XLIX National Congress on Magnetic Resonance

8-10 September 2021

GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

online

XLIX National Congress on Magnetic Resonance

Online, 8-10 September 2021

BOOK OF ABSTRACTS

SPONSORS

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Scientific Program

Tuesday September 7TH – PRE-MEETING EVENTS

10:00-12:00	GIDRM MASTER AND PhD GRADUATE AWARDS
13:10-14:50	JEOL SATELLITE MEETING
15:00-17:00	BRUKER SATELLITE MEETING

Wednesday September 8TH

9:15 - 9:30	OPENING
	CHAIRS: M. GEPPI, C. AIROLDI
9:30 - 10:20	GIDRM/GIRM GOLD MEDAL AWARD S. MAMMI MY BEST … TO BE PREPARED … TO SERVE
10:20 -10:50	INVITED LECTURE 1 G. PINTACUDA STRUCTURAL DYNAMICS OF MEMBRANE PROTEINS BY MAGIC-ANGLE SPINNING NMR

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11:10-11:30	**L. RUSSO** THE DARK SIDE OF THE HUMAN PRION PROTEIN
11:30-11:50	**F. FAVRETTO** THE ROLE OF PROLINE CIS/TRANS ISOMERIZATION AND CHAPERON ACTIVITY IN THE PROCESS OF ASYN AGGREGATION
11:50-12:10	**M. SPANO** HEMP PRODUCTS: NMR CHARACTERIZATION OF INFLORESCENCES AND HEMP SEED OILS
12:10-12:30	**BRACCO LECTURE - A. FRINGUELLO MINGO** PRE-CLINICAL IMPLEMENTATION AND VALIDATION OF A MR FINGERPRINTING SEQUENCE

12:30-14:00	**LUNCH BREAK**

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14:50-15:10	**M. E. DI PIETRO** GOING DEEPER WITH NMR: INTERACTIONS AND DYNAMICS OF DEEP EUTECTICS
15:10-15:30	**R. RIZZATO** NMR SPECTROSCOPY WITH NV DEFECTS IN DIAMOND: FROM SURFACE-NMR TO HYPERPOLARIZATION
15:30-15-50	**T. B. R. ROBERTSON** SIGNAL AMPLIFICATION BY REVERSIBLE EXCHANGE (SABRE) HYPERPOLARISATION OF MIRFENTANIL IN THE PRESENCE OF HEROIN
15:50-16-10	**BRUKER LECTURE - J. KEMPF** DNP DEVELOPMENTS IN SOLIDS & DISSOLUTION DNP AT BRUKER

	CHAIRS: S. BORSACCHI, K. SCHMIDT-ROHR
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17:10-17-40	**INVITED LECTURE 3** **A. DE ANGELIS** SOLUTION AND SOLID-STATE NMR STUDIES OF SARS-COV-2

THURSDAY SEPTEMBER 9TH

	CHAIRS: M. GEPPI, F. SEPAROVIC
9:30-10:00	**INVITED LECTURE 4** **L. FRYDMAN** IN VIVO ^{2}H AND ^{13}C METABOLIC IMAGING AS COMPLEMENT TO 1H MRI IN THE CHARACTERIZATION OF HEALTH AND DISEASE
10:00-10:40	**UNDER 35 GIDRM AWARD** **C. CIARAMELLI** NMR-DRIVEN IDENTIFICATION OF MOLECULES WITH BENEFICIAL EFFECTS ON HUMAN HEALTH IN COMPLEX MATRICES

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11:20-11:40	**T. CARTLIDGE** TOWARDS A SIMULATION FRAMEWORK FOR MAGNETIC SUSCEPTIBILITY INDUCED RELAXATION OF SPINS DIFFUSING IN POROUS MEDIA
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15:10-15:30	**G. CONTA** NMR-BASED METABOLOMICS AND MULTI-BLOCK OMIC APPROACH TO INVESTIGATE THE ROLE OF GUT MICROBIOME IN CHILDREN WITH TYPE 1 DIABETES
15:30-15:50	**V. GHINI** NMR AS A TOOL FOR THE BIOCHEMICAL CHARACTERIZATION OF MICROORGANISMS OF BIOTECHNOLOGICAL INTEREST
15:50-16:20	**INVITED LECTURE 7** **V. MICHAELIS** UNDERSTANDING TIN-CONTAINING PEROVSKITE MATERIALS WITH HELP FROM SOLID-STATE NMR SPECTROSCOPY

16:20	**GIDRM ASSEMBLY**

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16:50-17:20	**INVITED LECTURE 11** **G. T. MONTELIONE** ASSESSMENT OF PREDICTION METHODS FOR PROTEIN STRUCTURES DETERMINED BY NMR IN CASP: IMPACT OF ALPHAFOLD2

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PL8 - STRUCTURE DETERMINATION OF ANTIMICROBIAL PEPTIDES IN MODEL MEMBRANES AND LIVE BACTERIA (F. Separovic)

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SP3 - JASON: A NOVEL NMR TOOL (M. Perez)

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OC4 - HEMP PRODUCTS: NMR CHARACTERIZATION OF INFLORESCENCES AND HEMP SEED OILS (M. Spano)

OC5 - EXPLORING THE DYNAMICS OF ELASTOMERS BY 1H FIELD CYCLING NMR RELAXOMETRY (F. Nardelli)

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OC7 - NMR SPECTROSCOPY WITH NV DEFECTS IN DIAMOND: FROM SURFACE-NMR TO HYPERPOLARIZATION (R. Rizzato)

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OC20 - HYDROPHOBIC INTERACTIONS BETWEEN MACROCYCLIC GD-COMPLEXES AND POLYAROMATIC SYSTEMS AS ROUTE TO ENHANCE THE LONGITUDINAL WATER RELAXIVITY IN MAGNETIC RESONANCE IMAGING (G. Ferrauto)

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GIDRM/GIRM GOLD MEDAL AWARD

GMA

MY BEST … TO BE PREPARED … TO SERVE

S. Mammi

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Life continuously provides opportunities to learn and grow. The choice whether to accept the challenge and to adapt to the new situation is often ours and requires change, something that is not always easy or welcome but without which there is no growth. As the saying goes, *"Success comes in cans – not in cannots"*.
Since I was young, I tried to pursue happiness following what Baden-Powell suggested in his last message: *"the real way to get happiness is by giving out happiness to other people"*. Not consistently, often not successfully, at times reluctantly and at other times unintentionally, I tried my best to help other people to achieve their goals, to develop their potential and to fulfil their dreams. I did this as a father, as a Scout leader, as a professor and as a scientist.
People are (of course) a huge part of life. Relationships do not come easily to me. I tend to be "a bear", a loner, and the way I best express my love to people is through acts of service, preferably without direct interactions.
In this talk, I will describe part of my journey through Science and NMR, and I will try to highlight not only my few "claims to fame" but also some obstacles and failures that I encountered. My deepest gratitude for the successes is to the people that made them possible: mentors, colleagues and students without whom I would not be who I am today.

