GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XLV NATIONAL CONGRESS ON MAGNETIC RESONANCE

Frontiers of Nuclear Magnetic Resonance: Translational Aspects and Advanced Solutions to New Scientific, Technological, and Societal Challenges

Modena 5-7 September 2016

Dipartimento di Scienze Chimiche e Geologiche
Università degli Studi di Modena e Reggio Emilia

BOOK OF ABSTRACTS

UNDER THE AUSPICES OF

UNIMORE
UNIVERSITÀ DEGLI STUDI DI
MODENA E REGGIO EMILIA

AND WITH THE CONTRIBUTION OF THE

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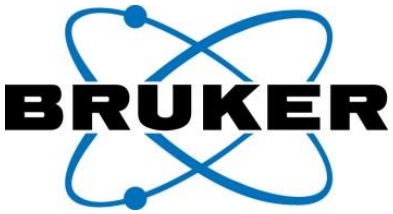

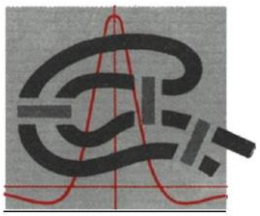

ACETAIA LEONARDI
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SCIENTIFIC PROGRAM

Monday September 5th

10:00-13:00	**Registration**
10:30-12:45	**Bruker workshop** **(main, but not exclusive, focus on solid-state NMR)**
12:45-14:00	**Bruker Lunch**
14:00-14:30	**Opening**
14:30-15:30	**Chair: V. Gallo and P. Turano** **GIDRM/GIRM gold medal award** **D. Capitani - Thirty years together with NMR:** **a long, enjoying, exciting, and sometimes hard walk**
15:30-15:50	**Annalaura Segre Fellowship 2015** **E. Carignani - Polymorphism and dynamics of glass forming alcohols studied by NMR spectroscopy and relaxometry**
15:50-16:35	**Plenary Lecture 1** **L. Emsley – DNP enhanced NMR crystallography**
16:35-17:40	**Coffee break + Poster session**

	Parallel session A **Chair: M. R. Chierotti**	**Parallel session B** **Chair: D. O. Cicero**
17:40-18:10	**A. Mucci -** Conjugated polymers for photovoltaics: from solution to solid-state NMR	**G. Parigi -** NMR relaxation to monitor dynamic phenomena
18:10-18:40	**C. Forte -** NMR for environmental sciences: selected applications	**L. Mollica -** Exploring the conformational space of an intrinsically disordered protein (IDP) via enhanced sampling and NMR spectroscopy
18:40-19:00	**F. Castiglione -** Transport phenomena in hydrogel systems: HR-MAS NMR investigation and modeling the anomalous diffusion effects	**C. Zucchelli -** Sp140 SUMOylation and DNA binding
19:00-19:45	**Plenary Lecture 2** **Chair: L. Calucci** **K. Saalwächter - Solid-state NMR investigations of elastomeric materials: high-end science at low field**	

Tuesday September 6th

8:45-9:30	**Plenary Lecture 3** **Chair: L. Schenetti** **M.L. Garcia Martin - Magnetic resonance in biomedical research**	
9:30-10:00	**Bruker Lecture** **S. Wegner - New opportunities for solid-state NMR using new technologies**	
10:00-11:20	**Coffee break + Poster session**	
	Parallel session A **Chair: A. Mucci**	**Parallel session B** **Chair: C. Marchioro**
11:20-11:50	**N. Proietti -** Unilateral NMR in cultural heritage: a tool for studying new eco-sustainable cleanings	**V. Righi -** HR-MAS NMR contribution to the comprehension of metabolic disorders in inflammatory and neoplastic hyperproliferative disease
11:50-12:10	**S. Mari -** Improving prediction accuracy and spectral assignment in public repositories	**A. Vignoli -** Serum metabolomic profiles identify ER-positive early breast cancer patients at increased risk of disease recurrence in a multicentre population
12:10-12:30	**D. Mammoli -** Triplet-singlet imbalance in pairs of magnetically equivalent spins	**G. Musco -** Integrating a prospective pilot trial and patient-derived xenografts to trace metabolic changes associated with acute myeloid leukemia
12:30-13:00	**GIRM assembly**	
13:00-14:15	**Jeol Lunch**	
14:15-15:00	**Plenary Lecture 4** **Chair: F. Arnesano** **Y. Cohen - Single and double encoding diffusion MRS and MRI: from model systems to imaging of the CNS**	
15:00-15:30	**Jeol Lecture** **K. Yazawa - Applications of ultra-fast MAS NMR**	
15:30-15:50	**Latest news from Jeol**	
15:50-17:00	**Coffee break + Poster session**	
17:00-20.00	**GIDRM assembly + announcement of poster competition winner**	
20:30	**Social dinner**	

Wednesday September 7th

8:45-9:15	**Lecture of Under 35 GIDRM prize 2016** **Chair: S. Mammi** **E. Ravera - Practical considerations over biomolecular solid-state NMR**	
9:15-10:00	**Plenary Lecture 5** **A. Piccolo - NMR-based metabolomics on agro-food products**	
	Parallel session A **Chair: M. Geppi**	**Parallel session B** **Chair: L. Mannina**
10:00-10:30	**M. Concistrè -** High resolution ^{14}N magic angle spinning SS-NMR	**F.P. Fanizzi -** NMR profiling as a tool for geographical origin assessment: the case of Italian extra virgin olive oil
10:30-10:50	**M. Mauri -** New travels of a noble explorer: ^{129}Xe in porous systems	**V. Gallo -** Validation of NMR fingerprinting methods: effects of processing on measure reproducibility and performance of the laboratories
10:50-11:20	**Coffee break**	
	Parallel session A **Chair: C. Airoldi**	**Parallel session B** **Chair: D. Capitani**
11:20-11:50	**D. Valensin -** Understanding the crucial role of metal ions in neurodegeneration by using NMR spectroscopy	**C. Zuccaccia -** Application of NMR in homogeneous organometallic catalysis: from olefin polymerization to water oxidation
11:50-12:10	**L. Mauri -** An automatic procedure for the HSQC quantification of heparin composition	**S. Zanzoni -** NMR footprints of upconverting nanoparticles mapped on the surface of transiently adsorbed proteins
12.10-12.25	**Chair: H. Molinari** **Poster competition winner**	
12.25-13-10	**Plenary Lecture 6** **C. G. Kalodimos – Molecular chaperones in action**	
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13:30-15:00	**Lunch**	

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GIDRM/GIRM Gold Medal Award

THIRTY YEARS TOGETHER WITH NMR: A LONG, ENJOYING, EXCITING, AND SOMETIMES HARD WALK

D. Capitani

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Since the beginning of my career I found NMR very fascinating because of the unbelievably wide variety of possible applications. Thirty years later I still see the application of NMR in the most various fields of research always growing and growing. In our laboratory we have always tried to exploit the versatility of NMR for giving an insight and a contribution in different fields of research.
In this lecture I wish to present some studies and results obtained by our group.
Some aspects concern the use of solid and liquid state NMR to elucidate the structure of polymers.
Other aspects deal with the study of gel and smectic phases with particular attention to the role of water, at this aim both high and low resolution NMR techniques were applied.
Other studies are focused on food science with the determination of the metabolite profile of food, the target analysis, and the application of statistical analysis to NMR data to get information on geographical origin, quality, safety, and food processing.
Finally, many studies are focused on cultural heritage. Among these, the evaluation of the efficiency of consolidating, protective, and cleaning treatments carried out on cultural manufacts, the evaluation of the state of conservation of cellulose-based materials, the structural characterization of clays, fired clays, and volcanic tuffs from ancient building and monuments, the non invasive and non-destructive monitoring in situ by portable NMR.
I have to say that during my walk with NMR I always enjoyed and I am not yet tired.

UNDER 35 GIDRM PRIZE 2016

U35GP

PRACTICAL CONSIDERATIONS OVER BIOMOLECULAR SOLID-STATE NMR

E. Ravera

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Biomolecular solid state NMR has seen a profound improvement from the theoretical and hardware standpoints; up to the point that sample preparation has become the key determinant for the success of SSNMR characterization, and sample preparation is all but a trivial accomplishment. Several approaches to SSNMR sample preparation have been proposed over the years, with varied results in terms of spectral quality: these aspects will be discussed, with a focus on sediments and immobilized proteins [1].
Among the different protein "solidification" strategies, we have proposed the use of sedimentation for obtaining samples with high sensitivity and resolution [2]. In this lecture we will cover the applications of NMR of sedimented solutes from its first description to the latest developments, including the implications towards the applicability of DNP. By examples we try to explain why such systems usually yield highly resolved spectra and show how sedimentation can contribute to the description of complex systems.
The other target of large interest that will be discussed are immobilized enzymes [3]. These systems are gaining great interest for industrial, medical and analytical applications, yet characterization of these systems have so far relied only upon activity tests or microscopic investigations. Among immobilization strategies, we have focused our attention on biosilica-entrapped enzymes: we have demonstrated that these enzymes yield high quality SSNMR spectra in their artificial but functional environment [4]. The NMR properties of immobilized enzymes will be thus analyzed for implications in ^{1}H detection and DNP.

This work has been supported by Ente Cassa di Risparmio di Firenze; by European Commission, Bio-NMR n. 261863, pNMR n. 317127 and COST action TD1103; the EU ESFRI Instruct through its Core Centre CERM/CIRMMP, Italy; FIRC though a triennial fellowship "Guglielmina Locatello e Gino Mazzega" (17941).

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ANNALAURA SEGRE FELLOWSHIP 2015

POLYMORPHISM AND DYNAMICS OF GLASS FORMING ALCOHOLS STUDIED BY NMR SPECTROSCOPY AND RELAXOMETRY

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The study of dynamic and thermodynamic properties of *glass forming* compounds plays a key role for understanding the mechanism of glass transition. Also *plastic crystals*, in which molecules have the average positions of the centres of mass ordered in a lattice, but dynamically disordered orientations, often present properties characteristic of the conventional molecular glass formers, and their study is an excellent way to focus on the role of the orientational degrees of freedom in glass transition.
In this context, neohexanol (2,2-dimethyl-1-butanol) and some of its isomers constitute an interesting class of compounds, since they have nearly globular shapes and show a rich polymorphism in the solid state including plastic crystalline and glass phases [1]. In particular, some isomers form orientationally disordered phases (ODIC) due to the ease of rotational motions in the solid state.
In this work, ^{1}H Fast Field-Cycling (FFC) NMR relaxometry (at Larmor frequencies from 10 kHz to 35 MHz) and static solid state ^{1}H and ^{13}C NMR spectroscopy have been applied to neohexanol and two of its isomers (3,3-dimethyl-1-butanol and 3,3-dimethyl-2-butanol) in the temperature range from -60 to 30 °C. Moreover, ^{1}H FFC NMR relaxometry and static ^{2}H NMR spectroscopy have been employed to investigate two selectively deuterated samples of 3,3-dimethyl-2-butanol. The spectroscopic and relaxation data acquired for the different isomers have been analyzed and compared to obtain information on the dynamic processes occurring in the different solid phases as well as to establish relationships between chemical structure and dynamic properties.

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PLENARY LECTURES

PL1

DNP ENHANCED NMR CRYSTALLOGRAPHY

L. Emsley

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When it can be applied, NMR spectroscopy is a riche source of structural information for both crystalline and non-crystalline materials. We show how enhanced NMR spectroscopy can be achieved by using dynamic nuclear polarization (DNP) for surfaces and for bulk solids. The approach yields over a hundred-fold signal enhancement in a variety of systems from mesoporous silicas to metal organic frameworks to nanoparticles and cements, microparticulate solids or intact pharmaceutical formulations.
By using incipient wetness impregnation of samples with a solution containing a polarizing radical, enhanced carbon-13, silicon-29, nitrogen-15, aluminum-27 or tin-119 DNP NMR spectra are obtained, allowing access to detailed structural features.
The most recent progress will be presented. In particular we will discuss results from high field (18.8 T) MAS DNP and from fast MAS. The role of new, highly efficient polarization sources will be discussed, as well as strategies to obtain the most efficient use of the microwave fields in the samples. In the best cases ^{1}H DNP enhancements of over 500 will be shown at 9.4 T and 100 K by combining degassing with incorporation of solid particles into the samples.

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PL2

SOLID-STATE NMR INVESTIGATIONS OF ELASTOMERIC MATERIALS: HIGH-END SCIENCE AT LOW FIELD

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Elastomeric materials play an important role in every-day life, rubber materials in tires or gels used in superabsorber or biomedial (e.g. drug delivery) applications being prominent examples. As these materials are disordered and consist of chemically or physically crosslinked chains, they are in most cases not soluble and thus pose challenges for their molecular-scale characterization. Understanding and being able to tune macroscopic properties such as stiffness or permeability for guest molecules requires characterization and control of the microscopic structure. Different NMR techniques have long been known to provide relevant qualitative information, but in recent years, proton multiple-quantum NMR [1] has evolved as the probably most powerful, quantitative and yet easy to use technique for that purpose. It relies on the measurement of ^{1}H-^{1}H residual dipole-dipole couplings (RDCs), and thereby provides not only information on the average crosslink density, which is proportional to the macroscopic mechanical elasticity moduls [2], but also on its spatial distribution (i.e. on inhomogeneities) and the content of non-elastic defects. Notably, the technique can be applied on rather simple low-field instrumentation [3].

In this contribution, I will discuss some instructive technical aspects of ^{1}H-^{1}H RDC quantification by double-quantum build-up analysis [1] and present some of our most recent applications of the technique addressing industrial problems as well as open questions in elastomer physics. Specifically, we have more recently started to study network deformation processes in molecular detail, as for instance relevant upon swelling [4] or stretching [5] of rubbers (see Fig. 1). We characterize the stretching on the single-chain level, and are thus able to test, augment, or even falsify established molecular theories of rubber elasticity.

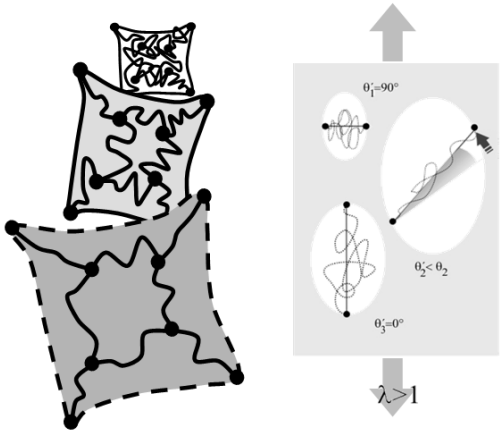

Fig. 1. Schematic representation of the microscopic deformation of network chains upon swelling (left) or uniaxial stretching (right).

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PL3

MAGNETIC RESONANCE IN BIOMEDICAL RESEARCH

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Magnetic Resonance imaging (MRI) and spectroscopy (MRS) have been extensively used in biomedical research to noninvasively study a wide range of biological processes. The first studies on spectroscopy applied to living organisms appeared in the early seventies [1, 2]. Almost at the same time, the first magnetic resonance image was obtained from a test tube containing two water-filled capillary tubes [3]. Few years later, the first MRI exam was performed on a human being [4]. Since then, the biomedical applications of MRI and MRS have grown enormously, not only in basic research [5], but also in the clinical environment, where they are currently used as routine tools for diagnosis and treatment follow-up.

Nowadays, with the technological advances over the last decades, together with more powerful computers and the development of advanced sequences, MRI and MRS have become very powerful techniques that can be used to investigate biological processes in very diverse systems, from single cells or biofluids to whole organisms. These techniques can provide us with anatomical, physiological and metabolic information, which highlights their great potential to contribute biomedical research. The aim of this presentation is to provide an overview of the different MRI and MRS techniques that can be used in biomedicine, with specific examples of preclinical and clinical applications.

In particular, the focus will be on: i) the characterization of the tumor microenvironment using both *in vivo* and *in vitro* techniques [6-9], and ii) MR molecular imaging using nanotechnology-based contrast agents [10, 11].

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PL4

SINGLE AND DOUBLE ENCODING DIFFUSION MRS AND MRI: FROM MODEL SYSTEMS TO IMAGING OF THE CNS

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Since the demonstration that diffusion-weighted MRI (DWI) is extremely sensitive to early ischemic events [1] and the finding that water diffusion is quite anisotropic in white matter of the CNS [2], DWI and more importantly diffusion tensor MR imaging (DTI) [3] have become important tools for studying CNS microstructures and pathologies [4]. All these applications, however, assume that water diffusion in the CNS is Gaussian; an assumption which is far from being correct in neuronal tissues especially when high diffusion weighting and long diffusion times are is used.
In the lecture after a short introduction on diffusion MRI we will first briefly described the application of q-space diffusion MRI (QSI) [5, 6]. We will show how QSI enables one to obtain micro-structural information in normal maturation and in different CNS pathologies. All these diffusion MRI applications can be classified a single diffusion encoding (SDE) MR experiments. In the lecture we will then introduce double diffusion encoding (DDE) MR and we will demonstrate the type of microstructural information that can be obtained from such double -PFG MR experiments [7]. First, the microstructural information obtained from complex phantoms where the ground truth is known will be described and then we will demonstrate how the same DDE methodologies can be used, through modeling, to provide microstructural information in cells, neuronal tissues and more [8,9].

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PL5

NMR-BASED METABOLOMICS ON AGRO-FOOD PRODUCTS

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The importance of NMR spectroscopy in the characterization of the metabolome of agro-food products has been progressively growing in recent years. While liquid-state NMR techniques are continued to be applied on extracts from plant and fruits, and on liquid materials, the HRMAS NMR investigation on the whole agro-food products is gaining interest for its capacity to provide high-resolution spectra capable to identify primary metabolites, and often secondary metabolites, without preliminary extraction.
This presentation will account for the recent works conducted at CERMANU to apply HRMAS and liquid-state techniques on different agro-food products such as wines, grape berries, cheeses, and tomato and maize leaves. Moreover, the elaboration of NMR results by multivariate analyses enabled to correlate the identification of the metabolome of the different agro-food materials to external parameters such as soil chemical and hydrological properties in the certification of the *terroir* of wines, the origines of production in the case of Campania mozzarella cheese, the bioorganic management practices in the production of maize, and the effect of microbes-derived biostimulants in the growth of tomato plants.
Besides metabolomics, the versatility of NMR spectroscopy becomes useful to an extended characterization of both food and agricultural materials. The High-Power Gradient Diffusion NMR spectroscopy was usefully applied to identify the degree of adulteration in extra-vergin olive oil. Furthermore, liquid-state ^{31}P-NMR spectra helped to study the influence of combined treatments of microbial bioeffectors and phosphorus amendments on the changes of phosphorous inorganic and organic forms in a soil under maize production.

PL6

MOLECULAR CHAPERONES IN ACTION

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Scarcity of high-resolution structural data has impeded an understanding of the recognition and anti-aggregation mechanisms of molecular chaperones. We recently reported the first ever structures of molecular chaperones in complex with unfolded proteins. We used advanced NMR spectroscopy techniques and isotope labeling approaches to determine the solution structure of the 50 kDa Alkaline Phosphatase (PhoA) captured in the unfolded state by three molecules of the Trigger Factor (TF) chaperone (~50 kDa), forming a ~200 kDa complex in solution [1]. We determined the high-resolution structure of each one of the TF molecules in complex with the corresponding unfolded PhoA region and reported how a chaperone dynamically engages its substrate. Very recently, we reported the structure of SecB, a chaperone that exhibits strong antifolding activity, in complex with PhoA and maltose binding protein (MBP) captured in their unfolded states [2]. The structural data revealed a unique complex architecture that explains the activity on the chaperone. Taken together, the data show how the different architectures of chaperones result in distinct binding modes with client proteins that ultimately define the activity of the chaperone.

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BRUKER & JEOL LECTURES

NEW OPPORTUNITIES FOR SOLID STATE NMR USING NEW TECHNOLOGIES

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Solid State NMR has made significant hardware progress in the last decade.
Remarkably espescially the maximum available spinning speed has been set nowadays to spees as high as 120 kHz.
This enables totally new and before not available pulse schemes, enabling solid state NMR to enter into the regime of high resolution spectra, as they have been seen beforehand only by high resolution NMR spectroscopists.
The talk will show and explain some of the benefits of the recent hardware progress and will also give a comparison of high speed and therefore high resolution MAS to older concept such as CRAMPS spectroscopy for high resolution proton NMR spectroscopy.

APPLICATIONS OF ULTRA FAST MAS NMR

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The Magic Angle Spinning is an essential technique to obtain high resolution solid state NMR spectra. We recently developed world's fastest ultra fast MAS system of 1 mm (up to 80 kHz) and 0.75 mm (up to 120 kHz). These give very attractive applications for structural analysis of organic and inorganic materials, particularly for samples with many spinning sideband (SSB) such as quadrupolar and paramagnetic materials, or with homogeneous broadening due to homonucelar dipolar coupling such as ^{1}H.
In this presentations, I introduce very recent applications using ultra fast MAS.
A first good example is a ^{7}Li spectrum of a cathode material for Lithium ion batteries. The conventional MAS rate (up to ca. 20 kHz) is insufficient to obtain clear center-band peaks due to many SSB caused by paramagnetic effect. By using ultra fast MAS, SSB goes away from center band peaks and consequently the positions of the peaks are confirmed.
The other good application is high resolution ^{1}H solid state NMR analysis. The fast MAS decouples ^{1}H-^{1}H dipolar network, and leads to high resolution ^{1}H solid state NMR.
^{1}H homonuclear correlation measurements can be powerful tools for structural analysis of pharmaceutical materials and biomolecules.

