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Gruppo Italiano Discussione Risonanze Magnetiche

XLVI NATIONAL CONGRESS ON MAGNETIC RESONANCE

Salerno 27-29 September 2017

Dipartimenti di Farmacia, Chimica e Fisica

Università degli Studi di Salerno

BOOK OF ABSTRACTS

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FISCIANO (SA) 27-29 SEPTEMBER 2017

SCIENTIFIC PROGRAM

Wednesday September 27th

10:00-13:00	**Registration**
10:30-12:30	**Bruker satellite meeting (registration starts at 10:00)**
12:45-14:00	**Bruker Lunch**
14:00-14:30	**Opening**
14:30-15:30	**Chair: M. Geppi** **GIDRM/GIRM gold medal award** **A. Mele** - MOLECULAR INTERACTIONS AND DYNAMICS AT A GLANCE: NMR AND BEYOND
15:30-16:15	**Plenary Lecture 1** **H. Molinari** – NMR STUDIES ON AMYLOID β PEPTIDES
16:15-17:20	**Coffee break + Poster session**

	Parallel session A **Chair: D. Lurie**	**Parallel session B** **Chair: M. Edén**
17:20-17:50	**S. Geninatti Crich** – RELAXOMETRY OF CANCER: EFFECT OF WATER MOBILITY AND MAGNETIC FIELD STRENGTH ON TISSUE AND CELL WATER PROTON T_1	**P. Thureau** - DNP-ENHANCED SOLID-STATE NMR FOR THE STUDY OF POWDERS AT NATURAL ISOTOPIC ABUNDANCE
17:50-18:10	**D. Di Censo** - MRI CHARACTERIZATION OF STRUCTURAL MODIFICATIONS IN STRIATUM OF PARKINSON'S DISEASE RAT MODEL	**S. Sykora** – THE UNBEARABLE FUZZINESS OF NMR DATA?
18:10-18:30	**M. C. Tourell** – PROBING THE LONG-TIME DIFFUSION LIMIT IN MACROPOROUS SPHERES PACKINGS VIA LONG LIVED SINGLET STATES	**R. Spadaccini** - DNP-SOLID-STATE NMR STUDIES ON THE ATP-DRIVEN LIPID FLIPPASE MSBA
18:30-18:50	**M. Fantasia** - NUMERICAL ANALYSIS OF THE NESTED BIRDCAGE RF COIL CONFIGURATION FOR $^{1}H/^{23}Na$ MRI AT 2.35 T AND WORKBENCH VALIDATION	**E. M. Vasini** - PCBC - A NOVEL AUTOMATIC PHASE & BASELINE CORRECTION ALGORITHM
18:50-19:35	**Chair: L. Calucci** **Plenary Lecture 2** **D. Sakellariou** – COMPACT MAGNETIC RESONANCE: FRONTIERS AND CHALLENGES	

Thursday September 28th

8:45-9:30	**Chair: E. Terreno** **Plenary Lecture 3** **D. Lurie -** FAST FIELD-CYCLING MAGNETIC RESONANCE IMAGING
9:30-10:00	**Bruker Lecture** **H. Kovacs -** AVANCE NEO – BREAKTHROUGH IN MULTI-RECEIVE NMR TECHNOLOGY
10:00-10:30	**Chair: M. Geppi** **Under 35 GIDRM award 2017** **G. Ferrauto -** ENHANCING SENSITIVITY OF CHEMICAL EXCHANGE SATURATION TRANSFER (CEST) MRI PROBES: FROM SMALL MOLECULES TO CELL-CEST AGENTS
10:30-11:20	**Coffee break + Poster session**

	Parallel session A **Chair: A. Mele**	**Parallel session B** **Chair: H. Molinari**
11:20-11:50	**M. Fragai -** CHARACTERIZATION OF L-ASPARAGINASE: NEW APPLICATIONS OF NMR IN BIOPHARMACEUTICAL RESEARCH	**A. Randazzo -** DNA G-QUADRUPLEX INTERACTING PROTEINS: FROM IDENTIFICATION TO CHARACTERIZATION
11:50-12:20	**S. Borsacchi –** SOLID STATE NMR SPECTROSCOPY AND ^{1}H RELAXOMETRY FOR A MULTI-SCALE INVESTIGATION OF INNOVATIVE MGO-BASED CEMENTS	**D. O. Cicero –** PLASMA PROTEIN BINDING OF DRUG CANDIDATES BY NMR
12:20-12:40	**F. Panattoni -** ^{1}H TIME-DOMAIN NMR ANALYSIS OF ELASTOMERIC NANOCOMPOSITES	**R. Marchetti -** NMR APPROACHES FOR THE STUDY OF THE INTERACTIONS BETWEEN HOST PROTEINS AND CELL SURFACE N-GLYCANS
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15:15-15:45	**Jeol Lecture** **A. Botana -** THE ROAD TO BETTER HFX EXPERIMENTS	
15:45-16:25	**Chair: D. Capitani** **Segre Fellowships 2016** **S. Bordignon -** 2D SOLID-STATE NMR EXPERIMENTS TO CHARACTERIZE THE HYDROGEN BOND NETWORK IN PHARMACEUTICAL COCRYSTALS **V. Mangini –** NMR-BASED APPROACHES FOR NANOTOXICITY ASSESSMENT: EFFECT OF SILVER NANOPARTICLES ON COPPER TRAFFICKING PROTEINS	
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9:30-10:00	**A. Rotondo -** DATA MINING FROM NMR SPECTRA OF VEGETABLE OILS	**F. Arnesano –** EFFECT OF CISPLATIN ON THE COPPER-DEPENDENT INTERACTION BETWEEN ATOX1 AND MNK1
10:00-10:20	**P. G. Takis -** DEVELOPING METHODOLOGIES FOR THE EFFECTIVE NMR BASED METABOLOMICS ANALYSIS OF BIOFLUIDS	**F. Carniato –** STRUCTURAL FEATURES OF Eu^{II}-CONTAINING CRYPTATES THAT INFLUENCE RELAXIVITY
10:20-10:40	**A. Rizzuti –** COMBINATION OF NUCLEAR MAGNETIC RESONANCE AND HYPERSPECTRAL REFLECTANCE FOR THE IDENTIFICATION OF ERWINIA AMYLOVORA SP. INFECTED LEAVE	**D. Alberti -** A THERANOSTIC APPROACH FOR BORON NEUTRON CAPTURE THERAPY (BNCT) TREATMENT BASED ON THE USE OF GD/B MULTIMODAL PROBES
10:40-11:10	**Coffee break**	
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11:10-11:40	**M. Halse -** HYPERPOLARISED LOW-FIELD NMR SPECTROSCOPY	**D. Picone -** PH INFLUENCE ON STRUCTURE AND STABILITY OF MNEI: A SWEET MODEL FOR PROTEIN FOLDING AND AGGREGATION STUDIES
11:40-12:00	**M. Tessari -** PARA-HYDROGEN HYPERPOLARIZATION FOR 1D AND 2D NMR CHEMICAL ANALYSIS AT SUB-MICROMOLAR CONCENTRATIONS	**L. Russo -** MECHANISTIC BASIS FOR THE RECOGNITION OF THE COACTIVATOR NCOA-1 BY STAT6
12:00-12:20	**E. Cavallari -** PARAHYDROGEN HYPERPOLARIZED METABOLITES FOR METABOLIC STUDIES ON BREAST CANCER CELLS	**V. Nasta -** STRUCTURAL INSIGHTS INTO THE MOLECULAR FUNCTION OF HUMAN [2FE-2S] BOLA3-GRX5 AND [2FE-2S] BOLA1-GRX5 COMPLEXES
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12:35-13:20	**Plenary Lecture 6** **T. Polenova –** INTEGRATIVE STRUCTURAL BIOLOGY OF BIOLOGICAL ASSEMBLIES: STRUCTURE AND DYNAMICS OF HIV-1 AND MICROTUBULE-ASSOCIATED PROTEIN ASSEMBLIES	
13:20-13:30	**Closing**	
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GMA

MOLECULAR INTERACTIONS AND DYNAMICS AT A GLANCE: NMR AND BEYOND

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The main, interconnecting and complementary motives inspiring this lecture are the investigation of the structural and dynamic properties of "novel" (relative to the publication age…) materials by using state-of-the-art NMR approaches and the strategy of gathering new information by the parallel and synergistic use of physical methods other than NMR. The multidisciplinary approach is a way to increase the collection of pieces for the description of matter as a mosaic composition.
Three different research lines will be presented, with paradigmatic cases where the merge of NMR and non-NMR data will make the difference.
Initially, the supramolecular assemblies of cyclodextrins with organic molecules, mostly active pharmaceutical components, will be introduced, starting from the early '90s research on host-guest complexation [1] up to the most recent HR-MAS NMR and solid state NMR on cyclodextrin-based polymers loaded with active principles for drug delivery. [2]
The second part will deal with NMR methods for ionic liquids (ILs). Ionic liquids are still an appealing field of research due to the fascinating complexity of their structure, the formation of nanosized polar and apolar domains without any phase separation, the possibility of modulating the type and intensity of intermolecular interactions by a suitable choice of the anion-cation pairs or, as recently underlined by the literature, blending different ILs. A brief overview of the NMR structural investigations starting from the pioneering ROE studies up to the most recent diffusivity investigations will be presented. [3]
Finally, but still in the ILs research theme, the applications of ^{129}Xe NMR spectroscopy will be presented as an highly innovative tool for exploring the liquid state and gaining information on the molecular voids within the liquid. In this particular example, the multidisciplinary approach turned to be the ace in the hole. [4]

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UNDER 35 GIDRM AWARD 2017

U35GA

ENHANCING SENSITIVITY OF CHEMICAL EXCHANGE SATURATION TRANSFER (CEST) MRI PROBES: FROM SMALL MOLECULES TO CELL-CEST AGENTS

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From their discovery in 2000, Chemical Exchange Saturation Transfer (CEST) MRI agents have been considered of great interest for diagnostic and theranostic applications. Their *in vivo* translation requires some important improvements such as: i) the increase of sensitivity, ii) the use of clinically approved compounds and iii) the application of new MRI sequences that increase the signal to noise ratio. The goal of my work mainly relies on the development of new CEST agents provided with a high sensitivity and safety and their possible application in biomedicine. In particular different paramagnetic complexes (paraCEST) have been investigated and CEST properties of Ln-HPDO3A chelates have been investigated by NMR/MRI. These Ln-based CEST agents have proposed both for the visualization of multiple cell populations resent in the same region (multicolour MRI)[1] and for *in vivo* tumour pH mapping (responsive agents)[2]. Since the intrinsic sensitivity of these paraCEST agents is not high (only one proton per molecule is present so giving a sensitivity in the millimolar range), the passage from single molecules to aggregate systems has been carried out; two examples have been reported, *i.e.* the use of Ln-HPDO3A-C16-based micelles and the use of Ln-DO3A-mesoporous silica nanoparticles. It has been shown that the sensitivity of this last system is two orders of magnitude higher than mono-molecular Ln-complexes. A further improvement of sensitivity has been reached by exploiting the large pool of water inside liposomes (LipoCESTs) [3]. Recently, for the first time, LipoCESTs agents responsive to enzymatic activity have been developed, able to provide quantitative information on the presence of enzyme in biological specimens (e.g. MMP-2 in blood serum). Finally, analogously to what reported for liposomes, Red Blood Cells have been loaded with paramagnetic shift reagents so generating a very sensitive CEST probe (detection threshold <1pM in term of cells concentration) [4]. Ln-loaded RBCs have been applied for mapping vascular volume in tumour region. Moreover the feasibility to use this approach with other cellular systems and the possibility to *in vivo* visualize labeled cells has been reported. The simultaneous use of multiple CEST probes has been shown to be useful for the multiparametric characterization of tumours thus providing insights into extracellular tumour pH, vascularization and perfusion.

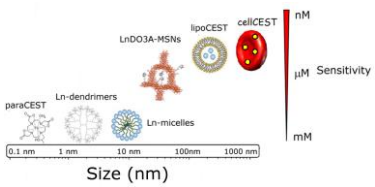

Fig. 1.Sensitivity of different CEST probes.

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ANNALAURA SEGRE FELLOWSHIPS 2016

ASF1

2D SOLID-STATE NMR EXPERIMENTS TO CHARACTERIZE THE HYDROGEN BOND NETWORK IN PHARMACEUTICAL COCRYSTALS

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Hydrogen bonds are among the main tools expendable to design supramolecular adducts such as cocrystals. A cocrystal is defined as a multi-component crystalline entity in which two or more different molecules occupy the same unit cell, linked by weak interactions.[1] Cocrystallization represents a strategy to successfully modulate the physicochemical properties of the components, such as mechanical properties, reactivity and dissolution rate.

Despite many efforts, it is not uncommon to encounter drawbacks in obtaining crystals suitable for X-ray analysis. When this happens, solid-state NMR (SSNMR) can step in to solve the issue. SSNMR has long proven to be a powerful means to study and characterize hydrogen bonds in supramolecular chemistry. In our case, several 1D (^{1}H MAS, ^{13}C CPMAS and ^{15}N CPMAS) and 2D (^{1}H DQ MAS, ^{13}C-^{1}H HETCOR, ^{14}N-^{1}H J- and D-HMQC) techniques were employed to investigate two samples: a co-drug of indomethacin and caffeine and a pharmaceutical cocrystal of ibuprofen and proline.

In the first case,[2] single crystal X-ray diffraction analysis was performed on a properly sized crystal, while SSNMR spectra were acquired to evaluate the ionic or neutral nature of the adduct which depends on the position of the hydrogen atom along the N···H···O interaction. As for the ibuprofen-proline cocrystal, owing to the lack of X-ray data, the experiments provided the stoichiometric ratio between the components, information about the ionic or neutral nature of the cocrystal and the hydrogen bond interactions at play in its crystal structure.

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ASF2

NMR-BASED APPROACHES FOR NANOTOXICITY ASSESSMENT: EFFECT OF SILVER NANOPARTICLES ON COPPER TRAFFICKING PROTEINS

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Despite the widespread use of silver nanoparticles (AgNPs) in an increasing number of everyday products and biomedical applications [1], large knowledge gaps exist with regard to the possible risks of long-term exposure to AgNPs. Only limited health effects of AgNPs themselves and of the silver ions released from them have been documented in humans [2]. Recent studies have shown how AgNPs can be internalized by eukaryotic cells through mechanisms of clathrin-dependent endocytosis or macropinocytosis [3]. This makes it necessary to understand and study toxicity, efficiency, transportation, and processing of AgNPs in biological systems. Furthermore, the native conformation of proteins interacting with AgNPs can be altered, sometimes resulting in the exposure of protein epitopes normally hidden within the protein and in unexpected responses [4, 5]. Lastly, AgNPs solution show intricate equilibrium with their Ag(I) ions. Since Ag(I) or Cu(I) have similar coordination properties, an impact on copper metabolism is expected, and proteins involved in copper homeostasis are among the leading candidates for the binding of Ag(I) and AgNPs within the cells [6]. In humans, the Cu chaperone Atox1 delivers Cu(I) to the metal-binding domains (MBDs) of two P_{1B}-type ATPases: the Menkes (Atp7a) and Wilson (Atp7b) disease proteins. Both Atox1 and the first MBD of Menkes (Mnk1), which are partners *in vivo*, can bind one Cu(I) through two Cys residues located in a conserved CXXC motif [7]. The main objective of this work is to gain direct evidence of the interaction of Atox1 and Mnk1 with AgNPs by NMR-based approaches. Although the two proteins have quite similar structure, their behavior with AgNPs is substantially different. Moreover, these Cu(I) transporters are ultra-sensitive *in vitro* and in-cell probes of Ag(I) ions released by the nanoparticles. The results produced in this work represent a first step towards the molecular understanding of potential risks, far too little considered, related to the use of AgNPs.

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

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PLENARY LECTURES

PL1

INTEGRATIVE STRUCTURAL BIOLOGY OF RNP MACHINERY IN SOLUTION

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Recent technical advancements have overcome the molecular-weight limits of traditional NMR methods. The most prominent example is the use of isoleucine, leucine and valine (ILV-) methyl groups as probes, which can provide inter-subunit distances even for molecular machines as large as hundreds of kDa. During the past decade, a handful of integrative computational tools have been introduced that can accurately translate such sparse information into structures [1]. However, as most of these tools were developed in a case-specific manner, there is an ongoing demand for a standardized integrative computational method. Here we present a general integrative modeling framework that can deal with molecules of different nature (e.g. proteins, nucleic acids) and make use of diverse distance and/or proximity information to determine the structures of supramolecular assemblies [2]. Our framework includes (i) collection of structural data from diverse complementary techniques that do not require crystallization; (ii) extension of the well-known integrative computational tool HADDOCK, such that it can address supramolecular complexes having >>6 components and consider extensive flexibility; (iii) a new approach to score different conformers of large assemblies with respect to the experimental data; (iii) a statistical analysis to evaluate the fitness of the data. This approach will be demonstrated on the example of the Box C/D RNP, which methylates rRNA at the 2'-O-ribose position in the nucleolus. The structure of the complex is obtained by a powerful combination of solution state NMR and small angle neutron scattering (SANS). We show that the active sRNP is a pseudo-tetrameric complex: by solving the structure of both the apo- and the holo-complex we are able to decipher the mechanisms of methylation and to explain the specificity of the enzyme. Furthermore, with an NMR detected activity assay we reveal that the methylation at different rRNA sites is regulated, which in turns offer implication for rRNA folding [3]. In the last part of the talk, I will show the application of ssNMR to study large RNP complexes. I will present the ssNMR-based structure of the 26mer box C/D RNA in complex with the protein L7Ae [4], together with the experimental strategy that we used to obtain it [5]. Lastly I will present first ssNMR spectra for the RNA part of a 400 kDa RNP complex.

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PL2

LOW-POWER SYMMETRY-BASED HOMONUCLEAR DIPOLAR RECOUPLING: DESIGN PRINCIPLES AND APPLICATIONS

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Homonuclear dipolar recoupling under magic-angle spinning (MAS) conditions is widely exploited for extracting structural information. However, a long-standing problem with such rotor-synchronized pulse sequences is to accomplish robust recoupling in the presence of large isotropic/anisotropic chemical shift dispersions at a reasonably low rf power: the spin nutation frequency, $\nu_{nut}=q\nu_r$, scales linearly with the MAS rate ν_r, where q is typically in the range of 3-8. This gives significant challenges for prolonged recoupling applications at (moderately) high MAS rates >15 kHz, with the risk of damaging the sample and/or the NMR probehead.

We will discuss our recent work on an open-ended series of two-fold symmetry pulse sequences for homonuclear recoupling: these "$SR2_2(p)$" schemes offer several beneficial features [1,2]: (*i*) they operate at the "2Q HORROR" condition $\nu_{nut}=\nu_r/2$ [3], which readily admits applications at the currently highest MAS rates >100 kHz; (*ii*) they are generated recursively, with each expansion stage p providing an improved compensation to resonance offsets and rf inhomogeneity; (*iii*) proton decoupling is redundant during recoupling at spinning rates >50kHz; (*iv*) they feature an excellent compensation to chemical shift anisotropy (CSA).

These properties will be demonstrated by ^{13}C double-quantum coherence (2QC) excitation and 2Q-1Q NMR correlations. The $SR2_2(p)$ schemes may also be tailored to provide either 2Q or zero-quantum recoupling, thereby providing robust magnetization transfers during "mixing" in 2D NMR correlation spectroscopy.

The $SR2_2$ pulse schemes were developed primarily for the fast-MAS regime, and they may only handle large resonance offsets of the recoupled spins at spinning frequencies >50 kHz. Yet, applications at relatively modest MAS rates are feasible for samples/nuclei with small offsets (<10 kHz), even in the presence of substantial chemical shift anisotropies. Such applications will be demonstrated in the contexts of high-order ^{31}P multiple quantum coherence excitation for "spin counting" purposes [4], and 2Q-1Q correlation NMR experimentation on various inorganic biomaterials intended for bone/tooth implants, encompassing the probing of the proton speciation at the surfaces of mesoporous bioactive glasses, and in phosphoserine-bearing calcium phosphate cements, as well as ^{11}B (spin-3/2) 2Q-1Q correlation NMR on bioactive P-bearing borosilicate glasses [5].

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PL3

FAST FIELD-CYCLING MAGNETIC RESONANCE IMAGING

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Most contrast in conventional MRI arises from differences in T_1 between normal and diseased tissues. Several studies on small tissue samples have shown that extra information could be obtained from T_1-dispersion measurements (plots of T_1 versus magnetic field), but this information is invisible to standard MRI scanners, which operate only at fixed magnetic field (e.g. 1.5 T, 3.0 T). We have developed Fast Field-Cycling Magnetic Resonance Imaging (FFC-MRI) to exploit T_1-dispersion as a potential biomarker, with the aim of increasing diagnostic potential [1]. T_1-dispersion is typically measured using FFC, by switching the magnetic field rapidly between levels during the pulse sequence [2]. In this way, a single instrument can be used to measure T_1 over a wide range of magnetic field strengths. FFC-MRI obtains spatially-resolved T_1-dispersion data, by collecting images at a range of evolution fields. In our lab we have built a range of FFC-MRI equipment, including two whole-body human sized scanners, operating at detection fields of 0.06 T [3] and 0.2 T [4]. The 0.06 T device uses a double magnet, with field-cycling being accomplished by switching on and off a resistive magnet inside the bore of a permanent magnet; this has the benefit of inherently high field stability during the detection period. The 0.2 T FFC-MRI system uses a single resistive magnet which has the advantage of increased flexibility in pulse sequence programming, at the expense of lower field stability during the detection period, necessitating more complex instrumentation. Our lab is investigating a range of applications of FFC relaxometry and FFC-MRI. We have demonstrated that FFC relaxometry can detect the formation of cross-linked fibrin protein from fibrinogen *in vitro*, via the measurement of ^{14}N-^{1}H cross-relaxation phenomena [5]. We have also shown that FFC-MRI can detect changes in human cartilage induced by osteoarthritis [6]. Recent work has focused on speeding up the collection of FFC-MRI images by incorporating rapid MRI scanning methods along with the use of improved pulse sequences and algorithms [7,8]. This presentation will cover the main techniques used in FFC-MRI and will summarise current and potential bio-medical applications of the methods.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 668119 (project "IDentIFY"). Funding from EPSRC grants EP/K020293/1 and EP/J500045/1 is also acknowledged.

