XLIII Convegno Nazionale delle Risonanze Magnetiche

BARI

GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

Atti del Convegno

Bari, 22 – 24 Settembre 2014

Aula Magna "Attilio Alto"

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Scientific program

Monday September 22nd

10:00-13:00 Registration

Satellite events

11:00-12:00 **Innovative Solutions**

Presentation of the project “Apulian Wheat Fingerprint”

12:00-13:00 **Parallel session**

Bruker users meeting | Agilent users meeting

13:00-14:00 Lunch

14:00-14:30 Opening

14:30-15:30 **GIDRM/GIRM gold medal award - Chair: M. Cremonini**

N. Niccolai: My long way from 90 MHz ... To few THz

15:30-15:50 **Annalaura Segre fellowship 2013 - Chair: H. Molinari**

S. Zanzoni: Set-up and optimization of electroporation protocols to achieve high cell viability and eukaryotic intracellular uptake of isotope-labeled proteins for in-cell NMR

15:50-16:35 **Plenary Lecture - Chair: V. Gallo**

P. Mastrorilli: Application of heteronuclear NMR spectroscopy for mechanistic and structural studies on platinum complexes [PL1]

16:35-17:40 Coffee break and Poster session

	Parallel session A	Parallel session B
	Chair: L. Mannina	**Chair: A. Spisni**
17:40-18:10	**F. Cesare Marincola:** From breast milk to cheese: some NMR-based metabolomics applications [OC1]	**F. Rastrelli:** NMR of monolayer-protected nanoparticles [OC6]
18:10-18:30	**N. Proietti:** Portable NMR as a non invasive tool for monitoring the water status of fruits [OC2]	**R. Luisi:** Nitrogen stereodynamics as key factor in the reactivity of small heterocycles [OC7]
18:30-18:50	**G. Praticò:** NMR-based metabolomics of breast milk to evaluate the effect of phenotype, lactation period and dietary intervention [OC3]	**C. Airoldi:** Identification of new anti-neurodegenerative compounds through the NMR screening of natural extracts [OC8]
18:50-19:10	**A. Rizzuti:** NMR based statistical hetero-spectroscopy for knowledge transfer in vine leaves quality assessment [OC4]	**C. R. Girelli:** Sterical and electronic effects in $[PtCl_2(\eta^2\text{-}CH_2{=}CH_2)(N\text{^}N)]$ complexes assessed by NMR [OC9]
19:10-19:30	**A. Ventrella:** Characterization of table grape quality by nuclear magnetic resonance coupled with chemometrics [OC5]	**P.Mazzei :** Quantitative evaluation of non covalent interactions between glyphosate and dissolved humic substances by NMR spectroscopy [OC10]

19:30-20:00 **GIRM Assembly**

Tuesday September 23rd

9:00-9:45	**Plenary Lecture - Chair: P. Turano**	
	M. Zweckstetter: NMR spectroscopy in neurodegeneration: Tau and TSPO [PL2]	
9:45-10:05	**Bruker Sponsor Lecture - Chair: P. Turano**	
	B. Perrone: When the speed matter(s): from very fast MAS to static solid state NMR [OC11]	
10:05-11:20	Coffee break and Poster session	
	Parallel session A	**Parallel session B**
	Chair: F. Arnesano	**Chair: M. Chierotti**
11:20-11:50	**L. Ragona:** Design of new anti-angiogenic agents targeting fibroblast growth factor-2 interactions with tyrosine-kinase receptor and heparan sulphate proteoglycans [OC12]	**G. Mollica:** Residual Dipolar Couplings: a tool for NMR crystallography [OC16]
11:50-12:10	**F. Favretto:** Characterization of bile salt binding to recombinant human liver fatty acid binding protein [OC13]	**F. Martini:** Crystallographic properties of zirconium phosphonates by means of ^{19}F and ^{1}H solid-state NMR [OC17]
12:10-12:30	**A. Lasorsa:** Cisplatin handover from the copper transporter ATOX1 to ATP7A (MBD1) in near physiological conditions [OC14]	**S. Todisco:** Application of solid state NMR spectroscopy for investigation the chemical shift anisotropy in dinuclear platinum complexes [OC18]
12:30-13:00	**D. O. Cicero:** The interaction of BA42, a new phosphatase from the antartict, with divalent metals ions [OC15]	**A. Bagno:** Predicting the NMR spectra of paramagnetic iron complexes by DFT [OC19]
13:00-14:30	Lunch with extra virgin olive oil tasting	

	Parallel session A	Parallel session B
	Chair: L. Calucci	**Chair: N. Niccolai**
14:30-15:00	**G. Pileio:** Long-lived localisation in MRI [OC20]	**G. Colafemmina:** NMR studies of internuclear interactions in micellar systems [OC23]
15:00-15:20	**A.Pagoto:** Lipid-based nanosystems targeting the activated endothelium for the in vivo visualization by MRI of localized inflammation [OC21]	**V. Di Tullio:** Single-sided NMR to study transport of water in agar gels used as cleaning tool in conservation of cultural heritage [OC24]
15:20-15:40	**M. Muñoz Ubeda:** Novel nanocarriers based on lipophilic mono- and disaccharides/DPPC encapsulating Gd(III) complexes as efficient MRI probes [OC22]	**C. Corsaro:** ^{1}H HR-MAS NMR investigation on the molecular degradation of ancient documents [OC25]
15:40-16:25	**Plenary Lecture – Chair: V. Gallo**	
	A. Macchioni: NMR as an essential tool for investigating molecular transition metal catalysts [PL3]	
16:25-17:30	Poster session and Coffee break offered by Euriso-top	
17:30-18:15	**Plenary Lecture - Chair: M. Gussoni**	
	D. Delli Castelli: Ln(III)HPDO3A complexes as paramagnetic MRI-CEST agents [PL4]	
18:15-18:35	**Agilent Sponsor Lecture - Chair: M. Gussoni**	
	M. Cremonini: NUS for everyone: not uniform sampling made easy [OC26]	
18:35-20:00	**GIDRM assembly + Announcement of poster competition winners**	
20:30	**Social Dinner**	

Wednesday September 24th

8:45-9:30 **Plenary Lecture - Chair: M. Geppi**

S. Ashbrook: Investigating disorder in the solid state using NMR spectroscopy [PL5]

9:30-10:15 **Lectures of poster competition winners - Chair: H. Molinari**

	Parallel session A	**Parallel session B**
	Chair: M. Geppi	**Chair: S. Mammi**
10:15-10:45	**S. Bracco:** Modulation of rotor dynamics and recognition of host-guest interactions in highly porous materials [OC27]	**M. Veronesi**: ^{19}F NMR for functional screening and fragment based approach [OC30]
10:45-11:05	**F. Grifasi:** Solid-state characterization of coordination polymers for electroluminescent applications [OC28]	**A. Mucci:** Assessment of freezing effects and diagnostic potential of biobank healthy and neoplastic breast tissues through HR-MAS NMR spectroscopy [OC31]
11:05-11:25	**G. Paul:** Zeolites for decontamination of polluted water: a combined solid-state NMR and FTIR study [OC29]	**M. Gussoni:** Perfusates and tissue analysis by NMR and EPR spectroscopy: a potential predictive tool for kidney graft outcome [OC32]

11:25-11:45 Coffee break

	Parallel session A	Parallel session B
	Chair: D. Cicero	**Chair: G. Musco**
11:45-12:15	**S. Ciofi Baffoni:** Copper and Fe/S cluster trafficking: two sides of the same coin [OC33]	**M. Tessari:** SABRE hyperpolarization and quantitative analysis of complex mixtures: application to coffee extracts [OC36]
12:15-12:35	**M. Gallo:** The N-terminal region of human frataxin [OC34]	**L. Del Coco:** Intercultivar and intracultivar variations of Apulian EVOOs studied by ^{1}H NMR metabolic profiling [OC37]
12:35-12-55	**C. Zucchelli:** NMR and Sp140 PHD finger: structure, phosphorylation and PIN1 interaction [OC35]	**A. Sobolev:** Metabolic identity and variability of endive and escarole cultivars grown in Lazio and Puglia [OC38]

12:55-13:40 **Plenary Lecture – Chair: P: Turano**

I.F. Duarte: NMR metabolomics: developments and applications in cancer profiling and toxicological studies [PL6]

13:40-15:00 Closure and lunch

GIDRM/GIRM Gold Medal Award

GMA

MY LONG WAY FROM 90 MHz ….. TO FEW THz

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Nuclear relaxation has been my first scientific love. After an initial manganous period [1], studying diamagnetic cross-relaxation among protons [2] and between ^{1}H and ^{13}C nuclei [3] has been my priority until Willie Gibbons put on my desk some soluble and stable spin-labels: it was the beginning of my post-nitroxide era [4]. Use, misuse and abuse of TEMPOL, the 4 hydroxy derivative of TEMPO nitroxide, has been, indeed, the main topic for many Conferences and papers of mine, see Fig. 1 and ref. [5].

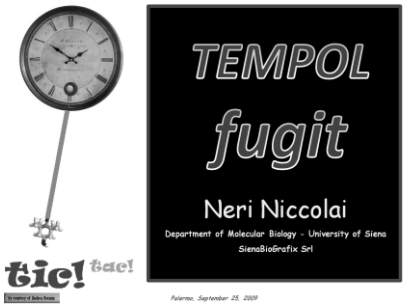

Figure 1. Conference given to honor Temussi's 50th birthday (Palermo 2009)

Trying to fit, unsuccessfully, paramagnetic attenuation profiles induced by TEMPOL to surface exposures of protein backbone atoms, I found a new way for calculating 3D atom depths [6]. However, something still prevented to get linear dependence of TEMPOL effects with protein structural features. Eventually, from combined analysis of MD simulations and paramagnetic attenuation profiles, the pivotal role of protein-water interactions to drive ligands to surface hot spots was established [7].

While TEMPOL was increasing its popularity for paramagnetic fragment-based NMR studies to investigate on protein surface accessibility [8], I was distracted by Bioinformatics. By using 3D depth algorithm to label atom insertion in protein structures, quantitative amino acid assignment to different structural layers was achieved. The average amino acid composition of each layer in a large subset of PDB structures has delineated new interesting features which suggest possible mechanisms for the preliminary steps of intermolecular interactions [9].

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ANNALAURA SEGRE FELLOWSHIP 2013

ASF

SET-UP AND OPTIMIZATION OF ELECTROPORATION PROTOCOLS TO ACHIEVE HIGH CELL VIABILITY AND EUKARYOTIC INTRACELLULAR UPTAKE OF ISOTOPE-LABELED PROTEINS FOR IN-CELL NMR

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In-cell NMR spectroscopy, in conjunction with specific isotope enrichment, represents a novel biophysical tool to investigate the interaction, conformational and functional characteristics of biomolecules at the atomic level in native environments inside living cells [1]. In bacteria, several in-cell NMR methods have been developed to detect the NMR signals derived from isotopically labeled overexpressed protein. The situation in eukaryotic cells appears more complex, given the lack of strong promoters that don't allow the expression of the appropriate amounts of isotopically enriched protein. Thus, the set-up and optimization of efficient protocols that achieve high cell viability and eukaryotic intracellular uptake of isotopically labeled proteins represents an essential prerequisite for in-cell NMR experiments. To date, several approaches, such as microinjection [2,3], cell-penetrating peptides (CPP) [1] and pore-forming bacterial toxin Streptolysin O (SLO) [4,5] have been attempted for introducing ^{15}N labeled proteins into cytosol of living cells. All these approaches however present some drawbacks and may not be universally applicable. Recently, the electroporation was proposed as suitable method to deliver proteins directly into the cytosol for in-cell high resolution NMR observation. In this perspective, the aim of project was to implement and evaluate electroporation as a generally applicable method for efficient delivery of biomolecules into the cytosol of different mammalian cell lines for in-cell NMR experiments. Small-scale experiments were carried out on different proteins and cell lines in order to achieve the highest protein uptake and cell viability. Unfortunately, electroporation procedure yielded very variable transduction efficiencies, ranging from 0.5% to 4%. Therefore, the concentration of the delivered protein for the observation of a *in-cell* NMR sample was not reproducible. This result suggests that the electroporation is not a suitable method for efficient delivery of protein molecules into mammalian cells for in-cell NMR observation.

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PLENARY LECTURES

PL1

APPLICATION OF HETERONUCLEAR NMR SPECTROSCOPY FOR MECHANISTIC AND STRUCTURAL STUDIES ON PLATINUM COMPLEXES

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Due to the presence of several types of spin ½ nuclei, NMR based platinum phosphine complexes are ideal candidates for mechanistic and structural studies in solution.
In our group we have studied the reactivity of phosphido complexes of platinum [1] in reactions of P-C bond formation [2], hydrogenation/deuteration [3], carbonylation [4] and C-H bond activation. In all cases, heteronuclear NMR spectroscopy gave precious information for detecting reaction intermediates and for assigning them the proper structure and, when applicable, dynamics [5].
For example, in the case of the carbonylation of the complex $[(PHCy_2)_2Pt(\mu\text{-}PCy_2)Mo(CO)_2Cp]$ (*Pt–Mo*), the reaction led to a mixture of *cis* and *trans* isomers (see Fig. 1) in equilibrium. Combination of $^{31}P\{^1H\}$ EXSY and $^{13}C\{^1H\}$ EXSY experiments carried out on such a mixture showed that the mechanism responsible for the *cis-trans* equilibrium envisages the dissociation-reassociation of the carbon monoxide, with the $PHCy_2$ constantly bonded to the Pt.

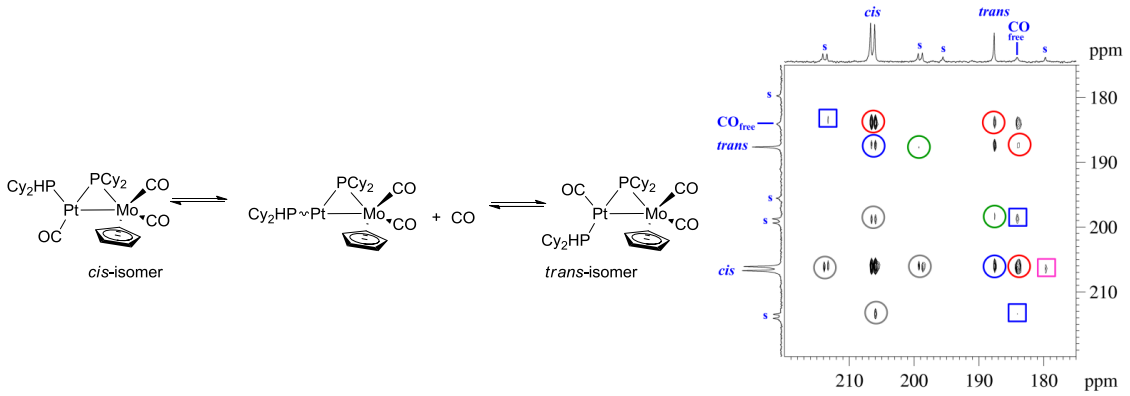

Fig. 1. $^{13}C\{^1H\}$ EXSY spectrum of the *cis-trans* mixture in the presence of free CO (CH_2Cl_2, 298 K).

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PL2

NMR SPECTROSCOPY IN NEURODEGERATION: Tau AND TSPO

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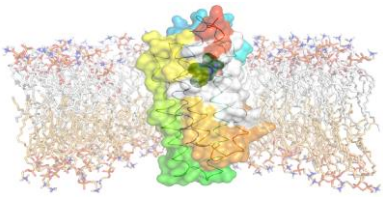

We are interested in the folding and misfolding of proteins and in particular in the molecular basis of the interaction of small molecules with proteins involved in neurodegenerative diseases. Our studies focus on the microtubule-associated protein Tau [1] and the mitochondrial membrane protein TSPO. Anti-aggregation drugs play an important role in Tau protein-based therapeutic approaches for Alzheimer´s disease. Although, several distinct classes of small-molecules have been identified, little is known about the mechanism of inhibition and the nature of generated Tau species. We have studied the binding of the pthalocyanine tetrasulfonate (PcTS), the phenothiazine methylene blue (MB) and its N-demethylated derivatives azure A and azure B to human Tau protein. Our studies showed that MB and its metabolites modify the native cysteines of Tau and retain it in a monomeric disordered state.[2] In contrast, PcTS binds to aromatic residues of tau and targets the protein into noncooperatively stabilized oligomers with a diameter of 30 to 40 nm.[3] Our results suggest that conformational modulation of Tau is a viable strategy for reduction of pathogenic Tau deposits and the development of novel therapeutics.The 18-kilodalton translocator protein TSPO is found in mitochondrial membranes and mediates the import of cholesterol and porphyrins into mitochondria.[4] In line with the role of TSPO in mitochondrial function, TSPO ligands are used for a variety of diagnostic and therapeutic applications in animals and humans. We determined the 3D high-resolution structure of mammalian TSPO in complex with its high-affinity ligand PK11195.[5] The TSPO-PK11195 structure is described by a tight bundle of five transmembrane a helices that form a hydrophobic pocket accepting PK11195. Ligand-induced stabilization of the structure of TSPO suggests a molecular mechanism for the stimulation of cholesterol transport into mitochondria. [5,6]

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PL3

NMR AS AN ESSENTIAL TOOL FOR INVESTIGATING MOLECULAR TRANSITION METAL CATALYSTS

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NMR has a key role in disclosing the structure in solution of homogeneous catalysts, allowing precious information about active species, intermediates, off-loop species, resting states, possible speciation and transformation of catalysts to be obtained.
Over the last years, we have been studying two main classes of molecular catalysts: early transition metal complexes for olefin polymerization and late transition metals for water oxidation. Investigation has been conducted taking advantage of the NMR methodologies developed in our group, mainly based on diffusion and NOE NMR experiments [1-3]. The latter have been applied to understand i) the real nature of the active species of industrially relevant metallocene and post-metallocene catalysts for olefin polymerization [4] and ii) how molecular catalysts for water oxidation transform when subjected to oxidative stress [5].
In this contribution, after having recalled the NMR methodologies used by us, an overview of our recent results will be provided.

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PL4

Ln(III)HPDO3A COMPLEXES AS PARAMAGNETIC MRI-CEST AGENTS

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CEST agents based on paramagnetic complexes have been reported to display improved properties with respect to the conventional probes (based on Gd(III) or iron oxide particles) especially for those medical applications like DCE-MRI, [1] cell tracking, [2] and measurements of diagnostically-relevant parameters (e.g. pH, enzyme, metabolites, …) [3] that benefit from the detection of MRI-CEST response generated by the frequency labeling of different protons in the same imaging experiment. Despite the great potential of the agents proposed so far, their clinical translation still appears rather difficult. Many issues need to be faced mostly related to both the overall safety of the MRI-CEST experiment (SAR limitations, probe toxicity) and the sensitivity/reliability of the CEST detection. The clinical use of CEST agents could be accelerated by considering clinically approved compounds potentially able to generate CEST contrast. A first example of this type of chemical is represented by the diamagnetic CT agent Iopamidol, whose ability to act as concentration-independent pH responsive CEST probe has been recently demonstrated in mice [4]. Following a similar guess, the paramagnetic analogues of the clinically approved MRI agent Gadoteridol can be considered as "clinically safe" CEST agents in virtue of the presence of the coordinating hydroxyl proton, whose exchange rate fulfills the necessary $\Delta\omega \geq k_{ex}$ condition. Interestingly, the peculiar conformational/configurational isomerism of Ln-HPDO3A complexes allows the selective saturation of two OH protons belonging to different diastereoisomers, thus making possible the application of the ratiometric method.
In this contribution, the CEST potential of this class of PARACEST agents will be presented.

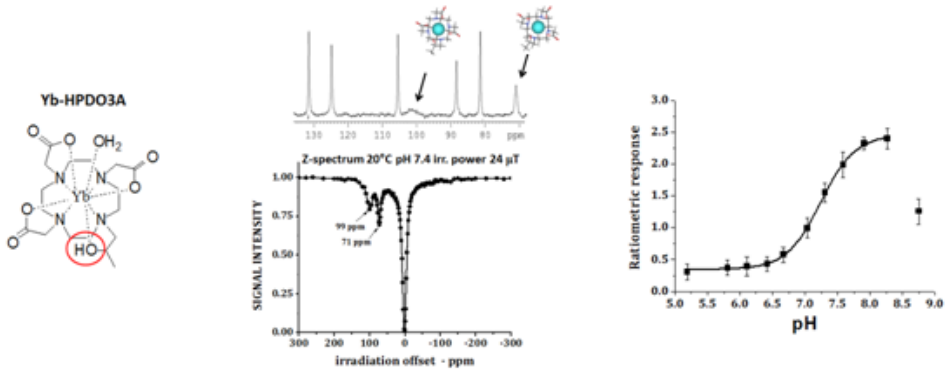

Fig. 1. YbHPDO3A NMR and CEST characterization

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PL5

INVESTIGATING DISORDER IN THE SOLID STATE USING NMR SPECTROSCOPY

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NMR spectroscopy provides an element-specific, sensitive probe of the local environment, enabling detailed information to be extracted. However, in the solid state the vast majority of this information remains unexploited, owing to the challenges associated with obtaining high-resolution spectra and the ease with which these can be interpreted. For inorganic solids, this problem is amplified by the large range of nuclides studied, the lack of prior information in the literature and the practical challenges of experimental implementation for species with long relaxation times, low sensitivity and large quadrupolar broadening.

