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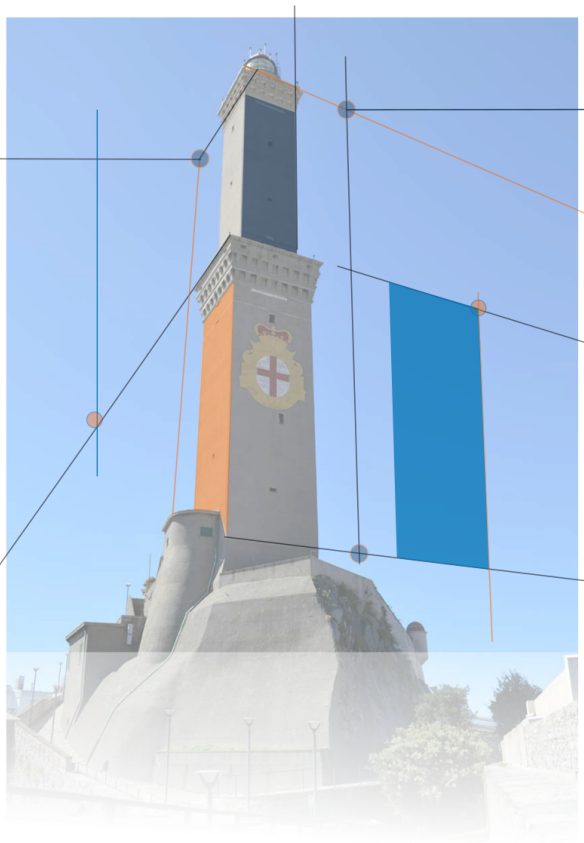

Italian Mass Spectrometry Society

www.imass.it - imass@biomedia.net

2nd IMaSS Network

10-12 Maggio 2017

Istituto Italiano di Tecnologia, Genova

Keynote speakers

Roland Thissen - *CNRS - University of Paris Sud*
Davide De Pietri Tonelli - *IIT, Genova*
Fabio Benfenati - *IIT & University of Genova*
Paola Manini - *EFSA, Parma*
Giada Furlan - *RIS di Parma*

Informazioni e Iscrizioni

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	2nd IMaSS Network: Program Timetable		
May 10, 2017. Day 1 Afternoon - Chair: E.Davoli, P. Franceschi			
1430	**E.Davoli, R. Cingolani**		Introduction
1500	**R.Thissen**	Universitè Paris Sud	*Mass Spectrometry for In-Situ Exploration of the Solar System*
1545	**Angela Bachi**	FIRC Institute of Molecular Oncology, Italy	*Combining shotgun and targeted proteomics to quantitate the missing proteome*
1615	**Coffee Break**		
1635	**Clarissa Braccia**	Istituto Italiano di Tecnologia	*Panax ginseng C.A. MEYER AND KOREAN RED GINSENG: A PROTEOMIC ANALYSIS*
1700	**Claudio Medana**	Università di Torino	*Mass spectrometry as a tool in the elucidation of enzymatic metabolism of bioactive molecules*
1725	**Zeeshan Shah**	Scuola Superiore Sant'Anna	*A DEEP, COMBINED OMICS EXPLORATION OF THE FAAH-/- BRAIN LIPIDOME*
1750	**Andrea Carretta**	SRA Instruments	*SIFT-MS: A New Approach to real-Time Mass Spectrometry*
1815	*Round Table: Wrap-up and Discussion - End of Day 1*		
May 11, 2017. Day 2 Morning. Chair: A.Armirotti, O.Curcuruto, R.Zecchi			
830	**Davide De Pietri Tonelli**	Istituto Italiano di Tecnologia	*An integrative approach to identify microRNA-dependent and independent regulatory networks in neurogenesis*
915	**Tom Knapman**	Sciex - UK	*Translating multi-OMICS Research into Precision Medicine with Mass Spectrometry-based clinical Sample Mapping*
940	**Lucilla Nobbio**	University of Genoa - DINOGMI	*Lipid impairment in Charcot-Marie-Tooth type 1A (CMT1A) neuropathy: identification of potential therapeutic targets.*
1005	**Michele Bianchi**	Università del Piemonte Orientale	*Quantitative evaluation of adenosine 5'-tetraphosphate and other five analytes related to nicotinamide phosphoribosyltransferase by LC-ESI-MSn in Melanoma cells and mouse plasma*
1030	**Elena Michelucci**	Università di Firenze, CISM	*Mass Spectrometry and Metallomics: binding site location in the Cyt c-CDDP model system*
1055	**Coffee Break**		
1115	**Veronica Mainini**	Shimadzu Italia	*In situ metabolomics: insights into tissue metabolism revealed from high resolution MALDI Imaging mass spectrometry*
1140	**Adriana Calderaro**	Università di Parma	*MALDI-TOF mass spectrometry applied to microbiology and virology*
1205	**Enrico Davoli**	IRCCS Istituto Mario Negri	*Challenges in quantitative MSI of drugs in tissues*
1230	**Barbara Cardinali**	IRCCS AOU San Martino IST	*Breast cancer subtyping by MALDI Imaging Mass Spectrometry*
1300	**Lunch Break + Poster session**		
May 11, 2017. Day 2 Afternoon. Chair: Silvia Catinella			
14:30	**Paola Manini**	European Food and Safety Authority	*The role of EFSA in the european food safety system*
15:15	**Annual IMaSS Members Meeting**		
17:30			
19:00	**SOCIAL DINNER - ACQUARIO DI GENOVA**		

May 12, 2017. Day 3 Morning. Chairman: C. Medana ,P. Franceschi, M. Roverso			
830	**Fabio Benfenati**	Istituto Italiano di Tecnologia	*Neuronal Interfaces: innovative therapies and neuroprosthethics*
915	**Mikhail Sheindlin**	Joint Institute for High Temperatures of Russian Academy of Sciences	*Advances in the Mass Spectrometric Study of the Laser-Induced Vaporization of Graphite and Zirconium Carbide*
940	**Dipali Kale**	Fondazione Istituto Italiano di Tecnologia	*Comparative lipidomic analysis of Astrocytes upon graphene and graphene oxide treatment by UPLC-Q-TOF-MS*
1005	**Samantha Riccadonna**	Fondazione Edmund Mach	*Why yellow raspberries are not red: an untargeted metabolomics approach*
1030	**Sara Stead**	Waters Inc.	*Rapid Evaporative Ionisation Mass Spectrometry – an emerging disruptive technology for the food testing industry?*
1055	**Coffee Break**		
1115	**Veronica Termopoli**	Università di Urbino	*The Analytical Scientist Innovation Awards (TASIA) 2016: Effortless introduction of liquid streams into an unmodified electron ionization source of a mass spectrometer (LEI interface).*
1140	*Special Session & Round Table: Data Mining in MS-Based Metabolomics.*		
	Chair & Introduction: Pietro Franceschi, Fondazione Edmund Mach		
1300	**Lunch Break**		
May 12, 2017. Day 3 Afternoon. Chairman: G. Pieraccini			
1430	**Cap. Giada Furlan**	R.I.S. Carabinieri	*Chemistry and Mass Spectrometry in a Forensic perspective*
1515	**Marco Pazzi**	Università di Torino	*GC-MS strategy combined with Chemometrics for fire debris investigations purposes*
1540	**Marco Modarelli**	MM SpA, Milan Water Management	*Mass spectrometer for on-line gas analysis of volatile compounds in Milan water management system*
1605	Best Poster - Oral communication		
1630	*Wrap-up and Meeting Closure*		

Mass Spectrometry for In Situ Exploration of the Solar System

Roland Thissen, PhD *Paris-Sud University, Physical Chemistry Laboratory (LCP)*

It is a major goal of space exploration to decipher the origin and evolution of Solar System bodies in relation to the primordial conditions of the solar nebula, the chemical fingerprints of the formation of the Solar System, the evolutionary processing of minerals, volatiles and organic compounds, as well as to elucidate the source and evolution of organic matter, its relevance for the origin of life, and to explore other possible modern habitats with suitable conditions to sustain life. The knowledge of the molecular, elemental and isotopic composition of Solar System bodies and of their environments is therefore central in space research. For instance, to understand the evolutionary processes, planetary sciences require in situ measurements of noble gas concentrations, their isotopic abundances, and the distribution of volatiles species such as H2O, HCN and others. Recently, in the frame of in situ measurements at bodies with potential relevance for astrobiology (Titan, Europa, Enceladus, comets, and asteroids), the need to develop a new generation of in situ space instruments able to handle and analyse the diversity and complexity of organics, their isotopic composition, and their potential interaction with inorganic material has emerged. This fosters the continuous effort to develop in situ analytical tools that are up to the analytical challenges of space exploration. My talk will present few historical results, describe key results determined recently by mass spectrometry, and will elaborate on the development efforts for new instruments, as well as the associated laboratory developments.

BIO

Roland Thissen is now a full time researcher at the French CNRS. He received his Ph. D. in Chemistry at the Liege University, Belgium, in 1993, for a work on the gas phase dissociation dynamics of doubly charged molecular ions by synchrotron radiation, performed at Orsay. He then went for post-doctoral stages in Japan during which he developed experimental skills in dispersed fluorescence, photoelectron spectroscopy, and VUV monochromator commissioning. He received a permanent position in CNRS in 1999 to work in the field of gas phase ion reactivity and fragmentation dynamics.He has been developing a project of High resolution mass spectrometry as a facility for extraterrestrial material analysis in the Astrophysics Institute of Grenoble (IPAG) from 2007 to 2016. He now works in the group of Christian Alcaraz in Paris-sud University, Physical Chemistry Laboratory (LCP) on a project to produce state selected molecular ions and study their fragmentation or reaction dynamics, as a function of their internal or kinetic energy.

