DiGeorge syndrome

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DiGeorge Syndrome (22q11.2DS) is a rare genetic disorder occurring in approximately 1 in 2500 live births. As the most common genetic microdeletion syndrome, it manifests with a wide range of congenital anomalies, affecting the heart, craniofacial development, and immune function. Individuals with 22q11.2DS have an elevated risk for neurodevelopmental and neuropsychiatric disorders, including cognitive deficits, anxiety, and a markedly increased risk of schizophrenia during adolescence and adulthood. Given that many of the deleted genes (approximately 10%) are involved in metabolic processes, alterations in brain metabolism may contribute to these phenotypes. The chromosomal region typically deleted in 22q11.2DS contains multiple genes whose haploinsufficiency may influence brain metabolism. Among these, Tbx1 has been the most extensively studied. However, the precise relationship between these genetic deletions and the resulting neurobiological changes remains unclear. In this study, we explored proteomic alterations in the brain of a well-established mouse model of DiGeorge Syndrome. This model is characterized by a loss-of-function mutation in the Tbx1 gene, which encodes the T-box transcription factor TBX1 (Tbx1+/- model), while wild-type (WT) mice served as controls. Proteomic analyses were performed on brain tissues collected at different developmental stages to identify both early and late molecular changes associated with the pathological phenotype. Total proteomes were extracted and analyzed using shotgun liquid chromatography-tandem mass spectrometry (LC-MS/MS). Comparative analyses were conducted at 2, 4, and 7 months of age between Tbx1+/- mice and their age-matched WT counterparts. The results revealed a distinct shift in the brain proteome at 7 months, characterized by pronounced disruptions in metabolic and energy-related pathways, which are strongly linked to neurodegeneration. Notably, mice with DiGeorge Syndrome exhibited an intermediate proteomic phenotype relative to other groups. Furthermore, early signs of dysregulation caused by Tbx1 haploinsufficiency were already detectable at 2 months, with significant alterations in neurotransmitter transport and synaptic signaling pathways. These early changes may be associated with neurodegenerative processes observed in adult affected mice. This study contributes to the identification of proteomic alterations linked to DiGeorge Syndrome and provides insight into quantitative and qualitative differences in brain protein expression in a mouse model of the disorder.

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# Mass spectrometry to investigate elexacaftor/tezacaftor/ivacaftor (ETI) impact on neurodevelopment

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Elexacaftor/tezacaftor/ivacaftor (ETI) is a drug which significantly improves expectancy and quality of life of individuals with cystic fibrosis (CF), a genetic disease caused by mutations in the cystic fibrosist transmembrane conductance regulator gene (CFTR), affecting approximately 100,000 people worldwide [1]. Previous studies from our group [2] [3] indicate that ETI induces accumulation of saturated sphingolipids (bearing d18:0 sphingoid base) in cells by inhibiting the delta-4 sphingolipid desaturase (DEGS1), the enzyme which converts dihydroceramides (dHCer) into ceramides (Cer). [4] Since saturated/unsaturated balance of sphingolipids is crucial for many biological functions, particularly for the proper development and function of myelin in the central and peripheral nervous systems (CNS and PNS) [5], disruptions in DEGS1 activity could have significant neurodevelopmental consequences. Genetic defects in DEGS1 are linked to severe neurodevelopmental disorders, including progressive leukodystrophy, characterized by hypomyelination, motor impairments, and cognitive dysfunctions [6]. Given the role of sphingolipid homeostasis in CNS and PNS development, ETI-mediated inhibition of DEGS1 raises concerns, especially regarding fetal exposure during pregnancy.

Despite the lack of specific approval for ETI use in pregnancy, many women with CF continue the treatment. This study aims to investigate the potential effects of ETI exposure on neurodevelopment.

To assess these effects, a long-term exposure study will be conducted in mice, with female mice receiving ETI through food before, during, and after pregnancy. Offspring neurological abilities and general well-being will be evaluated through behavioral tests, alongside morphological and functional assessments of the CNS and PNS. ETI interaction with DEGS1 will be characterized using LC-MS/MS to monitor dHCer-to-Cer conversion. ETI levels, as well as dHCer accumulation, will be quantified in both mothers and pups, with a particular focus on the brain. Targeted sphingolipid analysis and untargeted extended lipid profiling will be performed using targeted triple-quad based analysis, to explore broader metabolic changes associated with ETI exposure. Multivariate statistical analysis techniques will be applied to interpret the data and identify potential biomarkers of ETI- induced lipid dysregulation.

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# Functionalized Nanomaterials Based on 2D Nanocrystals and Metal Nanoparticles Activated by Radiation for Antitumoral Therapy

**Gloria Cenci**[1,2], Silvana Pinelli[3], Paola Mozzoni[3], Chiara Maccari[3], Melissa Santi[5], Nicola Rivi[1], Marco Villani[2], Marco Bormetti[1], Elena Ferrari[2], Valentina Sinisi[2], Paraskevi Papadopoulou[4], Francesco Bonaccorso[4], Filippo Fabbri[5], Franca Bigi[1], Giancarlo Salviati[2], and Francesca Rossi[2]

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Cancer remains one of the leading causes of mortality worldwide, affecting millions of individuals and accounting for approximately 10 million deaths annually [1].
Traditional antitumor clinical approaches, such as Photodynamic Therapy (PDT) and Radiotherapy, often have limitations related to low efficacy and significant side effects, highlighting the need for more effective cancer treatments. PDT traditionally relies on UV – near infrared light activation of a photosensitizer to generate Reactive Oxygen Species (ROS) that selectively destroy cancer cells. Recently, the use of X-rays as an external excitation source in PDT has proven effective due to their superior penetration capacity, extending the potential of conventional PDT to deeper tissues and overcoming the limitations of visible light penetration. Radiotherapy, on the other hand, kills cancer cells through the direct action of X-rays on biological tissues, leading to numerous side effects on healthy cells [2]. In recent years, nanotechnology-based therapies have attracted growing interest, in particular, 2D nanomaterials are considered promising candidates due to their physicochemical properties, making them ideal platforms for radiation-based cancer treatments [3, 4].
This study presents a novel biocompatible nanomaterial, consisting of functionalized 2D nanocrystals combined with metal nanoparticles, designed as radiosensitizer and PDT mediating agent. This nanomaterial can be internalized by cells and it can be activated by an external energy source (light, X-ray radiation), enabling the local killing of cancer cells through ROS production and the direct action of radiation on them.
In vitro experiments were conducted on cancer cells to evaluate the cytotoxicity and efficacy of the novel nanomaterial. Cell viability, colony-forming ability and cell cycle analysis were assessed at different time points before and after the treatment with increasing concentrations of the nanomaterial. Oxidative stress within the cells was assessed using multiple approaches, including lipid peroxidation analysis via the TBARS assay and the detection of oxidized nucleobases (8-oxo-7,8-dihydroguanosine) by UHPLC-MS/MS (sciex 6500+). Our findings revealed that this nanomaterial amplifies the effect of radiation in cancer cells, acting as an effective radiosensitizer.

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# Mitochondrial proteomics highlights structural and functional alterations in methylmalonic acidemias

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Methylmalonic acidemias (MMA) are severe inborn errors of metabolism characterized by pleiotropic metabolic perturbations and multiorgan pathology. The genetic defect includes diverse mutations in the methylmalonyl-CoA mutase gene (MUT), whose product isomerizes the methylmalonyl-CoA into succinyl-CoA for the Krebs cycle. As direct consequence, upstream metabolites like methylmalonyl-CoA, methylmalonic acid, and propionylcarnitine accumulate in body fluids. While earlier studies have focused on the potential direct toxicity of metabolites as a mechanism to explain the disease, the cellular and molecular defects underlying MMA pathophysiology are still obscure, thus treatment options are limited and non-curative [1,2]. To shed light on the mitochondrial unbalances in MMA, MUT-deficient cell lines were employed, including a CRISPR/Cas9-engineered HEK293 MUT-knockout cell model and fibroblasts derived from MMA patients [1]. Mitochondria were isolated from the cell models and the mitochondrial proteome of MMA was analyzed by LC-MS/MS and bioinformatic analysis to identify differentially regulated proteins and altered pathways. A severe dysregulation of the mitochondrial proteome was observed in combination with marked structural alterations that include a reduced mass and circular organelles. Mitochondria functionality was compromised as well, reporting increased ROS levels and reduced cell viability especially in propionate-enriched culture medium [3]. Finally, metabolome and respiration analysis revealed a clear compromission of the primary metabolism and energy flux in MMA cells, with a reduction of the total ATP production rate imputable to the mitochondrial ATP. These results underline severe aberrations in the mitochondrial structures and functions, bridging the gap in the mitochondrial dynamics of MMA.

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# Are we measuring the right peptides? Unveiling cross-reactivity in natriuretic peptides immunoassays

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Natriuretic peptides (NPs) are cardio-specific biomarkers routinely measured using immunoassays for the diagnosis and prognosis of heart failure (HF)1. However, immunoassays suffer from significant limitations, including cross-reactivity with truncated endogenous NP forms, lack of standardization across platforms, and variability in diagnostic cut-offs. In particular, B-type natriuretic peptide (BNP1-32) measurements are frequently affected by degraded forms, potentially leading to an overestimation of biologically active BNP and contributing to the "endocrine paradox" in HF diagnostics2. In this study, we evaluated the specificity of four immunoassay kits, two for Atrial natriuretic peptide (ANP1-28) and two for BNP1-32, by assessing their cross-reactivity with structurally similar NP fragments. Using a newly developed ultra-high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) platform3, we quantitatively determined the extent of cross-reactivity and its impact on immunoassay results. Our findings demonstrates that immunoassays may overestimate biologically active NP concentrations due to the presence of degraded peptides, underscoring the need for more precise methodologies in clinical diagnostics. Furthermore, the determination of half-maximal inhibitory concentration (IC50) values for ANP, BNP, and their degraded forms provides deeper insights into how cross-reactivity affects measurement accuracy. The novel mass spectrometry-based platform demonstrated superior specificity, effectively distinguishing active NPs from their truncated counterparts. This innovative approach offers a more reliable alternative for NP quantification, paving the way for enhanced accuracy in HF biomarker assessment and improved clinical decision-making.