UNDER 35 GIDRM AWARD 2021

U35GA

NMR-DRIVEN IDENTIFICATION OF MOLECULES WITH BENEFICIAL EFFECTS ON HUMAN HEALTH IN COMPLEX MATRICES

C. Ciaramelli

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The interest in edible products with beneficial effects on human health has largely increased over the last years, especially in the field of prevention against those diseases still lacking effective therapies. In many cases, the overall healthy properties of some natural products are reported, but little is known about the bioactive molecules involved and the molecular mechanisms through which these activities are carried out. NMR can be successfully employed to study the biological effects of molecules contained in complex mixtures – including natural extracts from edible sources – at molecular level.
In this context, our research group developed an NMR-based protocol to screen natural edible matrices for the identification of ligands of specific proteins representing potential pharmacological targets. NMR techniques, including STD (Saturation Transfer Difference)-NMR and tr-NOESY, are used for ligand-receptor interaction studies. Moreover, NMR data are complemented by other techniques, including *in vitro* biochemical and *ex vivo* cell assays and atomic force microscopy experiments. We applied this approach to screen different edible matrices for the presence of anti-Aβ molecules to be employed for the prevention of Alzheimer's disease, but also to identify possible ligands of Ataxin-3 oligomers and oncogenic Ras proteins. Regarding the preliminary chemical characterization required when analysing complex matrices, the metabolic profiling of the natural extracts was carried out by NMR. We set up a protocol for the rapid and semi-automatic identification and quantification of metabolites, using the Simple Mixture Analysis (SMA) tool of the MestReNova software, to be applied to the different complex mixtures.
In this communication, the most relevant works on this topic will be discussed. First, the study of beneficial effects of green and roasted coffee extracts on human health [1], with particular attention to the identification of ligands of Aβ oligomers and Ras proteins, will be presented. Then, the neuroprotective effect of cocoa extracts will be introduced [2], and lastly a preview on very recent results obtained on cinnamon buds and barks will be shared [3].
The identification of biomolecules with useful properties for human health can support their use as functional foods or in the preparation of dietary supplements and nutraceuticals, leading to potential improvements in the field of disease prevention.

References

[1] C. Ciaramelli, A. Palmioli, C. Airoldi. *Food Chemistry,* **278**, 47-55 (2019); C. Ciaramelli, A. Palmioli, A. De Luigi, L. Colombo, G. Sala, C. Riva, C. P. Zoia, M. Salmona, C. Airoldi, *Food Chemistry,* **252**, 171–180 (2018); A. Palmioli, C. Ciaramelli, R. Tisi, M. Spinelli, G. De Sanctis, E. Sacco, C. Airoldi, *Chem. Asian J.* **12**, 2457 – 2466 (2017); L. Amigoni, M. Stuknytė, C. Ciaramelli, C. Magoni, I. Bruni, I. De Noni, C. Airoldi, M. E. Regonesi, A. Palmioli, *J. Funct. Foods*, **33**, 297-306 (2017).
[2] C. Ciaramelli, A. Palmioli, A. De Luigi, L. Colombo, G. Sala, M. Salmona, C. Airoldi. *Food Chemistry*, **341**, 128249. (2021).
[3] C. Airoldi, C. Ciaramelli, A. Palmioli, *Mendeley Data*, V1, doi: 10.17632/v3kj6zn9tf.1 (2021).

PLENARY LECTURES

PL1

STRUCTURAL DYNAMICS OF MEMBRANE PROTEINS BY MAGIC- STRUCTURAL DYNAMICS OF MEMBRANE PROTEINS BY MAGIC-ANGLE SPINNING NMR

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In recent years, magic-angle spinning (MAS) NMR has developed as a powerful technique to investigate structure and dynamics of membrane proteins, enabling the study of these challenging systems in their native-like environment. Faster (100 kHz and above) MAS rates have paved the way for the direct detection of proton resonances, enabling a boost in sensitivity and resolution with respect to the more traditional approaches. In combination with high magnetic fields, this technical progress revolutionizes the atomic-level investigation of proteins i) by enlarging the molecular size of the systems that can be investigated with site specificity; ii) by reducing the requirements in terms of isotopic labeling, notably deuteration; iii) by speeding up the tedious processes of resonance assignment and acquisition of dynamical parameters.
Here we review the strategies underlying this leap forward and describe its potential for the detailed characterization of different transmembrane channels and transporters reconstituted in lipid bilayers. By measuring extended sets of site-specific dynamic observables involving both the backbone and the side chains, we unveil the presence of localized variations in conformational flexibility in regions responsible for substrate selectivity and transport and shed new light on the relation between conformational plasticity and function.

Fig. 1. A leap forward in sensitivity and resolution for studying structure and dynamics.

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PL2

QUANTIFICATION OF DOMAIN SIZES FROM 2 TO 200 nm BY SPIN DIFFUSION ANALYSIS

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The properties of many important synthetic or natural organic materials, including semicrystalline polymers [1], polymer capsules, polymer–MOF composites [2], amorphous pharmaceutical dispersions [3], pressure-synthesized carbon nanothreads [4], or wood, can be fully understood only if their structure is determined on both molecular and nanometer scales. Solid-state NMR is not only the best available method for characterizing the composition of such complex organic materials, but can often also be used to estimate the thickness of specific domains on the 2 to 200 nm scale, taking advantage of spin diffusion. This talk will give an overview of half a dozen ^{1}H and ^{13}C spin diffusion methods, with an emphasis on the analysis for domain size quantification.

Depending on the available ^{1}H chemical-shift or relaxation-time contrast, different techniques can be applied for domain-size analysis by ^{1}H spin diffusion. Stringent lower limits of domain sizes can be obtained from relaxation or zero-crossing times in simple ^{1}H inversion recovery with ^{13}C detection. Spin diffusion after inversion recovery (SDAIR) yields domain sizes from the relative exchange peak intensity (see Fig. 1). ^{1}H-^{13}C HetCor with spin diffusion and single-spectrum referencing can detect domains of more than 200 nm thickness, in favorable cases.