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PL4

NMR STUDIES ON AMYLOID β PEPTIDES

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Alzheimer's disease (AD) is a severe neurodegenerative disorder characterized by memory loss and progressive cognitive disability. One histologic hallmark of AD is the self-assembly of amyloid β peptide (Aβ) into insoluble amyloid aggregates [1]. Amyloid β peptides derive from the degradation, by endoproteinases, of the "amyloid precursor protein" at various sites, leading to critical cleavages that cause the N- and C-terminal release of the Aβ peptides [2]. These molecules are intrinsically disordered peptides, highly hydrophobic and with a high tendency to aggregate forming β–sheet rich assemblies. In general the conversion of random coil conformations into β–sheet rich structures is common to many other proteic systems [3]. It is now widely accepted that small soluble Aβ oligomers, and not insoluble aggregates or fibrils, formed along the aggregation pathway, represent the cytotoxic species [4]. The majority of aggregation studies of amyloid β peptides have been up to now performed on C-terminal truncated Aβ1-40 and Aβ1-42 [5].
The oligomerization studies here discussed have been extended to N-truncated pyroglutamate-modified amyloid-β peptides (AβpE3−42) and its mixtures with Aβ1-42 [6]. Indeed AβpE3−42 has been shown to be prominent in AD and has been postulated to initiate amyloid plaque formation and play a central role in triggering neurodegeneration and lethal neurological deficits [7].
We used NMR methods to understand the underlying aggregation molecular mechanism and discuss here the interactions of the Aβ peptides with the prion protein (PrP), which is one of the Aβ oligomers receptor that has been proposed to mediate Aβ toxicity [8].

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PL5

INTEGRATIVE STRUCTURAL BIOLOGY OF BIOLOGICAL ASSEMBLIES: STRUCTURE AND DYNAMICS OF HIV-1 AND MICROTUBULE ASSOCIATED PROTEIN ASSEMBLIES

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Recent methodological advances will be presented that enable atomic-level characterization of structure and dynamics of large biological assemblies by magic angle spinning NMR spectroscopy. I will discuss an integrative MAS NMR / MD simulations approach for i) determining structures and residue-specific dynamics, and ii) delineating intermolecular protein-protein interfaces. These approaches will be illustrated on two classes of systems: an HIV-1 capsid comprised of 1500 copies of 231-residue CA capsid protein, and microtubule-associated proteins bound to polymerized microtubules. Microtubules (MTs) and their associated proteins (MAPs) play important roles in vesicle and organelle transport, cell motility and cell division. Dynactin multisubunit assembly is the activator of the cytoplasmic microtubule-based dynein retrograde motor complex. CAP-Gly microtubule binding domain of dynactin's $p150^{Glued}$ subunit is critical for the regulation of dynein's motility. Mutations in the CAP-Gly domain are associated with neurological disorders, but the mechanism by which the CAP-Gly domain recognizes microtubules remains largely unknown, particularly at the atomic level. To determine the 3D structure and functional dynamics of CAP-Gly free and assembled on MTs and derive the intermolecular interfaces [1], a number of experimental strategies were implemented to overcome low sensitivity and resolution. HIV-1 capsids, assembled from ~1,500 copies of the capsid (CA) protein, are an integral part of mature virions. Conical in shape, capsids enclose the viral genetic material (two copies of RNA) together with several proteins that are essential for viral replication. In the assembled state, capsids are remarkably dynamic, with the CA residue motions occurring over a range of timescales from nano- to milliseconds [2-5]. These motions are functionally important for capsid's assembly, viral maturation, and interactions with host factors. In this talk, we present an integrated MAS NMR, DNP, MD, and DFT approach to probe the functionally important motions in assemblies of CA and their complexes with host factors Cyclophilin A (CypA) and TRIM5α, as well as assemblies of CA-SP1 maturation intermediates. We discuss the role of dynamic allosteric regulation in capsid's assembly, maturation, and escape from the CypA dependence. We demonstrate that the integration of experimental NMR and DNP methods and theory, at classical and quantum mechanical levels, yields quantitative, atomic-level insights into the dynamic processes that govern the capsid's function [2-5].

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PL6

COMPACT MAGNETIC RESONANCE: FRONTIERS AND CHALLENGES

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Magnetic Resonance spectroscopy (NMR) and imaging (MRI) have revolutionized science and medicine by their capacity to analyze matter in a non-invasive, three dimensional and localized manner. Applications cover very broad areas from chemical analysis, material characterization, bio-molecular structure and dynamics, anatomic and functional in vivo imaging. This extremely powerful technique has however several limitations, which reduce its widespread use in society, in view of industrial and biomedical applications. Low signal sensitivity, high-cost and limited availability are three of the main challenges for the next generation of modern magnetic resonance devices and technologies.
Several options for pushing the current limits will be discussed with particular emphasis on miniaturization for the magnet and the detector system. I will introduce fundamental ideas behind intuitive and optimized permanent magnet design, present and comment on several examples from single-sided portable magnetic resonance sensors and desktop NMR magnet systems. Several directions towards novel magnet technologies will be discussed with particular focus on MRI localized spectroscopy in the presence of rotating fields, on and on variable magnetic fields high-resolution relaxometry. These instrumental and methodological approaches are aim ing at applications in chemistry and chemical engineering for in situ analysis, in industry for on-line non-destructive monitoring and quality control, in personalized medicine for metabolic profiling of tissue extracts or organisms in vivo, in the study and optimization of contrast agents, and open the way for modern magnetic resonance devices for dedicated use.

BRUKER & JEOL LECTURES

SP1

AVANCE NEO – BREAKTHROUGH IN MULTI-RECEIVE NMR TECHNOLOGY

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NMR experiments involving multiple receivers provide a unique way of increasing the sensitivity and information content of data recorded in a given period of time [1-4].
AVANCE NEO represents the latest generation in the AVANCE series product line. The electronics is based on a 'transceive' principle, meaning each NMR channel has both transmitter and receiver capabilities. Thus, each channel is its own independent spectrometer with the full RF generation, transmission and receive infrastructure.
This architecture provides high flexibility and allows the implementation of multi-receive experiments in a straight-forward manner. Examples of multiple receiver operation are shown in routine analysis of small molecules and structure elucidation of biomolecular systems. The design of the experiments and the optimal applications for multiple receivers are discussed.

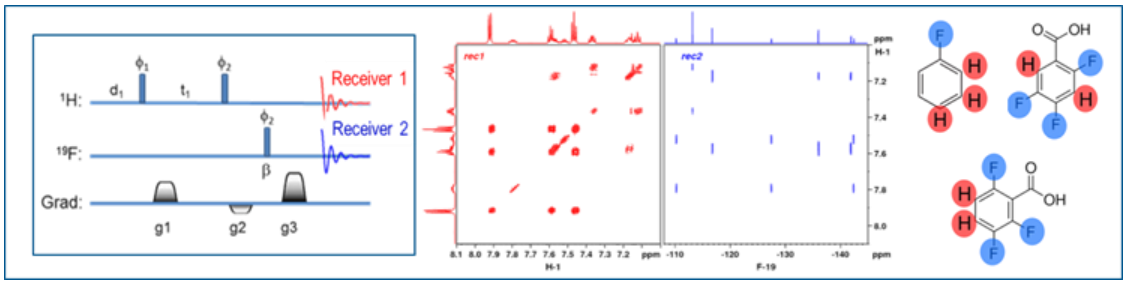

Fig. 1. 2D H-H COSY and F-H COSY spectra of a mixture of three ^{19}F-labelled aromatic compounds recorded in parallel using the pulse sequence shown.

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SP2

THE ROAD TO BETTER HFX EXPERIMENTS

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As NMR became a more and more popular technique, more and more hardware and software developments have been made. Unfortunately for most users, a large number of these developments were achieved in different ways, resulting in, for example, a large number of NMR probes available. The vast majority of these probes excel in some measurements but are suboptimal in others, thus forcing the procurement of several probes and a time wasted switching between them, or wasted machine time using a suboptimal solution.

One development done in order to simplify the situation was the development of the OneNMR probe. This probe had good sensitivity both for high frequency nuclei and for low frequency nuclei, thus eliminating the need to choose between direct and observe probes. Here we present the JEOL Royal HFX probe, a probe that like the OneNMR probe has good sensitivity both for high and low frequency nuclei, but in addition, it can operate either in double resonance mode or in triple resonance mode. This has been achieved thanks to a novel application of inductive coupling [1]. The JEOL Royal HFX is effectively equivalent to the OneNMR in double resonance mode, without losing sensitivity, and can operate in triple resonance mode to run experiments with any combination of ^{1}H, ^{19}F and heteronuclear nuclei. The probe automatically switches between these modes and eliminates the need to have a probe dedicated for the variety of fluorine experiments. Practical examples of its capabilities will be illustrated.

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ORAL COMMUNICATIONS

OC1

A THERANOSTIC APPROACH FOR BORON NEUTRON CAPTURE THERAPY (BNCT) TREATMENT BASED ON THE USE OF Gd/B MULTIMODAL PROBES

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This study aims at investigating a new theranostic approach for the treatment of primary tumours and metastasis based on the use of BNCT that combines low energy neutron irradiation with the presence of boron-containing compound at the targeted cells. This makes BNCT a promising option for the treatment of metastasis disseminated, for example, in the thoracic cavity that cannot be treated by methods requiring a precise localization, such as surgery or conventional radiotherapy. The innovation of this study lies on the development of novel theranostic agents, able to maximize the selective uptake of boron atoms in tumour cells and, at the same time, to quantify boron distribution in the tumour and in other tissues by Magnetic Resonance Imaging (MRI). The measurement of local boron concentration is crucial to determine the optimal neutron irradiation time, to calculate the delivered radiation dose and to evaluate the toxicity of the treatment by determining differences in boron concentration between tumour and healthy tissues. To this purpose a new dual BNCT/MRI agent has been synthesized and delivered to tumour cells using Low Density Lipoproteins as specific carriers. In particular, this study has been focused on the treatment of lung metastases generated by intravenous injection of a Her2 + breast cancer cell line (i.e. TUBO) in BALB/c mice, transgenic EML4-ALK mice used as primary lung tumor model [1] and of a subcutaneous tumour mouse model of Malignant Mesothelioma (MM). The latter is an aggressive tumour with a poor prognosis whose incidence and mortality is a function of past exposure to asbestos, after a latency period of 30-50 years. MM is a disseminated tumour against which conventional radiotherapy has limited effectiveness. Therefore, to improve both the clinical diagnostics and treatment, the discovery of new MM potential target molecules is of great interest. BNCT has been performed after MRI analysis at the TRIGA-Mark II reactor at the University of Pavia. With respect to controls, in boron treated group, tumour growth was significantly reduced.

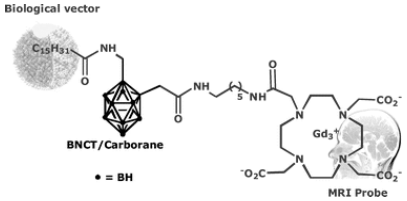

Fig. 1. Schematic representation of the dual *boron*/Gd agent (AT101).

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OC2

EFFECT OF CISPLATIN ON THE COPPER-DEPENDENT INTERACTION BETWEEN ATOX1 AND MNK1

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Copper (Cu) is an essential trace metal acting as cofactor of several key enzymes, which, in the free form can be extremely toxic [1]. Therefore, cells utilize highly conserved pathways to manage uptake, storage, and export of Cu, which generally involve Cu chaperones shuttling the metal ion between cellular targets with highly specific protein-protein interactions. In humans, the soluble cytosolic Cu chaperone Atox1 mediates Cu(I) delivery to the Cu-transporting P-type ATPases Atp7a and Atp7b (the Menkes and Wilson disease proteins, respectively), which are responsible for Cu release to the secretory pathway of the trans-Golgi network and Cu efflux [2]. Atox1 and soluble domains of Cu-transporting ATPases share a $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ ferredoxin-like structure and a highly conserved CxxC metal binding motif [3]. Cu(I) handover is believed to occur through the formation of an intermediate where a Cu ion is simultaneously linked to both proteins [4,5]. A growing number of studies has revealed that the same CxxC metal binding motif of Cu transporters can interact with and mediate the biological response to antitumor platinum (Pt) drugs, which are among the most used chemotherapeutics [6]. Thus, we deemed of crucial importance to investigate the interaction between cisplatin and the Atox1-Cu(I)-Mnk1 complex (Mnk1, first soluble metal binding domain of Atp7a). The experiments show that cisplatin can bind to the Cu(I) trafficking heterodimeric complex in fast exchange (on the NMR timescale) with the free proteins, thus leading to a kinetically stable adduct that has been structurally characterized by solution NMR and X-ray diffraction methods. The Cu(I) ion is displaced by Pt(II) bridging the two proteins and released to glutathione, a physiological reducing agent. The results indicate that cisplatin can affect Cu(I) homeostasis by interfering with the rapid equilibrium of Cu(I) exchange between Atox1 and Atp7a, an essential process related to cancer cell migration and metastasis [7].

Acknowledgments: We thank the University of Bari and the Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (C.I.R.C.M.S.B.) for support.

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OC3

SOLID STATE NMR SPECTROSCOPY AND ^{1}H RELAXOMETRY FOR A MULTI-SCALE INVESTIGATION OF INNOVATIVE MgO-BASED CEMENTS

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In the last years, MgO-based cementitious materials have attracted great interest because of the low CO_2 emissions associated with their production and especially for applications requiring relatively "low" pH of the cement pore solution, such as nuclear waste containment in deep repository [1]. The binder phase of MgO-based cements is magnesium silicate hydrate (M-S-H), the amorphous phase that forms from the reaction of MgO with a source of silica and water. Although a significant quantity of literature exists concerning the structure and nature of M−S−H [2-4], a full comprehension of properties, such as the hydration kinetics, the nature of the hydrated products and their multi-scale structure and organization, is still lacking. The investigation of these properties, as well as the research for new formulations with improved performances, is fundamental to achieve the industrial breakout of these materials. In this work we have combined Solid State NMR spectroscopy (SSNMR) and relaxometry, already proved to be powerful for the characterization of traditional cements, to obtain a detailed multi-scale description of novel MgO-based cements prepared by hydration of a 1:1 molar mixture of MgO and fumed silica (MgO/SiO_2) and of mixed formulations containing different amounts of MgO/SiO_2 and Portland cement. A ^{29}Si SSNMR investigation on samples freeze-dried at different hydration times allowed us to obtain quantitative information on the nature and structure of the binder phases at the nanometric level, as well as on their formation kinetics [5, 6]. The analysis of ^{1}H T_2 measured at low magnetic field and ^{1}H T_1 obtained by means of Fast Field Cycling relaxometry directly on cement pastes during their hydration provided a description of the state of water and of the evolution of the solid phases during the hydration process [7].

This work was financially supported by MIUR (FIR2013 Project RBFR132WSM).

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OC4

STRUCTURAL FEATURES OF Eu^{II}-CONTAINING CRYPTATES THAT INFLUENCE RELAXIVITY

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The present study describes a detailed analysis of the contribution of molecular parameters (water-exchange rate, electronic relaxation time, and rotational correlation time) to the relaxivity of three different Eu^{II}-containing cryptates (Fig. 1) over a wide range of field strengths using ^{1}H-NMR, ^{17}O-NMR, and EPR spectroscopies [1]. The results reported here demonstrate for the first time that it is possible to achieve a high relaxivity enhancement through non-covalent interactions between suitably functionalized Eu^{II}-containing complexes and slowly tumbling substrates like β-CD, poly-β-CD, and human serum albumin. Similarly to the case of well-known Gd^{III}-based complexes, the relaxivities of rapidly rotating Eu^{II} chelates are essentially limited by the value of the rotational correlation time. A lengthening of τ_R causes a considerable increase in the longitudinal relaxation rate of water protons. However, unlike what is often observed in the case of Gd^{III}-based complexes, the slow exchange process of the bound water molecules does not hamper the increase in relaxivity at the imaging fields of the supramolecular adducts. This is because the Eu^{II} complexes investigated to date have solvent exchange rates values in the range necessary to achieve high relaxivity. This property, in the case of macromolecular systems, largely compensates for the negative effect on r_1 of a greater distance between the water protons and the paramagnetic ion, thus enabling relaxivity values comparable to those of the analogous Gd^{III}-based systems. These preliminary results clearly indicate that in the case of Eu^{II}-containing complexes, it is possible to design and develop highly sensitive macromolecular or nano-sized probes for advanced MRI applications and we expect that our results will be instrumental in the design of future Eu^{II}-based contrast agents.

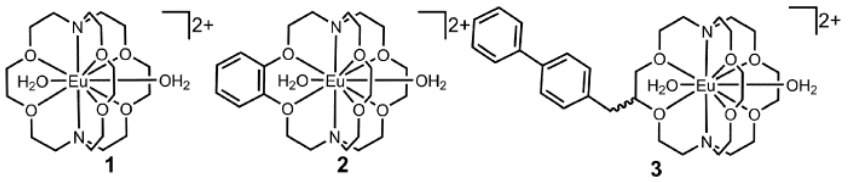

Fig. 1. Eu^{II}-containing cryptates used in this study. Complexes are drawn with two coordinated molecules of water, and counter anions are not shown for clarity.

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OC5

PARAHYDROGEN HYPERPOLARIZED METABOLITES FOR METABOLIC STUDIES ON BREAST CANCER CELLS

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Hyperpolarisation (HP) is a means to overcome the lack of sensitivity of MRS and MRI and has enabled real-time imaging of functional and metabolic processes. ParaHydrogen Induced Polarization is an affordable, easy to implement method that may allow wider application of MR hyperpolarized agents than the expensive and technically demanding gold-standard dissolution Dynamic Nuclear Polarization (dDNP). The introduction of PHIP-SAH (PHIP by means of Side Arm Hydrogenation) [1] allowed to considerably extend the applicability of parahydrogen to hyperpolarization of metabolites (pyruvate and lactate) that seemed unobtainable, before, using this hyperpolarization source.

This process relies on the following steps: 1) functionalization of the acid with an unsaturated alcohol (side-arm), 2) addition of parahydrogen to the side arm, 3) spin order transfer (SOT) from parahydrogen to the target ^{13}C carboxylate signal, 4) side arm removal (hydrolysis of the ester).

[1-^{13}C]-pyruvate is the most widely applied substrate for metabolic investigation, nevertheless the use of [1-^{13}C]-lactate for metabolic studies has some advantages [2]. Here we show the applications of these HP-metabolites for metabolic studies on breast cancer cells.

^{13}C hyperpolarized [1-^{13}C]-pyruvate and [1-^{13}C]-lactate have been obtained, according PHIP-SAH method from propargylic precursors. In order to test the metabolic conversion of HP-pyruvate into lactate, and vice versa for HP-lactate transformation into pyruvate, a cells lysate of breast cancer cells (TS/A) has been used.

These hyperpolarized [1-^{13}C]-molecules have been perfused to the cells suspension and a series of ^{13}C-NMR spectra has been acquired.

It has been demonstrated that the HP-pyruvate and HP-lactate by the PHIP-SAH method provide information about the rate of metabolic conversion that are consistent with results obtained with other conventional methods.

Therefore, this hyperpolarization method is a promising, affordable tool for metabolic studies that gives the opportunity of a wide application of ^{13}C hyperpolarized metabolites to cancer research.

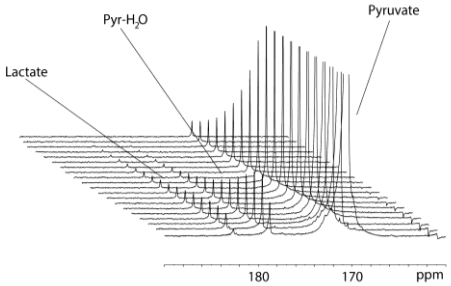

Fig. 1. Series of ^{13}C-NMR spectra acquired after perfusion of hyperpolarised pyruvate through of TS/A cells (20M) suspended in their grown medium.

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OC6

PLASMA PROTEIN BINDING OF DRUG CANDIDATES BY NMR

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Human serum albumin (HSA) is the most abundant protein in the blood circulation. Binding reversibly many endogenous and exogenous compounds with moderate to high affinity, HSA principally functions as a transport and depot protein. In the early 1940s, Bernard Davis published landmark papers in which he demonstrated that sulfonamide drugs bind to plasma albumin and that only the unbound drug is active. Since that initial study, more than 36 000 papers have been published that investigate albumin binding and drug action.

Most drugs have some affinity for human serum albumin (HSA) resulting in sequestration of them from plasma. Being the major protein component of human blood, if the K_D of the drug for albumin is 600 μM, ~50% of drug in the plasma compartment will be bound to HSA. Up to a point, this is an advantageous property as albumin binding increases the drug availability or stability by decreasing the clearance rate. However, when the K_D of binding decreases to the low micromolar range, >99% of drug in the plasma is albumin-bound and the *in vivo* efficacy can be significantly reduced. Additionaly, when the amount of free drug is very small compared to the amount that is protein-bound, any situation that disrupts albumin binding can significantly increase the concentration of free drug giving rise to potential toxicity.

These considerations make clear the importance of evaluating plasma protein binding of compounds in drug discovery . We present a medium-throughput NMR-based method to determine binding of compounds to both HSA and alpha-glycoprotein, the second most importand plasma binding protein.The method is complemented with competition-STD experiments for quantitative affinity measurement, and 2D ^{1}H-^{13}C correlation experiments to identify HSA binding sites . A comparison with equilibrium dialysis, the most frequently used technique to determine protein binding, will be presented.