Recent advances enabling accurate and efficient calculation of NMR parameters in periodic systems have revolutionized the application of such approaches in solid-state NMR spectroscopy, particularly among experimentalists. The use of first-principles calculations aids in the interpretation and assignment of the complex spectral lineshapes observed for solids. For materials with poorly characterized structures calculations provide a method for evaluating potential structural models against experimental measurements. As NMR is sensitive to the atomic-scale environment, it provides a potentially useful tool for studying disordered materials, and the combination of experiment with first-principles calculations offers a particularly attractive approach. I will illustrate the insight into local structure and disorder in inorganic materials that can be obtained when computational and experimental approaches are combined, using a variety of case studies including ceramic materials proposed for the encapsulation of radioactive waste and high-pressure silicate phases.

PL6

NMR METABOLOMICS: DEVELOPMENTS AND APPLICATIONS IN CANCER PROFILING AND TOXICOLOGICAL STUDIES

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Metabolomics entails the comprehensive analysis of endogenous metabolites present in cells, tissues or biofluids, using high-throughput profiling techniques, such as Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry (MS), combined with multivariate statistics. The general aim is to measure fluctuations in metabolite levels and metabolic pathways upon a given stimulus/perturbation. High resolution NMR is exquisitely powerful in this respect, since, despite inherent sensitivity limitations, it shows unparalleled analytical reproducibility and the ability to provide unequivocal structural and quantitative information on a wide range of metabolites. This communication will cover different aspects of NMR metabolomics applied to disease research and toxicological studies. In particular, examples of the work carried out in our group will be presented, concerning three main topics: 1) lung cancer metabolic signatures in human tissues and biofluids [1]; 2) modulation of cellular metabolism by anticancer drugs [2]; 3) metabolic effects of silver nanoparticles. Key developments and challenges in preclinical and clinical metabolomic approaches will be discussed.

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ORAL COMMUNICATIONS

OC1

FROM BREAST MILK TO CHEESE: SOME NMR-BASED METABOLOMICS APPLICATIONS

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The term *metabolomics* is often used to define a scientific study that measures, in a quantitative manner, all low molecular weight metabolites in complex biological samples. Metabolomics was initially applied in the fields of plant science [1] and toxicology [2] and has recently emerged as an important tool also in modern food science (*foodomics*) [3,4].
Metabolomics is ideal in the study of the low- molecular-weight compounds in milk and its derivatives. Currently, the metabolomics applications dealing with these food products can be divided into two main categories: those linking the metabolite profiling with nutritional aspects, and those aimed at linking the metabolite profile to processing and safety of raw materials and final products, as well as their identity. This presentation will illustrate two examples of these applications. The first one deals with the use of NMR-based metabolomics to investigate and compare the metabolic profiles of preterm human breast milk and preterm formula milk [5]. The second one regards the investigation of the kinetics of Fiore Sardo, a ‘‘Protected Designation of Origin’’ (PDO) cheese produced from raw ewe’s milk exclusively, by physicochemical parameters, microbial counting, and NMR metabolomics [6]. Both examples evidence the potential of metabolomics for the determination of the quality and the authenticity of dairy products.

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OC2

PORTABLE NMR AS A NON INVASIVE TOOL FOR MONITORING THE *WATER STATUS* OF FRUITS

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Water is probably the most important component of a food system because it influences many process variables and product characteristics [1]. It has been recognized that water content of a food can be related to the stability of the food. In fact the *nature* and the *availability* of the water also contributes to the development of deteriorative food processes. The water status is also related to the food texture. Both texture and water status are some of the key quality attributes used in the fresh and processed food industries to assess product quality and acceptability. Knowledge of textural properties is important in the food value chain including producers, marketers and consumers [2]. For fresh food such as fruit and vegetable, textural properties such as firmness are used as indices of readiness to harvest to meet requirement for long term handling, storage and acceptability by the consumer. For processed food understanding texture properties is important for the control of processing operations such as heating, drying and frying. Various approaches have been used to evaluate the sensory attributes of texture in food. Nuclear magnetic resonance (NMR) is one of the few instrumental techniques with high potential in the characterization of both water distribution and the anatomical interior of foods, both known to be critical for texture. Low field NMR and MRI has been used as a characterization and quality control tool for several food product [3]
Due to the increasing demand for rapid, cost effective and non-invasive measurements, the development of low-field NMR sensors for on-line monitoring of food quality is a challenge in the food industry. The main advantage of low-field single sided NMR technique is that it does not require any sampling and pre-treatment of the sample and once developed, standard protocols based on fast measurements can be easily transferred to quality control applications including texture, freshness and shelf-life monitoring [4,5]. NMR application to the water status of fresh and processed fruits will be shown to illustrate the potentialities of single sided NMR devices in the food quality control.

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OC3

NMR-BASED METABOLOMICS OF BREAST MILK TO EVALUATE THE EFFECT OF PHENOTYPE, LACTATION PERIOD AND DIETARY INTERVENTION

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Breast milk is the first functional food that human beings can assume, containing both nutritional components and non-nutritive bioactive factors, which promote infant growth and development. They include macronutrients, such as proteins, lipids and lactose, cells, anti-infectious and anti-inflammatory agents, growth factors, and prebiotcs, such as Human Milk Oligosaccharides (HMOs), which play a crucial role in the establishment and development of infant gut microbiota in early life, resulting in marked effects on immune and metabolic programming in infant. In the last few years, several dietary interventions have been proposed to modulate in a healthier way the composition and function of resident microbes in order to reduce the risk of disease.
In this scenario, a rapid characterization of milk composition, as the one obtained by NMR-based metabolic profiling, may contribute to the investigation of breast milk nutritional properties and its impact on infant health. In the present study, breast milk from healthy pregnant women was collected within the first three days (colostrum) and thirty days post-partum. High resolution ^{1}H NMR spectroscopy allowed to disentangle the complex mixture of metabolites present in both breast milk hydrosoluble (amino acids, simple and complex sugars and small organic acids) and liposoluble extracts (cholesterol, fatty acids and acyl-glycerol moieties). Four different milk classes were identified through the analysis of NMR spectral profiles, based on fucosylated HMOs content, associated with the expression of the secretor status (Se gene) and the Lewis blood epitopes (Le gene) of the lactating women. Changes in milk metabolome between "secretor" and "non secretor" individuals were evaluated during the first month of lactation. Finally, a combined approach of ^{1}H NMR-based metabolomics and PCR was applied to assess possible changes in breast milk metabolic and microbiological composition induced by perinatal oral supplementation of VSL#3 probiotic mixture, containing four strains of lactobacilli, three strains of bifidobacteria and one strain of streptococcus.
The combination of microbiological and metabolomic approach has proven to be a valuable tool for the evaluation of the effects of the interaction between diet, genotype and milk maturation process. Such analytical platform may contribute to study changes in milk composition induced by pathophysiological states (gestational diabetes), mode of delivery and gestational age, and to target appropriate nutritional interventions (prebiotics and probiotics).

OC4

NMR BASED STATISTICAL HETERO-SPECTROSCOPY FOR KNOWLEDGE TRANSFER IN VINE LEAVES QUALITY ASSESSMENT

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Development of quality assessment and authentication tools of vine leaves is gaining increasing interest due to the use of vine leaves as food products. In this framework, the combination of methods based on NMR spectroscopy, direct infusion High Resolution Mass Spectrometry (HRMS) and XRPD was developed as powerful tool for the characterization of brined and fresh leaves [1].

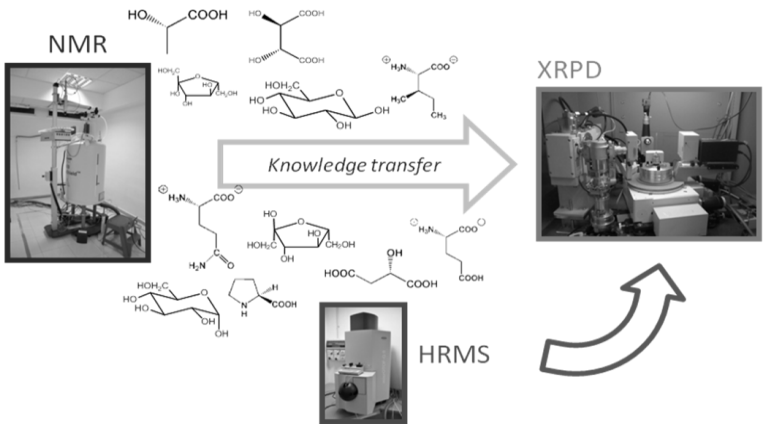

Fig. 1. Scheme representing NMR based statistical hetero-spectroscopy approach for knowledge transfer.

In this presentation primary and secondary metabolites of vine leave samples will be identified by comparing NMR data to chromatograms carried out by HPLC coupled to HRMS experiments. The NMR, HRMS and XRPD gave complementary information and their use in covariance analysis allowing to use NMR spectroscopy as support for XRPD and HRMS techniques for vine leaves quality assessment. This NMR based approach (Fig. 1) can be extended to other food products.

Acknowledgements

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OC5

CHARACTERIZATION OF TABLE GRAPE QUALITY BY NUCLEAR MAGNETIC RESONANCE COUPLED WITH CHEMOMETRICS

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In this work, the possibility to obtain information regarding the quality of table grapes by using Nuclear Magnetic Resonance (NMR) Spectroscopy was investigated. In particular, ^{1}H NMR was employed, adopting one-dimensional ^{1}H NOESY experiments, to characterize the geographical origin of Red Globe table grapes coming from Puglia, Sicilia, Spain and Portugal. The principal component analysis (PCA) was applied to gain an exploratory overview of the distribution of the samples based on their relevant spectral information; such analysis highlighted the necessity of classification techniques, so that the data were subjected to the Linear Discriminant Analysis (LDA), in order to build statistical models to classify table grapes based on the geographic origins. The classification performances obtained were remarkably satisfying, with an average recognition ability equal to 97.8 %.
The above mentioned analytical approach was also applied to characterize the agronomical practices used for Italia table grape farming, with the particular purpose to discriminate organic from conventional grapes. Even for this study the experimental data were elaborated by multivariate statistical techniques: after the exploratory overview of data by PCA, classification techniques were used with different strategies, obtaining interesting classification performances, even reaching a rate of 100 %.
All the models obtained within this work were subjected to cross-validation procedures, in order to get reliable results.

OC6

NMR OF MONOLAYER-PROTECTED NANOPARTICLES

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Owing to their peculiar size-related properties and their self-assembled nature, nanoparticles (NPs) protected by a monolayer of organic molecules offer a straightforward route to the construction of complex chemical systems, ranging from catalysts and sensors to theranostic agents and artificial vaccines [1]. Indeed, while such a complex self-organization has inspired several elegant applications in many fields, the structure of the coating monolayer, and its interaction with other molecules, is still largely unknown. In this challenging scenario we have investigated the properties of NPs monolayers by Paramagnetic Relaxation Enhancement [2] and relaxometry in the solid state under ultrafast magic-angle spinning (MAS) conditions. We have also exploited the spins located on the NPs monolayer as a magnetization source to be selectively transferred to interacting species, thus detecting small organic molecules in complex mixtures via NOE [3]. More recently, we have assessed the efficiency of NPs as pseudo-stationary phases in the context of "NMR chromatography" [4]. Selected examples taken from the aforementioned subjects will be presented in this communication.

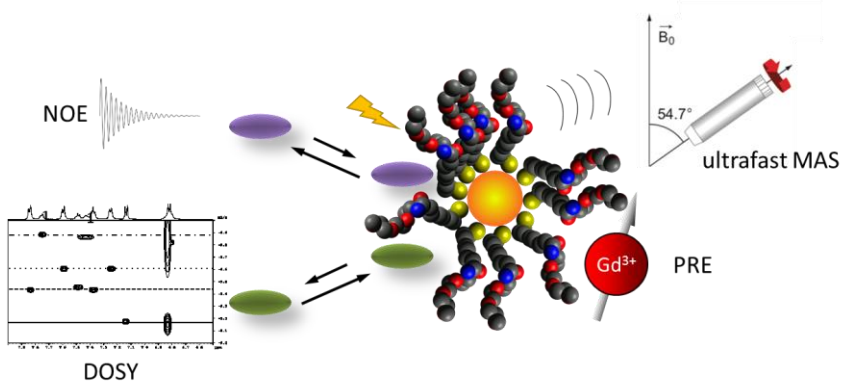

Fig. 1. Using NMR to study nanoparticles: from structural investigations to new applications

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OC7

NITROGEN STEREODYNAMICS AS KEY FACTOR IN THE REACTIVITY OF SMALL HETEROCYCLES

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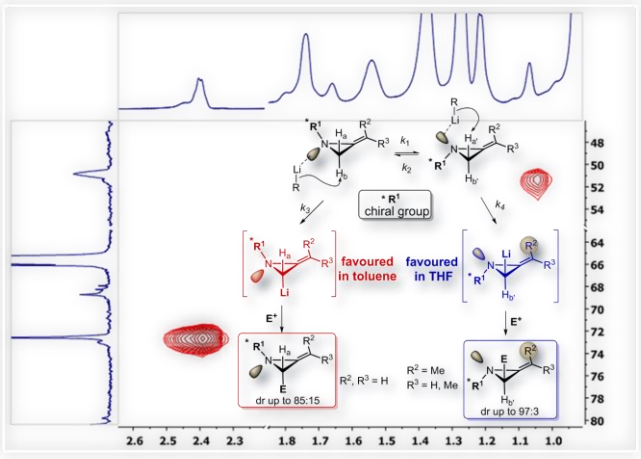
Chirality is a key feature of chemical entities, from small organic molecules to supramolecular assemblies, and it is at the heart of molecular and supramolecular chemistry. The chirality of small organic molecules in general originates from the configuration of stereocenters, while the chirality of supramolecular assemblies is often attributed to the global conformation and stereodynamics.[1] Recently we started a research project aimed at exploiting dynamic phenomena for regio- and stereoselective synthetic processes.[2] In particular, control on the nitrogen stereodynamics in small heterocycles, such as aziridines and azetidines, allows for the preparation of stereodefined and structurally different chemical entities starting from the same starting compounds. In this communication, the role of D-NMR in the study of stereodynamics in solution, and in the development of stereoselective syntheses will be reported.



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OC8

IDENTIFICATION OF NEW ANTI-NEURODEGENERATIVE COMPOUNDS THROUGH THE NMR SCREENING OF NATURAL EXTRACTS

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In this communication we show the potential of NMR approaches for the screening of natural product mixtures obtained from plant extracts, aimed at the discovery of new bioactive compounds.
In particular we focused our interest on the identification of new ligands of amyloidogenic peptides and proteins (Aβ1-42, PrP106-126 and Ataxin-3) involved in Alzheimer's [1], mammalian prion [2] and Machado-Joseph's neurodegenerative diseases [3]. Due to the severe impact of these pathologies on the quality of life of the patients and their families, their massive economic burden, and the lack of effective therapies and diagnostic tools, there is an urgent need for effective molecules for their treatment and diagnosis. In this context, the availability of new screening methods is strategic.
Exploiting STD NMR and trNOESY experiments we were able to identify ligands of amyloid peptides and proteins in *Salvia sclareoides* [4], *Genista tenera* [5] and green tea extracts [6]. The interaction of the best ones was further investigated at molecular level working on the purified molecules by combining NMR with other biophysical techniques such as AFM, CD, TEM and fluorescence microscopy.
Our data provide important information for the rational design of new compounds with higher affinity for Aβ1-42, PrP106-126 and Ataxin-3, to generate new anti-amyloidogenic molecules and/or molecular tools for the specific targeting of amyloid aggregates *in vivo*.

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OC9

STERICAL AND ELECTRONIC EFFECTS IN [$PtCl_2(\eta^2\text{-}CH_2{=}CH_2)(N\text{^}N)$] COMPLEXES ASSESSED BY NMR

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In this work we studied a series of pentacoordinate Pt(II) complexes, of the type [$PtCl_2(\eta^2\text{-}C_2H_4)(N\text{^}N)$], *N^N* = dinitrogen ligand, (see Fig. 1), with a variable sterical hindrance due to Me substituents, to evidence the changes in the platinum electron cloud due to the interaction of these substituents.

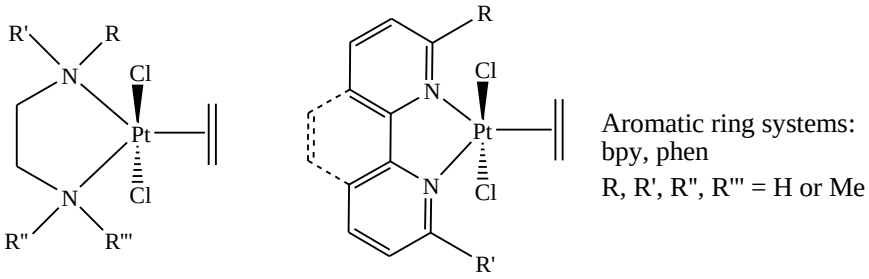

Fig. 1. General structures of the reported pentacoordinate complexes

In a previous work, we demonstrated that in Pt(II) pentacoordinate complexes the kinetic is not necessarily related to the thermodynamic stability, being the rotation around the Pt−N bonds, after spontaneous chelate ring opening, closely related to the complex decomposition rate [1]. Nevertheless, the relation between sterical hindrance of the *N^N* donors and thermodynamic stability of pentacoordinate complexes is not completely clear. We therefore analyzed by NMR the series of [$PtCl_2(\eta^2\text{-}C_2H_4)(N\text{^}N)$] complexes to verify the relation between the ability of the *N-N* dinitrogen ligands to donate electric charge to the metal and the thermodynamic stability of the complexes. An increase of ^{195}Pt NMR chemical shift values, proportional to the number of Mes germinal or vicinal to the *N*-donors was observed in [$PtCl_2(\eta^2\text{-}C_2H_4)(N\text{^}N)$] complexes. Accordingly, this reveals a progressive reduction of the electron density around the metal which correlates with the five coordinate stability. The updated main factors determining the stability of a pentacoordinate complex are (i) considerable steric hindrance, in the corresponding square planar complexes, originated by ethene loss [2]; (ii) stable chelation of the *N^N* ligand, being the Pt-halogen and Pt-olefin bonds, very stable in these systems [3]. (iii) Mes interacting with five coordinate Pt(II) electron density and causing the diagnostic deshielding of the ^{195}Pt NMR signals.

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OC10

QUANTITATIVE EVALUATION OF NONCOVALENT INTERACTIONS BETWEEN GLYPHOSATE AND DISSOLVED HUMIC SUBSTANCES BY NMR SPECTROSCOPY

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Interactions of glyphosate (N-phosphonomethylglycine) herbicide (GLY) with soluble fulvic acids (FAs) and humic acids (HAs) at pH 5.2 and 7 were studied by ^{1}H and ^{31}P NMR spectroscopy. Increasing concentrations of soluble humic matter determined broadening and chemical shift drifts of proton and phosphorus GLY signals, thereby indicating the occurrence of weak interactions between GLY and humic superstructures. Binding was larger for FAs and pH 5.2 than for HAs and pH 7, thus suggesting formation of hydrogen bonds between GLY carboxyl and phosphonate groups and protonated oxygen functions in humic matter. Changes in relaxation and correlation times of ^{1}H and ^{31}P signals and saturation transfer difference NMR experiments confirmed the noncovalent nature of GLY−humic interactions. Diffusion-ordered NMR spectra allowed calculation of the glyphosate fraction bound to humic superstructures and association constants (Ka) and Gibbs free energies of transfer for GLY−humic complex formation at both pH values. These values showed that noncovalent interactions occurred most effectively with FAs and at pH 5.2. Our findings indicated that glyphosate may spontaneously and significantly bind to soluble humic matter by noncovalent interactions at slightly acidic pH and, thus, potentially pollute natural water bodies by moving through soil profiles in complexes with dissolved humus.

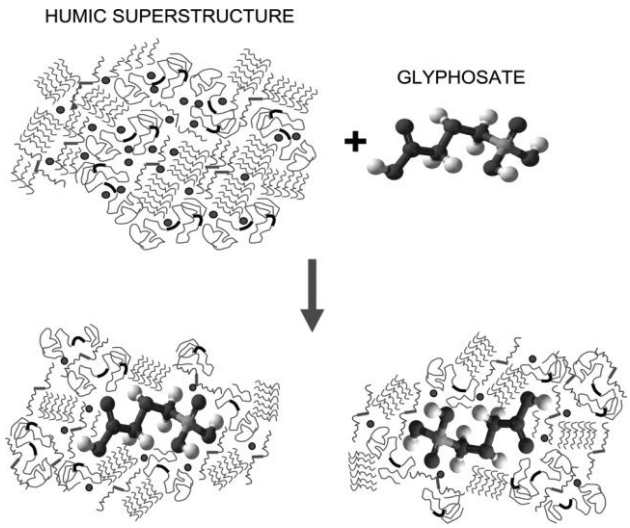

Fig. 1. Formation of humic-glyphosate host-guest complexes.