AN INTEGRATIVE APPROACH TO IDENTIFY MICRORNA-DEPENDENT AND INDEPENDENT REGULATORY NETWORKS IN NEUROGENESIS

Davide De Pietri Tonelli, PhD *Neurobiology of miRNA lab – Istituto Italiano di Tecnologia, Genoa, Italy*

MicroRNAs (miRNAs) are small noncoding RNAs, which have been shown to control virtually all physio-pathological aspects of mammalian brain. Uncovering miRNA functions in brain health and disease are amongst the main challenges in modern neuroscience and purpose of current pharmacological and technological R&D worldwide. Mammalian miRNAs, in complex with RNA Induced Silencing Complex (RISC) proteins, form imperfect base- pairing with sequences of target mRNAs. Once bound, miRNAs can either repress translation or induce the decay of target mRNAs, but a combination of both effects is also possible. Identification of miRNA targets has been the subject of a growing number of computational and experimental approaches, but it's still particularly challenging. Indeed, despite progress over the years, the imperfect association between a miRNA and its targets complicate this task. Moreover, the regulatory effect of a single miRNA on targets is generally very low both at the transcript and protein level. To compensate for their mild regulatory activity, miRNAs exert their functions in a highly combinatorial way: one miRNA can regulate several mRNAs in parallel and different miRNAs can target one mRNA simultaneously, thus regulating target expression more efficiently. It follows that alone, computational, transcriptomics, or proteomics are insufficient to identify the impact of miRNA-dependent gene regulatory networks. I will present recent evidence from our lab based on the integrative use of genetically modified rodents/cell models, bioinformatics, transcriptomics and proteomics to infer miRNA functions *in vivo/vitro*. This approach allowed us to identify miRNA-independent functions of miRNA biogenesis proteins [1] and miRNA-dependent regulatory networks [2], which control embryonic and adult mammalian neurogenesis.

References:

[1] Marinaro F, et al *"MicroRNA-independent functions of DGCR8 are essential for neocortical development and TBR1 expression"*. EMBO Rep. 2017;

[2] Pons-Espinal M. et al *"Synergic functions of miRNAs determines neuronal fate of adult neural stem cells"*. Stem Cell Rep. 2017

BIO

D. De Pietri Tonelli, is a Tenure Track Researcher and Head of the laboratory of "Neurobiology of microRNAs", in the Neuroscience and Brain Technologies dept. (NBT) of IIT, Central research Laboratories in Genova. He holds a MSc in Molecular and Cell Biology and a PhD in Neurobiology. He has long-standing interest in post-transcriptional control of gene expression, microRNAs and Neural Stem Cells Biology, acquired at multiple institutions (San Raffaele Inst. Milan, Italy; Max-Planck Inst. Dresden, Germany). He is author of many scientific articles in peer-reviewed international journals (among which Development, EMBO Reports, Nature Comm., Nature Neurosci., Nucleic Acids Res., Stem Cell Reports); one book and two book-chapters. Current research in his lab focuses on the role of miRNAs and other noncoding RNAs in neurogenesis (embryonic and adult) and circadian biology in rodents. The ultimate goal of his research is to develop RNA-based drugs for brain therapy and repair.

Link to Neurobiology of microRNAs: https://iit.it/research/lines/neurobiology-of-mirna

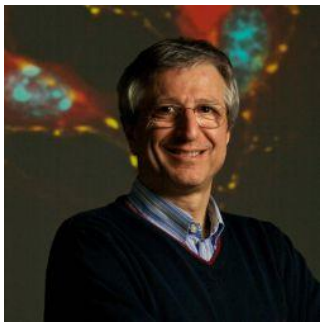
The role of EFSA in the european food safety system

Paola Manini, PhD *European Food Safety Authority*

EFSA is an independent European agency funded by the European Union (EU) budget that operates separately from the European Commission, European Parliament and EU Member States. EFSA was set up in January 2002 as part of a comprehensive programme to improve EU food safety. In 2005 EFSA moved from Brussels to Parma. Currently, EFSA counts over 450 staff and 1500 experts, appointed through an open selection procedure on the basis of proven scientific excellence. EFSA is the reference body for risk assessment of food and feed in the European Union. Its work covers all risks associated with the food chain ("from field to fork"), including food and feed safety, nutrition, animal health and welfare, plant protection and plant health. In all these fields, EFSA provides independent scientific advice and support for EU risk managers and policy makers, and provides independent and clear risk communication. EFSA also promotes scientific cooperation. The development of European policies and legislation, authorisation for the marketing of new products and control activities are outside EFSA's remit. EFSA's main task is to carry out scientific risk assessments, in response to requests from the European Commission, European Parliament and EU Member States. Scientific risk assessment is mainly carried out by EFSA's Scientific Panels, composed of highly qualified and independent scientific experts, with the support of EFSA staff. EFSA Panels are tasked to draft and adopt scientific outputs on general health issues and regulated products. Risk communication is part of EFSA's mandate. All scientific outputs (about 500/year) are published in the EFSA Journal and are made publicly available through the EFSA website. To bridge the gap between science and the consumer, EFSA develops a suitable and tailored communications approach, e.g. profiling the issue on the EFSA website, media activities, webinars, or discussion at scientific events.

BIO

Dr. Manini graduated in Chemistry at the University of Parma in 1992 summa cum laude and received her PhD in Analytical Chemistry from the same University in 1997, under the supervision of Prof. Alessandro Mangia. She presented a dissertation on the application of LC-MS in several fields, including food analysis and the characterization of organometallic compounds. During her PhD, she spent six months as visiting scientist at the Free University of Amsterdam, in the laboratory of Udo Brinkmann, where she contributed to research on the LC-MS quantitation of pesticides in water and vegetable samples. From 1997-2009, she worked as contract researcher at the Laboratory of Occupational and Environmental Toxicology of the University of Parma, in the research group of Prof. Antonio Mutti. She brought her experience with MS-hyphenated techniques in the biomedical field. Her research activity was aimed at characterizing the metabolism of industrial chemicals (e.g., n-hexane, styrene, naphthalene) and environmental pollutants (benzene, toluene) and at developing of new biomarkers of exposure and early effect. In 2009 Dr. Manini joined the European Food Safety Authority (EFSA) as Senior Scientific Officer in the FEED Unit, where she coordinates the working group on feed flavourings. Paola Manini is author of 92 peer-reviewed publications and 4 book chapters.



NEURONAL INTERFACES: INNOVATIVE THERAPIES AND NEURO PROSTHETICS

Fabio Benfenati, PhD, MD *Center for Synaptic Neuroscience and Technology - Istituto Italiano di Tecnologia, Genova, Italy*

The brain is characterized by highly complex organization, parallel computation, integration of afferent information, emergent properties and functional/structural adaptation. Recent research in the field of neuroscience have stimulated the creation of biomimetic hybrid devices, in which neurons are interfaced with electronic chips, organic electronics or have been genetically modified to generate opto-neural interfaces. By creating these interfaces, one can monitor and change the altered neuronal activity in experimental models of brain diseases and create hybrid devices capable of regulating the excitability and plasticity of neural networks. We recently engineered an optogenetic probe capable, upon illumination, to stimulate transcription in pathological neurons in which the expression of neuronal genes is depressed. Another interesting field is the direct reprogramming of neurons from skin cells and their subsequent grafing to compensate for the neuronal degeneration that accompanies many diseases of the nervous system. We were able to reprogram dopaminergic and GABAergic neurons and studied their incorporation in the host neuronal networks after transplantation in experimental models of Parkinson's disease and epilepsy. Finally, we successfully interfaced organic electronics with nerve tissue. Using photovoltaic polymers, we were able to stimulate or inhibit the activity of neurons with light, mimicking the process that occurs in retinal phototransduction. We have also shown that this bioorganic interface restores light sensitivity in the retina affected by photoreceptor degeneration, suggesting an important future in the field of retinal prostheses. Optogenetics, cellular reprogramming and hybrid interfaces therefore represent concrete and promising therapeutic approaches to diseases of the nervous system.

BIO

Prof. Benfenati is the Director of the Synaptic Neuroscience and Technology Center at the Italian Institute of Technology, Full professor of Neurophysiology at the University of Genova Medical School and Adjunct Professor at the Rockefeller University, New York. Prof. Benfenati is author of over 280 research articles. In his scientific activity, he has addressed the mechanisms of neural and synaptic communication using a variety of experimental models of human diseases by using a combination of experimental techniques. The main topics are: (i) Pathogenetic mechanisms of synaptopathies in genetically altered mice as models of human neurological disorders; (ii) Generation of engineered neuronal networks, neuro-electronic and opto-neural interfaces by exploiting optogenetics and photovoltaic interfaces; (v) Study of graphene-neuron interfaces.