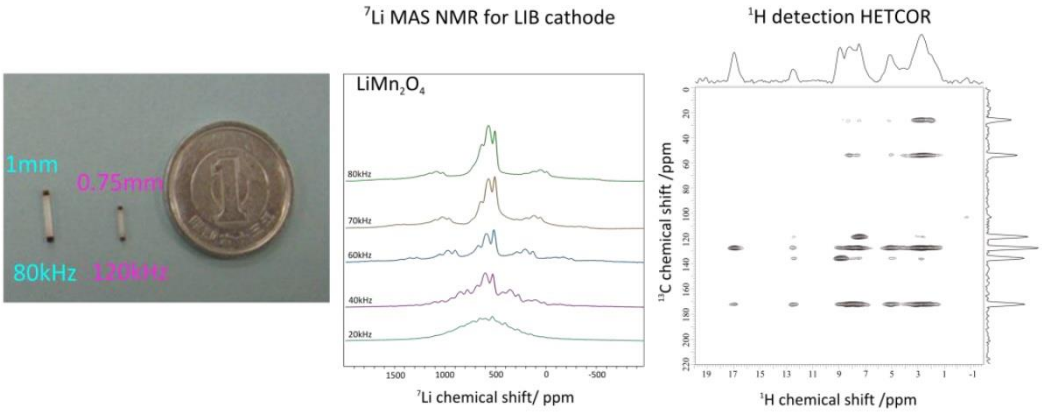

Fig. 1. A picture of 1mm and 0.75mm rotors for ultra fast MAS (left) and their typical applications, 7Li MAS spectrum for a LIB cathode (center and 1H detection 2D HETCOR spectrum for a pharmaceutical material (right)

ORAL COMMUNICATIONS

OC1

CONJUGATED POLYMERS FOR PHOTOVOLTAICS: FROM SOLUTION TO SOLID-STATE NMR

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Conjugated polymers are widely studied for applications in organic electronics.[1] The structural scenery is varied and the properties of polymers and copolymers can change heavily with the structure. The characterization of organic polymers used in organic electronics, and in particular in polymer solar cells (PSCs), is strongly multidisciplinary. It requires expertise within different branches of chemistry and at the border among chemistry, physics and engineering.
Here, the contribution of solution NMR to the study of self-aggregation properties of thiophene based conjugated polymers will be presented. NMR findings on polymers in solution appear to be correlated to morphological properties and solvatochromism. Since one of the most actively investigated application of conjugated polymers is as donors in bulk hetero junction PSCs, also some results obtained with solid-state NMR on polymer/fullerene blends will be reported.

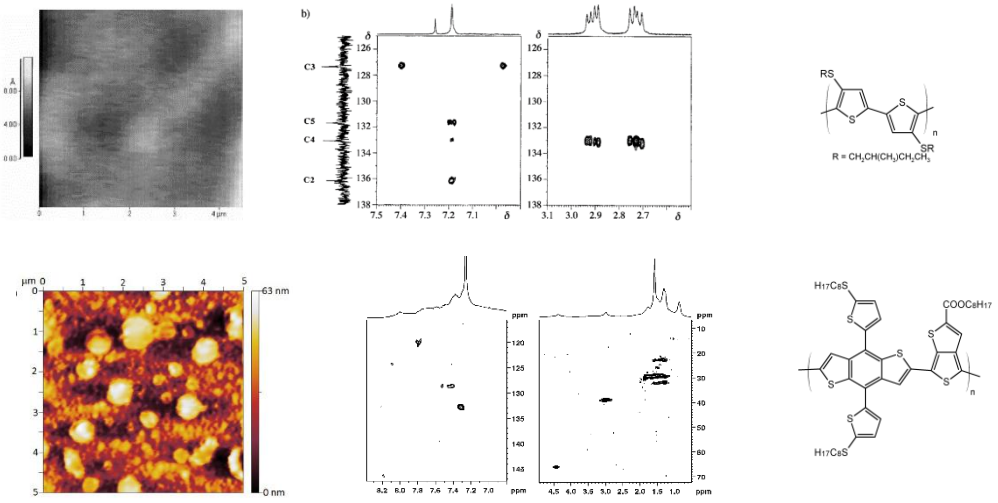

Fig. 1. Comparison between aggregation in thin film and NMR spectral appearance of two structurally different conjugated polymers.

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OC2

NMR FOR ENVIRONMENTAL SCIENCES: SELECTED APPLICATIONS

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Climate change and sustainable land use are among the most pressing environmental problems that modern society is facing, and to which other important issues, such as renewable energy and waste management, are directly related. These problems mainly involve environmental matrices, such as air particulate, soil, ocean sediments, and water dissolved matter, and, in order to be able to face them, a deep understanding of the chemistry and the key processes that take place in such matrices is required. Given the complexity of these systems, where different components (organic matter, sand, clay, minerals, roots, microbes, etc.) and different phases (solid, soft and liquid) coexist, NMR is often the only tool that can provide information on the molecular-level framework that underlies the problem. Furthermore, thanks to the combined application of solution and solid state techniques and the use of different types of pulse sequences, NMR allows environmental samples in a relatively unaltered state to be studied. This is extremely important since the structure, morphology and physical arrangement of the investigated material in part determines its environmental reactivity.
Selected examples of the application of NMR in two different fields of environmental sciences, taken from the experience of the Magnetic Resonance Laboratory (MarsLab) at ICCOM-CNR, will be shown.
The first one concerns the application of ^{13}C solid state NMR for the investigation of soil quality in relation to land management practices. Organic carbon is one of the indicators of soil quality and NMR gives a detailed indication of the speciation and reactivity of carbon in soils. The former is fundamental to evaluate the degree of humification and to assess the effects of agricultural practices or other anthropic activities on soils; the latter is of great relevance since transformations of soil organic matter directly influence the production of greenhouse gases, with consequences on climate.
The second example is the application of solid state NMR techniques to investigate the structure of biochars obtained from the carbonization of biomass through different types of thermochemical processes. Biochar is gaining much interest because it has a higher energy content than the biomass it is obtained from, and because, if used in soils, it contributes to reduce greenhouse gas emissions thanks to its long-term stability. Furthermore, biochar can be efficiently used for soil amendment thanks to its porosity, which can be studied by means of ^{1}H NMR relaxometry, a technique that also gives insight into the interaction of biochar with water.

OC3

TRANSPORT PHENOMENA IN HYDROGEL SYSTEMS: HR-MAS NMR INVESTIGATION AND MODELING THE ANOMALOUS DIFFUSION EFFECTS

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Hydrogels are amorphous polymeric matrices, able to retain up to 99% of water in weight, widely used for drug delivery applications, tissue engineering and regenerative medicine fields. The description of the transport phenomena of small molecules dissolved in hydrogel systems is a key step for drug-delivery applications. In particular, the contributions of solute-solute and polymer-solute interactions [1,2] on the drug diffusional mobility, related to the polymer network mesh size, plays an important role in designing new systems. We use High Resolution Magic Angle Spinning (HR-MAS) NMR experimental technique for understanding the mechanisms, which govern the transport motion of several drug molecules dissolved in the bulk phase of these materials. The data provide direct experimental evidence that anomalous diffusion take place inside the polymer matrices [3]. Indeed, performing experiments at different diffusion time Δ the molecular mean square displacement (msd) is estimated and consequently the equation of motion. Starting from the experimental data a complete overview on the transport properties of several drug molecules is provided, in particular a theoretical model that describes and rationalizes the differences between hydrogel and water environments is presented.

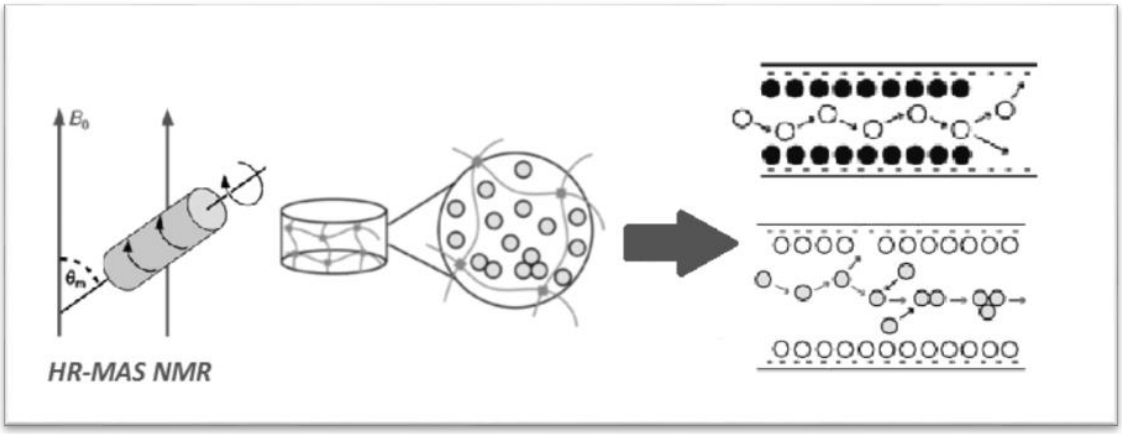

Fig. 1. Transport mechanism in hydrogel matrix using HR-MAS NMR.

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OC4

NMR RELAXATION TO MONITOR DYNAMIC PHENOMENA

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Nuclear Magnetic Relaxation Dispersion (NMRD) is a well-established technique to obtain information on molecular dynamics in biological systems, through the correlation times of different kinds of motions. ^{1}H NMRD profiles can detect motions occurring on time scales from 10^{-6} to 10^{-9} s, thus allowing for the detection of reorientation times of proteins from a few kDa up to MDa [1,2]. Using NMRD measurements we have shown that the intrinsically disordered protein (IDP) α-synuclein as well as a variety of other IDPs undergoes slow reorientations at time scales comparable to folded proteins. The slow motions are not perturbed by mutations in α-synuclein, which are related to genetic forms of Parkinson's disease, and do not depend on secondary and tertiary structural propensities. Our study indicates that long-range correlated dynamics are an intrinsic property of IDPs and offers a general physical mechanism of correlated motions in highly flexible biomolecular systems [3].

Novel promising magnetic resonance imaging (MRI) applications may be offered by paramagnetic nanostructures providing synthetic ease, versatility and good capability of enhancing the contrast in MRI. Nanocrystals coated with Gd(III) chelates present the advantage of a rigid core minimizing internal degrees of freedom. We have shown that gold nanostars can provide exceptionally high relaxivity, and that their efficacy is higher than spherical nanoparticles (Fig. 1) [4]. We demonstrated that this high efficiency is the result of optimized inner-sphere water exchange kinetics and particle surface-mediated elongation of second-sphere water residence lifetimes (and therefore enhanced second-sphere relaxivity). These results show that particle shape and second-sphere relaxivity are important considerations in the design of Gd(III) nanoconjugate contrast agents for MRI.

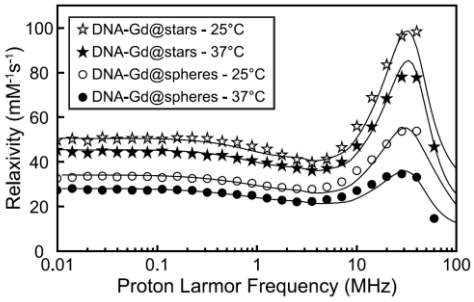

Fig. 1. ^{1}H relaxivity and its field dependence for water solutions of Gd(III)-DNA attached to gold spherical nanoparticles and gold nanostars.

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OC5

EXPLORING THE CONFORMATIONAL SPACE OF AN INTRINSICALLY DISORDERED PROTEIN (IDP) VIA ENHANCED SAMPLING AND NMR SPECTROSCOPY

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It is becoming increasingly evident that a significant fraction of the eukaryotic proteins is unstructured under physiological conditions. The term Intrinsically disordered proteins (IDPs) has been coined to refer to this class, which may involve the overall amino acid sequence or just specific domains [1]. Thus, rather than existing as a most probable state at equilibrium, IDPs are well described by a dynamic conformational ensemble of states among which they can easily interconvert. Due to their nature, a typical feature of IDPs is their ability to interact with multiple targets, thus being involved in diverse key cellular functions, including signaling and regulation, and pathological conditions, such as neurodegenerative disorders and cancer. Understanding and characterizing IDPs behavior is therefore of pivotal interest.
The C-terminal domain of the Sendai virus nucleoprotein (nTail) is intrinsically disordered. Experimental studies on this 26-residues-long peptide identified a central region with high alpha helical propensity, which is responsible for differently folded states [2]. Therefore, nTail represents a suitable prototype for computational studies of IDPs as well as of transient folding events. A major limitation, while attempting to simulate and adequately sample the wide conformational space of IDPs, is the regime of timescales in which rare events, including unfolding and refolding, typically occur. Herein, we exploited two enhanced sampling technique, namely the Parallel Tempering Metadynamics in the Well-Tempered Ensemble (PTMetaD-WTE) [3] and the Scaled Molecular Dynamics (SMD) [4], to explore the conformational space of nTail. The methods allowed an extensive sampling of the different states of the system, as all of the structures reported by experimental studies were successfully visited in our simulations and analyzed by direct comparison between calculated and experimental values of Chemical Shifts (CSs) and Residual Dipolar Couplings (RDCs).

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OC6

SP140 SUMOYLATION AND DNA BINDING

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Sp140 is an IFNγ-inducible leukocyte-specific nuclear protein, with unknown structure and function [1, 2]. Since 2008 an increasing number of evidences implicate Sp140 in the etiology of blood tumors (CLL [3-6], MLL [7, 8]). Sp140 harbours chromatin interacting domains (SAND domain, PHD finger, BRD) and indeed we proved that Sp140 binds to chromatin (IF, ChIP-seq) and interacts with proteins involved in RNA splicing, transcription and chromatin remodeling (interactome study by co-IP and mass spcetrometry). Structural and functional study of Sp140 domains (NMR and other biophysical and biochemical techniques), show that: PHD_{Sp140} finger does not bind to histones but to the PPIase Pin1 [9]; BRD_{Sp140} recognizes acetylated histone H4 tail; $SAND_{Sp140}$ interacts with DNA. We are currently focusing on: a) Sp140 binding to DNA: we are solving the solution NMR SAND structure and studying both the structural determinants for DNA interaction and the sequence specificity; b) Sp140 SUMOylation: our hypothesis that PHD_{Sp140} finger works as intramolecular SUMO E3 ligase for the adjacent BRD is supported by the facts that: PHD_{Sp140} and BRD_{Sp140} form a structural unit, named here "PB" (sequence alignment, NMR spectra); PB_{Sp140} SUMOylation is achieved through in vitro reactions; full length Sp140 is SUMOylated in DAUDI cells and four of the SUMOylation sites we identified by mass spectrometry are in BRD_{Sp140}; Ubc9 (SUMO E2 ligase) binds to PHD_{Sp140} and PB_{Sp140} (NMR titrations). Using as substrate a peptide containing one of the BRD_{Sp140} SUMOylation sites, we are performing in vitro SUMO1 conjugation assays and rate calculation [10] with and without PHD_{Sp140}, to prove an E3 SUMO ligase activity of PHD_{Sp140}.

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OC7

UNILATERAL NMR IN CULTURAL HERITAGE: A TOOL FOR STUDYING NEW ECO-SUSTAINABLE CLEANINGS

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Cleaning is an operation carried out to remove undesired layers formed on the surface of the manufacts and that may induce degradation to the manufacts. An important improvement to "classical" cleaning systems is the formulation of solution confined in host systems like physical or chemical gels. With respect to organic solvents, hydro-gel systems offer several advantages for the cleaning of cultural heritage artifacts in terms of selectivity, controllable and gradable penetration rate at every stage [1]. The study of water transport is a fundamental step to develop and improve with complexant and enzymes the effectiveness of hydrogels as cleaning methods [2,3]. In this study a cleaning method based on agar gel applied on stone was studied by single-sided NMR. Relaxation times and self-diffusion measurements were carried out on hydrogel systems with different agar concentrations. Hydrogels were put in contact with the stone and the kinetics of water penetration from the hydrogel through the stone was studied. Transport phenomena and penetration rate of water throughout the porous matrix were studied in a fully non-invasive and non-destructive way.
^{1}H depth profiles collected on stones after absorbing water from the gel gave information on the amount of absorbed water and on its penetration depth inside the specimen after water capillary absorption. The measurement of the self-diffusion coefficient of water entrapped in the gel structure provided evidence of the obstructive effect to the water molecules caused by the agar gel network. Portable NMR can be applied to tune the best condition of application of the cleaning system (agar concentration and time of application) in a non-destructive and non-invasive way.

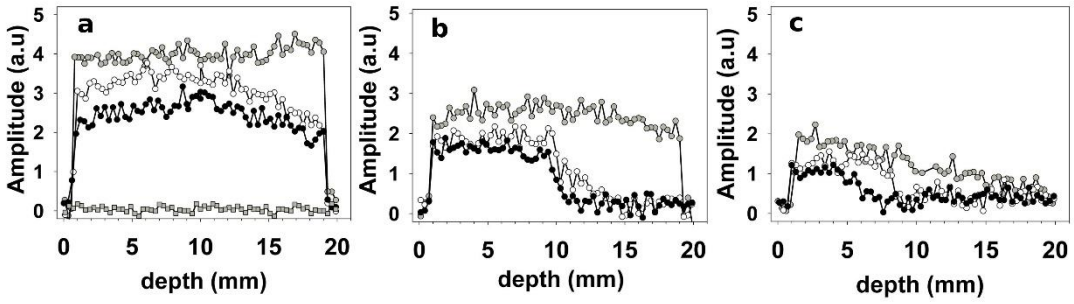

Fig 1. ^{1}H depth profiles of Noto specimens dry (square) and after absorbing water from the gel for 30 min (black circles), 1h (white circles) and 4 h (grey circles). ^{1}H depth profiles of Noto stone treated with a) agar 1%, b) agar 3%, and c) agar 5%.

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OC8

IMPROVING PREDICTION ACCURACY AND SPECTRAL ASSIGNMENT IN PUBLIC REPOSITORIES

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NMR spectral profiling and metabolite identification is one of the key bottlenecks in metabolomics and in general in mixture analysis.[1,2] Unlike Magnetic Resonance Imaging (MRI) which provides a straight visual representation of the objects (e.g. organs) under analysis, High Resolution NMR does not produce a direct 'picture' of the molecule. Resolving a molecular structure on the basis of the measured NMR spectra can be classified as an inverse problem where the goal is to recover 'hidden' information (i.e. the molecule) from 'outside' noisy data (NMR data). This is an ill-posed problem for which unique solutions can only be selected by imposing some constraints. 2D NMR experiments have proven to be extremely effective at providing "hard" proof of through-bond atom connectivities and hence narrowing down the 'acceptable' hypotheses set. However, this information may not lead to an unambiguous structure and additional knowledge is usually required.

An alternative, complementary tool to regularize the problem is given by chemical shift prediction. Leaving aside slow ab initio (i.e. DFT) prediction methods, most existing fast prediction methods are dependent in one way or another on a large database of assigned data which can be used to train a machine learning type of algorithm or used to search for similar chemical shift environments (e.g. increments and HOSE-code approaches).

However, regardless of the size and classification of the molecules selected for the training set, it will cover a very tiny fraction of the whole universe of possible compounds. For example, considering structures of molecular weight up to 500, the best guess for the number of plausible compounds is around 1060 which is effectively an endless frontier.[3] In order to come up with a general purpose 1H Prediction engine that covers as much chemical space as possible with reliable prediction error estimates, we have developed a Bayesian algorithm that combines the various partial estimates yielded by different prediction procedures (ideally as orthogonal as possible) and derive the statistics characteristics of the final outcome. In this work, we have used 3 prediction methods, Modgraph NMRPredict (Increments and HOSE code-based), Mestrelab Random Forests and MolApps Prediction (PLS method).

Furthermore, since only a fraction of existing metabolites and their spectral fingerprints are known, we decided to include into the Mestrelab Random Forests prediction model a set of metabolites from the Human Metabolome Database.[4] We selected 898 records where NMR spectroscopic data were available. We clustered these according to their maximum common substructure similarity scores and chemical class. A subset of those structures were selected to be included into the Random Forests prediction model. The predictive performances of the overall 1H Prediction engine will be illustrated with different data sets.

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OC9

TRIPLET-SINGLET IMBALANCE IN PAIRS OF MAGNETICALLY EQUIVALENT SPINS

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Long-Lived States (LLS)[1] are spin states with lifetimes T_{LLS} that can be much longer than the longitudinal relaxation time constant T_1. In a two-spin system, they correspond to a "Triplet/Singlet Imbalance" (TSI)[2], induced by perturbing the high-temperature equilibrium between the average of the populations of the three triplet states and the population of the singlet state. In recent years, it has been shown that it is possible to create a TSI by lowering the spin temperature well below the Zeeman splitting. This can be readily achieved by using Dissolution Dynamic Nuclear Polarization (d-DNP), either in pairs of inequivalent spins[3] or in pairs of magnetically equivalent spins[4,5].
In our laboratories, we have generated TSI's in partly deuterated ethanol[4] and in fumaric acid[5]. In $CD_3{}^{13}CH_2OD$ (with ^{13}C in natural abundance), a TSI was observed through crossrelaxation into observable transitions of both ^1H and ^{13}C spins, in analogy to cross-relaxation in ^{13}C-bearing methyl groups[2]. In fumarate, a symmetry-breaking addition of D_2O catalyzed by fumarase made the two protons magnetically inequivalent, so that the TSI became observable.
In principle, our strategies should be applicable to H_2O, possibly for generating an excess of either *ortho*- or *para*-water with respect to the 3:1 ratio that prevails in the high temperature regime. However, H_2O appears more challenging because the TSI can relax through spin rotation and dissipate through fast proton exchange. We have found suitable conditions where proton exchange is sufficiently slowed down by dilution in aprotic solvents[6] and we have adapted our d-DNP equipment to allow dissolution with such solvents. We have also studied longitudinal relaxation of H_2O in gas-phase[7], where spin-rotation is the dominant relaxation mechanism, finding relaxation times T_1 on the order of tens of milliseconds.
We will report here our latest results in this quest for the creation of *ortho*- or *para*-water, which we like to refer to as "forbidden fruits" of spectroscopy.