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# A Proteomic Workflow that Combines Mass-Spectrometry and Drug-Repurposing to Find New Candidate Drugs for Soft-tissue Sarcoma

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Soft-tissue sarcoma (STS) is a rare and aggressive cancer that originates from mesenchymal tissue.
Its aggressiveness is mostly related to its high metastasis rate and the unsatisfactory efficacy of traditional therapies [1]. Thus, there is an urgent need to find new therapeutic strategies. In this context, drug repurposing represents a valid alternative approach that allows to reduce the time and costs of pharmaceutical research in which new applications are identified for drugs already approved or under investigation for different diseases [2].
In this research, new therapeutic strategies for the treatment of STS were discovered using a combined proteomic-bioinformatic approach. The proteome of 26 STS and paired healthy tissue were obtained with a nano Ultimate 3000 coupled to an Orbitrap Exploris 480 equipped with FAIMS. The comparison of cancer/healthy proteome allowed the identification of specific over-expressed/under-expressed proteins and pathways. Druggable proteome was explored using Drug Bank database. Promising drugs were selected based on localization of protein targets, type of molecule and pharmacological activity, and on previous clinical and biological data on cancer. Molecular docking performed on six small molecules that had not been previously tested for sarcoma treatment identified promising novel putative therapeutic approaches, while the in vitro validation on sarcoma cancer cell line showed that three drugs are able to kill sarcoma cancer cells also in vitro. Finally, using a proteomic approach performed on treated sarcoma cells, the mechanisms of action of the three putative drugs were elucidated. In conclusion, here we showed that a combined proteomic-bioinformatic approach is a valuable tool for the discovery of new therapeutic strategies.

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# Enhancing proteomics characterization of thyroid nodules: a pixel-classifier for mass spectrometry imaging analyses

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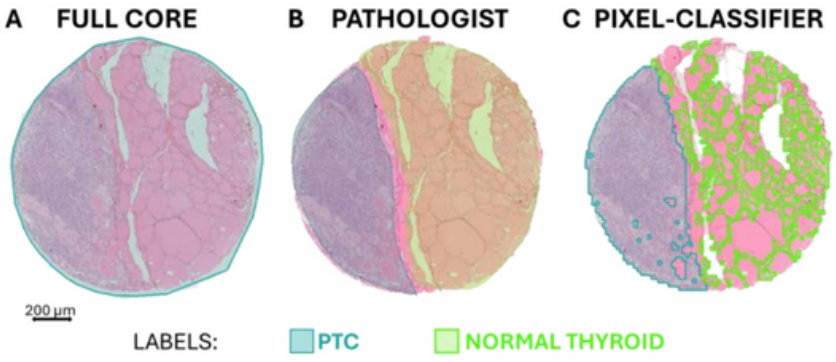
Matrix-assisted Laser-desorption Ionization Mass spectrometry imaging (MALDI-MSI) has shown promise in the molecular characterization of thyroid neoplasms [1]. Yet challenges remain in minimizing signal interferents and improving diagnostic discrimination. In this study, we propose an interdisciplinary approach integrating digital pathology with spatial proteomics to enhance MALDI-MSI analysis of thyroid lesions from formalin fixed-paraffin embedded (FFPE) tissue sections. To do so, a pixel-classifier [2] was built in QuPath [3] and employed to automatically select cell-rich regions of interest (ROIs) from hematoxylin and eosin (H&E) stained tissue microarrays (TMA), reducing interference from colloid-rich areas. Then we compared proteomics signals obtained from the full core (FC) areas, manually annotated from the pathologist (PAT) and those obtained with the pixel-classifier (PC). Hence, compared to conventional manual annotation approaches (PAT) and FC data, PC ROIs significantly decreased interfering signals (~15%) while increasing the S/N of tryptic peptides (≈ +37%). This resulted in a greater number of detected m/z signals (+9-24\%) and improved spectral clustering when performing Principal component analysis (PCA) to distinguish different histopathological regions. Receiver operating characteristic (ROC) analysis further confirmed the improved classification power, with a 50% increase in discriminatory m/z features among across different thyroid nodules diagnosis compared to conventional FC and PAT data.

Unsing a pixel-classifier to select cell-specific regions globally enhances reproducibility, reduces operator workload, and optimizes MALDI-MSI workflows. Altogether, the approach proposed paves the way for more accurate molecular characterization of thyroid neoplasms and holds potential for improving biomarker discovery and diagnostic precision in clinical pathology.

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# Integration of mass cytometry imaging and glyco-imaging to characterize B cell follicle dynamics

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The use of spatial mapping tools allows for a better understanding of tissue processes in pathophysiology. However, due to specific platform-to-platform incompatibility, protocols that maximize output from a single tissue section are lacking. Moreover, a central mechanism for regulating immunoglobulin (Ig) effector functions is through Fc glycosylation.

Here, we present a workflow for the sequential imaging of the same tissue section using matrix assisted laser desorption ionization (MALDI) and multiplexed ion beam imaging (MIBI). We first profiled N-glycosylation on human lymphatic tissues to investigate how Ig Fc glycosylation is regulated within B cell subpopulations. We stained the same tissue sections to quantify approximately 30 protein markers, targeting the single-cell composition of B cell follicles. After spatial registration, the imaging data was combined into a single dataset where each pixel retains bimodal information (N-glycans and MIBI probes) from the same tissue section. By using MIBI markers to guide the analysis throughout the follicular regions (i.e. dark/light zone, mantle zone), we studied the dynamic changes in mannosylation, fucosylation and sialylation across unique B cell populations in situ.

Overall, these findings streamline a strategy that allows mass spectrometry imaging by MALDI, high-definition spatial proteomics by MIBI and H&E on the same tissue section. Moreover, it clarifies how Ig glycosylation is structurally organized during the follicular reaction.

# Metabolomic approach for unraveling novel Gaucher disease biomarkers on dried blood spot

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Gaucher disease (GD) is a rare, inherited, and autosomal recessive disorder caused by mutations in the GBA1 gene leading to a deficiency of a lysosomal enzyme, acid β-glucocerebrosidase, and glucosylsphingosine (Lyso-Gb1) toxic overload. GD diagnosis and its subsequent management are very challenging due to the wide spectrum of symptoms and complications. The introduction of enzyme replacement therapy (ERT) 30 years ago was considered a great revolution in the treatment of GD patients. However, the exact pathogenetic mechanisms are not completely known, thus a deeper comprehension is required to provide valuable alternatives for its management. In this context, we performed an untargeted metabolomics approach to enable novel biomarker discovery and to profile the phenotypical status produced by the genetic background directly on dried blood spot (DBS) of patients.
Metabolites from DBS specimens of a GD patient carrying a homozygous c.1448 T>C mutation, before and after ERT, and a healthy control at pediatric age were analysed with Orbitrap ExplorisTM 120 for untargeted metabolomics strategy. The molecular features have been investigated first for statistics through Compound Discoverer, then for further combined functional analysis using Ingenuity Pathway Analysis (IPA).
Overall, more than 500 metabolites were robustly identified and relatively quantified. Among the known GD biomarkers, routinely used in the clinical practice, LysoGb1 was confirmed as overexpressed at baseline before ERT, while they decreased after ERT, demonstrating the robustness of our method as proof of concept. Simultaneously, among the other features with the same trend, a new putative biomarker emerged from the metabolomics approach, that is likely compatible with 2-deoxy-2,3-dihydro-N-acetylneuraminic acid, a ganglioside derivative.
Moreover, functional analysis revealed the modulation of inflammatory pathways involving several classes of sphingolipids, which are reverted by ERT. In conclusion, the DBS untargeted metabolomics strategy, can help unravel the pathogenesis of GD, the most common sphingolipidosis in the Caucasian population, by identifying new biomarkers and therapeutical targets, and supporting the disease management over time.

# Using the power of timsTOF fleX to study the N-glycome extracted from dried blood spot

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The study of N-glycans has emerged as crucial in the context of human health and medicine, considering the role of this class of biomolecules in determining protein folding, localization and degradation.
Due to their composition (a small group of monosaccharides with two possible anomeric configurations and different potential linking sites), N-glycans are structurally heterogeneous molecules. They exhibit a strong structure-function correlation, so it's fundamental to study them in their native configuration, keeping in mind that they are often found as isomers [1]. The timsTOF fleX is by logic one of the best instruments to study the N-glycome, thanks to its high sensitivity and specificity. Coupling nano-flow untargeted liquid chromatography (nanoLC), trapped ion mobility and mass spectrometry give us a very strong analytical power, not to mention the PASEF technology we have available, which significantly increases the number of annotated features [2].
We adapted a nanoLC method found in literature [3] to a shorter analytical time suitable for our needs, and we are currently working on optimizing the ion mobility-mass spectrometry parameters. Once the method is proven solid on N-glycans NIST standards, the first goal of the project will be creating a N-glycans library from dried blood spot (DBS) samples, a type of microsampling devices that has proven to be effective and cost-reductive in multi-omic studies [4]. The following step will consist in the multi-omic integration of glycomics data with lipidomics, metabolomics and proteomics datasets derived from the same samples, in order to open a new multi-omic path for personalized medicine.

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Life Science

# From birth to term: an integrated proteomic and lipidomic profiling of preterm neonatal development

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Background: Preterm birth disrupts key stages of fetal development and forces newborns to adapt to extrauterine life before full physiological maturation [1]. This early transition increases the risk of neurological and metabolic complications, while activating complex compensatory processes that remain poorly characterized. In this study, we applied a mass spectrometry-based multi-omics approach to longitudinal plasma samples from very preterm infants (< 32 weeks of gestation), aiming to identify molecular signatures of early postnatal development that could guide personalized care and improve clinical outcomes.