OC7

MRI CHARACTERIZATION OF STRUCTURAL MODIFICATIONS IN STRIATUM OF PARKINSON'S DISEASE RAT MODEL

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, and its pathogenesis is still poorly understood. Current research aims at establishing the MRI role in characterizing the evolution of PD in humans as well as in rat models [1]. In a previous study, MRI showed the presence of striatal structural changes correlated with task switching inabilities induced by unilateral injury of the nigrostriatal pathway [2]. Here we report ex vivo MRI evidence of potential markers of PD evolution in the unilaterally 6-hydroxydopamine intranigral injected rat. Comparison of lesioned and sham-lesioned rats aimed to detect structural changes occurring soon after the injury (1 day) and later (up to 21 days). Ex vivo MRI was carried out on formaldehyde fixed whole brains using a 2.35 T Bruker Biospec scanner. T_1 and T_2 maps coupled with high-resolution (52 μm in plane, thickness 1 mm) T_2*-weighted structural images of the Substantia Nigra pars compacta (SNpc) and Striatum (ST) were acquired with Paravision 4.0 and processed using MATLAB-based software. TH+ immunostaining was used to assess the extension and progression of the induced neurodegeneration. The ST images reveal a complex pattern of white/grey matter ratio, showing an ipsilateral texture change in the lesioned rats (n=4); while sham-lesioned (n=4) brains do not present inter-hemispheric differentiation of T_1, T_2, and T_2* values. All lesioned brains reveal a progressive increase of T_1 in the whole ipsilateral ST with respect to the contralateral one, reaching a maximum difference of about 7% at 21 days post-lesion. In the same ROIs, the T_2 value in the ipsilateral ST with respect to the contralateral one showed an increase of about 3% at 1 day post-lesion, with a small decrease for longer times. TH^+ immunostaining analysis confirms that these findings are consistent with the temporal evolution of the neurodegeration. Our hypothesis is that ipsilateral ST structural changes could be associated to both neurotransmission and vasomotor action of DA in the terminal field of the nigrostriatal axons [3, 4]. This approach could be useful to characterize the early evolution of PD development in the 6-OHDA rat model.

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OC8

NUMERICAL ANALYSIS OF THE NESTED BIRDCAGE RF COIL CONFIGURATION FOR ^{1}H/^{23}NA MRI AT 2.35T AND WORKBENCH VALIDATION

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The availability of high field Magnetic Resonance Imaging (MRI) scanners permits a wide range of X-nuclei imaging, such as, for example, sodium (^{23}Na) useful to monitor chemotherapeutic response in rat glioma models [1]. To this purpose, several specific configurations of double-tuned RF coil have been described [2-5]. For transceiver double-tuned RF coil, the use of passive decoupling method (trap) is often preferred [2]. In this work, we present both a numerical modelling and workbench test of dual-tuned ^{1}H/^{23}Na nested birdcages RF transceiver configuration suitable for a pre-clinical 2.35T scanner. Using a numerical FEM model (Ansys HFSS v.17.2) the ^{1}H (High Pass (HP): 8 copper legs, length 16 cm, diameter 10.5 cm) and ^{23}Na (Low Pass (LP): 8 copper legs, length 13cm and 80 cm, diameter 9cm) birdcages have first been individually tuned, respectively 100.3 MHz and 26.6 MHz. Later the RF coil decoupling in the nested configuration was studied as a function of the relative azimuthal angle between the legs rotating the LP around its axis from 0° to 45° (complete superposition between HP and LP legs) by 3.75° steps. The decoupling was measured, considering the frequency shift of the useful resonant mode of the HP birdcage, for different length ratios of the two birdcages as well as without and in presence of an RF shield for various ratios of the shield to HP birdcage diameters: $k = D_{\text{shield}} / D_{\text{HP}}$. We observe that, for all configurations, the maximum ^{1}H frequency shift is at 0° (maximum coupling), while the minimum shift is at 22.5° (maximum decoupling). In the maximum decoupling configuration the ^{1}H frequency shift changes from about 20% when the shield is not present to zero for $k = 1.04$ thus suggesting that choosing an appropriate shield distance, the electrical coupling can cancel the magnetic one. For the ^{23}Na frequency, as expected, the shift is much smaller and compatible with the simulation uncertainness. To validate the simulations a set of HP, LP and shield were build, tuned and tested on the workbench showing a frequency shift behavior very close to the numerical model.

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OC9

CHARACTERIZATION OF L-ASPARAGINASE: NEW APPLICATIONS OF NMR IN BIOPHARMACEUTICAL RESEARCH

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An increasing number of biological drugs are approved every year to treat human diseases. Several biological drugs are proteins that can be structurally characterized by NMR spectroscopy, x-ray crystallography and cryo-electron microscopy. The conjugation of proteins with polymers/biopolymers to improve pharmacokinetics and safety makes it problematic the investigation of these "stealth" biologics. An integrated use of solid-state and solution NMR and x-ray crystallography can be useful for a more detailed structural characterization of these systems.

OC10

RELAXOMERY OF CANCER: EFFECT OF WATER MOBILITY AND MAGNETIC FIELD STRENGHT ON TISSUE AND CELL WATER PROTON T_1

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Conventional diagnostic magnetic resonance imaging (MRI) has focused on the improvement of the spatial resolution by using high magnetic fields (1-7T). High field allows the visualization of small tumour mass but lacks to give a precise evaluation of tumour grading, oxygenation, pH and metastasization. The presence of hypoxic or necrotic regions as well as the interstitial pressure are important hallmarks of the disease as they may affect the type of therapy to be chosen. This work aims at developing an innovative diagnostic strategy, based on the measurements of low fields $1/T_1$ NMRD profiles (0.01-16MHz) with Fast Field Cycling FFC-NMR to obtain quantitative information on tumour characteristics, due to different water content and mobility that is invisible to standard high field MRI. To this purposes different types of mammary tumours cells have been injected in the leg muscle to generate a tumour xenograft suitable for "in vivo" studies. The Stelar relaxometer used in this study is equipped, for the first time anywhere in the world, with a 40mm 0.5T magnet with a dedicated 11 mm detection coil allowing to acquire FFC-NMR profiles "in vivo"(Figure 1). Preliminary results show significant differences in FFC-NMR profiles reporting on different tumour characteristics. Cell swelling, caused by hypoxia or necrosis, increases both the amount of cytoplasmatic water and its mobility causing the increase of T_1 of tumour tissues. We can conclude that FFC relaxometry may be a paradigm-shifting technology which will generate new, quantitative disease biomarkers, directly informing and improving clinical diagnosis, treatment decisions and monitoring in oncology. Despite this prototype FFC-NMR instrumentation is not endowed with spatial resolution, fundamental knowledge that will be obtained, will open the route for the development of new diagnostic horizons in oncology until now uncharted and easily transferable to the increasing number of FFC-MRI scanners already present around in the world.

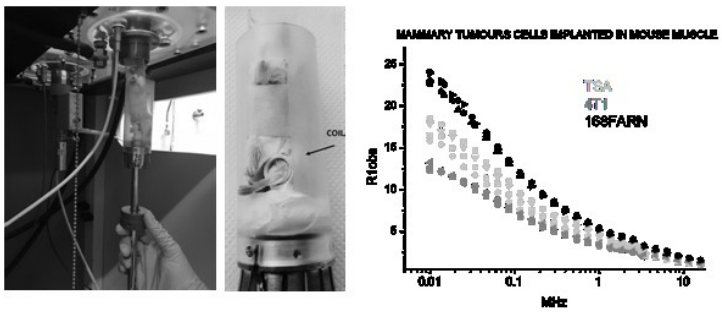

Fig 1. A) Photographs of the “in vivo” 11mm solenoid detection coil of FFC-NMR; B) “In vivo” $1/T_1$ NMRD profiles of tumours obtained from transplantation of three mammary adenocarcinoma cell lines.

OC11

HYPERPOLARISED LOW-FIELD NMR SPECTROSCOPY

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The development of robust, high-resolution, low-field NMR spectrometers presents the opportunity for exciting new applications of NMR spectroscopy outside of the typical laboratory environment. One of the significant barriers to progress in this field is the relatively low sensitivity of these devices, which is a direct consequence of the lower magnetic fields (< 2 T) when compared to standard NMR spectrometers (7 - 23.5 T). Therefore low-field NMR is an area where hyperpolarisation techniques, which can boost NMR sensitivity by several orders of magnitude, can have a transformative impact. Here we focus on the use of the parahydrogen (p-H_2) induced polarisation (PHIP) approach [1] as p-H_2 is relatively cheap and easy to produce and so does not significantly compromise the portability and affordability of the these NMR devices when compared to other hyperpolarisation methods. PHIP can be achieved either using a hydrogenation reaction or through catalytic transfer of polarisation at a metal centre, the so-called SABRE approach.[2] In this work, we demonstrate that high levels of hyperpolarisation can be observed on a benchtop (1 T) NMR spectrometer using both hydrogenative PHIP and SABRE and show how a fully integrated flow system can be used to generate reproducible and renewable SABRE hyperpolarisation in seconds. In addition, we explore how hyperpolarisation can facilitate the observation of chemically diagnostic information in even lower fields, such as the Earth's magnetic field.

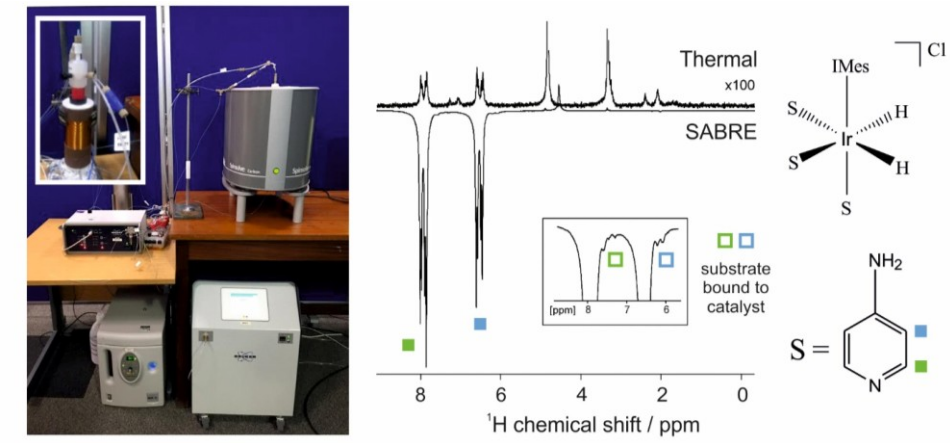

Fig. 1. Integrated flow system for SABRE hyperpolarization on a 1 T (43 MHz) benchtop NMR spectrometer (left) with example single-scan ^{1}H NMR spectra of 50 mM 4-aminopyridine acquired with and without SABRE hyperpolarization (right).

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OC12

NMR APPROACHES FOR THE STUDY OF THE INTERACTIONS BETWEEN HOST PROTEINS AND CELL SURFACE N-GLYCANS

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N-glycosylation is the most common post-translational modification present on proteins. Although built on a pentasaccharide core linked to an asparagine residue, the N-glycans exhibit variations of structures associated with tissue and development, but also with diseases, which make them attractive biomarkers. The recognition of cell surface N-glycans by proteins belonging to the host immune system, including dectins and siglecs [1], play a versatile role in human physiology, mediating a wide range of biological processes (see Fig. 1). It is worth to note that aberrant N-glycans – host immune proteins interactions have been associated with an increasing number of pathologies, including infections, autoimmunity and cancer and, therefore, they represent an emerging target to prevent or counteract the course of several diseases.

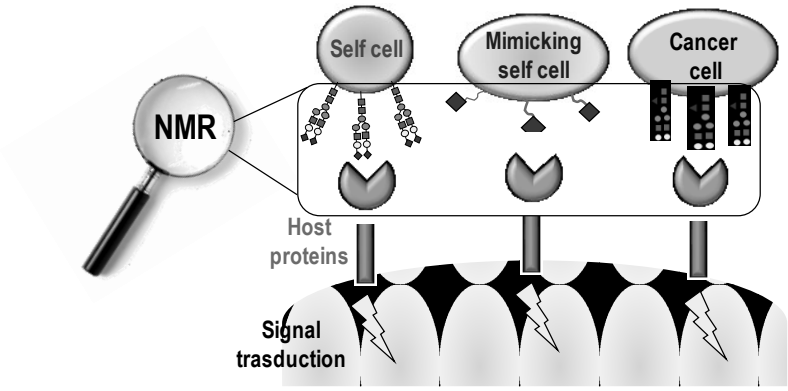

Fig. 1. Different mechanisms of interactions between host proteins and cell surface N-glycans.

Within this frame, we are currently investigating the binding between different N-glycans recognizing host proteins and *ad hoc* synthesized complex carbohydrates [2] mainly by means of NMR spectroscopy [3]. A combination of advanced ligand-based NMR techniques allowed us to define the ligands bioactive conformation and to map the interacting epitope of naturally occurring complex-type N-glycans in the interaction with different proteins of the host immune system. These crucial information on binding mechanisms are particularly relevant for the development of novel inhibitors or cell-directed therapeutics.

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OC13

STRUCTURAL INSIGHTS INTO THE MOLECULAR FUNCTION OF HUMAN [2Fe-2S] BOLA3-GRX5 AND [2Fe-2S] BOLA1-GRX5 COMPLEXES

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Assembly of mitochondrial iron-sulfur (Fe/S) proteins requires a dynamic interplay of protein:protein interactions and defects of this process cause many rare diseases. In this frame, members of the monothiol glutaredoxin (Grxs) and of the BolA-like protein families have recently emerged as specific interacting partners [1,2]. We solved the 3D structures of human BOLA3 (hBOLA3) and human BOLA1 (hBOLA1) proteins using triple resonance experiments. This structural knowledge has been the starting point to investigate by NMR the interaction between apo hBOLA3 (or apo hBOLA1) and both apo and [2Fe-2S] GRX5 form, performing ^{1}H-^{15}N HSQC experiments at different protein-protein ratios [3].
Exploiting site directed mutagenesis experiments, we characterized the [2Fe-2S] cluster coordination motif in the human BOLA3-GRX5 and BOLA1-GRX5 complexes and we investigated redox properties of the [2Fe-2S] bound cluster by NMR, EPR, UV/vis and CD spectroscopies. Collectively, these data allow us to obtain experimentally driven docking models of the two heterocomplexes.
The BOLA1-GRX5 complex coordinates a reduced, Rieske-type [2Fe-2S] cluster, while an oxidized, ferredoxin-like [2Fe-2S] cluster is present in the BOLA3-GRX5 complex. The differences in the structural rearrangement and in the cluster redox properties provide the first indications for discriminating the functional roles of the two heterocomplexes.

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OC14

^{1}H TIME-DOMAIN NMR ANALYSIS OF ELASTOMERIC NANOCOMPOSITES

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The addition of silica nanofillers to elastomers is generally associated with an improvement of the mechanical properties of the pristine polymeric material [1]. Several studies have demonstrated that this improvement is not only due to the addition of rigid domains to the polymer, but also a consequence of the filler-rubber interactions at the interface. The exact mechanism by which these interactions on the microscopic scale affect macroscopic properties, such as polymer resistance, is still mostly unknown, but it is generally associated with the formation of closely linked regions at the filler-rubber interface, where part of the rubber becomes immobilized [2]. This suggests the use of nanofillers able to increase the amount of immobilized rubber chains to improve elastomer performances.
In this work, SBR nanocomposites based on two different types of fillers, sepiolites and POSS-silica, were analyzed using ^{1}H time-domain NMR techniques.
The combination of solid echo and CPMG experiments, together with a suitable strategy of data analysis, allowed us to draw a complete picture of the dynamic properties of the nanocomposites and, in particular, to obtain a quantitative evaluation of the bound rubber content. This approach was applied to several sepiolite-SBR composites in order to investigate the effects of the chemical treatment of the filler (untreated, activated by acid or silanized) and of the vulcanization of the rubber. A preliminary study of the effects of physical aging on the dynamic properties of the nanocomposites was also performed.
Moreover, the same ^{1}H relaxometric approach was applied to the study of the dynamic properties of nanocomposites based on SiO_2–POSS "hybrid" fillers, in the attempt of understanding the effects of both the POSS cross-linking action and the mechanical reinforcement mainly determined by silica [3]. To this aim, a complete characterization of the dynamically distinguishable regions of the nanocomposites and, in particular, the determination of the bound rubber content was carried out on both SBR composites filled with SiO_2 nanoparticles previously functionalized with POSS, and SBR composites physically mixed with silica and POSS.

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OC15

PH INFLUENCE ON STRUCTURE AND STABILITY OF MNEI: A SWEET MODEL FOR PROTEIN FOLDING AND AGGREGATION STUDIES

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MNEI is a single chain derivative of monellin, a natural sweet plant protein made-up by two polypeptide chains (A and B), of 44 and 50 residues respectively, held together by non-covalent interactions. The protein was engineered to improve resistance to thermal denaturation by linking the C-terminal of chain B with the N-terminal of chain A through a Gly-Phe dipeptide. While retaining the same sweetening power of monellin, MNEI has higher thermal stability (above 70°C) and can recover the native fold when cooled down around 60 °C [1]. It also has several major advantages that make it a suitable candidate for potential application as artificial sweetener: it can be produced by heterologous expression *in vitro* with high yield [2] it is highly soluble at acidic pH and does not contain any disulfide bridge. The main drawback of the protein is the lower solubility accompanied by increased aggregation propensity at neutral to alkaline pH [3]. Besides the interest linked to their sweetness, monellin and its single chain derivatives have been widely used as models for folding, stability and self-assembly investigations. Although MNEI is very resistant at acidic pH, exposure to neutral or alkaline pH strongly affects its stability. We have performed a thorough NMR study of the dynamic properties of MNEI as a function of pH [4]. The results demonstrate that, at physiological temperature, exposure to higher pH increases MNEI plasticity. Furthermore, a survey on temperature and ionic strength has been pursued. The structural changes, originating in a well-defined region, are transmitted to the whole structure and are likely to trigger unfolding processes and amyloid oligomerization.

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OC16

DNA G-QUADRUPLEX INTERACTING PROTEINS: FROM IDENTIFICATION TO CHARACTERIZATION

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Telomeres are DNA–protein complexes at the ends of eukaryotic chromosomes. Human telomeric DNA, which consists of tandem repeats of the short TTAGGG sequence, can form secondary structures named G-quadruplexes (G4s). Telomeric G4s have been shown to have regulatory roles in telomere extension and maintenance. Furthermore, the interest in the more general significance of G4s has expanded during the past decade to include G4 structures in oncogene promoter sequences, 5'-UTR regions and introns, as well as in a number of fragile/breakpoint regions.

The analysis of the G4–protein interaction network can be considered a crucial point to clarify the elusive biological mechanisms in which such relevant DNA structures could be implicated. Some proteins are able to recognize G4 structures and some are also able to unfold them. The discovery of these proteins raises interesting questions regarding the dynamic nature and function of such structures within the genome, especially at telomeres. In this frame, we decided to search for proteins able to recognize G4-forming truncations of human telomeric DNA sequence [1]. In particular, we employed a chemoproteomic-driven approach, where the molecule of interest is used as a bait to fish out its interactors from nuclear extracts. In particular, we have used different G4 conformations, namely the parallel and the anti-parallel folds. Very interestingly, different sets of proteins were identified. In this communication, a number of unpublished results will be presented, among which a preliminary structural study of the interaction between the HMGB1 protein and the parallel telomeric G4 structure.

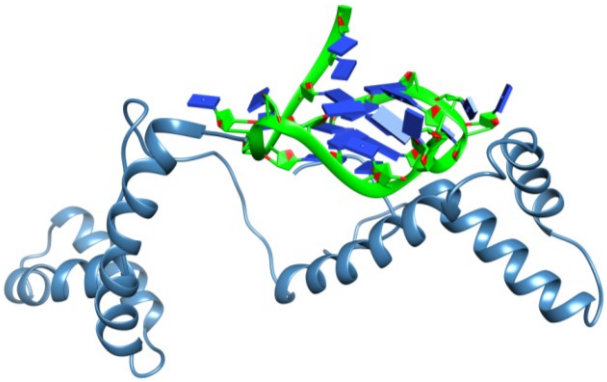

Fig. 1. Model of the HMGB1/G-quadruplex complex.

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OC17

COMBINATION OF NUCLEAR MAGNETIC RESONANCE AND HYPERSPECTRAL REFLECTANCE FOR THE IDENTIFICATION OF *ERWINIA AMYLOVORA* SP. INFECTED PEAR LEAVES

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In this contribution the effects of infections by *Erwinia amylovora (Ea)*, the agent of fire blight, on the metabolic composition of pear leaves are highlighted.
In search for a combined approach [1] that could give a comprehensive information on the metabolome of infected pear leaves, we focused our attention on NMR and Hyperspectral Reflectance (HR) techniques, as NMR easily provides information on molecules with relatively high concentration (typically, such molecules correspond to primary metabolites) in the plants while HR supplies a rapid information on the plant status.
HR-NMR covariance analysis allowed to transfer metabolome information obtained by NMR to HR bands through a knowledge transfer approach, and permitted to obtain maps (Fig. 1) which represent the specific infection status of the pear plants where HR bands can be determined as infection markers.

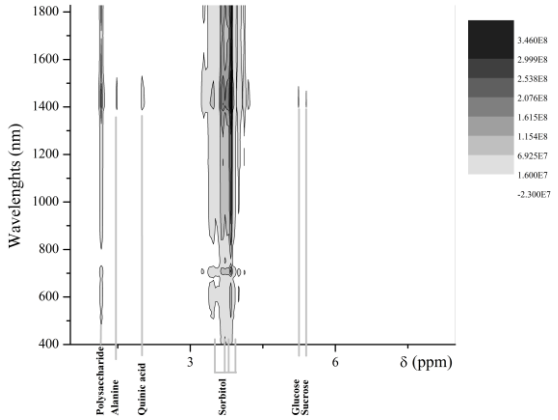

Fig. 1. Contour plot resulting from the HR-NMR covariance analysis applied to *Ea* infected pear leaves.

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OC18

DATA MINING FROM NMR SPECTRA OF VEGETABLE OILS

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NMR analysis of vegetable oils seems to be an evergreen topic because of the great amount of information quickly provided by ^{1}H and hetero-nuclear NMR spectra, especially run on liquid matter [1, 2]. Indeed, beyond the characterization of the fatty acid mixture, detectable by the main signals integrations, many other minor components are known to be detectable and quantifiable [3, 4]. These specific analyses took advantage from many sophisticated techniques and pulse sequences available within the "NMR toolbox". Our idea is to exploit the whole human history of the NMR of oils, combined with our specific experience, to improve the rapid characterization panel for any sample. This work conveys the different findings concerning three classes of compounds: aldehydes, di-acylglycerols and squalene. The proper knowledge of the specific resonances belonging to these species enables their possible quantification keeping into account also the possible signal overlap leading to overestimation of other species [5].