In uniformly ^{13}C-enriched materials, the build-up of ^{13}C cross peaks at long mixing times can contain domain-size information from 2 to 5 nm. CODEX ^{13}C NMR shows that the magnetization spreads from the source ^{13}C spin to >100 neighbors within about 1 s, corresponding to a ^{13}C spin diffusion coefficient of 0.3 – 1 nm^2/s. On this basis and in combination with composition information from NMR spectra and domain shapes suggested by microscopy, detailed spin diffusion simulations can be used to determine valuable structural information, for instance cellulose fibril sizes in wood.

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PL3

SOLUTION AND SOLID-STATE NMR STUDIES OF SARS-COV-2

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SARS-CoV-2 is the novel coronavirus that is the causative agent of COVID-19, an infectious disease responsible for a world-wide pandemic. Three out of the four structural proteins encoded in the viral genome are integral membrane proteins, and understanding their structure, dynamics, interactions, and function in a membrane- environment is key to developing new classes of antiviral drugs. We describe solution and solid-state NMR studies of the SARS-CoV-2 envelope (E) protein, a structural 75-residue integral membrane protein whose transmembrane domain exhibits ion channel activity under some conditions and whose cytoplasmic domain participates in protein-protein interactions that contribute to several aspects of the viral replication-cycle. NMR studies and NMR-guided antiviral assays that provide molecular guidance for the design of inhibitors targeting the E protein in the viral envelope will be described.

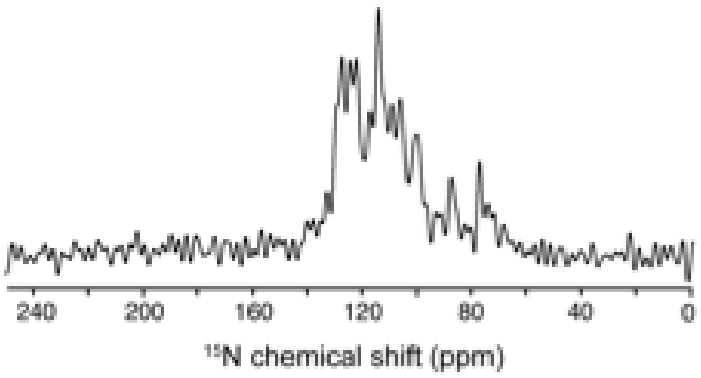

Fig. 1. Oriented sample ^{15}N chemical shift solid-state NMR spectrum of the transmembrane domain of E protein from SARS-CoV-2 embedded in 1,2-dimyristoyl-sn-glycero-phosphocholine bilayers oriented with the lipid bilayer normal perpendicular to the applied magnetic field. Fast uniaxial rotational diffusion of the protein about the bilayer normal yielded motionally averaged single line resonances. The tilt angle of the transmembrane helix of E protein can be estimated to be about 45°.

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PL4

IN VIVO ^{2}H AND ^{13}C METABOLIC IMAGING AS COMPLEMENT TO ^{1}H MRI IN THE CHARACTERIZATION OF HEALTH AND DISEASE

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NMR spectroscopy is well known for its ability to resolve chemically distinct sites in complex media. When combined with its ability to localize the spins' positions using field gradients (MRI), this opens unique opportunities to monitor the chemistry and biochemistry of functioning organs in living organisms –including humans. A particularly valuable window opened by such magnetic resonance spectroscopic imaging (MRSI) approaches concerns the characterization of *in vivo* metabolic processes; i.e., the changes undergone by the metabolites supporting life, in the presence of health and disease. NMR-based metabolic imaging methods could provide complements to more established techniques such as ^{1}H MRI, which can provide contrast based on parameters such as water's T1, T2 and diffusivity. The fate of MRSI-based metabolic techniques, however, hinges in overcoming NMR's sensitivity problem. The present talk will focus on two complementary approaches that we and others are pursuing to alleviate this challenge; one based on the use of hyperpolarized ^{13}C MRSI, and the other on reliance of ^{2}H MRSI at ultrahigh fields. The former has the advantage of very high sensitivity but of short lifetimes; the latter has a much lower sensitivity, but ^{2}H's quadrupolar character enables the use of rapid repetitions making up for this deficit. Both of these methods were utilized to target two apparently different –but biologically related and equally hard to tackle– problems, involving growth of masses in the abdomen: one concerns the development of embryos and foetuses in normal and in impaired pregnancies; the other the characterization of pancreatic cancer tumors and their distinction from other diseases like pancreatitis. In both instances, special spectroscopic imaging methods had to be developed to maximize the power of the emerging techniques, but as a results of this new information and biological insight difficult or altogether impossible to obtain by other methods, could thus be obtained. The flexibility of these methods also enabled their application, in combination with ^{1}H-based MRI and MRSI observations, to distinguish the behaviour of different phenotypes in wildtype and knockout animal models. The prospects of these methods for further biological research, as well as for potential human translation, will be briefly discussed.

Acknowledgments: This research was made possible by grants from the Israel Science Foundation, the US NIH, the Thompson Family Foundation, and the Clore and Kimmel Institutes at the Weizmann.

PL5

SOLID-STATE NMR IN CRYSTAL ENGINEERING: A SIMPLE TITLE FOR A COMPLEX TOPIC

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Crystal Engineering deals with the rational design and synthesis of novel crystalline molecular materials with desired properties [1]. The design leverages the strength, directionality, and robustness of supramolecular synthons to obtain specific and peculiar molecular arrangements. In this context, the structural characterization is extremely important because the outcome properties strongly depend on the 3D arrangement of the molecules. Single crystal X-Ray diffraction is the best technique for structural studies but sometimes it encounters difficulties with crystallization and crystal size. Over the last decades, solid-state NMR (SSNMR) has developed into an indispensable and complementary tool in Crystal Engineering for investigating the structure of crystalline supramolecular adducts. In particular, its ability to locate hydrogen atoms makes it a unique technique for characterization of the weak interactions. Here we present some examples of SSNMR applications on co-crystals and molecular salts focusing on the possibility of:
- investigating hydrogen bonds also in terms of proton transfer [2];
- determining the tautomeric character of molecules [3];
- probing atom-atom proximities and distances [4].
The complementarity of the technique with X-Ray diffraction and computational tools will be highlighted.