Methods: We collected 74 plasma samples from 16 preterm neonates at five time points from birth to term-equivalent age (TEA). Using only 5 µL of plasma for proteomics and 20 µL for lipidomics, we enriched low-abundance proteins via the Mag-Net method [2] and extracted lipids using a single-phase butanol/acetonitrile protocol [3]. Data were acquired on high-resolution mass spectrometers and processed with dedicated software. Time-dependent molecular changes and their associations with clinical variables were explored through statistical tests and network-based analyses, including Weighted Gene Co-expression Network Analysis (WGCNA) [4].

Results: Over 1500 proteins and 600 lipids were identified, showing clear temporal trends. Proteomics revealed progressive maturation of immune-related functions and metabolic pathways. Lipidomics showed dynamic changes in sphingolipids, phospholipids, and acylcarnitines, reflecting evolving energy demands and inflammatory states. Co-expression analysis identified modules correlating with developmental stage and key clinical traits such as the presence of brain lesions.

Conclusions: This is, to our knowledge, the first study to integrate untargeted proteomics and lipidomics on longitudinal plasma samples from very preterm infants using such minimal volumes. Despite the technical challenges, we successfully captured coordinated molecular adaptations during early postnatal development. This integrated, systems-level view highlights potential biomarkers and points to new opportunities for more personalized care — including nutritional and metabolic support — to help improve long-term outcomes in this highly vulnerable population.

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Life Science

# An in-depth mapping of CSF protein complexes in ALS

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The development of effective therapeutic strategies for Amyotrophic Lateral Sclerosis (ALS) is a challenge for translational research. The identification of new molecular features characterizing ALS clinical forms could be the starting point to generate novel pathogenic hypotheses, that could then be exploited for the development of new effective drugs. In neurodegenerative diseases like ALS, the uncorrected protein folding, and the formation of aggregates is one of the most important features. In particular, the loss of proteostasis and the wrong assembly of the protein in their native form leads to toxic molecules that might cause an overload of the degradation machinery. Given this, the misfolding of CSF proteins could lead not only to a wrong protein assembly, which results in the formation of toxic complexes, but also to the absence of complexes fundamental for the physiological activities. However, due to the difficulties in performing global analysis of protein complexes, the literature on in vivo studies is still limited or lacking. The possibility to detect these molecular interactions would open new perspectives for the study of the pathogenesis and the progression of this deadly disease. For this reason, the present work aims to map CSF protein complexes from ALS patients and subjects without neurodegenerative conditions, with the aim of identifying new molecular targets involved in ALS pathogenesis and progression.

We have recently developed a new method that uses size exclusion chromatography under native conditions, mass spectrometry and bioinformatic analysis to untargeted investigate CSF complexes. The method was applied to our cohort of patients, allowing the detection of key protein complexes involved in neurodegeneration, neuro-inflammation, oxidative stress, synaptogenesis, protein aggregates 'clearance and stress-induced response, which were significantly altered in ALS. In addition, several newly inferred protein complexes were detected.

In conclusion, our preliminary data could provide a better understanding of ALS pathogenesis, and, importantly, the identification of new therapeutic targets.

# Advances in thyroglobulin measurement: exploring dried blood spot mass spectrometry for enhanced clinical utility

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In contemporary medical practice, human thyroglobulin (Tg) represents the primary tumour biomarker for detecting the recurrence of differentiated thyroid carcinoma (DTC) in patients who have undergone thyroidectomy[1]. Tg is a large and highly glycosylated tissue specific protein exclusively produced by both healthy and tumour thyroid follicular cells in the thyroid gland [2]. Different techniques, including immunometric assays (IMA) and radioimmunoassays (RIA), have been implemented in clinical settings to gauge Tg levels in blood samples collected through venipuncture [3]. However, the reliability of these methods is compromised by the presence of antibodies, including antithyroglobulin antibodies (TgAbs) and heterophile antibodies (HAs), resulting in frequent inaccuracies in the quantification of T due to either the under or overestimation of the actual values [4,5]. In recent years, liquid chromatography tandem mass spectrometry (LC-MS/MS) has emerged as a distinctive and alternative tool aimed at overcoming the challenges posed by antibody interference [6]. Despite its potential, the effectiveness of LC-MS/MS has yet to be fully explored and, if performed, could improve our knowledge regarding the potentiality of this tool for the detection of Tg [3,6].

In this work, we present a workflow, based upon LC-MS/MS and Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA®) technology, to quantify serum Tg, indicating greater sensitivity and specificity compared to the routinely available protocols. Moreover, this workflow is also currently being translated and tested for use with samples obtained with dried blood spot (DBS) devices, offering a simple, cost-effective, and minimally invasive alternative to venipuncture [7]. Based upon these findings, this LC-MS/MS and SISCAPA®-based approach not only shows the potential for improving the accuracy of Tg quantification but may also simplify this process for patients living in remote areas who could independently collect DBS samples for Tg monitoring.

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# Lipidome of extracellular vescicles in hypoxic pancreatic cancer

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Pancreatic cancer has the worst prognosis among all cancers. Some cancer types, including pancreatic cancer, remain asymptomatic in early stages, making early diagnosis challenging with established screening methods. Pancreatic ductal adenocarcinoma (PDAC), which accounts for approximately 90% of pancreatic cancers, is predominantly diagnosed at an advanced stage, resulting in the lowest five-year survival rate (7%) among all cancers [1]. Different ways are used to diagnose pancreatic cancer in clinical practice like magnetic resonance imaging, computed tomography, endoscopic ultrasound, and positron emission tomography. Additionally several types of blood tests were considered for PDAC screening [2], such as carbohydrate antigen (CA) 19-9, either alone or in combination with other blood proteins. Recent studies suggest that the analysis of circulating tumour DNA and extracellular vesicles shows a potential for the diagnosis of PDAC, which are currently under investigation.
This work focuses on the lipidome of extracellular vescicles (EVs), paying particular attention to exosomes. EV is defined as a lipid bilayer particle that is naturally secreted by cells into extracellular microenvironment without replication capability. EVs are generally divided into three groups: exosomes (diameter from 30 to 150nm), microvesicles (diameter from 50 to 1000nm) and apoptotic bodies (diameter from 500 over 1000nm) [3]. To identify and characterize biomarkers for PDAC, lipid levels and their composition in exosomes have been investigated. The study has been performed on samples obtained from cellular line PANC1 (immortalized PDAC cells), collected and filtered through 100kDa MWCO filters. A total exosome isolation solution was added and left to react overnight and then vesicles were collected through exospin columns. To cell pellet, lipid extraction following the Bligh & Dyer protocol was performed and the samples were analysed by hydrophilic interaction liquid chromatography (HILIC) coupled with electrospray ionization high-resolution Fourier-transform mass spectrometry (ESI-FTMS). Furthermore, a mild alkaline hydrolysis (1 h at 37 °C) was carried out to study ceramides and cerebrosides by separation on reversed phase liquid chromatography (RPLC)-ESI-MS. In this contribution, preliminary results on the lipidome characterization of EV from PANC1 are reported.

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Life Science

# Proteomic insights into Therapy-Induced Senescence in lung cancer: a DIA-PASEF Mass Spectrometry approach

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Therapy-induced senescence (TIS) is a common response to chemotherapy, exerting both pro- and anti-tumor effects [1,2]. It is well established that the accumulation of senescent cells triggers the production of the senescence-associated secretory phenotype (SASP), which can create an immunosuppressive and pro-tumorigenic environment. [3,4]. Understanding these mechanisms and identifying therapy-specific biomarkers and functional signatures could help in developing strategies to eliminate senescent cancer cells and prevent therapy-induced tumor progression.

This study employed a mass spectrometry (MS) approach combining Data-Independent Acquisition (DIA) and ion mobility techniques to analyse the proteome of lung adenocarcinoma A549 cells and IMR-90 fibroblasts, used as a non-tumor control, following treatment with two senescence-inducing chemotherapeutics: Doxorubicin and Cisplatin.

Following Doxorubicin treatment, we excluded from the statistical analysis possible confounding factors related to senescence in non-tumoral cells and we identified 146 and 175 proteins specifically overexpressed in proliferating and senescent A549 cells, respectively. Among the latter, STAT1 and STAT2 were notable due to their involvement in interferon signaling, a key modulator of tumor growth. Additionally, the study explored connections between senescence, Doxorubicin resistance, and immune evasion, identifying four lysosome-associated proteins (NPC2, SAP, PLD3 and SPHM) with potential roles in autophagy.

In the next phase, the same approach was used to investigate Cisplatin-induced senescence, revealing 175 and 208 proteins specifically upregulated in proliferating and senescent A549 cells, respectively. The role of these proteins in TIS, as well as similarities and differences between Doxorubicin- and Cisplatin-induced senescence, will be explored.

These findings will provide deeper insights into lung cancer senescence, increasing our understanding of drug-specific mechanisms and opening the way for biomarker discovery and personalised therapeutic strategies.

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# Unraveling the Plasma Lipidome: How Physical Activity Shapes Lipid Metabolism in Cancer Patients

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Background: Despite the known health benefits of physical activity (PA) [1-3], its molecular mechanisms are not fully understood. While cytokines, miRNAs released from contracting muscles, and inter-organ communication via extracellular vesicles have been proposed [4,5], the role of circulating lipids in mediating PA benefits remains unexplored [6].
Aim: To investigate the impact of PA on the plasma lipidome in active and non-active adult male individuals with gastric cancer.
Methods: Mass spectrometry-based lipidomics approaches were applied to the analysis of plasma from 20 cancer patients with different fitness levels.
Results: No differences in plasma cholesterol and triacylglycerol (TG) levels were observed between active and non-active individuals, but variations in lipidome profiles were noted, particularly in the abundance of neutral lipids and phosphatidylcholines (PC). Key discriminators of PA included 3 fatty acylcarnitines (AcCar), 1 diacylglycerol, 15 TG species, 2 PC species, 2 lysophosphatidylcholines (LPC), 1 ceramide (Cer), and 1 sphingomyelin species. Levels of TG and AcCar decreased with PA, indicating their use to support its energetic demands, as well as pro-inflammatory lipids such as Cer and LPC.
Conclusions: Our findings may uncover new pathways through which lipid metabolism mediates the benefits of PA in cancer patients, supporting the implementation of exercise programs in cancer management.