OC11

WHEN THE SPEED MATTER(S): FROM VERY FAST MAS TO STATIC SOLID STATE NMR

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Solid state NMR is a technique that is often associated with Magic Angle Spinning (MAS). In recent years, many efforts have been done in order to increment the maximum achievable spinning rate and using state-of-the-art technology is now possible to reach spinning speed of 60 kHz and beyond. The striving toward faster speeds is driven by the fact that the homonuclear and dipolar broadening caused by the strongly coupled proton network are weakened by MAS. Very fast MAS allows to reveal phenomena otherwise obscured by proton spin diffusion, like the local dynamics in polymeric chains. We will show an example of relaxation measurements of monolayer-protected nanoparticles performed at 60 kHz magic angle spinning.
Although going towards faster spinning speeds present some advantages, not all samples can sustain the large centrifugal force and friction exerted at high spinning speeds. Nevertheless, precious information can still be accessible under static and (ultra)slow-spinning conditions. As we will show, the (pseudo) static powder pattern line-shape reflects the tensor alignment of the nucleus in its local environment and it can be used to determine the topology of antimicrobial peptides in fragile membranes systems [1-3].

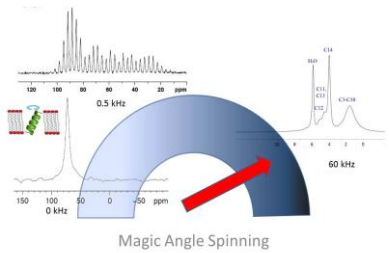

Fig. 1. Regimes of magic angle spinning and examples of possible experiments.

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OC12

DESIGN OF NEW ANTIANGIOGENIC AGENTS TARGETING FIBROBLAST GROWTH FACTOR-2 INTERACTIONS WITH TYROSINE-KINASE RECEPTOR AND HEPARAN SULPHATE PROTEOGLYCANS

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Fibroblast growth factor-2 (FGF2) plays a major role in angiogenesis, the process of generating new capillary blood vessels from pre-existing ones, an important natural phenomenon used for healing and reproduction. In healthy tissues the body controls angiogenesis by producing a precise balance of growth and inhibitory factors. Pathological angiogenesis underlies a wide range of diseases, including cancer, and FGF2 thus represents a target for anti-angiogenic therapies.
FGF2 needs to set up a productive ternary complex with the tyrosine-kinase receptors (FGFRs) and the heparan sulphate proteoglycans (HSPG) to exert its pro-angiogenic activity. Natural and synthetic molecules, able to interfere with HSPG/FGF2/FGFR interaction, have been designed starting from endogenous inhibitors of angiogenesis, such as Long Pentraxin-3 and Thrombospondin-1, which regulate angiogenesis through different mechanisms, including binding and sequestration of FGF2. A combination of NMR approaches were used to characterize the structural and dynamical features of protein/inhibitor interactions. NMR and MD studies allowed to propose the molecular basis of inhibitors anti-angiogenic activity and the functional consequences of their effective action were evaluated through *in vitro* and *in vivo* studies [1-4].

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OC13

CHARACTERIZATION OF BILE SALT BINDING TO RECOMBINANT HUMAN LIVER FATTY ACID BINDING PROTEIN

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Human Liver Fatty Acid Binding Protein (HLFABP) is a small protein belonging to the FABP family, a class of soluble 14-15KDa cytoplasmic proteins involved in the regulation of normal lipid homeostasis. Despite the exact role of HLFABP has not been clarified yet, a large number of experimental evidences suggest a function in facilitating the cellular uptake and metabolism of poorly soluble compounds. It has been proposed that HLFABP could bind long chain fatty acids, but also bulkier ligands such as fatty acyl-CoA, thioesters lysophosphatidic acid, heme, eicosanoids, bilirubin and bile acids (BAs) [1]. Thus, HLFABP has been proposed as BAs carrier during the enterohepatic circulation of these precious molecules [2].

The aim of the present study is a deep spectroscopic investigation of BA binding to HLFABP. A small library of BAs was presented to [^{1}H-^{15}N] HLFABP to rationalize the determinants of the interaction. Our NMR data show that HLFABP can interact with a wide range of bile salts, through a complex binding path, involving at least one binding-competent intermediate. These data are in agreement with previous results dealing with the interaction between HLFABP and glycocholic acid (GCA) [3]. We demonstrated that the presence of GCA substantially increased slow motions throughout the protein backbone at variance with other proteins of the family. Therefore, slow motions were further characterized by acquiring CPMG relaxation dispersion experiments. Furthermore, changes in free energy of opening indicated that HLFABP is more stable in the presence of BAs than in its free form and that the global hydrogen bond network was not significantly perturbed upon ligand addition. Then, a detailed analysis of NMR data showed that the unbound protein exists as an ensemble of conformers in fast exchange on an NMR time scale. Fluorescence competition experiments were used to integrate NMR data. HLFABP exhibited an affinity in the μM range toward all bile acids screened, as it was also confirmed by NMR titration experiments.

In conclusion, we demonstrated through different spectroscopic techniques, that HLFABP is able to accommodate a variety of BAs with a 1:1 stoichiometry, as it is also supported by mass spectroscopy experiments. In addition, slow motions were substantially increased upon BA addition, indicating that the partial occupation of the large hydrophobic cavity of HLFABP significantly increased conformational exchange phenomena. This property could be ascribed to the protein's promiscuous binding and introduces an intriguing question, related to whether the internal dynamics can be considered a mechanical initiator of ligand dissociation.

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OC14

CISPLATIN HANDOVER FROM THE COPPER TRANSPORTER ATOX1 TO ATP7A (MBD1) IN NEAR PHYSIOLOGICAL CONDITIONS

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Manifold experimental studies show that copper (Cu) transporters are involved in the uptake, transport and efflux of platinum (Pt)-based drugs, among the world's best-selling antitumor drugs [1]. The soluble chaperone ATOX1, which physiologically promotes the handover of Cu(I) from CTR1 to the metal binding domains (MBDs) of the ATPases ATP7A and ATP7B, is able to bind cisplatin (cis-$PtCl_2(NH_3)_2$) and take part to the intracellular distribution of Pt-drugs [2]. ATP7A and ATP7B can also bind and efflux Pt-drugs through the vesicles of the trans-Golgi network, hence contributing to the enhancement of tumor cell resistance [3]. Here we report a tandem ESI-MS and 2D NMR spectroscopic characterization of cisplatin binding to ATOX1 and MNK1, the first MBD of ATP7A. In the absence of any reducing agent, we found that Pt binds to the metal binding motif (CXXC) of both proteins, even though pregnant differences. In particular, MNK1 forms a quite long lasting monofunctional adduct (cis-$[PtCl(NH_3)_2]^+$-MNK1) which evolves to the corresponding bifunctional one (cis-$[Pt(NH_3)_2]^{2+}$-MNK1); while ATOX1 reacts slowly by forming directly a chelate adduct (cis-$[Pt(NH_3)_2]^{2+}$-ATOX1). We also studied the reactivity of both proteins towards cisplatin in conditions mimicking the cellular environment that is millimolar concentration of the physiological reducing agent glutathione (GSH). In this regard, we proved that MNK1, but not ATOX1, competes successfully with GSH for binding to cisplatin. Finally, we found that no transfer of platinum-moiety from ATOX1 to MNK1 occurs in our experimental setting. This latter result appears to be in contrast with literature data reporting the occurrence of such an exchange, although an exogenous reducing agent, such as tris(2-carboxyethyl)phosphine (TCEP), exerting a strong trans-labilizing effect, was always used. Our study highlights the complexity of Pt-loading reactions with a special focus on how different platinophiles can influence each other: only a careful molecular investigation of the speciation, taking place under physiological conditions, can clarify key issues around resistance of tumor cells to cisplatin.

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OC15

THE INTERACTION OF BA42, A NEW PHOSPHATASE FROM THE ANTARTICT, WITH DIVALENT METALS IONS

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In the context of the structural genomics project *White Genome,* the structure of a new protein belonging to the Antarctic flavobacterium *Bizionia argentinensis* [1,2], BA42, was recently determined [3]. This is the first structure of a member of the PF04536 family comprised of a stand-alone TPM domain. The BA42 structure reveals a new topological variant of the four β-strands constituting the central β- sheet of the αβα architecture and a double metal binding site stabilizing a pair of crossing loops, not observed in previous structures of proteins belonging to this family. The only protein in the TPM family whose function is known is AtTLP18.3 from *Arabidopsis thaliana* that was classified as a thylakoid acid phosphatase [4]. BA42 also presents phosphatase activity and this activity is regulated by the presence of divalent metals. BA42 shows differences in structure, stability and dynamics in the presence or absence of bound metals. The affinity for divalent metal ions is close to that observed in proteins that modulate their activity as a function of metal concentration, anticipating a possible role for BA42.

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OC16

RESIDUAL DIPOLAR COUPLINGS: A TOOL FOR NMR CRYSTALLOGRAPHY

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The dipolar coupling is one of the most important spin interactions in NMR as it provides a means for determining intermolecular distances, and from these, the geometry and conformation of molecules. In solid-state NMR, dipolar couplings are usually eliminated by spinning the sample around an axis oriented at the magic-angle with respect to the external magnetic field, but they can be purposely reintroduced during selected periods of the experiment using carefully designed pulse sequences.
In particular, the possibility of accessing weak homonuclear dipolar couplings, corresponding to long-range interactions, is of the utmost importance for crystal structure determination since they provide unique information about the conformational and crystal packing properties of the material. However, the measurement of weak homonuclear couplings remains difficult in the case where the sample contains clusters of many magnetic nuclei, for example carbon nuclei in uniformly labeled molecules, or proton nuclei in almost any organic material [1], because of the concomitant presence of stronger couplings dominating the spin dynamics.
Recently, a new methodology combining off-magic-angle spinning, frequency-selective pulses, and multiple quantum filtering has been introduced for estimating weak homonuclear dipolar couplings in multiple-spin coupled networks.[2-4] Here we show how ^{13}C-^{13}C and ^{1}H-^{1}H residual dipolar couplings obtained through this methodology can be used as constraints for NMR crystallography to probe the crystal packing of small powdered organic molecules. Specifically, we focus on how this method could be complementary to powder diffraction in the identification of the space group and the determination of the unit-cell parameters.

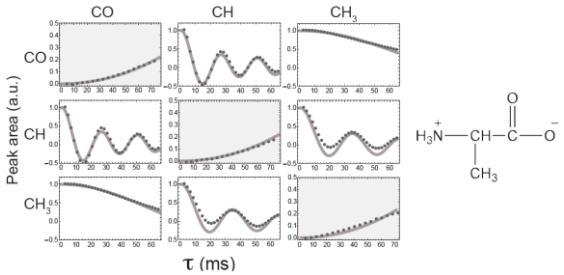

Fig. 1. ^{13}C-^{13}C dipolar couplings are measured using frequency-selective spin echoe experiments under slight off-MAS conditions

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OC17

CRYSTALLOGRAPHIC PROPERTIES OF ZIRCONIUM PHOSPHONATES BY MEANS OF ^{19}F AND ^{1}H SOLID-STATE NMR

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Zirconium phosphonates, where zirconium centres with octahedral symmetry are coordinated to a variable number of surrounding phosphonic ligands, are very versatile materials, which can be used for many different applications (proton exchange membranes, heterogeneous catalysts, fillers for polymeric materials). Their macroscopic properties are strongly related to the crystal structure on a molecular and supramolecular scale that can be easily tuned by varying the chemical composition and/or the preparation conditions. Solid-state NMR (SSNMR) has proven to be very powerful to extract crystallographic information, thanks to the sensitivity of both isotropic and anisotropic components of internal spin interactions to many different structural parameters [1].
In this work SSNMR was used to refine the crystallographic structures, obtained by Powder X-Ray Diffraction (PXRD), of different zirconium phosphonates [2]. The sensitivity of different ^{19}F anf ^{1}H nuclear properties to various crystallographic features was also explored. From ^{19}F MAS spectra recorded at high MAS frequencies (65 kHz) it was possible to detect slight differences in the ^{19}F isotropic chemical shifts ascribable to different conformations around the Zr centers in the unit cell. ^{19}F chemical shift anisotropies (CSA) were also measured by analysing the spinning sideband profiles obtained at low MAS frequencies. Advanced simmetry-based R recoupling sequences were applied to measure ^{1}H CSA and ^{1}H-^{19}F dipolar couplings under ultra-fast MAS conditions [3], through which a detailed characterization of the inter-molecular hydrogen-bond interactions, not achievable by PXRD, was possible.

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OC18

APPLICATION OF SOLID STATE NMR SPECTROSCOPY FOR INVESTIGATION THE CHEMICAL SHIFT ANISOTROPY IN DINUCLEAR PLATINUM COMPLEXES

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In the framework of our recent researches, several phosphido bridged dinuclear platinum complexes have been characterized by ^{195}Pt NMR.[1-3] Evaluating the NMR features and the crystallographic data of the complexes, a correlation between ^{195}Pt chemical shift (CS) values and the geometry of the platinum center was found.[4] In order to find a possible explanation for such a relationship, data on chemical shift anisotropy (CSA) and CS tensor magnitude were extracted by solid state NMR spectra. Due to large anisotropy exhibited by platinum in the solid state and due to the presence of others NMR active nuclei, ^{195}Pt NMR spectra (see Fig.1) were acquired by using the CP/CPMG pulse sequence developed by Schurko and coworkers.[5] In this contribution, the results of our investigations by solid state ^{195}Pt NMR measurements on several dinuclear Pt species will be presented and discussed.

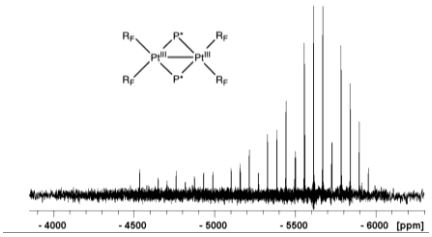

Fig. 1. Static ^{195}Pt NMR spikelet spectra of a Pt(III) diplatinum complex obtained using CP/CPMG pulse sequence.

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OC19

PREDICTING THE NMR SPECTRA OF PARAMAGNETIC IRON COMPLEXES BY DFT

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^{1}H paramagnetic shifts and line widths have been calculated with DFT methods in a variety of paramagnetic Fe (II, III, IV) complexes. A promising area of application is Fe(II) and Fe(III) complexes known to exhibit spin crossover, where the spin-state energetics must also be predicted. It is shown that each spin state leads to quite different spectral features, so that calculated NMR spectra can also be used to infer both the spin state and the associated structural information (see Fig. 1) [1]. Results are also presented for reactive Fe(IV) oxo species [2].
DFT-predicted paramagnetic shifts can assist in obtaining and understanding the NMR spectra of paramagnetic molecules, which generally require different experimental strategies and exhibit problems in detection and assignment.

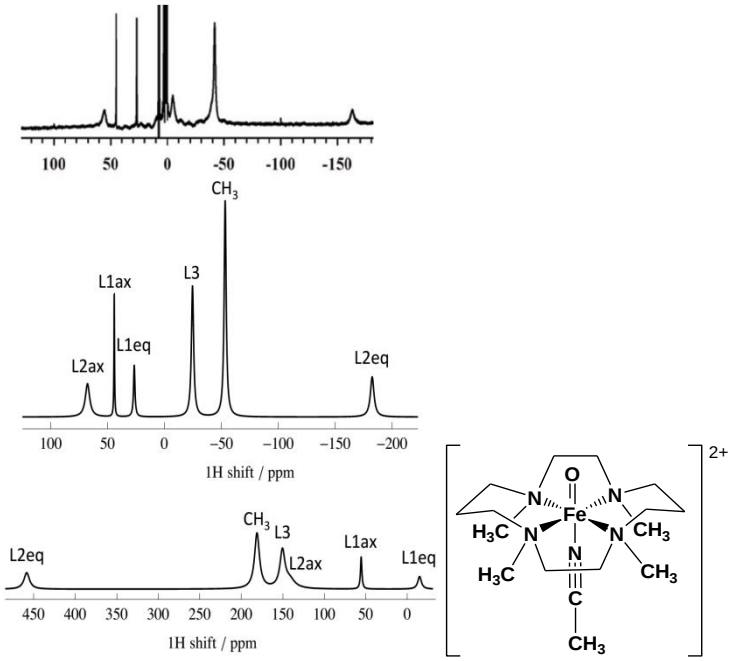

Fig. 1. Top panel: experimental ^{1}H NMR spectrum of the Fe(IV) oxo complex represented; middle panel: calculated spectrum with $S = 1$; bottom panel: calculated spectrum with $S = 2$.

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OC20

LONG-LIVED LOCALIZATION IN MRI

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The longitudinal nuclear relaxation time, T_1, sets a stringent limit on the range of information that can be obtained from MRI experiments. Hyperpolarised metabolic imaging, for example, has been used in oncology to grade tumours or to detect treatment response by following the flux between 1-^{13}C-pyruvate and lactate. However, the relatively short lifetime of spin polarisation limits these studies to fast metabolic reactions and prevents observations of extensive migrations through the metabolic network. Similarly, MRI techniques used in MR angiography fail to visualise areas with slow flow such as regions with large aneurisms.
Long-lived nuclear spin states provide a possibility to extend the timescale over which information can be encoded in magnetic resonance. By developing MRI pulse sequences that allow the spatially-selective conversion between magnetisation and long-lived spin order it is now possible to localise an ensemble of molecules for a remarkably extended duration (>50 times longer than T_1 in the case discussed). The fact that long-lived singlet spin order is insensitive to radiofrequency and gradient fields (unless highly specific pulse sequences are used) is used here to demonstrate the possibility to perform a multi-slice experiments where different portions of the same initial (thermal or hyper-) polarisation are imaged at different time intervals, each much longer than T_1. An object can be then loaded with hyperpolarised long-lived order and different portions of this order can be used to image different parts of the same object at different times. This idea of *singlet tagging* rises interesting opportunities to study very slow flow and diffusion on a macroscopic scale. Example of these applications are discussed

OC21

LIPID-BASED NANOSYSTEMS TARGETING THE ACTIVATED ENDOTHELIUM FOR THE IN VIVO VISUALIZATION BY MRI OF LOCALIZED INFIAMMATION

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Introduction

The inflammation is a complex phenomenoncharacterized by changes in the vasculature (increased blood flow and vascular permeability), activation of resident immune competent cells and infiltration of mobile cells of the immune system (neutrophils, macrophages, lymphocytes). The exposure of several endothelial epitopes allows the recruiting of these immune cells into the inflamed area.These molecules (e.g. VCAM, ICAM, Selectin) are suitable markers, because directly exposed to the blood flow, for MRI-based detection of inflamed zone. Considering that MRI is a minimally invasive technique with an excellent spatio-temporal resolution, but quite poor sensitivity, is necessary to design highly sensitive probes to ride over this weak point.Lipid-based nanosystems are excellent candidates in virtue of their biocompatibility, ability to deliver a high number of imaging units at the target site and for the easy incorporation of targeting vectors. This work was aimed at assessing the potential of lipid-based nanoparticles targeting VCAM-1 receptors as MRI agents for the visualization of activated endothelium.

Methods

The targeting cyclic nonapeptide with sequence CNNSKSHTCwere synthesized using Solid-Phase Peptide Synthesis, purified by LC-MS and characterized by conventional 1D and 2D protonNMR. The peptide was conjugated with DSPE-PEG(2000)-Amine using DSG (DisuccinimidylGlutarate) as cross-linker. The same procedure was followed for the scrambled peptide with sequence HSCNKNSCT. Micelles (diameter ca 20 nm) contained DSPE-PEG2000 (57.5% in moles), Gd(III)-DOTAMA$(C16)_2$ (40%), DSPE-PEG(2000)-peptide (2%),and Rhodamine-labeled DSPE (0.5%). Liposomes (diameter 130 nm) were formulated reducing to 5% DSPE-PEG2000 and incorporating 75% of DPPC. Targeted and non-targeted nanoparticles were then i.v. injected in C57Bl/6J mice bearing skeletal muscle inflammation and imaged by MRI at 1 T.

Results

Peptide-conjugated phospholipids were obtained with yields around 30% and purity around 70%. The vectorized micelles displayed longitudinal relaxivity (0.5 T, 25°C)of 35.0 $s^{-1}mmol_{Gd}^{-1}$, much higher than targeted liposomes (25.0 $s^{-1}mmol_{Gd}^{-1}$). Both systems displayed good stability (>2 weeks) in serum at 37°C.

Due to the higher relaxivity,the micellar aggregates were tested on a model of peripheral inflammation induced by LPS injection. MRI experiment was performed on mice (n=6) after 48h of LPS injection, corresponding to the strongest inflammation response, which was confirmed by histological analysis. A T_1 contrast enhancement of around 45% was

observed on the inflamed leg only 4h post injection. This value was ca. 4-foldhigher than the enhancement measured in the contralateral healthy leg. Moreover the same condition was maintained for the control experiments performed with nanoparticles exposing scramble peptide. In this case was observed a ca. 2-fold lower contrast enhancement respect to the targeting nanoparticles, which is almost comparable with the healthy leg.

Conclusions

Gd-loaded soft lipid-based nanoparticles vectorized with a peptide recognizing the endothelial inflammation marker VCAM-1 were prepared and characterized both *in vitro* and *in vivo*. The targeting nanoparticles, in contrast with the non-targeted ones, displayed the ability to interact with VCAM-1, giving the possibility to detect the inflammation sites *in vivo* by MRI.