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PL6

NMR TITRATIONS SHED LIGHT ON THE MECHANISM OF NEGATIVE COOPERATIVITY AND ON THE STRUCTURAL LONG-DISTANCE CONFORMATIONAL EFFECTS OF TRANSTHYRETIN LIGAND BINDING

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Binding of thyroxine analogues to transthyretin (TTR) stabilizes the protein and is used to treat TTR amyloidosis. However, X-ray crystallography has not shown significant conformational changes induced by ligand binding. The structural basis of the known negative cooperativity between the two identical ligand binding pockets of TTR is also unclear. Analysis of NMR experiments identified long-distance effects induced by ligand binding that explain the structural basis of protection from amyloidogenic proteolytic cleavage of TTR by bound ligands. Moreover, occupation of only the first ligand-binding pocket populates a modified intermediate state that likely explains the structural basis of negative cooperativity.

PL7

UNDERSTANDING TIN-CONTAINING PEROVSKITE MATERIALS WITH HELP FROM SOLID-STATE NMR SPECTROSCOPY

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Hybrid lead halide perovskite materials are rapidly developing as effective photovoltaic semiconducting materials. One area that has drawn considerable attention is their fast-paced development in photoconversion efficiency (25 - 30%) when used in conventional or tandem-based light-harvesting solar cells. The unique feature of these solids involves their highly flexible ABX_3 structure, which can readily accommodate a vast array of ion doping or ion substitution of the A, B or X positions of their lattice. We will discuss progress in unravelling the structure-property relationship in these materials, examining their atomic-level structural changes and electronic properties. Solid-state nuclear magnetic resonance (NMR) spectroscopy has rapidly advanced as a robust analytical characterization method to understand these complex structural changes. We will discuss a multinuclear magnetic resonance approach to reveal unique NMR signatures for structure and dynamics. Recent results on the greener tin-containing compounds in both hybrid and non-hybrid perovskites and perovskite-like will be featured. The unique signatures appear to be sensitive to both composition and synthetic conditions, imparting unique electronic properties. Solid-solution behaviour supported by solid-state NMR is complemented using DFT computations and diffraction techniques, enabling a complete picture of their short-, medium- and long-range structure.

PL8

STRUCTURE DETERMINATION OF ANTIMICROBIAL PEPTIDES IN MODEL MEMBRANES AND LIVE BACTERIA

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Antimicrobial peptides (AMPs) have been extensively studied as promising alternatives to traditional antibiotics. Solid-state NMR has been used to characterise their effect on lipid bilayers, their primary target. Such studies are important to provide high-resolution details within a model system but correlation with *in vivo* situations remains speculative, especially in view of the complex modulation observed with slight changes in conditions such as pH, temperature, lipid composition or peptide concentration. Studying AMPs in live bacteria is, therefore, important but presents several challenges, such as sensitivity and bacterial lifetime. Studies of AMPs in live *E. coli* or *S. aureus* bacteria using solid-state NMR techniques will be presented. The impact of the AMP maculatin 1.1 (Mac1) on bacteria was monitored by ^{31}P while structural details on the peptide were obtained using dynamic nuclear polarization (DNP) enhanced ^{13}C and ^{15}N solid-state NMR experiments. Finally, a novel strategy to perform in-cell DNP NMR experiments was established by using spin-labelled peptides; and $\{^{15}N\}^{13}C$ REDOR measurements have been performed to measure the distance between several pairs of ^{13}C=O and ^{15}NH within the Mac1 amino acid sequence, which indicate a transmembrane helical structure in bacteria.

PL9

APPLICATIONS OF MAGNETIC NANOPARTICLES IN BIOMEDICINE: PAST, PRESENT AND FUTURE TRENDS

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Starting from the mid-1990s, several iron-oxide Magnetic Nanoparticles (NPs) were developed as MRI contrast agents. Though an unfavorable cost/benefit ratio has led to their withdrawal from the market as simple diagnostic agents, innovative applications have recently prompted a renewal of interest in these NPs.
Nowadays, magnetic NPs are regarded as valuable diagnostic and therapeutic tools. In the field of cell therapies, for example, they allow labeling and in vivo tracking of cells, as for example stem cells [1] or exosomes [2], transplanted in living organisms. In the field of cancer immunotherapy, they allow noninvasive monitoring of immune response in tumor tissue and at the same time, they can alter macrophage polarization, so producing a therapeutic effect. In addition, they can be agents for magnetic fluid hyperthermia [3]. Magnetic NPs are applied also as drug delivery systems, since they are deliverable to the target tissue by using magnetic gradient actuation forces. Moreover, such NPs are detectable in an innovative tomographic diagnostic imaging modality, namely Magnetic Particle Imaging (MPI) and are therefore multimodal contrast agents for MRI and MPI.
In this contribution, taking a cue from the research performed in our laboratory, I will present current preclinical/clinical applications of magnetic NPs, as well as emerging trends, in the study and application of these materials.

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PL10

LIFE AT THE BOTTOM: CONTEMPORARY MILLITESLA NMR AND MRI

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A promising approach to portable MRI is operation at ultra-low magnetic field where cost-effective electromagnets become practical. MRI in the ultra-low field (ULF) regime - when the magnetic field used for signal detection is below 10 mT - is inherently challenging mainly due to intrinsically low Boltzmann polarization. We will discuss signal acquisition approaches and hardware methods to improve attainable SNR in the Johnson-noise-dominated Larmor frequency of 276 kHz (6.5 mT) [1, 2]. We will also discuss our work to reduce noise and increase attainable information per unit time using compute-based approaches that leverage low-cost GPU. These include magnetic resonance fingerprinting (MRF) to enable multiple quantitative contrasts at ULF [3], and the use of our neural network deep learning approach, AUTOMAP (AUtomated TransfOrm by Manifold APproximation), to reconstruct highly-undersampled low SNR imaging data [4, 5]. In addition, we will discuss several classes of NMR and MRI experiments enabled by operation at low magnetic field, which can outperform what can be done with high-field instruments [6–15].