Link to Synaptic Neuroscience and Technology Center: https://www.iit.it/it/centers/nsyn-unige

Chemistry and Mass Spectrometry in a Forensic perspective

Giada Furlan, Capt., PhD *Chemistry Section, Reparto Carabinieri Investigazioni Scientifiche Parma, Italy*

The Carabinieri Scientific Investigation Department, known as RIS, is a section of the Carabinieri Army that deals with every scientific activity connected to a crime scene, from the first walk through to the court trial, throughout the laboratory activities. Chemistry in particular contribute not only in itself, but also through all the chemical techniques that are applied in the other forensic fields. Chemistry in itself is maybe one of the older and best known forensic science, since it started in the 17th century with forensic toxicology. As many people know, mass spectrometry and, in particular, gas-chromatography - mass spectrometry hyphenated techniques are widely applied in several forensic fields, first of all toxicology, followed by explosive and arson analysis. Nowadays, some new perspectives for these "old" analytical techniques come from the association with other methods or devices, such as solid phase micro-extraction (SPME) and liquid chromatography, new sampling devices and increase of the sensitivity. It is, furthermore, fundamental to stress the fact that, forensic environment is a melting-pot of techniques and skills that, all together, contribute to the "resolution" of a single case and this can't be fully understand without giving a glance to real cases. The aim of this presentation is to give a general overview on chemical applications into the forensic field, since chemistry in the forensics is not only forensic chemistry, but deals with -and can affect also- other forensic branches, such as biology, fingerprints, ballistics and so on; and to give a glance on possible future perspective as well.

BIO

Capt. Giada Furlan, is the Chemistry Section Director of the Carabinieri Scientific Investigation Department in Parma and coordinates the activities of five laboratories, namely toxicology, explosives and arson, material analysis and SEM-EDX laboratory. She graduated in Chemistry at the University of Trieste in 2003 summa cum laude and received her PhD in Organic Chemistry from the same University in 2006, for a work on the chemoenzymatic synthesis and activity of enatiomerically pure derivatives of paraconic acid. Carabinieri officer from 2007, she worked first in Rome as Second officer of the Forensic Training Department and then, in the same position, in the Forensic Chemistry Section both in Rome and Parma. Teacher at the Center of Excellence for the Stability Police Units (Vicenza) and at the Institute for the Investigative Techniques (Velletri), she is the italian Carabinieri delegate in the European Network of Forensic Science Institute (material analysis) and in the EU-US Explosives Expert's group.

Combining shotgun and targeted proteomics to quantitate the missing proteome

Angela Bachi

IFOM, the FIRC Institute of Molecular Oncology, Milan, Italy.

Proteins abundances span in the proteome at vastly different ranges; yeast proteome, for example, is estimated to fluctuate from fewer than 50 to more than 106 molecules per cell. Many of these molecules, including cell cycle proteins, are present at levels not readily detectable by traditional data dependent proteomic methods (DDA). Moreover, the DDA runs are plenty of missing values among the replicates due to the stochastic nature of the method, thus hampering the robustness of the analysis. We designed a robust workflow named Missing values Monitoring (MvM)1 that combines DDA and Data Independent Acquisition (DIA) in order to quantitate low abundant proteins. To demonstrate the potential of this method we focused on the quantitation of cell cycle proteins in mitosis versus G1 phase in S. cerevisiae. We obtained successful quantitation for cell cycle related proteins till ~50 molecules per cell comparable to an SRM based method, demonstrating the potential of MvM workflow to quantitate dynamically regulated proteins.

References

[1] Matafora V, Corno A, Ciliberto A, Bachi A. Missing Value Monitoring Enhances the Robustness in Proteomics Quantitation. J Proteome Res. 2017 Apr 7;16(4):1719-1727.

Title Breast cancer subtyping by MALDI Imaging Mass Spectrometry

B.Cardinali[1], L. Del Mastro[1] , Ben Neely[2] , T.W.Powers[2], , F.Carli[3] , Aldo Profumo[4] , Peggi Angel[2], and R.R.Drake[2]

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[4] Biopolymer and Proteomics Unit, IRCCS AOU San Martino- IST, Largo R. Benzi 10, 16132 Genoa, Italy

The treatment of HR+, G2, HER2- breast tumors, currently considered at intermediate risk of recurrence, is still challenging, since the response to the therapy, the disease free and overall survival, are not the same for patients with these tumor histological characteristics. The analysis of differences in the proteomic profile could help to improve breast carcinoma sub-classification. In this study formalin fixed paraffin embedded (FFPE) breast cancer tissues were analyzed using MALDI Imaging mass spectrometry (IMS). Experiments were conducted at MUSC Proteomics Center on 30 samples (25 G2 and 5 G3 - more aggressive) selected at IRCCS AOU San Martino-IST: samples preparation (antigen retrieval, in situ trypsinization and matrix coating) was optimized and IMS data were collected on a AutoflexIII Smartbeam mass spectrometer. Statistical analysis identified 55 significantly different signals and grouped samples in two clusters. Tissues from patients who relapsed were classified in the same group, suggesting a possible classification in risk categories based on FFPE tissues proteomic profile. A validation set of 11 samples was analyzed and classified using the 55 peaks more significantly expressed in the training set: samples from patients with recurrence were “correctly” classified in the high risk group. To improve the classifications further analysis on a larger training set of 70 FFPE samples have been performed on a Bruker Solaris FT mass spectrometer. The statistical analysis and MS/MS analysis of the tryptic digests from selected samples are in progress to redefine the classifier and identify those peptides detected as differently expressed by IMS. This work could provide a better description of breast cancer heterogeneity.

Quantitative evaluation of adenosine 5'-tetraphosphate and other five analytes related to nicotinamide phosphoribosyltransferase by LC-ESI-MS^n in Melanoma cells and mouse plasma

Michele Bianchi[1], Adolfo Amici[3], Ambra A. Grolla[2], Erika Del Grosso[2], Roberta Bellini[2], Cristina Travelli[2], Silvia Garavaglia[2], Leonardo Sorci[3], Nadia Raffaelli[4], Silverio Ruggieri[4], Armando A. Genazzani[2], Giuseppe Orsomando[3]

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[4] Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Via Brecce Bianche 10, 60131 Ancona, Italy

Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD) synthesis, that is an essential coenzyme for maintaining the cellular homeostasis1,2. Adenosine 5'-tetraphosphate (Ap4) is a natural nucleotide known as the most potent vasoactive purinergic mediator in mammals3. Preliminary in vitro4 studies shown that Ap4 production is related to NAMPT activity. However, it has never been reported whether NAMPT can catalyze the synthesis of Ap4. The main aim of the work was to develop a new bioanalytical LC-ESI-MSn method to quantify Ap4 in engineered B16 Melanoma cells and mice plasma. Secondly, to quantify with the same method all the analytes (adenosine 5'-diphosphate, adenosine 5'-triphosphate, nicotinamide, nicotinamide mononucleotide and NAD) involved in NAD homeostasis. In order to correlate NAMPT to Ap4 production, various cells lines were analyzed which differ each other for intracellular NAMPT levels. As result, intracellular Ap4 levels were increased more than two times in cells over-expressing NAMPT (B16 FLAG-NAMPT: 1.07 ± 0.18 nmol/mg protein v. B16 WT: 0.53 ± 0.07 nmol/mg protein; $p<0.05$) and were significantly reduced in cells silenced for the enzyme (ShNAMPTLow: 0.33 ± 0.08 nmol/mg protein; $p<0.05$)5. It has been confirmed that Ap4 is present in murine plasma, suggesting that extracellular functions of NAMPT could be based on its enzymatic synthesis. The data obtained allow us to determine that Ap4 production in melanoma cells is dependent on NAMPT expression. It highlights novel mechanisms by which this enzyme could exert the plethora of actions that are attributed to it.

References

[1] A. A. Grolla, et al., *Pigment Cell & Melanoma Research* **28**, 718-729 (2015).
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[3] J. Pintor, et al. *The Journal of Pharmacology and Experimental Therapeutics* **308**, 468-473 (2003).
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[5] A. Amici, A. A. Grolla, et al., *Cell Chemical Biology,* Accepted Manuscript.

Panax ginseng C.A. MEYER AND KOREAN RED GINSENG: A PROTEOMIC ANALYSIS

Clarissa Braccia[1], Mara Colzani[2] PhD, Prof. Giancarlo Aldini[3].

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Panax ginseng is a slow-maturing perennial herb which has been commonly used in the Far East for over 2000 years because of its therapeutic effects. Panax is a genre of plants deriving from *Araliaceae* family; it is divided in different species, in which *Panax ginseng* is collocated.
Korean Red Ginseng is a form of *Panax ginseng*, typically produced in Korea, where the roots are exposed to a steaming process at about 90 °C for 3 hours and then to a drying process. After the steaming treatment, the roots become reddish: that is the origin of the name Red Ginseng.
Several clinical trials demonstrate pharmacological effects on: central nervous system, cancer, cardiovascular system and diabetes. So far, the biological effects of *Panax ginseng* have been mainly attributed to its steroidal saponins, called ginsenosides. Probably for this reason, the protein content of this plant has been less characterized; moreover, its genome has not been fully sequenced, making the proteome incomplete. The exact mechanism of action of ginseng components, both ginsenosides and proteins, is still unidentified. Although many reports have been published about the pharmacological effects of *P. ginseng,* little is known about the its biochemical pathways. Proteomics analysis could be useful to elucidate the molecular mechanism underlying its beneficial effects.The aim of this work was to apply high resolution-mass spectrometry (via Q-Exactive) to characterize the proteome of the Korean Red Ginseng, compared to that of White Ginseng (before the steaming process). Mass spectrometry-based proteomics is the ideal approach to detect and identify the proteins present in complex samples, because it is sensitive, unbiased and allows large-scale analysis. The analysis is difficult because of the presence of different compounds such as phenols, polysaccharides and terpenes that can bind the proteins and thus interferes with the SDS-PAGE. To identify not only the most abundant proteins, it is necessary to establish a dedicated proteomic workflow based on protein extraction strategy that would enable protein fractionation as well as the removal of the interfering compounds. An advanced protocol was necessary to efficiently extract and fractionate proteins by preparative SDS-PAGE. A broad protein profile was obtained only for White Ginseng; Red Ginseng did not produce a satisfying SDS-PAGE pattern and it was not possible to identify proteins either by in-gel and in-solution digestion. We speculated that the protein profile in Red Ginseng was altered by the thermal treatment.The White Ginseng proteome was analyzed both with in-gel digestion and in-solution digestion. An exploration of protein-protein interactions of the 344 identified proteins was performed, building an interaction map. The functional analysis showed that the most represented functional classes are: catalytic activity (46.2%) and binding (21.2%). Analyzing the KEGG pathways in which the identified proteins are involved, it is emerged that the majority of proteins belongs to signaling pathway (9.29%), in particular apoptosis signaling and fibroblast growth factor signaling.