OC22

NOVEL NANOCARRIERS BASED ON LIPOPHILIC MONO- and DISACCHARIDES/DPPC ENCAPSULATING Gd(III) COMPLEXES AS EFFICIENT MRI PROBES

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The great progresses achieved in the last decade in the fields of molecular imaging and nanomedicine has led the development of a large number of nanosized systems for applications in a series of biomedical areas. Lipids based nanoparticles (LNs), like micelles or liposomes, are among the most considered nanocarriers for drug delivery, based on supramolecular aggregates obtained by spontaneous assembling in aqueous solution of amphiphilic molecules consisting of a hydrophobic and a hydrophilic moiety.[1,2] The use of these LNs as magnetic resonance imaging (MRI) nanoprobes has allowed to deliver to the site of interest a large number of Gd^{III} complexes, thus increasing the sensitivity of the technique.[3]
In this work, liposomes were prepared by using phosphocoline DPPC and newly synthesized glycolipids, where the carbohydrate moiety is represented by a mono or disaccharide (glucose, maltose) and the hydrophobic part is a branched fatty acid derivative covalently linked to the sugar residue. The glycolipids allow to expose a large number of saccharide residues on LNs surface that are not recognized by the immune system and provide improved stealthiness, higher hydrophilicity and high biodegradability. These liposomes were prepared by TLE method with purification by dialysis,[4] followed by a complete characterization with PCS, Zeta Potential, DSC and stability tests. Commercial contrast agent ProHance® (Gd-HPDO3A) was encapsulated within the aqueous interior of the liposome and monitored measuring the variation of longitudinal relaxation rate ($R_1 = 1/T_1$) of water protons as a function of magnetic field strength and temperature (0.01-70 MHz, at 283, 298 and 310 K). The relaxivity values of these liposomes are greatly reduced in comparison with the free metal chelate due to the reduced membrane permeability and thus reduced water exchange.[5] Finally, we have carried out a comparison of the two glycolipids with glucose or maltose at different ratio between saccharide and the phosphocoline DPPC (100 %, 50%, 40 % and 30 % of glycolipid).

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OC23

NMR STUDIES OF INTERNUCLEAR INTERACTIONS IN MICELLAR SYSTEMS

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Triton X-100 is one of nonionic surfactants widely used in biochemical and chemical processes for a long time. Although that, the issue about the micelle size and shape is still open since Robson[1] proposed two possible arrangements, one spherical multilayered and one oblate. It is generally accepted that the non polar hydrocarbon chains are packed in the micellar core, whereas the hydrophilic polyoxyethylene chains stay outside the core and move relatively free in the solvent. Some authors have investigated the structure of the simple system TX100/water [2] to stabilize the correct structure of the micelles [2,3].
We have studied binary and different ternary systems to determine the effect of the several oils on the structure of the binary system TX100/water using DLS and NMR techniques. Self-diffusion and DLS measurements are made to obtain the dimensions of micelles and evaluate the effect of the oils in the ternary systems. Oil solubility is different if the oil have a linear o cyclic chain.
It is well known that NMR methods, such as 1D and 2D NOE, are effective tools to study the interactions intra or intermolecular between different parts of molecules that are involved in dipolar interactions among spatially near spins. We can extract valuable information about internuclear distances in the micelles from intensity of the signals. Moreover can be hypothesize a possible distribution of components into the micelle. The results show that TX-100 micelles are spherical with no-well marked hydrophilic-hydrophobic boundary. In this way we have obtained hints about the position and dynamics of the mobility of the hydrophilic and hydrophobic chains of TX-100 in the different systems.

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OC24

SINGLE-SIDED NMR TO STUDY TRANSPORT OF WATER IN AGAR GELS USED AS CLEANING TOOL IN CONSERVATION OF CULTURAL HERITAGE

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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].
In this study a cleaning method based on agar gel applied on stone was studied by single-sided NMR. Transport phenomena and penetration rate of water throughout the porous matrix were studied in a fully non-invasive and non-destructive way.
Relaxation times and self-diffusion measurements were carried out on hydrogel systems with different concentrations of agar (1-5%). Hydrogels were put in contact with the stone and the kinetics of water penetration from the hydrogel through the stone was studied. All experiments were conducted using a portable single-sided NMR operating at a radiofrequency of 16 MHz.
^{1}H depth profiles of the stone gave important information about the amount of absorbed water and its penetration depth inside the specimen after water capillary absorption. The time-dependent self-diffusion coefficient of water measured by stimulated echo pulse sequence provided clear evidence of the interaction of water molecules with the agar gel network.

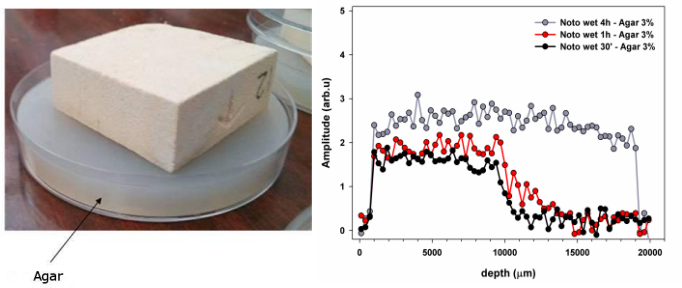

Fig. 1. Left, agar gel in contact with the stone. Right, ^{1}H depth profiles of the stone obtained after making the stone absorb water from the hydrogel for 30 minute, 1 and 4 hours respectively.

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OC25

^{1}H HR-MAS NMR INVESTIGATION ON THE MOLECULAR DEGRADATION OF ANCIENT DOCUMENTS

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The detailed knowledge of products arising from cellulose degradation is essential in understanding deterioration pathways and in improving conservation strategies and durability of cellulosic cultural heritage [1]. In this study, we use proton HR-MAS NMR spectroscopy (mono and bi-dimensional) in order to detect the products of cellulose degradation in solid paper samples [2]. Our results evidence the presence of carboxylic acids in all not aged and artificially aged model samples made of pure cellulose as well as in naturally aged samples from ancient paper. This can be accounted for the ring opening of β-D-glucopyranose, followed by oxidation. Since these products can catalyze further degradation, their knowledge is fundamental to plan conservation strategies of historical documents. Furthermore, in all ancient samples we detect some amino acids whose presence is associated to animal glue, a material that was usually used in the production of European paper in the Middle Ages as sizing compound, and could suggest for artifacts dating, authentication and provenance. Finally, by measuring the proton relaxation times as a function of the environmental humidity and samples' aging, we investigate the interaction between cellulose and water and find evidence of the cellulose supermolecular structure modification provoked by the hydration process.

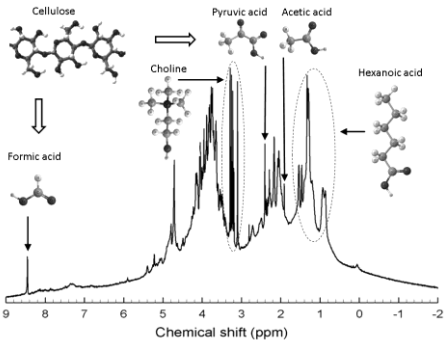

Fig. 1. Proton HR-MAS NMR spectrum of a sample produced in Milan (Italy) in 1430 together with the chemical structure of some identified compounds.

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OC26

NUS FOR EVERYONE: NON UNIFORM SAMPLING MADE EASY

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The measurement of NMR spectra commonly requires a compromise between a number of important experimental and spectral parameters, in particular sensitivity, resolution, and available measurement time. Over the years, a great deal of NMR research has been concerned with the development of techniques for circumventing NMR's inherent limitations and, in particular, reducing measurement time required for multidimensional NMR data collection. However, despite the proliferation of other methodologies for recording and processing NMR data, the Fourier Transform (FT) is still by far the most widely used technique for collecting and processing NMR data, owing to its ease-of-use and robustness. The FT needs no adjustable parameters, it is rigorously quantitative, and the results of an FT are not dependent on the details of the particular algorithm employed to perform it.

There has recently been significant attention paid to the development and application of sparse or non-uniform sampling (NUS) techniques to NMR as a way of significantly speeding up the measurement of multidimensional datasets. With NUS, only a subset of the usual linearly sampled data is recorded, according to a user-definable sampling schedule.

In this talk it is demonstrated that there is much to be gained by applying NUS techniques to small molecule 2D NMR measurements, and that the acquisition and processing framework in VnmrJ 4 provides the advantages of NUS with the same ease-of-use as for a conventional measurement.

OC27

MODULATION OF ROTOR DYNAMICS AND RECOGNITION OF HOST-GUEST INTERACTIONS IN HIGHLY POROUS MATERIALS

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Highly porous materials are attracting large attention in the recent literature for their applications in the field of gas storage, selective recognition and molecular confinement, but the dynamics of the structural elements have never been explored. Spin-lattice relaxation times ($T_1(^{13}C)$) and spin-echo ^2H NMR are the most powerful methods for describing the reorientation frequencies and the trajectories travelled by the mobile elements in solids. The discovery of ultra-fast molecular rotors (10^6 Hz at 225 K) in porous aromatic frameworks (PAF) and porous molecular crystals allowed us to look at them from a new perspective [1,2]. The unusual combination of remarkable porosity with fast dynamics enabled the reversible speed-regulation of matrix rotors by the interaction with I_2 vapors entering the galleries of the porous compounds (Fig. 1a).

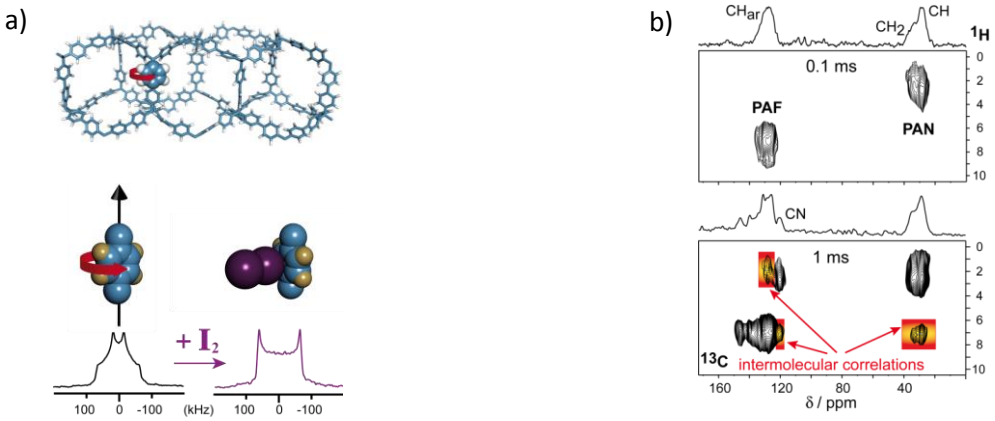

Fig. 1. a) Idealized structure of a PAF where a representative p-phenylene rotor is highlighted. ^2H spin-echo NMR spectra at 250 K of porous host and I_2-loaded host. b) 2D ^1H–^{13}C HETCOR PMLG MAS NMR of PAF-polyacrylonitrile (PAN) at contact times of 0.1 and 1 ms.

Multinuclear NMR approach is a method of choice for characterizing nanostructured materials and their interfaces. 2D PMLG HETCOR NMR experiments, designed for high-resolution both in hydrogen and heteronuclear domains, were applied successfully for identify polymer-matrix interpenetrated nanocomposites compounds realized by in-situ polymerization in PAFs of 4500 m^2/g (Fig. 1b). This resulted in the observation of host-guest intermolecular interactions across the extremely extended interfaces [3].

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OC28

SOLID-STATE CHARACTERIZATION OF COORDINATION POLYMERS FOR ELECTROLUMINESCENT APPLICATIONS.

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In this communication we report the synthesis and the characterization of a series of coordination polymers with luminescent properties and specific three-dimensional structure. These materials are interesting not only for their intriguing architectures, but also because of their potential applications, for instance as catalysts, molecular based magnets, electrical conductors, luminescence devices, and porous materials. [1] The presented coordination polymers are based on copper cyanide (CuCN) or thiocyanate (CuSCN) with a diaza-ligand (dabco) or sulphurated ligands, as thiourea (tu), methyl-thiourea (mtu), phenyl-thiourea (ptu), diphenyl-thiourea (dptu) and fluorophenyl-thiourea (fptu). The syntheses have been carried out through traditional techniques such as solvothermal/hydrothermal and crystallization methods, but solid-state "solvent-free" approaches (*mechanochemistry*) were also applied. Owing to the microcrystalline powdered nature of the compounds, the structural characterization was based mainly on solid-state NMR analysis, through ^{13}C CPMAS and NQS to evaluate the ligands coordination, ^{15}N CPMAS to investigate copper-nitrogen interactions and ^{1}H MAS to clarify the weak interactions involving hydrogen atoms between ligands and metal salts. Other solid-state techniques completed the structural characterization: RAMAN and Infrared Spectroscopies and X-Ray Powder Diffraction. Single Crystal X-Ray Diffraction was suitable for one compound (CuCN*tu). In addition, Differential Scanning Calorimetry (DSC) and Thermo-Gravimetric Analysis (TGA) conveyed the thermal characterization. Luminescence properties (emission/excitation spectra and lifetimes) were explored through solid-state UV and Fluorescence, showing outstanding results (Fig. 1) which represent a very important challenge because of the lack of data reported in literature about copper-based compounds [2,3].

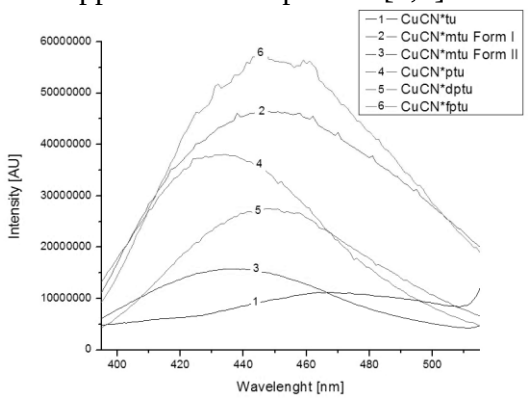

Fig. 1. Fluorescence measurements (emission) of thiourea-based compounds

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OC29

ZEOLITES FOR DECONTAMINATION OF POLLUTED WATER: A COMBINED SOLID-STATE NMR AND FTIR STUDY

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The effective removal of fuel-based pollutants from waste waters is one of the most important and expensive environmental problems that have to be faced. The use of porous materials as sorbents to remove pollutants is receiving increasing interest. Among all contaminants that can pollute ground waters, methyl *ter*-butyl ether, toluene and *n*-hexane were selected as model molecules that are representative of different hydrocarbon compound families. Zeolites with different textural and surface features were selected as adsorbents and the effect of their physicochemical properties (i.e. pore size architecture and type and amount of surface OH sites) on sorption capacity were studied. High silica mordenite (MOR) and Y zeolites (both with a SiO_2/Al_2O_3 ratio of 200) and ZSM-5 solid (SiO_2/Al_2O_3 ratio of 280) were selected as model sorbents. By combining FTIR and SSNMR (both ^{1}H MAS and ^{13}C CPMAS NMR) spectroscopies, it was possible to follow accurately the pollutant adsorption process on various zeolites characterized by a low amount of bridged silanols as well as high concentration of surface SiOH groups. The adsorption process is found to occur in different steps, and to involve isolated silanol sites, weakly interacting silanols, bridged silanols and the siloxane network of the zeolites through H-Bonding and van der Waals interactions. Furthermore, the interaction strength and sorption capacities of all three zeolites will be discussed in detail [1].

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OC30

^{19}F NMR FOR FUNCTIONAL SCREENING AND FRAGMENT BASED APPROACH

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^{19}F NMR spectroscopy is nowadays well-recognized as a powerful tool for the hit identification and hit-to-lead optimization phase of the drug discovery process. Both binding and functional screening can be used for identifying enzyme and/or protein–protein, protein–DNA, and protein–RNA inhibitors and for the accurate determination of their potency (K_D or IC_{50}). Given the success of ^{19}F NMR screening approaches, new libraries of fluorinated compounds have been generated with the aim of increasing the screening hit rate. An insight into ^{19}F libraries construction and ^{19}F NMR-based biochemical assays on recombinant purified enzymes and/or more complex biological system for the identification and validation of inhibitors of target proteins will be presented.

OC31

ASSESSMENT OF FREEZING EFFECTS AND DIAGNOSTIC POTENTIAL OF BIOBANK HEALTHY AND NEOPLASTIC BREAST TISSUES THROUGH HR-MAS NMR SPECTROSCOPY

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HR-MAS NMR spectroscopy was employed to monitor the metabolic profiles of Modena BioBank breast samples over one year of freezing at -80 °C. The study includes 22 adult female patients living in Modena and its hinterland, who underwent total mastectomy or quadrantectomy in 2011 - 2012. Variations occur, especially affecting phosphocholine and choline. This is not a trivial finding, since many studies base the distinction between neoplastic and healthy tissues or the assessment of tumor grade on the analysis of choline metabolites [1,2].
Despite the changes observed, we established that the diagnostic power of the HR-MAS NMR spectra of frozen samples is preserved, at least as far as the distinction between neoplastic and healthy samples is concerned. Lactate, phosphocholine, phosphoethanolamine, taurine, myo-inositol and glucose are biomarkers that can be used to distinguish healthy from neoplastic tissues, whereas some metabolite ratios, such as Lac+PE+Tau/Glc+Myo, seem to have even higher discrimination potential.

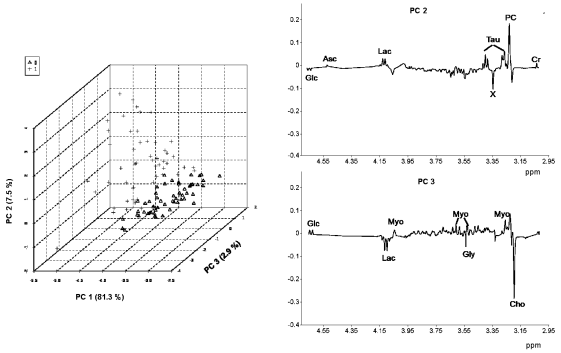

Fig. 1. Scores plot of PCA on neoplastic (crosses) *vs* healthy (triangles) samples. Loadings profiles (right) of PC2 and PC3.

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OC32

PERFUSATES AND TISSUE ANALYSIS BY NMR AND EPR SPECTROSCOPY: A POTENTIAL PREDICTIVE TOOL FOR KIDNEY GRAFT OUTCOME

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The possibility of establishing graft quality plays great importance in organ transplantation. Proper preservation needs deep analysis of all mechanisms involved in the ischemia/reperfusion process. This study deals with a combined NMR and EPR spectroscopy approach to provide a platform for dynamic assessment of donor kidney viability and metabolism on a ex vivo perfusion circuit. The use of different protocols to preserve the organs led to different outcomes. Particularly on porcine kidney model the effect of pulsatile Machine Perfusion (RM3^{TM}-MP) compared with the traditionally used static storage solutions was examined. Moreover to assess whether providing oxygen during storage/reperfusion is helpful in limiting organ damage, "Hemarin-M101", a respiratory molecule extracted from a marine invertebrate was added to the solution.
^{1}H-NMR spectra were collected at 11.4 T in 5mm NMR tube with a capillary (TSP 10mM) for absolute quantitative determination. On kidney perfusates and tissues the kinetic trend of metabolites: aminoacids (valine, alanine, glutamate, glycine), creatine, creatinine, trimetylamine-N-oxide (TMAO), glutathione and lactate was monitored.
A X-band EPR instrument was used for real-time quantification of ROS production in perfusate solution by a recently developed EPR method [1] and in biopsies at 77 K. Tissue damage was measured by Thiobarbituric Acid Reactive Substances (TBARS) and Protein Carbonyl (PC) enzymatic assays. High correlation between NMR and EPR data was found ($R^2 = 0.79$; $p < 0.05$); TBARS and PC level increase ($p < 0.01$) showed a delay. In conclusion we propose a protocol to evaluate the quality of preserved kidney to reduce the number of discarded organs and optimize patient management.

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OC33

COPPER AND FE/S CLUSTER TRAFFICKING: TWO SIDES OF THE SAME COIN

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In a living cell the maturation of copper and Fe/S proteins is a catalyzed process involving the participation of a surprisingly large number of proteins. Both copper and Fe/S clusters need indeed to reach vital destinations without inflicting damage or becoming trapped in adventitious binding sites. Fe/S clusters are first assembled on scaffold proteins and then transported, as it occurs for copper ions, where they are required at cellular level. Metallochaperones assist this trafficking function specifically releasing metal cargo upon contact with protein partners. Copper and Fe/S trafficking pathways will be described and compared at the molecular level by an integrated structural biology approach. Specifically, mitochondrial and cytoplasmic trafficking pathways involving monothiol [2Fe-2S]-binding glutaredoxins [1-3] and copper(I) chaperones, will be presented. The presented data show that monothiol glutaredoxins act as cluster transfer proteins following a specific and cluster-mediated protein-protein recognition mechanism [4], as it occurs also with copper chaperones. This mechanism guarantees a safe transfer at the cellular level of the potentially harmful copper ions and Fe/S clusters from one protein to another, up to its final target protein.