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OC10

HR-MAS NMR CONTRIBUTION TO THE COMPREHENSION OF METABOLIC DISORDERS IN INFLAMMATORY AND NEOPLASTIC HYPERPROLIFERATIVE DISEASE

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HR-MAS NMR spectroscopy allows the detailed metabolic analysis of whole biopsy samples, of normal and alterated tissues, to be performed. The NMR spectra constitute a "fingerprint" of the whole metabolome. Correlations between some metabolites and proliferative markers allow gaining insight into the relationship between cellular proliferation and the metabolic changes associated with the presence and tumour aggressiveness. Because metabolites represent the downstream expression of genome, transcriptome and proteome, they can closely reflect the phenotype of an organism at a specific time. Analyzing metabolic differences between normal and perturbed pathways could also provide insight into disease prognosis and diagnosis. Metabolomics could reveal novel cancer biomarkers that might expand our current understanding of this multi-factorial disease. The clinical role of proton NMR in the evaluation of brain tumours, as well as breast and prostate cancer, has been under investigation for several years.[1] During last years our interest was focused on metabolism of colonrectal and brain tissues by using the HR-MAS NMR technique. Some results coming for our research on glioma and linfoma brain tumours and the metabolic correlation existing between colorectal cancer and polyps will be presented.

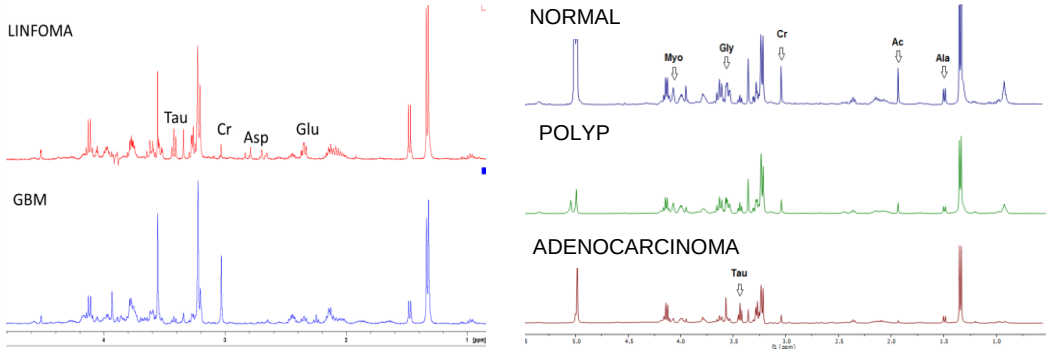

Fig 1. 1D ^{1}H cpmg spectra of A) brain tumors: linfoma and glioma and B) colorectal tissues: normal, polyp and adenocarcinoma

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OC11

SERUM METABOLOMIC PROFILES IDENTIFY ER-POSITIVE EARLY BREAST CANCER PATIENTS AT INCREASED RISK OF DISEASE RECURRENCE IN A MULTICENTRE POPULATION

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Detecting signals of micrometastatic disease in early breast cancer (EBC) patients could improve risk stratification and allow better tailoring of adjuvant therapies. We have previously shown that postoperative serum metabolomic profiles are predictive of relapse in a single-centre cohort of ER-negative EBC patients [1,2]. Here, we investigated this further using pre-operative serum samples from ER-positive, premenopausal women with EBC who were enrolled in an international phase III trial [3].
Methods: Proton nuclear magnetic resonance (NMR) spectroscopy of 590 EBC samples (319 with relapse or ≥ 6 years clinical follow up) and 109 metastatic breast cancer (MBC) samples was performed. A Random Forest (RF) classification model was built using a training set of 85 EBC and all MBC samples. The model was then applied to a test set of 234 EBC samples, and a risk of recurrence score was generated based on the likelihood of the sample being misclassified as metastatic.
Results: In the training set, the RF model separated EBC from MBC with discrimination accuracy of 84.9%. In the test set, the RF recurrence risk score correlated with relapse, with an area under the curve of 0.747 in receiver operator characteristics analysis. Accuracy was maximised at 71.3% (sensitivity 70.8%, specificity 71.4%). The model performed independently of age, tumor size, grade, HER2 status and nodal status, and also of AdjuvantOnline risk of relapse score.
Conclusions: In a multicentre group of EBC patients, we developed a model based on preoperative serum metabolomic profiles that was prognostic for disease recurrence, independent of traditional clinicopathological risk factors.

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OC12

INTEGRATING A PROSPECTIVE PILOT TRIAL AND PATIENT-DERIVED XENOGRAFTS TO TRACE METABOLIC CHANGES ASSOCIATED WITH ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a life-threatening hematological disease. Different prognostic parameters have been identified in the last decades, however novel, more sensitive and specific prognostic factors based on global biological features are required to define the best treatment strategy. Herein metabolomics, combined to the other "-omics" disciplines appears to hold promise for giving important contributions in identifying prognostic factors in the different AMLs. In the present study we took advantage of two complementary strategies to trace AML associated metabolic trajectory. On one hand, we designed a prospective observational clinical trial to identify metabolic changes associated with blast clearance during the first two cycles of intensive CT, to this aim we enrolled a total of nine AML patients and their PB, BM and urines and their metabolic changes from diagnosis to remission were followed by Nuclear Magnetic Resonance (NMR). On the other hand, we used NMR to analyze the metabolic changes occurring in the plasma of immunocompromised mice upon engraftment of primary human AML blasts. The mouse model allowed to monitor the metabolic trajectory associated with disease progression in a controlled system, thus reducing the intrinsic source of variability associated with human samples and AML genetic heterogeneity. Combining the two longitudinal approaches to trace AML either from diagnosis to remission in patients or from healthy to full-blown AML in mice, we narrowed our screen to 7 common metabolites, for which we observed a mirror-like trajectory in mice and humans, faithfully tracing AML progression and remission, respectively. We interpreted this set of metabolites as a dynamic fingerprint of AML evolution. To the best of our knowledge this is the first integrated longitudinal study reporting on the metabolic changes associated with Acute Myeloid Leukemia that merges human and patient-derived xenografts. Overall these NMR-based metabolics data, to be consolidated in larger cohorts, hold promise for providing valuable and non redundant information on the systemic effects of leukemia and appear to be well suited to be integrated in more comprehensive system biology approaches, to further advance our growing understanding on the complex interaction between leukemia genome, phenotype, and clinical manifestations.

OC13

HIGH RESOLUTION ^{14}N MAGIC ANGLE SPINNING SS-NMR

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Here we report on our latest progress towards making the exploitation of ^{14}N solid-state NMR routine for biomolecular systems, pharmaceuticals and natural materials.
The moderate quadrupolar constant of ^{14}N provides a wealth of structural and dynamic information although ad-hoc methodology is required in order to obtain good sensitivity and resolution. Two approaches have recently become popular: the indirect detection of ^{14}N fundamental transition via 'spy' nuclei [1-3] and the direct detection of ^{14}N overtone (OT) transition [4-7].
The first method enable ^{14}N spectral properties to be determined through detection along another channel, typically ^{13}C or ^{1}H. A variant of this method is here proposed and discussed. In our approach, coherences between the ^{14}N site and the spy nucleus are mediated by the application of a moderate rf field on the ^{14}N channel. Our approach allows looking at backbone quadrupolar couplings to extract structural information and to work on natural abundance materials to obtain fingerprint of their molecular structure.
The second approach relies on the detection of the overtone (OT) transitions: a double quantum transition with direct detection at twice the Larmor frequency. Because OT transitions are not affected by first-order quadrupolar coupling interactions, ^{14}N-OT-NMR results in very narrow (few kHz) lines. This huge gain in resolution is balanced by a poor sensitivity in the direct observation of OT transition. Here we discuss the use of different techniques [7] to improve sensitivity in ^{14}N-OT-NMR. The analysis of results has been facilitated by the use of a new simulation strategy implemented within the *Spinach* library [8].

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OC14

NEW TRAVELS OF A NOBLE EXPLORER: ^{129}Xe IN POROUS SYSTEMS

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Xenon is a "noble gas" and easily travels through most porous materials. The resonance of the spin active isotope ^{129}Xe is shifted by confinement: smaller pores produce greater chemical shift. In systems such as zeolites and nanoclays, where pore size is small, fixed, and homogeneous, simple equations relate chemical shift and nanopore size.[1]
More advanced NMR techniques opened up the study of mesoporous and hierarchical systems, and even natural materials such as rocks and soils where wide distributions of pore size exist. For example, variable temperature measurements allow estimating the size of mesopores by modeling chemical shift as the result of fast dynamic equilibrium between xenon bound on the pore walls and unbound xenon within the pore itself. [2]

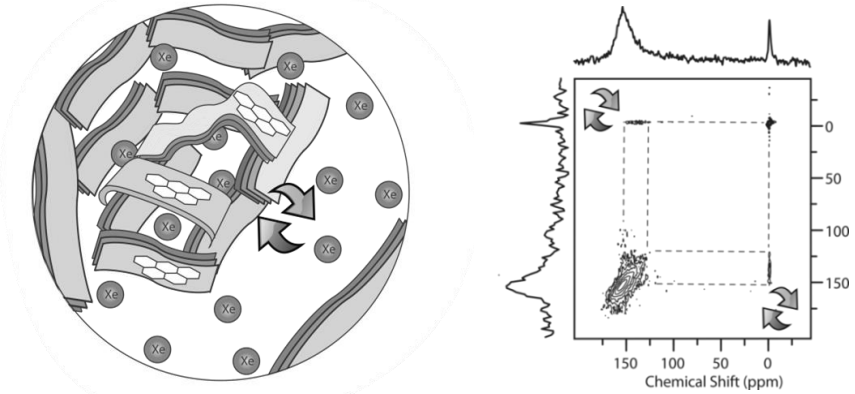

Fig. 1. Left: depiction on xenon in heterogeneous porosity, with exchange between micro and macroporous environments marked by arrows. The same arrows also mark the out of diagonal peaks in the 2D EXSY spectrum on the right, acquired with exchange time of 400 ms

Generalizing this approach, the combination of chemical shift at variable pressure and temperature, T_1 relaxation measurement and 2D exchange spectroscopy (EXSY) provides information on the different pore systems and their respective connections at different time scales. Fig. 1 depicts a mixed porosity system, a biochar [3,4] formed by graphitic layers that form mesopores with microporous walls. The timescale of the exchange is such that two separate chemical shifts appear, but exchange is detected by NMR EXSY at the tenths of second range as a function of thermal activation parameters. Enhancement of this technique by hyperpolarization even provides evidence of directional diffusion.[5]

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OC15

NMR PROFILING AS A TOOL FOR GEOGRAPHICAL ORIGIN ASSESSMENT: THE CASE OF ITALIAN EXTRA VIRGIN OLIVE OIL

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A key issue related to Extra Virgin Olive Oil (EVOO) evaluation and pricing is its geographical origin, being the Italian product in increasingly high demand on the market. NMR-based metabolomic profiling, a combination of NMR spectroscopy and multivariate analysis, has been often used for EVOOs characterization purposes [1]. We have recently profitably applied such a method to study inter and intra-cultivar variations of EVOOs with geographic and genetic identification of samples, even at individual olive tree level. Interesting results have been obtained specially for products from Apulia (the leader EVOOs producer region in Italy) [2]

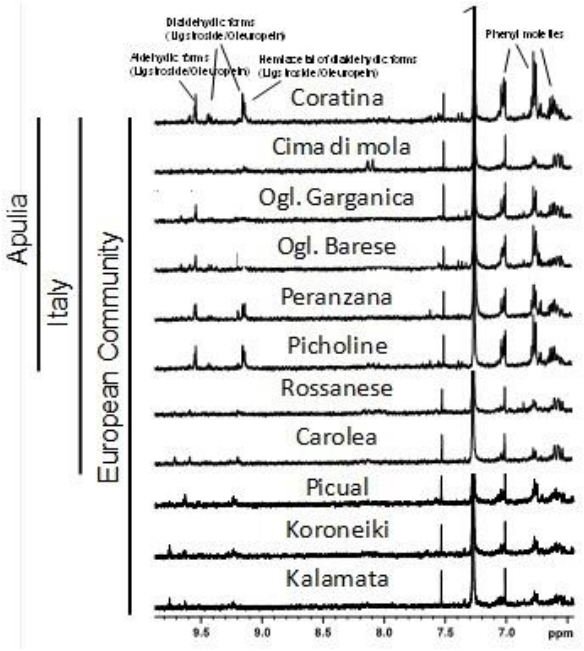

Fig. ^{1}H NMR spectra of different EVOOs in the region indicative for polyphenols content.

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OC16

VALIDATION OF NMR FINGERPRINTING METHODS: EFFECTS OF PROCESSING ON MEASURE REPRODUCIBILITY AND PERFORMANCE OF THE LABORATORIES

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The goal of this work was to set up new quality control parameter suitable for performance assessment in NMR fingerprinting methods. In order to achieve the goal, an inter-laboratory comparison (ILCs) was organized. It consisted in the analysis of wheat and flours aqueous extracts (4 samples) and was aimed to ascertain the statistical equivalence of the scaled NMR spectra. 780 NMR spectra were produced by 32 participants using 39 different NMR spectrometers. Seven NMR signals were selected for elaboration by univariate internationally agreed statistics typically applied in performance assessment of ILC participants. NMR data were generated submitting the 780 NMR spectra to 4 processing sessions differing for number of operators, processing procedure, software and integration mode. In the following table the details of the four processing sessions are listed.

Session	Number of operators	Processing procedure	Software	Integration mode
1[1]	many	phase and baseline correction according to operator expertise	no limitation	no limitation
2	one	manual phase correction and automatic baseline correction	Mestre Nova	integral
3	one (the same as in session 2)	manual phase correction and automatic baseline correction	Mestre Nova	peak
4	one (different from sessions 2 and 3)	manual phase correction and automatic baseline correction	AMIX	integral

The results will be described and commented with particular attention to the factor affecting measure reproducibility and performance of the laboratory.Participants to ILC are gratefully acknowledged.

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OC17

UNDERSTANDING THE CRUCIAL ROLE OF METAL IONS IN NEURODEGENERATION BY USING NMR SPECTROSCOPY

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Neurodegenerative diseases are extremely debilitating age-dependent disorders, characterized by cognitive impairment [1]. They affect millions of people worldwide and their incidence will enormously grow in the next years. The main hallmark of neurodegeneration is the presence of insoluble proteinaceous deposits in the brain, formed by the aggregation of misfolded proteins. Transition metal ions, like copper, zinc and iron are able to interact with these proteins, promoting either aggregation or production of reactive oxygen species (ROS). Understanding metal interactions with amyloidogenic proteins is, therefore, essential to evaluate the role of metal ions in neurodegeneration. NMR spectroscopy is a powerful tool for the structural characterization of metalloproteins [2] and it is largely applied to identify metal binding modes in amyloidogenic proteins [3-5]. We have investigated Cu(II) and Cu(I) interactions with α-Synuclein (Figure 1), Amyloid β and Prion Protein, which are the proteins involved in Alzheimer's, Parkinson's and Prion diseases, respectively. Our approach consists in combining NMR spectroscopy with other techniques, such as CD, EPR, XAS spectroscopies, potentiometry and computational methods, with the final aim to get a detailed description of the copper coordination sphere, copper binding affinity and copper-induced structural rearrangements.

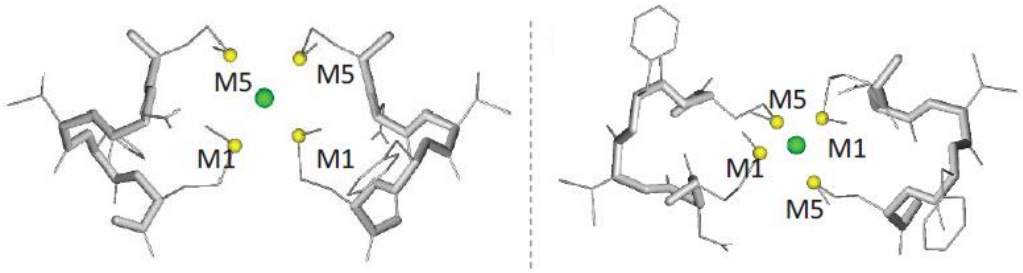

Fig. 1. Proposed Cu(I) site in the parallel (left) or antiparallel (right) dimeric Ac-αS1-15 structures

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OC18

AN AUTOMATIC PROCEDURE FOR THE HSQC QUANTIFICATION OF HEPARIN COMPOSITION

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Heparin, a widely used anticoagulant drug of biological origin, is a polyanionic polysaccharide, composed of a repeating disaccharide of an uronic acid and a glucosamine, variously substituted [1]. The complexity of heparin is mostly due to its biosynthetic pathway [2], which comprises, during the growth of the polymeric chain, a series of structural modifications consisting of partial N-deacetylation/N-sulfation, epimerization of glucuronic acid to iduronic acid and sulfation at different positions of the glucosamine and of the uronic acid residues. Moreover, during the process of extraction and purification of heparin from the animal tissue, some further modifications of the structure may occur, such as epoxidation of the iduronate residue or oxidation of the reducing terminal residues. ^{1}H-^{13}C HSQC NMR experiment was demonstrated to be one of the most successful approaches to the analysis and quantification of the mono- and di-saccharide composition of heparin and low molecular weight heparins (LMWH), through the integration of diagnostic signals [3,4,5]. The signal separation in the HSQC experiment allowed the quantitative evaluation of many signals that overlap in the corresponding monodimensional spectra, thereby allowing the quantification, with acceptable error of major and minor components [6]. The method was recently applied to distinguish samples from different animal/organ source [7] and also the brand drug from generics [8].A wider applicability of the method is limited by the need of having specifically trained operators. In the present work, an automatic processing method was introduced within the framework of Bruker Assure software. System suitability tests were included to guarantee instrument performance and an acceptance criterium in terms of signal to noise ratio of each spectrum was established. The automatic integration of the HSQC signals was optimized to minimize the differences with the manual integration by an expert operator. Calculation and reporting of the mono- and di-saccharide composition was implemented in the software. The comparison of the composition obtained by manual and automatic analysis of a library of porcine mucosa heparin HSQC spectra showed good similarities. This automated procedure is an important step to make the method available to a wider audience and sets the basis for an interlaboratory study.

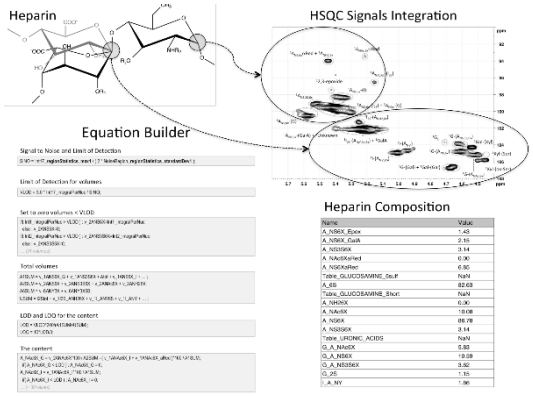

Fig. 1. Automatic processing, integration, calculations and reporting of quantitative HSQC analysis.

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OC19

APPLICATION OF NMR IN HOMOGENEOUS ORGANOMETALLIC CATALYSIS: FROM OLEFIN POLYMERIZATION TO WATER OXIDATION

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The comprehension of catalyst structure-reactivity relationships is a fundamental step toward a rational optimization of homogeneous transition metal catalysts. However, "catalytic recipes" frequently include the use of additives (activators, scavengers, etc...) in addition to (pre)catalyst(s) and substrates. Dissecting and understanding the various reactive/unreactive interactions between all these components, which play a pivotal role in determining the overall activity and selectivity of the catalysts, is thus of primary importance to rationalize the overall catalytic performances. Thanks to the high content of detailed information at the molecular level, NMR spectroscopy is one of the leading spectroscopic techniques to directly face this problem. Combining several information from multinuclear and multidimensional experiments, NMR methods can be successfully exploited to precisely determining the molecular and/or supramolecular structure in solution of pre-catalysts, catalytic active species, (off-loop) intermediates and/or species deriving from catalyst transformation or deactivation.
In this communication, the application of 1D and 2D NMR methodologies in the fields of transition metal catalyzed olefin polymerization and water oxidation will be illustrated using selected examples. In a first example, the role of Hf-C_{Aryl} and Hf-C_{Alkyl} bonds in determining activation and self-modification of pyridyl-amido and metallocene olefin polymerization catalysts will be discussed [1-2]. In a second example, it will be shown how the interaction of $[Cp^*IrL_1L_2L_3]X_n$ water oxidation catalysts with sacrificial oxidants, such as cerium ammonium nitrate, sodium periodate or hydrogen peroxide, determines the progressive oxidative transformation of the catalyst [3] and how this transformation is modulated by the nature of ancillary ligands (L) and experimental conditions.