MALDI-TOF mass spectrometry applied to microbiology and virology

Adriana Calderaro[1], Sara Montecchini, Mirko Buttrini, Sabina Rossi, Giovanna Piccolo, Maria Cristina Arcangeletti, Maria Cristina Medici, Federica Motta, Isabella Rodighiero, Carlo Chezzi, Flora De Conto.

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is increasingly utilized as a rapid technique to identify microorganisms by their molecular fingerprint and/or by biomarker detection. Although the massive number of entries in the available Maldi Biotyper database Bruker Daltonics, the absence of reference spectra of some species as dermatophyte fungi or bacteria such as spirochaetes, does not allow their identification.
Moreover, very few reports are available about its application in parasitology and virology.
In this study, in a first approach, the commercial database of the spectrometer used in our laboratory was supplemented with additional spectra. Initially, the profiles of several *Brachyspira*, *Borrelia*, *Leptospira* and dermatophyte species were included without any intervention on setting; subsequently, the versatility of the system allowed us also to supplement the database with the spectra of the parasite *Trichomonas vaginalis* and with that of respiratory viruses infected cells, extensively modifying the parameters commonly set for the routine identification of bacteria and fungi.
In a second approach, when needed, the identification of other parasites, such as *Entamoeba histolytica* and *E. dispar*, and of other viruses (poliovirus) at serotype level was achieved by protein biomarker detection.

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Breast cancer subtyping by MALDI Imaging Mass Spectrometry

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The treatment of HR+, G2, HER2- breast tumors, currently considered at intermediate risk of recurrence, is still challenging, since the response to the therapy, the disease free and overall survival, are not the same for patients with these tumor histological characteristics. The analysis of differences in the proteomic profile could help to improve breast carcinoma sub-classification. In this study formalin fixed paraffin embedded (FFPE) breast cancer tissues were analyzed using MALDI Imaging mass spectrometry (IMS). Experiments were conducted at MUSC Proteomics Center on 30 samples (25 G2 and 5 G3 - more aggressive) selected at IRCCS AOU San Martino-IST: samples preparation (antigen retrieval, in situ trypsinization and matrix coating) was optimized and IMS data were collected on a AutoflexIII Smartbeam mass spectrometer. Statistical analysis identified 55 significantly different signals and grouped samples in two clusters. Tissues from patients who relapsed were classified in the same group, suggesting a possible classification in risk categories based on FFPE tissues proteomic profile. A validation set of 11 samples was analyzed and classified using the 55 peaks more significantly expressed in the training set: samples from patients with recurrence were “correctly” classified in the high risk group. To improve the classifications further analysis on a larger training set of 70 FFPE samples have been performed on a Bruker Solaris FT mass spectrometer. The statistical analysis and MS/MS analysis of the tryptic digests from selected samples are in progress to redefine the classifier and identify those peptides detected as differently expressed by IMS. This work could provide a better description of breast cancer heterogeneity.

MALDI-TOF mass spectrometry applied to microbiology and virology

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is increasingly utilized as a rapid technique to identify microorganisms by their molecular fingerprint and/or by biomarker detection. Although the massive number of entries in the available Maldi Biotyper database Bruker Daltonics, the absence of reference spectra of some species as dermatophyte fungi or bacteria such as spirochaetes, does not allow their identification.
Moreover, very few reports are available about its application in parasitology and virology.
In this study, in a first approach, the commercial database of the spectrometer used in our laboratory was supplemented with additional spectra. Initially, the profiles of several *Brachyspira*, *Borrelia*, *Leptospira* and dermatophyte species were included without any intervention on setting; subsequently, the versatility of the system allowed us also to supplement the database with the spectra of the parasite *Trichomonas vaginalis* and with that of respiratory viruses infected cells, extensively modifying the parameters commonly set for the routine identification of bacteria and fungi.
In a second approach, when needed, the identification of other parasites, such as *Entamoeba histolytica* and *E. dispar*, and of other viruses (poliovirus) at serotype level was achieved by protein biomarker detection.

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SIFT-MS: A New Approach to real-Time Mass Spectrometry

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Volatile Organic Compounds (VOCs) analysis is of the outmost importance since a long time in a wide range of areas: environmental/workplaces monitoring, food and flavor products characterization, breath analysis for diagnostic purposes, material emissions, geochemistry mapping and classic HS analysis are only a few examples.
The currently used approach involves GC-MS analysis, often coupled with thermodesorbtion techniques.
SYFT Technologies has recently introduced VOICE200, a mass spectrometer based on a soft ionisation technique, which is able to deliver comparable results to those obtained with traditional approaches with, at least, two important improvements: analysis time is dramatically reduced (within a few minutes), neither gas chromatographic separation nor thermal desorption is required.
The idea behind the Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is to use a series of reagent ions to induce specific chemical ionizations; by exploiting the different affinities of the various analytes of interest with ionizing species, it is possible to obtain qualitative and quantitative data in real time.

SPHINGOSINE-1-PHOSPHATE DETERMINATION IN LIVERS MICE BY UPLC-MRM MS

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In the last decades the study about human diseases have had some new key players: the lipids. These latter were recognized not only as the bricks of cellular membrane, but also, and more notably, as the fundamental players in a broad range of biochemical processes, such as calcium homeostasis and membrane trafficking [1, 2].
It was recently understood that aberration in lipid metabolism caused many human diseases, and the most studied pathologies related to a high fat content diet are obesity and prediabetes syndromes [3, 4]. In particular ceramides and phosphosphingolipids, both belonged to the sphingolipids family, are involved in such disorders [5]. There are four major pathways to synthetize ceramides that involve many enzyme families: from glycosphingolipids, from sphingomyelin, from ceramide-1-phosphate and finally a de novo synthesis that involves serine and palmitate. Phosphosphingolipids are synthetized from ceramide, through the alkanolamine sphingosine (2-amino-4-octadecene-1,3-diol). All of the mentioned enzyme-catalyzed reactions are reversible.
Pathological diseases disquiet the enzymatic equilibrium between ceramides, sphingosines and phosphosphingolipids leading to oxidative stress and metabolic cellular damage. Some vitamins, such as pyridoxamine, are able to bring back the disequilibrium.
In our project livers from mice fed with standard (SD), standard plus pyridoxamine (SD+P), high fat (HFAT) and high fat plus pyridoxamine (HFAT+P) diets were analyzed using UPLC coupled with a triple quadrupole mass spectrometry (MRM mode). We focused on a peculiar biomarker of hepatic steatosis, the sphingosine-1-phosphate, and monitored its concentration in different diets with the aim to characterize lipidomic of prediabetic disease.

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E-cigarettes emissions. Critical aspects in producing and reporting data as from the EC Tobacco Product Directives requests.

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The WHO Tobacco Free Initiative recommends parties to propose guidelines for testing and measuring the contents and emissions of tobacco products and Article 10 recommends to implement effective legislative, executive, administrative measures requiring tobacco companies to disclose contents and emissions of tobacco products. Article 20 of the Tobacco Product Directive (TPD) requires that also e-cigarettes undergo the regulations on ingredients and emissions adopted for tobacco products.
Accordingly, the TPD included several Articles to regulate ingredients and emissions of various tobacco products and to oblige tobacco manufacturers to declare all ingredients and additives being used in tobacco product. In particular, Article 7 prohibits the placing on the market of those products containing additives or that have CMR properties in unburnt form.
For these reasons manufacturers must declare e-liquids and emissions components. While direct analysis of liquids does not present particular difficulties, no standardized methods are available to determine emission components. Mass spectrometry probably is the most accurate method but large uncertainties relies in emissions.
We present here the development of a GC/MS method to produce and sample vapours from different e-cigarettes and a critical evaluation of results from a large number of available e-liquids emissions.
In our preliminary analysis, we analyzed about 200 emissions by as many samples with 3 different e-cig hardware. Our results confirmed formation of toxic substances like acrolein, formaldehyde, diacetyl and acetaldehyde. Furthermore, our results showed as these molecules ranged depending on the used hardware and the parameters of hardware itself.

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Challenges in quantitative MSI of drugs in tissues

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Mass spectrometry imaging (MSI) allows spatial visualization, at a molecular level, of compounds on surfaces. This approach is used also to describe drug penetration in tissues, allowing 2d and 3d spatial information (1) of drug concentration and tissue molecular markers.
MSI data is quantitative on a relative scale, i.e. ion intensities are proportional to the analyte concentration but, on an absolute scale, assigning a "true" concentration value of drug in tissues is still challenging. Matrix effects, due to tissue composition, affects ionization efficiency, changing the absolute signal. The use of a homogeneous layer of internal standard, deposited on the tissue, compensates tissue derived matrix effects, proved by the linearity of calibration lines built on the same tissue. Absolute quantitative analysis can be confirmed using a gold standard technique, like HPLC-UV or HPLC-MS, analyzing parts of the same tissue (2).
While absolute quantitative data seems to be possible in principle, the fact that there is no chromatographic separation of tissue components from the analyte leaves a very high chemical noise in the signal that needs to be taken in account, especially when low levels of analyte are present in the spectrum.
Drugs in tissues typically are in the low pg/g to μg/g range, ppb to ppm, and an efficient quantitative MSI experiments should be able to detect as low concentration as possible. For this reason it is necessary to perform pre-processing of full scan data, to identify precisely the analyte ions and integrate them.
Different competences in the team are therefore needed to obtain accurate localization and quantitative information of drugs in tissues.