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OC34

THE N-TERMINAL REGION OF HUMAN FRATAXIN

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Friedreich ataxia (FRDA) is the most common hereditary autosomal recessive ataxia. FRDA has been linked to a deficiency of frataxin (FXN), an essential protein involved in intracellular iron homeostasis, especially iron-sulfur cluster and heme synthesis. FXN is a small evolutionarily conserved mitochondrial protein encoded in the nucleus and targeted to mitochondria, where a two-step proteolysis removes the targeting sequence to produce different shorter products. Mitochondrial processing peptidase (MPP) cleaves frataxin converting the pro-peptide into different variants, being the most abundant a 19 kDa intermediate, FXN42-210, and then into the 17 kDa shortest form, FXN81-210. Previous reports suggested that FXN42-210 is a transient processing intermediate, whereas FXN81-210 represents the mature protein. However, recent studies have shown that both FXN42-210 and FXN81-210 may be isoforms of FXN that are normally present in cultured cells and tissues and have strikingly different biochemical properties [1].
Structurally, FXN presents a conserved globular C-terminal domain (residues 90-210) that has been thoroughly characterized. In contrast, the characterization of the N-terminal region, which is poorly conserved even among closely related mammals, has remained elusive. This is due to complications from proteolysis and degradation of the protein's N-terminus that hindered the characterization beyond residues 88-210. Furthermore, the biological role of the N-terminal region of hFXN is not clear. Recently, it has been isolated a stable human frataxin intermediate spanning residues 45-210 and the nearly complete resonance assignment by NMR spectroscopy has been reported (BMRB 15736) [2]. With the aim of providing a structural insight to better understand the role of the N-terminal residues of FXN, we have undertaken an NMR study comprising the fragment FXN45-210 as a model of the human isoform FXN42-210. Here we present the results and discuss the metal binding properties of the longer isoform of FXN.

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OC35

NMR AND Sp140 PHD FINGER: STRUCTURE, PHOSPHORYLATION AND PIN1 INTERACTION

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Sp140 is a IFNγ-inducible, leukocyte-specific putative transcription factor, member of the Sp100 family. It is a recently identified protein, unknown and unexplored at both structural and functional levels [1-3]. Since 2008 increasing evidences are emerging showing its involvement in the etiology of chronic lymphocytic leukemia (CLL) [4-6]. Sp140 sequence indicates a modular structure with a "Sp100-like" domain for putative dimerization, a SAND domain, a PHD finger and a bromodomain. SAND domains bind to DNA in a sequence specific manner while PHD fingers and Bromodomains recognize histone epigenetic marks. We solved by solution NMR the Sp140 PHD finger structure [7], revealing an unexpected switch from an α/β- to an all α- fold and an unprecedented (in the PHD finger family) cis/trans isomerization of a peptidyl-prolyl bond (T726-P727). In line with its structural peculiarity, *in vitro* binding assays (fluorescence and NMR titrations, histone peptide arrays) indicated no binding to un- or modified histone tails. Prompted by the cis/trans isomerization, we asked whether Sp140 PHD finger could be a substrate for Pin1, a unique human PPIase able to catalyze cis/trans isomerization of phosphorylated S/T-P bonds in a wide range of targets. Indeed, Pin1 binds to Sp140 PHD finger, as revealed by NMR titrations, and it catalyzes isomerization of a synthetic peptide containing the phosphorylated Sp140 PHD finger T-P bond (^{1}H-^{1}H ROESY NMR experiments). Co-immunoprecipitation in HEK293T cells confirmed *in vivo* Sp140 protein as a new Pin1 target. Then by in-extract NMR experiments we showed that the PHD finger is phosphorylated on T726 upon addition of lysate from MEC1 cells (a CLL line) and that after phosphorylation the trans pT-P bond is converted into cis pT-P, likely through Pin1 catalytic activity. To identify the Thr kinase specific for Sp140 PHD finger we are performing NMR monitoring of *in vitro* phosphorylation reactions with different kinases, in-extract NMR experiments in presence of kinase inhibitors and *in vivo* co-immunoprecipitation assays. Although Sp140 function needs further investigations, our data include Sp140 in the list of Pin1 targets and suggest a Pin1-regulated modulation of Sp140 PHD finger biological role.

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OC36

SABRE HYPERPOLARIZATION AND QUANTITATIVE ANALYSIS OF COMPLEX MIXTURES: APPLICATION TO COFFEE EXTRACTS

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SABRE is a nuclear spin hyperpolarization technique based on the reversible association of a substrate molecule and parahydrogen (p-H_2) to a metal complex. A transient scalar coupling network within this complex allows the transfer of the spin-order from p-H_2 to the nuclear spin of the substrate molecules, resulting in NMR signals enhanced by one or two orders of magnitude. This sensitivity increase allowed the detection of NMR signals under 1 micromolar concentration in a single scan. [1] We have recently applied this technique to complex mixtures, showing that it is possible to quantify analytes in the low micromolar concentration regime. Applications of SABRE for the detection and quantification of several aroma components at low micromolar concentration in coffee extracts will be presented.

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OC37

INTERCULTIVAR AND INTRACULTIVAR VARIATIONS OF APULIAN EVOOS STUDIED BY ^{1}H NMR METABOLIC PROFILING

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We are currently involved in several studies aimed to assess intercultivar and intracultivar variations of apulian EVOOs by ^{1}H NMR metabolic profiling. Here we report on 93 authentic EVOO samples collected during the harvesting period 2012-2013 from different microareas of Salento area (Lecce, Italy) which were investigated by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and chemometrics (OPLS DA). Focusing the attention on the two cultivars of "Terra d'Otranto" PDO production, [1] Cellina di Nardò and Ogliarola Leccese, the potential effects of cultivar and cultivation area on samples of monovarietal extra virgin olive oils were studied. In particular, the aim was to investigate potential differences of Salento EVOOs, originating from specific microareas, related to the chemical composition of major and minor components. In this regard, the distance from the sea was taken into account to find potential influence of microclimate on the specific studied cultivars [2]. Higher polyphenols and aldehydes content was found for EVOOs coming from coastal areas, as well as for saturated fatty acid content. On the contrary, higher polyunsaturated fatty acid content was found for samples coming from inner areas of Salento peninsula.

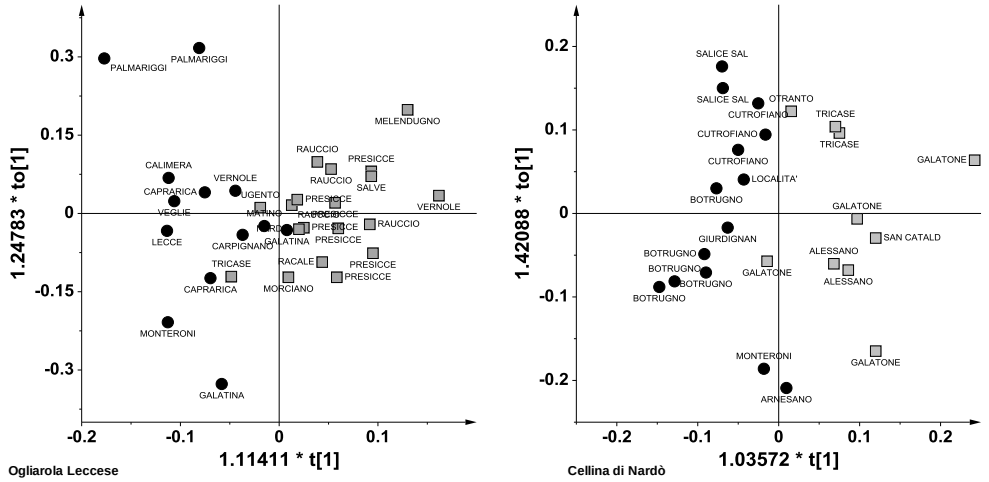

Fig. 1. OPLS-DA scoreplot for Ogliarola Leccese and Cellina di Nardò EVOOs. OPLS DA was used to investigate how the distance from the sea can affect the olive oil quality of a specific cultivar.

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OC38

METABOLIC IDENTITY AND VARIABILITY OF ENDIVE AND ESCAROLE CULTIVARS GROWN IN LAZIO AND PUGLIA

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Italy is the leader in the production of leafy crops such as endive and escarole (*Cichorium endivia* var. *latifolium* and *crispum*), which are consumed as fresh or minimally processed food. The valorisation of "made in Italy" produce was achieved by assessing cultivar identity and traceability and by enhancing the nutritive and economic value using genomic, transcriptomic and metabolomic approaches in collaboration with public and private enterprises. Within the CISIA project [1], the NMR spectroscopy was chosen to monitor the metabolic profiles of endive and escarole cultivars.
Thirty hydro-soluble metabolites (amino acids, organic acids, sugars, polyols, phenols) from leaves of curly- and smooth- leafed endives (seven cultivars) were identified and quantified at transplanting and harvesting (22 and 86 days after sowing, respectively). Four cultivars ('Domari', 'Myrna', 'Flester', 'Confiance') were monitored in the Lazio region during two production cycles (2011-2012) and two of them ('Domari' and 'Flester') further tested in Puglia for a one year cycle (2013). The multivariate statistical analysis of NMR profiles pointed at the leaf developmental stages and environmental conditions as the major factors of metabolite variability, although cultivar-specific profiles could be assessed.

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POSTERS

P1

INFLUENCE OF GEOGRAPHICAL ORIGIN ON COCOA BEAN METABOLIC PROFILE DETERMINED BY HR MAS 1H NMR

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Chocolate and cocoa-based products are among the goods with higher added value. Cocoa is produced through a multi step process involving, as key steps, cocoa beans fermentation and roasting. Cocoa beans fermentation is performed according to traditional procedures characteristic of the country of origin. Local or regional variations in cocoa plant materials, fermentation procedures and drying processes lead to a traded good typical of the country of origin. Because commercial samples usually lack information on fermentation and drying practices as well as on planting material used, a secure method for the quality control of traded cocoa beans would be desirable. Moreover, today's consumers increasingly require high-quality cocoa products, as mono-origin and premium chocolates, but few cocoa producers declare the real geographical origin and scarce studies are present in literature about the analytical methods for determining the geographical origin of cocoa products.
In this work, 61 fermented and dried cocoa beans of 23 different geographical origins coming from the three major crop-growing areas (Africa, Central/South America, Asia/Oceania) were collected and studied. Metabolic profile was determined by ^{1}H HRMAS-NMR directly on cocoa powder and after the method optimization. In fact the metabolite profile that can be obtained in ^{1}H HRMAS NMR spectra strongly depends on the cocoa amount in the disposable respect to D_2O (ratio cocoa:D_2O) and the optimization of the method is a prerequisite to obtain reliable results.
^{1}H HRMAS-NMR analysis allowed the simultaneous detection and quantification of amino acids, polyalcohols, organic acids, sugars, methylxanthines, catechins, and lipids, by assigning the major signals of the spectra. The data set obtained, representative of all classes of soluble compounds of cocoa, was very useful to characterize the metabolic profile of fermented cocoa beans. The whole HR MAS spectra were also utilized as fingerprint of the samples and elaborated with multivariate statistical methods (PCA). Results showed a discrimination of the cocoa origins. HR MAS gives the advantages to obtain a very rapid picture of the samples, comprising both lipofilic and hydrophilic components, avoiding any sample manipulation.

P2

INFECTIONS IN PEAR PLANTS: EARLY STAGE DETECTION OF *ERWINIA AMYLOVORA* AND *PSEUDOMONAS SYRINGAE PV. SYRINGAE* BY NUCLEAR MAGNETIC RESONANCE, HYPERSPECTRAL REFLECTANCE AND HIGH RESOLUTION MASS SPECTROMETRY

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Italy is the biggest producer of pear in Europe, ranking among the major producing countries of this crop in the world.
Among the major bacterial diseases that affect pear production are fire blight and bacterial canker caused by *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae*, respectively. Fire blight has been responsible for serious economical losses in many countries, especially for pear, apple, and quince species. Thus, there has been a growing concern about eradication measures and detection techniques to reduce the dissemination of this pathogen [1]. Detection of the disease requires regular inspections during the growing season, when the symptoms become visible. Appropriate inspection period depends on the host species and the geographical location. Since the symptoms of fire blight may be confused with those of other diseases and, in some cases, infection is latent, unequivocal detection of fire blight needs isolation and laboratory tests [2].
This study aims to explore the potentiality of Nuclear Magnetic Resonance (NMR) combined with High Resolution Mass Spectrometry (HRMS) and Hyperspectral Reflectance (HR) for early detection of fire blight disease on pear.
In the greenhouse, pear seedlings were inoculated by *Erwinia amylovora* (*Ea*) strains, *Pseudomonas syringae* pv. *syringae* (*Pss*) and sterilized distilled water, the latter being used as negative control. All plants were grown in sterilized growing media and regularly irrigated and fertilized to avoid any possible biotic or abiotic external stress. HR, NMR and HRMS measurements were carried out at four different stages of plant growing with intervals of about 10 days.
In this presentation, we show the results of our combined analytical approach in the evaluation of infections caused by *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae* on pear leaves. In particular, the effects on the primary and secondary metabolite composition of the leaves will be highlighted. Moreover, the results of covariance analysis allowing to transfer metabolome information obtained by NMR spectroscopy to HR bands will be reported. This "knowledge transfer" approach [3] was consolidated and validated by HRMS.

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P3

NUMERICAL SIMULATION OF 7 T RADIO FREQUENCY NECK SURFACE COILS

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Ultra-High-Field (7-9.4T) human MRI scanners have been developed with the aims to improve SNR, resolution both for the proton signal and other nuclei. This requires the development of novel Radio Frequency (RF) coils [1-5]. We report the numerical design of proton RF surface coils made with microstrip technology suitable for neck MRI applications at 7T.
The adopted neck coil design is made by microstrip elements [3] disposed along the z-axis (B_0) tuned at the relevant 7T frequency by means of chip capacitors connected at each end. Using the software HFSS14.0 a FEM numerical model of the proposed coils design was developed. The eigenvalue method was used to characterize the EM field distributions of the resonant modes. The first EM model comprised 3 microstrip copper elements (length 100 mm, width 5 mm, separation 5 mm) positioned above a segmented copper plane (length 100 mm, width 80 mm) used to maintain RF ground continuity. A dielectric Teflon slab (length 100 mm, width 80 mm, tickness 15 mm) was positioned between the microstrip and the ground plane. With the aim to study the extent of the penetration depth two additional FEM models were made by 5 microstrip elements with separation between the elements set to 5.0 or 2.5 mm. The FEM modelling of the 3 elements neck coil gives a S11 spectrum with 3 resonant frequencies (295, 413, 489 MHz). The RF B1 field of the first mode is suitable for MRI applications at 7 T. The RF B1 penetration in the direction perpendicular to the coil plane (sample x-axis) is about 23 mm. The RF B1 field homogeneity (-20% of maximum) in the direction perpendicular to the microstrip elements (sample y-axis) is about 49 mm, to be compared to the total strip width of 25 mm. The 5 elements model (separation 5.0 mm) gives a spectrum with 5 resonant frequencies (298, 389, 468, 525, 552 MHz). The RF B1 penetration in the sample x-axis is about 26 mm and the B1 homogeneity along the sample y-axis improves to 74 mm. The reduction of the microstrip separation to 2.5 mm gives a much better B1 x-axis penetration (41mm) and y-axis B1 homogeneity (95 mm).
In conclusion, we have designed a 7T RF surface coils suitable for MRI applications in the neck of human. The simulations showed that with a suitable selection of the microstrip geometry (number, separation) it is possible to optimize the RF B1 field penetration and homogeneity. Work is in progress to test a microstrip RF coil prototype with phantoms and volunteers using a 7T human MRI scanner. We expect improvements useful for clinically relevant information in imaging the human neck, in particular in case of neurodegenerative diseases [6].

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P4

PROGRESSION OF WHOLE RAT BRAIN FORMALDEHYDE FIXATION BY 2.35 T MRI

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MRI of fixed brains allows to accurately map a number of biophysical parameters and to relate these to the microscopic structure of the tissues in normal and pathological conditions [1-2]. MRI has been used to study the formaldehyde fixation of whole human brains both theoretically and experimentally [3-5]. Recently, we reported about the fixation process of whole mouse brains over 18hours using the standard perfusion methods [6].
In this MRI study we have investigated the progression of whole rat brain formaldehyde fixation by 2.35T MRI for a longer time interval. In particular, the time course of the MR signal variation in specific ROIs was quantified over 200hours from sacrifice by means of high-resolution GE images. Animal experiments were performed in compliance with the national law 116/95. Adult rats (n=2; 589±20g) were sacrificed under deep anaesthesia (chloral hydrate 400mg/kg i.p.) and perfused through the heart with saline at 4°C containing heparin (50000IU/ml). Then the brains were carefully removed from the skull and immersed in 20ml of 4% formaldehyde buffered at pH 7 before the MRI scans. Each brain was then positioned in a 8ml tube containing the perfusion solution and inserted in the 2.35T MRI Bruker Biospec equipped with a TX/RX birdcage volume coil (8rungs, diameter 65mm, length 10cm) tuned at the proton frequency of 100.3MHz. High-resolution axial GE image (TR=4500ms; TE=46ms; FOV=2.7cm^2; 512*512pixels; 53µm resolution; slice thickness=1mm; slice number=25; NEX=18; TACQ=11h30min) were used to assess anatomical details and signal variation over time from specific ROIs (left and right Cortex (CX)/Striatum (STR)). The MRI images were analysed with Paravision 4.0. The signal in the tissue ROIs was fitted with a generalised logistic function [7]. The fitting parameters for the CX and STR were, respectively, (A=1;K=1.44;Q=υ=0.6;B=4;M=-0.2) and (A=1;K=1.25;Q=υ=0.6; B=1;M=1.6). The signal of the formaldehyde solution was used as a standard to account for instrumental variation over time. The superficial layers of the rat brain (CX) showed a fast increase of the signal with a total variation of about 7 % within the first 23 hours from perfusion. The signal intensity in the STR showed an initial lag of about 20 hours followed by a 25 % increase reaching the plateau after about 200 hours from perfusion. These results can be interpreted as the differential effect of formaldehyde diffusion and fixation kinetics in the superficial and deep tissues.
In conclusion, in this MRI study we have investigated, for the first time, the progression of whole rat brain fixation over an extended time window. A maturation process characterizes the deeper perfused-fixed tissue reaching a steady value within 200 hours from perfusion, and it should be taken into account for subsequent optimization of MRI experiments and histology.

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P5

NMR INVESTIGATION OF RANDOM COIL REGIONS: CLUES TO ASSESS THE DIFFERENT BIOLOGICAL ACTIVITY OF TWO CERATO PLATANIN FAMILY MEMBERS

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Plant pathogenic fungi secrete several non-catalytic proteins involved in various aspects of the pathogenesis process. Amongst these, cerato-platanin (CP) was first identified and characterized as a PAMP (pathogen-associated molecular pattern) [1,2]. A sequence homology search revealed a set of fungal Cys-rich secreted proteins that have been grouped in the CP family. They induce synthesis of phytoalexins, overexpression of defense-related genes, H_2O_2 and NO production, markers of defense activation [3].
The core member of this family, CP (from *C. platani*) shows a double ψ–β barrel fold [2]. Here we present the results of a CP orthologue with 73% of similarity, cerato-populin (Pop1), produced by *C. populicola*. Though both CP and Pop1 are host defense inducers, Pop1 shows a slower and weaker defense induction capacity than CP [4].
The aim of the present investigation is to define the basis of the different biological activity of the two proteins at a molecular level. Pop1 ^{15}N, ^{13}C and ^{1}H resonances have been assigned [5]. The analysis of Pop1 structure obtained by homology modelling, in comparison with the CP NMR structure, will be presented. A detailed analysis of the NMR-derived protein dynamics (fast and slow regime) and NOE data indicated differences between the two proteins, mainly located in the random coil region. Interestingly, this region was proposed to have an important role in oligosaccharides binding and in necrosis induction of leaves' cells [6]; therefore, the different pattern of residues' interactions might be the leading cause of their diverse biological activity. To address this hypothesis we have performed NMR experiments in the course of a titration of both Pop1 and CP with oligosaccharides.
The high dependence on chemical pesticides in Europe poses large risks to both the environmental and human health; thus, reducing the use of those chemicals in crop production is one of the major objectives in sustainable agriculture. We expect that our results, besides providing new hints on the molecular mechanisms operating in plants induced resistance, will contribute to reach the major goal of environment protection. Besides that, from a basic science perspective, this work illustrates a time saving approach for the investigation of a protein structure-function relationship when the high-resolution structure of an orthologous protein is already available.

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P6

DYNAMICS AND POLYMORPHISM OF ANHYDROUS Na-IBUPROFEN BY SOLID STATE NMR SPECTROSCOPY AND RELAXOMETRY

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Sodium Ibuprofen, a well-known anti-inflammatory drug, is stable at room temperature in a di-hydrated crystalline phase, which has been widely studied and characterized [1,2].
The di-hydrated form undergoes dehydration when heated above 80°C or exposed to P_2O_5 or to N_2 atmosphere, giving rise to an anhydrous form named form 1. Form 1 has been observed before [3] and recently its structural properties have been investigated by X-Ray Powder Diffraction (XRPD), Differential Scanning Calorimetry (DSC) and Solid State NMR (SS NMR) [4].
In this work form 1 has been studied by SS NMR techniques in the temperature range from 20 to 80°C. By variable temperature ^{13}C CP-MAS spectra, we have found that form 1 is not stable upon heating and it transforms into a new phase, named form 2, which on the contrary results to be stable after further cooling and heating steps.
The aim of the study was the characterization of the dynamic processes occurring in the two anhydrous forms. The dynamics was first investigated by Fast Field Cycling (FFC) ^1H NMR relaxation measurements for both form 1 and form 2 at room temperature, and interesting differences have been observed in the T_1 dispersion curves obtained. Moreover, the dynamics of form 2 has been studied by variable temperature SS NMR experiments, in particular, in addition to ^{13}C CP-MAS spectra, we performed ^{13}C and ^1H T_1 and ^1H $T_{1\rho}$ measurements from 20 to 80°C.
All the relaxation data have been simultaneously analysed with suitable mathematical models in order to determine motional parameters, such as activation energies and correlation times.
The results have been also compared with those previously obtained by our group for the di-hydrated form.