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PL11

ASSESSMENT OF PREDICTION METHODS FOR PROTEIN STRUCTURES DETERMINED BY NMR IN CASP: IMPACT OF ALPHAFOLD2

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The remarkable performance of some protein structure prediction groups in the 2020 Critical Assessment of Protein Structure Prediction experiment (CASP14) has set a new standard for protein structure modeling. These breakthrough technologies exploit advances in attention-based machine learning, contact prediction based on sequence co-variance analysis, the massive data bases of genomic sequence data, and the rapidly growing database of experimental protein structures. The best deep learning methods, like Google DeepMind AlphaFold2 (AF2) [1], provided amazingly accurate protein structure predictions. The methods are particularly successful in predicting static X-ray crystal structures. NMR studies can provide unique information about protein conformations in solution. In CASP14, three reference structures provided by solution NMR methods were available (targets T1027, T1029, and T1055), as well as a fourth data set of NMR-derived contacts for an integral membrane protein (T1088). For the three targets with NMR-based structures, the best prediction results ranged from excellent (GDT_TS = 0.90, backbone rmsd ~ 1.5 Å, for T1055) to poor (GDT_TS = 0.47, backbone rmsd ~ 6 Å, for T1029). We explored the basis of these results by comparing all CASP14 prediction models against experimental NOESY and chemical shift NMR data. For T1055, both the NMR structure and the AF2 model are excellent fits to these NMR data; remarkably, the AF2 model is a slightly better fit to these data than the reported NMR structure. For T1027, nuclear relaxation measurements reveal extensive internal dynamics, presenting a challenge for current protein structure prediction methods. The analysis of target T1029 motivated exploration of a novel method of "inverse structure determination", in which an AF2 model was used to guide NMR data analysis. NMR data provided to CASP predictor groups for the fourth target T1088, a 238-residue integral membrane porin, was also used to assess several NMR-assisted prediction methods. Most groups involved in this exercise generated similar beta-barrel models, with good agreement with the experimental data. However, as was also observed in the 2018 CASP13 NMR-assisted experiment, some pure prediction groups that did not use any NMR data generated models for T1088 that better fit the NMR data than the models generated using these experimental data [2]. These results demonstrate the remarkable power of modern methods to predict structures of proteins with accuracies rivaling solution NMR structures, and that it is now possible to reliably use prediction models to guide and complement experimental NMR data analysis.

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BRACCO, BRUKER AND JEOL LECTURES

SP1

PRE-CLINICAL IMPLEMENTATION AND VALIDATION OF A MR FINGERPRINTING SEQUENCE

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Magnetic resonance imaging is a powerful and versatile tomography technique commonly used in clinic for diagnostic purposes. Many different acquisition methods have been developed to visualize or to extract quantitative information from an investigated sample or from a biological tissue of interest. In particular, Fast Imaging methods allow to obtain weighted images with good temporal efficiency via gradient echo sequences. This family of methods has been quite recently adapted to a novel quantitative acquisition paradigm, called *MRI Fingerprinting* (MRF) [1], which allows the non-invasive and concomitant quantification of multiple properties of a sample through a novel approach to data acquisition, post-processing and visualization [1]. The MRF technology is based (i) on the acquisition of the MR signal under pseudorandom variations of acquisition parameters and (ii) on matching the obtained signal with a large dictionary of theoretical signal profiles to extract the values of intrinsic parameters of the sample [2].
The present work fits in this context, aiming to implement an MRI method able to manage a priori pseudorandom variations in both the flip angle (FA) and the repetition time (T_R). The simultaneous estimation of T_1 and T_2 relaxation times can be achieved through the best matching algorithmic procedure between the measured MRF signal and all the simulated signals contained within a dictionary simulated using the Extended Phase Graphs (EPG) tool for depicting the magnetization response to the implemented MRF method. The described method, not included among the standard methods released by Bruker, was implemented on a Pharmascan preclinical scanner operating at 7 T, adapting the standard Bruker method (*i.e.* FISP, FID-mode) to the MRF paradigma [2]. The implementation required to modify the Bruker pulse and gradient programming file (ppg), which contains all the operational instructions for the device to run an MRI sequence. The developed MRF sequence was then validated and optimized through *in vitro* measurements and concluded with a preliminary *in vivo* experimental session carried out on a rat healthy brain. The method has proved to be sensible to different relaxation times of *in vitro* phantoms, and accordingly the *in vivo* data confirmed the sensibility to the rat brain relaxometry variations. The obtained results suggested that the implemented MRF method can simultaneously estimate T_1 and T_2 with a good agreement with respect to the standard MRI sequence commonly used and in a much shorter time (*i.e.* t_{MRF} of approx. 15 minutes *v.s* approx. hours), confirming the potential of MRF as an interesting tool for quantitative imaging.
Furthermore, the MRF approach may offer the opportunity to simultaneously quantify many MRI parameters besides T_1 and T_2, for a wide variety of imaging applications.

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SP2

DNP DEVELOPMENTS IN SOLIDS AND DISSOLUTION DNP AT BRUKER

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Ordinary signals from conventional nuclear magnetic resonance (NMR) and imaging (MRI) experiments are known to be intrinsically weak due to exceedingly small (ppm-level) nuclear spin polarization levels. Dynamic Nuclear Polarization (DNP) circumvents this disadvantage by transferring high electron spin polarization levels at low temperature and high field into the nuclear spins of a sample. In recent years DNP has been developed and utilized in many different technological "flavors", with two particular approaches gaining the most widespread use: solid-state Low-Temperature MAS DNP (LT-MAS DNP), and, more recently, dissolution DNP (d-DNP) for applications in solution-state NMR and MRI.
In this talk, we will present the state of the art and recent development of both LT-MAS and d-DNP, including overview of the latest available technologies and coming developments, as well as highlight to a few of the newest applications.
LT-MAS DNP is now a well-established product, with about 50 Bruker systems installed worldwide, ranging from 400 to 900 MHz. Continual push for improved hardware recently resulted in our release of a new compact microwave source for 400 MHz NMR, the 263 GHz Klystron developed along with CPI Canada. We also present developments towards DNP in standard-bore magnets that can extend the reach of DNP for high-field applications. At the same time, academic labs have driven development of new radicals [1] to improve DNP enhancement, especially at high field.
d-DNP is a much newer approach, but rapidly maturing and gaining applications popularity. Ardenkjær-Larsen and co-workers introduced the method in 2003 [2], dramatically boosting the polarization level of nuclear spins for enhancements >104 vs. room-temperature solution-state signal levels. At Bruker, we developed and installed three early prototype systems [4] with academic collaborators in France, and continue with development of a new system with aiming for zero-consumption cryogenic performance for samples near 1 K and at 7 T field. All of our systems enable cross-polarized (CP) DNP [3]. Due to origins of the polarization from 1H DNP, CP enables 13C hyperpolarization in just 10 min (20x faster buildup) with state-of-the-art hyperpolarization levels (>60%). We will present an overview of early and next-generation d-DNP polarizers as well as the value of these key methods for both liquid state NMR and MRI applications.