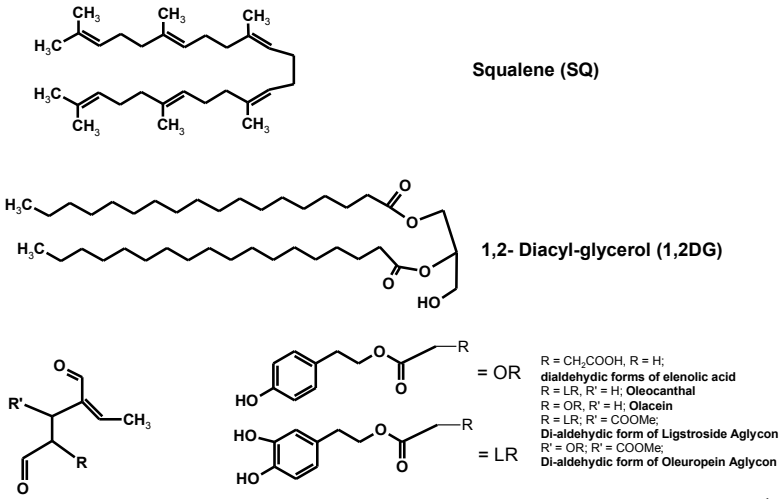

Fig. 1. Standard addition of squalene demonsrates possible quantification by ^{1}H-NMR.

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OC19

MECHANISTIC BASIS FOR THE RECOGNITION OF THE COACTIVATOR NCOA-1 BY STAT6

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STAT6 belongs to a family of transcription factors known as the signal transducers and activators of transcription (STAT). STAT family members share a similar protein structure, which is essential for their activation and function. STAT proteins mediate signaling from activated cytokine receptors to the nucleus[1]. After phosphorylation at a specific tyrosine by a receptor associated Janus kinase, STATs form homo- or heterodimers and translocate into the nucleus where they modulate transcription by specific DNA sequence elements [1]. STAT6 becomes activated in response to IL-4 and IL-13 and mediates most of the gene expression regulated by these cytokines. By direct interaction with specific parts of its transactivation domain, STAT6 recruits the co-activators p300/CDP and NCoA1 (also called steroid receptor coactivator-1, SRC-1), which are essential for transcriptional activation by IL-4 [2]. In particular, the interaction between STAT6 and NCoA1 is modulated by a short region of the transactivation domain that includes the motif LXXLL (where X is any amino acid). The crystal structure of a STAT6-derived peptide (Leu794-Gly814) in complex with the NCoA1 PAS-B domain257-385 revealed that the Leucine side-chains of the motif (Leu802, Leu805 and Leu806), are deeply embedded into a hydrophobic groove of the surface of NCoA1 [3]. More recently, it has been demonstrated by a fluorescence polarization binding assay that additional residues (Leu794, Pro797 and Thr798), flanking the LXXLL motif in STAT6, play an important role in stabilizing the protein binding to NCoA1 [4]. Here, we report the structural characterization of the complex between a STAT6-derived peptide encompassing the region from Gly783 to Gly814 and the NCoA1 PAS-B domain$^{257\text{-}385}$ using Nuclear Magnetic Resonance (NMR) and X-ray crystallography. The structural characterization of the STAT6$^{783\text{-}814}$/NCoA1$^{257\text{-}385}$ complex demonstrates that STAT6$^{783\text{-}814}$ peptide binds the NCoA1 PAS-B domain$^{257\text{-}385}$ by additional amino acid interactions from its N-terminal region resulting in a more extended binding interface with NCoA1 compared to that identified before in the crystal structure with the STAT6$^{794\text{-}814}$ peptide.

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OC20

DNP-SOLID-STATE NMR STUDIES ON THE ATP-DRIVEN LIPID FLIPPASE MsbA

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MsbA is a 65 kDa homodimeric ABC transporter from *E. coli*. Although its primary function is Lipid A translocation across the cytoplasmic membrane in *E. coli* using ATP hydrolysis as the energy source, it has also been shown to translocate a variety of lipophilic substrates. Its structure resembles and shares 46% sequence similarity with Sav1866 [1] and 30% identity with the human multi-drug resistance protein P-glycoprotein [2]. Given its favorable expression yields in *E. coli* and its stability makes it a good model for an ABC efflux pump. Solid-state NMR and in particular magic angle sample spinning (MAS) offers the unique possibility to probe structure and dynamics of the full-length transporter within proteoliposomes. MsbA has been prepared in quantities required for solid-state NMR by optimized expression conditions enabling isotope labeling [3]. Here we present a DNP enhanced magic angle spinning solid-state NMR based study on MsbA reconstituted in liposomes. We show that, using selectively labelled MsbA samples, it is possible to follow conformational changes in the transmembrane domain of MsbA upon Vanadate-ATP trapping and drug binding. Moreover we are able to show that different drug interact with different regions of the transmembrane domain.

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OC21

THE UNBEARABLE FUZZINES OF NMR DATA?

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It is almost 10 years since I started working with Mestrelab Research (Santiago de Compostela) on some aspects of their Mnova software NMR plug-in. One of the tasks I am handling is the automatic structure verification (ASV) which consists in answering the question "to which degree is this proposed molecular structure compatible with this set of NMR data". This means "teaching" the computer to "think NMR" or even to "think NMR the way chemists do". Something that brings up an amazing number of almost philosophical dilemmas and even grotesque situations!
Here, I do not want to present any specifics of Mnova, this is not a Company presentation. Rather, I will attempt a very personal reflection about the lessons that I have personally learned so far from my work. Lessons that regard the *unavoidable fuzziness of any experimental data* and the *propagation of the fuzziness to higher logical levels* in any system complex enough to resemble an artificial intelligence. It is much like studying error propagation in complex calculations, but applied to logical systems.

While teaching a computer to dab in human logic is a hard proposition, teaching it to apply human logic to real data is something that can drive a programmer insane.

In NMR many of the problems arise from the fact that no spectrum is really fully defined – there is noise, field inhomogeneity and instability, baseline roll, massive overlap of quantum transition, mathematical ill-definiteness of spectral decomposition into peaks, etc. On top of this there are also imperfections (errors) in predictions of NMR parameters, unexpected interactions between sample components, weird behavior of real molecules (as opposed to their structural formulas), and the impossibility to fully rely on chemical NMR 'rules' because they are either too bland to be useful, or else have quite frequent 'exceptions'.
I will also attempt to answer the hard question of *how much can we really trust the information deduced from NMR spectra* and which are the principle traps for novices, experienced NMR spectroscopists, and AI's alike. They are all apt to err – but in ways different enough to start charting some characteristic patters.

Note: I do collaborate quite closely with many people at Mestrelab Research, Santiago de Compostela, Spain. Carlos Cobas, Felipe Seoane, Mike Bernstein, Vadim Zorin, Esther Vaz, Maruxa Sordo, to name just a few.
However, the opinions I express here are rather personal; I am not quite sure whether my colleagues would approve them (well, probably yes). A Company should tell you that the Verification wizard is a marvel of unchallengeable logic, while I will try and show you that such a dream is impossible. Yet, we try hard to keep improving it.

After all, we humans are not perfect, too, and we also all try hard to improve, don't we?

OC22

DEVELOPING METHODOLOGIES FOR THE EFFECTIVE NMR BASED METABOLOMICS ANALYSIS OF BIOFLUIDS

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Nuclear magnetic resonance (NMR) spectroscopy is an emerging clinical tool for studying metabolism in detail, allowing for early diagnosis of several diseases [1,2]. NMR based metabolomics has high reproducibility and intrinsic "untargetedness" [3], which provide high precision and accuracy for quantification of many metabolites in biofluids, presupposing their accurate assignment in the biofluids NMR profile as well as the quite high abundance of the biofluid. The vast majority of NMR based metabolomics studies is carried out by solution ^{1}H NMR, focusing, mainly, on urine and serum/plasma. However, the use of high resolution magic angle spinning (HRMAS) NMR yields access to solid or semisolid specimens, such as tissues [4].
Here, an alternative, effective protocol for metabolomic characterization of biofluids based on their gelification and subsequent application of HRMAS ^{1}H NMR is introduced [5]. This approach satisfies all the requirements for performing metabolomics studies (i.e. reproducibility and analytical sensitivity), produces similar statistical/metabolic profiling results to solution NMR, and improves storage up to 24 hours [5]. This strategy complements the standard procedures of NMR-based metabolomics (assignment/quantification of many metabolites fingerprint in the NMR profile), either in laboratory settings - for instance in case of low biofluid availability (much less than 40 µl of neat urine are needed to obtain a sample), as it may be the case for metabolomics on small animals; or in clinical settings, for instance devices for urine transportation or domestic collection/short term storage, without the need of cold-storage and reducing the risks of leakage (Fig. 1).

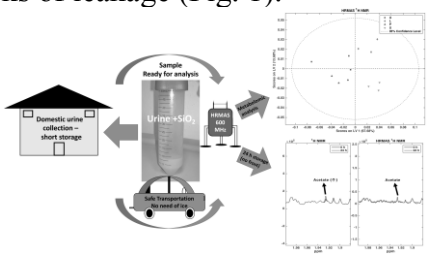

Fig. 1. From biofluids collection-gelification to fast, accurate metabolomic analysis.

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OC23

PARA-HYDROGEN HYPERPOLARIZATION FOR 1D AND 2D NMR CHEMICAL ANALYSIS AT SUB-MICROMOLAR CONCENTRATIONS

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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 determines the transfer of the spin-order from p-H_2 to the nuclear spin of the substrate molecules, resulting in NMR signals enhanced by two orders of magnitude. SABRE has so far been reported for nitrogenous heteroaromatics, sulfur heteroaromatic compounds, nitriles, Schiff bases and diazirines. Several such SABRE-active moieties appear in the structures of drugs, odorants and metabolites, giving rise to an interest in devising analytical approaches for their detection. We have recently presented an approach for continuous SABRE hyperpolarization at high magnetic field, that can be straightforwardly incorporated in multidimensional NMR experiments [1]. This method was implemented in a 2D correlation experiment that allows detection and quantification of dilute solutes at nanomolar concentration. Applications of this technique for the NMR detection and quantification of dilute analytes in natural extracts and body fluids will be presented.

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OC24

DNP-ENHANCED SOLID-STATE NMR FOR THE STUDY OF POWDERS AT NATURAL ISOTOPIC ABUNDANCE

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In solid-state NMR, the high density of spins for abundant nuclei in organic compounds (^{1}H, or ^{13}C for uniformly enriched samples) drastically complicates the spin dynamics and, hence, prevents the measurement NMR of molecular information based on long-range dipolar couplings. Investigating distances between rare spins, e.g. ^{13}C, in samples at natural isotopic abundance (NA) would constitute a potential solution to this problem but, unfortunately, the low sensitivity of NMR makes it impracticable.
Recently, it has been shown, however, that NMR sensitivity could be enhanced by high-field low-temperature magic-angle spinning dynamic nuclear polarization (DNP) [1]. Interestingly, DNP provides NMR signal enhancement by one or two orders of magnitude and hence allows ^{13}C-^{13}C scalar or dipolar correlation experiments to be recorded on NA samples within practicable experimental times [2]. Here, the potential of DNP-enhanced experiments will be illustrated for the study of powders at natural abundance, which cannot be studied using standard characterization techniques, such as X-ray diffraction [3].

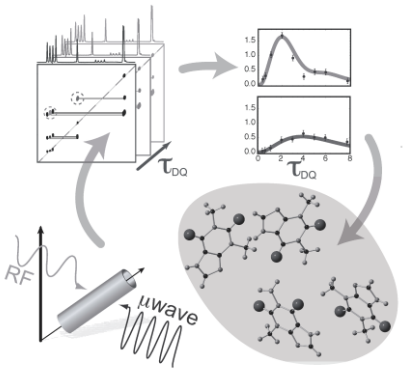

Fig. 1. Experimental protocol to study powders at natural abundance

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OC25

PROBING THE LONG-TIME DIFFUSION LIMIT IN MACROPOROUS SPHERE PACKINGS VIA LONG LIVED SINGLET STATES

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Magnetic resonance (MR) diffusion experiments are routinely used to characterize the microscopic morphology of porous media. The walls of the solid matrix impose restrictions on the diffusing molecules, resulting in a decrease in the apparent diffusion coefficient, $D(\Delta)$, as a function of measurement time, Δ. At long times, when the displacement of the diffusing species is much larger than the characteristic pore size, $D(\Delta)$ reaches an asymptotic value, independent of the measurement time and partially determined by the geometry of the pores. From this value, characteristic parameters of the porous media, such as the pore-size distribution, porosity (Φ) and tortuosity (α) can be determined [1].

The time-scale over which diffusion can be measured is determined by the lifetime of the NMR signal of the diffusing species. In traditional MR diffusion experiments, the longitudinal relaxation time, T_1, sets an upper limit on the measurement time (a few seconds) and, consequently the length scales that can be investigated (~ 50 - 100 µm). However, this length-scale can be superseded by the use of long-lived singlet states, which allow diffusion to be measured over several minutes [2] and the determination of compartment sizes of up to 0.8 mm in diffusion-diffraction experiments [3].

In this work, we exploit long-lived singlet states to measure the long-time diffusion coefficient in beds of randomly packed spheres with diameter > 500 µm, and average pore size > 150 µm. The onset of the long-diffusion limit was reached at measurement times longer than 60 s (Fig 1.). At this time the ratio of the apparent diffusion coefficient to the unrestricted value, $D(\Delta)/D_0$, was 0.60, corresponding to $\alpha = 1.65$ and $\Phi = 0.36$, consistent with literature values for randomly packed, mono-sized spheres [4].

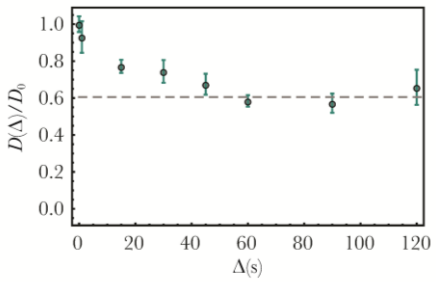

Fig. 1. The apparent diffusion coefficient of the singlet molecule in randomly packed spheres of diameter 500 – 600 um. The dashed line indicates the long-time, asymptotic value of the diffusion coefficient.

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OC26

PCBC - A NOVEL AUTOMATIC PHASE & BASELINE CORRECTION ALGORITHM

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Manually, phase (PC) and baseline (BC) corrections in 1D NMR spectra are done sequentially one after another. In addition, the Bc is always done only on the "real" part of the spectrum where the spectral peaks are much narrower than in the imaginary part and therefore interfere less with the visual assessment of the final quality. However, even when the computer is used just as a display tool, one notices that there is a mutual interference between the two corrections, especially close to the final solution. One often hesitates between a range of possible "results", uncertain which is the best one (or the most correct one). Moreover, the fact that baseline correction is not done on the imaginary part means that if one tries to iterate the whole process, any change in phase parameters brings back into the displayed real part the uncorrected baseline artifacts present in the imaginary part, thus making iterations of the whole procedure problematic.

In the past, much work was done on automatic (i.e., objective) phase and baseline corrections of NMR spectra. As a result, there exists a number of algorithms that work quite well, but sometimes exhibit with residual problems. So far, such algorithms were emulating the manual procedure. Basically, they all 'fit' the parameters which describe the phase (ph0, ph1) and the baseline (various parametrized models) so as to maximize some 'final quality' assessment of the corrected spectrum. Historically, the "quality functions" included peak heights, negative peak lobes, DISPA pattern symmetry, selected baseline points, peak ablation, etc. Clearly, the quality function Q(spectrum) is the heart of the problem because its maximum should match our subjective (and therefore ill defined) expectations about how a corrected spectrum should look. A typical case of fuzzy mathematical modelling!

Here we present a new type of the quality function Q, one based on the characteristics of a spectrum's histogram, which are very 'sensitive' to both phase and baseline distortions. On this basis we have devised a new algorithm which has also other strong points: first of all, it handles BOTH corrections simultaneously (PcBc rather than Pc + Bc), and it applies the Bc to BOTH the real and the imaginary parts of the spectrum.

We will describe the fully automatic algorithm we have developed starting from these premises, and illustrate the results achieved on a number of 1D spectra of various kinds.

References

A full collection of references would take up a couple of pages. We think that the audience of this presentation is well aware of the practical problem, and also of some of its current solutions. On the other hand, the idea of using histograms for a joint PcBc adjustment did not so far appear anywhere in the literature. We will make up for the lack of references as soon as possible, probably in a regular paper.

POSTERS

P1

NEW INSIGHTS INTO BOMBESIN INTERACTION WITH TUMOR CELLS EXPRESSING GRP RECEPTOR

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Bombesin (BN) is a 14-residue peptide originally isolated from the amphibian *Bombina bombina* [1]. It belongs to a family of peptides showing a variety of biological activities in numerous tissues and cell types, [2] exerted through their interaction with the Gastrin-Releasing Peptide Receptors (GRPR), transmembrane G-proteins coupled receptors triggering different signaling transduction pathways, resulting, among which, in the stimulation of cell proliferation. GRPRs are significantly involved in the pathogenesis of different human cancers [3], and are recently emerged as tumoral markers in early prostate and breast cancers diagnosis [4]. For these reasons, the research of new GRPR ligands as antagonists or carriers for cytotoxic and imaging molecular tools might be a promising strategy for the treatment and diagnosis of human tumoral malignancies [5]. In this scenario, structural data about BN binding to GRPR are required for the design and synthesis of high affinity receptor ligands, but, unfortunately, they are not yet available. BN conformation has been studied in various solvents demonstrating that it adopts an unordered structure in aqueous media and in dimethyl sulfoxide [6], while a partial helical structure has been observed in aqueous solutions containing TFE [7]. According to proposed models, this is the conformation that, probably, BN presents when anchored to biological membranes.
With the aim to verify the truthfulness of this hypothesis, we studied the effect of d_{25}-SDS (a biological membrane mimetic) on BN by CD and NMR spectroscopy. As for BN-GPCR interaction, the heptapeptide BN(8–14) has been shown to be the minimal carboxyl fragment interacting with the receptor, the same experiment were performed also on the BN C-terminal heptapeptide. Moreover, to discover the structural determinants of BN interaction with GRPR, the binding of both BN and BN(8-14) to human prostate carcinoma cell line (PC-3) over-expressing the receptor has been studied thought on-cell STD-NMR experiments.

Acknowledgements: Authors acknowledge AIRC for funding project 17030 - Targeting of Gastrin-Releasing Peptide receptor expressing tumors: NMR characterization of Bombesin/GRP-R interaction

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P2

LONG PLASMA HALF-LIVES OF LIPIDATED PEPTIDES: OLIGOMERIZATION OR ALBUMIN BINDING?

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Despite the biological potency of peptides as drugs, their effectiveness is limited by their short half-lives in plasma. Lipidation is a common strategy used in the design of peptide drugs for improving their pharmaceutical properties.
The presence of a lipid group in peptides modulates hydrophobicity, tendency to self-associate, and binding to albumin. The balance of these properties affects the circulating duration of lipidated pharmaceutical peptides. However, the molecular basis of the prolonged action of acylated peptides with long-chain saturated lipids is poorly understood.
Glucagon-like peptide-1 (GLP-1) is an incretin hormone that induces glucose-dependent stimulation of insulin and reduction in glucagon secretion, delays gastric emptying and decreases appetite. Liraglutide is a palmitoylated GLP-1 analogue approved for once daily treatment for type II diabetes. On the other hand, Semaglutide, an octadecandioic acid GLP-1 conjugate, is in phase 3 clinical trials for once weekly dosing.
In this work, we have investigated the oligomerization propensity and the serum albumin binding properties of lira and semaglutide analogues conjugated with fatty acids of different length. The aim is to better understand the equilibrium between oligomerized peptides, peptide monomers and albumin binding and try to find a correlation with the plasma half-life and the nature of the fatty acid in these GLP-1 analogues.

P3

TAU PROTEIN: ASSAYS FOR ENZYMATIC UBIQUITINATION

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The deposition of protein Tau in intracellular neurofibrillary tangles is a major hallmark of Alzheimer's disease (AD) and other tauopathies. Tau is a microtubule associated protein that modulates the stability of axonal microtubules. A large variety of post-translational modifications were found in Tau, including phosphorylation, glycosylation, acetylation, truncation, ubiquitination and prolyl-isomerization [1, 2]. Recent evidence indicated that, besides phosphorylation, polyubiquitin also marks Tau in paired helical filaments purified from AD brains [3]. Polyubiquitination regulates fundamental cellular pathways, including protein turnover by proteasomal degradation. Due to its role in the clearance of misfolded proteins, dysfunction of the ubiquitin-proteasome system was proposed to be one of the key mechanisms of neurodegeneration. Indeed, a failure of proteasome function may cause the accumulation of ubiquitinated proteins such as Tau, exacerbating aggregation and neurotoxicity. In this context, we aim to define the effect of polyubiquitination on the structural propensities of Tau, on its aggregation pathway to fibrils, and on its clearance. The implementation of this research requires the obtainment of high amounts of Tau modified at specific lysine residues with polyubiquitin chains. We are currently focusing our attention on a enzymatic method. Ubiquitination of substrates is catalyzed in vivo by three enzymes: ubiquitin-activating (E1s), ubiquitin-conjugating (E2s), and ubiquitin ligase (E3s). Substrate specificity is given by recognition of the target protein by E3. CHIP, an E3 enzyme which targets misfolded proteins towards proteasomal degradation, can ubiquitinate Tau in vitro in combination with Ubch5 (an E2) and E1 enzymes [4]. We tried to use Ubc13, an alternative E2 enzyme that was shown to interact with CHIP [5]. Our preliminary results show that both combinations of E1-Ubch5b-CHIP, and E1-Ubc13-CHIP can ubiquitinate Tau. In order to determine the ubiquitination sites on Tau, we plan to perform mass spectrometry analysis. The work is in progress to control the enzymatic reaction in order to obtain homogenously modified samples, and to extend the polyubiquitin chain.