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P7

COMBINATION OF NMR SPECTROSCOPY AND HIGH RESOLUTION MASS SPECTROMETRY TO STUDY THE EFFECTS OF ETHEPHON (ETH) AS ABSCISING AGENT FOR TABLE GRAPES *CV. CRIMSON SEEDLESS*

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Table grapes has considerable commercial value worldwide, USA, Brazil, Italy, South Africa, China, Chile, India and Australia being the most important producers. Italy is one of the greatest world producers and exporters of table grapes to be consumed as fresh products.In recent years, interest in the marketing of "fresh cut" table grapes stimulated the use of fruit abscising (loosening) agents to enhancing berry detachment from the stem. In fact, abscising agent applications decrease the fruit detachment force (FDF) required to separate berries from stem and allow automated harvesting of undamaged individual grape berries. Abscission agents have been used in different fruit crops to decrease FDF with positive results [1]. In particular, Ethephon (2-chloroethylphosphonic acid, ETH), an ethylene-releasing agent originally used as plant-growth regulator, has been extensively evaluated also as a potential fruit loosening agents for fruits such as citrus (Citrus sinensis L.), grapes (Vitis vinifera L.), olives (Olea europaea L.) and cherry (Prunus avium L.).
In the framework of our studies on metabolomics of table grapes [2-5], the use of Ethephon (ETH) as abscising agent in the cultivation of table grapes cv. Crimson Seedless has been investigated. In this presentation, we show the results obtained following a combined metabolomic approach based on NMR and high-resolution mass spectrometry (HRMS) and a traditional approach measuring drop and fruit detachment force (FDF) of the berries. The NMR-HRMS covariance analysis allowed to correlate the quantity of tartaric acid (revealed by NMR) with that of secondary metabolites influenced by treatment with ETH (revealed through HRMS).

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P8

INFLUENCE OF Ce^{3+} CONCENTRATION ON DOPED Si-BASED ORGANIC/INORGANIC SOL-GEL LAYERS FOR CORROSION PROTECTION: A SOLID STATE NMR STUDY

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The unique properties achievable by hybrid organic/inorganic (O/I) materials make them attractive for a wide variety of applications including resistance to scratching, abrasion, heat stability, as well as other mechanical properties [1]. Moreover, the protection against corrosion conferred to the metallic substrate by hybrid silsesquioxane layers is noteworthy [2,3]. In this field, hybrid O/I films doped with Ce^{3+} ions have been successfully used [4] as anticorrosion coating and resistant interlayers between metal surfaces and paints. However, key critical points as cerium ions distribution and structural effects on the silsesquioxane matrix are still matter of debate.
The acquisition of NMR spectra of hybrid materials doped with paramagnetic ions is often difficult or even impossible. [5,6] However, we observed that Ce^{3+} effects are weaker than those of other lanthanides, allowing the study by ^{29}Si, ^{13}C, ^{1}H and relaxation solid state NMR of the [Ce^{3+}] effect on the structural features of the silsesquioxane networks, prepared from methyl-triethoxysilane and glycidoxypropyl-trimethoxysilane. The samples were prepared by sol-gel chemistry in aqueous medium under acidic catalysis, adding different amounts of $Ce(NO_3)_3$ in order to obtain a doping with Ce/Si mol% ranging from 0.004 up to 4. Moreover, a Ce-rich sample was also prepared (Ce/Si mol% = 20). The homogeneous sols were deposited onto teflon substrates and the obtained thick layers were detached after drying at 160°C for 30 min. Our NMR results show that siloxane condensation degree, water coordination and relaxation phenomena are concentration dependent parameters. The increase of Ce^{3+} up to 4 mol % leads to the reduction of adsorbed water, probably hindering the formation of hydrogen bonds. The T^2/T^3 ratio, calculated from ^{29}Si SP spectra, increases with Ce^{3+} loads up to 0.2% mol and then remains constant up to 4%. The 20% Ce-doped sample shows a very different behavior. The comparison between the hybrid matrix without cerium doping and that sample shows clearly an increase in the degree of condensation of the silsesquioxane network.

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P9

POLYMER DYNAMICS IN LDPE/LAYERED SILICATE NANO-COMPOSITES BY FFC ^{1}H NMR

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Polymer/clay mineral nano-composites represent a class of materials of great interest for many practical applications. In particular, low density polyethylene (LDPE) based nanocomposites are widely used for packaging due to their gas barrier properties, flame retardancy, high stiffness and toughness [1]. The aim of this study was to investigate the effects of doping with montmorillonite on the dynamics of LDPE, this microscopic level property being strongly correlated to the macroscopic properties at the basis of nano-composite applications. To this end, Fast Field Cycling (FFC) ^{1}H NMR relaxometry was applied on LDPE and LDPE/layered silicate nano-composite samples [2] at three different temperatures between the glass transition temperature and the melting point. FFC NMR relaxometry is indeed one of the most useful methods for the investigation of polymer segmental and collective chain dynamics [3,4]. To obtain dynamic information, the ^{1}H FFC NMR dispersion profiles, that is the frequency dependence of spin lattice relaxation rates $1/T_1(\omega)$, were transformed in susceptibility curves ($\omega/T_1(\omega)$ vs ω) and analysed in terms of the Cole-Davidson model for segmental motions. The curves obtained at different temperatures were combined in master curves assuming the frequency-temperature superposition (FTS) and were used to estimate the contribution of collective dynamics to relaxation.

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P10

SOLID STATE NMR INVESTIGATION OF POLYMER-MODIFIED CALCIUM SILICATE HYDRATE (C-S-H)

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Calcium silicate hydrate (C-S-H) is the main constituent of fully hydrated cement paste and is responsible of the mechanical properties of ordinary Portland cement [1]. Various organic polymers are added to cement formulations in order to improve the final performance. The effects of different comb-shaped superplasticizers (Fig. 1a) on the microstructure of C-S-H have been investigated by Solid State NMR methods. ^{29}Si MAS NMR spectra of polymer-modified C–S–H systems (Fig. 1b) show that the hydration and hardening are affected by the presence of the organic molecules [2]. These effects depend on the molecular architecture and the concentration of the additives in the batch. The spin-lattice relaxation times (T_1) of protons have been measured via saturation recovery and analyzed through a purposely developed model in order to obtain information on the mixing degree of the different additives with the inorganic matrix and the amount of organic/inorganic interface

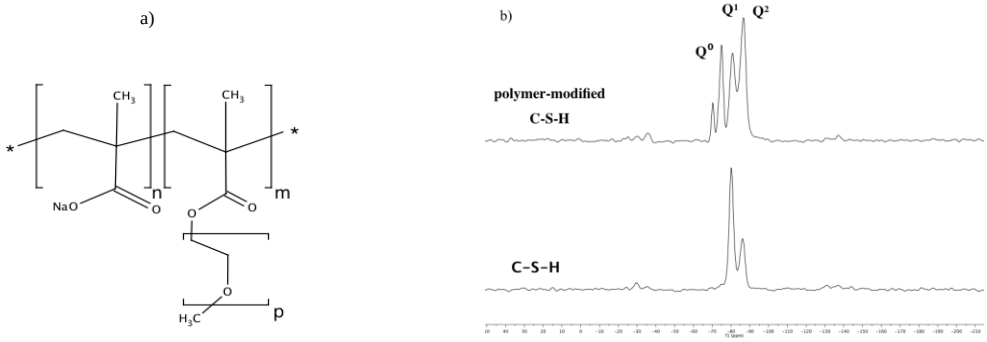

Fig. 1. a) Sketch of the superplasticizers molecular formula. b) ^{29}Si MAS NMR spectra of C-S-H (bottom) and a polymer-modified C-S-H.

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P11

POLYMORPHISM AND DYNAMICS OF NEOHEXANOLS STUDIED BY FAST FIELD CYCLING AND SOLID-STATE NMR

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The study of the dynamic and thermodynamic properties of *glass-forming* compounds may be of relevance for understanding the mechanism of glass transition. *Plastic crystals*, in which the average position of the centers of mass are ordered on a lattice while the orientations are dynamically disordered, often present many properties characteristic of the conventional molecular glass formers. Therefore, in addition to orientationally disordered crystals (ODIC) they may show up conventional isotropic glass phases. Their study is an excellent way to highlight the role of the orientational degrees of freedom in glass transitions.
For plastic crystals glass-formers, the investigation of the relationship between chemical structure and dynamic properties may greatly benefit from thorough comparative studies on compounds belonging to the same family. In this context, neohexanol (2,2-dimethyl-1-butanol, $CH_3CH_2C(CH_3)_2CH_2OH$) and some of its isomers constitute a very interesting class of compounds, since they have nearly globular shapes, and give rise to both plastic crystalline phases and glasses. In particular, these compounds show a rich polymorphism with several solid-solid phase transitions and, in some cases, ODIC phases due to the ease of rotational motions of the moelcules in the solid state [1].
Fast field cycling (FFC) NMR relaxometry is very important for obtaining information on dynamic properties, allowing motions over a wide frequency range to be investigated. In particular, FFC NMR relaxometry has been successfully applied in the study of the glass transition process in viscous liquids and polymers [2]. On the other hand, solid-state NMR is a very powerful technique for the study of dynamics, providing, through a combination of different experiments, complementary information with respect to FFC NMR.
In this work ^{1}H FFC NMR relaxometry and solid-state ^{1}H and ^{13}C NMR experiments have been applied to neohexanol and three of its isomers (3,3-dimethyl-2-butanol, 2,3-dimethyl-2-butanol and 3,3-dimethyl-1-butanol) in the temperature range from -50 to 30°C. In particular, ^{1}H spin lattice relaxation times (T_1) have been measured at Larmor frequencies from 10 kHz to 35 MHz with FFC techniques and at 400 MHz with Saturation-Recovery experiments. Moreover, static ^{13}C spectra have been recorded under ^{1}H high-power decoupling. The behaviors of the different isomers have been analyzed and compared giving information on the dynamic processes occurring in the different solid phases.

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P12

ANALYSIS OF MEMBRANE TYPE 1 MATRIX METALLOPROTEINASE (MT1-MMP) BEHAVIOR AT THE CELL SURFACE

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Collagenolysis is a critical biological process and the elucidation of its molecular details has been a great challenge for structural biologists for more than two decades.
Membrane type 1 matrix metalloproteinase (MT-MMP1) is a membrane-anchored collagenase that mainly confines its activity at the cellular surface, therefor its mechanism of action need to be characterized in the presence of membrane-mimicking environments.
Here the interaction of the extracellular isolated domains of MT1-MMP and of the extracellular full-length protein with different membrane-like environments (micelles, bicelles and liposomes) has been investigated through solution NMR. The simultaneous interaction of the hemopexin-like (HPX) domain and of the extracellular full-length protein with bicelles and a collagen-like triple helical peptide (THP) has been also explored and the binding regions highlighted. The comparison of the interaction mode of MT1-MMP with THP with respect to that of the soluble collagenase MMP-1 [1] provides clues about the different mechanisms of collagenolysis occurring for these two enzymes.

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P13

SOLID-STATE NUCLEAR MAGNETIC RESONANCE FOR THE CHARACTERIZATION OF HALOGEN BONDING IN CO-CRYSTALS

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We report the use of Solid-state NMR to detect the structural features of halogen-bonded co-crystals in powder form. In particular, we investigated the correlations (Fig. 1) between ^{13}C or ^{15}N chemical shifts and structural features (C—X, X···N or X···X⁻ distances) probing the well-documented I···N and I···Br interactions [1, 2]. Shifts of the ^{13}C—Br and ^{13}C—I resonances are a clear indication of the presence and strength of the halogen bond, while ^{15}N data (Fig. 2) provide complementary source of information. Moreover, ^{13}C nuclei directly bonded to quadrupolar nuclei (such as $^{79/81}Br$ and ^{127}I) show bandshapes with characteristic multiplets arising from second-order quadrupolar effects *via* residual dipolar interactions: these effects complicate the SSNMR spectra, but are in principle a new source of information on the halogen bond [3].
In order to unambiguously correlate the spectral data with the halogen bonding environment, we tried different experimental setups to observe the resonance for the carbon directly involved in the interaction. First of all we performed NQS pulse sequence, with several dephasing delays, in 1H/13C{1H} CPMAS experiments. However the best signal to noise ratio was achieved through 19F/13C{19F} CPMAS experiments.
The latter has made possible to improve the CP transfer efficiency, enhancing the resonance peaks we were interested in.

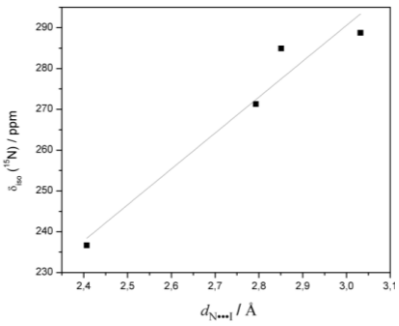

Fig. 1: Plot of experimental values of δ_{iso} (^{15}N) as a function of the corresponding n···i distance, for nitrogen atoms involved in halogen bonding.

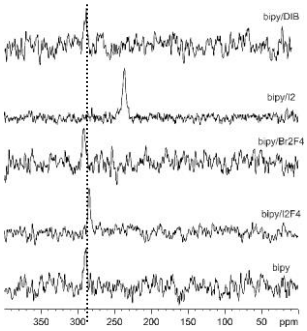

Fig. 2: $^{1}H/^{15}N\{^{1}H\}$ CPMAS spectra acquired at ambient temperature at B_0 = 9.4T.

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P14

QUANTIFICATION OF FATTY ACIDS COMPOSITION IN SICILIAN EXTRA VIRGIN OLIVE OILS BY MEANS OF ^{1}H HR-MAS NMR SPECTROSCOPY

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We study the fat composition of more than twenty monovarietal extra Virgin Olive Oils (VOOs) produced in Sicily by means of High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR) spectroscopy. In particular, we consider two different but almost equivalent NMR methodologies (based on peak integration) [1,2] and compared the obtained results with the traditional analytical technique of Gas Chromatography (GC). The obtained percentage of principal fatty acids confirms that ^{1}H NMR spectroscopy offers new opportunities for assessing virgin olive oil quality and genuineness. The NMR technique is a rapid (few minutes of signal acquisition), non-destructive (no need of sample treatment) and reliable methodology to be used in an official method in conjunction with other traditional analytical techniques such as GC, as demonstrated here [3].
The fatty acid composition in extra Virgin Olive Oils (VOOs) is the major factor influencing their chemical and physical properties and consequently their overall quality in terms of organolepetic and nutritional factors. In particular, health effects have been attributed to certain fatty acids such as oleic and linolenic acids [4]. Indeed a balanced proportion of fatty acyl chains in the diet is of primary importance for human health. The composition of fatty acids in extra VOOs changes from cultivar to cultivar. Our aim is to characterize the individual composition allowing the identification of the geographical origin of each cultivar. In doing so it is possible to determine the extra VOO authenticity and to prevent the adulteration of high-value extra VOOs [5].
Finally, we want to stress that the consequence of insisting on NMR spectroscopy for extra VOOs characterization leads to the reduction of chemical consumption and waste production, which is important from both the economic and environmental points of view. All these characteristics also make the methodology very attractive for industry.

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P15

SYNTHESIS AND MULTINUCLEAR NMR CHARACTERIZATION OF PLATINUM(II) AND PALLADIUM(II) COMPLEXES OF NATURAL ANTICANCER SUBSTANCES

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Flavonoids constitute a group of natural antioxidant substances which have been studied intensively, due in part to their therapeutical properties, such as antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral and anticancer [1]. A well-known flavonoid is quercetin (Fig. 1), found in abundance in onions, apples, broccoli, and berries. Herein we report the synthesis and the NMR characterization of new Pt(II) and Pd(II) quercetin based complexes (Fig. 1), with the aim to evaluate their anticancer activity.

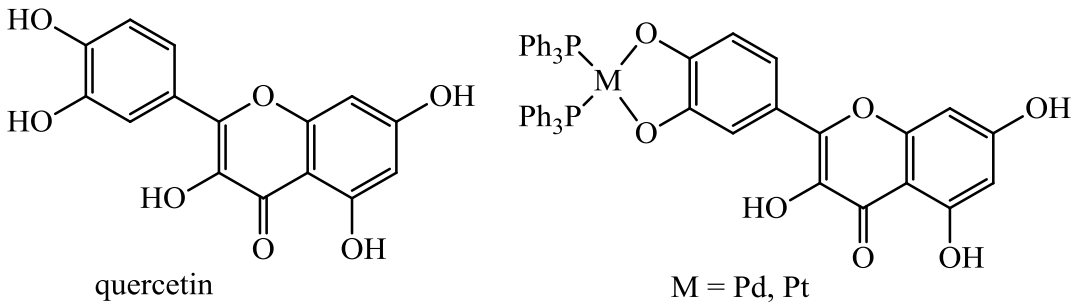

Fig. 1. Quercetin and some new Pt(II) and Pd(II) quercetin complexes.

In this presentation, we also report on the synthesis and the NMR characterization of new Pt(II) and Pd(II) complexes containing curcumin (Fig. 2), a naturally occurring pigment in Turmeric with many biological and pharmacological properties [2].

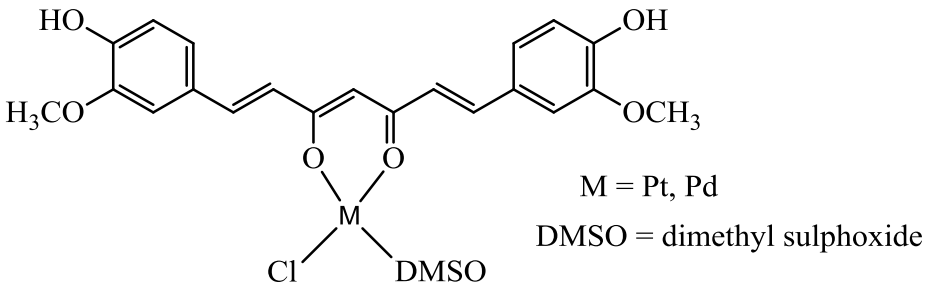

Fig. 2. Some new Pt(II) and Pd(II) curcumin complexes

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P16

THE METABOLIC EFFECT OF PHYSICAL EXERCISE

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The physical exercise induces changes in metabolic profile of human bio-fluids [1-6]. In this study we want to investigate the metabolic changes induced by physical exercise in three different bio-fluids: saliva, urine, serum.
For this preliminary study we collected saliva, urine, and serum samples of six professional soccer players before and after a physical exercise of Yo-Yo intermitted recovery test. The bio-fluids were analyzed by NMR spectroscopy. The ^{1}H NMR spectra provided the input data for targeted and untargeted analysis. For the targeted analysis we assigned 50 metabolites in serum spectra, 40 metabolites in urine spectra, and 30 metabolites in salive spectra. For the untargeted analysis ^{1}H NMR spectra were analyzed by principal component analysis (PCA).
The major metabolic changes were observed in serum samples with variation of concentration of lactate, alanine, glucose, pyruvate, Krebs cycle intermediates like citrate, succinate, fumarate.
Serum PCA analysis shows that is possible to cluster pre- serum samples from post.

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P17

MULTINUCLEAR NMR RELAXOMETRIC STUDY OF PICOLINATE CONTAINING MACROCYCLIC Mn^{II} COMPLEXES

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Stable high-spin Mn^{2+} complexes represent an attractive alternative to the classical Gd^{3+}-based contrast agents (CAs) for application in Magnetic Resonance Imaging (MRI) [1]. In fact, Mn^{2+} is an essential element present in the human body, and thus humans have developed efficient mechanisms to manage an excess of this ion in organs and tissues. On the contrary, Gd^{3+} is extremely toxic, and the release of the metal ion after the administration of certain Gd^{3+}-based CAs to patients suffering from severe renal failure has been shown to trigger a potentially fatal disease called Nephrogenic Systemic Fibrosis [2]. The main drawback of Mn^{2+}-based CAs is their lower effective magnetic moments, which often results in lower relaxivities (r_1). Our strategy to improve the relaxivity of Mn^{2+}-based contrast agents is twofold: a) to increase the number of water molecules coordinated to the metal ion; b) decrease the tumbling rate of the complexes. To this aim, we have investigated the potential of the Mn^{2+} complex of $dpama^{2-}$ as a CA (Fig 1). We have performed a complete ^1H (T_1) and ^{17}O (T_2 and shift) NMR relaxometric study as a function of field strength, temperature and pH. The relaxivity measured for [Mn(dpama)] at 20 MHz and 298 K (pH = 7.3) amounts to 5.32 $mM^{-1}\cdot s^{-1}$, a value that is *ca.* 60% higher than those measured under the same conditions for small Mn^{2+} complexes containing one coordinated water molecule (i. e. r_{1p} = 3.3 mM^{-1} s^{-1} for $[Mn(edta)]^{2-}$) [3]. We have then prepared the corresponding di- and trinuclear complexes, which show a further improvement of the relaxivity, and measured their affinity to HSA.