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# Exploring 6-Aza-2-Thiothymine as a MALDI-MSI Matrix for Lipid Mapping in FFPE Samples

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Proteins and nucleic acids have historically been the primary research focus on human pathology mechanisms. However, recent studies have highlighted the critical role of lipids as regulators in various disease processes, including cancer. While lipids are well-known for their function as components of cell membranes and energy sources, their involvement in cancer pathogenesis, progression, and outcomes has become increasingly recognized [1]. This has led to the emergence of cancer spatial lipidomics as a rapidly growing research field. Matrix-Assisted Laser Desorption/Ionisation- Mass Spectrometry Imaging (MALDI-MSI) has further expanded, enabling the mapping of spatial distribution and relative abundance of analytes within tissues, including lipids in solid tumours [2]. Formalin fixation and paraffin embedding (FFPE) is the standard method for preserving tissue samples, enhancing histological quality. However, this process results in the depletion of several lipid species, complicating lipid analysis [3]. Nevertheless, recent advancements in the MALDI-MSI technique hinted the possibility to still extract and map solvent-resistant lipids, mainly represented by phospholipids, which still hold biomedically relevant information [4]. The choice of MALDI matrix plays a crucial role in lipid extraction and ionization, influencing molecular coverage and sensitivity. While matrices like 2',5'-dihydroxybenzoic acid (DHB) and Norharmane (NOR) are commonly used for lipidomic analysis, the optimal matrix depends on the specific research objectives [5]. In this study, we aim to investigate the potential of using 6-aza-2-thiothymine (ATT) as the matrix for mapping lipid species with MALDI-MSI, given that ATT has already proven to be an optimal matrix for peptide analysis [6]. As a proof-of-concept, we evaluated ATT's performance in lipid analysis on FFPE mouse brain sections using both positive and negative ion modes, comparing the results with those obtained from other widely used dual-polarity matrices. These analyses demonstrate that ATT matrix could be a possible alternative to traditional matrix employed for lipidomics MALDI- MSI analysis. Moreover, we applied ATT on tissue microarrays of various cancer types, including colorectal cancer (CRC), breast cancer (BRCA), clear cell Renal Cell Carcinoma (ccRCC), and glioblastoma (GB), to assess the capability of ATT to ionise lipids in different pathological tissue types.

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# Mass Spectrometry Approach reveals differential ER remodeling by FAM134B isoforms during myogenesis

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The endoplasmic reticulum (ER) in muscle cells, also known as sarcoplasmic reticulum, undergoes significant remodeling to form a compact, organized, and specialized membrane network throughout the entire length of the myotubes [1]. ER-phagy, a catabolic process, profoundly impacts ER morphology through the selective elimination of discrete ER portions via lysosomal degradation. This selective form of autophagy is crucial for maintaining basal cellular homeostasis and is further upregulated by stressors such as protein misfolding or calcium imbalance [2].

ER plasticity and ER-phagy are intertwined processes essential for maintaining ER dynamics. The formation of an extensive ER network with highly specialized structures is one of the hallmarks of muscle differentiation thus suggesting that myogenesis involves ER remodeling. However, molecular mechanisms of this process remain poorly characterized.

To achieve this, we employed an integrated approach that leverages C2C12 myoblasts as a cell model, combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) proteomics, to generate a quantitative landscape of ER proteome reshape during myogenesis. Our findings emphasize the crucial role of ER-phagy in redesigning ER membranes during myoblast differentiation, with ER-phagy receptor FAM134B emerging as a critical player in maintaining ER proteostasis and facilitating ER remodeling. Notably, we identify FAM134B2, a poorly characterized isoform of FAM134B as essential in ensuring proper ER homeostasis in muscle cells.

During differentiation, myoblasts downregulate FAM134B1, via lysosomal degradation while transcriptionally upregulating FAM134B2, highlighting its role in maintaining ER dynamics and promoting myotube formation. The shift favoring FAM134B2 plays a crucial role in regulating ER morphology during myogenesis.

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# Metabolic and Lipidomic Responses to Hyperoxic recovery during Hypoxic Training in Elite Alpine Skiers

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Metabolomics is a powerful approach to explore molecular adaptation to physical exercise, enabling physiological responses evaluation and training/recovery strategies optimization, especially in elite athletes.

This study investigates the effects of hyperoxic recovery after interval training in hypoxic conditions on plasma metabolome and lipidome in alpine ski athletes, focusing on how oxygen availability influences metabolic responses and recovery. Eleven male alpine ski athletes completed two different training on two separated days, interspersed by a two-weeks wash-out period. After an adaptation phase that simulated 3500m altitude, the participants completed interval training exercises on both days, but with two different recovery strategies: the subjects either breathed 100 % oxygen (HS) or hypoxic air (NHS) on day 1 and day 2, respectively. Plasma samples were collected before exercise, immediately post-exercise, and seven days post-exercise, and analyzed with untargeted MS-based metabolomics and lipidomics approaches.

Immediately after training, fatty acid and branched-chain aminoacid oxidation, TCA cycle, and carnitine synthesis were upregulated in HS, indicating a shift in energy metabolism, while in NHS gluconeogenesis and glucose-alanine cycle pathways increased, suggesting a sustained reliance on glucose in hypoxia. After one week, differences between HS and NHS became more pronounced, particularly in NAD metabolism-related pathways, reflecting an adaptive response to hypoxic exercise modulated by oxygen supplementation.

Lipidomics results showed that sphingolipid metabolism decreased immediately after training but increased after one week, suggesting a protective role in hypoxic conditions. As expected, diacylglycerols (DG) and triacylglycerols (TG) decreased immediately post-exercise independently from oxygen concentration. Seven days after training TG returned to baseline level in NHS but not in HS, suggesting hyperoxia may dysregulate lipid release in blood stream. Taken together, the results indicate that oxygen availability significantly impacts metabolic and lipidomic adaptations to high-intensity exercise, highlighting the need for careful evaluation of oxygen supplementation in altitude training.

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# Metabolomic Investigation on Tear Fluid for the Identification of New Biomarkers of Glaucoma Progression

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Glaucoma is a progressive optic neuropathy characterized by the degeneration of retinal ganglion cells, which can lead to irreversible blindness. Monitoring the disease Rate of Progression (ROP) is an important tool in the clinical setting to continuously reconsider the therapy prescribed to the patient 1. The lack of effective prognostic tests compels us towards the necessity to find new possible biomarkers of disease progression.

Here we implemented an untargeted exploratory metabolomics strategy via LC-MS/MS to highlight potential new molecular biomarkers of ROP in tears of glaucoma patients; in particular, the tear fluid of 48 patients was analyzed, 25 patients with slow ROP and 23 with fast progression. Tear metabolites were extracted from imbibed Schirmer strips using a solution of ACN/MeOH/H2O in a ratio of 2:2:1. After collecting the supernatant, it was dried using the Speedvac and frozen at -80°C. Each dry residue was resuspended using a solution containing 95:5 H2O/ACN + 0.1% HCOOH and injected into the mass spectrometer. Chromatographic separation was performed on a C18 column, running for 30 minutes at a constant flow rate of 0.300 ml/min. The analytical batch was preceded by the acquisition of pooled samples using the DeepScan AcquireX tool, which allows the identification and effective characterization of tear sample compounds by means fragmentation spectra library implementation through artificial intelligence algorithm. After, tear samples were acquired individually using Full Scan method, without fragmenting precursor ions, in both ionization modes. Raw files were processed by Compound Discoverer software to perform both statistics and quantification of the identified features by applying fast over slow ROP groups ratio.

The metabolites annotated through the database were 282, but the dataset upload to the bioinformatic software Ingenuity Pathway Analysis (IPA) was possible for only 212 metabolites, which could be associated with unique codes usable by the software (HMDB, KEGG, LipidMaps). IPA was used to perform functional analysis of metabolite expression, which granted us highlight modulated molecular pathways and potential prognostic ROP biomarkers. In particular, an alteration of fatty acid metabolism was observed in patients with fast/dramatic ROP. Moreover, pharmacological therapy in patients with slow ROP appears to be not very successful because the mechanisms of neurodegeneration in the nervous tissue are more evident. The confirmation of these preliminary results could open the door to new possible biomarkers of disease progression push toward the implementation of a personalized therapy for patients.

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MS Technology

# Synthesis of novel reactive MALDI matrices for the analysis of low molecular weight compounds

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Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) is a powerful and easy-to-perform analysis technique that proved to be useful in a vast field of applications. However, the application of this technique to low molecular weight compounds has stayed challenging due to the spectral interferences produced by conventional organic matrices in the low m/z range. To overcome this issue, alternative methodologies based on rationally designed organic matrices have been proposed[1].
The purpose of this study was to synthesize a new functionalized MALDI matrix able to react with small compounds, such as amines or alcohols, with the aim of analyzing these low-mass compounds in a biological sample avoiding interfering matrix background. The reaction was carried out by nucleophilic substitution of two different p-hydroxy compounds, 4-hydroxybenzoic acid (4-HBA) and alpha-cyano-4-hydroxycinnamic acid (CHCA), with a 2,4,6-trichloro-1,3,5-triazine (TCT) in the presence of a base[2]. Trichloro-triazine, also known as cyanuric chloride, has reported a marked reactivity toward hydroxyl and amine groups[3]. The resulting product of the reaction was characterized by ATR-IR and NMR spectroscopies and MS analysis. Thus, the other two chlorines on TCT-CHCA or TCT-HBA can further react with amines or alcohols allowing to increase the molecular weight of these small compounds towards higher m/z ratio.
The newly obtained compounds, TCT-CHCA or TCT-HBA, were tested with different amino acids, such as phenylalanine, glycine, isoleucine and cysteine. Compared to conventional CHCA, clean spectra without interfering matrix-related ions were obtained. The novel matrix was also applied to complex sample mixtures such as amino acids-based supplements and a blood/plasma extract to verify its reactive properties for LMW compounds detection. Preliminary results are reported in this communication.