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SP3

JASON: A NOVEL NMR TOOL

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A complete processing, analysis and reporting NMR suite has been developed to facilitate NMR interpretation. A detailed explanation of the engine and capabilities of this new tool will be provided, with special emphasis on automation and optimized workflows.

ORAL COMMUNICATIONS

OC1

QUANTUM CHEMICAL METHODS AND PARAMAGNETIC NMR IN (BIO)INORGANIC CHEMISTRY

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NMR is an invaluable tool for the atomic-level structural characterization of molecules and materials. The presence of paramagnetic metals causes perturbations to the spectra (shifts and broadening) the interpretation of which is attracting more and more interest: these perturbations require more complex data acquisition and analysis, but encode relevant structural information. First of all, they can be related to the relative position of the probe nucleus with respect to the paramagnetic center, and this information has long been used in several fields. Furthermore, the accessibility and quality of Quantum Chemical methods for the calculation of paramagnetic NMR observables have increased so much that now it is feasible to extract reliable information about the metal coordination sphere. We have tested the performance of QC methods in the prediction of proton hyperfine shifts of small complexes [1] as well as in a metalloprotein [2]. Structural information is obtained at a resolution inaccessible to other techniques.

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Acknowledgements: This work has been supported by the Fondazione Cassa di Risparmio di Firenze, and the Italian Ministero dell'Istruzione, dell'Università e della Ricerca through the "Progetto Dipartimenti di Eccellenza 2018-2022" to the Department of Chemistry "Ugo Schiff" of the University of Florence, and the University of Florence through the "Progetti Competitivi per Ricercatori" to ER. We acknowledge the CINECA award to ER under the ISCRA initiative, for the availability of high-performance computing resources and support. The authors acknowledge the support and the use of resources of Instruct-ERIC, a landmark ESFRI project, and specifically the CERM/CIRMMP Italy center.

OC2

THE DARK SIDE OF THE HUMAN PRION PROTEIN

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The conformational conversion of the prion protein (PrP) from its normal cellular form, PrPC, to the unsoluble scrapie form, PrPSc, is at the basis of the pathogenesis of the transmissible spongiform encephalopathies (TSE) [1,2,3]. The misfolding of PrPC into PrPSc may occur due to genetic mutations of the PrP gene enhancing the aggregation propensity of the protein or through infection by diseased PrPSc forms, which then act as a template for PrPC-PrPSc autocatalytic conversion [4]. Nonetheless, most reported prionpathies are the results of spontaneous conversion of PrPC into PrPSc whose mechanism has been not yet elucidated, despite the fact that several in vitro and computational studies suggest PrP high conformational flexibility as a crucial factor in aggregation mechanism [5,6]. As a matter of fact, the capability of PrPC to populate partially unfolded state (usually termed as PUFs) in equilibrium with the native state appears to be an essential step prior to convert to the β-structured toxic oligomers and successively to the fibrillar unsoluble forms. In spite of this wealth of knowledge, a high resolution description of the initial stages of the conformational transition from PrPC to PrPSc is not yet available, as well as a detailed molecular picture of PrPC folding mechanism. Here, in order to understand the structural and dynamics determinants controlling the formation of intermediate states involved in fibril assembly, we report a high-resolution exhaustive NMR-Based investigation of conformational equilibria and folding mechanisms for full length and 90-231 human prion proteins.

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OC3

THE ROLE OF PROLINE CIS/TRANS ISOMERIZATION AND CHAPERON ACTIVITY IN THE PROCESS OF αSYN AGGREGATION

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Aberrant aggregation of the protein α-Synuclein (αSyn) into fibrils is the hallmark of Parkinson's disease (PD) [1]. The core of the fibrils is formed by the hydrophobic non-Amyloid-β component (NAC) localized in the central part of αSyn, while the preNAC region, which bears most of the point mutations that lead to sever forms of familial PD, establishes the interface between the two protofilaments [2]. The C-terminal part of αSyn contains five prolines residues, that influence significantly its aggregation properties [3].
Catalysis of proline cis/trans isomerization is important for the activity and misfolding of many intrinsically disordered proteins [4] and, within cells, the reaction is catalyzed by enzymes belonging to the class of peptidyl-prolyl isomerases (PPIases), such as cyclophilins [5].
It was previously noted that cyclophilin A (CypA) colocalizes and directly interacts with αSyn in vivo [6]. In this work, we characterized the interaction between CypA and αSyn at an atomic level of resolution using NMR spectroscopy and we established the role of proline isomerization in the aggregation process of αSyn. Our data clearly demonstrate that the catalysis of proline isomerization by CypA lowers the energy barrier for αSyn misfolding facilitating fibril formation, while the binding of CypA to the preNAC region opposes αSyn aggregation. In fact, removal of the prolin-reach region enhances the chaperon activity of CypA that strongly inhibits αSyn aberrant aggregation [7]. The data reveal the presence of a dual functionality of PPIases, consisting i) in proline cis/trans isomerization and ii) chaperoning activity.

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OC4

HEMP PRODUCTS: NMR CHARACTERIZATION OF INFLORESCENCES AND HEMP SEED OILS

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Industrial hemp (a Cannabis sativa L. chemotype) has been cultivated all over the world for centuries being the raw material in different industrial fields. However, due to the association with narcotic Cannabis, the industrial hemp cultivation almost disappeared in the 20th century. Recently, the EU published a regulation for the reintroduction of selected varieties of this crop in Europe to valorize hemp multiple applications [1]. The present study, developed in the frame of the Lazio Region project "Industrial Hemp: development and valorization of a sustainable new agro-food chain" aimed at the valorization of Cannabis sativa L. products of Lazio Region. In this context, an NMR analysis of inflorescences and hemp seed oils was applied. Bligh-Dyer extracts of inflorescences [2] from Ferimon hemp cultivar grown with different agronomical practices (irrigation, fertilization) and harvested at different periods were analyzed by high resolution NMR experiments (^{1}H, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC), allowing to define the metabolomic profile. Sugars, amino acids, organic acids, and other metabolites were detected, suggesting the potential use of this crop in the food field. Moreover, the application of Principal Component Analysis (PCA) on the obtained results allowed to observe the effect of the agronomical practices, mainly irrigation and harvesting, on the inflorescences chemical variability. The ^{1}H NMR analysis of hemp seed oils allowed to define the lipophilic profile (fatty chains, ω-6:ω-3 ratio, β-sitosterol, and aldehydes) of this foodstuff whose use is spreading widely. Moreover, the applied NMR protocol showed to be a powerful tool for the determination of some quality parameters of hemp seed oils since a specific official regulation does not exist. In this context, the NMR analysis of some commercial hemp seed oils [3] with a declared ω-6:ω-3 ratio of 3:1 (optimal for healthy human nutrition) showed that the effective values of this quality parameter. This study showed how NMR methodology represents an important tool for the study of innovative matrices such as hemp derived products, whose use is rapidly growing.