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OC20

NMR FOOTPRINTS OF UPCONVERTING NANOPARTICLES MAPPED ON THE SURFACE OF TRANSIENTLY ADSORBED PROTEINS

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In recent years, nanoparticles (NPs) have been attracted huge interest in many fields. Their successful application in biomedicine necessitates understanding and controlling the interactions of NPs with biomolecules such as proteins, nucleic acids, carbohydrates, and lipids. Of particular importance is the adsorption of proteins on the NPs surface, as they may significantly affect protein structures and functions thereby influencing cellular activities [1].

Here we report the study of interactions between Ubiquitin (Ub), a small cytosolic protein involved in a large variety of cellular pathways [2], and lanthanide-doped upconverting SrF_2 nanoparticles (UCNPs). These UCNPs show attractive applications in biomedical luminescence due to their ability to produce upconversion emission. Upconversion is extremely useful for in vivo imaging, enabling deep tissue penetration, minimal tissue scattering, and high optical imaging resolution [3]. NMR spectroscopy, upconversion luminescence measurements and isothermal titration calorimetry were used to probe and characterize the Ub-UCNPs interactions. Besides, the incorporation of paramagnetic ions into NPs allowed us to exploit paramagnetic NMR as an additional, or alternative, method to conventional approaches for the investigation of proteins contacting NPs [4]. Paramagnetic centers close to the surface of NPs imprint metal nucleus distance information on NMR spectra of adsorbing proteins. PRE mapping improves the sensitivity of binding-site detection and has the unique advantage over alternative strategies that NP-contacting sites can also be detected when the test protein experiences competing interactions with itself (aggregation) or in the presence of a competing protein−protein association equilibrium, exemplifying possible interactions taking place in complex macromolecular mixtures (e.g., biological media).

The data indicate that the used UCNPs are not biologically inert but rather are capable of biomolecular recognition. In addition, the proposed approach can be successfully applied to all NP systems in which paramagnetic ions, or organic spin labels can be introduced, as a new tool to discriminate specific NP-protein interactions in complex biomolecular mixture.

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POSTERS

P1

^{1}H NMR METABOLOMICS APPROACH AS AN AID TO UNDERSTAND BIOCHEMICAL CHANGES OCCURRING DURING COCOA FERMENTATION

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Fermentation is a crucial stage in cocoa processing, because the biochemical reactions occurring on the main constituents of raw cocoa will lead to the development of the sensorial characteristics. ^{1}H NMR and HR-MAS ^{1}H NMR were previously found as useful tools to assess the geographical origin of cocoa and the fermentation level, the last influencing the metabolite profile of cocoa to a higher level in respect to other factors [1,2]. Cocoa beans fermentation level is at present evaluated by bean color (unfermented, slaty; underfermented, violet and fermented, brown). What actually happens during fermentation has been the subject of much research and the biochemical processes at a molecular level are still not entirely clear, hence, the global changes on cocoa bean chemical composition from the start to the end of fermentation process were explored by ^{1}H NMR metabolomics approach.

The experimental plan involved cocoa beans from 1500 pods, which were subjected to spontaneous fermentation in Ivory Coast. Samples were collected and dried at 0, 2, 3, 4 and 6 days of fermentation. Because generally not all the beans inside a sample reach the same fermentation level at the same time, the beans in each sample were further divided in slaty, violet and brown subgroups.

Metabolomics approach by ^{1}H NMR led to the simultaneous detection and quantification of several groups of metabolites (amino acids, sugars, organic acids, alcohols, catechins, methylxanthines), allowing to follow the global modifications of all the principal chemical classes of macromolecules during cocoa fermentation. Data on the whole samples showed that polyphenols, sugars and citric acids decrease during fermentation, methylxanthines remain constant, while amino acids, 2,3-butanediol, lactic, acetic and succinic acids increase. Interesting, when the beans of different colors in each sample were considered, a different metabolite distribution was observed. As an example, amino acids are more abundant in violet beans with respect to brown beans, even if they are considered poor fermented. This might indicate that violet beans have good potential to generate cocoa aroma through Maillard reaction during bean roasting.

Comprehensive and simultaneous metabolite profile of cocoa beans obtained by ^{1}H NMR can contribute to provide new insights on the comprehension of the biochemical changes occurring during fermentation.

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P2

^{1}H-NMR TO EXPLORE THE METABOLOME OF EXHALED BREATH CONDENSATE IN α_1-ANTITRYPSIN DEFICIENT PATIENTS: A PILOT STUDY

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So far, efforts to find laboratory markers characteristic of α_1 antitrypsin deficiency (AATD) have failed to produce convincing results that can be translated into clinical practice. Among the emerging technologies, the study of the complete set of low molecular weight metabolites within a biological fluid, may be useful to identify a signature that discriminates patients from controls, helping in the diagnosis and treatment of patients. Given its high reproducibility and requirement of minimal sample preparation, NMR spectroscopy is a very attractive tool.
Assuming that the metabolic activity of AATD patients would differ from that of healthy controls, the metabolic profiles of exhaled breath condensate (EBC) from 11 patients and 11 controls have been compared in an experiment in which the researchers who carried out the analyses and interpreted the results were blinded regarding who was in the control and in the experimental groups.
Although the spectral profiles of both groups were dominated by the signals of the same 20-25 compounds (which included monocarboxylic acids, alcohols, ketones and aminoacids), differences between controls and patients could be evidenced. In particular, the amount of different metabolites, in particular propionic acid, acetate, butyrate, benzoate, butanediol, fatty acid, alanine, acetone, acetoin, ethanol, lactate, methanol, was significant different in patient than in control's profile. When profiles were matched to individuals, not surprisingly each single subject was unambiguously assigned to one of the two cohorts, thus allowing to confirm the hypothesis that differences in the metabolite content could correlate with the disease status.

P3

INHIBITION OF THE NRF2-KEAP1 INTERACTION: SEARCHING FOR NEW PHARMACOPHORES

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The Keap1-Nrf2 pathway is the major regulator of cytoprotective responses against reactive oxygen species (ROS) and electrophiles in eukaryotic cells. Nrf2 is the transcription factor that regulates cytoprotective gene expression and Keap1 is the negative regulator of Nrf2, which mediates the proteasomal degradation of Nrf2. The inhibition of the Keap1-Nrf2 interaction may constitute a valuable therapeutic strategy for improving the outcome of a wide variety of diseases in which reduction of oxidative stress by protective genes plays an important therapeutic role such as cancer, neurodegenerative, cardiovascular, metabolic, and inflammatory diseases [1].
We have characterized the interaction of the Kelch domain of the Keap1 protein (KK1) with Nrf2 derived peptides with the aim of understanding the individual contributions of each amino acid to the binding mode to KK1. The hope is that the discovery of alternative binding modes to Nrf2 can lead to a different pharmacophore for the development of novel inhibitors.
We have used different shorter peptides derived from the 9mer minimal Nrf2 sequence required for high affinity Keap1 binding [2]. Using chemical shift perturbation and SPR experiments we have determined the role of each amino acid in Keap1 binding. The possible presence of different binding modes of shorter peptides was also investigated. In addition, we have explored the possible presence of an allosteric site, which might constitute an alternative region of the protein for targeting a new class of Nrf2-KK1 inhibitors, by studying a KK1 point mutant and its interaction with the Nrf2 derived peptides.
These results provide new insights for the rational drug design of peptidomimetic inhibitors of the Keap1-Nrf2 interaction.

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P4

DETERMINATION OF SOY ISOFLAVONES IN FOOD SUPPLEMENTS BY ^{1}H-qNMR

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Isoflavones are a class of compounds with estrogenic activity and they are found in small amounts in a number of legumes, grains, and vegetables, but soybeans are by far the most concentrated source of isoflavones in the human diet. Soy products that are gaining popularity in Western countries include soy-based meat substitutes, soy milk, soy cheese, and yogurt and many different food supplements, in which, soy extracts may be present alone or in association with others natural compounds. Soy isoflavone extracts are available as dietary supplements without a prescription in the majority of countries and also in Italy. These products are not usually standardized, and the amounts of isoflavones they provide may vary considerably. Moreover, quality control may be an issue with some of these products [1]. When isoflavone supplements were tested for their isoflavone content, the isoflavone content in the product differed by more than 10% from the amount claimed on the label in approximately 50% of the products tested [2]. For this reason ^{1}H NMR spectra of different methanolic extracts of soy-based food supplements were recorded using a Bruker FT-NMR AVANCE III HD 600 MHz spectrometer. It was possible to identify signals belonging to the soy isoflavones in the aromatic zone (from 6.5 to 8.5 ppm). Genistein, daidzein and glycitein signals were assigned also using ^{13}C, JRes and heteronuclear experiments such as HSQC and HMQC. The identified compounds were tentatively quantified using ERETIC 2 method. The obtained results further demonstrate the potential of the NMR technique in food chemistry.

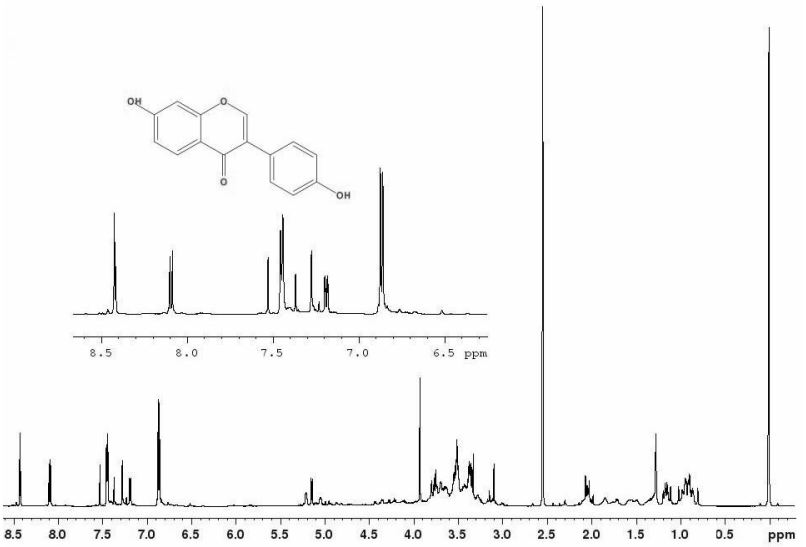

Fig. 1. A typical soy supplement spectrum.

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P5

NMR STUDY OF AN ANTIMICROBIAL PEPTIDE AND ITS INTERACTIONS WITH MODEL CELL MEMBRANES

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The appearance of drug resistant strains, also known as "antibiotic resistance", is a global health problem which causes the failure of common treatments and an increase in health costs. It is therefore necessary to find new anti-microbial agents. In this context antimicrobial peptides (AMPs), which are present in the innate immune systems of many organisms, seem particularly promising. In fact, their mechanism of action involves directly the cell membrane of pathogen microorganism, causing its permeability and rupture. The relation between the structure of these peptides and their mode of action towards the different kinds of cell membranes is a key aspect in the design of new antibiotic drugs, in order to increase the antimicrobial activity and the selectivity. NMR spectroscopy is a valuable technique for the study of the interaction of guest molecules with model cell membrane at a molecular level. On one hand, solution NMR experiments provide detailed information on the structure and conformation of peptides in solution, on structural changes occurring in the presence of micelles of phospholipids and on spatial proximities between peptides and phospholipids. On the other hand solid state NMR (SSNMR) techniques can provide valuable information about the mechanism of action of AMPs in phospholipids bilayer lamellar phase, which is more similar to the real cell environment [1].
The peptide studied in this work, YI13C, is a synthetic amphipatic peptide which has been designed to be selective towards Gram-negative bacteria and to have an antiendotoxin activity (i.e. neutralization of lipopolysaccharide, LPS)[2]. The aim of the study was to investigate the interactions of YI13C with model cell membranes and its conformation in the presence of phospholipid aggregates. The peptide has been obtained in its dimeric form by solid phase synthesis on resin support. Solution NMR spectra of the peptide in the presence of DPC (dodecylphosphocholine) micelles have been registered in order to determine the conformation of the peptide. This has been compared with previous results obtained in the presence of LPS [2]. In order to study the interactions with phospholipid bilayers, multilamellar vescicles (MLVs) of phospholipids and peptides at different concentrations have been prepared. The observation of ^{31}P - both in static and in MAS experiments- and the measurements of relaxation times allowed us to observe the effect of YI13C on the phase properties of the bilayer and on the dynamics of head groups of phospholipids. Moreover, the ^{2}H spectra in deuterated water provided information about the hydration state of the bilayers.

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P6

IMPROVING THE PERFORMANCE OF DIFMETRÈ® BY CAFFEINE-INDOMETHACIN CO-DRUG FORMATION

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A co-crystal is defined as a multi-component crystalline entity in which two or more different molecules occupy the same unit cell while linked together through weak interactions. Co-crystallization represents a strategy to modulate the physicochemical properties of the components. As for pharmaceutical co-crystals, in which one of the constituent molecules is an API (Active Pharmaceutical Ingredient), the main objective is to improve the bioavailability of hardly soluble active ingredients.
We synthesized a caffeine-indomethacin co-crystal. The sample was characterized by IR and Raman spectroscopy (the spectra are not reported in this poster), XRPD, SSNMR. Lastly, we evaluated its thermal and solubility performances through TGA, DSC and DKT tests.
As for the SSNMR analysis, we conducted ^{13}C CPMAS, ^{15}N CPMAS and ^{1}H MAS experiments and obtained information about the following aspects:

- ^{13}C CPMAS: purity and degree of crystallinity, number of independent molecules in the unit cell, nature of the adduct (salt or co-crystal)
- ^{15}N CPMAS: confirmation of number of independent molecules in the unit cell, involvement of nitrogen atoms in weak interactions (i.e. hydrogen bonds)
- ^{1}H MAS: presence and strength of hydrogen bonds

The adduct was revealed to be a 1:1 co-crystal of the two APIs, with one molecule of each in the unit cell. We observed the formation of a single hydrogen bond interaction between the carboxylic group of indomethacin and the purinic nitrogen atom of caffeine.

P7

COMBINING NMR METABOLIC PROFILING AND MICROBIOME ANALYSIS OF CANNONAU WINE

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Wine is a food product of remarkable commercial interest for a lot of countries, among which Italy of course. The chemical composition of grapes and their wines is significantly influenced by the environmental conditions of the vineyard. The ^{1}H-NMR metabolic approach can provide interesting information regarding the variety, geographical origin and behaviour of fermentation [1].By this approach we are performing a qualitative and quantitative analysis of the metabolome of grapes pulp and skins, musts (before and after fermentation) and wines produced by different vineyards that deal with the vinification of Cannonau, a typical Sardinian wine, perhaps the oldest in the Mediterranean Basin.

Wine	**Place of harvest**
Cannonau AHO	Alghero S.Maria La Palma- microv. AGRIS
Cannonau Mores	Mores- microv. AGRIS
Cannonau Santadi	Santadi- microv. AGRIS
Cannonau EF	Santadi- microv. EF
Cannonau Sedilesu	Mamoiada- microv. AGRIS
Cannonau Sedilesu CS	Mamoiada- microv. Sedilesu

Table 1. List of the origins of the different samples

The antioxidant activity of these extracts was also evaluated by assessing their reductive potential (Folin-Ciocalteu method) and their ability to quench radical species (ABTS-TEAC and DPPH methods). As a matter of facts, a correlation between a moderate wine consumption and a reduction of the frequency of neurodegenerative diseases, cardiovascular disease, cancer, brain dysfunction and inflammation, has been observed [2]. The NMR analysis of the different types of samples deriving from different locations has allowed identifying the metabolites most representative of the grape geographical origin. In addition, the influence, on the must composition, of the fermentation processes, as well as the correlation to the geographical location and the winery of production have been investigated.
At the same time we are performing, through genomic analysis, the characterization of the microbiome, present in the different kinds of samples. Once identified different populations of bacteria, yeasts and fungi, we will investigate the correlation between their metabolism and the NMR metabolic profile of the samples [3].

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P8

DEVELOPMENT OF QUANTITATIVE ^{13}C AND ^{1}H NMR METHODS FOR THE CHARACTERIZATION OF PHLOROTANNINS FROM MACRO-ALGAE

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Phlorotannins are physiologically active components from brown macro algae. They are oligomeric or polymeric phloroglucinol (1,3,5- trihydroxybenzene) derivatives where monomer connection can be obtained *via* three main bonds: aryl-aryl (fucols), aryl-ether (phlorethols) and dioxin bonds (eckols) [1]. In order to correlate bioactivity data to the presence of phlorotannins in algal extracts, reliable methods for their quantitative determination are necessary. Although phlorotannins have different structures and molecular weights, their chemical properties are extremely similar; consequently, the characterization and quantitative determination of single phlorotannins in a complex algal extract remains a challenge. However, in some cases, it can be useful to characterize them at the functional level, quantifying specific phlorotannin functionalities as groups rather than attempting to compute each individual substructure.
In this context, the present research was performed to evaluate the feasibility of ^{13}C and ^{1}H NMR methods for the quantitative analysis of total phlorotannins extracted from *Laminaria digitata* and for the determination of the relative content of different linkage typologies. Both spectra were recorded in quantitative conditions utilizing sodium trimethylsylilpropionate-*d4* (TSP) as internal standard. In the case of ^{13}C NMR, spectra were registered with an Inverse Gated Decoupling pulse sequence to suppress NOE effect and maintain proton decoupling. Chromium (III) acetylacetonate was added as relaxation agent, in order to obtain quantitative ^{13}C NMR spectra in relatively short times [2].
Results showed that ^{13}C NMR in quantitative conditions allows to determine the percentage of each carbon typology (aryl-aryl bonds, aryl-ether bonds and dioxin bonds), in particular of those involved or not involved in linkage. Moreover, the mean number of hydrogen for each aryl unit can be calculated. This allows an accurate quantitative analysis by ^{1}H NMR, with high precision and purities found comparable to the Folin-Ciocalteu assay and 2,4-dimethoxybenzaldehyde assay [3]. The knowledge of the mean number of hydrogens for aryl units in fucol and fucophloretol type phlorotannins allowed also calculating the mean degree of polymerization in the phlorotannin mixture.

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P9

UNRAVELING ELASTOMERS-OLIGOSILSESQUIOXANES INTERACTIONS BY NMR SPECTROSCOPY AND DYNAMIC MECHANICAL ANALYSIS

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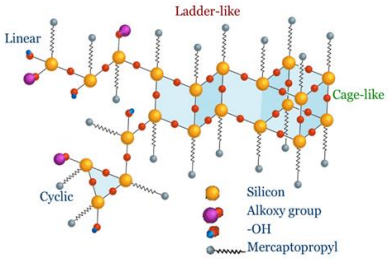
SiO_2-based nanofillers are commonly used to enhance the mechanical properties of rubber composites. Tuning size, shape and surface functionalization of the nanofillers allows the formation of continuous percolative networks within the rubber matrix, ultimately driving the filler-rubber interactions and providing effective reinforcement. Moreover, it has been recently reported that the addition to polymers of small amount of silsesquioxanes with tailored cage-like or ladder-like structures and highly reactive groups leads to a dramatic improvement of the mechanical properties.
With this aim, we have synthetized by controlled sol-gel hydrolysis-condensation reactions, mercaptopropyl-functionalized oligosilsesquioxanes (NBBs) with cage- and ladder-like structures, which have been incorporated (up to 10%wt) into polybutadiene rubber (PBD) by a swelling technique. The NBBs synthesis has been optimized in order to maximize the yield in cage-like or ladder-like oligomers, by tuning reaction duration and amount of water produced in situ, as proved by ^{29}Si NMR. [1]



Scheme 1. Different architectures of thiol-functionalized oligosilsesquioxanes (NBBs).

By means of ^{13}C and ^{1}H solid state NMR experiments two main effects due to filler-rubber interaction were observed: the NBBs loading-dependent change in PBD configuration from cis to trans, according to the ^{13}C spectra; the small shift and lineshape changes of PBD proton resonances, which appeared dependent mainly on filler structure. Moreover, homogeneous filler dispersion and increased molecular rigidity could be proved through ^{13}C VCT experiments. Dynamic mechanical analysis revealed that even very small NBBs amounts (<4%wt) provide significant reinforcement, thus indicating that the adopted approach may be promising for effectively improving the mechanical properties and for a potential reduction of silica utilization in rubber compounding.

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P10

FEATURE EXTRACTION FROM ^{1}H-NMR SPECTRA FOR FOOD CHARACTERIZATION

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During the last decade the awareness of consumers and society in general towards all aspects that concern food consumption has strongly increased. This has led to the new-concept restaurants, food production techniques and ways to enjoy and pair foodstuff, having high quality standards as drivers. The present work is part of a larger project, which is based on the fundamental idea of using analytical chemistry and advanced data analysis to build new tools to aid consumers when choosing foodstuff. A first benchmark, here explored, concerns the classification of different types of beer for assessing the linkage between the "objective" chemical analytical information and the "subjective" consumer's taste.
Analytical data were acquired on a set of one-hundred beer samples differing by brewery, alcohol content, yeast, brew type, etc. In a fingerprint-approach perspective, ^{1}H-NMR spectra were recorded together with NIR, GC-MS and fluorescence spectra, but also the relative sensory and marketing data were collected.
In particular, this work focuses on the analysis of NMR spectra aiming at linking gastronomic preference to feature-based beer patterns. Both fingerprinting and metabolic profiling approaches have been used in unsupervised analysis to reveal similarities and peculiarities among the different types of beer.
Peaks assignment, on the basis of literature references [1, 2, 3] and brewing process knowledge, have also been carried out in order to give "chemical" names to the new variables, to ease the fruition for non-insiders too.
Different chemometric tools (after proper preprocessing) have been applied in both approaches: PCA and ICA for fingerprinting; while a semi-automated strategy has been used for profiling which integrates depicting spectral intervals and decomposition (PCA) / resolution (MCR) techniques to resolve overlapping peaks and finally obtaining a peaks' features table.