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P4

STRUCTURAL BASIS OF THE LINEAGE-SPECIFIC ADAPTATIONS OF THE FOLD IN A NEW TRX-RELATED PROTEIN FROM *ECHINOCOCCUS GRANULOSUS* ABLE TO BIND Fe/S CLUSTERS

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Echinococcus granulosus, is a cyclophyllid cestode that parasitizes the small intestine of canids as an adult, but which has important intermediate hosts such as livestock and humans, where it causes cystic echinococcosis, also known as hydatid disease [1].
A unique aspect of flatworm metabolism is that these parasites are entirely dependent, for the control of redox homeostasis, on the enzyme thioredoxin glutathione reductase which provides electrons to thioredoxins (Trxs) and glutaredoxins (Grxs) at the expenses of NADPH [2]. This enzyme replaces the canonical thioredoxin reductase and glutathione reductase system. Parasitic tapeworm genomes have revealed an unexpected diversity of Trxs and Grxs [3], but the functions of most of these proteins have not been studied. A new Trx-fold protein in the tapeworm *Echinococcus granulosus* has been recently characterized and designated Iron-sulfur Trx-related protein (IsTRP) [4]. IsTRP lacks canonical reductase activity and the dimeric form of IsTRP coordinates Fe_2S_2 in a glutathione independent manner, relying on two adjacent cysteine residues highly conserved in the active site of Trxs. Interestingly classical Trxs do not coordinate Fe/S cluster and, as a matter of fact, this binding would be detrimental for the enzymatic activity. To allow the coordination of the cluster there must be some adaptation because the second cysteine is partially occluded in classical Trxs. With the aim to unveil the structure adjustment responsible, in IsTRP, for the replacement of canonical oxidoreductase activity for Fe/S binding, we have solved the high-resolution NMR structure of the apo-IsTRP and analyzed it with different computational tools including molecular dynamic simulations of the wt proteins and some of its mutants. Our analysis showed that, differently from what observed for classical Trxs, the two conserved cysteines are both exposed and therefore available to coordinate the Fe/S cluster. Furthermore, the replacement in IsTRP sequence of two highly conserved charged residues with two bulky hydrophobic sidechains occlude an internal water cavity that has be shown to be important for the catalytic activity of classical Trxs [5].

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P5

NMR-BASED METABOLIC PROFILING AND BIOLOGICAL CHARACTERIZATION OF *Peucedanum ostruthium*

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Peucedanum is a large and heterogeneous plant genus of the Apiaceae family that includes more than 120 species distributed in Europe, Africa Asia, and North America [1]. We focused on the study of the specie *Peucedanum ostruthium*, well known in the ethnopharmacological history for its antioxidant, antimycobacterial, antifungal, anti-inflammatory and healing activities. *P. ostruthium* is traditionally employed in Austria and Italy where it is called "Masterwort" because of its various applications as medicinal plant. The leaves and the rhizome are used for the preparation of teas, liqueurs and bitters or as powders applied on the skin [2]. According to this preliminar information, we decided to explore three extractive typologies. We selected extractions that were as close as possible to the traditional use of the plant: decoction, hydroalcoholic and Soxhlet extraction of leaves and rhizome. Then, we exploited NMR spectroscopy for the untargeted metabolic profiling of the extracts and for the quantification of the most representative metabolites. Among the metabolites identified in *P. ostruthium* extracts, we focused on the presence of a specific class of phenolic acid: chlorogenic acids (CGAs), secondary metabolites commonly found in plants with a various spectra of biological actions. CGAs exhibit anti-inflammatory, anti-oxidant and anti-carcinogenic activity and protection in neurodegenerative diseases [3]. Moving from these experimental evidences, we started to investigate the potential biological activities of our extracts through different approaches and from a molecular perspective. Firstly, the antioxidant activity of the crude extracts was evaluated by assessing their reductive potential (Folin-Ciocalteu method) and their ability to quench radical species (ABTS-TEAC and DPPH methods). Moreover, to verify the potential wound healing property of our samples, we are evaluating cell proliferation and migration *in vitro* in a scratch assay with NIH-3T3 murine fibroblasts. Finally, we are studying the molecular interaction of crude extract components with a neurodegenerative amyloid oligomer model (Aβ1-42 oligomers). In addition, we are testing their ability to inhibit Aβ1-42 oligomer-induced neurotoxicity by specific cellular assays on SH-SY5Y.

Acknowledgment: We thank Fondazione CARIPLO and Regione Lombardia for funding project 2015-0763.

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P6

INTERACTION STUDIES OF CXCR4-PEPTIDE COMPLEX IN LIVING CANCER CELLS BY NMR SPECTROSCOPY

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Chemokines are a large family of 8 to 12 kDa peptides that serve as chemoattractants for cellular activation, differentiation and trafficking. To date, about 50 chemokines have been identified in humans, and these have been grouped into four families - CXC, CC, CX3C, and XC - based on the arrangement of cysteine residues involved in the formation of disulfide bonds. The biological activities of chemokines are exerted via seven transmembrane domain G-protein coupled chemokine receptors [1]. The chemokine CXCL12 (stromal cell-derived factor-1a) binds to the CXCR4 and CXCR7 receptors, initiating divergent signaling pathways that result in chemotaxis, cell survival and/or proliferation, increased intracellular calcium and transcription of genes critical for cell inflammation and cancer metastases [2]. Hence, the CXCR4-CXCL12 pathway is considered an important potential cancer therapeutic target. Nevertheless CXCR4 inhibitors suitable for prolonged use as required for anticancer therapy are not available. In this respect, we have recently developed a few peptides able to bind CXCR4 and to inhibit CXCL12-dependent migration at low nanomolar concentration. Intraperitoneal treatment with these peptides drastically reduced the number of B16-CXCR4-derived lung metastases in C57/BL mice.
Here we present an in-depth NMR investigation of the interaction of selected peptides of that series, and some newly developed peptides with living cells expressing the CXCR4 receptor. To target membrane-bound receptors, several homonuclear NMR experiments, such as saturation transfer difference (STD), WaterLogsy (WL) and transferred NOESY (trNOESY) can be used on living cells, detecting binding events and providing information on the bound conformation of the ligands [3]. In fact, the data will enable us to identify (by STD and WL) the portions of the ligand in closest contact with the protein and to define (by trNOESY) the preferred conformations of the bound ligand.

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P7

NMR-BASED METABOLIC PROFILING OF HOP EXTRACTS AS POTENTIAL ANTI- AMYLOIDOGENIC AGENTS

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Beer is a widely consumed alcoholic beverage made from natural ingredients, malt, yeast, hop and water. The female inflorescence of hops (*Humulus lupulus* L.) is an indispensable ingredient of beer, containing bioactive molecules, which have been found to be useful for pharmacology or food industry [1]. Hop is characterized by a high content in aromatic compounds, among which several flavonoids, known to exert various biological activities and presenting a significant antioxidant capacity. We decided to screen different hop varieties employed in artisanal brewing (namely Cascade, Tettnang, Saaz and Summit) to obtain their NMR metabolic profiling, focusing in particular on their aromatic content. NMR-based molecular recognition studies [2] have been performed to verify the presence of Aβ1-42 ligands in hop extracts. Aβ1-42 is an amyloidogenic peptide whose oligomers have been described as the most toxic Aβ species *in vivo* and represent one of the major molecular target to develop potential drugs and diagnostic tools for Alzheimer's Disease (AD). Moreover, antioxidant and anti-amyloidogenic activities were evaluated by cellular and biochemical assays using differently enriched fractions of hops extract. The results show that the biological activities of different hops correlate with their content in aromatic compounds. This evidence is supported by ligand-receptor interaction studies performed on Aβ1-42 and by cellular assays, highlighting flavonoids and polyphenols as the main interactors. Furthermore, the very high antioxidant activity of hop extracts supports also an indirect beneficial action consisting in the reduction of the oxidative stress induced by the same amyloids on neurons. All together, these data confirm the potential of hops as source of health-promoting compounds, showing a great nutraceutical potential, and suggest its employment in the development of dietary supplements able in preventing neurodegeneration.

Acknowledgment: We thank Fondazione CARIPLO and Regione Lombardia for funding project 2015-0763

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P8

NMR-BASED METABOLOMICS OF SERUM: AN AUTOMATIC APPROACH FOR METABOLITE IDENTIFICATION AND QUANTIFICATION

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The metabolic profiling of serum can provide important information about physiological and pathological states and may aid understanding of the mechanism of disease occurrence [1]. A quantitative analysis of the metabolites contained in serum samples can be performed using ^{1}H NMR spectroscopy [2]. However, due to the complexity of this matrix, NMR-based profiling has remained manual, resulting in a slow, expensive, and error-prone procedures that have hindered clinical and industrial adoption of metabolomics via this analytical technique. Here we present an approach that exploits MestreNova software, and in particular its SMA (Simple Mixture Analysis) plugin [3], which can quickly, accurately, and autonomously produce serum sample metabolic profile in a very short time. In particular, we have implemented a library of about 40 compounds which, given a 1D ^{1}H NMR spectrum, can autonomously determine and quantify the metabolic profile in less than 1 minute. One of the greatest limitations related the NMR analysis serum samples depends on the fact that spectra include both sharp/narrow peaks, from small molecule metabolites, and broad peaks, from proteins and lipids. The size difference between macromolecules and small molecule metabolites provides an excellent basis for ultrafiltration of the serum. Nowadays, the easiest and most reliable method is to pass the sample through a 3 kDa molecular weight cut off (MWCO) micro-centrifuge filter in order to separate metabolites from proteins and other large molecules [4]. Nevertheless, this is a time-demanding procedure that implies risks in sample manipulation and loose of information regarding macromolecules contained therein. For this reason, we are exploring the possibility to filter NMR peaks *a posteriori* on the basis of peak linewidths as determined by globals spectral deconvolution [5]. This approach will allow to save time in sample preparation and to retain information from the high molecular weight components of serum.

Acknowledgements
We thank Fondazione CARIPLO and Regione Lombardia for funding project 2015-0763 and Regione Lombardia for funding project “VIBRO”.

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P9

ONE-POT MODIFICATION OF SEPIOLITE FOR TUNING FILLER-POLYMER INTERFACIAL INTERACTION

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The development of nanofillers capable to enhance the mechanical robustness of polymer nanocomposites depends on the ability of tailoring both structure and functionalities of inorganic fillers. Indeed, the reinforcement effect is produced not only by the filler network structure but also by the filler-polymer interfacial interaction. Among clay fillers, sepiolite is used due to easy availability, low cost, high specific surface area, mechanical and chemical stability and anisotropic particle shape. However, its hydrophilic surface leads to low affinity towards polymers and high self-aggregation, thus requiring an appropriate chemical modification. In this study, clay nanostructures functionalized with a silane-coupling agent (TESPT) are obtained starting from both pure and organically modified sepiolite through a simple one-pot route. The contemporary etching and silanization lead to the production of size-controlled needle-shaped nanofibers, preserving the clay anisotropic features. The effectiveness of sepiolite modification is proved by the excellent reinforcement properties obtained in styrene-butadiene rubber nanocomposites [1].

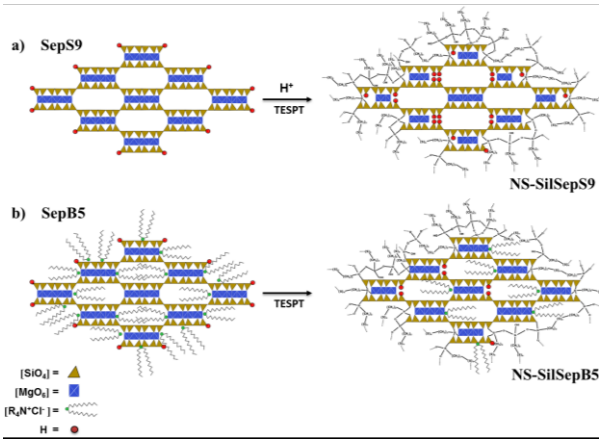

Fig. 1. Scheme for one-pot hybrid filler preparation from pure (sepS9) and organically modified sepiolite (SepB5) [1].

The changes of sepiolite structural features depending on the chemical treatment are assessed in particular by ^{29}Si, ^{13}C and ^{1}H MAS NMR with the complementary information obtained from FTIR, XRD and TEM analyses.

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P10

HYDRODYNAMICS OF β2-MICROGLOBULIN IN PRESENCE OF TWO MOLECULAR CHAPERONES BY NMR

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Protein aggregation into insoluble fibrils is nowadays considered the cause of many serious diseases [1]. A natural rescue system is represented by molecular chaperones which are able to prevent protein aggregation and misfolding [2].
Here we present a NMR investigation of β2-microglobulin (β2m) hydrodynamic properties in the presence of two molecular chaperones, namely clusterin and αB-crystallin. β2m is a paradigmatic amyloidogenic protein and it is itself responsible for dialysis-related amyloidosis (DRA) in patients affected by chronic renal failure [3]. ^{15}N NMR relaxation experiments were carried out in the absence and in the presence of the two molecular chaperones to measure longitudinal (R_1), transverse (R_2), rotating frame ($R_{1\rho}$) relaxation rates and $^{15}N\{^1H\}$ nuclear Overhauser effect (NOE) for the individual backbone amide nitrogens. In addition, also DOSY spectra were recorded to obtain the translational diffusion coefficients under the different conditions. The decrease of the average NOE values and of the rotational correlation time estimated from the relaxation rates [4] along with the increase of the translational diffusion coefficient of β2m in the presence of the molecular chaperones suggest that clusterin and αB-crystallin are capable of decreasing the average association extent that characterizes the actual intermolecular dynamics of β2m in agreement with previous observations [5] and experimental findings [6].

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P11

SOLID STATE NMR OF PEROVSKITES: CHARACTERIZATION OF DIFFERENT SOLID FORMS OF METHYLAMMONIUM LEAD IODIDE

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Halide Perovskites-based Solar Cells (PSCs) are rapidly emerging as a serious alternative to rival the established silicon photovoltaic technologies. Power conversion efficiencies have jumped from 3% to over 20% in few years of academic research [1, 2]. The class of methylammonium lead halide perovskites ($CH_3NH_3PbX_3$, X = I, Br, Cl) is one of the most studied and, within this class, methylammonium lead iodide, $CH_3NH_3PbI_3$, has particularly desirable properties, such as high optical absorption coefficients, long balanced charge carrier diffusion lengths, as well as the low-cost solution-processing techniques used to incorporate it in solar cells [3].
In this contribution we present a Solid State NMR (SSNMR) study of two forms of $CH_3NH_3PbI_3$ obtained by different preparation methods. We performed a multinuclear NMR characterization by the acquisition of high-resolution ^{207}Pb, ^{13}C, and ^{1}H Magic Angle Spinning (MAS) spectra. Moreover the relaxation properties of ^{1}H nuclei were investigated by low-resolution experiments, and proton longitudinal (T_1) and transverse (T_2^*) relaxation times were determined. Interesting differences between the two samples were highlighted and interpreted in terms of homogeneity/dis-homogeneity of the solid phases, structural and dynamic properties.

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P12

SYNTHESIS OF NEW GADOLINIUM COMPLEXES FOR MAGNETIC RESONANCE IMAGING WITH IMPROVED RELAXIVITY

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Prototropic exchange of mobile protons can play a significant role in enhancing relaxivity of gadolinium-based contrast agents (GBCAs) [1, 2], especially when the mobile protons are present on the ligand in proximity of the Gd-ion.
As evidence of this, the relaxivity of **Gd-Phen-DO3A** (9.09 ± 0.01 $mM^{-1}s^{-1}$) was proved to be twice the value of the commercial complexes Gd-DOTA and Gd-HP-DO3A (4.52 ± 0.03 $mM^{-1}s^{-1}$ and 4.90 ± 0.05 $mM^{-1}s^{-1}$ respectively) [1]. Interestingly, the effect of the prototropic exchange reaction occurs at physiological pH and a moderate protein binding property was also observed, which is known to give an additive contribution in enhancing the relaxivity value.
The coordination cage of this novel GBCA is the same of Gd-HP-DO3A but with an additional phenol moiety, which is essential to both prototropic exchange and protein binding (Fig. 1**)**.
A new series of GBCAs derived from Gd-Phen-DO3A was designed in order to rationalise the role of the phenol moiety in the determination of the prototropic effect. In particular, three novel macrocyclic Gd-complexes were synthesized in order to modify the distance between mobile protons and Gd ion (compounds **1** and **2**, Fig. 1), and to tune the pH value at which the prototropic effect is exploited (compound **3**, Fig. 1).

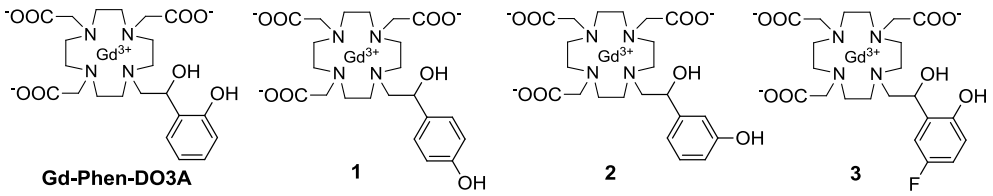

Fig. 1. Chemical structures of Gd-Phen-DO3A and new derivatives.

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P13

ISOLATION, PURIFICATION AND STRUCTURE ELUCIDATION OF TEN COMPOUNDS PRESENT IN AN ENDEMIC TOXIC PLANT OF ALBANIA, *Gymnospermium maloi*

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Gymnospermium maloi is an endemic toxic plant located in Gjirokaster, south region of Albania. From the botanic point of view *maloi* is determined as a new species and its chemical composition is poorly studied [1]. Until now, only some molecules are reported while the rest of components remain still largely unknown [2]. Here we present the chemical analyses of dichloromethane extract of root and aerial part of *Gymnsopermium maloi*. This investigation led on isolation and elucidation of ten compounds, mainly alkaloids and triterpenes. For what it concerns the separation, purification and isolation of these compounds, different chromatographic techniques such as MPLC, UHPLC, HPLC, analytical and semi preparative, equipped with a variety of detectors, UV, ELSD, TOF, Orbitrap, were used. While 2D homonuclear and heteronuclear NMR spectroscopy was applied to achieve their chemical structures elucidation. The major part of these pure compounds were biologically evaluated showing interesting anticancer and antibacterial effects. The presence of these compounds in *G.maloi* is reported here for the first time. The research work on this plant is ongoing in our lab attempting to elucidate the structure of possible new other molecules.

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P14

USING DYNAMIC NUCLEAR POLARIZATION-ENHANCED SOLID-STATE NMR TO STUDY THE POLYMORPHISM OF SMALL ORGANIC MOLECULES

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The first report on the phenomenon of polymorphism made its appearance in the scientific literature 185 years ago, when Wöhler and von Liebig described the changing nature of a mass of solid benzamide. Today, despite a large number of contributions aimed at understanding its main chemical and physical aspects, polymorphism is still enigmatic [1], yet it draws a lot of interest from both the academic and the industrial world.

One of the great challenges in this field is to understand how and by what means molecules interact to form periodic arrangements. In this context, natural-abundance ^{13}C-^{13}C double-quantum (DQ) experiments have been recently used to obtain quantitative structural data to detect small changes in the molecular conformation of theophylline polymorphs, and to discern among multiple crystal packings [2].

Here, we show how both standard 1D and advanced 2D ^{13}C-^{13}C DQ solid-state NMR experiments have been successfully employed to elucidate the polymorphic changes of a series of small organic molecules exhibiting multiple crystal forms. The DNP-enhanced ^{13}C CPMAS spectra were collected with different polarization times to reveal differences between the bulk and the surface of crystallites; 2D ^{13}C-^{13}C DQ spectra were useful to characterize the π–π stacking and assess subtle changes in the crystal packing of polymorphs.

This method ultimately offers structural constraints which can be coupled with powder X-ray diffraction data and crystal structure prediction calculations to solve powder structures.

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PROBING THE CO_2 BINDING SITES IN A POROUS-ORGANIC POLYMER BY COMBINING SOLID-STATE NMR AND CALCULATIONS

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Solid-state NMR is particularly suited to investigate gas adsorption in several types of materials [1,2]. Indeed, depending on the type of experiment, it can either highlight signals arising from the host structure through cross-polarization (CP) experiments or the gas signal through direct excitation experiments. However, this approach is yet to be applied to compounds which lack long-range order such as porous organic polymers (POPs).

We report on the utilization of 1D and 2D ^{13}C CPMAS and MAS SSNMR spectroscopy in probing the binding sites and dynamical processes of ^{13}C-enriched CO_2 insides the pores of a pyridine-containing porous organic polymer (POP) (Fig. 1) [3]. Our findings from the 1D ^{13}C CPMAS and ^{1}H-^{13}C off-resonance FSLG-HETCOR NMR (Fig. 2) spectra of the sample dosed with $^{13}CO_2$ both at 30 and 800 mbar indicated preferential adsorption at the nitrogen pyridine rings of the POP. Further information on dynamic processes among CO_2 species within and outside the POP cavities is also achieved from ^{13}C static and MAS spectra. The results are discussed in terms of fast-exchange processes between physisorbed CO_2 and free CO_2 coexisting in the nanospace within the POP or in the outer space near the entrance.

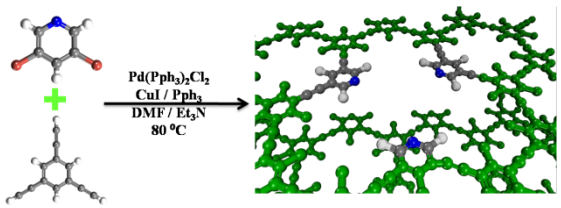

Fig. 1. Synthesis for the Pyridine POP with a structural model of a potential oligomer chain. Carbon (grey), oxygen (red), nitrogen (blue), hydrogen (white), repetitive part of the structure is shown in green.

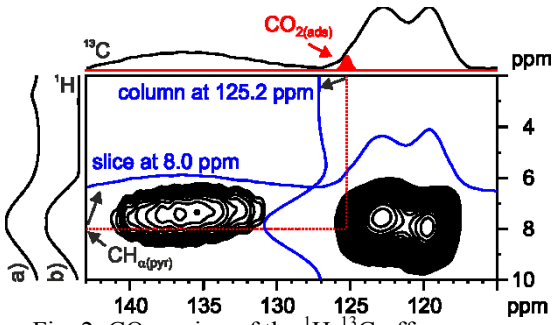

Fig. 2. CO_2 region of the ^{1}H-^{13}C off-resonance FSLG HETCOR spectrum of POP loaded with 800 mbar of CO_2 highlighting the correlation between pyridinic CH_α and adsorbed CO_2.