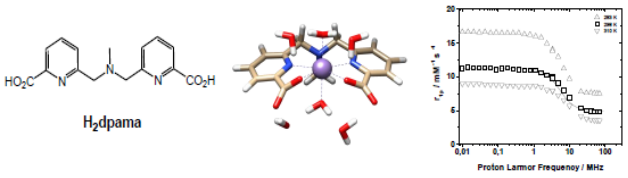

Fig. 1. The ligand H_2dpama (left), the optimized geometry (middle) and the NMRD profiles (right) of the complex $[Mn(dpama)(H_2O)_2]\cdot 4H_2O$.

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P18

IS THE DNA NICK SEQUENCE THE ONLY DETERMINANT FOR THE CAMPTOTHECIN TOPOISOMERASE IB INHIBITORS SPECIFICITY?

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Human topoisomerase I is a ubiquitous enzyme belonging to the class of DNA topoisomerases that has been recognized as a valuable target for the development of effective antitumor agents. Camptothecin (CPT) derivatives are topoisomerase IB (TopIB) selective inhibitors that are currently used in cancer therapy. CPT intercalates between the base pairs in position -1 and +1 with respect to the cleavage site, slowing down the DNA relaxation and religation. TopIB does not have a consensus sequence for DNA binding and cleavage, but in the presence of CPT the cleavage pattern of TopIB shows a preference for a guanine in position+1 and the requirement of a thymine in position -1 [1]. The structural basis for this CTP selectivity remains controversial. Some studies suggest that apolar nonbonding interaction between CPT and DNA bases at the cleavage site plays a role in the stabilization of the complex [2]. On the other hand, the analysis of available crystallographic structures of TopIB ternary complexes as well as bioinformatics calculations have shown that the stabilization of the inhibitors is also related to the formation of a hydrogen bond network between the protein, the DNA and the drug [3]. This indicates that the role of the protein must be taken into account to fully understand the mechanism of CPT sequence specificity.
In this work, we have studied using NMR spectroscopy the interaction in solution between topotecan, a water soluble derivative of camptothecin, and two dumbbell oligonucleotides with a difference sequence at the cleavage site, TG or CG.
We detected a selective interaction of the topotecan with the DNA at the cleavage site for both DNAs. However, in the excess of the drug, topotecan interacts rather unspecifically also with other sites of the DNA sequences. Furthermore, our results show a structural difference at the level of the nick, where the switch of the nucleotide in position -1 is reflected on the conformational stability of the residue at position +1. Still, this dissimilarity is not translated in a differential affinity for the drug of both DNA sequences, suggesting that the presence of the enzyme is required for the CPT sequence specificity.

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P19

DOES GLYCOSYLATION MODULATE ANTI-ALZHEIMER'S ACTIVITY OF FLAVONOIDS?

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Flavonoids are phytochemicals present in almost all terrestrial plants and, as a consequence, in plant-based foods, and thus assumed by humans through the diet. Recent evidences suggest that several flavonoids have positive effects against dementia and Alzheimer's disease, reversing age-related declines in neurocognitive performances [1,2,3,4].
Flavonoid glycosylation occurs very frequently in Nature and influences their solubility, chemical stability, bioavailability and pharmacokinetic. By combining NMR ligand-receptor interaction studies and TEM analysis we are investigating the effect of flavonoid glycosylation on their ability to interact and inhibit Aβ peptide aggregation.
This information will be essential for the design and development of new anti-Alzheimer's drugs.

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P20

NMR METABOLOMIC STUDIES ON S. CEREVISIAE: PROTOCOL OPTIMIZATION FOR EXTRACTION AND ANALYSIS OF INTRACELLULAR METABOLITES

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Saccharomyces cerevisiae is one of the most intensively studied eukaryotic model organisms in molecular and cell biology. Thanks to its relative simplicity and easy handling, it has been widely used to elucidate fundamental aspects of cellular processes such as signalling, cell cycle and metabolism, and it is now used in pioneering studies on several human diseases.
NMR spectroscopy represents a rapid, non-destructive, high throughput method for the analysis of metabolites, which requires minimal sample preparation. Nevertheless, when the analysis of yeast intracellular metabolites has to be performed, a very carefully set up of the protocol for the extraction of intracellular metabolites is necessary. As a matter of fact, yeast cells present a cell wall whose composition and resistance to disruption can be modulated by several factors including the growth conditions, the growth phase and specific mutations. In this communication we present our results concerning the optimization of intracellular metabolite extraction from *S. cerevisiae* cells and their metabolic profiling by NMR spectroscopy [1].

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[1] Manuscript in preparation

Acknowledgements

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P21

UNTARGETED METABOLOMIC INVESTIGATION OF *TRYPANOSOMA BRUCEI*

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Human African trypanosomiasis (HAT) or sleeping sickness is a parasitic infection in sub-Saharan Africa transmitted by tsetse flies. Its causative agent is the flagellated protozoan *Trypanosoma brucei*. Current treatments for HAT are inadequate because of high toxicity and complex administration regimens, so discovering new drugs and understanding their mode of action is of great importance.
A non-targeted metabolomics-based approach by measuring how the levels of small molecules comprising the metabolome of the parasite are perturbed when exposed to drugs, will be presented. This approach typically employs high-resolution mass spectrometry to provide a fingerprint of biological samples [1-4].
Unlike previous studies such untargeted analysis as well as the identification of the HAT metabolites was performed by ^{1}H and bidimensional HSQC NMR spectroscopic methods.

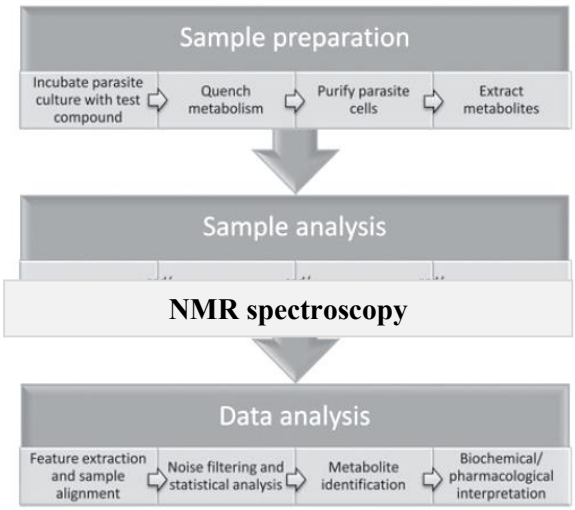

Fig. 1. General outline of methodology for metabolomics studies of HAT parasite in cell culture.

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P22

METHODS FOR OBTAINING NUCLEAR RELAXATION PARAMETERS IN SOLID SAMPLES: A COMPARISON BETWEEN CONTINUUM AND DISCRETE APPROACHES

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Determination of NMR parameters from relaxation data is often carried out with numerical instable approaches, like multiexponential fitting or classical least squares methods. In some cases a more effective approach to obtain these parameters might be the numerical inversion of real-value Laplace equation [1]. This is known to be an example of ill-posed problem and needs a treatment known as regularization. The aim of this work is studying the application of a continuum method for inverting solid state relaxation data in order to obtain NMR parameters like distributions of relaxation times (T_1, T_2, $T_{1\rho}$) and population weighted rate averages (PWRA) [2]. The continuum and discrete approaches have been comparatively applied to several sets of both synthetic and experimental data, representative of most situations encountered in a wide range of solid materials, with the aim of finding, case by case, the best data analysis strategy.

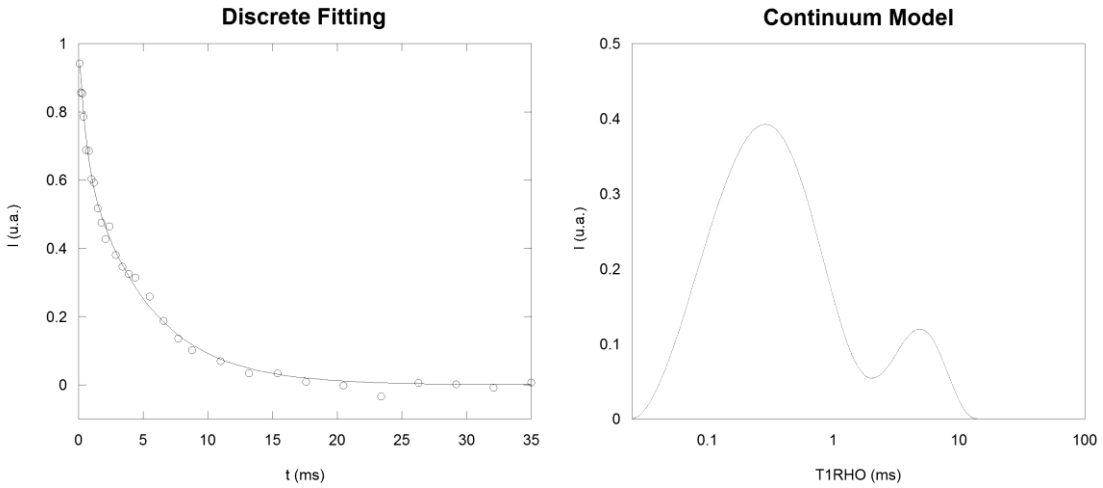

Fig. 1. Representation of discrete and continuum approaches on the same $T_{1\rho}$ decay data set.

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P23

METABOLIC DIFFERENCES BETWEEN THE PGI INTERDONATO LEMON OF MESSINA AND INTERDONATO LEMON OF TURKEY REVEALED BY ^{1}H HR-MAS NMR

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We have studied the metabolic profile of the Sicilian lemon known as "PGI (Protected Geographical Indication) Interdonato Lemon of Messina" by means of High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR). There is a growing interest to develop analytical techniques able to reveal the origin of a protected foodstuff [1-3]. We have performed a quantitative determination of the main metabolites constituting the lemon juice of two citrus lemon hybrids: the PGI Interdonato lemon of Messina and the Interdonato lemon of Turkey.
As a title of example figure 1 shows the comparison between two typical proton HR-MAS NMR spectra of the PGI Interdonato lemon of Messina and of Interdonato lemon of Turkey in the chemical shift region of amino acids.
Essentially, the two hybrids of lemon have the same content of citric acid, isocitric acid, vitamin C, glutamic acid, glutamine, scyllo-inositol, serine, stachydrine, sucrose, gallic acid and trigonelline. For what concerns the major metabolite differences, the PGI Interdonato lemon of Messina is richer in asparagine, fructose, -glucose, malic acid and myo-inositol with respect to the Interdonato Turkish lemon which is richer in fatty acids, GABA, arginine, choline, isoleucine, leucine, lactic acid, methanol, proline, tryptophan and valine.

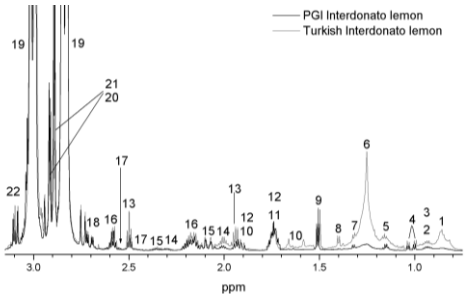

Fig. 1. 1:Satured Fatty Acids; 2:Isoleucine; 3:Leucine; 4:Valine; 5:Ethanol; 6:Unsatered Fatty acids; 7:Threonine; 8:Lactic acid; 9:Alanine; 10:Arginine; 11:DSS; 12:Lysine; 13:GABA; 14:Proline; 15:Glutamic acid; 16:Glutamine; 17:Isocitric acid; 18:Aspartic compound; 19: Citric acid; 20:Malic acid; 21:Asparagine; 22:Stachydrine.

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P24

PRELIMINARY CHARACTERIZATION OF UNIFLORAL COFFEA SPP. HONEY

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It is well known that coffee is one of the most consumed and appreciated beverage in the world. However, in addition to provide coffee beans to be roasted for our pleasure, coffee plant has large floral display with strong scent and produces more fruits and seeds when pollinated by bees. In view of these aspects it is not surprising to encounter coffee honey as a side-product, albeit rare and almost unexploited, of coffee production. In the framework of a project of Ernesto Illy Foundation in Colombia, a sample of coffee honey has been preliminary characterized by NMR and liquid chromatography. Two different *Citrus* spp. honeys have also been studied, for comparison. According to a determined *Coffea* pollen frequency higher than 60% the coffee honey may be considered unifloral. Purine and pyridine alkaloids have been identified and quantified. Caffeine and theobromine are particularly abundant if compared with the content of these methylxanthines in unifloral *Citrus* spp honey. The caffeine content is about 10-20 times higher than that of lemon or orange honeys. In view of its lower solubility, theobromine determination by NMR is affected by solvent selection, in all cases, however, its content is remarkably higher than that reported for honey from *Citrus* spp. [1]. As far as pyridine alkaloids is concerned, trigonelline has been detected. This compound, well know constituent of coffee beans, has been recently identified for the first time in several European honeys [2]. The content of trigonelline in coffee honey is about 2.5 times higher than that determined in both lemon and orange honeys. Caffeine has been recently proposed as a fingerprint marker for the description of citrus honey [3]. On the basis of the present data, caffeine and theobromine may be suggested as reliable markers to trace coffee honey authenticity, however further studies on a wider number of samples are necessary to confirm this hypothesis.

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P25

QUANTIFICATION OF CAFFEINE IN HUMAN SALIVA BY NUCLEAR MAGNETIC RESONANCE AS AN ALTERNATIVE METHOD FOR CYP1A2 PHENOTYPING

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The first step in caffeine metabolism is mediated for over 95% by the CYP1A2 isoform of cytochrome P450. Therefore, CYP1A2 activity is most conveniently measured through the determination of caffeine clearance [1,2]. The HPLC quantification of caffeine is fully validated and is the most widely used method. It can be performed on saliva, which is gaining importance as a diagnostic bio-fluid and permits easy and low invasive sampling [3].
Here, we present a quantitative ^{1}H nuclear magnetic resonance (NMR) method to analyze caffeine in human saliva. The procedure is simpler than the HPLC analysis because it involves an ultra-filtration step and a direct extraction in deuterated solvent, yielding a matrix that is then analyzed. This NMR method was demonstrated to be reliable in terms of linearity, accuracy, recovery, and limits of detection (LOD). Good precision (RSD%), recovery and LOD were obtained compared to those reported for HPLC methods [4,5,6,7]. The method was applied to samples collected from different volunteers over 24 h following a single oral dose of about 100 mg of caffeine administered with coffee beverage and capsule.

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P26

STRUCTURAL BASIS OF LIGAND BINDING AND SELECTION IN TRYPANOSOMAL REDOX-ACTIVE GLUTAREDOXIN

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Glutaredoxins (Grx) are small proteins belonging to the thioredoxin-fold family with functions as thiol/disulfide oxidoreductases in glutathione (GSH)-dependent reaction. Some Grx are able to coordinate iron-sulfur (FeS)-clusters, forming holoprotein with essential roles in FeS-cluster biogenesis and trafficking. This requires the use of GSH for redox and non-redox processes, despite the fact that in both cases the thiol is bound to the protein using the same conserved residues. The redox metabolism of pathogenic trypanosomes substantially differs from others because this organism uses trypanothione ($T(SH)_2$, bis-glutathionylspermidine) instead of GSH [1]. This affects also Grx-dependent reactions. Trypanosomes possess two dithiolic (class I, CXXC active site) and three monothiolic (class II, CXXS) Grx. 2-C-Grx1 is class I redox-active, FeS cluster protein that use both GSH and $T(SH)_2$ as substrates [2]. In this work, we use NMR to investigate the non-covalent binding of ligands to this protein with the aim to address structural basis of: i) substrate discrimination in trypanosomal Grx and ii) redox *vs* non-redox use of thiols in Grx mechanism. For this, we solve the 3D structure of the protein by NMR (Stefani *et al.*, this conference) and use chemical shift perturbation [3] to study the interaction of 2-C-Grx1 with GSH, $T(SH)_2$ and GSH analogues under reducing and non-reducing conditions. The peaks undergoing significant shifts in the in the HSQC spectra are the same for the two thiols but different broadening of the signals has been observed during the titration for the two thiols suggesting different kinetics in the recognition. Several unusual features observed during the titrations, such us deviation from linearity, unusual changes in line shape and splitting of the peaks clearly indicate that the observed process involves at least one intermediate state in addition to the free and bound states. This result has been complemented with CD and fluorescence spectroscopy titration on wild-type and mutant versions of the protein and compared to our previous data obtained for a class II trypanosomal Grx [4,5]. Our results suggest that thiol binding to trypanosomal Grx is adapted to the use of $T(SH)_2$ for dithiolic reactions.

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P27

REORIENTATIONAL MOTIONS IN PLA FILMS: A SOLID-STATE NMR STUDY

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The investigation of the dynamics in solid polymeric materials has been the subject of numerous studies because of the possibility of correlating it with their physical properties. To this aim, solid-state NMR is one of the most powerful techniques, since it can give information on both local and global dynamic processes thanks to the possibility of choosing among different nuclei and NMR nuclear properties, which are sensitive to molecular motions over a wide range of characteristic frequencies (Hz-GHz) [1,2,3].
In this work, variable temperature SSNMR experiments were used to investigate the dynamic properties in the kHz regime of poly(lactic acid) (PLA) films around their glass transition temperature ($T_g \simeq 40$ °C). A characteristic line broadening in the ^{13}C spectra, due to the interference between the ^{1}H decoupling field and a reorientational motion with a characteristic frequency in the kHz regime, was observed around 70-85 °C. Around the same temperature a minimum of ^{1}H $T_{1\rho}$ relaxation time was also found. The simultaneous fitting of the experimental curves of ^{13}C line widths and ^{1}H $T_{1\rho}$ as functions of temperature to a suitable dynamic model allowed a detailed characterization of PLA main chain segmental reorientation to be performed.

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P28

NMR STUDIES OF AN EPHA2 SAM DOMAIN MUTANT INVOLVED IN THE PATHOGENESIS OF CATARACT

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Ephs form the major identified subfamily of receptor tyrosine kinases (RTKs) and are involved in several biological activities, including cell migration and proliferation [1]. Ephs intracellular portion includes a Sam (Sterile alpha motif) domain, which is a small protein binding module made up of roughly 70-80 residues arranged to form a five helix bundle [2]. Among Eph receptors, EphA2 has been widely investigated and its crucial role in cancer extensively characterized [1]. Heterotypic Sam-Sam interactions involving EphA2-Sam are important to regulate receptor endocytosis along with its consequent degradation and may influence pro-oncogenic activities [3,4].
However, recently a few studies have also demonstrated a correlation in between EphA2-Sam and the pathogenesis of cataract. In major detail, several mutations affecting EphA2-Sam have been described and associated to cataract formation in humans [5]. These mutations influence protein stability through changes of solubility and folding efficiency, which may cause cellular disorganization and lens opacity [5].
We generated one of these EphA2-Sam mutants as a recombinant protein in *E.coli*. Moreover, we adopted heteronuclear NMR techniques to evaluate how the mutation could affect EphA2-Sam structural features and its ability to interact with Sam-domain containing binding partners. Our preliminary studies pave the way for a better understanding of molecular mechanisms affecting EphA2 receptor network during the cataract pathogenesis.

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P29

STRUCTURAL STUDIES ON C-TERMINAL REGION OF KIAA0323: A NEDD8 BINDING PROTEIN.

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The Kazusa cDNA sequencing project aims at characterizing unidentified human genes that encode large proteins (>50 kDa). These genes are named as "KIAA" plus a four-digit number [1]. Thus far, the project has identified several proteins but their functions remain largely elusive. Lately, biochemical studies focused on KIAA0323 demonstrated that an "atypical" CUE domain in this protein shows a preference for NEDD8 binding [2]. The region spanning residues 597-678, corresponding to the very C-terminal end of the amino acid sequence, seems to be primarily involved in such interaction. In this study, we report the structural studies designed to determine the tri-dimensional structure of this region. The C-terminal end of KIAA0323 was obtained by expression in *E. Coli* cells fused to the GST protein in order to facilitate its purification. The production of protein fully marked with ^{15}N was also successfully achieved. The protein purity was checked by several chromatographic and electrophoretic techniques. The NMR and CD spectroscopy were used in order to determine the structure of this region. 2D NMR experiments such as ^{1}H-^{1}H TOCSY, ^{1}H-^{15}N HSQC along with 3D NMR experiments such TOSCY-HSQC were acquired for a better and precise peak assignment. After the assignment, the structure was generated by the collection of the detected NOEs, limited for the structured region of the protein and the solution structure was determined by restrained molecular dynamics.

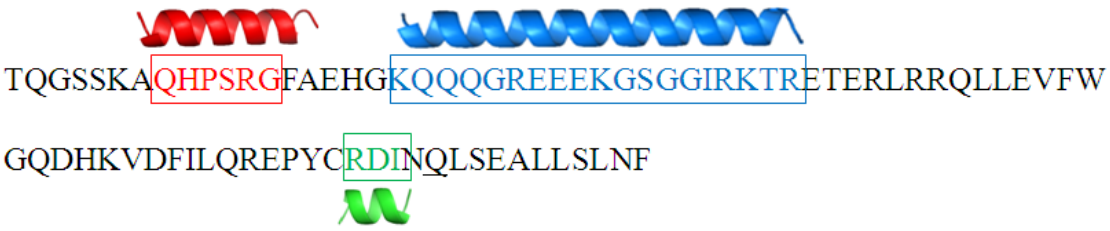

Fig. 1. KIAA0323, structured regions assigned for the very C-terminal end of the protein.