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P11

METABOLITE PROFILING OF *GYMNSOPERMIUM MALOI* AND *GYMNOSPERMIUM SCIPETARIUM* USING NMR SPECTROSCOPY AND BIOLOGICAL TESTS

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Gymnospermium maloi and *Gymnospermium scipetarium* are two endemic species located in the south region of Albania, respectively in Gjirokaster and Elbasan [1]. Most of the work done until now was directed to their botanical and biological characterization while little is known about the metabolic profile and chemical composition of these closely related species. The macroscopic view of both metabolomes was obtained by the bucketing technique of ^{1}H NMR spectra of bulbs, stems and leaves and by further analyses by PCA, PLSDA, OPLSDA. The results obtained indicated that there is possible to differentiate these species further from their different spectra profiles. This study is focused on different classes of primary and secondary metabolites, including: aminoacids, carbohydrates, and alkaloids. Moreover the biological activity of the crude extracts of *Gymnospermium maloi* and *Gymnospermium scipetarium* were evaluated *in vitro* on human chronic myeloid leukemia cell line K562 and the results are reported.

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P12

NUCLEAR QUADRUPOLE RESONANCE CHARACTERIZATION OF HALOGEN BONDING

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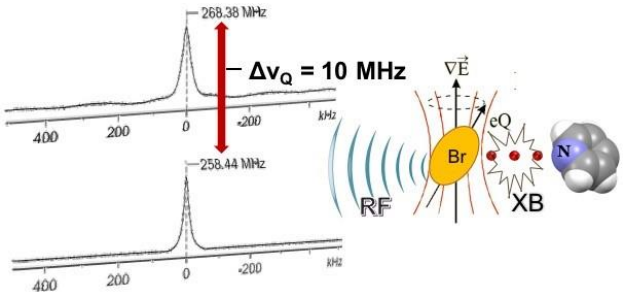
The halogen bonding (XB) is an attractive interaction between an electrophilic region associated with an halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity [1]. The XB is exploited as driving force to build supramolecular architectures with many promising applications in medicinal chemistry, catalysis, and conductive materials [2]. Solid State Nuclear Magnetic Resonance (SSNMR) has already proven to be an efficient tool to detect and characterize XB [3]; however, in the case of covalently bonded halogens, SSNMR still remains highly impractical. In the present work, we aim to fill the gap left by SSNMR with Nuclear Quadrupole Resonance (NQR), an old technique which has experienced a renaissance over the last 10 years due to the increased need of detecting illicit substances (*e.g.*, narcotics, explosives) [4]. Here, we report the successful characterization of a series of halogen-bonded compounds using $^{79/81}$Br NQR to probe Br···N motifs of different strength. The quadrupolar coupling constant C_Q and the asymmetry parameter η of the EFG tensor were experimentally determined by $^{79/81}$Br Nutation NQR. Overall, NQR is very sensitive to the occurrence of the halogen bonding: the pure resonant quadrupole frequency and the C_Q of bromine nuclei are both shifted upon XB formation by tens of MHz. NQR therefore enables direct insight of XB by probing covalently bonded bromine nuclei involved in XB, which are prohibitive for SSNMR. $^{79/81}$Br NQR frequencies may be used to detect the XB occurrence, whereas the quadrupolar interaction parameters provide an indirect measure of the strength of the XB.



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P13

CRYSTAL STRUCTURE AND TAUTOMERISM OF PIGMENT YELLOW 138 DETERMINED BY X-RAY POWDER DIFFRACTION AND SOLID-STATE NMR

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Pigment Yellow 138 (P.Y. 138) is a commercial greenish-yellow pigment based on quinophthalone. It exhibits three possible tautomeric forms, which denoted as CH-form (**1a**), OH-form (**1b**) and NH-form (**1c**), Fig. 1.
Due to the lack of good crystals for the single crystal X-ray diffraction analysis, the crystal structure of P.Y. 138 was determined by combining X-ray powder diffraction data (using real-space methods with subsequent Rietveld refinements) solid-state NMR and computational data. The tautomeric state was investigated by solid-state 1D and 2D multinuclear NMR experiments.
In the crystals, the compound exhibits the NH-tautomer with a hydrogen atom situated at the nitrogen of the quinoline moiety. Direct evidence of the presence of the NH-tautomer is provided by ^{1}H-^{14}N HMQC solid-state NMR at very fast MAS (70 kHz). Solid-state dispersion-corrected density functional theory calculations with BLYP-D3 confirm the correctness of the crystal structure and support the NH-tautomer. The NH hydrogen atom forms an intramolecular resonance-assisted N-H···O hydrogen bond to the neighbouring indandione moiety.

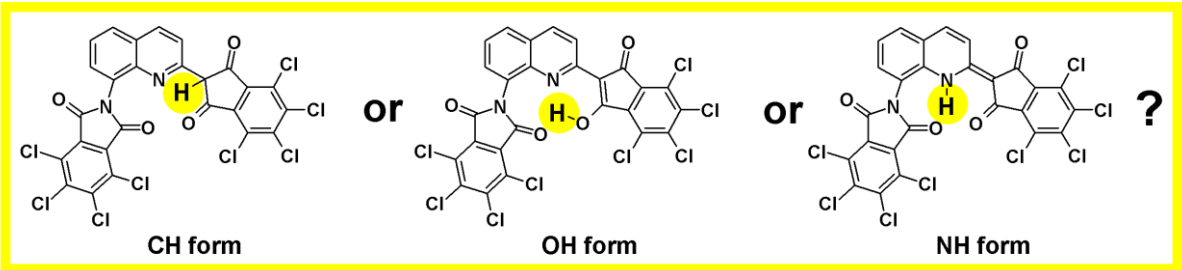

Fig. 1. Possible tautomeric forms of P.Y. 138

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P14

NMR-BASED METABOLOMICS OF BRONCHO ALVEOLAR LAVAGE FLUID (BALF) TO INVESTIGATE CHRONIC LUNG REJECTION.

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Bronchiolitis obliterans syndrome (BOS) is the main phenotype of an irreversible obstructive graft dysfunction known as chronic lung allograft dysfunction (CLAD), which challenges patient survival after lung transplantation. CLAD diagnosis relies on functional parameters and presents a significant heterogeneity in the pathology evolution. Tools that will help unraveling the complexity of the disease and identifying useful predictive markers are urgently needed. Metabolomics is indeed a platform capable of capturing disease-relevant metabolic profile changes and molecular signatures of disease processes.

To this purpose, we applied NMR-based metabolomics analysis to broncho alveolar lavage fluid (BALf) samples obtained during fiberoptic bronchoscopy from lung transplant stable patients or patients suffering from BOS or other respiratory pathologies (sarcoidosis and extrinsic allergic alveolitis).

In order to design an efficient and reproducible protocol to study BOS by NMR metabolomics, experimental conditions were optimized: several sample concentrations, different sample temperatures and times of storage and variations of spectra acquisition modes were evaluated. Exploiting the combination of mono and bi-dimensional NMR spectra, 37 polar metabolites, including amino acids, Krebs cycle intermediates, mono and di-saccharides, nucleotides and phospholipid precursors, were unequivocally identified and quantified.[1] The resonances of 5 additional metabolites have not been assigned yet. A comparison among ^{1}H-NMR spectra of BALf samples from stable individuals, BOS patients and people suffering from other respiratory pathologies was performed, showing interesting difference in the metabolic profiles.

The NMR data pointed out the potential of this methodology for the identification of predictive biomarkers to unravel serious post-transplant lung pathologies, including BOS. These preliminary results strongly support the possibility to afford a metabolic signature of BOS from NMR BALf analysis.

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This work was financially supported by the Cariplo (Milan, Italy) grant # 2013-0820-Bando 2013-Ricerca Medica.

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P15

BENEFICAL PROPERTIES OF COFFEE EXTRACTS: AN NMR STUDY TO ACCOUNT FOR POTENTIAL NEUROPROTECTIVE ACTIVITY

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Coffee is one of the most diffused beverages all over the world and recently interest in green coffee has increased too. Among the metabolites present in coffee extracts, most studies have been focused on chlorogenic acids (CGAs), quinic acid esters of hydroxycinammic derivatives – mainly caffeic and ferulic acid. CGAs represent the most abundant family of polyphenols in green coffee beans and occur ubiquitously in food.[1] A number of beneficial biological effects have been described for this family of polyphenols, including anti-inflammatory activity, anti-carcinogenic activity and protection in neurodegenerative diseases.[2] However, the molecular mechanisms through which these biological activities are carried out have not been completely elucidated yet.

Here, the screening of coffee extracts for the presence of metabolites responsible for neuroprotective activity is reported. Six coffee varieties, with different geographical origins, have been selected: three of them belong to the specie Arabica (Brazil, Colombia, Burundi) and the others to the specie Robusta (Tanzania, Uganda, Vietnam). For each of them both green coffee beans and roasted coffee were analysed. The metabolic profiles of the extracts obtained from the different coffee beans were characterized and compared by NMR spectroscopy.

Both the coffee extracts and 5-CGA, the most abundant phenolic constituent from CGAs family found in coffee beans, were tested for their biological activities. In particular, the molecular interaction with a neurodegenerative amyloid oligomers model (Aβ1-42 oligomers) was evaluated by means of STD-NMR and trNOESY-NMR spectroscopy.[3] Moreover, their antioxidant and anti-amyloidogenic activities were evaluated by cellular and biochemical assays, validating the existence of a correlation among the recognition of the molecular targets and the biological responses. Altogether, our results highlight how the biological efficacy of the different extracts can be related to their metabolic profiles.

Acknowledgements

Coffee samples were kindly provided by Beyers Koffie, Belgium.

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P16

THE EFFECT OF CORONARY OCCLUSION ON ARTERIAL SERUM METABOLITES

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Coronary artery disease (CAD) is a group of diseases that includes stable angina (SA), unstable angina (UA) and myocardial infarction (MI). It is caused by coronary arteries flow impairment due to vessels thrombosis (UA and MI) or to atherosclerotic plaques (SA). CAD it is the leading cause of death in the developed countries [1]. The accuracy of current diagnostic tools and markers, are still insufficient.
The aim of this study is to identify new biomarkers that can be used as diagnostic tool of CAD. Here we present the results of a metabolic study performed, using NMR, on arterial blood of patients with SA and UA.
Changes in arterial serum of SA patients collected before and after an angioplasty, a medical procedure that removes the occlusion, were analyzed. Angioplasty could be a model for the first moments of a MI, because, during this procedure, blood flow in coronary artery is blocked for one minute using a balloon. In the case of UA patients, serum was collected before and after a medical procedure that removes the thrombus and restores the correct blood flow.
Our preliminary result show a quick answer of the body to the presence of coronary artery occlusion, an answer that involves metabolites related to energy metabolism.

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P17

NMR-BASED METABOLIC APPROACH TO CHARACTERIZE STOOL SAMPLES OF PATIENTS WITH LIVER CIRRHOSIS

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Cirrhosis is a chronic disease that affects the liver by altering its structure and functions. This disease is extremely widespread in our country and it is one of the ten leading causes of death. In this work, we report the NMR-based metabolomic study of stool samples [1-2] of patients with cirrhosis disease. The focus on fecal samples is due to the growing attention to the intestinal microbiota environment, now considered as a real and essential "organ" in our body [3]. The gastrointestinal tract is, in fact, the habitat of a dynamic and varied microbial community mainly composed of bacterial species. The activity of these bacteria have effects on human health through the metabolism of nutrients introduced with the diet and the possible production of metabolites biomarkers of diseases [4-5].
In this study, we analyzed by ^{1}H-NMR spectroscopy 21 stool samples of patients with liver cirrhosis and 7 stool samples from healthy control subjects. The ^{1}H NMR spectra were investigated using the Principal Component Analysis (PCA) to detect possible metabolites whose levels were significantly different in sick and healthy groups and therefore to provide useful information regarding the interaction between the pathology and the human gut microbiota. An higher content of short chain fatty acids (valeric and butirric acids) was observed in control samples with respect to cirrhosis patients ones whereas branched chain amino acids content was higher in cirrhotic patients samples.

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P18

^{1}H-NMR BASED METABOLIC PROFILING AS A TOOL TO STUDY THE EFFECTS OF A PHENOLIC EXTRACT ON A HEPATOBLASTOMA CELL LINE

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The polyphenols have been the subject of intense scientific interest in recent years for their multiple protective and therapeutic effects. These molecules can affect metabolic pathways depending on the organ or cell type, on the cell state, and on the duration and dosage of the treatment. Recently we evaluated the effect of a phenolic extract from chestnut shell on a hepatoblastoma cell line, HepG2, evidencing that it was able after 48h of treatment to induce a decrease of the cell proliferation, an increase of the percentage of cell apoptosis and a mild elongation in the cells percentage at G0/G1 phase.
To evaluate the metabolic pathways affected from the treatment with this phenolic extract on HepG2 cells, we analysed the metabolic modifications using 1H-NMR spectroscopy based metabolomics approach. Firstly we performed an extraction of polar and apolar phases in untreated and treated cells by chloroform-methanol mixture, and, then, we acquired 1H spectra, assigned the metabolites present in the cells by chemical shifts and analysed statistically the spectra by fold change, principal component analysis and OPLS-DA. The obtained results showed that: i) the cells cultured in the presence or absence of this extract displayed different metabolic profiles, ii) a decrease of some amino acids and other molecules such as alpha- and beta-glucose, lactate and uracil (Figure 1).
Overall these data evidenced that the treatment with the phenolic extract from chestnut affected amino acid and glycolytic metabolisms, that are strictly correlated to AMPK representing both cellular sensor and glucose sensor. Therefore a glucose decrease can induce a decrease of AMPK activation able to lead cell death in according to the experimental results, obtained previously.

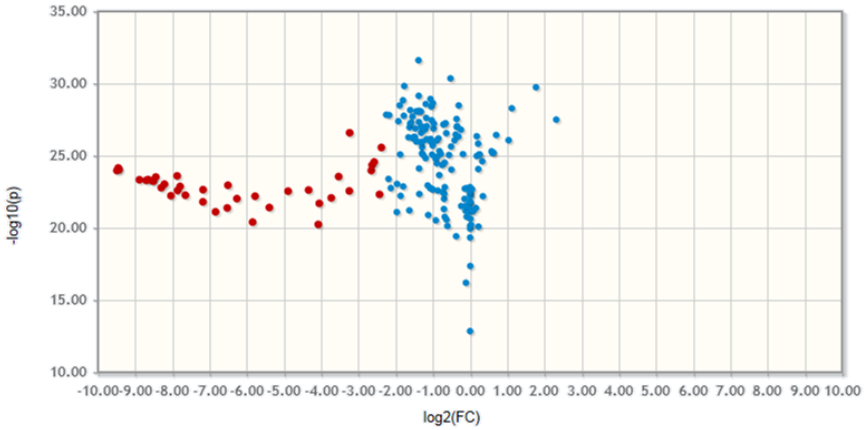

Fig. 1. Volcano plot obtained comparing the metabolite profile evaluated in the treated cells vs untreated cells. We report in red the metabolite protons for which the p-values are statistically significant and, hence, lesser than 0.05

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DIFFERENT SPECTROSCOPIC APPROACHES FOR THE ANALYSIS OF SECONDARY METABOLITES DERIVED FROM DIFFERENT TISSUES OF *Crocus sativus* L. FLOWER CULTIVATED IN ITALY

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In this study, several spectroscopic and chromatographic techniques were employed to investigate the metabolic profile of different tissues of *Crocus sativus* L. flower cultivated in Italy.
The volatile profiling of stigmas was characterized by GC-MS [1] for samples with the same genetic and geographical origin, revealing some differences associated with different drying processes applied.
LC-DAD, NMR [2], FT-IR [3], and Raman were used to explore quality parameters of saffron while other *C. sativus* flower parts (i.e. tepals and stamens) were also investigated. The metabolic profiling of saffron samples with different genetic origin but produced in the same region was also examined. *C. sativus* stamens, a possible bioadulterant, were studied to investigate potential marker compounds useful for their identification. The results obtained highlight some new aspects of genuine saffron sold in Italy.

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P20

NMR CHARACTERIZATION OF A VARIANT OF THE HUMAN ILEAL BILE ACID BINDING PROTEIN

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Ileal Bile Acid Binding Protein (IBABP) is a cytosolic protein known to bind and transport bile acids in the ileum. Recently, a N-terminal 49 amino acids longer variant of the IBABP, arising from an alternative transcription start site, has been identified in humans. Interestingly, this new long variant (IBABP-L) has been shown to be up-regulated in colorectal cancer, whereas the expression of the canonical short IBABP is unchanged [1].
A database search revealed that the sequence of IBABP-L is highly conserved among primates, and the first 24 amino acids are predicted to be a potential signal peptide for the secretion pathway. In order to assess the subcellular localization of the two variants, the cDNAs coding for IBABP and IBABP-$L_{(1\text{-}177)}$ (including the signal peptide) have been cloned and expressed in mammalian cells as GFP fusion proteins. Confocal microscopy images of Hek293 cells transfected with the two constructs showed that, whereas IBABP localizes to the cytoplasm as expected, the long isoform is mainly found in the ER/Golgi apparatus, indicating targeting to the secretory pathway.
The construct of mature IBABP-$L_{(25\text{-}177)}$ has been expressed in *E. coli* and purified. NMR characterization has been performed on the ^{15}N-IBABP-$L_{(25\text{-}177)}$ in order to assess whether the additional residues influence the structure and dynamics of the protein. NMR titration studies have been performed using ^{15}N-glycocholic and ^{15}N-glycochenodeoxycholic acids, the two most abundant bile acids in the ileum, and the results compared with those obtained for IBABP [2, 3].

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P21

LOW FIELD TIME-DOMAIN ^{1}H-NMR AS AN INNOVATIVE TOOL FOR POLYMER CHARACTERIZATION

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Recently, several pulse sequences originally formulated for high-field NMR spectrometers have been successfully tested out and/or readapted for low-field instruments: in such cases, the magnetization evolution is directly analyzed in the domain of time (TD) and the different families of protons are separated accordingly to their different mobility. The technique proven effectiveness, its high versability (i.e no experimental restrictions on sample phase) and the relatively low price make low field ^{1}H-TD-NMR a valuable characterization tool, particularly in polymer science. On top, low-field *double-quantum* NMR experiments allows to measure D_{res} distributions in vulcanized elastomers [1]. Since recent theoretical studies have demonstrated a direct connection between the microscopical NMR-accessible D_{res} and the macroscopical network crosslink density, an analysis of the network evolution with time in both undercuring and overcuring conditions of vulcanized IR and BR rubber for tyre-applications can be performed (fig.1) [2]. A similar correlation between microscopical and macroscopical properties have been investigated on BEVA ®731-substitute ethylene-butyl acrylate/tackifier/wax ternary and binary mixtures for cultural inheritage applications as well: the comparison between the NMR-accessible, measurable temperature-dependent proton rigid fraction (fig.2) in mixtures and the expected theoretical values can clarify the miscibility of the single mixture phases and, in case, the presence of interphase effects which may lead to improved stickiness properties. Lastly, similar rigid fraction measurements performed on poly(*n*-butyl acrylate)/polystyrene (PBA/PS) core-shell nanoparticles (NPs) shed lights on the different evolution of the two phases themselves and directly quantify the relative polymer amount in the two phases [3]. Particularly, at PBA and PS characteristic T_g significant changes in the rigid fraction have been measured: a strong phase separation preserving the single polymer peculiar properties and the absence of significant interfaces between them have thus beeen demonstrated.

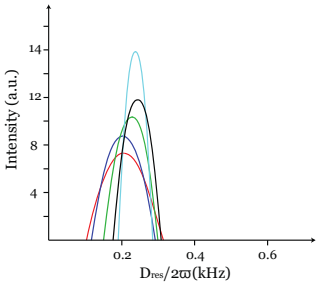

Fig.1. IR D_{res} distributions at different vulcanizing times.

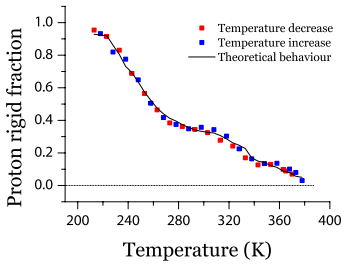

Fig.2. Ternary mixture rigid-fraction temperature dependence: experimental data and theoretical behaviour.

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P22

NMR APPLICATIONS IN THE DEVELOPMENT OF CURCUMIN-BASED RADIOTRACERS FOR EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

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Curcumin and curcuminoid complexes with metals have been subjected to a large number of studies due to their interesting potential as therapeutics in varying diseases. Curcumin is a phyto-compound and dietary spice extracted from the rhizome of the herb *Curcuma longa* L., commonly known as turmeric. It is commonly used in traditional medicines of eastern world countries thanks to its properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [1].
The features and the coordination properties of trivalent gallium are well-known from its traditional"cold" chemistry and can be exploited for developing new radiotracers. Notable attention has been focused on innovative biomolecules labeled with gallium-68 since it is a generator-produced radionuclide, a positron emitter with suitable energy and half-life (89%β^+, maximum energy = 1.92 MeV, T1/2 = 67.7 min) useful for many applications in nuclear medicine.
The aim of the present study is to investigate the feasibility of the labeling of curcumin and its derivatives with gallium-68 to obtain potential diagnostic tools for Alzheimer's disease. For this purpose, a complete characterization of the equivalent ^{nat}Ga complexes structure and properties was performed by means of nuclear magnetic resonance on a large variety of curcumin derivatives, applying both 1D and 2D homo/heterocorrelated techniques (Fig. 1).