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High resolution AP-MALDI MS imaging as an aid to develop new strategies to improve ALS therapy

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Amyotrophic lateral sclerosis is a neurodegenerative disorder characterized by the loss of upper and lower motoneurons (MNs). Sigma-1 receptor (S1R) is implicated in the pathophysiology of all major CNS disorders and recently it has been demonstrated that a S1R agonist has potent beneficial actions in the SOD1 G93A animal model of ALS. A novel selective S1R ligand named RC-33, has been recently discovered. Its physicochemical characterization and in vitro metabolic stability suggest that this compound is a promising novel neuroprotective drug candidate.
Mass spectrometry imaging is a technique that allows direct determination of drug residues in tissues (2,3) allowing 2d and 3d spatial information on drug penetration in tissues. SOD1 G93A mice have been treated with the S1R agonist RC-33 and atmospheric pressure MALDI MSI (AP-MALDI-MSI) has been used to study the distribution of this candidate drug in spinal cord slices (ex vivo). In order to improve the protocols for preclinical investigations of drug candidates S1R agonists in animal models of ALS, we developed a versatile AP-MALDI-based imaging method to assess (ex vivo), in spinal cord slices, drug distribution, efficacy and mechanism of action. This study might offer an effective help in choosing suitable drug dosage to face pathologies related to MNs degeneration.
The method was based on AP-MALDI MSI and has been used to study the distribution inside tissues both of drugs to ascertain if they reach the intended target site during the pathology progression.
AP-MALDI MSI quantitative determination is ongoing and deuterated RC-33 has been synthesized to be used as a reference compound for direct, on-tissue, quantitation.

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In vitro and in vivo metabolism of the selective antagonist of the Eph-ephrin system UniPR1331

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The Eph receptors, together with their membrane-bound ligands, the ephrins, represent a key cell–cell communication system essential for the majority of the morphogenetic processes occurring during the embryonic development, as well as for the maintenance of cellular architecture of various epithelial tissues in the adult [1]. Moreover, it has been shown that a few specific subtypes of Eph receptors, i.e. EphA2, EphB2 and EphB4, are involved in tumor and vascular functions during carcinogenesis [2]. We recently identified N-(3β-hydroxy-Δ5-cholen-24-oyl)-l-tryptophan (UniPR1331) as the first small molecule antagonist of the Eph–ephrin system effective as an anti-angiogenic agent in endothelial cells, bioavailable in mice by the oral route [3]. We hereby present in vitro (mouse and human liver microsomes) and in vivo (mouse, p.o., i.p.) studies focused on the qualitative (HR-MS) and quantitative (HPLC-ESI-MS/MS) analysis of Phase I and Phase II metabolism of UniPR1331. Hydroxylation in multiple sites, oxidation of hydroxyl group in position 3 to ketone, glucuronidation were the main routes of biotransformation in mouse liver fractions, whereas oxidation to ketone and sulfation were the main routes in human liver fractions. After oral administration of UniPR1331 to mice, a previously unidentified isobaric metabolite was detected in plasma and in bile fluid. In vitro faecal fermentation yielded the same chemical entity suggesting a chief role of the intestinal microbiota in the in vivo biotrasformation of UniPR1331.

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MALDI-MS imaging distribution and quantitation study of imatinib inside tumor tissues using gold nanoparticles as matrix.

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MALDI-MS imaging is a useful technique to study drugs distribution inside tissues. The use of nanoparticles as matrices has opened new opportunities reducing background signals in the low mass range and increasing the spatial resolution. Among several nanomaterials, gold nanoparticles (AuNPs) have received attention for their suitability, offering large surface areas, simple sample preparation, flexibility, and selectivity.
We used AuNPs as matrix to study the distribution of the chemotherapeutic drug imatinib (IMT) inside mesothelioma, ovarian, lung and gastric xenograft tumor models. For drug concentration measurement inside the treated tumors, IMT was spotted at increasing concentrations on an untreated tissue slice, to build a calibration curve. AuNPs of 20nm diameter were synthesized and homogenously sprayed with D8-IMT as internal standard. Images were generated with an AB MALDI-TOF/TOF 4800, by plotting the sodium adduct of the drug molecule at *m/z* 516.3. An automatic signal processing algorithm, developed in Python was used to quantify IMT in each pixel after extraction, noise correction, internal standard calibration and normalization, correlating the different normalized ion signal intensity in each pixel to the calibration curve.
The AuNPs matrix allows to efficiently ionize IMT inside different tumor tissues harvested from treated mice, with a low background signals from matrix degradation and overcoming the problem of the ion suppression effect of the biological matrix. The study highlighted the heterogeneous distribution of this drug that poorly penetrates inside these tumors, suggesting the concept of pseudo-resistance as a further explanation for ineffective therapies and tumors relapse.

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A DEEP, COMBINED OMICS EXPLORATION OF THE FAAH$^{-/-}$ BRAIN LIPIDOME

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Fatty acid amide hydrolase (FAAH) is a serine protease, which hydrolyzes bioactive endocannabinoids (ECs). ECs are lipid mediators that bind to and activate cannabinoid receptors CB1 and CB2. Upon binding ECs initiate a number of signaling pathways which control diverse set of biological functions including memory, learning, social behavior, appetite and inflammation. Modulating the metabolism of ECs by inhibiting FAAH holds therapeutic promise in a wide range of neurological diseases. Since the discovery of FAAH, it has been a very good target for pharmacological studies, many natural and synthetic analogues have been designed to target this enzyme. Given its large number of biological functions, a deeper understanding of its lipidome and proteome would generate useful information for future pharmacological strategies to combat neurological disorders. The aim of our work is to investigate the difference in lipidome of FAAH-/- mice brain compared to its wild type using Ion mobility mass spectrometry, difference in proteome using TMT labelled quantitative proteomics and a deeper targeted lipidomics of all the biosynthetic intermediates of anandamide pathway which is the most important signaling lipid in FAAH knockout mice.
By using ion-mobility assisted, untargeted mass spectrometry, we discovered the upregulation of pro-apoptotic ceramides, besides previously known fatty acid ethanolamides and acyl taurines. Further we extended our study to TMT labelled quantitative proteomics to explore the effect of upregulated bioactive lipids on the proteome of FAAH -/- brain. The experiment generated relative expression data on 243 mouse proteins, out of which the majority of significantly upregulated proteins are related to cell growth, apoptosis, transport processes, cell to cell adhesion and RNA splicing. Our targeted lipidomic analysis of anandamide pathway showed a downregulation of its metabolic precursors, which could be a possible feedback inhibition to fine tune the levels of anandamide. Overall our study gives a deeper understanding of the lipidome and proteome of FAAH knockout mice brain.

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Comparative lipidomic analysis of Astrocytes upon graphene and graphene oxide treatment by UPLC-Q-TOF-MS

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During the past decade, graphene (GR) and graphene oxide (GO) have been increasingly investigated for their biomedical applications such as neuroprosthetic devices. This possible use of GO/GR make them important to determine their bio-compatibility with neuronal cells. However, the biological interaction of neuronal cells such as astrocytes with GR/GO is largely unknown. Here, we have investigated the effect of GR and GO treatment on astrocyte cells to understand the intracellular metabolic pathways affected. Untargeted lipidome analysis based on high-resolution UPLC-Q-TOF-MS allowed us to observe substantial changes in the lipid composition of astrocyte cells treated with GO and GR flakes (compared to their respective vehicles). Dysregulated lipids indicate that glycerolipid metabolism, glycerophospholipid metabolism, cholesterol biosynthesis, triglyceride metabolism are perturbed by GO/GR. Interestingly, un-targeted Lipidomics data was co-related with untargeted proteomics data. These finding provide further insights into the altered lipid profile across a wide range of biochemical pathways in astrocyte upon GO/GR treatment.

Translating multi-OMICS Research into Precision Medicine with Mass Spectrometry-based clinical Sample Mapping

Tom Knapman

Sciex-UK

Modern analysis methods allow us to probe further into an individual's genome, proteome and metabolome than ever before. Multi-OMICS approaches to profile and compare differences between individuals in the presence of a disease or treatment aim to integrate data from each of these OMICS areas to provide an overall biological understanding of a population, or to uncover markers for particular phenomic trait. One of the challenges in an OMICS approach, is the increasingly advanced methodologies that are necessary to collect OMICS data to the required depth and quality. This talk will focus on how advanced mass spectrometry-based sample mapping techniques can be applied in a simple, routine fashion to allow large scale multidimensional mapping of proteomes and metabolomes that can be subsequently integrated with genomic data and interrogated using sophisticated healthcare informatics to provide a powerful multi-OMICS approach for Precision Medicine.