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P16

DFT-NMR METHOD AND COMPUTATIONAL STUDIES FOR THE IDENTIFICATION OF LIMONOL DERIVATIVES AS HEAT SHOCK PROTEIN 90 (HSP90) INHIBITORS

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The identification of inhibitors of Hsp90 is currently a primary goal in the development of more effective drugs for the treatment of various types of multi-drug resistant malignancies. In the attempt to identify new small molecules modulating the activity of Hsp90, we screened a small tetranortriterpenes library from *Cedrela odorata* L. (Meliaceae) [1]. Their structures were determined via spectroscopic analyses and chemical methods. The Hsp90 inhibitory activity of compounds **1**-**16** was evaluated by means of a panel of chemical and biological approaches. Three limonol derivatives (**1**, **3** and **5**) were shown to bind Hsp90, to inhibit its ATPase and chaperone activities and to cause a depletion of some Hsp90 client proteins. To completely elucidate the mechanism of action of these compounds, computational methods (DFT-NMR method, molecular docking and molecular dynamics) were used to identify the Hsp90 region involved in the molecular interaction with these plant small molecules (Fig. 1) [2].

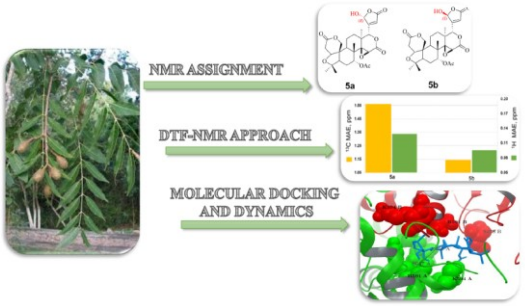

Fig. 1. Combined approach to identify limonol derivatives as Hsp90 inhibitors.

To rationalize the biological effects of limonol derivatives, molecular docking and molecular dynamic studies between **1**–**5** and Hsp90a protein were performed (Schrödinger Suite). Structural data provided for the Hsp90a/**1** and Hsp90a/**5** complexes have allowed us to identify some key interactions required for the binding of small molecules to the C-terminus of the protein. Our results could be crucial for the design of new C-terminus inhibitors of Hsp90.

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P17

NMR-BASED METABOLOMICS STUDY OF BRONCHOALVEOLAR LAVAGE FLUID (BALF) FROM BRONCHIOLITIS OBLITERANS SYNDROME (BOS) PATIENTS

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Bronchiolitis obliterans syndrome (BOS) is the main phenotype of an irreversible obstructive graft dysfunction known as chronic lung allograft dysfunction (CLAD), which challenges patient survival after lung transplantation. CLAD diagnosis relies on functional parameters and presents a significant heterogeneity in the pathology evolution. Therefore, tools that will help unravel the complexity of the disease and identify useful predictive markers are urgently needed. Metabolomics is a platform capable of capturing disease-relevant metabolic profile changes and molecular signatures of disease processes. To this purpose, NMR spectroscopy was employed for the metabolic profiling of bronchoalveolar lavage fluid (BALf) from lung transplant recipients without BOS (stable subjects), and with BOS at different degree of severity: potential BOS (BOS 0p) and established BOS (BOS I) [1]. The tuning of several parameters was performed to design an efficient and reproducible protocol for the screening of metabolites in a pulmonary fluid that should reflect the status of airway inflammation/injury. Exploiting the combination of mono and bi-dimensional NMR experiments, 38 polar metabolites, including amino acids, Krebs cycle intermediates, mono- and di-saccharides, nucleotides and phospholipid precursors were unequivocally identified, in substantial agreement with previously reported assignments [2]. In order to correlate the metabolic signature with the onset of BOS, metabolites' content of the above recipients was analyzed by multivariate (PCA and OPLS-DA) statistical methods. PCA analysis differentiated stable subjects from BOS I patients and this discrimination was significantly improved by the application of OPLS-DA. The analysis of stable vs BOS 0p and of BOS 0p vs BOS I samples showed a clear discrimination of considered cohorts, although with a poorer efficiency compared to those measured for stable vs BOS I patients, these results being in agreement with the development of the disease. The NMR data pointed out the potential of this methodology for the identification of predictive biomarkers to unravel and monitor serious post-transplant lung conditions, including BOS. These preliminary results strongly support the possibility to afford a metabolic signature of BOS from NMR BALf analysis.

Acknowledgements. This work was financially supported by Fondazione CARIPLO grant #2013-0820 and Fondazione Cariplo - Regione Lombardia grant #2015-0763.

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P18

POTENTIAL NEUROPROTECTIVE ACTIVITY OF COFFEE EXTRACTS AND THEIR PRINCIPAL COMPONENTS: THE NMR POINT OF VIEW

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A number of beneficial biological properties have been described for coffee and, in particular, for chlorogenic acids (CGAs), the most abundant family of polyphenols contained in green coffee extracts [1]. They include anti-oxidant, anti-inflammatory, anti-aging activity and also protection in neurodegenerative diseases, such as Alzheimer's disease [2]. Also melanoidins, the high molecular weight macromolecules produced during coffee roasting, are known for their anti-oxidant, metalchelating and antimicrobial properties. However, the molecular mechanisms through which these biological activities are carried out have not been completely elucidated.
Here, the NMR-based screening of green and roasted coffee extracts for the presence of compounds responsible for neuroprotective activity is presented. Six coffee varieties, with different geographical origins, have been selected: three of them belonged to the specie Arabica (Brazil, Colombia, Burundi) and the others to the specie Robusta (Tanzania, Uganda, Vietnam). The metabolic profiles of the extracts obtained from the different coffee beans were characterized and compared by NMR spectroscopy.
The biological activity of green and roasted coffee extracts, fraction enriched in high molecular weight melanoidins and pure 5-CGA, the most abundant phenolic constituent from CGAs family found in coffee beans, was evaluated. In particular, the molecular interaction with a neurodegenerative amyloid oligomers model (Aβ1-42 oligomers) was evaluated by means of STD-NMR and trNOESY-NMR spectroscopy [3]. Moreover, their antioxidant and anti-amyloidogenic activities were evaluated by cellular and biochemical assays, validating the existence of a correlation among the recognition of the molecular targets and the biological responses. Altogether, our results highlight how the biological efficacy of the different extracts can be related to their metabolic profiles.

Acknowledgements
Coffee samples were kindly provided by Beyers Koffie, Belgium.
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P19

NMR CHARACTERIZATION OF HEMP PRODUCTS

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For many decades, industrial cannabis (*Cannabis sativa L.*) has been an important crop in Italy both for agronomic and for economic reasons. In fact, Italy was the second largest hemp producer, second only to Russia, and the largest exporter. Since the late 1970s cannabis cultivation was prohibited because of a psychotropic substance, tetrahydrocannabinol (THC), contained in the inflorescences, and consequently all the varieties disappeared from the territory, including the traditional varieties of Italian origin appreciated all over the world to be the best. The rediscovery of hemp in Italy is closely related to the Circular of Ministry of Agricultural and Forestry Policies of 2 December 1997 (G.U. n. 62 of 16-3-1998) [1] which disciplinate *Cannabis sativa* cultivation.
In the project *e*-ALIERB, we proposed a NMR characterization of hemp inflorescences and hemp processing products, namely hempseed oil, hemp flour, hemp flavored beer. Both ^{1}H NMR spectra and 2D experiments (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) were carried out. In hemp inflorescences study, four different cultivar (Uso31, Felina32, Ferimon and Fedora17) at four different harvest times (on 16 June 2016, on 12 and 28 July 2016, and on 1 September 2016) were analyzed. Various hemp processing products were also analyzed: two hemp flavored beers produced in 2016, two hempseed oils obtained by cold pressing of seeds coming from mixed cultivar (mainly Uso31) and produced on November of two successive years (2015 and 2016), a sample of hemp flour obtained by grinding Uso31 cultivar hempseeds and produced in 2016. Hempseed oil samples and hemp flour organic extract showed comparable spectra, containing characteristic lipophilic compounds such as fatty acids, triglycerids and sterols. Inflorescences and flour hydroalcoholic extracts are also comparable because of their similar content in carbohydrates, amino acids, organic acids and other compounds. June-harvested inflorescences of 3 different cultivar (Uso31, Felina32, Ferimon) were compared and Ferimon showed to have the highest concentrations of metabolites. Ferimon inflorecences of 3 different harvest times were analyzed to understand the trasformation of metabolic profile during the ripening of the plant. It showed that amino acids and organic acids content decreased among the time while glucose had an inverse trend.

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P20

^{1}H NMR METABOLOMIC PROFILING APPROACH TO IMPROVE THE OUTCOME PREDICTION FOR CANCER TREATMENTS

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The evaluation of the metabolomic profiling by Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful and reliable tool for the identification of total metabolites present in different biological fluids such as sera under a given physiological condition and cancer and in a defined temporal state, and is able to identify new diagnostic/prognostic biomarkers. NMR is the only non-destructive technique that can identify and quantify complex mixtures of metabolites using small sample volumes and little sample preparation.
Tacking advantage of a 600-MHz NMR spectrometer with cryoprobe, equipped with an automation system, we performed metabolomic profiling on liquid biopsy samples collected in melanoma and colorectal cancer patients at different time points before and during treatment. In detail, metabolomic profiling was evaluated on metastatic colorectal patients subjected to first line bevacizumab plus chemotherapy and on metastatic melanoma patients subjected to different immunotherapy treatments.
Using Orthogonal Projections to Latent Structures discriminant analysis (OPLS-DA) and Loading Plot, we were able to group in separate clusters the patients with different outcome and to identify a set of metabolites that, either before or during treatments, can discriminate patients with favorable than those with worst outcome.
Overall, the obtained results suggest that metabolomic profiling represents a useful approach to improve outcome prediction for cancer treatment.

P21

METAL IONS: INFLUENCES ON PROTEIN STRUCTURE, FOLDING MECHANISM AND FIBRIL FORMATION

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Metal ions are known to take part in many aggregation processes directly related to the pathogenesis of numerous neurodegenerative disorders [1]. Furthermore, metal cofactors can radically modify the folding pathway of a protein even when the native structure itself does not a priori require such a cofactor.
We demonstrate how Ml4 (a metal-free prokaryotic zinc finger) folds via an apparently classic two-state cooperative transition while Ros87 (the metal-binding structural homologue) shows a two steps folding mechanism that features a well-structured zinc-binding intermediate [2]. A delicate barrier-less downhill transition connects the intermediate to the native state. We describe also the amyloidogenic propensities of these two iso-structural globular proteins. The results obtained show that within 168 hours amyloid formation has already started in Ros87, while Ml4 is still monomeric in solution. Overall, this study proves how different folding mechanisms, here induced by metal binding, significantly affect amyloid fibril formation propensity of highly homologous proteins.

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P22

A THEORETICAL DECOUPLING METHOD FOR PARALLEL TRANSMISSION RF COILS AND WORKBENCH TEST

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The availability of high B_0 field MRI scanners and consequent shortening of the working wavelength up to values comparable with the sample size [1] is one of the reason for the increasing popularity of Parallel Transmission (PTx) [2], a technique that permits a fine control of the exciting field B_1^+ [3]. To this purpose a specific hardware with a plurality of RF coils is usually engineered such as to reduce mutual coupling, by geometrical or electrical methods, at a negligible level [4-6]. The decoupling procedure becomes increasingly cumbersome when the RF coil number increases and their sensitivity profiles overlap considerably. In this work we present a novel approach to PTx RF coils design based on a simple geometrical design and an analytical decoupling procedure. We have designed and built an 8 channels TEM array suitable for pre-clinical scanner (mice/rat application) at 2.35 T with the PTx elements disposed like the legs of a birdcage and connected to a ground shield. Using the system geometrical symmetries we demonstrate for the first time, by FEM numerical simulation (HFSS v.17.2) and workbench measurements that it is possible to tune and match the eight transmitters using the same tuning/matching capacitor values with a considerably constructive simplification. We have developed an analytical decoupling method based on the calculation of the N*N complex impedance Z matrix, relating the voltage on the ports and current of each transmitter. Such matrix can be obtained by the measurement of the coupling between elements of the RF array and by the knowledge of the lumped impedances. The efficacy of our analytical decoupling method is fully demonstrated by numerical modelling. By using RF signals of appropriate phase and amplitude on each port, it is possible to null the currents induced from one RF coil element into the others. Workbench measurements demonstrate the practical feasibility of this approach and its stability was tested against load presence and small variations of the lumped elements values. Finally the coil efficiency was evaluated and compared to the one of a geometrically equivalent high-pass birdcage RF coil. The comparison shows that the latter is more efficient in transmission but theoretical arguments are given to suggest that, when used in receive-mode, the two approaches are substantially equivalent.

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P23

T_1 AND T_2 TO ASSESS MEMBRANE WATER PERMEABILITY AND HEMOZOIN GENERATION AS BIOMARKERS IN *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELLS

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Invasion and progress in the asexual intra-erythrocytic maturation of *P. falciparum* induce stage-dependent changes of biophysical properties of the host cell, such as membrane stiffness and transmembrane water exchange. Additionally, the parasite generates growing amounts of hemozoin (HZ) during its maturation. Transmembrane water exchange and HZ generation can be considered malaria biomarkers as they mirror the parasite developmental stage (Rings, trophozoites and schizontes) to and can act as reporters for monitoring the effect of antimalarial molecules. Herein, we propose to analyze *in vitro* cultures of synchronized *P. falciparum*-infected RBCs by using MR relaxometry (MRR). The study includes the use of i) T_1 as quantitative biomarker to assess changes in membranes' permeability to water molecules by adding Gd-HPDO3A in the extracellular compartment [1] and ii) T_2 as quantitative biomarker to assess HZ formation. MRR can be used to assess the stage of parasites' maturation and the effect of antiparasitic drugs. In fact, the combination of both MRR parameters T_1 and T_2 allows to determine whether a molecule produces i) maturation delay of *P.falciparum* in the host cell; ii) intracellular inhibition of hemozoin generation; iii) changes in RBC and parasite membrane water permeability. Preliminary results have shown that water exchange rate (Kex) decreases by 50% on passing from not-parasitized RBC to ring stage (first stage of parasites' maturation), then Kex increases to reach the value of not-parasitized RBC by passing from rings to trophozoites and schizontes state (Fig.1).
T_2 is an excellent reporter of the presence of Hz crystals. We have found that measurements at different magnetic field strength (20- 80 MHz range) yields much more reliable results when compared to values obtained at a single field.The method was used to test the effect of dihydroartemisinin (DHA) and chloroquine. A different effect of the two molecules has been found. DHA modifies water membrane permeability and slows down the parasite maturation. Conversely, chloroquine acts by reducing HZ formation (T_2 effect) but does not affect the water membrane permeability.In conclusion, MRR is an efficient tool to investigate *P. falciparum infection making possible to i)* determine the maturation state in intact host cells without further manipulation and ii) monitor the effect of drugs (faster and more objective than. microscopy).

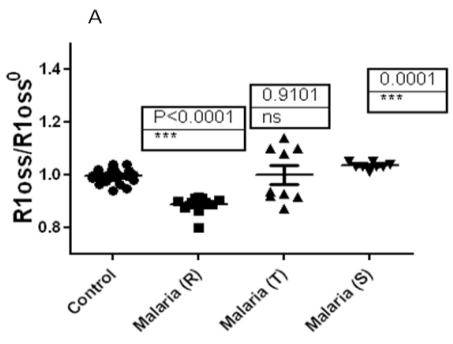

Fig. 1. Normalized R_{1obs} for cRBCs and iRBCs at three stages of malaria infection (R= Rings, T=Trophozoites, S=Schizonts).

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P24

DEVELOPMENT AND IMPLEMENTATION OF A LOW-FIELD NMR SYSTEM

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The aim of this research is to develop a small size low-field portable NMR system to be used in industrial and research on site applications.
A permanent magnet system, designed using finite-element modelling software, has been built to produce a static field of $\vec{B}_0 = 0.48$ Tesla. A single coil was used for both transmitting the excitation pulses and detecting the NMR signal. The probe circuit is essentially an LC tank with a tunable resonant frequency. Signal transmission and post-processing are all managed by programming Field Programmable Gate Array (FPGA) devices.
All electronics circuits were made using modern techniques, design and testing of printed circuit boards at the NEMES_LAB (Nuclear Electric Measurement & Electronics System Lab) Laboratory of the Physics Department.

P25

VALIDATING NMR METHODS FOR FINGERPRINTING AND SIMULTANEOUS MULTICOMPONENT QUANTITATIVE ANALYSIS

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The goal of this work was to set up validation procedures and new quality control parameters suitable for performance assessment in simultaneous multi component quantitative NMR analysis and NMR fingerprinting methods. In order to achieve the goal, three inter-laboratory comparisons (ILCs) were organized. The first one [1,2] consisted in the analysis of wheat and flours aqueous extracts (4 samples) and was aimed to ascertain the statistical equivalence of the scaled NMR spectra. Seven signals were submitted to univariate internationally agreed statistics typically applied in performance assessment of ILC participants. The second ILC [3] regarded a model mixture made up of five compounds. In particular, a model mixture made up of five compounds [Aldicarb, Methamidophos, Oxadixyl, Pirimicarb and 3-(trimethylsilyl)-2,2,3,3-tetradeutero-propionic acid sodium salt (TSP)] dissolved in deuterated water was submitted to NMR analyses. The analytical target of the second ILC was the quantification of analytes by the calibration line method. Such a method was chosen as it allows for identification of a theoretical line to be taken as reference in performance assessment. The third ILC consisted in the analysis of wine grape juice (7 samples) and was aimed to confirm the statistical equivalence of the scaled NMR spectra and to quantify several metabolites by standard addition method. Results show that quantitative NMR is a robust quantification tool. Performance assessment was carried out on single component quantification, by the popular and traditional z-score, and on multi-component analyses by means of a new performance index (named Q_p-score) which is related to the difference between the experimental and the consensus values of the slope of the calibration lines. Qp-score is a parameter suitable for harmonization of fingerprinting protocols and simultaneous quantitative multi component analysis. Such parameter, that was designed considering consolidated internationally agreed statistics, represents an unbiased evaluation tools for NMR method validations.

Acknowledgments: All the researchers listed in references 1 and 2 as participants to ILCs and the participants to the third ILC are gratefully acknowledged.

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P26

NMR STUDY OF NEW FARNESYL PIROPHOSPHATE SYNTHASE INHIBITORS

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Farnesyl Pirophosphate Synthase (FPPS) is a key enzyme in the mevalonate, biosynthesis [1]. FPPS catalyzes the reaction where isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are coupled to produce geranyl pyrophosphate (GPP), which is then condensed with an additional IPP to produce farnesyl pyrophosphate (FPP) [2]. N6-Isopentenyladenosine (i6A) is a modified nucleoside exhibiting anti-tumor effects on human and murine cells.[3] Previously we demonstrated the structural interaction of i6A with the enzymatic pocket of FPPS by recording saturation transfer difference (STD) NMR experiments. The data extracted from the STD experiments indicate that the isopentenyl moiety and the purine ring of i6A are directly involved in the interaction with FPPS binding pocket. Later we made changes on the N6 adenosine position of i6A, in order to make a real probing of FPPS enzyme and to obtain new potential FPPS inhibitors [4]. Here we present the NMR study of interaction of new i6A analogs with FPPS enzyme. Additionally, we report an innovative NMR based approach to measure the FPPS inhibitory activity of the newly synthesized inhibitors.

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INSIGHTS INTO THE MATURATION OF IRON REGULATORY PROTEIN 1

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Aconitase is a citrate/isocitrate hydrolase containing a [4Fe-4S] cluster as catalytic center. The [4Fe-4S] cluster acts as a Lewis-acid in the elimination of the hydroxyl from the substrate. The apo form of cytosolic aconitase, known as IRP1 (iron regulatory protein 1), binds to iron-responsive elements (IREs), untranslated regions of mRNAs of proteins involved in iron homeostasis and trafficking [1].
The in vivo biosynthesis of Fe/S clusters and their insertion into various apo target proteins is a process that requires complex proteinaceous machineries [2]. Depending on the cluster types and the organisms, several specialized assembly systems have evolved. In particular, the CIA (cytosolic iron–sulfur protein assembly) machinery in the cytosol of eukaryotic cells is essential for generating [4Fe-4S] clusters for both cytosolic and nuclear Fe/S proteins. Numerous proteins involved in the CIA machinery have been recently identified and much progress has been made in understanding their molecular function [3, 4]. Within this frame, it has been recently suggested that human cytosolic CIA2A in complex with CIAO1 protein has a role in the conversion of apo IRP1 to cytosolic aconitase [4]. The latter process has not yet been investigated in detail and the mechanism by which the two CIA components (CIA2A and CIAO1) assist the IRP1 assembly process is currently unknown.
We investigated IRP1 maturation process by characterizing the role of the CIA2A-CIAO1 complex in transferring the Fe/S cluster to IRP1 via a combination of NMR, UV−vis and electron paramagnetic resonance (EPR) spectroscopy, unraveling the function of the complex as a dedicated maturation factor of IRP1.

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P28

THE STRUCTURAL DETERMINANTS OF SELECTIVITY IN PROTEIN-PROTEIN INTERACTION: THE STEPWISE CSP NMR REVEALS THE BINDING PREFERENCE OF CUBAN DOMAIN FOR NEDD8

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NMR spectroscopy and molecular dynamics were applied to determine the structure of CUBAN domain in solution and its interaction with NEDD8 [1]. The results reported the structure of this domain in solution and in the interaction with NEDD8. The goal of the new results are addressed to elucidate the determinants of the selectivity in the mechanism of binding of CUBAN domain (597- 678) toward NEDD8 with respect to ubiquitin. The results indicated that in this protein-protein recognition several interactions take place of different nature creating a complex interplay between hydrophobic and polar interactions thus leading to a different recognition of the substrates. These interactions appear different in the interaction of CUBAN with NEDD8 and ubiquitin respectively and, thus, appear the basis of the sclectivity and of the structural preference. The use of stepwise chemical shift perturbation of the ^{15}N HSQC NMR spectrum of the protein upon increasing the molar ratio of the partner involved helps to give the picture of this complex number of interactions.