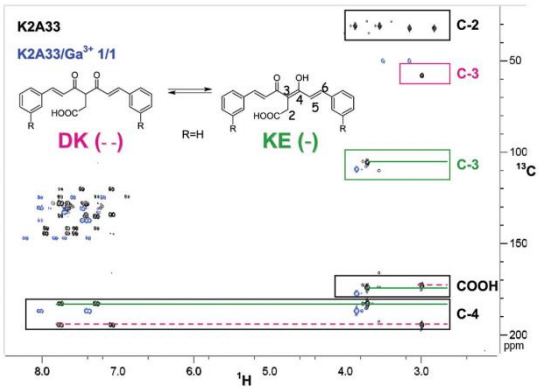

Fig. 1. ^{1}H,^{13}C HMBC spectra of curcumin derivative K2A33 (R = H; black) and K2A33:Ga^{3+}1:1 (blue). The typical cross-peaks of aliphatic chains in the tautomeric forms of the free ligand are highlighted in square boxes–magenta for DK and green for KE–for correlation peaks of COOH and C-4 a dotted and a solid line are respectively used to clearly distinguish the two species.

Acknowledgements: This work was funded by the "Fondazione di Vignola" (Project: "Diagnosi precoce della malattia di Alzheimer: nuovi agenti diagnostici bi-modali per imaging: RAD - radiotracers for Alzheimer's Disease)".

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P23

BIS-HYDRATED Mn(II) COMPLEXES AS POTENTIAL MRI PROBES

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The application of Mn^{2+} complexes as MRI CAs was already envisaged in the early times of MRI in the late 1980s [1]. An important advantage of Mn^{2+} CAs over the traditional Gd^{3+} counterparts is the lower toxicity of free Mn^{2+}. On the other hand, the lower effective magnetic moment of Mn^{2+} complexes with respect to Gd^{3+} analogues generally results in lower relaxivities of the Mn^{2+} complexes [2]. An obvious strategy to increase relaxivity is to increase the number of water molecules coordinated to the paramagnetic ion (q). In our work we report a series of ligands containing pentadentate 6,6'-((methylazanediyl)bis(methylene))dipicolinic acid binding units that form complexes with Mn^{2+}. The protonation constants of the ligands and the stability constants of the Mn^{2+} complexes were determined using potentiometric titrations in 0.15 M NaCl. A detailed ^{1}H NMRD and ^{17}O NMR study provided insight into the parameters that govern the relaxivity for these systems. The exchange rate of the coordinated water molecules in [Mn(dpama)] is rather fast: $k_{ex}^{298} = 306\ (\pm 16) \times 10^6\ s^{-1}$. The mononuclear derivative binds human serum albumin (HSA) with an association constant K_A of 3372 M^{-1}, which results in the replacement of the coordinated water molecules by donor atoms of protein residues. The dinuclear analogue also binds HSA while leaving one of the Mn^{2+} centres exposed to the solvent with two coordinated water molecules. Thus, this complex shows remarkably high relaxivities upon protein binding (39.0 $mM^{-1}\ s^{-1}$ per Mn, at 20 MHz and 37 °C). The trinuclear [mX(Mn(dpama)$(H_2O)_2)_3$] complex also binds HSA with a K_A of 1286 ± 55 M^{-1} and a relaxivity of the adduct of 45.2 ± 0.6 $mM^{-1}\ s^{-1}$. This property is very interesting for MRI visualization of blood vessels, as well as to improve the residence time of the agent in the blood pool.

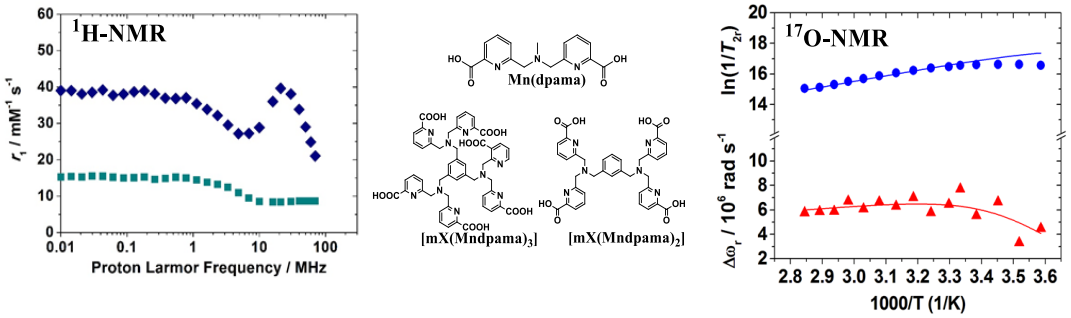

Fig.1. The ^{1}H NMRD profiles for [mX(Mn(dpama)$(H_2O)_2)_3$] free (*bottom*) and fully bound to HSA (*top*) at 310 K and pH = 7.2 (left), the structure of the ligands (middle) and reduced transverse (blue •) ^{17}O NMR relaxation rates and ^{17}O NMR chemical shifts (red ▲) measured for [Mn(dpama)] at 11.74 T (right).

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P24

ABSOLUTE POLAR-METABOLITE CONCENTRATION MEASUREMENTS, USING ^{1}H *q*-NMR, IN MATRICES CONTAINING INTERFERING PROTEIN SIGNALS

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Absolute analyte quantification by NMR spectroscopy is rarely pursued in metabolomics, even though this would allow researchers to compare results obtained by other analytical techniques and to create an integrated platform that could be the successful strategy in new biomarkers discovery. The presence, in biological matrices, of interfering protein signals (e.g. the serum in cell culture medium) can seriously affect the results of absolute quantification by ^{1}H *q*-NMR (quantitative NMR). We comprehensively explored all the strategies commonly used to cope with the issue, such as CPMG (Carr-Purcell-Meiboom-Gill) or applying post process filtering algorithms to remove the protein signal contribution, from the ^{1}H *q*-NMR spectra (Assure™ Bruker and MestRe Nova line-fitting), and we compared the absolute concentrations measured by PULCON method [1] to those obtained after a new procedure for pH-controlled serum protein removal [2], [3]. The overall accuracy of methods was assessed by means of a newly devised figure of merit, while the percent error (in absolute value) of every measurement for all the analytes, returns a punctual estimate of accuracy degradation, that may help to choose the most suitable approach for protein removal in the absolute quantification.

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NMR STUDY OF Gp36-MPER

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The feline immunodeficiency virus (FIV) is a lentivirus that resembles the human immunodeficiency virus (HIV). Increasing evidence suggest a common structural framework for these glycoproteins, corresponding to similar roles in virus cell fusion. FIV is studied as a model system for anti-HIV vaccines and anti-HIV drugs development.[1] We previously demonstrated that several short synthetic peptides that mimic the MPER of Gp36 reduce the infectivity of FIV. In particular, an octapeptide (^{770}WEDWVGWI^{777}), dubbed C8, elicited antiviral activity as result of blocking cell entry, as observed for HIV fusion inhibitors, depending on the presence of regularly spaced Trp residues.[2,3] In the hypothesis that C8, similarly to T20 peptide,[4] behaves as a fusion inhibitor peptide, we studied C8 for its ability to interact with the MPER region of Gp36 named Gp36-MPER. We acquired 3D-NMR experiments, on a ^{13}C and ^{15}N double labeled protein sample, at Bruker 900 MHz of Bijvoet Center for Biomolecular Research, in dodecylphosphocoline/sodium dodecyl sulfate (DPC/SDS) mixed micelles at molar ratio of 90/10,[5] thanks to a grant funded from Instruct-UB FP7. NMR structure of Gp36-MPER was calculated on the basis of NOEs data evidencing the presence of α-helical and β-turn conformations. Interaction of Gp36-MPER with C8 peptide was demonstrated on the basis of the observation of chemical shift perturbation in ^{15}N-NHQC spectra.[6] Our results show that C8 binds Gp36-MPER through the involvement of several residues. We demonstrated that the antiviral activity of C8 is possibly related to its ability of modifying the structural properties of Gp36-MPER.

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CHARACTERIZATION OF JUICES FROM ANCIENT DANISH APPLE CULTIVARS BY ^{1}H-NMR METABOLOMICS AND CHEMOMETRICS

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Apple juices of over 200 old Danish cultivars were, for the first time, characterized using a NMR-based metabolomics approach and chemometrics. The study was conducted in collaboration with The Pometum, an experimental orchard and gene bank of the University of Copenhagen that hosts the national and international collection of fruit genotypes. This work is part of a wider project that aims at promoting the utilisation of ancient Danish apple cultivars for niche products since they may have unique flavour qualities that can be attractive in juices.
High-field proton NMR spectroscopy was applied for samples characterization. 1D ^{1}H NMR spectra were acquired to determine the metabolic fingerprint of the juices, while 2D homonuclear experiments were acquired for assignments purposes. A total of 26 metabolites were quantified by using Bruker's Spin Generated Fingerprint (SGF) Profiling ™ [1]. Classical chemical analysis and sensory evaluation of the juices were also performed in order to have a full overview of the studied samples. Chemometric tools such as Principal Component Analysis (PCA), and the recently developed Recursive weighted partial least squares (rPLS) [2] were employed. The first allowed to explore the whole NMR spectral dataset seeking for valid metabolic signatures related to different varieties, while the second was able to find the correlation between the
NMR spectrum and the sensory evaluation.
The results have shown no particular clustering among the different cultivars, however a great variation in chemical composition was observed. In particular, the analysis of the aromatic region of the NMR spectrum showed that some juices have an interesting composition in polyphenolic content suggesting the use of their respective cultivars for the formulation of precisely characterized niche products.

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P27

INTERACTIONS OF Cu(II) AND Ni(II) IONS WITH HEMOPRESSIN

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The endocannabinoid system consists of cannabinoid receptors (e.g. CB_1 expressed mainly in central nervous system and CB_2 found mainly in immune cells) and endogenous ligands. The endocannabinoid system may influence many biological processes, like pain sensation, memory and addictions. It is also involved in many pathological conditions including Alzheimer's disease, Parkinson's disease, depression and obesity [1].
Hempressin, a peptide with the sequence PVNFKFLSH, is an example of endogenous ligand for the endocannabinoid system. It derives from α(1)-chain of hemoglobin and it was first found in rat brain homogenate. Studies of Heimann and co-workers have shown that hemopressin selectively binds CB_1 cannabinoid receptors blocking its signaling, *in vitro* and *in vivo* [2]. In fact, hemopressin causes nonopioid antinociceptive effects. Additionally, it shows hypotensive effect. Recently, studies with mices demonstrated that hemopressin inhibits food intake in normal and obese animals [3]. Subsequent analysis of rat brain homogenate results in finding elongated analog of hemopressin, peptide RVD-PVNFKLLSH, which in turn acts as a CB_1 cannabinoid receptors agonist, while hemopressin is its antagonist [4] An immunoaffinity mass spectrometry studies revealed existing of a whole family of hemopressin-elongated peptides, designated pepcans, in rodent brain extracts and human and mouse plasma, which show negative allosteric modulation of CB_1 cannabinoid receptors [5].
Because of the presence of a His residue and a free N-terminus in the hemopressin sequence, pepcans might be attractive ligands of endogenous divalent metal ions. Therefore, we decided to study metal ions binding ability of hemopressin and its analogs. We were able to characterize interactions of Cu(II) ions with three peptides: human and mouse hemopressin (PVNFKLLSH), N-terminally extended human and mouse hemopressin (RVD-PVNFKLLSH), and rat hemopressin (PVNFKFLSH). In case of NMR analysis, Ni(II) ions were used as diamagnetic probe for Cu(II) ions.

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P28

INVESTIGATIONS OF CATALYSTS WITH DYNAMIC NUCLEAR POLARIZATION SOLID-STATE NMR SPECTROSCOPY

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Solid-state nuclear magnetic resonance is a powerful method for studying the molecular structure and dynamics of a broad range of systems. Solid-state NMR suffers from low sensitivity, because of the small nuclear spin polarizations involved even with high magnetic fields, and so long acquisition times are required. The problem of sensitivity becomes overwhelming for dilute species, so that measurements of adsorbates on surfaces, molecules at interfaces or isotopes with low natural abundance are often impossible. Weak NMR signals can be enhanced by dynamic nuclear polarization, which involves transfer of electron spin polarization from radicals implanted in the sample to nearby nuclei. Recent developments in the design of gyrotrons have made high-field DNP NMR spectrometers possible. [1] The substantial enhancements obtained with DNP make NMR studies of dilute species feasible for the first time and have already prompted new NMR applications to surfaces and materials which are porous or particulate on the micro- to nanoscale. In the future DNP-enhanced experiments will dramatically transform solid-state NMR studies of a broad range of technologically useful materials with applications in gas storage and sequestration, therapeutic delivery, heterogeneous catalysis, and tissue engineering. [2] The eventual aim of this project is to apply DNP-enhanced solid-state NMR to structural and mechanistic studies of catalysts consisting of transition metals supported on oxide surfaces. Catalysts are challenging systems to study by solid-state NMR because of the low concentration of active sites and adsorbate molecules on the support surface. The substantial signal enhancements obtained with DNP allow ^{27}Al NMR investigations of surface sites in alumina supports and ^{13}C studies of functionalizing molecules in functionalized silica.

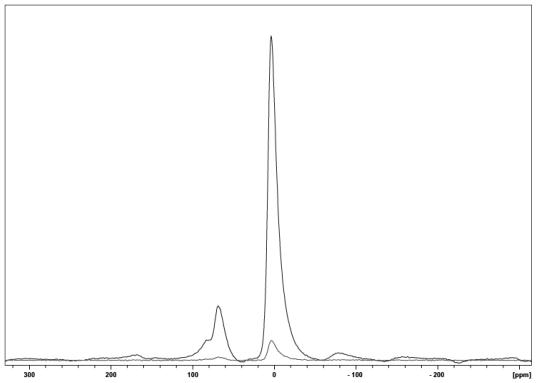

Fig. 1. 27Al CP spectra of gamma alumina sample, enhanced by DNP.

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P29

THE HYDRATION PROCESS OF CELLULOSE IN AGED PAPER STUDIED BY MEANS OF ^{1}H HR-MAS NMR SPECTROSCOPY

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The detailed knowledge of hydration mechanisms of cellulose-based materials is of great importance in many fields and especially for the preservation and restoration of cultural heritage such as ancient paper. The interactions between water and cellulose at the accessible sites within the fibers' complex structure is responsible for the rupture of hydrogen bonds and the consequent consumption of the amorphous regions enhancing the degradation processes [1, 2]. In this study, we have measured the longitudinal T_1 (spin-lattice) and transverse T_2 (spin-spin) relaxation times of the macroscopic magnetization by means of proton Nuclear Magnetic Resonance (NMR) experiments. Experiments were performed on model paper with different hydration levels artificially aged at 90°C and 59 % of relative humidity for several days. The behavior of T_1 and T_2 is quite complex and strictly dependent on the water content of paper samples: this has been interpreted as due to the occurrence of different mechanisms regulating the water-cellulose interaction within the fibers. Furthermore, we have measured T_1 as a function of the artificial aging time comparing the results with those measured on three paper samples dated back to the 15th century. We found that the evolution of T_1 in model papers artificially aged correlated with that of ancient paper providing therefore a way for estimating the degradation of cellulosic materials in terms of an equivalent time of artificial aging.

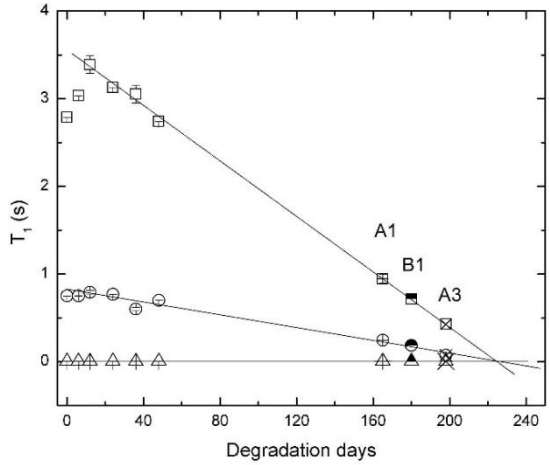

Fig. 1. The spin-lattice relaxation time (T_1) of the three different contributions identified as a function of the artificial aging time including data from model aged samples (empty symbols) and the extrapolated value for ancient samples (filled symbols labeled A1, B1 and A3). Lines cross at about 225 degradation days defining the limit of the mechanical stability for paper.

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P30

THE BIOLOGICAL MECHANISM OF PEPTIDIC HORMONE THYMOSIN α1 INVOLVE INTERACTION WITH EXPOSED PHOSPHATIDYLSERINE ON MEMBRANE AND USES AS CARRIER HUMAN SERUM ALBUMIN

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Thymosin α1 is a peptidic hormone used in the therapy of several diseases [1].
It is unstructured in water solution and interacts with negative regions of micelles and vesicles assuming two tracts of helical conformation with a structural break in between. Previous work reported that during the interaction of Thymosin α1 with sodium dodecylsulphate micelles, mixed dodecylphosphocholine – sodium dodecylsulphate micelles and with mixed dipalmitoylphosphatidylcholine- dipalmitoylphosphatidylserine vesicles the peptide assumes structural elements in the binding process [2, 3].
The results indicate that the preferred interactions are those where the membrane is negatively charged. The direct interaction of Thymosin-α1 with K562 cells before and after inducing apoptosis with resveratrol is reported herewith. Preliminary studies on the possible role of Human Serum Albumin to deliver the peptide to target districts are also reported. The release of Thymosin-α1 mediated by HSA to membrane regions with phosphatisdylserine exposure is hypnotized as a step in the biological mechanism of the peptide.

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P31

NMR AS A TOOL FOR MEASURING THE STORAGE POTENTIAL OF TABLE GRAPES

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In this presentation we will describe a chemical method based on the measurement of relative concentrations of three analytes which allows the determination of the storage potential of table grapes.
Once defined the storage propensity index SPI as the numeric parameter that represents on a scale, for example from 0 to 100, the propensity of the fruit to preserve the organoleptic and nutritional properties of a healthy and marketable product, we related this index to the fraction of three metabolites involved in the ripening of the fruit.
The chosen metabolites are arginine, ethanol and 1,2-propanediol,[1] and their determination was made by integration of their signals in the ^{1}H NOESY spectra recorded with suppression of the residual signal of water.
By relating the SPI indexes with time for a defined cultivar stored in a cold storage room it is possible to draw a line (see Figure 1) that permits to predict the storage potential of unknown fresh grapes of the same cv and stored in the same cold storage conditions.

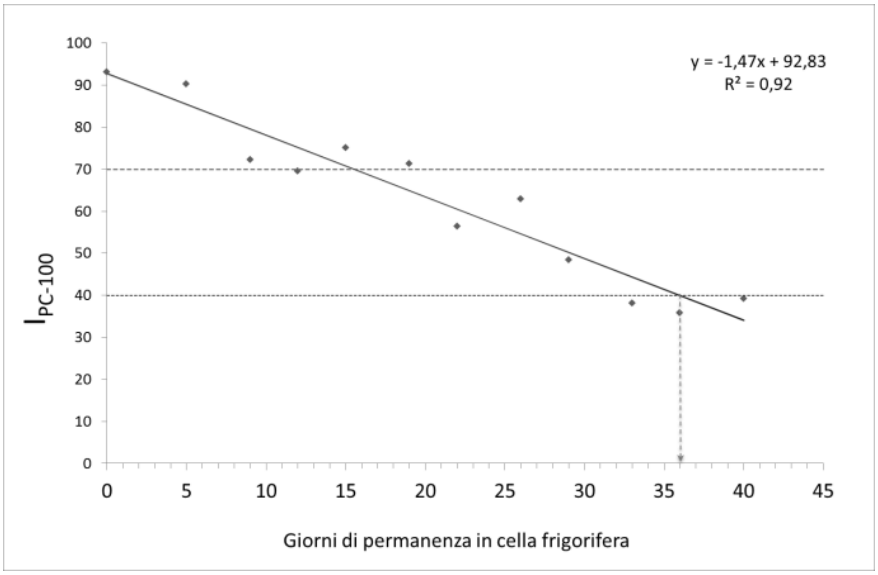

Fig. 1. The experimental line obtained for cv Palieri stored in a cold storage room in Noicattaro (BA)

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P 32

HOP EXTRACTS AS POTENTIAL ANTI-NEURODEGENERATIVE AGENTS: FROM NMR-BASED METABOLIC PROFILING TO INVESTIGATION OF THEIR ACTIVITY

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Beer is one of the most worldwide consumed drink and hop represents one of its main ingredients. It is characterized by a high content in aromatic compounds, among which several flavonoids, known to exert various biological activities and presenting a significant antioxidant capacity.
In the light of these evidences, we decided to screen different hop varieties employed in artisanal brewing (namely *Cascade, Tettnang, Saaz* and *Summit*) for the presence of ligands of amyloidogenic peptides and proteins (Aβ1-42 and Ataxin-3), responsible for etiology of Azheimer's [1] and Machado-Joseph's neurodegenerative diseases [2].
As a matter of fact, these pathologies have a high impact on global public health and social care challenges. The lack of effective therapies and diagnostic tools increase the need for new molecules for their treatment and diagnosis.
To date, among the anti-neurodegenerative molecules identified, aromatic compounds are the most abundant class and hop can represent an important edible source. Here we have exploited NMR spectroscopy to obtain the metabolic profiling of the four hop varieties previously mentioned, focusing in particular on their aromatic content. NMR-based molecular recognition studies [3] have been performed to verify the presence in hop extracts of Aβ1-42 ligands, and others are ongoing to support preliminary biochemical data obtained for Ataxin-3. Moreover, their antioxidant and anti-amyloidogenic activities were evaluated by cellular and biochemical assays [4]. These activities correlate with their content in aromatic compounds and for Aβ1-42 are supported by ligand-receptor interaction studies, highlighting flavonoids as the main interactors. In addition to all data collected, current analyses are leading to the identification of compounds involved in anti-amyloidogenic acitivities. Furthermore, extracts have shown a very high antioxidant activity, supporting also an indirect beneficial action consisting in the reduction of the oxidative stress induced by the same amyloids on neurons.
All together these data point out the great nutraceutical potential of hop, suggesting its employment in the development of diets effective in preventing neurodegeneration.