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# Bruker GlycoTyper™: A novel MALDI-MS-based solution for exploiting the power of targeted glycomic analysis as a new source of biomarkers in liquid biopsies -- Systemic Lupus Erythematosus disease course classification as a case-in-point

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Systemic lupus erythematosus (SLE) is a chronic autoimmune condition characterized by autoantibody-driven immune-complex formation. Lupus nephritis (LN) results from immune-complex deposition in the glomeruli, leading to inflammation, tissue damage, and kidney failure. Early diagnosis and immunosuppressant treatment of LN are crucial to prevent end-stage renal disease. While renal biopsy is the standard for LN diagnosis, alternative liquid-biopsy approaches are urgently needed.

To study changes in immunoglobulin N-glycan expression during lupus, the GlycoTyper™ platform, a MALDI-MS-based method for N-glycan analysis, was applied to urine samples from 114 healthy controls (HC), 116 SLE patients, and 210 LN patients. Anti-IgG antibody arrays were printed on amine-reactive slides, and multi-well modules enabled the analysis of 16 patient samples per slide. N-glycans were released from captured IgG using PNGase F and analyzed by MALDI-MS. A quality strategy consisting of pre-acquisition system suitability tests and on-slide QC arrays ensured robustness of the platform that demonstrated linearity down to low-double-digit picomolar abundancies of individual glycan moieties.

A multiclass random forest classifier, using abundancy-profiles of 26 N-glycans and 2 demographic characteristics (age and sex), achieved an area under the receiver operating characteristic curve of 0.89 for differentiating LN patients from HCs and SLE patients without kidney disease. The model showed 91% sensitivity and 90% specificity, respectively, on a held-back testing/ validation sample set. N-glycan intensities were normalized and transformed to centered log ratios (CLRs). While the classifier differentiated LN patients from HC and SLE patients using IgG-derived N-glycans from urine samples, N-glycan profiles from HC and SLE patients showed no significant differences. Further analyses of longitudinally collected samples identified sets of N-glycans associated with disease course as well as with response to immunosuppressive treatment, demonstrating the potential of using these N-glycan profiles for both disease progression monitoring as well as therapy-guidance, in addition to early risk assessment.

We conclude that MALDI-MS-based glycoproteomic characterization of urinary IgG offers a novel tool to differentiate patients with indolent SLE from those likely to progress to develop LN, to monitor disease course, and potentially to prognosticate treatment response in LN patients.

MS Technology

# 20years of Biphenyl

**Emanuele Ceccon**

*Restek S.p.A.*

On the occasion of the 20th anniversary of Restek's creation of the biphenyl stationary phase, we will delve into the mechanisms of Pi retention, Polarizability and dispersion through the analytical key: Is the biphenyl phase the new c18?
Starting with the chemistry underlying the many different c18 phases we will move forward in understanding how much and if the biphenyl phase can make our reverse phase analytical approach easier and faster, especially when it come about mass spectrometry ,we will do so by delving into the chemistry underlying the biphenyl phase itself, so as to ensure the best understanding.

# The PerkinElmer QSight 500, Ultimate Robustness for Dirty Matrices - Unique Source Design for Reduced Sample Preparation

**Alessandro Fabiani**

*Perkin Elmer*

The PerkinElmer QSight® 500 LC/MS/MS System offers a highly sensitive and productivity-oriented triple quadrupole mass spectrometry solution, specifically engineered to meet both routine and regulatory analytical demands across food safety, environmental, and industrial applications. In combination with the QSight LX50 UHPLC, the system addresses the growing need for robust, high-throughput analysis in complex and contaminant-rich sample matrices.

At the core of QSight's performance is its unique dual-source configuration, enabling flexible combinations of Electrospray Ionization (ESI), Enhanced Desolvation and Ion Transport (EDIT), and Atmospheric Pressure Chemical Ionization (APCI), with automated probe switching and rapid polarity transitions. This versatility supports simultaneous detection of a broad polarity range of analytes and facilitates fast method development.

Crucially, QSight is designed to deliver ultimate robustness for dirty matrices, thanks to its innovative StayClean™ Technology. This system integrates a coaxial flow electrospray source, the Hot-Surface-Induced Desolvation (HSID™) interface, and Laminar Flow Ion Guides™, collectively minimizing contamination from matrix components. The coaxial design reduces ion suppression and turbulence, while the HSID™ ensures efficient desolvation with minimal fouling—drastically reducing the need for extensive sample cleanup and routine maintenance.

The result is a system that can tolerate high-matrix loads while maintaining consistent performance.

Laboratories benefit from up to 15% increased instrument uptime, translating to approximately 35 additional operational days per year, without sacrificing sensitivity or precision. Moreover, ion transfer through laminar gas flow instead of electric fields eliminates susceptibility to drift and reduces re-optimization requirements.

Finally, the UniField™ Detector enables rapid polarity switching in microseconds and near-simultaneous detection of positive and negative ions—critical for fast-paced, multi-target workflows.

Together, these features establish QSight as a leading platform for high-throughput laboratories seeking exceptional robustness, reduced sample preparation, and minimal downtime when analyzing complex or "dirty" matrices.

# Ultra-trace elemental analysis is carried out using ICP-MS and HR-ICP-MS, with future advancements expected through the integration of a laser ablation system for direct solid sample analysis

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Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is among the most suitable techniques for material screening due to its versatility and numerous advantages. These include ultra-high sensitivity (at the parts-per-trillion level), short analysis times (within days), minimal sample mass requirements (on the order of milligrams), the ability to simultaneously detect multiple elements, and the availability of well-established, efficient analytical methods.

The production of raw materials typically involves a series of chemical processes and treatments. To optimize production protocols from a radiopurity perspective, it is essential to understand the impact of each processing step on the final product. ICP-MS is uniquely capable of monitoring contamination across all stages of production. For instance, through progressive chemical digestions, it can distinguish between bulk and surface contamination—something other screening techniques cannot achieve.

To obtain accurate and reproducible results, maintaining a contamination-free working environment is critical. Even trace levels of environmental pollutants can compromise the analysis. For this reason, cleanroom environments are ideal for both sample preparation and ICP-MS measurements.

Advanced capabilities are provided by High-Resolution ICP-MS (HR-ICP-MS), which can effectively resolve spectral interferences caused by overlapping isotopes, oxides, and doubly charged ions, leading to more precise elemental quantification.

Furthermore, with the recent installation of Laser Ablation ICP-MS (LA-ICP-MS) at LNGS, it is now possible to perform direct analyses on solid samples. In this technique, a focused laser beam ablates the sample surface, and the resulting aerosol is transported into the ICP-MS system. This allows for spatially resolved measurements and significantly reduces the need for complex chemical sample preparation.

Environment and Plant Science

# The comparison between Proton Transfer Reaction and Adduct Ionization Mechanism for volatile organic compound analysis in Pisum sativum L.

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Plants are members of communities inhabiting an environment where they can share or compete for water and nutrients, and exchange information with neighboring plants1,2. Plants have evolved various mechanisms to interact with the environment, including the release of volatile organic compounds (VOCs), which can be constitutively or in response to abiotic and biotic stresses2. VOCs are key mediators of plant-plant communication, and their analysis and comprehension provide a promising avenue for real-time health status monitoring, and early disease diagnosis in plants3. While real-time VOC monitoring is well established and commonly performed using Proton Transfer Reaction – Time of Flight – Mass Spectrometry (PTR-TOF-MS)3, its application in crop improvement remains limited. In this study, we investigated constitutive VOC emissions of the pea plant (Pisum sativum L. var. saccharatum) and their variation during early growth stages by using a TOF-MS, which can hold two different reactors: the most common Proton transfer reactor (PTR), and the newly introduced Adduct ionization mechanism – ion molecule reactor (AIM-IMR), (Vocus 2R, Tofwerk)4. The work aims to compare the two different technologies for ionizing sampled VOCs in order to establish which is the most effective for assessing the health status of our model plants. Pea plants were grown in a phytotron with eight climatic chambers. In each growth chamber, two seedlings were potted at a 10 cm distance from a support. Sample air was drawn from the chambers into the Vocus 2R, using perfluoroalkoxy Teflon tubing. VOC variation during early growth stages was investigated using an untargeted metabolomics approach, comparing emissions from day 7 (chosen as baseline) with emissions from subsequent days. Features (i.e., m/z values) with significant changes in abundances were identified imposing an adjusted P-value of t-test lower than 0.05 (5% false discovery rate) and minimum Log2-transformed fold change (FC) of 1.3 in at least one comparison. Results demonstrated that AIM-IMR allowed to acquire a greater number of m/z values than the PTR, considering the total number of extracted m/z values and the significant m/z values resulting from the comparisons. The annotation features with significant changes in abundance showed C7, C10, and C11 compounds and nitrogen species as the most prominent. These results show that AIM-IMR is a suitable approach for real-time VOC monitoring, enhancing the understanding of plant emissions and interactions. This knowledge could contribute to the development of novel strategies for crop protection.