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P29

^{1}H NMR RELAXOMETRIC STUDY OF THE INTERCALATION OF Gd(III) COMPLEXES INTO SYNTHETIC SAPONITES

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This project is aimed at the study of a novel class of paramagnetic clays obtained by intercalation of Gd^{III}-complexes in the interlamellar space of synthetic sodium-saponite. A sodium-saponite sample, (Na-SAP-110) with dimensions in the range of 50-100 nm (as evidenced by TEM and DLS measurements) was prepared as a starting material that shows a good suspendability in water. The cationic exchange capacity (CEC) of the Na-SAP-110 was determined by UV-Vis-NIR spectrometry and corresponds to 37 meq/100 g. Two Gd^{III}-complexes, selected for their good thermodynamic and kinetic stability, have been synthesized with ligands based on DOTA (GdL1, Fig. 1) and AAZTA (GdL2, Fig. 1) macrocycles. The complexes GdL1 and GdL2 were introduced into the saponite through a cationic exchange procedure in acidic aqueous medium: the final products were named as GdL*n*/SAP-110 (Fig. 1). XRD, HRTEM and TGA studies confirmed the success of the intercalation process of the complexes in the interlamellar space of saponite. In particular, TGA study allowed to observe that when intercalated both Gd(III) complexes are stabilized from a thermal point of view and this suggests that they are located in the interlamellar space of the saponite clay. After the intercalation, the suspendability of the paramagnetic clays nanoparticles was very good, maintaining the nanometric size of the starting sodium saponite. A detailed NMR relaxometric study was performed on both complexes and on the intercalated materials. By analyzing the relaxivity values as a function of the applied magnetic field and temperature, we observed that the introduction of the paramagnetic complexes into interlayer of saponite causes a substantial change of their rotational dynamics, slowing down their tumbling. One consequence of the limited molecular motion is that relaxivity values at high magnetic field of GdL*n*/SAP-110 materials increased significantly compared to the isolated GdL*n* complex (Fig. 1). The water exchange in the GdL*n*/SAP-110 samples decreases compared to the relative isolated complexes, probably due to diffusional problems of water in the interlamellar layer of saponite. The stability of GdL*n*/SAP-110, finally, was very good, both in aqueous solution and in physiological medium (Seronorm™ matrix) and this opens the way for their possible use as probes for applications in biomedical research.

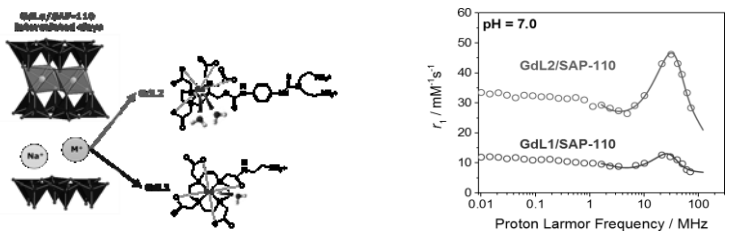

Fig. 1. *(sx)* Structure of GdL*n*/SAP-110 paramagnetic clays with the intercalated Gd^{3+}-based complexes GdL1 and GdL2. *(dx)* $1/T_1$ ^{1}H-NMRD profiles of GdL*n*/SAP-110.

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GIFFONINS, CYCLIZED DIARYLHEPTANOIDS, FROM DIFFERENT PARTS OF HAZELNUT TREE (*CORYLUS AVELLANA*), SOURCE OF THE ITALIAN PGI PRODUCT, "NOCCIOLA DI GIFFONI"

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Diarylheptanoids are a class of natural products based on the 1,7-diphenylheptane skeleton, known to possess various biological activities, including leishmanicidal and antiprotozoal, antitumor, anti-inflammatory, and inhibitory on nitric oxide production [1]. There exists a smaller number of cyclic diarylheptanoids that are formed from the corresponding linear type by phenolic oxidative coupling, either C−C coupling leading to meta,meta-bridged biaryls or C−O coupling leading to bridged diaryl ethers. Diarylheptanoid derivatives occur frequently in plants belonging to the Betulaceae family, but so far only one report deals with their presence in the leaves of *Corylus avellana* [2]. With the aim to valorize the species of *C. avellana* source of the Italian "Nocciola di Giffoni", also known as "Tonda di Giffoni", a labeled PGI (protected geographical indication) product of the Campania region, the phytochemical investigations of leaves, flowers and leafy covers were carried out. From the MeOH extract of the leaves 16 new phenolic compounds were isolated. Their structures, elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, TOCSY, ROESY) experiments as well as ESIMS analysis, resulted to be diarylheptanoid-type molecules, characterized by oxygenated functions at different positions of the heptanoid chain, named giffonins A-P [2,3]. Chemically, giffonins A-H are characterized by the presence of only one stereogenic centre on the heptyl moiety; for these compounds the absolute configuration was established through the application of the modified Mosher's method [2]. For giffonins J-P, identified as highly hydroxylated cyclized diarylheptanoids by 1D- and 2D-NMR experiments, and possessing at least two stereogenic centres on the heptyl unit, the relative configurations were established by a combined QM/NMR approach, comparing the experimental ^{13}C/^{1}H NMR chemical shift data and the related predicted values [3]. From the MeOH extract of the leafy covers of *C. avellana*, two new cyclic diarylheptanoids, giffonins T and U, along with the diarylheptanoid carpinontriol B were isolated. The absolute configurations of carpinontriol B and giffonins T and U were assigned by comparison of their experimentally electronic circular dichroism curves with the TDDFT-predicted curves after the determination of their relative configurations performed as reported for giffonins J-P [4]. Furthermore, from the methanolic extract of the male flowers of *C. avellana* were identified three previously undescribed diarylheptanoids, named giffonins Q-S [5].

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NMR STRUCTURE ELUCIDATION OF BIOACTIVE METABOLITES ISOLATED FROM NORTH AFRICA SPECIES

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Plant-based remedies continue to play a key role in the health care of people in north Africa (central Sahara, Fig. 1), where wild indigenous medicinal plants have been identified, and are currently used by the local population for various illnesses [1]. In the last years, an international cooperation between Italy and Algeria states has been developed to provide phytochemical and biological investigations on saharian endemic plants, supporting their traditional uses with scientific information.
The studies led to the isolation of known and new bioactive molecules, and their chemical structures were elucidated by 1D and 2D homonuclear and heteronuclear NMR spectroscopy. From medicinal species belong to Thymelaeaceae, Myrtaceae, Cystaceae, Asteraceae family were identified for the first time several sub-classes of secondary metabolites, including terpenoids and polyphenol derivatives [2, 3] and their antioxidant, antiinflammatory, antimicrobial, anticancer properties were verified.

Fig. 1. Tassili N'Aijer region in Central Sahara

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P32

NMR-BASED METABOLOMICS TO UNDERSTAND THE ABSCISSION PHENOMENON OF OLIVES AND FOR THE CHARACTERIZATION OF WINES

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Metabolomics, is one of the latest 'omics' sciences and deals with the study of global changes in the entire metabolite set of certain cells, tissues and organisms. The application of metabolomics in food systems, named "foodomics", can be applied to all food system processes from farm to human, including food resource production, industrial food processing and food intake by humans. The metabolites present in food matrices are affected by multiple factors such as botanical or geographical origins, cultivars, climates, storage conditions, biological and physiochemical processing. Therefore, the comprehensive and quantitative analysis of metabolites, can provides the detailed features of foods. Nuclear Magnetic Resonance (NMR) is one of the most suitable techniques to obtain "high-throughput" analytical data in metabolomics, and it gives a complete view of the foodstuff (e.g., olive oil, wine, fruit juice, honey) metabolites. The distinguished advantages of NMR are its nondestructive and noninvasive nature and its ability to detect quantitatively and reproducibly a broad range of substances with different chemical properties such as sugars, lipids, organic acids and so on. Multivariate and univariate statistical analyses not only define the differences among food matrices based on their NMR spectra, but also suggest candidate biomarkers contributing to the features of food. Regarding the application of NMR in food metabolomics, herein I will present two different studies. The first one aims to identify mechanisms responsible for olive fruitlet drop of the cv Frantoio, a widely cultivated Italian olive cultivar. *Olea europaea* is one of the most important and widespread fruit trees in Mediterranean basin. Olives are a product of great economic importance for EU (about 73% of the global olive oil production). The low yield (and the high costs of the grove management) makes olive growing as a less profitable cultivation if compared with other crops with the risk of land abandonment. Since no specific information is available to understand the molecular mechanism and metabolic processes inducing fruitlet abscission in olive tree, this project aims to use metabolomics as an essential step to find possible practical solutions to reduce the incidence of fruitlet shedding and increase the final yield. The second one is a research work related to applications of NMR spectroscopy in combination with multivariate statistical analysis for the characterization and authentication of Tuscan wines. In this study, we demonstrate that NMR metabolomics is a very powerful analytical tool to investigate the variability of the metabolic profile of wines, not only to assess the grape variety, vintage and geographical origin, but also to distinguish wines produced in terracotta jars or in steel barrels.

P33

EVALUATION OF HELICAL PEPTIDES AS INHIBITORS OF SAM-SAM INTERACTIONS INVOLVING THE EPHA2 RECEPTOR

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EphA2 is a tyrosine kinase receptor involved in different physiological and pathological conditions; in particular, EphA2 is related to cancer, where it is often up-regulated [1]. Improved receptor endocytosis and consequent degradation can be exploited as an appealing strategy to decrease EphA2 over-expression in cancer cells and reduce tumor malignancy [2]. The cytosolic Sam domain of EphA2 (EphA2-Sam) can be considered a target for a rational drug-design of anti-cancer compounds since it engages protein modulators of receptor endocytosis (such as the lipid phosphatase Ship2) and stability (like the adaptor protein Odin) through heterotypic Sam-Sam interactions [3]. EphA2-Sam heterotypic complexes are highly driven by electrostatic contacts [4], thus we have designed several peptide sequences with high predicted helical propensities and enriched in charged residues, to investigate their PPI (Protein-Protein Interactions) inhibitory potentials. Peptides conformational and binding studies were performed by NMR (Nuclear Magnetic Resonance), CD (Circular Dichroism), and MD (molecular dynamics simulations) along with interaction studies through SPR (Surface Plasmon Resonance) and MST (Microscale Thermophoresis) [5].

Acknowledgement: Our work on the Sam domain of EphA2 is funded by Airc (Italian Association for Cancer Research): grant MFAG15831.

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P34

STORAGE OF NUCLEAR SPIN ORDER IN LOW VISCOSITY MEDIA

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Extending the storage time of nuclear spin order is an important issue in modern NMR spectroscopy since many NMR and MRI applications are limited by the lifetime of spin states. Under normal circumstances the survival of spin magnetisation is limited by longitudinal relaxation (T_1) which brings the magnetisation back to equilibrium within a few seconds. The conversion of longitudinal into singlet order extends that lifetime to tens of minutes up to an hour[1,2].

Most nuclear spin relaxation mechanisms in liquids are a function of the correlation time τ_c of the molecule which is linked to the molecular tumbling and inversely proportional to viscosity. Therefore a lower viscosity would reduce the influence of those mechanisms. We show the increase in the lifetime of longitudinal (T_1) and singlet order (T_S) as the viscosity of the dissolving media decreases.

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P35

NMR STUDIES OF PROTEIN ADSORPTION ON SILVER NANOPARTICLES

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Protein interaction with metal surfaces is a crucial aspect of biological responses taking place when a metallic entity enters in a biological environment. Over the past 20 years, we have witnessed an increase in nanoparticle (NP) research. Silver nanoparticles (AgNPs) are an example of widely used nano-formulations (435 products, 24% in 2015) [1], essentially due to their declared antimicrobial properties. NPs have the potentiality to adsorb proteins and other biomolecules from complex matrices, such as biological fluids and cellular environment. The main interaction between NPs and protein is the formation of a protein-corona [2], which changes with the environment and time, and alters the biophysical properties of NPs [3].
We investigated the interaction of AgNPs, produced by laser ablation, with human ubiquitin, a protein essential for degradative processes in cells, in view of their possible combined use and synergistic action in the wound healing process. The interaction of ubiquitin with uncoated AgNPs showed that the system rapidly evolves into AgNP clusters held together by an amyloid form of the protein [4,5]. In contrast, in the presence of sodium citrate, ubiquitin is rapidly adsorbed on the surface of AgNPs and a stable protein corona is formed and monitored by NMR and SPR. Furthermore, the evolution of protein corona is studied in the presence of albumin, the most abundant blood protein, and with total human serum, showing that albumin and serum proteins displace ubiquitin from the nanoparticle surface and that released ubiquitin retains its native structure. Finally, we designed an ubiquitin mutant (K48C) with a single cysteine residue able to convert the protein interaction with AgNPs from soft (mainly electrostatic) to hard (covalent) corona. Such mutation is sufficient to prevent ubiquitin release in an extracellular medium, thus establishing a proof-of-concept for controlling the evolution of a protein-corona on a rational basis.

Acknowledgements
We thank the University of Bari, the Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (CIRCMSB), and CNR-Nanotech.

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P36

A SOLID STATE NMR STUDY OF LIMONENE-ENCAPSULATING TREHALOSE SYSTEMS

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Aroma encapsulation at micro- or nano-scale in carbohydrate matrices is nowadays representing a principal strategy to enhance and protect the functionality of flavors and to control their delivery and release, modulating their perception. Flavor encapsulation is increasingly gaining importance in food processing, as it offers several advantages such as getting stable functional ingredients by conferring physical and chemical protections during storage and manufacturing processes [1]. The achievement of a glassy state is critical for encapsulation of volatile compounds and conditions of processing and storage can affect the physical properties of the amorphous materials used for encapsulation [2]. Improvements in this field requires the understanding of the interactions occurring at nano-scale between the entrapped aroma and the sugar matrix.
In this study, a combination of low- and high-resolution solid state NMR techniques has been applied to investigate the structural and dynamic properties of samples made by trehalose as model carbohydrate and (R)-limonene as aroma compound and limonene-trehalose interactions at nano-scale. In particular, samples prepared by different techniques (spray-drying and ball milling) and with different aroma content were investigated. . To this aim, TD-NMR techniques have been applied for the study of ^{1}H spin-spin and spin-lattice relaxation processes. Solid echo and CPMG pulse sequences, with subsequent FID and CPMG signal analyses, were applied for the characterization of the dynamically distinguishable regions of the samples, while the measure of ^{1}H T_1's, affected by the spin diffusion process, was carried out to obtain information on both the global dynamics of the systems in the MHz regime and the degree of homogeneity of the sample on a spatial scale of about 100 Å.
High-resolution techniques were used as complementary experiments to investigate structural properties of the trehalose samples, such as conformational structure, identification of different phases and structural changes in crystalline polymorphs.

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DYNAMICS OF POLY(VINYL BUTYRAL) STUDIED BY DIELECTRIC SPECTROSCOPY AND ^{1}H NMR RELAXOMETRY

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The combined use of Dielectric Spectroscopy (DS) and ^{1}H Fast Field-Cycling (FFC) NMR relaxometry allowed a detailed understanding of the dynamic behavior of the amorphous ter-polymer poly(vinyl butyral) (PVB) to be achieved across the glass transition temperature (T_g=70 °C by Differential Scanning Calorimetry). Above T_g, main chain segmental motions (α relaxation) were detected and characterized by both DS and FFC NMR relaxometry. The α process showed a Vogel Fulcher Tammann temperature dependence, with an associated T_g of 69 °C and a fragility of 155 for PVB glass. Below T_g, DS revealed a β relaxation ascribed to reorientations of the vinyl alcohol dipoles due to local twisting motions and allowed an activation barrier of 11 kcal/mol to be determined. NMR relaxometry data supported the occurrence of this motion, although ^{1}H relaxation could also be affected by reorientations of propyl side chains.

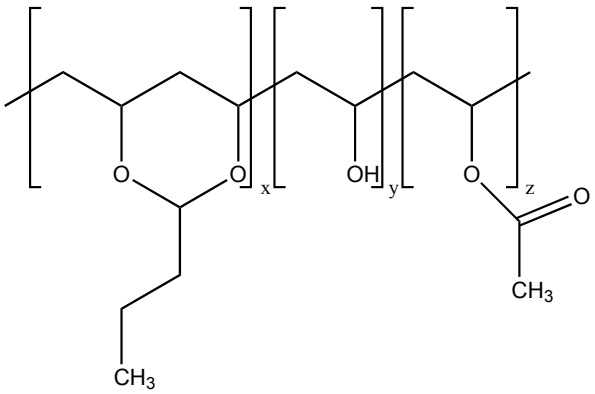

Fig. 1. Chemical structure of PVB. For the sample investigated, the molar fractions of vinyl butyral (x), vinyl alcohol (y), and vinyl acetate (z) units are 0.55-0.57, 0.41-0.45, and 0-0.02, respectively.

This study was partially financed by the Regional project SELFIE (Sistema di Elementi avanzati multi Layer basato su superFici e materiali Innovativi nanostrutturati per una Edilizia sostenibile ed energeticamente efficiente), bando FAR-FAS 2014, Regione Toscana.

P38

DEVELOPMENT OF FUNCTIONAL MRI PROTOCOL IN A MULTIPLE SCLEROSIS MOUSE MODEL

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Functional MRI (fMRI) studies have demonstrated brain plasticity with altered activation patterns in the performance of specific tasks in multiple sclerosis (MS) patients [1]. fMRI in the resting state (rsfMRI) has recently emerged as a valuable tool to identify functional abnormalities in MS patients. To identify imaging biomarkers in the preclinical mouse model of MS, experimental autoimmune encephalomyelitis (EAE), high-resolution T2w morphological images and rsfMRI Images in the brain of mice and relative controls were acquired. Anesthesia was initially induced by isofluorane and then by medetomidine administration [2]. rsfMRI images were acquired using EPI with TR=600ms, TE=10ms, EPI segments=2, for a total scan time of 6 minutes. T2w imaging was performed with a RARE sequence with TR=5220ms, TE=76ms, RARE factor=16. Data were analyzed by means of FSL software [3]. In figure 1 some of the resting state networks identified are reported. Indipendent component analysis decomposes a two-dimensional data matrix (time×voxels) into a set of time-courses and associated spatial maps. Resting state imaging analysis describe the temporal and spatial characteristics of underlying hidden signals, i.e. components. Obtained brain networks reflect brain activated connectivity areas in the EAE model that can be further investigated for unravel demyelination mechanism and neurophysiological function associated to disease progression or testing of new therapeutic strategies.

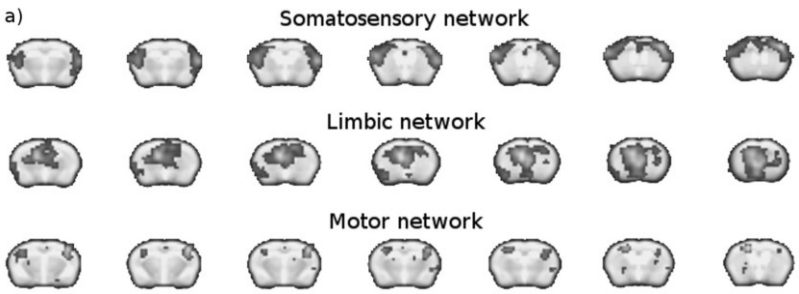

Fig. 1. Somatosensory, motor and limbic networks detected by Independent Component Analysis (ICA) of rsfMRI data.

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P39

A MULTI-ANALYTICAL STUDY OF THE CONSTITUTIVE MATERIALS AND THE STATE OF CONSERVATION OF A 16th CENTURY ITALIAN WALL PAINTING

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In this study a multi-analytical approach was carried out to characterize original and non-original materials, the stratigraphy structure and state of conservation of the wall paintings (16th century) painted by Antonio del Massaro da Viterbo, nicknamed il Pastura in the Vitelleschi Chapel in the Tarquinia Chatedral. Using unilateral NMR [1] a non-invasive monitoring was carried out directly in situ to evaluate the performance of different traditional and innovative cleaning treatments [2]. By high resolution NMR spectroscopy in liquid and solid state [3] and ATR-IR spectroscopy performed on micro-samples the full characterization of the organic materials was obtained. Portable XRF and mid reflection FT-IR spectroscopy allowed us to study the composition of the pictorial layer evidencing original pigments, restoration materials and alteration compounds

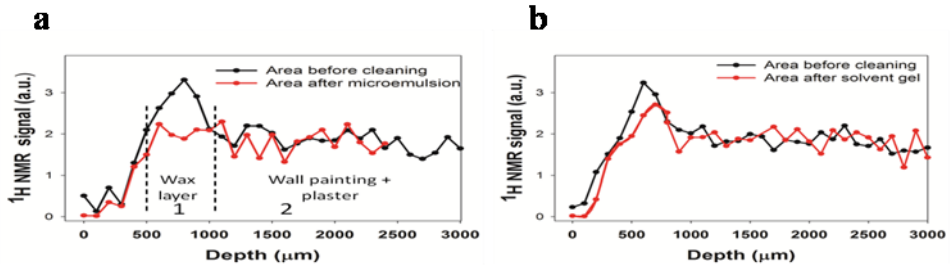

Fig. 1. Unilateral NMR was applied to evaluate the removal of the wax layer by two cleaning products: a micro-emulsion and a solvent gel. In figures 1a and 1b , the ^{1}H NMR depth profiles collected from two areas of painting were showed. In both areas before the cleaning treatments the NMR profile encoded two regions with a different hydrogen content.

Acknowledgements: This study is part of the project "BIO-REMP (BioREstoration of Mural Paintings)" supported by the Italian Research Infrastructure for Heritage Science IPERION CH.it.

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METABOLIC PROFILING OF AUTOCHTHONOUS APULIAN WINE GRAPE JUICES

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Wine grapes are food products of considerable commercial value for several countries. In the framework of project "Recupero del Germoplasma Viticolo Pugliese" (Re.Ge.Vi.P.), with the aim to valorize Apulian grape biodiversity, we used ^{1}H NMR spectroscopy to obtain the metabolic fingerprinting of wine grape juices, belonging to 10 representative Apulian cultivars (Primitivo, Negroamaro, Verdeca, Bianco D'Alessano, Bombino Bianco, Minutolo, Malvasia Nera, Uva Di Troia, Susumaniello, Bombino Nero). Grape samples were collected in three years (2013, 2014, 2016); they were harvested at similar level of ripening. Different approaches of multivariate statistical analysis were applied to spectral data in order to find specific metabolites discriminating for variety and/or year (Fig. 1).
In this presentation, metabolic profiling of wine grapes from different cultivars along with multivariate statistical analysis and performances of classification models will be shown.

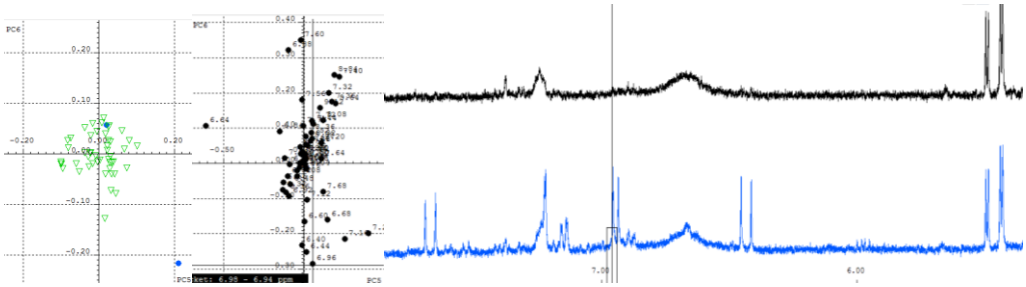

Fig. 1. Classification model built on wine grape juice samples *cv* Primitivo, used to predict unknown samples.