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P33

A SERUM METABOLOMIC ANALYSIS OF HCV-INFECTED PATIENTS SUCCESSFULLY TREATED WITH IFN-FREE DAA REGIMENS

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HCV infects about 170 million of subjects worldwide. The virus has a high propensity to persist in the host, leading to cirrhosis and liver cancer. Metabolomics is the study of metabolic changes in biological systems and may identify specific profiles associated with subtle alterations induced by diseases. Few studies are available on metabolic changes in liver injuries, and since none of which was focused on HCV-infected patients before and after reaching a SVR following treatment with direct acting antivirals (DAAs), the aim of this study was to perform a serum metabolomics analysis in this setting. Sera were collected from 52 HCV patients (18 men, mean age 65 ±9,7) successfully undergoing different IFN-free DAA regimens, before therapy (baseline) and at posttreatment week 12 (SVR12). HCV genotype was 1a/1b in 70%, 2a/2c in 23%, 3 in 4.7% and 4 in 2.3%. METAVIR score indicated F3-F4 score in 55% of patients, the remaining 45% had F0-F2. We also analyzed a small group of 12 sera from healthy subjects in order to localize them in the PLS plot respect to baseline and SVR12 as a preliminary negative control for both groups. Samples were analyzed using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). Partial Least Squares (PLS) and the canonical analysis (CA) were applied, demonstrating a significant pair-wise discrimination (96% of accuracy) for the two time-points of each patient and highlighting a metabolic shift. Several metabolites with unequivocal assignment (i.e. amino acids, organic acids, creatine, creatinine, lactate and choline) differed comparing baseline with SVR12. Baseline featured higher level of formate and acetate ($p<0.05$) and methionine was higher in SVR12 ($p<0.05$). Preliminary analysis revealed also a progressive nearing of SVR12 to the healthy fingerprint. The serum metabolomic analysis of pre- and post-treatment samples showed remarkable profile changes from baseline to SVR12. We found variations, with opposite directions, in formate (produced in adults only by hepatocytes) and methionine. These metabolites take part in biochemical ways (i.e. glucose metabolism) also involving acetate and other amino acids, going through the synthesis of folates. These alterations could be implied in the metabolic impairment previously observed in chronically infected HCV-patients. Also, since methionine is the major source of methyl groups, an understanding of its variation, could reveal important dysfunctions in liver essential pathways requiring methylation (i.e. epigenetic regulation of DNA) in HCV-related chronic infection.

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INTERACTION STUDIES OF FERRITIN WITH LIPIDS

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Maxiferritins are a family of well-characterized iron storage proteins with an essentially ubiquitous distribution in all life forms [1, 2]. They are formed by 24 identical or similar subunits self-assembled into a globular shell containing an iron core consisting of a ferric oxy-hydroxide mineral similar to ferrihydrite [3]. The structure of each subunit is a helix bundle composed of four α-helices, and a fifth C-terminal short α-helix. The subunits are assembled into a spherical-shape structure with 4-3-2 symmetry, which forms a hollow complex with an approximately 8 nm diameter cavity capable of storing up to 4500 iron atoms.
In vertebrates, native cytosolic ferritins are the product of self-assembly of two types of highly homologous subunits: "light" (L, 20 kDa), and "heavy" (H, 22.8 kDa) subunits, with up to 53% identity. The H subunit is characterized by the presence of a ferroxidase di-iron binding site which is responsible for the enzymatic oxidation of Fe^{2+} to Fe^{3+}. The L subunit, without a ferroxidase center, still assists Fe^{2+} oxidation inside the cage, but at a much slower rate, and plays a role in iron nucleation and mineralization [4]. Ferritins display a dynamic iron-storage function, central to cellular iron homeostasis. It has been suggested that, besides its iron-storage function, ferritin plays a role in lipid metabolism [5]. The crystal structure of the HoSF-arachidonate complex (PDB id 4DE6) revealed that the lipid is bound in the pocket at the 2-fold intersubunit contacts. Thus, ferritin may as well protect unsaturated fatty acids from oxidation, limiting their participation in the lipid peroxidation chain reaction, thus reducing their inflammatory effect [5].
Intrigued by the possible role of ferritin as a new fatty acid binding protein we have here investigated the interactions of apo horse spleen ferritin (HoSF) with a pool of lipids, employing a series of 1D ^{1}H-NMR, diffusion (DOSY) and saturation transfer (STD) NMR experiments. Unsaturated fatty acids (arachidonate and oleate) exhibited better binding properties with respect to saturated fatty acids (lauric acid), detergents (sodium dodecyl sulphate) and bile acids (chenodeoxycholic acid). These studies have been accompanied by mineralization assays through UV/visible measurements, aimed at clarifying whether a functional coupling exists between mineralization and ferritin/lipid interactions.

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P35

PHYTOCHEMICALS DIRECTLY DETECTED BY HIGH-RESOLUTION NMR ON KLAMATH (*Aphanizomenon Flos-Aquae*) BLUE-GREEN ALGAE

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Aphanizomenon flos-aquae (AFA) is a wild freshwater unicellular microalga that spontaneously grows in copious amounts in Upper Klamath Lake (OR, USA) a volcanic lake with hot, deep and mineral rich waters. These algae are considered as a "superfood", due to their complete nutritional profile that has proved to have important therapeutic effects.[1-4] Here we report on the results obtained applying high-resolution NMR directly on AFA powder suspension. Klamath algae metabolome revealed to be quite complex. The most peculiar phytochemicals that can be detected directly on algae by NMR are mycosporine-like amino acids and low molecular weight glyceryl glycosides.

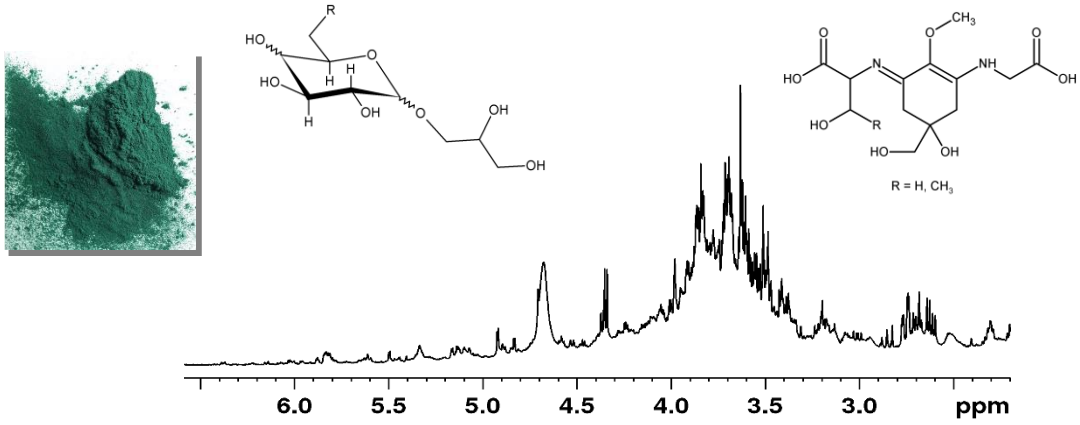

Fig. 1. AFA powder ^{1}H NMR profile.

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P36

NMR STUDIES ON COPPER TRANSPORT PROTEINS INTERACTING WITH SILVER NANOPARTICLES

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Despite the widespread use of silver nanoparticles, little is known about the potential risks arising from the silver nanoparticles (AgNPs) themselves and from the silver ions released from them. Since Ag(I) or Cu(I) have similar coordination properties, an impact on copper metabolism is expected. Cells utilize several pathways to ensure uptake, storage and export of copper. In humans, the Cu chaperone Atox1 delivers Cu(I) to the metal-binding domains (MBDs) of two P_{1B}-type ATPases: the Menkes (Atp7a) and Wilson (Atp7b) disease proteins.[1;2] Both Atox1 and the first MBD of Menkes (Mnk1) bind one Cu(I) through two Cys residues located in a conserved CXXC motif.[3] Thus, the aim of our work has been to gain direct evidence by NMR of the interaction of Atox1 and Mnk1 with Ag(I) or AgNPs. Although the two proteins have quite similar structure, their behaviors is substantially different.
AgNPs are also characterized by a remarkable antibacterial effect, thus monitoring the interactions of AgNPs with bacterial cells can be crucial for elucidating the origin of the bactericidal activity and for expanding their biomedical and environmental applications. Therefore, we have investigated the Ag metabolism in gram-negative bacteria, by a forefront technique called in-cell NMR.[4] *E. coli* cells overexpressing Atox1 were treated with Ag salts. In-cell NMR revealed that, within treated cells, Atox1 undergoes only minor changes. In contrast, after the lysis of the cells, the protein appears to be bound to the metal. From these data, it can be inferred that the scarce reactivity of Atox1 with Ag(I) inside the cell can be due to compartmentalization (e.g. in the periplasm) and/or sequestration of the metal ion. Further studies are ongoing to trace the fate of Ag(I) ions within *E. coli* cells and to unravel the mechanisms used by gram-negative bacteria to become resistant to xenobiotics. This latter is a particularly hot issue, especially after the recent discovery of an *E. coli* strain resistant to the last-resort antibiotic Colistin.[5]

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Acknowledgment

This work was supported by the Italian "Ministero dell'Università e della Ricerca" (FIRB 2011-RINAME, RBAP114AMK). We gratefully acknowledge "Anna Laura Segre" fellowship for support to V.M. We also thank the University of Bari and the Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (C.I.R.C.M.S.B.).

P37

APPLICATION OF NMR IN THE DEVELOPMENT OF CURCUMIN-BASED LIGANDS FOR EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by the formation of amyloid plaques and neurofibrillary tangles bringing the patient to a neurological disorder. Plaques are mainly made by amyloid-beta (Aβ) fibrils that interact each other forming extended structures. To date, the only way to assess definitively the presence of AD is post-mortem and there is no valuable cure for the disease. Curcumin attracts considerable attention as a food component thanks to its properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [1]. In recent years, curcuminoids and their metal complexes have been subjected to a large number of studies due to their potentially preventing activity in the pathogenesis of different diseases among which AD [2]. Curcumin could find applications both in the treatment of AD, due to its ability to inhibit Aβ aggregation, and in the early diagnosis. Curcuminoids labelled with ^{18}F or ^{99m}Tc have recently shown their potential as diagnostic tools for Alzheimer's disease. Nevertheless, ^{68}Ga is a positron emitter, generator produced radionuclide and its properties can be exploited also in medical facilities without a cyclotron *in situ* [3]. Herein, ^{nat}Ga-labelled complexes with curcuminoids were synthesized and characterized by means of NMR spectroscopy, which is a powerful technique, able to identified and characterize all the synthesized products and intermediates, follow the formation of isomeric species, if in slow exchange in NMR time scale, and to define the coordinating site of metal complexes. ^{1}H and ^{13}C NMR chemical shifts of free ligands and their Ga^{3+} complexes shed light in the coordinating mode of the ligands (see Fig.1). NMR data confirmed the formation of Ga^{3+} complexes.

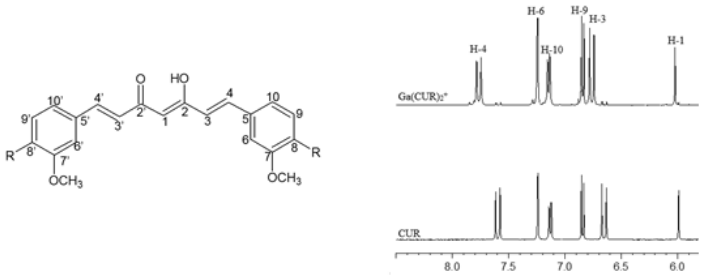

Fig.1: General structure of Curcuminoid compound and 1HNMR of L:M 1:2 and L

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P38

A SERUM NUCLEAR MAGNETIC RESONANCE-BASED METABOLOMIC SIGNATURE OF ANTIPHOSPHOLIPID SYNDROME

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Antiphospholipid syndrome (APS) is a rheumatic inflammatory chronic autoimmune disease characterized by the presence of auto-antibodies circulating in the blood directed against phospholipids. The autoimmune reaction induces inflammatory processes on the vessel walls, so that APS patients suffer for a hypercoagulable state associated with vascular thrombosis and pregnancy loss in women. Cardiac, cerebral and vascular strokes in these patients are responsible for a significant reduction in life expectancy Timely diagnosis and accurate monitoring of disease course is pivotal to improve accuracy of therapy decisions,

In the present work, using ^{1}H NMR spectroscopy, we analyzed the blood sera metabolomic profile of APS patients. Our NMR-based metabolomic study revealed significant differences among serum metabolic profiles of patients affected by APS compared with the profiles of age, gender, and sampling-date matched control individuals. Several metabolites have been identified as potential biomarkers of APS, many of which have been linked to autoimmune systemic inflammatory disease. Interestingly, gender based analysis indicates that there are both shared and unique metabolic markers for females and APS pathology.

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P39

INVESTIGATION OF POLYMER DYNAMICS IN *PVB-ATO* NANOCOMPOSITES BY LOW-FIELD AND FAST FIELD-CYCLING ^{1}H NMR RELAXOMETRY

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Nanocomposites of polymers and inorganic fillers are materials of great interest for many practical applications. Polyvinylbutyral (PVB) is an amorphous polymer employed as an inter-layer film in laminated safety-glass manufacture industry thanks to its high optical clarity and good adhesion to glass. Additional interesting properties can be obtained by adding a filler to the PVB matrix. Indeed, PVB loaded with Antimony doped Tin Oxide (ATO) nanoparticles is able to filter out the near infrared waves of sunlight, while maintaining transmission in the visible region [1]. Given the worldwide energy saving and environmental preservation policies, this property seems promising for applications in building and vehicle glass construction.
In view of the optimization of PVB-ATO nanocomposites for applicative purposes, a thorough characterization of its structural and dynamic microscopic properties is necessary. To this aim, we studied PVB films loaded with 0, 5 and 23 wt% of ATO nanoparticles across the polymer glass transition (T_g = 70 ^{o}C) by means of low-field (20 MHz) and fast field-cycling (FFC) ^{1}H NMR relaxometry. Both uncoated and surface modified (with methacryloxypropyltrimethoxysilane) nanoparticles were dispersed in the polymer matrix.
Analysis of the FIDs recorded in solid-echo experiments with different echo delays and of the dispersion curves obtained by measuring ^{1}H longitudinal relaxation times by FFC in the 0.01 to 35 MHz frequency range allowed us to investigate the effects of the nanoparticles on the polymer dynamics and to quantitatively determine the fractions of polymer with different mobility. In particular, it was observed that the surface-modified particles do not affect the dynamics of the polymer matrix at any loading level in the whole temperature range investigated. On the contrary, the uncoated particles induce a slowing down of the polymer motions above the glass transition but do not significantly affect them below it. Our data demonstrate the crucial role played by the particle surface in influencing the polymer dynamics.

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P40

THE METABOLIC PROFILE OF *ASPERGILLUS* EXTRACTS BY HR-MAS NMR SPECTROSCOPY COMBINED WITH MULTIVARIATE STATISTICAL ANALYSIS

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Aspergillus is an ubiquitary fungal genus whose conidia are present in the air we breathe, but does not normally cause illness. In those people with a weakened immune system [1] *Aspergillus* is a true opportunistic filamentous fungus. Common *Aspergillus* infections include Invasive Aspergillosis (IA), Allergic BronchoPulmonary Aspergillosis (ABPA) and aspergilloma. Unfortunately, today the overall mortality for aspergillosis in immunosuppressed patients is high, till 60%.[2] This phenomenon is also connected to the resistance of some strains to specific antifungal. In this scenario the importance to know the metabolic content of different isolated *Aspergillus* strains comes out clearly in order to understand the resistance mechanism to certain drugs. The present work is part of the preliminary stage of the study on the antifungal-resistant *Aspergillus*. The work is focused on the characterization, based on the metabolic profile, of phenotypes of the isolated *Aspergillus* species using ^{1}H High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy.[3,4] Four different *Aspergillus* species were studied: *A. niger*, *A. fumigatus*, *A. flavus* and *A. terreus*. A significative number of replicates of *Aspergillus* extracts were characterized by conventional ^{1}H HR-MAS spectra. Moreover, in order to discriminate the metabolic profile of each *Aspergillus* strain, multivariate statistical analysis (PCA, PLS-DA) was also performed on the ^{1}H-NMR data. This combined approach is useful to identify peculiar strain-specific biomarkers. The results show some evidence of metabolic differences in the studied strains.

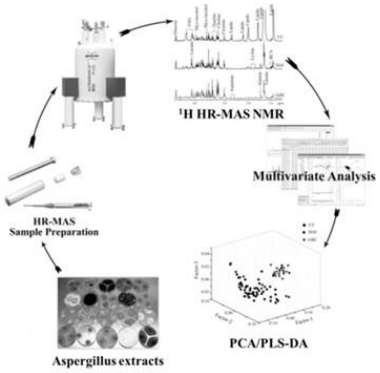

Fig. 1. ^{1}H HR-MAS NMR / multivariate analysis for the *Aspergillus* extracts characterization.

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STRUCTURAL AND DYNAMICAL CHARACTERIZATION OF BPSL1445 FROM *BURKHOLDERIA PSEUDOMALLEI*

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Burkholderia pseudomallei (Bp) is a pathogenic bacterium responsible for melioidosis, a severe endemic disease in South-East Asia and an emerging threat in Australia, in the Indian subcontinent and in South America [1]. Melioidosis causes septicemia and organ failure, with a high mortality rate. Antibiotic treatments are largely ineffective due to multi-drug resistance. Due to its rapid diffusion in the tropical area Bp is considered as a dangerous pathogen, also with potential use in biological terrorism. The mechanisms of virulence and host resistance of Bp are still poorly characterized, thus limiting the availability and efficacy of suitable vaccines against melioidosis [2]. Vaccines derived from attenuated Bp mutants induce protective immunity in a murine model of melioidosis, suggesting that some mechanisms of organism protection against the disease exist [2]. The high risks related to these vaccines, though, strongly limit their application. In the framework of a multidisciplinary project tackling Meliodosis through reverse and structural vaccinology approaches [3] we focused on the candidate protein antigen BPSL1445 (Lipo1). Bioinformatics investigations predict that Lipo1 belongs to the SYLF superfamily characterized by the presence of a C-terminal lipid-binding module, DUF500, highly conserved from bacteria to mammals. Members of the SYLF superfamily are generally poorly characterized and their biological function is still unknown and it is hypothisized to bind phosphoinositide lipids [4]. Here we present the solution structure of BPSL1445, its dynamical characterization obtained by 15N spin relaxation analysis and the analysis of the putative interaction with phosphatidylinositol. The structure has been used as starting point for a molecular dynamics which has been performed to identify possible epitopes suitable for non-living vaccines production [5].

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This work was supported by Fondazione Cariplo (contract number 2009-3577)

P42

ELECTROSPUN LIPID BINDING PROTEINS COMPOSITE NANOFIBERS WITH ANTIBACTERIAL PROPERTIES AS POTENTIAL WOUND DRESSING

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One of the promising new techniques in the production of biomaterials is the electrospinning process, a simple and versatile method for the fabrication of fibrous materials with a variable pore structure and fibre diameters (scalable down to a few nanometers), for the production of drug delivery vehicles [1]. Electrospun scaffolds have been shown to mimic the natural extracellular matrix (ECM) and enhance the cell migration and proliferation, with a wide range of applications in biomedical fields such as tissue engineering scaffolds, wound dressing materials and carriers for drug delivery [1]. Many types of electrospun nanofibers have been reported, employing silk fibroin, polysaccharides, collagen and blends of synthetic biocompatible and biodegradable polymeric materials, which can actively support and supplement a quick deposition of healthy tissue [2]. The main strategy employed to develop interactive systems consists in the combination of synthetic polymers, which are easily processable and ensure the good mechanical properties of the final product, and natural polymers, which favour the healing process and increase skin compatibility [1].
In the present work we investigate the possibility of uploading into polymer nanofibers a carrier protein capable of forming host-guest complexes with water insoluble functional molecules. The chosen polymer nanofiber is a blend of keratin and poly(ethylene oxide) (PEO) [3]. Keratin has many useful properties such as biocompatibility and biodegradability and it was shown that the mouse fibroblast cells could proliferate well on keratin films [3]. We show here the feasibility of electrospinning a keratin/PEO mixture containing a bile acid binding protein (BABP), hosting into its internal pocket Irgasan (IRG), a broad-spectrum antimicrobial agent, for the production of biomembranes with excellent antibacterial properties. NMR spectroscopic approaches were employed to test BABP binding ability and characterize the IRG binding site. The obtained nanofibers may have important application in the field of wound dressing.