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Environment and Plant Science

# Phthalates diesters in Mediterranean zooplankton

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Phthalates (PAEs) are plasticizers impacting on environment, ecosystems, human and animal beings. PAEs have several applications in many plastic products such as food packaging, medical equipment, electrical wiring, architecture, and construction materials [1].
Identification and quantitative detection of 6 dimethyl phthalate (DMP), di-ethyl phthalate (DEP), di-n-butyl phthalate (DNBP), butylbenzyl phthalate (BBP), di-2-ethylesyl phthalate (DHEP), di-n-octyl phthalate (DnOP) were performed in Mediterranean zooplankton samples. Quantification of the 6 PAEs in the extract was performed with a gas chromatograph (GC TRACE 1310 GC Thermo, USA) coupled a triple-quadrupole mass spectrometer (TSQ 8000 EVO Thermo, USA). The separation takes place through a capillary column and the method employed QuEChERS [2] coupled to ultrasound extraction followed by dispersive solid-phase clean-up with PSA.
LOQ was 20 ng g-1 w.w. for DBP end DEHP and 10 ng g-1 w.w.) for DMP, DEP, BzBP, DOP.
DEHP was recovered in all samples in the range 100 - 5840 ng g-1 wet weight and a mean value of 1753 ng g-1. DMP, DEP, DNBP, BBP, DOP were found < LOQ.
According to literature, DEHP is the prevalent plastic additive found in water environments with documented adverse effects on aquatic organisms [3].

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Environment and Plant Science

# Optimization of LC-Q-TOF Mass Spectrometry and Chromatographic Parameters for the analysis of PFAS: a multivariate approach to maximize sensitivity and resolution

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals, which can exert a range of toxic effects, including a behaviour as 'endocrine disruptor chemicals' (EDCs). Despite the ban under the Stockholm Convention, considering their high persistence in the environment, detectable concentrations can be found in different matrices like water, food, etc. In addition, to replace long-chain perfluoroalkyl acids, ether organofluorinated substances (ether-PFAS) have been synthesised, including GEN-X ($C_6H_4F_{11}NO_3$) and ADONA ($C_7H_2F_{12}O_4$), with the aim of increasing the molecule's solubility and thus degradability by introducing the ether functional group. Indeed, toxicological studies have shown that these synthesised 'emerging PFAS' also have a non-negligible toxicity towards humans and animals, so their detection, quantification and regulation will be necessary in the future [1]. To develop innovative and improved methods aimed at the detection and quantification of PFAS in different matrices, mass spectrometry and liquid chromatography parameters were optimized by employing Design of Experiments (DoE). The selected responses to be optimized were chromatographic peak area (related to sensitivity) and resolution (chromatographic separation), while variables were the following: on the chromatographic side, flow, gradient ramp and column temperature; on the mass spectrometry side, capillary voltage, sheath gas flow and fragmentor voltage. Considering the number of variables, the Placket-Burman (PB) design was chosen using a standard mix of 20 PFAS to identify the more influent variables, with the minimum number of experiments (16, including replicates). In order to reduce the computational effort, a Principal Component Analysis (PCA) was performed on the matrix containing the peak area response. The loading plot of the first two components shows different correlations between the responses, highlighting 4 different clusters of analytes. Thus, the computation of one model for each cluster was performed instead of 20 models for the individual analytes. Coefficient plots show that most variables are significant; in particular, the sheath gas flow was significant with a positive sign for all 4 clusters. The positive effect on the response may be due to either the easier generation of smaller droplets in the ionization process, or the improved focalization of the ions into the spectrometer inlet. In terms of resolution response, unresolved 'critical pairs' were identified, for a total of 9 pairs. Also in this case most variables were significant. Specifically, the flow showed a positive sign: higher mobile phase flow rates probably reduce the phenomenon of longitudinal diffusion, producing narrower peaks and thus higher resolution. The validation of the models was performed by the execution of the experiment at coded level 0. For both peak area and resolution responses, validation by Student's t-test was confirmed for some models, but not for most of them, suggesting non-linear models. Therefore, a response surface DoE will be set, considering the significant variables from PB results in order to optimize sensitivity and resolution. By working in this way, it will be possible to apply the method optimized for analysis of various matrices like water, food or packaging, upon development of an appropriate pre-treatment technique [2].

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Environment and Plant Science

# VOCs communication shapes the kinematic behavior of pea plants in individual and social contexts

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Plants are crucial components of ecological communities, where they engage in both competitive and cooperative interactions 1. They have evolved diverse strategies to explore their surroundings and interact with neighboring plants and insects such as pollinators. Among these strategies, the emission of volatile organic compounds (VOCs) from leaves, flowers, fruits, and roots serves as a key mechanism for communication and resource management1,2. In climbers, the climbing behavior itself may represent an additional adaptive strategy, exhibiting distinct characteristics for individual or social context (i.e., a single plant or two plants potted together), which indicates that competitive and cooperative tendencies are reflected in the way they move 3,4.

In this study, we investigated the climbing behavior of pea plants (Pisum sativum L. var. saccharatum) while moving towards and clasp a potential support under two conditions: (i) an individual condition, where a single plant was potted with a support nearby, and (ii) a social condition, where two plants were potted together with the support positioned equidistantly from them. Plants were cultivated in a phytotron with eight climatic chambers. To assess their responses, we employed an untargeted metabolomics approach based on an adduct ionization mechanism-Time of Flight-Mass Spectrometry (AIM-TOF-MS, Vocus 2R, Tofwerk) alongside 3D kinematic analysis. The latter involved images recorded by two infrared cameras allowing to reconstruct the 3D trajectories of plants movement.

Preliminary results indicate significant differences in VOC emissions for the individual and social conditions, which correlates with distinct kinematic features. This study represents the first integration of behavioral and metabolomic analyses to elucidate the physiological and molecular mechanisms underlying plant interactions. We contend that this novel approach, will provide novel insights into the dynamic processes underlying plants communication and interaction.

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Environment and Plant Science

# Optimization of Mass Spectrometry parameters for Emerging Contaminants detection: A comparative study of Triple Quadrupole and Q-TOF analizers

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Emerging contaminants (ECs) are a broad group of substances whose presence in the environment has attracted the attention of the scientific community over the last two decades [1]. Given the extremely low concentrations of ECs and the presence of interfering substances in environmental matrices, sophisticated instrumentation, such as liquid chromatographic coupled to mass spectrometry, is often required to achieve the necessary levels of specificity, accuracy, sensitivity and precision [2]. In this work, we propose the optimization of MS parameters for the consequent comparison of two HPLC-MS methods involving different tandem mass spectrometry (MS/MS) configurations: QqQ (Triple quadrupole) and Q-TOF (Quadrupole-Time of Flight). A total of 45 analytes were investigated, and due to their diverse properties, a multivariate experimental design was employed to assess multiple factors influencing sensitivity [3]. Chromatographic peak area was used as the response variable. For QqQ method optimization, a Face-Centered Composite Design (FCCD) was applied after a targeted study of MRM conditions for each analyte. Gas Temperature, Gas Flow, and Capillary Voltage were varied within the experimental domain. Model validation confirmed that all variables significantly influenced ionization efficiency, albeit to different extents. Q-TOF optimization required a broader set of variables, since the instrument is equipped with a Jet-Stream source, including Gas Temperature, Gas Flow, Sheath Gas Flow, Capillary Voltage, Fragmentor, and Mobile Phase Flow. Given the high number of factors, a Plackett-Burman screening was first conducted, followed by a FCCD. The most significant variables were Sheath Gas Flow, Capillary Voltage, and Fragmentor. The optimized methods were compared in terms of specificity, sensitivity and precision. The Q-TOF, operating in MS/MS mode, exhibited the highest specificity, which is crucial for complex matrices to minimize interferences. However, the QqQ, while maintaining high specificity, demonstrated significantly lower Limits Of Detection (LOD) and superior precision. The results of this comparison indicate that the QqQ is the optimal choice for the quantitative analysis of ECs in complex matrices. However, it is important to highlight the significant potential of the Q-TOF for suspect screening or untargeted analyses. Therefore, the optimized methods will be applied to the analysis of real samples for different purposes: the Q-TOF will be used for suspect screening, while the QqQ will enable targeted quantification of specific ECs. In conclusion, the experimental design approach proves to be fundamental to optimize the parameters in the two mass spectrometry configurations, allowing a more precise comparison of their performances. This ensures the selection of the most sensitive, specific and robust method for detecting emerging contaminants, offering more effective tools to address environmental challenges.

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Environment and Plant Science

# U-Pb geochronology by mass spectrometry: new insights to the evolution of the Pinerolo-Sanfront Unit, Dora-Maira Massif (Western Alps)

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Mass spectrometry is a highly versatile technique that can be applied to many scientific disciplines and represents an extremely useful tool in geosciences, where it is used to investigate various aspects of Earth's evolution and composition through the chemical and isotopic analysis of rock sample. Its applications include radiometric dating of rocks and minerals, isotopic analysis for reconstructing geodynamic histories, provenance studies of geological and archaeological materials, and environmental monitoring [1]. Among the available techniques, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become an essential tool for deciphering the geological evolution of orogenic chain, due to the high-resolution geochronological in-situ analysis, offering precise constraints on the timing of magmatic and metamorphic events [2]. In this study, we apply LA-ICP-MS U-Pb geochronology on zircon crystals to constrain the crystallization age of a mafic magmatic body from the Pinerolo-Sanfront Unit, the lowermost tectonic unit of the Dora-Maira Massif [3], one of the Internal Crystalline Massifs of the Western Alps. The Pinerolo-Sanfront Unit was classically interpreted as a Permo-Carboniferous metasedimentary sequence (~300 Ma) [4], but our data reveal a significantly older Cambrian magmatic event (~530 Ma), suggesting the presence of either a previously unrecognized fragment of continental basement or an exotic block incorporated during later tectonic processes. Whether it reflects an exotic body or a preserved portion of ancient basement, this finding prompts a partial re-evaluation of the evolutions and characterization of the Pinerolo-Sanfront Unit. The identification of this Cambrian magmatism provides new insights on the tectono-metamorphic evolution of both the Pinerolo-Sanfront Unit and the Dora-Maira Massif as a whole, and enhances both, the characterization and our understanding of the geological history of this portion of the Western Alps. To clarify between these two hypotheses, further U-Pb geochronological studies on both additional mafic bodies and their host rocks will be essential.