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COMPREHENSION OF METABOLIC DISORDERS IN INFLAMMATORY AND NEOPLASTIC HYPER-PROLIFERATIVE DISEASES: A NMR CONTRIBUTION

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A progression model based on genetic alterations has been proposed for several types of tumors and in skin diseases, the concept of cancerization field involves cluster of genetically altered cells in a chronically photo damaged skin without clinical evidence of neoplastic lesions [1]. Psoriasis is a chronic, inflammatory skin disease associated with significant morbidity and mortality. Cutaneous Squamous Cell Carcinoma (CSCC) is a common skin cancer characterized by malignant proliferation of keratinocytes. CSCC usually arises from precursor lesions such as actinic keratosis (AKs), but can also grow de novo or on chronically inflamed skin. AKs are the most common skin lesion of disordered keratinocyte proliferation in the disease continuum of photo-damaged skin that may lead to invasive CSCC. In addition, patients with psoriasis can be developed AK or CSCC. Exploring the metabolome of cancer, precancerous lesions and inflammatory disease seems to be a parallel and effective way to understand the phenotypic changes associated with cancer progression. Metabolomics could reveal novel cancer biomarkers that might expand our current understanding of the multi-factorial disease. Nuclear Magnetic Resonance (NMR) spectroscopy, giving an accurate description of the molecular composition of a human tissue, provides a "fingerprint" of the whole metabolome [2]. Correlations between some metabolites and proliferative markers allow gaining insight into the relationship between cellular proliferation and metabolic changes associated with the presence of tumor and its aggressiveness.

Acknowledgments
Thanks are due to "Natalino Corazza Foundation" for financial support.

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P42

STUDY OF DRYING PROCESSES OF PUMPKIN USING "QUANTITATIVE" IMAGING BY NUCLEAR MAGNETIC RESONANCE

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The drying processes of pumpkin *(Cucurbita Maxima Duchesne)* samples is investigated through Nuclear Magnetic Resonance Imaging (MRI). The effects of hot-air drying (50°C - 70°C) on the physical properties of samples were determined. The behaviours of decrease in mass due to water loss obtained by both the standard gravimetric and MRI methods are in good agreement. The T_2 weighted images obtained by MRI, at given temperatures, show that the moisture distribution and T_2 values are related. This approach and data analysis show that the non-invasive and non-destructive MRI technique offers the possibility to study water distribution in food during drying processes. The quantitative moisture distribution maps allow future development and verification of models for prediction of mass transport phenomena in foods.

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SOLID-STATE NMR CHARACTERIZATION OF VENLAFAXINE MOLECULAR SALTS WITH IMPROVED SOLUBILITY PROPERTIES

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The search for multicomponent crystals, especially molecular and ionic co-crystals and salts is at the forefront of crystal engineering studies. [1] The main goal is to tune and/or improve the physicochemical properties (e.g. physical stability, solubility, bioavailability…) by binding together through weak interactions and within the same unit cell an API (Active Pharmaceutical Ingredient) and one or more partner molecules, whether active or "innocent".[2] Structural characterization of co-crystals is extremely important because the outcome properties depend on the 3D arrangement of the molecules in the lattice. We report on the synthesis and characterization of a series of molecular salts between the antidepressant drug venlafaxine hydrochloride and six different organic acids accepted by the Pharmacopeia, i.e. coumaric, ferulic, oxalic, salicylic, fumaric and citric acids. All compounds have been characterized by single crystal and powder X-ray diffraction and solid-state NMR spectroscopy. All these experiments have been fundamental to completely describe the molecular structures and the intermolecular interactions. Several advanced 2D SSNMR spectra such as ^{1}H DQ MAS, ^{13}C-^{1}H HETCOR and ^{14}N-^{1}H J-HMQC (Figure 1) were acquired taking advantage of the resolution and sensitivity improvement provided by indirect detection pulse sequences and very fast MAS at 70 kHz. These experiments have been fundamental to definitively confirm the molecular salt formation for all compounds and to provide useful insights on crystal packing and hydrogen bond network of [VenH][HFumarate], thus supporting and confirming the structure solved by XRPD. Concerning [venH][H_2citrate], whose X-ray structure was not available, 1D and 2D SSNMR experiments have been pivotal to provide structural information and to clearly indicate the presence of a N^+-H group.

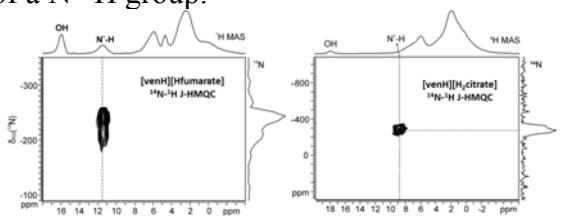

Fig. 1. 2D ^{14}N-^{1}H J-HMQC spectra of [venH][Hfumarate] and [venH][H_2citrate] (^{1}H Larmor frequency 600 MHz; MAS frequency 70 kHz)

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P44

FERRITIN DECORATED PLGA/PACLITAXEL LOADED NANOPARTICLES ENDOWED WITH AN ENHANCED TOXICITY TOWARDS MCF-7 BREAST TUMOUR CELLS

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Targeting tumours with theranostic systems, that combine delivery of drugs and imaging agents within a single vector, represents one of the most important innovation for cancer treatment. Moreover, nanotechnology is under intense scrutiny in the design of new medical protocols, as a significant improvement of diagnoses and therapy is expected from the application of nanosized drug delivery systems, in particular of cancer and cardiovascular diseases. [1] In this contest, liposomes, solid-lipid nanoparticles, and biodegradable polymeric nanoparticles are the most widely investigated systems. Poly (D, L-lactide-co-glicolide) (PLGA) has attracted great attention in the design of nanosized delivery systems because of its excellent biocompatibility and biodegradability. [2] Therefore, surface modification and coating of PLGA nanoparticles is the subject of studies aimed at prolonging their circulation lifetime. Here, we propose the use of ferritin as simultaneous targeting and coating agent for PLGA nanoparticles (Fig. 1). In addition to act as a hydrophilic cover, Ferritin may endow the nanoparticle with a potential targeting capability thus improving the uptake by the tumour cells through specific ferritin receptors. The involved receptors belong to scavenger receptor class A member 5 (SCARA5) for L-ferritin and to TIM-2 and TfR-1 for H-ferritin, in mice and human, respectively[3]. It is well known that, many cancer cells reprogram iron metabolism in ways that result in net iron influx through the up-regulation of the expression of proteins that are involved in iron uptake (i.e. TfR1, SCARA5) and the decreased expression of iron efflux proteins, such as ferroportin. [3] On this basis herein Horse spleen ferritin has been covalently conjugated to the external surface of PLGA nanoparticles exploiting NHS activated carboxylic groups. Moreover, PLGA nanoparticles have been loaded with a Gd based imaging agent (Gd-DOTAMA), in order to monitor their distribution with MRI. The targeting and cytotoxic capabilities of ferritin functionalized PLGA nanoparticles has been compared with the corresponding albumin coated ones as well as with unfunctionalized particles in MCF7 breast cancer cell line expressing SCARA5.

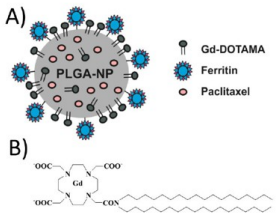

Fig. 1. A) PLGA-NPs-Ferr nanoparticles; B) Gd-DOTAMA.

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P45

INNOVATIVE QNMR METHODOLOGY FOR THE CARBOHYDRATES QUANTIFICATION IN COMPLEX MIXTURES. A CHALLENGE ON HONEY

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The knowledge on carbohydrates composition is of great importance to determine natural matrix properties. Due to their structural similarity and to the high number of isomers they present, carbohydrate analysis is complex. There is a growing interest in the development of new analytical methods, both for raw materials and for finished products, that do not require pre-treatment of the sample and that are, at the same time, specific and highly accurate in identification and quantification of sugars. Here, we have devised an innovative qNMR analytical procedure [1]. The method was developed on honey samples previously dissolved in water and filtered with no other sample treatment. Twenty two main sugars present in honey were simultaneously quantified: four monosaccharides (glucose, fructose, mannose, ramnose), eleven disaccharides (sucrose, threalose, turanose, maltose, maltulose, palatinose, melibiose, melezitose, isomaltose, gentiobiose nigerose and kojibiosio) and seven trisaccharides (raffinose, isomaltotriose, erlose, melezitose, maltotriose, panose and 1-kestose). Satisfactory results in term of limit of quantification precision, trueness and recovery were obtained. An accurate control of instrumental temperature and of the sample pH, assure optimum chemical shift reproducibility, making the procedure potentially automatable and suitable to routine analysis. This innovative approach demonstrated on honey that is the most complex natural matrix in saccharides composition, can be easily transferred to other natural matrices

Reference

[1] Italian patent application filed “Metodo per l’identificazione e quantificazione di sostanze in miscele complesse” 102017000071682

P46

HEPARAN SULPHATE HEXASACCHARIDES: ROLE OF SULPHATION OF THE IDURONIC ACID RESIDUE ON BINDING PROPERTIES TO ANTITHROMBIN

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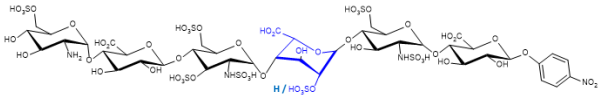
Heparan sulphate (HS) is ubiquitously present in all mammal cell surface and plays a key role in different physiological and pathological biological events, including regulating embryonic development, inflammatory responses, blood coagulation and viral/bacterial infections, by interacting with different proteins [1]. HS is a linear highly sulphated polysaccharide, comprising disaccharide repeating units of a D-glucuronic (GlcA) or L-iduronic acid (IdoA) 1-4 linked to a glucosamine (GlcN).
The IdoA residue in HS is a unique structural component, as the pyranose ring may assume in solution three equienergetic conformations (mostly between the $^{1}C_{4}$-chair and $^{2}S_{0}$-skew boat), of which the $^{2}S_{0}$ was demonstrated to be the "active" form in the binding to antithrombin (AT) [2]. Notably, two chemoenzymatically synthesized hexasaccharides containing AT binding region (ATBR) and differing by only 2-O sulphation of the IdoA, were shown to possess comparable affinity to AT [3]. Given the growing interest to the synthetic analogues of the FDA approved drug Fondaprinux and chemoenzymatic methods of their synthesis, we focused our interest on studying the binding of these hexasaccharides to AT by NMR and molecular modelling. The aim of this study was to establish the spatial configuration of hexasaccharidic backbone of two compounds during their binding with protein highlighting the role of conformational flexibility of the iduronic acid within the interaction [4], particularly in the specific case of IdoA containing hexasaccharides, never considered as active form of heparan-like compounds. We obtained the binding epitope by Saturation Transfer Difference experiments. The AT-bound conformation of the two hexasaccharides was determined by quantitative analysis of tr-NOESY (transferred NOESY) supported by molecular modelling and dynamic simulations. Additionally, MicroITC (Isothermal Thermal Calorimetry) was used to determine the affinity constants (Kd) of two hexasaccharides uncovering their similar behaviour in terms of thermodynamic parameters in solution during the interaction with AT.



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P47

STRUCTURAL STUDIES OF β-AMYLOID(1-42)-ACETYLCHOLINE MOLECULAR INTERACTION

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in adults. Based on the amyloid cascade hypothesis, AD is caused by the accumulation of the amyloid beta peptide (Aβ), which promotes neuronal loss and impairs neuronal function. In view of this hypothesis, thousands of small-molecules are under scrutiny to identify new substances able to control the formation of β-amyloid fibrils, either stabilizing the soluble peptide conformation or solving aggregates.[1-4]
Following a different very accredited idea, Alzheimer's disease is dependent on cholinergic deficit, implicating dysfunction of acetylcholine (Ach) transmission. In agreement with both the mentioned hypotheses, we experimented the possibility that, Ach, in addition to its role of cholinergic neurotransmitter, may exert its action as an anti-Alzheimer agent, through a direct interaction with β-amyloid fragment Aβ(25–35).[5]
Extending these investigations to the full length β-amyloid peptide, here we present the results of CD and NMR conformational analysis of Aβ(1-42) in presence of Ach. This study aimed at the determination of structural interactions between Aβ(1-42) and Ach molecule, is carried out in an innovative perspective that, new lead compounds may be identified, to design poly-functional pharmacological tools that synergically modulate cholinergic transmission and Aβ aggregation.

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P48

FLUORINATED NANOPARTICLES FOR THE IN-VIVO TRACKING OF INFLAMMATION IN A MOUSE MODEL OF SPINAL CORD INJURY

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This work aimed at developing a ^{19}F-MRI/MRS procedure to visualize the time-dependent cellular events (*e.g.* immune cells recruitment) following a spinal cord injury (SCI), with the ultimate goal of designing improved pharmacological and/or cell-based therapeutic schemes. The strategy used was based on the preparation of PerFluoro-15-Crown-5-Ether-based nanoemulsions (PFCE-NEs) by exploiting their ability to be quickly internalized by circulating immune cells after intravenous injection.
PFCE-NEs (hydrodynamic diameter of 170±20 nm) were obtained upon emulsification (by direct sonication) of PFCE with a phospholipid labeleld with a fluorescent dye (Rhodamine-DOPE) and the surfactant Pluronic F-68. The efficiency of the system to label immune cells was investigated first *in vitro*, in polarized (M1, M2) or non-polarized (M0) primary macrophage subsets, and then *in vivo* in a mouse model of SCI. The half-life time of the system was determined by ^{19}F MRS. The *in vivo* monitoring of the inflammation response to the injury was determined by both ^{19}F MRI and MRS (by Point RESolved Spectroscopy).
In vitro experiments proved the strong avidity of M0, M1 and M2 phenotypes towards PFCE-NE (95% labeling efficiency). In vivo, the ^{19}F-MRI signal in blood displayed a bi-exponential decay with very different time constants (t_1=70 min, t_2=20 hours). The recruitment of macrophages at the lesion site was successfully followed for 2 weeks, both by ^{19}F PRESS and MRI (Fig. 1), displaying the higher signal enhancement in the first days post injury even if, when multiple administrations of the contrast agent were repeated, a constant and faint fluorine accumulation was detected throughout the 14 days of monitoring. *Ex-vivo* immunohistology displayed a massive accumulation of particles in the injured region.

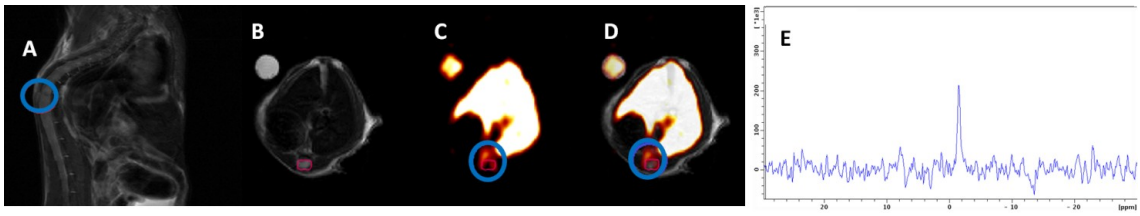

Fig. 1. A) ^{1}H high resolution MR sagittal image of the SCI; B) ^{1}H and C) ^{19}F MR axial image of the SCI; D) merge of ^{1}H and ^{19}F MRI of SCI; E) PRESS of a 43 mm^3 voxel located in the site of injury. Blue circles indicate the lesion site, red line delineates the vertebral column.

P49

FEATURES OF ^{195}Pt AND ^{31}P CHEMICAL SHIFT TENSOR IN PLATINUM DIORGANOPHOSPHANIDO COMPLEXES

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In our recent work we reported a spectroscopic and theoretical study on two dinuclear phosphanido-bridged Pt(+2), and Pt(+3) complexes in which we demonstrated that the strong difference in NMR chemical shift displayed by ^{31}P and ^{195}Pt nuclei is caused mainly by one of the three principal components of the chemical shift (CS) tensor.
The theoretical calculations permitted to determine the orientation of the three principal components of the CS tensor, pointing out that, for both complexes, the distinct component is the one oriented along the direction perpendicular to the Pt_2P_2 plane. Moreover, the main contribution to the distinct component of the CS tensor was found to stem mainly from the paramagnetic term (σ_p) for both ^{195}Pt and ^{31}P [1], thus pointing out a key role of the molecular orbital energies of the molecules for the values of the distinct component of the CS tensor.
Based on these results, we decided to extend this kind of study on ^{31}P and ^{195}Pt NMR in the solid state to others two dinuclear Pt complexes in which the formal oxidation states of Pt are +2 and +1 and in this contribution we report the results.

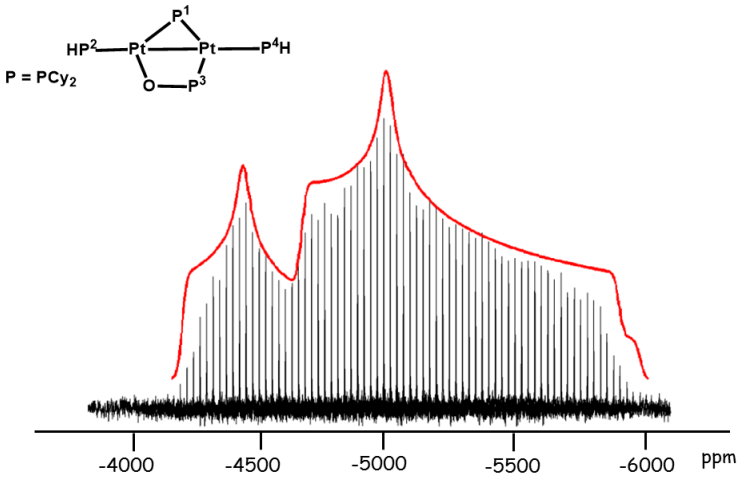

Fig. 1. ^{1}H-^{195}Pt CP/CPMG spectra (bottom trace) and simulation of powder patterns (top trace in red) of [(PHCy$_2$)Pt(μ-PCy$_2$)(κ^2P,O-μ-POCy$_2$)Pt(PHCy$_2$)] (Pt-Pt)

Reference

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P50

PERFIDI FILTERS: A SUMMARY

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In the field of Nuclear Magnetic Resonance, the capability to distinguish/filter the signal of different part of a sample plays a key role in many applications. For example, in the medical field such possibility allows to improve the image contrast and the identification of the different tissue inside the human body. In the oil and gas field, the characterization of the different saturating reservoir fluids grants for an optimized exploitation [1, 2].
Regarding T_1 filtering, literature reports many sequences dedicated to signal selection such as Short Time Inversion Recovery and Multiple Inversion Recovery [3], but usually these techniques allow the selection of only a discrete number of T_1 values.
Parametrically Enabled Relaxation Filters with Double and Multiple Inversion [4, 5, 6] (PERFIDI) is an innovative technique which implements T_1 filters, but differently to the standard selective sequences, it allows to filter the signal of a selected range of T_1 values. PERFIDI filters, de facto, act like electronic band-pass, low-pass and high-pass filters.
PERFIDI filters have been developed and tested both by Nuclear Magnetic Resonance Relaxometry and Imaging. Here we present a panoramic summary of this innovative filter technique, describing the validation of the method and the application on different kind of samples all characterized by a continuous T_1 distribution, such as biological tissue and oil-water saturated porous media.

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P51

SMALL MOLECULE SCREENING AGAINST EPHA2-SAM: A ROUTE TO DISCOVER POTENTIAL ANTICANCER AGENTS?

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Ephrin A2 receptor (EphA2) plays a pivotal function in cancer due to its over-expression in several types of tumors, including prostate and breast cancers [1]. The process of ligand-induced receptor endocytosis, followed by degradation, has recently attracted a lot of attention as a likely path to diminish tumor malignancy. The Sterile alpha motif (Sam) domain of EphA2 (EphA2-Sam) is the site where protein inhibitors of endocytosis and modulators of receptor stability are engaged by means of heterotypic Sam-Sam associations [2]. In this context, small molecules able to block EphA2-Sam mediated interactions may be provided with anticancer potential. With this in mind, we have first conducted a virtual screening -through computational docking studies- to predict possible EphA2-Sam ligands. To achieve this task we started from a 3D NMR structure of EphA2-Sam (pdb code: 2E8N), several databases of virtual molecules, and a variety of bioinformatic tools. Among the best in silico hits, a few drug-like compounds, respecting Lipinski's "rule of five" [3], were purchased and experimentally tested against EphA2-Sam by means of NMR chemical shift perturbation (CSP) studies with ^{15}N labeled protein and HSQC experiments.

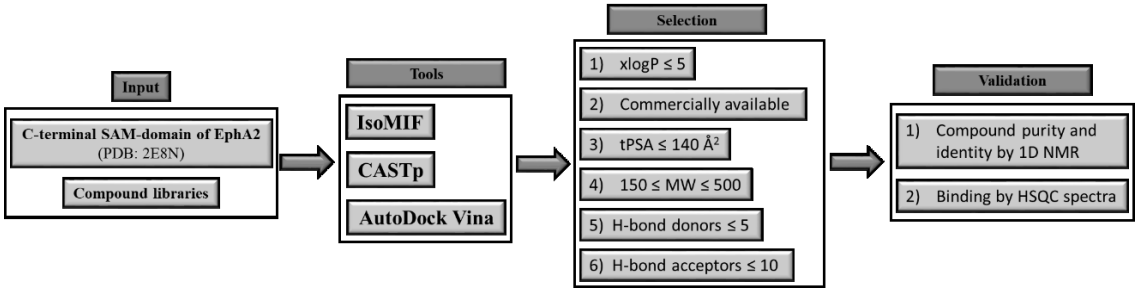

Fig. 1. Workflow of the implemented searching protocol for small molecules potentially able to bind EphA2-Sam.

Acknowledgements
M. Vincenzi is supported by a Fellowship from Fondazione Umberto Veronesi (grant 2017).

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