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P43

EXTRACTION AND STRUCTURAL CHARACTERIZATION OF BIOACTIVE NATURAL ISOFLAVONES

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Natural flavonoids represent a wide, well-documented class of biologically active compounds that have been studied for their potential application in a number of fields. Osajin (1) and pomiferin (2) are two isoflavones extracted from *Maclura pomifera* (Osage Orange) [1,2] that were shown to express anticancer, antibacterial and antidiabetic activity [3-5]. Kupeli *et al.* reported that, starting from the same *M. pomifera* plant but changing extraction solvents and process conditions, it is possible to isolate two isomers of osajin (1) and pomiferin (2), namely warangalone/scandenone (3) and auriculasin (4), presented as anti-inflammatory and antinociceptive agents (Figure 1) [6]. Basing on the procedures reported in the literature, we optimized the extraction conditions for osajin (1) and pomiferin (2) and for warangalone/scandenone (3) and auriculasin (4) from the fruits of *M. pomifera*. We designed a set of experiments to verify and directly compare the structures; HRMS confirmed the molecular weight of the isolated compounds, while an HPLC method resolving the couples of isomers is now being set up. Full NMR characterization (^{1}H, ^{13}C, 2D experiments) will help in attributing the correct molecular structures. In particular, NOESY NMR experiments are being used to assess the positioning of the substituents on the scaffold basing on non-scalar interactions between proton groups.

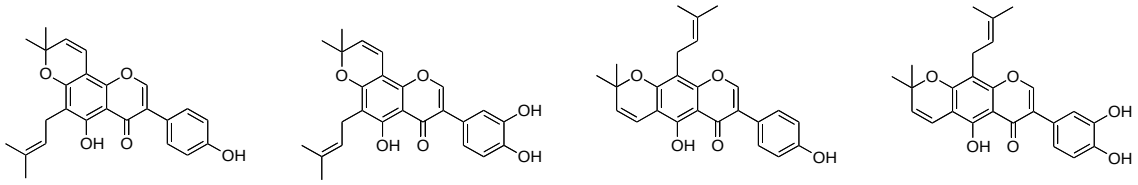

Fig. 1. Structures of the cited compounds

Besides these structural insights, our research work is also oriented at providing details on the biological activity of such compounds in the anticancer and cardiovascular fields of medicinal chemistry [7].

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P44

NMR EVALUATION OF MITOCHONDRIAL DYSFUNCTION IN LOW-PROTEIN DIET IN COLLAGEN VI-RELATED MYOPATHIES

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A pilot clinical trial based on nutritional modulation was designed to assess the efficacy of one-year low-protein diet (LPD) in activating autophagy in skeletal muscle of patients affected by COL6/collagen VI-related myopathies. Ullrich congenital muscular dystrophy and Bethlem myopathy are rare inherited muscle disorders caused by mutations of COL6 genes and for which no cure is available yet. Studies in COL6 null mice revealed that myofiber degeneration involves autophagy defects and that forced activation of autophagy results in the amelioration of muscle pathology. Seven adult patients affected by COL6 myopathies underwent a controlled low-protein diet for twelve months and the alterated metabolites pathway was evaluated in blood samples. Safety measures were assessed, including muscle strength, motor and respiratory function, and metabolic parameters. The treatment resulted safe as shown by preservation of lean-fat percentage of body composition, muscle strength and function. Patients displayed a reduction in lactate (ranging from 10% to 60%) and a decrease in acetate serum levels after one year, as revealed by NMR analysis. The reduced concentration of lactate and acetate after LPD suggests an improvement in mitochondrial aerobic energy production through the tricarboxylic acid cycle, with a reduced conversion of pyruvate to lactate. These metabolic changes point to an improved mitochondrial function. The data provide evidence that LPD is able to activate autophagy and is safe and tolerable in patients with COL6 myopathies, indicating autophagy activation as a potential target for therapeutic applications.

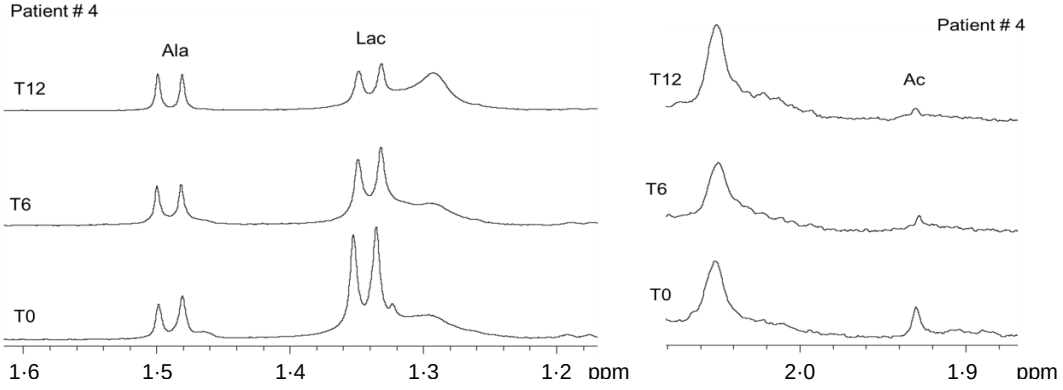

Fig. 1. Representative Carr-Purcell-Meiboom-Gill 1H NMR spectra of serum of patient 4 at T0, T6 and T12, showing the methyl resonance of alanine (Ala), lactate (Lac) and acetate (Ac)

P45

CISPLATIN EFFECT ON COPPER-DEPENDENT INTERACTION BETWEEN Atox1 AND Mnk1: A DEEP STRUCTURAL AND FUNCTIONAL STUDY

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Copper (Cu) is an essential trace metal acting as cofactor of several key enzymes, which, in the free form can be extremely toxic [1]. Therefore, cells utilize highly conserved pathways to manage uptake, storage, and export of Cu,that generally involve Cu chaperones shuttling the metal ion between cellular targets with highly specific protein-protein interactions. In humans, the soluble cytosolic Cu chaperone Atox1 mediates Cu(I) delivery to the Cu-transporting P-type ATPases Atp7a and Atp7b (the Menkes and Wilson disease proteins, respectively), which are responsible for Cu release to the secretory pathway of the trans-Golgi network [2]. Atox1 has a β1α1β2β3α2β4 ferredoxin-like structure and a CXXC heavy metal binding motif, which is highly conserved among metallochaperones and soluble domains of Cu-transporting ATPases [3], [4]. Cu(I) handover is believed to occur through the formation of an intermediate where the metal ion is simultaneously linked to both proteins [5]. A growing number of studies has revealed that same CXXC metal binding motif of Cu transporters can interact and mediate the biological response of antitumor platinum (Pt) drugs, which are among the most used chemotherapeutics [6]. Thus, we deemed of crucial importance to investigate the interaction between cisplatin and the Atox1-Cu(I)-Mnk1 complex (Mnk1 is the first metal binding domain of Atp7a). Initially, we performed an NMR investigation of the Atox1-Cu(I)-Mnk1 complex in order to characterise the protein residues that are involved in Cu(I) binding. Then, we monitored the reaction between cisplatin and the the Atox1-Cu(I)-Mnk1 complex by means of LC-MS and ^{15}N-^{1}H NMR (the latter using ^{15}N – cisplatin). Finally, we investigated by UV-Vis spectroscopy the reaction between cisplatin and the Atox1-Cu(I)-Mnk1 complex in the presence of BCS (a Cu(I) - chelator) in anaerobic conditions or in the presence of the reducing tripeptide GSH, a physiological constituent.

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UNRAVELING HYDROGEN BOND NETWORK IN THEOPHILLINEPYRIDOXINE CO-CRYSTAL BY SOLID-STATE NMR

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Co-crystallization is an effective method to tune the physicochemical properties (e.g. physical stability, solubility and bioavailability…) of the components forming a cocrystal. A co-crystal is a multi-component crystalline form characterized by two or more different molecules linked together through weak interactions and lying in the same unit cell. A pharmaceutical co-crystal is characterized by the presence of an API (Active Pharmaceutical Ingredient) as a starting material.
Here we report the synthesis and characterization of a theophylline-pyridoxine co-drug, a co-crystal formed by two APIs usually given in co-therapy. The co-drug has been characterized by single crystal XRD, IR and Raman spectroscopy. Solid-state NMR (SSNMR) has been instrumental to the characterization of the hydrogen bond (HB) network which is not well resolved in the X-ray structure, due to intrinsic limitation of the technique. Several advanced 2D SSNMR spectra such as ^{1}H DQ MAS, ^{1}H-^{14}N HMQC, ^{1}H-^{13}C and ^{1}H-^{15}N HETCOR were acquired, taking advantage of the resolution and sensitivity improvement provided by indirect detection experiments acquired at 70 kHz of spinning speed. These were fundamental in determining the position of hydrogen atoms along the HB interactions and thus, in defining the ionic or neutral character of the co-crystal.

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POTENTIAL OF SOLID-STATE ^{13}C NMR IN THE CHARACTERIZATION OF THE MAIN ORGANIC COMPONENTS IN PLANT BIOMASSES

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The knowledge of the roughly composition of plant biopolymers is an important *matter* in the view of their possible reuse as feedstock in the field of renewable energy production.
Solid-state ^{13}C CPMAS NMR has proven to be invaluable in the study of plant composition because it allows to overcome all the solubility problems and the structural uncertainties associated with the dissolution process. In this way, solid-state NMR spectroscopy permits a detailed analysis of the plant components in their natural state.
Having at disposal samples of different plant biomasses, at first we developed a method, based on published studies (1), for the determination of the ratio between cellulose and lignin. All the spectra reported in this work were obtained from plant samples ground prior to record the spectrum in order to facilitate the spinning; no extraction or separation of the individual components was performed. The spectra have been deconvoluted divided in two regions: the aromatic region, containing lignin resonances and the aliphatic region containing mainly cellulose and emicellulose resonances.
Characteristic peaks are observed in the ^{13}C CP-MAS spectrum for lignin: guaiacyl structural units (G), more abundant in softwood, have a spectral pattern slightly different from syringyl structural units (S), typical of hardwood. The relative proportion of syringyl and guaiacyl lignin can therefore be detected (2). We observed that after an alkaline extraction performed on some plant samples, the S-unit content in the lignin skeletal decreases more than the G-unit content, thus evidencing that the alkaline treatment preferentially removes the less condensed S-lignin units (rif. Paglia). Similar spectral changes leading to the same conclusions are also observed in the FT-IR spectra of the same samples.
A good deal of attention was then focused on cellulose and in particular on its crystalline and amorphous forms, as these two forms can be distinguished in CP-MAS spectra. There are spectral variations that can be connected to differences in the crystallinity and organization of the microfibril structure of the cellulose polymer.

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NMR BASED ENZYMATIC ASSAY OF FARNESYL PIROPHOSPHATE SYNTHASE

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Farnesyl Pirophosphate Synthase (FPPS) catalyzes the two step synthesis of the C15 isoprenoid farnesyl pyrophosphate (FPP) in the mevalonate, isoprenoid biosynthesis pathway. In this reaction isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are coupled to produce geranyl pyrophosphate (GPP), which is then condensed with an additional IPP to produce farnesyl pyrophosphate (FPP) [1]
FPPS activity is generally measured via a radioenzymatic assays. [2] However to avoid the use of radiochemicals Gao et al.[3] developed a new method based on the colorimetric detection of PPi or Pi, the byproduct of FPPS catalyzed reaction. Unfortunately, this method suffers of low sensitivity and may produce ambiguous results.
Here we present a new FPPS enzymatic method based on the ^{1}H NMR quantitative determination of the substrates - IPP and DMAPP - vs - the product – GPP. Monodimensional ^{1}H NMR experiments of IPP and DMAPP are recorded in the presence of FPPS protein at the time T=0 (sample preparation) and after 1 h of incubation (T=60'). The intensities of IPP, DMAPP and GPP signals are measured and their variation is plotted as function of the time. A quantitative estimation of FPPS activity is possible by measuring the relative intensities of IPP, DMAPP and GPP in presence of known FPPS inhibitors such as zoledronate and etidronate. Our data show that NMR can be a suitable technique to measure FPPS enzymatic activity.

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COMBINING QUANTUM MECHANICAL CALCULATION AND CHEMICAL SHIFT PERTURBATIONS TO GAIN DETAILED INFORMATION ON LIGAND-PROTEIN COMPLEX CONFORMATION

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The understanding of protein-ligand recognition events relies on the availability of high-quality structural information. In particular, in drug discovery the rational design of new bioactive chemical entities benefits from detailed descriptions, at atomic level, to characterize the binding mode of small organic molecules.
Nuclear Magnetic Resonance (NMR) is well known to be a suitable method for the investigation of protein-ligand 3D structures in solution. However, the complete determination of the high resolution structure of the complex is expensive and time-consuming. Computational techniques are widely used to predict ligand-protein complex conformation (e.g. Molecular Docking, Molecular Dynamics, etc). These computational approaches can be very rapid and efficient but they are particularly prone to false positive. Here we will present a novel approach that combines the strong points of these two approaches, by taking advantage of the speed of computational strategies and the reliability of NMR data, to investigate the structure of protein-ligand complexes in a fast and reliable manner. Chemical Shift Perturbation (CSP) is a NMR measurement containing valuable information about the ligand effect on the protein chemical environment. The use of CSPs in ligand-protein studies is widely adopted [1], in particular of those originating from the backbone amide in ^{15}N-labeled protein, because they can be rapidly measured. To automatically extract structural information from CSPs an innovative routine based on quantum mechanical calculation was implemented and combined to molecular docking.

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PARAMAGNETIC CONTRINBUTION TO ^{195}Pt AND ^{31}P CHEMICAL SHIFTS IN DIORGANOPHOSPHANIDO COMPLEXES

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In the course of our research on platinum diorganophosphanido complexes we found that the presence or absence of metal-metal bond have a pronounced effect on ^{31}P and ^{195}Pt chemical shift. [1]
The chemical shift tensor for ^{31}P and ^{195}Pt shows a anisotropic distribution which can be described by the three principal components. With the aim to compare the principal components of ^{31}P and ^{195}Pt chemical shield tensor in presence and in absence of a metal-metal bond, we carried a Multinuclear Solid State NMR characterization on two platinum complexes. The solid state measurements revealed that only one of three component is influenced by the presence of metal-metal bond.
In order to evaluate the orientation and the paramagnetic contribution to each of three principal components, the DFT calculations were performed and in this contribution we report the results. [2]

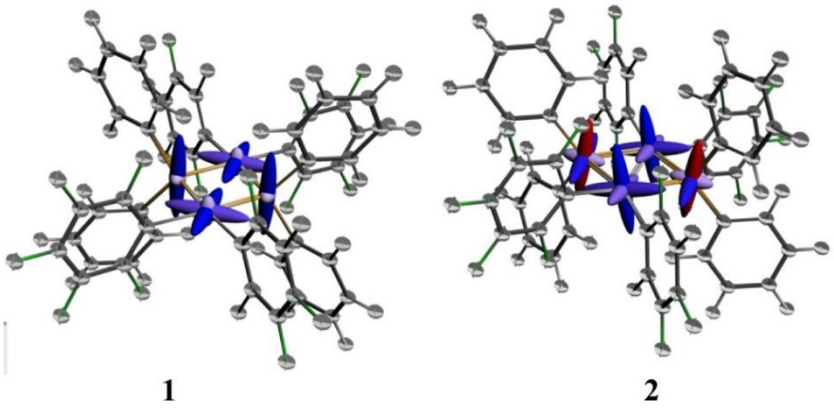

Fig. 1 Orientation of the principal components of the shielding tensors of ^{195}Pt and ^{31}P in $[(C_6F_5)_2Pt(\mu\text{-}PPh_2)_2Pt(C_6F_5)_2]^{2-}$ (**1**) (left), $[(C_6F_5)_2Pt(\mu\text{-}PPh_2)_2Pt(C_6F_5)_2](Pt{-}Pt)$ (**2**) (right).

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P51

Cu^{2+} COMPLEXATION OF TRACINE-S-ALLYL-CYSTEINE DERIVATIVE FOR POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative age-related disease characterized by dementia, cognitive impairment, memory loss and the extracellular deposition of amyloid plaques in the brain tissue formed by the aggregated amyloid β peptide (Aβ). It is well known that physiological levels of metal ions such as Cu^{2+} and Zn^{2+} cause Aβ to aggregate and therefore metal chelators can interfere in metal-induced Aβ aggregation and neurotoxicity [1]. On the other hand, Tacrine (TAC) was a first drug approved by the U.S. FDA for the palliative treatment of AD. However, it soon exhibited hepatotoxicity, resulting in limited clinical application. In this regard, quite a number of tacrine derivatives, have been developed to improve its activity, to combine metal-binding capacity, and inhibition of Aβ aggregation, respectively [2]. In particular, it has been shown the chelating ability towards Cu^{2+} of tracine-S-allyl-cysteine derivative compound with potential role for the treatment of AD [3, 4]. It is widely accepted that the hydrophilic fragment containing the first 16 residues of the Aβ peptide (Aβ16) is highly representative of the coordination behavior of the wild peptide toward metal ions [1]. Hence, Aβ28 peptide is a good model for the longer Aβ40 and Aβ42 peptides. With this in mind, NMR spectroscopy has been used to evaluate the effect of tracine-S-allyl-cysteine derivative compound on Cu^{2+} chelation and Cu^{2+}-induced Aβ1-28 aggregation in order to study its capacity against Alzheimer's disease.

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P52

α-SYNLUCLEIN AND SMALL MOLECULES INTERACTIONS

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Amyloid fibrils of α-Synuclein (aS) are the main constituent of Lewy bodies and are largely investigated for their involvement in the neurodegenerative Parkinson's disease. aS oligomerization seems to have a crucial role in neurons toxicity, altering the cellular metabolism, the synaptic vesicles pools and causing mitochondrial damage. An insight of the binding of some cellular metabolites and α-Synuclein oligomers will be presented and their implication on neurons toxicity will be discussed.

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DYNAMIC NUCLEAR POLARIZATION SOLID-STATE NMR FOR ANALYZING SYNTHETIC POLYMERS

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Description of microstructure/morphology/properties relationships in polymeric materials, including accurate structural elucidation of chain-ends, is classically required to finely tune their macroscopic properties. In this context, solid-state NMR (SSNMR) is typically regarded as one of the techniques of choice but its low sensitivity usually precludes elucidation of subtle structural features in polymers. Dynamic nuclear polarization (DNP) [1,2] could potentially circumvent this difficulty by boosting SSNMR sensitivity. Recently, we have shown that DNP SSNMR at 9.4 T could prove highly relevant for the structural elucidation of chain-ends in high molecular weight polymers, hereby allowing chemical reactions involving such large molecular weight samples to be effectively monitored [3]. We then showed that optimizing the DNP sample preparation method could further improve the overall sensitivity enhancement provided by DNP, reaching up to 50-fold enhancement in the most favorable cases [4].
This communication describes our recent efforts in using DNP SSNMR for the structural characterization of polymers. In particular, we have investigated the impact of polymer deuteriation on the DNP signal enhancement and the overall sensitivity gain for a series of poly(styrene) samples obtained by nitroxide-mediated polymerization (NMP) or atom-transfer radical polymerization (ATRP). Results indicate that sample deuteriation does increase the DNP signal enhancement, but this does not necessarily translate into a net gain in overall sensitivity owing to a reduced cross-polarization efficiency that results from a reduced amount of protons in the sample. We also show that, in agreement with the literature, removing molecular oxygen from polymer DNP samples may lead in some cases to significant improvements in terms of overall sensitivity gain. We finally describe preliminary results regarding the analysis of block copolymers samples, in an attempt to correlate DNP signal and sensitivity enhancements with the morphology of nanostructured polymers.

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MULTIPLE-PULSE DECOUPLING *VS* 60 KHz MAGIC ANGLE SPINNING

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Modern high-resolution solid-state NMR experiments increasingly rely on proton–proton homonuclear dipolar decoupling. Since the pioneering works by Lee and Goldburg [1] and Waugh et al. [2], many developments have been proposed to design homonuclear decoupling sequences that are less sensitive to experimental imperfections, so as to improve both spectral resolution and sensitivity in homonuclear-decoupled spectra. [3]
Importantly, in such experiments, the precession frequencies are changed because the spins precess around a tilted effective field and not only around the direction of the external field, which means that the observed chemical shifts are changed. The corresponding scaling factor depends on the pulse sequence in use. It can be calculated from the tilt angle but is also slightly dependent on the offset and the effective radiofrequency (RF) field. As such, changes in RLC circuitry of the NMR probehead, as observed for instance when analyzing different types of samples (owing to different inductance values), may influence the scaling factor and compromise the comparison of the chemical shifts in ^{1}H NMR spectra recorded on different samples (although using the same experimental conditions).
To circumvent this issue, one possibility is to use ultra-fast MAS (>60 kHz) probe heads in order to obtain reproducible chemical shifts and hence to allow the ^{1}H spectra of different samples to be properly compared. In addition, using the ^{1}H chemical shifts measured in these conditions, one can calibrate the scaling factor of homonuclear-decoupled experiments. In this work, various examples along these lines will be presented.

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