Mass spectrometry as a tool in the elucidation of enzymatic metabolism of bioactive molecules

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Bioactive molecules, in particular drugs and toxic compounds, are generally organic substances endowed with limited water solubility. Both metabolic transformation and environmental degradation reactions provide solubility enhancement by oxidation (above all hydroxylation), reduction and hydrolysis. Molecular characterization of transformation products allows to quantitatively follow the molecular fate of bioactive and toxic compounds. In our research, untargeted analysis was performed by the means of HPLC-HRMS with mass defect filtering. For the elucidation of metabolism reaction, heterogeneous photocatalysis represents a valuable in vitro model to rapidly generate transformation products. This model was used to identify the main redox degradation pathways of some bioactive molecules and to compare the found degradants with known metabolites [1].
The biotransformation of drugs and toxic molecules involve many enzymatic systems. In this presentation some examples of drug molecules will be described, initially focusing on the complete human metabolic pathways of nicotine, compared with the photocatalysis approach [2].
Then metabolic transformation by uncommon enzymes will be reported: *i)* the effect of Baeyer-Villiger (BV) monooxigenase responsible of antibiotic resistance on *imipenem* drug [3]; *ii)* the N-oxidation by human hepatic flavin-containing monooxygenase on the doping drugs *selegiline* and *clomiphene* [4] and *iii)* the different approach followed in the case of hydrophobic compounds (fatty acids) metabolized by fungal enzymes and studied by GC-MS after derivatization.

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In situ metabolomics: insights into tissue metabolism revealed from high resolution MALDI Imaging mass spectrometry

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Over the last years, several research groups worldwide have implemented metabolomics approaches in order to investigate more deeply the dynamic changes involved in specific cellular processes. Indeed, each type of cell and tissue has a "metabolic fingerprint" providing information about organ or tissue-specific processes related to physiological and pathological state. Traditional methods for the detection and characterization of metabolites of interest on tissue samples involve their extraction and eventually purification prior analysis, thus leading to a loss of information on their specific in situ localization. MALDI Imaging Mass Spectrometry (MALDI-IMS) represents a unique tool for its capability of generating maps able to localize molecules in tissue. Nevertheless, it also allows to generate MSn spectra, able to elucidate the structure of the molecules of interest. We are reporting high spatial resolution images of metabolites obtained on brain tissue sections. First of all, we will show MALDI imaging analysis of phospholipids performed by high energy CID MS/MS. Results demonstrate that this is a very efficient method, able to elucidate the structure of isobaric species, confirming the potential of this technology to bridge between spatial and structural information on this class of compounds. Then, we will report the possibility of using MALDI-IMS to investigate the metabolic response of a GM2 gangliosidoses mouse model treated with enzyme replacement therapy.

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Mass Spectrometry and Metallomics: binding site location in the Cyt *c*-CDDP model system

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Filter aided sample preparation (FASP)/bottom-up high resolution mass spectrometry (HR-MS) approach was applied to the cytochrome *c*-*cis*-diamminedichloridoplatinum(II) (Cyt *c*-CDDP) model system in order to identify the Pt binding sites on this protein. The binding site location was accomplished in an automated way by using Mascot search engine: the potential coordinating amino acid residues M, C, H, K, W, T, S, E, D and Y (S-, N- and O-donors) were included in the search files as modified residues with mass gains relative to the possible CDDP fragments Pt^{2+}, $[Pt(NH_3)]^{2+}$, $Pt[(NH_3)_2]^{2+}$ and $[Pt(NH_3)_2Cl]^{+}$ and considering the charge brought by each of them. The platinated peptides found with Mascot were manually assessed to positively confirm the presence of the characteristic Pt isotopic profile in the HR-MS full scans of the precursor ions and in their MS/MS spectra.[1-3] The following ten binding sites were identified: T58, W59, K60, E61, E62, T63, M65, E66, Y67, M80. Among them, E61, E62, T63, M65 and M80 have already been reported in literature[4-7] while T58, W59, K60, E66 and Y67 have been detected for the first time. Due to the small number of characteristic b and y fragments obtained, probably related to Pt binding on protein, all these binding sites result equally probable and no one of them can be excluded at this level of our investigation. Anyway, the FASP/bottom-up approach used here has demonstrated its ability to highlight the remarkable selectivity of Cyt *c*-CDDP binding since only two specific portions of the protein (T58, W59, K60, E61, E62, T63, M65, E66, Y67 in peptide 56-73 and M80 in peptide 80-86) resulted involved. The critical issues of FASP/bottom-up HR-MS approach applied to metallomics are highlighted.

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Mass spectrometer for on-line gas analysis of volatile compounds in Milan water management system

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In order to monitor real time levels of volatile pollutants in water distributed in Milan, MM SpA is implementing an on-line analysis system. A Hiden analytical mass spectrometer with single quadrupole for direct analysis of gases has been tested for the first time in measuring several pollutants at ppb levels in water. The system has a permeable membrane inlet for the direct analysis of dissolved species. Parameters like water pressure, flow and temperature have to be taken in account, as well as the materials used to carry water from the piping to the instrument.

NEW FUNCTIONALISED SLIDES FOR IMAGING MS EXPERIMENTS: A PROMISING NANOSTRUCTURED TiO_2 SUPPORT

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Sample preparation is a fundamental step in Imaging Mass Spectrometry experiments to realize a precise and reproducible analysis among an entire sample set. A key role is played by the homogenous adhesion of tissue sections on the slides. Various research groups spend their time studying new workflows and also evaluate innovative supports bringing functionalized surfaces. In this contest, we tested new slides on which a layer of nanostructured titanium dioxide (ns-TiO_2) was present. We compared the performances of these slides to standard histology glasses and we employed them to perform Imaging-MS experiments using MALDI-TOF/TOF and vacuum MALDI-LTQ-Orbitrap instruments. In our experience, ns-TiO_2 coated slides provide many advantages: first of all, we obtained a better adhesion of tissue sections on the slides, also working with difficult samples as eye and lung, that improves uniformity of detection among tissue surface and prevents tissue folding and detachments. We also observed the formation of smaller matrix crystals during coating with automated spray device and a more homogenous layer that enhances spatial resolution. We observed a reduced delocalization of analytes, compared to the standard slides for Imaging-MS, which were prepared in parallel for the same samples, as well as no loss of sensitivity and comparable signal-to-noise ratios. We used ns-TiO_2 coated slides in Imaging-MS experiments of various types of animal samples and a wide range of small weight analytes. In conclusion, ns-TiO_2 coated slides showed remarkable advantages with respect to standard slides and suggested additional applications, which are currently investigated in different types of MALDI-TOF analyses and Imaging-MS experiments.

Lipid impairment in Charcot-Marie-Tooth type 1A (CMT1A) neuropathy: identification of potential therapeutic targets.

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Active myelination requires the high demand of lipid biosynthesis in Schwann cell (SC) processes, and the disruption of lipid biosynthesis may result in abnormal myelin and Peripheral Nervous System pathology. In experimental models of CMT1A disease, the most frequent form among hereditary neuropathies, we found a down-regulation of genes primarily related to lipid metabolism, a striking and early reduction of sphingomyelin content and a significant up-regulation of acid sphingomyelinase activity, which mirror the dysmyelinating phenotype observed in this disease. To examine in depth the role of lipids in CMT1A, we performed either targeted and untargeted analysis by LC MS/MS in sciatic nerves, enriched myelin fraction and biological fluids. We found an overall derangement of both sphingo- and phospholipid synthesis and recycling. Moreover, in CMT1A rat cerebrospinal fluid and serum, the whole lipidome heavily differed from that of wild-type littermates to suggest a systemic metabolic derangement. As sphingo- and phospholipid pathways are pharmacologically targetable, we treated CMT1A myelinating dorsal root ganglia (DRG) cultures with compounds that were able to restore the lipid homeostasis in SC and improve in vitro myelination. We are confident that refining our knowledge on the lipid pathways altered in CMT1A may contribute to the development of pharmacological approaches ready to be used in future clinical trials.

Mass spectrometry and dry-cured ham processing

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Parma dry-cured ham is a protected designation of origin (P.D.O) Italian food product. According to the traditional Parma ham council recipe, hams are processed via salting, drying and aging steps under controlled temperature and humidity conditions. During the salting phase a rich protein exudate is generated from ham. In order to reduce the salt content several strategies were proposed such as the replacement of sodium chloride with other salts or application of pressure. We used 2D-PAGE coupled to and MALDI TOF-TOF to characterize the protein content of exudates collected during salting phase from pressed and unpressed hams, at three time points, 1 day, 5 day and 18 days. Identification and quantitative protein content were confirmed by LC-mass analysis. We found that exudates proteome changes over time and pressure impacts on muscle texture accelerating extraction of myofibrillar proteins. Moreover, the release of myoglobin, that is involved in color development of Parma ham, is affected by pressure. This study expands the proteomic approach in the characterization of modifications that meat muscle undergoes during technological processes[1, 2] like the production of cooked ham[3].

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GC-MS strategy combined with Chemometrics for fire debris investigations purposes

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In the sphere of fire debris investigations, the possibility to recognize whether the collected evidences trigger towards the occurrence of an arson or not is a largely debated issue[1-3]. As a matter of fact, the results of GC-MS analyses performed on collected fire debris may be compared in a rather easy way to the ones from ignitable liquids, e.g. found in possession of a suspect arsonist. With the aim of evaluating the use of gasoline as a fire accelerant, different gasolines sampled from different oil stations located within the area of the city of Turin were analysed by SPME-GC-MS. The use of Multivariate Data Analysis approaches allowed us to build classification and likelihood ratio models indicating the probabilities that fire accelerants have been employed. Fresh and weathered samples were analysed and compared to standard mixtures (ASTM1618). Once the chromatograms have been collected, different chemometric approaches have been tested both on raw and semi-quantitative data. In particular, Principal Component Analysis, Self-Organizing Maps (SOM) and Partial Least Squares – Discriminant Analysis, as well as N-way strategies like Tucker3, were employed with the goal of recognizing the usage of fire accelerant. Likelihood ratio approaches have been evaluated for the same purpose, too. Even if in a preliminary stage, this work seems to emphasize the employment of multivariate strategies aimed to potentially help the interpretative process of fire debris investigations.