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P30

THYMOSINα1 INSERTS IN MODEL MEMBRANES BY THE N TERMINAL REGION

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The solution structure of Thymosin α1 has been determined by NMR spectroscopy. After the complete assignment of the resonances and the collection of the detected NOEs the solution structure was determined by restrained molecular dynamics. Results indicated that Thymosin α1 is completely unstructured in water solution and assumes a helicoidal conformation in presence of model membranes both when added directly in the preparation of the solution and, when added to a solution containing already formed SDS micelles. This result was found to be very similar to that obtained in phospholipid vesicles by Circular Dichroism spectroscopy, indicating that Thymosin α1 assumes a secondary structure. The solution structure by NMR indicates that Thymosin α1 shows a 3_{10} helix secondary structure with a disordered structural break around residues 9 and 14. Thymosin α1 is deeply inserted into the hydrophobic region of the micelles by the residues 1-5 of its acetylated N-terminal end. These results suggest that Thymosin α1 which has a strong propensity to insert into the membrane, behaves as a self-structuring peptide on the membrane and, then, may be able to interact with nearby proteins and/or receptors, acting as effector(s) and triggering a biological signaling cascade.

P31

SPECTROSCOPIC CHARACTERIZATION OF THE ANIONIC SURFACTANTS INFLUENCE ON THE β AMYLOID "–KLVFF–" FRAGMENT

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Neurodegenerative diseases, which include Alzheimer's (AD), are highly prevalent. These diseases are characterized by disorders in protein folding causing specific protein rearrangement leading to the self-aggregation which finally promote the deposition of insoluble protein aggregates known as amyloid plaques [1,2]. Alzheimer's disease (AD) is the main cause of dementia in the elderly. Although the causes of AD are not yet known, age is considered to be the main risk factor triggering the disease [3]. According to the amyloid hypothesis the amyloid plaques are mainly composed of β-amyloid peptide (Aβ). Many amyloidogenic proteins contain simple repetitions of 6-8 amino acids involved in protein misfolding and aggregation [4]. The KLVFFAE sequence represents the amyloidogenic fragment of the Aβ peptide which has been extensively studied as it shows a behavior similar to the β-peptide from which it derives [5]. Phospholipid bilayers are known to affect the aggregation phenomena of amyloidogenic fragments [6]. Anionic surfactancts like Sodium Dodecyl Sulfate (SDS) are usually employed to mimic cell membrane during NMR experiments to study amyloidogenic peptide and moreover Aβ-membrane interaction [7, 8]. RGKLVFFGRNH$_2$ sequence, known as OR2 peptide, is a short synthetic peptide based on the -KLVFF- amyloidogenic fragment sequence with added RG-/-GR residues at the N- and C-terminal ends to aid solubility. OR2 has been studied, at physiological pH, in absence and in presence of different concentration of SDS. Using different techniques we demonstrate that the residue-KLVFF- strongly influence OR2 behavior. Particularly the peptide adopts different conformations in presence of SDS under and above critical micelle concentration (cmc). SDS concentration under cmc leads and accelerates OR2 oligomerization. A similar behavior has been observed for Aβ$_{1\text{-}40}$ [7, 8].

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P32

ARTIFICIAL CELLULAR SYSTEMS: APPLICATIONS TO SYNTHETIC BIOLOGY AND TO BIOMOLECULAR NMR SPECTROSCOPY

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Synthetic biology approaches usually develop cellular design using "bottom-up" strategies, inserting and deleting genes from existing organisms. Instead, "Top-down" approaches could allow to control living cells in a manner that does not require direct genetic modification [1]. We have exploited artificial cellular mimics that send specific chemical messages to natural cells. Since the chemical message is only sent in the presence of a specific, orthogonal molecule that the artificial cell but not the natural cell can detect, the described system expands the senses of natural cells without genetic intervention. Besides this, the synthetic biology approach of using artificial cells inspired further application aimed at understanding protein chemistry in cytomimetic systems. "Bottom-up" approaches based on simplified cell mimics could provide important insights into protein chemistry in native-like environments. Water-in-oil emulsions (simple to prepare) were exploited as in-vehicle systems for NMR spectroscopy investigations. This mimic cells allow to exclude interference from extra-vesicular macromolecules (a protein would normally not be dissolved in the oil phase). In addition to building a biomembrane-like boundary to generate a confined aqueous milieu, we also investigated the possibility to mimic intracellular, non-bounded compartments. Aqueous two-phase systems, ATPS, are good imitations of compartmentalized cytoplasm. When two or more incompatible polymers are mixed at appropriate concentrations in aqueous solution, phase separation occurs resulting in two aqueous phases, or microcompartments [2]. Other particular feature of biological living systems is that their intracellular living conditions is deeply crowded with raised concentrations of macromolecules (50-400mg/mL) which affect several protein attributes (i.e. ligand binding, protein-protein interaction, folding, etc.) [3,4]. These crowded conditions are being also studied by NMR spectroscopy. We mimic the crowding conditions including synthetic crowding agents such a PEG, Ficoll, BSA or Lysozyme. Model proteins chosen for these approaches are [^{15}N]-Ubiquitin for in-vehicle and compartmentalization studies, and [^{15}N]-labelled human liver fatty acid binding protein [4] for experiments in crowded environments.

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P33

METABOLOMIC ANALYSIS OF RED BLOOD CELLS DURING STORAGE IN BLOOD BANK CONDITIONS

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Blood transfusion is a fundamental therapy in many pathological conditions. Regrettably, many clinical evidences describe several adverse effects of transfusion, due to red blood cell (RBC) alteration during storage. Thus, the possibility for a blood bank to evaluate the quality of RBC concentrates units appears crucial to improve clinical results and reduce transfusion adverse effects.
We believe that changes of RBCs metabolism is one of the primary cause. Aim of this work is a) to study the metabolic profile of RBCs during storage b) to verify if leukoreduction before storage modifies RBCs metabolic profile. We use ^{1}H-NMR spectroscopy as it requires little or no sample treatment and it allows identification of more than 40 metabolites in a single measurement.
Whole blood collected from 16 donors was prepared as prestorage-leukoreduced or non-leukoreduced RBC concentrates. Aliquots of the two concentrates were analyzed weekly (up to 42 days). Each of them was centrifuged to separate RBCs from the conservation medium and then analyzed separately: 224 samples were assayed. We could monitor the consumption of additives present in the conservation medium. In addition, up to 30 metabolites, excreted by RBCs, were identified and quantified. Hypoxanthine, a known uremic toxin and substrate for oxidative stress, and 5-oxoproline, a side product of the glutathione cycle, were found to accumulate inside and outside the cells. We propose this last molecule as a biomarker of RBCs protection level against oxidative stress [1]. Chemometric analysis revealed differences in metabolites concentrations between leukoreduced and nonleukoreduced RBCs. In particular, leukoreduction lowers haemolysis and hypoxanthine accumulation during storage. In conclusion, our data prove that a detailed knowledge of RBCs metabolic changes during storage may open the way to develop more effective protocols for blood conservation and patient oriented transfusion therapy.

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P34

EX VIVO HR-MAS NMR, IN VIVO MRS-MRI AND MULTIVARIATE ANALYSIS TO HIGHLIGHT BIOMARKERS IN GLIOMAS

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Gliomas account for about 40% of total primitive brain tumors, and discrimination between high and low glioma grade remains a vital diagnostic decision, determining the most effective treatment and having an important impact on patient management and outcome. The *ex vivo* HR-MAS NMR spectra provide more details about metabolites than *in vivo* MRS and permits to produce a metabolic picture of the tissues. Accurate biochemical assignment of metabolites will improve our interpretation of HR-MAS data and the translation of NMR tumor biomarkers to *in vivo* studies. We developed this project on gliomas with the aim to gain a better insight into the discrimination among different grades and subtypes using *ex vivo* HR-MAS NMR, *in vivo* MRS, MRI, clinical data, chemometrics and statistical analysis. We performed experiments on 20 specimen from different grade glioma. After a chemometric analysis some small metabolites such as alanine, lactate, myo-inositol and glycine seems to be able to discriminate between high and low grade glioma. The same chemometric analysis was performed on *in vivo* MR spectrum. A number of metabolites have been identified as potential biomarkers of tumor type; now we need to combine all the *in vivo*, *ex vivo*, histological and clinical data to obtain a unique tumor fingerprints. Results gathered from this study should lead to the development of tools that can facilitate the distinction of tumor types and grade that cannot be readily distinguished by histopathology or by routine neuroimaging.

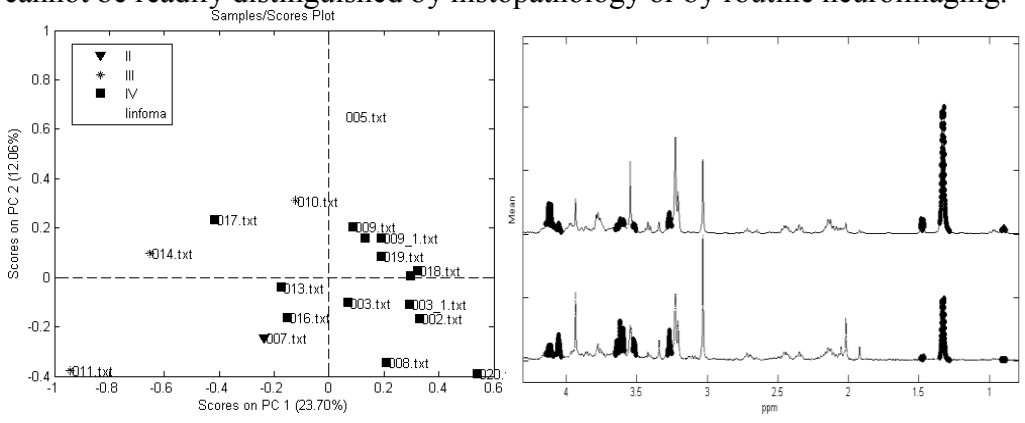

Fig. 1. Scores plot of PCA different glioma grade samples. Average spectra from grade IV and grade III glioma and the selected discriminant metabolites

P35

NMR ASSIGNMENT AND INTERACTION STUDIES ON TRYPAREDOXIN, A VALIDATED DRUG TARGET AGAINST TRYPANOSOMATID PARASITES

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Trypanosomatids are parasitic protozoa responsible of severe tropical diseases affecting millions of people in (sub)tropical regions of the world. Available drugs suffer from limited efficiency, unaffordable costs and high toxicity. Metabolic pathways that are unique to, and of vital importance for these parasites promise a chance to create efficient and safe therapeutics by rational inhibitor design. Trypanosomatids have evolved a unique thiol metabolism based on trypanothione [$T(SH)_2$], a bis-glutathionyl conjugate of spermidine [1]. The absence of the trypanothione system in mammals, the lack of a functional redundancy within the parasite thiol system together with the sensitivity of trypanosomes against oxidative stress, render the components of this redox system attractive drug target molecules. Tryparedoxin (Tpx), a distant member of the thioredoxin protein family, plays a central role in most of T(SH)2-dependent parasite pathway [2]. It has been shown to be essential for *T.brucei*, thus fulfilling a crucial prerequisite for a drug target molecule and it is sufficiently distinct from homologous mammalian proteins.
Fragment Based Drug Discovery (FBDD) represents the approach of choice to identify new scaffolds. It has several advantages over conventional high-throughput screening (HTS). In particular, NMR spectroscopy is ideally suited for fragment based screening because it can reliably detect low affinity binding, is less prone to yield false positives and false negatives and it can be used to quantify binding affinities[3].
Recently few compounds were found that inactivate Tpx in vitro and in the intact parasite demonstrating that Tpx is a druggable target [4] but the HTS approach conducted on this protein proved to be rather unsatisfactory and NMR-based FBDD methods could be a valid alternative to discover new scaffolds.
With the aim to set up an NMR based screening of ligands, we have achieved the resonance assignment for *T. brucei* Tpx. The dihedral angles predicted from backbone chemical shifts were in good agreement with the available X-ray structure of the protein[5]. Finally, this information has been used to study by SOFAST-HMQC the interaction of Tpx with compounds that have been previously shown to inhibit the peroxidase system in trypanosomes[4].

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P36

NEW NMR METHODS ALLOWING THE DETECTION AND QUANTIFICATION OF ANALYTES: SENSING AND CHROMATOGRAPHY

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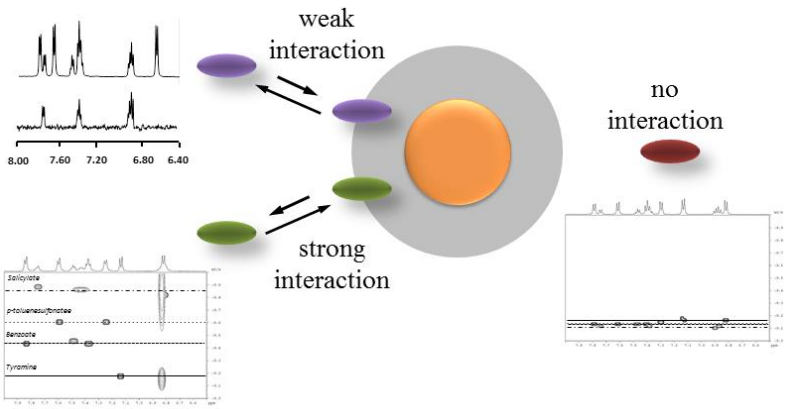
Chemosensing is a well-developed method to detect a target analyte in a sample. In such systems, the chemosensor selectively binds a substance and consequently undergoes a change in its properties (fluorescence emission, absorbance, redox potential…). Unfortunately, this method is prone to give false positives when interfering species are present in the sample. To overcome this problem we have developed new methods based on a combination of NMR and monolayer-protected nanoparticles (NPs) as receptors [1].
Different functional groups can be introduced in the NPs monolayer, resulting in the formation of self- and pre-organized binding sites that give rise to hydrophobic, ion paring and ion-dipole interactions with target analytes.
When the interactions are strong, a variation of the analytes' diffusion coefficient is observed. However, differences between binding and nonbinding species are generally not observed for weak interactions. In this case, we show that the "NOE pumping" experiment [2] can be employed as a viable alternative.



Various monolayer-protected nanoparticles were tested in combination with different analytes such as organic anions (carboxylates, sulfonates, boronates) and cations (ammonium salts). The selectivity and the sensitivity of the method are presented for significant systems.

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P37

CHARACTERIZATION AND VALORIZATION OF TANGERINE HONEY FROM CALABRIA

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Citrus honey is one of the most important and valuable honey production in Calabria. Not only nationally, but also worldwide, it is one of the most popular honeys because of the intensity and delicacy of the aroma and thus it is one of the few Italian honeys which is exported. This production, however, suffers the competition from other similar products coming from other areas of the world where citrus cultivation is widespread, especially Spain, but also Mexico, Israel, the United States, and Brazil. Both mixed citrus honeys and monofloral honeys composed either of orange or tangerine are labelled with the generic name "Citrus". The specific name "Tangerine honey" often hides a counterfeit product, since it does not appear distinguishable from the generic "Citrus honey" or the more common "Orange honey" at the level of official control. Recently, with the aim of enhancing the special features of Tangerine, a research identified some parameters to distinguish Tangerine citrus honey from the generic honey: Tangerine citrus honey has a higher percentage of pollen Citrus and a value of methyl anthranilate between 0.70 and 1.30 mg/kg^{-1}.

The purpose of this work is to apply a recent, extremely reliable method [1] for the identification of botanical origin, to citrus honeys with the aim of determining the special features of Tangerine. The method is based on Nuclear Magnetic Resonance combined with multivariate statistical analysis [2] and aims to identify markers that can strengthen the distinction between orange and tangerine.

Fifty-one samples were collected in tangerine production areas located in the province of Cosenza. The samples were compared with 31 citrus honeys from all over Italy where generally the prevailing colture is orange. The NMR analysis of these products, combined with multivariate statistical analysis, allowed the distinction of honeys through some signals arising from molecules present in both varieties in different amounts or to specific marker compounds of tangerine honey.

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P38

MODEL COMPOUNDS FOR HYDROTHERMAL LIQUEFACTION: A DETAILED CHARACTERIZATION OF THE SOLID RESIDUES BY ^{13}C CP-MAS NMR

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The increasing demand for energy as well as related environmental concerns, and the predicted shortage of fossil fuels, are strongly pushing for search of new sources of crude liquid (*bio-oil*), as suitable alternatives to petroleum. In this field, the hydrothermal liquefaction (*HTL*) of biomasses (mainly composed of carbohydrates, lipids and proteins) has got particular attention, as they are a completely renewable resource. Together with the bio-oil, HTL process also produces a carbon rich solid residue [1] with interesting properties. These carbon materials have found a large number of applications in different domains ranging from environmental science to energy storage.

Based on the observation of the complex composition of the bio-oil [2] and solid residue, we carried out a thorough study on the HTL treatment of model compounds. In particular we used carbohydrates (glucose, cyclodextrin, cellulose, and 5-HMF) and protein (glycine, BSA) model compounds as well as their binary mixtures (cellulose/BSA).

Aqueous solutions of model compounds were treated at different times under HTL conditions and the filtration of the reaction mixture allowed to recover of the solid residue. A detailed investigation of HTL transformation mechanism for solid residue production has been carried out by recording ^{13}C CP-MAS NMR spectra. Variable contact times CP MAS spectra and cross-polarization polarization inversion (CPPI) spectra were recorded in order to better differentiate the numerous components underlying the broad ^{13}C NMR signals of the solid residues. Carbohydrates solid residues at different reaction times show similar ^{13}C CP-MAS NMR spectra, with a strong aromatic component due to polyfuranic chains domains together with graphitic-like domains. Raising the reaction time, the percentage of aromatic carbons increases while the components assigned to the aliphatic carbons decrease.

Cellulose/BSA mixture solid residues still show spectra with an aromatic motif predominance, but, in this case, just a negligible amount of polyfuranic chains domains is detected and, conversely, condensed nitrogen containing structures appears.

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P39

STRUCTURE OF THE DITHIOLIC GLUTAREDOXIN 2-C-GRX1 FROM THE PATHOGEN *TRYPANOSOMA BRUCEI BRUCEI*

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Trypanosomatids are protozoan pathogen of the order Kinetoplastida which causes severe diseases in (sub)tropical regions of the world. Among then, *Trypanosoma brucei* and related species are responsible for african trypanosomiasis, also known as sleeping sickness in humans and "Nagana" in animals. The parasite thiol metabolism is based on trypanothione [bis(glutathionyl)spermidine] and the flavoenzyme trypanothione reductase [1] rather than on the corresponding glutathione and glutathione reductase that are used by the large majority of the organisms.
Glutaredoxins (Grx) are small ubiquitous proteins that can catalyze glutathione-dependent redox reactions (class I Grx) or can be involved in the biogenesis of iron sulfur cluster (class II Grx). The genome of trypanosomes encodes for three class II Grx with monothiol active site (1-C-Grx1, 1-C-Grx2 and 1-C-Grx3) and two classical dithiol Grx (2-C-Grx1 and2-C- Grx2) [2]. Recently we have solved the structure of 1-C-Grx1 highlighting some peculiarities of this parasitic protein [3,4].
T. brucei 2-C-Grx1 present the classical CPYC active site and it is able to coordinate an iron-sulfur cluster [5] despite it was previously proposed that the presence of the proline in position 2 prevents the formation of the cluster.
Here, we present the solution structure of the apo form of 2-C-Grx1 and we compare it with the monothiolic 1-C-Grx1 from the same organism and with orthologous human proteins.

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P40

NMR OF TRANSIENT IRON COORDINATION SITES IN PROTEINS

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We will present an overview of solution and solid state NMR approaches suitable to monitor transient iron cofactor binding sites and trafficking routes in bacterial and fungal heme acquisition systems and in the eukaryotic iron-storage ferritin [1,2].

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P41

APPLICATIONS OF METABOLOMICS IN BIOMEDICINE: PROSPECTIVE LONGITUDINAL STUDY OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Metabolomics has been introduced for molecular fingerprinting of biosamples in a wide variety of clinical disorders, including the Chronic Obstructive Pulmonary Disease (COPD) [1,2]. COPD is generally treated with inhaled bronchodilators and corticosteroids, but only 10% of patients show an effective reduction of the inflammatory response and symptoms [3].
The aim of this study was to investigate, through proton nuclear magnetic resonance (^{1}H NMR)-based metabolomics combined with multivariate statistical analysis and spirometry, whether the suspension and subsequent re-introduction of inhaled corticosteroid therapy induced changes in the health status and in the metabolic profile of COPD patients. The sample examined in this study includes 14 subjects aged between 64 and 83 years old, all with spirometric diagnosis of stable COPD. The study includes 4 visits in which every time was varied the pharmacological therapy. At each visit have been performed study of the lung function and collected samples of urine, serum and exhaled breath condensate (EBC).
The multivariate statistical analysis of the acquired data allowed to show that the variation of the inhaled drug therapy doesn't altered clearly the metabolic profile of the patients, or at least that the intra-individual variations related to therapy are much less relevant than those interindividual. Hypothesis partially supported by the fact that pairwise comparisons between visits show some statistically significant discrimination ($p<0.05$) on the data relating to serum and exhaled breath condensate. In particular, the absolute best discrimination is found on serum samples between visits 2 and 4, visits that reflect the same drug therapy but are separated by the period of suspension of corticosteroid, and the analysis of metabolites has highlighted changes statistically significant ($p<0.05$) between the levels of four metabolites: a decrease of serine's level and an increase levels of VLDL, isoleucine and leucine.

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AUTHOR INDEX

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