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Environment and Plant Science

# Mass Spectrometry and QuEChERS-based Extraction: Tracing Emerging Contaminants in Antarctic marine organisms

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The definition of emerging contaminants (ECs) encompasses a broad range of chemical compounds that are not traditionally monitored in the environment but have been recognised for their potential to cause ecological and human health risks. Despite their possible adverse effects, these compounds have not yet undergone international regulation. Recent scientific studies have confirmed the widespread presence of ECs even in remote areas such as Antarctica, where various classes, including pesticides, pharmaceutical and personal care products and hormones, have been detected [1,2]. The presence of these compounds in polar environments causes concern as the region's extreme environmental conditions (low temperatures, prolonged darkness, and freezing waters) combined with the chemical species' characteristics of poor degradability may lead to prolonged environmental persistence or pseudo-persistence [3]. The accumulation of these contaminants poses significant risks to the Antarctic biota, including potential mutagenic, genotoxic, and endocrine-disrupting effects [4]. The simplicity of the region's trophic chain further exacerbates these risks, as even minor fluctuations in key species populations can trigger cascading effects throughout the Antarctic marine ecosystem [5]. Given the complexity of biological matrices, detecting trace levels of ECs in Antarctic marine organisms presents a significant analytical challenge. This study aimed to develop and optimise an analytical method for detecting ECs at trace levels in Antarctic biota. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method was optimised using samples of Adamussium colbecki, an Antarctic bivalve, as a model organism. Following validation of the methodology with A. colbecki, the protocol was extended to other Antarctic marine species, including Sphaerotylus antarcticus, Odontaster Validus, Trematomus bernacchii, and Laternula elliptica to assess its robustness and applicability. Recovery (R%) and matrix effect (ME%) were used as key performance indicators. The final method demonstrated satisfactory recoveries (42-143%) and acceptable matrix effects (62-108%) across all tested species, confirming its suitability for complex biota samples. The optimised procedure was subsequently applied to environmental specimens collected during Antarctic expeditions from 2001 to 2022, successfully identifying trace levels of contaminants such as triclosan (TCS), perfluorooctanoic acid (PFOA), and octyl-dimethyl p-aminobenzoic acid (OD-PABA). This study presents the first validated QuEChERS method for ECs analysis in Antarctic biota, offering a simple and reliable tool for long-term environmental monitoring and contamination assessment in the Antarctic marine ecosystem.

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# Optimized HPLC-DAD-ESI-QTOF-MS method for simultaneous detection and quantification of abscisic acid and its metabolites in hemp (Cannabis sativa L.) leaf extracts

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ABA (abscisic acid) is a key phytohormone that plays a crucial role in plant development, regulating seed dormancy, germination, growth, and fruit abscission. ABA is also classified as a key phytohormone of plant drought response, where it modulates stomatal closure to reduce water loss and maintain plant turgor. Numerous studies have highlighted the significance of ABA, its conjugate ABA-glucose ester (ABAGE), and its degradation products, phaseic acid (PA) and dihydrophaseic acid (DPA) in plant responses to drought. For this reason, the simultaneous identification of ABA, ABAGE, PA, and DPA is essential for a complete understanding of how plant responses to drought are regulated. Several analytical methods exist for determining ABA and its metabolites/catabolites, including GC-MS, ELISA, LC-MS, HPLC-UV, HPLC-fluorescence or HPLC-chemiluminescence, Raman spectroscopy, and quantitative real time immuno-PCR [1]. However, these techniques have limitations, such as reliance on radiolabeled compounds or hazardous derivatization reagents, poor sensitivity, low resolution, and long run times. Additionally, many phytohormones occur at extremely low concentrations, and do not ionize efficiently in mass spectrometers.

To address these challenges, we propose an optimized HPLC-DAD-ESI-QTOF-MS analytical method that enhances sensitivity through optimized mass spectrometry parameters. This method was validated in hemp (Cannabis sativa L.) subjected to water deficit.

Leaves were sampled, freeze-dried, and extracted for the simultaneous detection of ABA, ABAGE, PA, and DPA. Collision and ionization energies were optimized for each metabolite, and calibration curves were generated using deuterated analytical reference standard solutions at varying concentrations. This approach significantly improves sensitivity and enables accurate quantification of ABA and its metabolites/catabolites at trace levels. Further advancements in mass spectrometry and sample preparation will be essential to extend profiling to deeply understand the hemp response to environmental stresses known to raise endogenous phytohormones levels in plants.

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Environment and Plant Science

# Advancements in Mass Spectrometry for DNA Adductomics: Method Development for Biological Effect Monitoring in Wildlife

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In the Hut of Omics Sciences, recent advancements in mass spectrometry (MS) analysis offer new opportunities for a comprehensive study of DNA modifications, known as DNA adductomics [1]. These modifications, known as DNA adducts, can be used to assess the effects of exposure at the genome level, providing a tool for monitoring biological effects [2].
This study presents a workflow for environmental DNA adductomics, encompassing sample preparation, screening of adducts related to genotoxicity, oxidative stress, and epigenetic alterations, and its application to target species (benthic amphipods) from the Baltic Sea. A high resolution mass spectrometry based method was developed to detect potential DNA adducts taking advantage of the accurate mass data and MS/MS fragmentation. A novelty was the nontargeted screening of nucleoside adducts by using the characteristic neutral loss of 2′-deoxyribose (116.0473 Da) from the fragmentation of nucleoside adduct ions. Initial data analysis, performed manually, identified 23 putative DNA adducts in the amphipods, including markers for epigenetic modifications and oxidative/nitrosative adducts [3]. The introduction of nLossFinder, an in-house program, automated the detection of DNA adducts, greatly improving processing time and efficiency. This tool uses a peak detection algorithm to identify putative adducts based on their fragmentation pattern, recognizing specific neutral loss between MS1 and MS2 [4]. The detection process was further enhanced through batch quantification using TraceFinder software, which confirmed and quantified the putative adducts detected by nLossFinder. In total, 350 low-mass and 516 high-mass adducts were identified in the amphipods [2]. Finally, a simultaneous approach was developed for detecting both DNA and RNA adducts in the same animal and the same sample injection by leveraging the neutral loss of ribose for the RNA adducts. This method enabled the reprocessing of archived Data-Independent Acquisition (DIA) data to detect RNA modifications, resulting in the identification of 60 putative RNA adducts in amphipods. Some of these adducts were structurally confirmed by comparison with reference standards [5]. Overall, this work represents significant advancements in the automated detection and quantification of DNA and RNA adducts, demonstrating the potential of reprocessing archived data to gain new insights into adductomics.

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# Glucosinolate variation among organs and growth stages in Eruca sativa cultivated in a closed system

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The global nutraceutical market is continuously developing and there is an increasing demand for more sustainable, accessible, and nutritious plant supplies. In this context, indoor agriculture offers a promising solution, particularly for cultivating microgreen - young vegetables that are gaining popularity for their sensory attributes and nutritional benefits [1]. This study investigates the variation of glucosinolates and other bioactive compounds in Eruca sativa among organs and different growth stages, including seeds, sprouts, microgreens, plants in full vegetative phase and flowering plants. A newly developed closed system called 'Lampada ventilante' was used to obtain microgreens, plants in full vegetative phase and flowering plants. This device reproduces the existing ecosystem around a plant and makes it possible to cultivate plants without the use of pesticides and with good production yields thanks to precision lighting and targeted ventilation [2]. Ultrasound Assisted Extractions (UAE) were performed using ethanol/water (7:3) solution. The different extracts were analyzed by liquid chromatography coupled with high-resolution mass spectrometry, using a chemometric approach, to identify biomarker metabolites. The negative ion mode UHPLC-Q- Exactive-MS/MS profiles were processed using Compound DiscovererTM 3.3 software to obtain the identification of a big number of known bioactive compounds such as glucosinolates, glycosylated flavonoids, fatty acids, and lipids. The LC-MS data were processed using Partial Least Square - Discriminant Analysis (PLS-DA) and revealed a significant separation among the samples, suggesting metabolic differences and highlighting most glucosinolates as key markers of the microgreen stage. The present work develops controllable and reproducible environmental conditions, which are difficult to obtain in field agriculture, intending to understand the evolution of the metabolome of E. sativa during plant growth by LC-HRMS and LC-HRMS/MS combined with multivariate data analysis. Furthermore, results could be a potential reference for applications of microgreens in the functional food and nutraceutical industry.

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Environment and Plant Science

# Per- and polyfluoroalkyl substances (PFASs) and Brominated flame retardants (BFRs): background levels in fish of Central Adriatic Sea

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Introduction: PFASs and BFRs (PBDEs, HBCDs, and e-BFRs) are persistent and toxic environmental contaminants associated with endocrine dysfunction, immunosuppression, and cancer [1]. They tend to bioaccumulate in fish, thus entering the human food chain. EU has set limits in food only for PFASs [2]. This study aims to evaluate the background contamination levels of PFASs and BFRs in fish samples belonging to commercial species.

Materials and Methods: Forty-eight fish samples were analysed, selecting the species based on their trophic position, feeding habits and habitat: Mullus barbatus (n=17), Solea solea (n=15), and Merluccius merluccius (n=16). The study area was the Central Adriatic Sea (Civitanova Marche and Ancona), in summer and winter 2023-2024. All the analysis were conducted in isotopic dilution. 19 PFASs were analysed by LC-MS/MS [2] and 9 PBDEs congeners, 3 HBCDs isomers and 9 e-BFRs by a single sample preparation and dual detection method: GC-MS/MS for PBDEs and e-BFRs; LC-MS/MS for HBCDs [3].