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OC5

EXPLORING THE DYNAMICS OF ELASTOMERS BY ^{1}H FIELD CYCLING NMR RELAXOMETRY

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Elastomers are polymeric materials extensively used for manufacturing a wide range of products for industrial applications. For this reason, great attention is focused on the production of new improved materials with well-defined mechanical properties. For this purpose, elastomers are usually characterized by mechanical measurements (modulus, strain at stress, etc.), which only provide macroscopic observables; however, in order to precisely guide the design of optimized materials with specific requirements, it is also crucial to obtain a description of the topology and dynamics of the polymer network at the molecular scale. Indeed, the mechanical properties of the material are strictly related to the mobility of the polymeric chains, which can be modulated for example by changing the amount of chemical cross-links or by adding reinforcing fillers (*e.g.* carbon black, nanosilica).
In this field, ^{1}H field-cycling (FC) NMR relaxometry represents an ideal technique to investigate complex polymer dynamics at the molecular level [1]. In fact, it allows the characterization of molecular motions over a broad time scale, by measuring ^{1}H spin-lattice relaxation rates ($R_1 = 1/T_1$) on a wide range of Larmor frequencies (from 10 kHz to 35 MHz); this range can be further enlarged by combining experiments at different temperatures based on the frequency-temperature superposition (FTS) principle [2].
In this work, ^{1}H FC NMR relaxometry was applied to characterize the effect of cross-linking [3] and the effect of filler [4] on polymer chain dynamics in a range of elastomeric materials of interest in the tire industry. The study allowed molecular dynamics to be carefully investigated over a quite broad time scale, ranging from local segmental motions to longer-range motions of the polymeric chains.

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OC6

GOING *DEEPER* WITH NMR: INTERACTIONS AND DYNAMICS OF *DEEP* EUTECTICS

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Deep Eutectic Systems (DESs) are an emerging class of sustainable materials of great interest in green chemistry [1]. Next to the desirable physicochemical properties (good stability, low flammability, exceptional dissolution capabilities) and the environmental and economic advantages (ease of preparation from largely available and safe raw materials, low cost and high sustainability), an added value of DESs is their potential as tunable solvents. Not only the constituents can be mixed in a very high number of combinations, but the mixtures are also compatible with the addition of a third component, opening up many new opportunities in terms of applications.

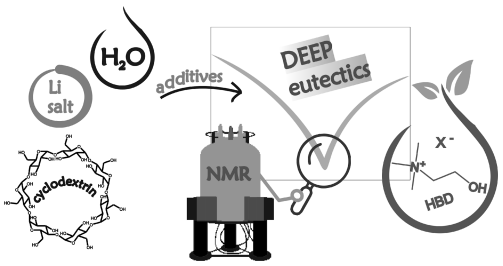

Fig. 1. Application of NMR spectroscopy to deepen the structure and dynamics of DESs and their mixtures with different additives.

In view of their technological and industrial exploitation, a proper fundamental understanding of DESs basics at the molecular level is imperative. Although hydrogen bonding is postulated as the root cause of DESs' unusual physicochemical properties, more efforts are needed to elucidate the intermolecular network of the mixtures and how they are affected by the replacement of one or more species or by the presence of additives. Here we illustrate the potential of multiple NMR techniques to get unique insights into structure and dynamics of choline-based type III DESs and their mixtures with water, cyclodextrins or lithium salts (Fig. 1) [2]. We demonstrate that the results obtained by chemical shift analysis, correlation experiments, and relaxation and diffusion measurements, acquired on different nuclei, all converge to a detailed and consistent picture of the systems. Noteworthy is also the multifaceted and all-embracing description that can be achieved when NMR spectroscopy is used jointly with other techniques (X-ray, UV Resonance Raman, MD and Monte Carlo simulations).

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OC7

NMR SPECTROSCOPY WITH NV DEFECTS IN DIAMOND: FROM SURFACE-NMR TO HYPERPOLARIZATION

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Nitrogen-Vacancy (NV) centers in diamond constitutes a unique nanoscale spin system which has attracted great interest for its potential in a variety of research fields, from quantum information processing to magnetic field sensing and magnetic resonance imaging at the nanoscale. Recently, a promising approach has emerged on utilizing such defects as room temperature NMR sensors capable of unprecedented performances in terms of sensitivity. The ability of these devices to optically read-out NMR signals allows for sample volumes by several orders of magnitude smaller than what is necessary for conventional inductive NMR detection [1–3]. Furthermore, such defects can be used as hyperpolarization tools for NMR signal enhancement since: i) they exhibit electronic spin states which can be highly polarized by laser pulses; ii) they can be coherently manipulated iii) their spin polarization can be transferred to nuclei outside the diamond. And all this under ambient conditions.

In this talk, I will introduce the field of NV centers, present some techniques used for their manipulation and describe how they can be used as NMR sensors as well as spin hyperpolarizers for NMR sensitivity enhancement.

Specifically, I will describe our recent work on the development of a novel technique called “surface NV-NMR” which enables performing NMR at surfaces and interfaces on the microscopic length-scale and under ambient conditions with far greater sensitivity than conventional NMR. Surface NV-NMR is able for instance to detect signals from a monolayer deposited on the diamond surface, indicate chemical binding, quantify molecular coverage and monitor in real-time the formation kinetics at the solid-liquid interface [4].

Finally, I will talk about our latest results on studying spin-polarization transfer between ensembles of shallow NV-centers in diamond and nuclear targets inside and outside the diamond lattice. I will show how pulsed protocols based on spinlock pulses have shown to be efficient and how these can be improved for future developments with a view to practical applications in microscale NV-NMR, bulk NMR or biomedical imaging [5].