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Untargeted LC-MS analysis reveals new metabolic perturbations in children with type 1 diabetes

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Type 1 diabetes mellitus (T1D), characterized by defects in insulin secretion or action, is one of the most common chronic diseases in childhood[1], that influences the entire life of the patient. Despite the good glycemic control that is possible to obtain with insulin replacement, T1D is still associated with an excess of mortality in adulthood population[2], in particular for cardiovascular events, suggesting that several metabolic disorders persist in T1D patients in insulin treatment. The aim of our study is to investigate, on the basis of the untargeted analysis, if and which different metabolic alterations already present in T1D childhood population, not far from diabetes onset.
The analysis was conducted on the urine specimens through high-definition mass spectrometry. The samples were collected from 56 children of the pediatric population with the diagnosis of type 1 diabetes and in insulin replacement therapy and from 32 healthy volunteers comparable for age, sex and puberty.
The samples were analyzed through a Q-TOF mass spectrometer coupled with the UPLC in reverse phase column. The acquisition operated in ESI + and in ESI - ions, in scan and in MS^E mode. The data, were processed with multivariate (PCA, PLS-DA[3]) and univariate statistical analysis (ROC curves, t-test). After the validation of the models we were able to build robust models that clearly separate T1D children from healthy peers, and to extract a panel of variables discriminating between the two groups. The metabolites extracted showed that several pathways (i.e. steroids hormones, tryptophan metabolism) are already altered in a T1D pediatric cohort, even if the age of the onset is not far from the samples collection and all enrolled children are in good glycemic control.

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Why yellow raspberries are not red: an untargeted metabolomics approach

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MS based metabolomics technologies can drive the discovery of new compounds characterizing biological diversity. However, new bioinformatics approaches are needed to support analysts in the compound identification phase, which is usually manually handled and time consuming. Indeed, in untargeted experiments, each metabolite is broken into fragments during ionization and the identification of group of ions belonging to the same metabolite is usually non trivial.
To tackle this problem we have recently proposed a new algorithm to semi-automatically identify metabolite pseudospectra [1]. Its central idea is that the features belonging to the same metabolite should have highly correlated extracted ion traces (EICs) in the surrounding of the metabolite chromatographic peak.
In the contribution, we will illustrate the effectiveness of the proposed approach, which was used to identify in yellow raspberries the pseudospectra of a new group of compounds similar to A-type procyanidins. This specific "case study" will also give the opportunity to highlight peculiar characteristics of MS based metabolomics in presence of complex biological matrices.

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Rapid Evaporative Ionisation Mass Spectrometry – an emerging disruptive technology for the food testing industry?

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Mass spectrometry has traditionally been one of the 'last resorts' for food quality and composition analysis. While gas chromatography-MS (GC-MS), GC isotope ratio-MS (GC IR-MS), and liquid chromatography-MS (LC-MS) are widely used for food and agricultural product analysis, MS methods (including these) are generally considered to be slow, expensive and not amenable for routine application, mostly due to laborious sample preparation procedures. The advent of ambient ionization mass spectrometric methods remove most of the constraints associated with sample preparation and opened new opportunities for point-of-control monitoring.

Since ambient ionization MS (AIMS) methods require minimal or no sample preparation, the use of internal standards (or even external calibrators) is often impossible, resulting in the lack of quantitative information provided by these methods. Nevertheless, the spectral profiles are highly characteristic of the type, origin, age, etc. of the sample, which makes these approaches excellent for rapid profiling analysis. In these cases the MS spectral information is used as a 'fingerprint' for the identification of critical attributes associated with both the genetic origin and environmental exposure of the sample.

Rapid Evaporative Ionization MS (REIMS) was originally developed as a direct combination of electrosurgery (surgical diathermy) and MS, for the intraoperative identification of cancerous tissue and surgical margin control [1]. However, it has become clear from extensive collaborative studies with the food testing industry that the method can equally be used for the instantaneous characterisation of meat and fish as well as practically any water-containing food commodity and has potential for the development of an automated at-line testing platform [2].

Proof-of-principle applications have been developed addressing various food quality and composition testing requirements, e.g. detection of undeclared ingredients in processed foods and establishing authenticity of various products, e.g. Protected Designation of Origin (PDO) status dairy products, processed meats, farming production method (organic vs. conventional), geographical origin of pistachio nuts and botanical origin of monofloral honey.

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Innovative analytical methodologies for the development of new antitubercular agents

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Tuberculosis (TB) still constitutes a considerable threat for human health [1]. A strategy to fight TB could be the design of a glycoconjugate vaccine composed of carbohydrate moieties coupled to an immunogenic carrier molecule able to induce both humoral and T cell-mediated immune responses [2]. Ag85B was selected as a carrier protein since it is one of the most potent antigen species expressed by *Mycobacterium tuberculosis* yet identified [3]. The protein was conjugated with simple saccharidic moieties activated with the iminomethoxyethyl functional group [4], which selectively reacts with the ε-amino group of lysine residues [2]. Some Ag85B mutants at 23 and 275 were also planned in order to prevent the glycosylation in these positions and to preserve the immunogenicity of the glycoconjugates.
One of the main problems in vaccine development is the lack of a clear correlation between design and protection [5]. For this reason, the pharmaceutical analysis played a crucial role at all the stages of the research.
At first, MALDI-TOF analyses of the intact protein and its glycosylated forms were performed to define identity and purity of the samples and to assess the glycosylation profile. In addition, a peptide mapping was carried out on Ag85B, which was enzymatically digested with trypsin and chymotrypsin.
Furthermore, SPR technology was employed to determine vaccine affinity for IgG antibodies. The affinity was investigated comparing the wild type protein with its mutant and glycosylated forms. Analyses allowed to obtain a dissociation constant in the nanomolar range for Ag85B and to justify the mutagenesis approach, since wild type Ag85B and its mutants bind to the antibody more strongly than the glycosylated protein.

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Towards automation of MALDI/ToF experimental data pre-processing

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Reproducibility of bioinformatics analysis is essential for the quality of results. We have developed Geena 2 [1], a public tool for the automation of MALDI/ToF spectra pre-processing.

Its input consists in lists of peaks. It supports the following computations:

a) isotopic abundances for the same molecule are summed up,
b) data are normalized against a standard peak,
c) background noise is limited by applying a threshold modulated on the spectra profile,
d) an average spectrum representative of a given sample is defined by aligning replicate spectra and by computing the average intensities,
e) final alignment of average spectra is computed.

Various parameters are available in order to cope with the user needs. The output includes the average spectra and their alignment. All intermediate results may be downloaded.

Geena 2 has been used for the evaluation of the effects of long-term cryopreservation on serum samples [2], and in two recently published retrospective studies on the correlation between serum peptidomic profiles and cancer. In the first study [3] we found out a correlation between C3f serum level and risk to develop a breast cancer in patient affected by gross cystic disease of the breast. Second study showed, in women who undergone surgery for a breast cancer, a higher risk of relapse in patients having high serum level of angiotensin II [4].

In order to introduce more flexibility and statistical power, GeenaR, an implementation of Geena 2 able to exploit the richness of R modules, is under development. GeenaR will be able to leverage a greater number of analyses, e.g. for biomarker identification, through a workflow-based interface.

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Phenolic Compounds and Antioxidative Activity of Extracts of Berries Cultivated in NorthWestern Italy

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Berry fruits popularity has rapidly increased in Western countries due to their composition and health effects. The aim of this study was to compare the phenolic compounds and antioxidant activities of methanolic extracts of cultivated berry fruits harvested during the 2016 summer season in the Piedmont Region (NW Italy). Samples of raspberries (*Rubus idaeus*), black currants (*Ribes nigrum*), red currants (*Ribes rubrum*), white currants (*Ribes pallidum*), white and red gooseberries (*Ribes grossularia* L.), blackberry (*Rubus fruticosus*), goji (*Lycium barbarum* L.), and three Cvs. (*Duke, Blue Ray and Misty*) of blueberries (*Vaccinium corymbosum*) were extracted using 80:20 (v/v) methanol:water with formic acid (1%) at a solids to solvent ratio of 1:5 (w/v) and evaluated for total phenolic contents (TPC) [1] and their radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical [2]. All the extracts were characterized by HPLC-DAD-ESI-HRMS in positive and negative ion mode. On the basis of the information obtained from the UV-VIS spectrum and of the accurate mass of precursor ions and tandem MS experiments, the main polyphenols were identified and quantified. The berry extracts were classified by the content of anthocyans and other flavonoid classes. Results showed that the highest and lowest TPC values were recorded in black currants and goji samples, respectively. Maximum and minimum DPPH radical scavenging activities confirmed the same trend recorded for TPC values.

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Advances in the Mass Spectrometric Study of the Laser-Induced Vaporization of Graphite and Zirconium Carbide

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First extensive studies of vaporization of carbon at very high temperatures are dated back to the middle of 70's [1] when power lasers became available for the laboratory use and graphite vaporization data was highly demanded due to potential use of carbon materials in aerospace applications. Nevertheless, in most of the studies performed by the mass spectrometry of the laser-produced carbon vapors the molecular composition was poorly correlated with a surface temperature if any reliable temperature measurements were ever done at all.