Results and discussion: In all the samples, BFRs were detected at very low levels and in almost all samples e-BFR < LOQ. PBDE congeners 49, 47, 100 and 99 were the most frequently quantified among the nine included in the method. Only α-HBCD was measured in M. merluccius and M. barbatus (β and γ isomers were <LOQ). PFAS were present in all samples, with M. barbatus showing the highest average concentration of Σ19PFAS (0.902 ± 0.620 µg/kg), followed by S. solea (0.757 ± 0.382 µg/kg) and M. merluccius (0.595 ± 0.364 µg/kg) (Fig.1). PFOS was the principally detected analyte in all species, followed by long-chain perfluorocarboxylic acids (C9-C14) (Fig.2). None of the samples exceeded the maximum limits set for Σ4PFAS (PFOA, PFNA, PFHxS, tot-PFOS) in fish products (Regulation 915/2023). However, due to the limited number of samples, no final conclusions can be drawn, highlighting the need for further study and monitoring.

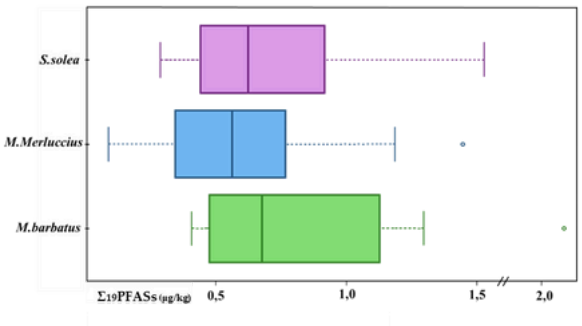


**Fig.1** Box plot reporting Σ19PFASs (µg/kg)

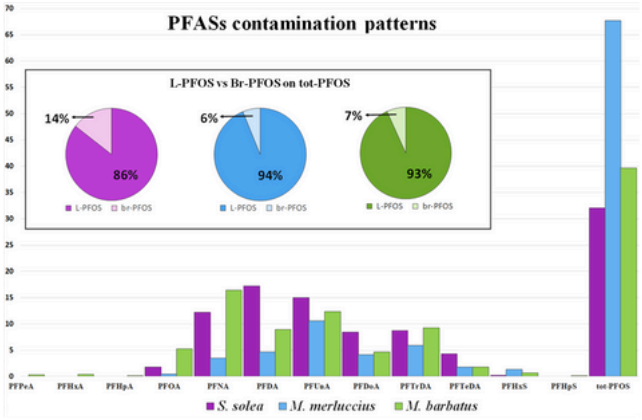


**Fig.2** PFASs contamination patterns (only quantified PFASs are reported)

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Environment and Plant Science

# The Role of Ophrys Orchids as Bioindicators of Pristine Environments

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Orchidaceae represent one of the most diverse families of angiosperms, with over 25,000 species characterized by specialized interactions with pollinators [1]. The genus Ophrys represents a unique case in plant-pollinator interactions, making these orchids excellent bioindicators of unpolluted and ecologically stable environments. These species engage in highly specialized relationships with specific pollinators, guided by the emission of precise chemical compounds—such as alkanes, alkenes, and alkynes —that mimic the sexual pheromones of the insects they attract. This intricate ecological mechanism depends on the integrity of the surrounding ecosystem and the presence of a stable pollinator population[2].

Moreover, Ophrys orchids form essential symbiotic relationships with mycorrhizal fungi and microorganisms present in the soil, which are crucial for seed germination and nutrient absorption. In order to better understand the chemical basis of these interactions, scent analyses have been performed using Gas Chromatography-Mass Spectrometry (GC/MS)[3]. To resolve the issue of the double bond position in alkenes—a key factor in pollinator specificity—dimethyl disulfide (DMDS) derivatization was applied[4]. This approach allowed the precise identification of specific alkenes involved in the orchid-bee interaction, further confirming the role of Ophrys as fine-tuned ecological indicators.

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# Biomass burning tracers in polar and alpine ice cores: high-resolution analyses with the novel Fast Liquid Chromatography – tandem Mass Spectrometry (FLC – MS/MS) coupling with a Continuous Flow Analysis (CFA) system

**Azzurra Spagnesi**[2], Elena Barbaro[1,2], Matteo Feltracco[2], Fabrizio De Blasi[1,2], Daniele Zannoni[2], Federico Dallo[1,2], Jacopo Gabrieli[1,2], Andrea Spolaor[1,2], Andrea Gambaro[2,1] Carlo Barbante[2,1]

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Continuous Flow Analysis (CFA) systems, originally designed for field analyses of trace chemical species in ice cores, have seen significant innovations over the past decade, particularly through the integration of novel spectrometric techniques, which has expanded the range of measureble analytes in polar and alpine ice. Mass spectrometric techniques combined with melter systems allow to achieve continuous concentration records of elements and chemical species in ice cores at parts per billion (ppb) and parts per trillion (ppt) levels. This approach significantly reduces sample handling by CFA, which directly inject the decontaminated meltwater stream into the analytical instrumentation within a few minutes, ensuring high-depth-resolution measurements and reducing analytical efforts [1].
Tandem mass spectrometry, based on a Triple Quadrupole Mass Analyser System, is particularly effective in addressing interferences within meltwater matix. Leveraging the high selectivity and sensitivity of tandem mass spectrometry, we recently developed a novel CFA configuration at the National Research Council – Institute of Polar Science in Venice (CNR-ISP), in collaboration with Ca' Foscari, University of Venice. For the first time, we coupled a melting unit with a Fast Liquid Chromatography tandem Mass Spectrometry (FLC-MS/MS) system to quantify specific biomass burning tracers in ice cores (i.e., vanillic acid, syringic acid, and levoglucosan) in a semi-continuous manner (one measurement every 30 sec.).
Unlike Fast Ion Chromatography (FIC), which is limited to ion determination [2], FLC-MS/MS allows for the detection of a wide range of organic compounds after proper optimization of chromatographic and mass spectrometric parameters. Using this validated method, detection limits of 3.6 and 4.6 pg mL-1 for vanillic and syringic acids, respectively, were achieved [3]; whilst 66 ng L-1 of detection limit was achieved for levoglucosan, demonstrating a net improvement over the previous LOD of 600 ng L-1 obtained with discrete analyses [4]. Proved the great potentialities of this hyphenated technique, we aim to develop a unique CFA system configuration integrating FLC-MS/MS and additional analytical tools, including Ion Chromatography, Cavity Ring-Down Spectrometry, Inductively Coupled Plasma Mass Spectometry, and laser particle counter, allowing for high-resolution, multi-component analyses of organic compounds, major ions, water stable isotopes ($\partial$18O, $\partial$2H), heavy metals, and insoluble particles in alpine and polar ice cores.

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Environment and Plant Science

# Thanatometabolomics in wildlife: Identifying potential metabolic markers of post-mortem intervals in wild animals by ambient mass spectrometry

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Veterinarians, particularly in the fields of veterinary forensics and veterinary epidemiology, need to be able to estimate the amount of time that has elapsed since an animal died. A reliable estimate of post-mortem interval (PMI) of the earliest cadavers found would inform veterinary services about the possible time since the index case of a disease and the possible range of the epidemic front.
We pictured, in a non-targeted manner, the metabolic changes that occurred in four wild boar cadavers placed in a forested area of the North-Eastern Italy. We sampled and extracted the decomposing tissues from the hind limb adductor muscles at nine different time points. The metabolic signatures were then analyzed by direct analysis in real time high resolution mass spectrometry (DART-HRMS). A non-parametric ANOVA identified a suitable number of metabolic markers that were able to describe the post-mortem changes correlated to the time of death. In order to go beyond a simple tentative annotation achieved by DART-HRMS, these metabolites were identified by liquid chromatography tandem high resolution mass spectrometry (LC-HRMS/MS). We were able to identify specific biomolecules that have potential for use in PMI estimation, such as dipeptides, homocarnosine, hypoxanthine, and amino acids. Next, pathway analysis confirmed the extinction of energetic metabolism and a switch towards another source of fuel for the on-going decomposition processes. Our findings suggest that, by targeting a combination of compounds with different post-mortem stabilities, the decomposition of wild boars could be tracked by using an appropriate set of metabolites as revealed by our DART-HRMS study. [1]

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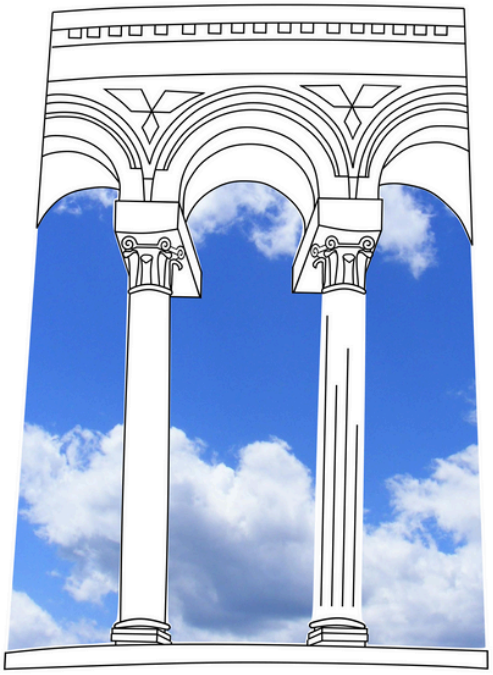

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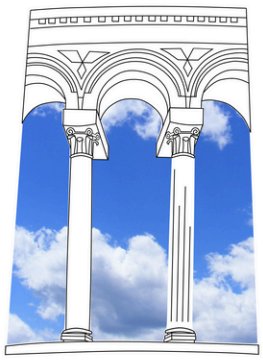

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