In the present study the method and apparatus used for determination of the composition of carbon vapor presented in [2] were significantly improved in order to make a further considerable extension towards extremely high temperatures. Due to some major improvements in the design of the TOF mass spectrometer, time-shape of the laser pulse and pyrometer time resolution different carbon species from C1 to C7 and their temperature evolution were reliably recorded up to 4500 K. It turned out that the relative partial pressures of the different carbon species as well as the corresponding enthalpies were in a very good agreement with thermodynamic predictions. It was in particular confirmed that the molecules C3 and C5 constituted the main species in carbon vapor in the vicinity of its melting temperature.

The same approach was applied in the present work to examine the vapor composition of zirconium carbide at temperatures much above the earlier limit of ca. 3000 K reached in [3]. Evaporation of samples of various starting composition within the homogeneity domain ranging from ZrC0.65 to ZrC1.0 were investigated up to 4200 K. Therefore molecular composition of the evaporating liquid ZrC was obtained for the first time.

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Detection of Trace Impurities in Refractory Solids by TOF Mass Spectrometry with Laser-Induced Evaporation

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A new method for the determination of trace impurities of tens of ppm in solid materials is proposed, based on laser-induced evaporation of the substance in a vacuum in the forced congruence mode with simultaneous analysis of the evaporation products using a TOF mass spectrometer. The method was initially developed for determination of trace impurities in highly refined alumina powder.

In the present work, laser-induced evaporation with a laser power density on the order of 10^5 W/cm^2, sufficient for the intensive evaporation of the matrix oxide, was used for the mass spectrometric analysis of trace impurities. In distinction from the LIMS method, the evaporation of the substance into a vacuum is carried out by a pulse of the CW CO_2-laser with duration of tens of milliseconds without formation of a plasma jet. The neutral vapor particles enter the TOF mass spectrometer and pass the ionizer, where they are ionized by the electron impact, and then the space–time separation of ions occurs in a flight tube of the mass spectrometer. Thus, the processes of evaporation and subsequent ionization of the material are unrelated to each other, which enable the optimization of the regimes of laser vaporization and ionization by the electron beam.

The first results of measurements of the relative composition of impurities in α-corundum are presented. The comparative analysis of the results was inspired by substantial difference in the measurement results for impurities in the initial material obtained by a few conventional methods. The reported values of the relative composition of impurities were in a good agreement with the results of inductively coupled plasma mass spectrometry (ICP-MS).

The Analytical Scientist Innovation Awards (TASIA) 2016: Effortless introduction of liquid streams into an unmodified electron ionization source of a mass spectrometer (LEI interface).

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We have combined, for the first time, an atmospheric pressure gas-phase conversion mechanism with new ceramic coatings to create an innovative interface, called Liquid-EI (LEI)1. LEI is based on electron ionization (EI) but differs from previous attempts; the vaporization of solutes and mobile phase takes place at atmospheric pressure into a specifically designed region, called the "vaporization micro-channel", before entering the high-vacuum ion source. The interface is completely independent from the rest of the instrumentation, and can be adapted to any gas chromatography-mass spectrometry (GC-MS) system, as an add-on for a rapid LC-MS conversion. A ceramic liner, placed inside the vaporization micro-channel, acts as an inert, 'non-stick' vaporization surface, speeding up the gas-phase conversion of large molecules while lessening possible memory effects.

EI is an unparalleled, well-established tool for the identification of unknown gas-phase molecules. Its extension to a liquid phase, without the drawbacks and limitations that troubled this hybrid combination to date, provide the same unique advantages (library searchable mass spectra, robustness, negligible matrix effects) to LC amenable compounds, opening the door to new, challenging LC-MS applications. Deactivated silica coatings help to release the heaviest compounds to the gas-phase, improving vaporization efficiency and reducing high-temperature contact time for the most labile substances, bridging the gap between the world of classic LC-MS and GC-MS.

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Biomarkers to monitor myelin loss and remodeling

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The identification of de-remyelination biomarkers specific and sensitive to monitor disease progression and myelin remodeling, to test putative pro-remyelinating compounds and thereby select specific treatments is a prime goal within the Multiple Sclerosis (MS) community. Sphingolipids are major components of the myelin membrane and they are essential and rate limiting for its correct development, arrangement and function. Even more, sphingomyelin pathway is triggered by different stimulants and participates in pathogenic processes of MS. Therefore, we performed sphingolipid-targeted analysis by LC-MS/MS on tissue, cerebrospinal fluid (CSF) and serum of different CNS demyelinating experimental models to verify whether the levels of these lipids correlate with the amount of myelin damage and disease status. In particular, the cuprizone-fed model in which de- and remyelination can be exactly and reliably monitored was used to verify the specificity and sensitivity of sphingolipids as putative myelin biomarkers, while experimental autoimmune encephalomyelitis (EAE) mice were used to correlate biochemical data with disease scoring. As we found altered expression pattern of sphingolipids either in tissue homogenates and biological fluids of both the animal models, we are confident to finalize the identification of biomarker/s to monitor demyelination and remyelination that can be readily translated to human patients.

Environmental odor pollution. A GC-MS/O study with OdorPrep sampling approach

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Nowadays, odor emissions are an environmental pollution problem, therefore during the last decades scientific activity in this research field has been focused on the identification of the odor active compounds and on their correlation with the human perception [1].

Conventional approaches coupling the sampling on fiber or adsorbent substrates with GC/MS have been widely developed in order to characterize odorants [2-4]. A notable improvement in odor identification was gained with the development of GC-MS with olfactometric detection (GC-MS/O) that allows to associate the identification and quantification of odor compounds with their human perception [5,6]. Local regional normative describe GC/MS approaches for odor characterization [4], while standardized new sampling devices, OdorPrep, are currently under development under EC

This study shows the potentialities of an innovative methodological approach to identify odor active compounds responsible of odor annoyance coming from different industrial activities such as landfills, wastewater treatment plants and refineries. When the odor nuisance was perceived by population and when the VOCs concentration exceeded the threshold value, the prototype OdorPrep (LabService s.r.l.) started to collect air in Nalophan bags. The qualitative characterization of samples was then carried out by AirServer- TD-GC/ MS-O. The application of the aforementioned methodology during the nuisance events allowed to overcome the limitations of the conventional approaches related to the lack of instrumental sensitivity and to identify in a more accurate way the chemical compounds contributing to the annoyance.

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Combining shotgun and targeted proteomics to quantitate the missing proteome. Angela Bachi

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Quantitative evaluation of adenosine 5'-tetraphosphate and other five analytes related to nicotinamide phosphoribosyltransferase by LC-ESI-MS^n in Melanoma cells and mouse plasma. Michele Bianchi, Adolfo Amici, Ambra A. Grolla, Erika Del Grosso, Roberta Bellini, Cristina Travelli, Silvia Garavaglia, Leonardo Sorci, Nadia Raffaelli, Silverio Ruggieri, Armando A. Genazzani, Giuseppe Orsomando

Panax ginseng C.A. MEYER AND KOREAN RED GINSENG: A PROTEOMIC ANALYSIS. Clarissa Braccia, Mara Colzani PhD, Prof. Giancarlo Aldini.

MALDI-TOF mass spectrometry applied to microbiology and virology. Adriana Calderaro, Sara Montecchini, Mirko Buttrini, Sabina Rossi, Giovanna Piccolo, Maria Cristina Arcangeletti, Maria Cristina Medici, Federica Motta, Isabella Rodighiero, Carlo Chezzi, Flora De Conto.

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MALDI-TOF mass spectrometry applied to microbiology and virology. Adriana Calderaro, Sara Montecchini, Mirko Buttrini, Sabina Rossi, Giovanna Piccolo, Maria Cristina Arcangeletti, Maria Cristina Medici, Federica Motta, Isabella Rodighiero, Carlo Chezzi, Flora De Conto.

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High resolution AP-MALDI MS imaging as an aid to develop new strategies to improve ALS therapy. Enrico Davoli, Silvia Giordano, Caterina Bendotti, Simona Collina, Daniela Curti[1.] In vitro and in vivo metabolism of the selective antagonist of the Eph-ephrin system UniPR. Francesca Ferlenghi, Riccardo Castelli, Giorgio Carmine, Massimiliano Tognolini, Marco Mor, Alessio Lodola, Federica Vacondio.

MALDI-MS imaging distribution and quantitation study of imatinib inside tumor tissues using gold nanoparticles as matrix. Silvia Giordano; Lavinia Morosi; Francesca Falcetta; Mridula Prasad; Ilaria Fuso Nerini; Simonetta Andrea Licandro; Roberta Frapolli; Paolo Ubezio; Maurizio D'Incalci; Massimo Zucchetti; Pietro Franceschi; Sonja Visentin; Enrico Davoli

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GC-MS strategy combined with Chemometrics for fire debris investigations purposes. Eugenio Alladio', Marco Pazzi, Laura Pacifici, Fabrizio Malaspina, Marco Vincenti'

Untargeted LC-MS analysis reveals new metabolic perturbations in children with type diabetes. Paola Pirillo, Alfonso Galderisi, Matteo Stocchero, Antonina Gucciardi, Vittoria Moret, Elena Donazzolo, Mauro Naturale, Eugenio Baraldi, Giuseppe Giordano

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The Analytical Scientist Innovation Awards (TASIA) 0: Effortless introduction of liquid streams into an unmodified electron ionization source of a mass spectrometer (LEI interface).. Veronica Termopoli, Pierangela Palma, Giorgio Famiglini, Maurizio Piergiovanni and Achille Cappiello.

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Environmental odor pollution. A GC-MS/O study with OdorPrep sampling approach. Ivano Battaglia et al.

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