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OC8

SIGNAL AMPLIFICATION BY REVERSIBLE EXCHANGE (SABRE) HYPERPOLARISATION OF MIRFENTANIL IN THE PRESENCE OF HEROIN

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Signal Amplification By Reversible Exchange (SABRE), first reported in 2009 [1], allows for the creation of a hyperpolarised state in solution without chemical change to the substrate. For example, World Health Organisation essential medicines have been polarised without chemical modification [2].

Despite also being a World Health Organisation essential medicine, fentanyl has recently become a drug of abuse with an estimated potency ~500 times that of morphine. This poses a serious threat to public health with quantities as low as ~2 milligrams enough to induce overdose. In particular, the lacing of heroin samples with fentanyl for increased potency and decreased production cost has been a major contributing factor to thousands of deaths per year in the US and an increasing number across Europe.

A range of fentanyl analogues (fentalogues), some of which are more potent than fentanyl, have recently appeared on the international drugs market however, due to the small quantities typically present, existing detection technology struggles to rapidly detect low concentrations of fentanyl or fentalogues. In this work four fentalogues were prepared for testing. Three contained a pyridyl ring, as this functionality has been the focus of multiple SABRE reports [3], and a further fentalogue, Mirfentanil, has been reported to have human *in vivo* activity [4].

We present results for the successful hyperpolarisation of Mirfentanil and a range of fentalogues produced through the systematic derivatisation of fentanyl and these results are discussed in relation to concentrations commensurate with the lethal dose.

The application of SABRE to a more realistic sample of Mirfentanil spiked heroin was tested and in a Minfentanil/heroin mixture with 3% Mirfentanil, hyperpolarisation is observed [5]. It is envisaged the application of SABRE could enable fentalogue detection on a benchtop instrument in a single scan, increasing the accessibility and speed, of detection with a view towards a forensic application.

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OC9

NMR APPROACH FOR INVESTIGATING TWO NEW OXALILPLATIN-PYROPHOSPHATO ANALOGS

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Cisplatin is one of the most potent drugs used in the treatment of a variety of cancers but severe side effects, such as nephro-, neuro-, and oto-toxicity, limit its use. One strategy to reduce the systemic side effects of platinum(Pt)-based drugs is to choose carrier ligands that promote specific accumulation of the drug in target organs or cells. A notable example is represented by *phosphaplatins*, where the Pt(II) ion is coordinated to two *cis* N-donors and to a single bidentate pyrophosphate ligand, which can deliver the therapeutic agent specifically to bone tissue. In this study, two new Pt(II)-pyrophosphato complexes containing the carrier ligands *cis*-1,3- diaminocyclohexane (*cis*-1,3-DACH, **1**) and *trans*-1,2-diamine-4-cyclohexene (1,2-DACHEX, **2**), variants of the 1*R*,2*R*-diaminocyclohexane ligand present in the clinically used drug oxaliplatin, have been synthesized with the aim of developing new potential antitumor drugs with high bone tropism. Complexes **1** and **2** have been characterized by heteronuclear NMR spectroscopy (^{1}H, ^{13}C, ^{31}P, and ^{195}Pt). Moreover, ^{31}P{^{1}H} NMR experiments at different pH values allowed the determination of the acidity constants of **1** and **2** and an estimate of their stability under physiological (Figure 1) and acidic conditions to mimic the environment of cancer tissues. ^{31}P NMR was also used to investigate the reactivity of the new complexes towards 5'-GMP in order to have a preliminary indication of their mechanism of action. Finally, the two Pt(II)-pyrophosphato complexes were tested to evaluate their cytotoxicity *in vitro* against a panel of human tumor cell lines [1].

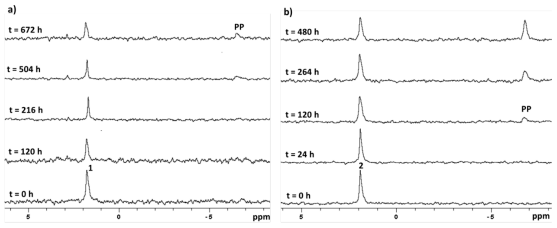

Fig. 1. ^{31}P NMR (121.5 MHz) spectra of **1** (**a**) and **2** (**b**) at different incubation times in physiological conditions (D_2O, HEPES buffer 50 mM, pH* = 7.4, 120 mM NaCl, 37 °C).

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OC10

STRUCTURE AND DYNAMICS OF "COOL" ORGANIC PIGMENTS BY SOLID STATE NMR

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Nowadays, painting building roof and façades with "cool" pigments, showing transparency and reflective properties towards the near infrared (NIR) radiation, is one of the most affordable strategies to mitigate the Urban Heat Island (UHI) effect [1,2], responsible for worming and consequent sharp raise of energy demand in city areas. Recently, perylene bis-imide derivatives (PBIs) have attracted a considerable interest as "cool" organic pigments [3], representing a more versatile and lower-cost alternative to the currently used inorganic pigments based on rare-earth oxides. NIR reflectance and transparency of PBIs were found to be strongly related to the nature and position of the substituents at the PBI core. Supramolecular packing, crystallinity and particle size also play a role, but a full comprehension is still far from being achieved [3,4]. Solid state NMR spectroscopy (SSNMR) can be very helpful to disclose information on molecular and supramolecular features of PBIs structure, complementary to those achievable by other techniques such as X-ray diffraction (XRD) and electron microscopies. In this work SSNMR has been used in combination with density functional theory (DFT) calculations to investigate the structural and dynamic properties of the PBI pigment Paliogen Black L0086 (P-black) (Fig. 1) [5], which has shown promising "cooling" properties in acrylic coatings [4]. The comparison with DFT predictions permitted the full assignment of the ^{13}C SSNMR spectrum, pointing out correlations between ^{13}C isotropic chemical shifts and supramolecular packing. Moreover, variable temperature ^{13}C experiments provided information on the π-flip motion involving the methoxybenzyl substituents bonded to the imide nitrogen atoms,

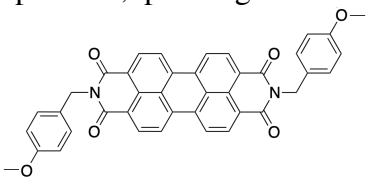

Fig.1 Structure of P-black.

which was found to depend on structural order as well as on the length of the alkyl linker between the substituent and the PBI core. Finally, a preliminary investigation on PBI pigments similar to P-black has been carried out, which highlighted the utility of ^{13}C SSNMR for the characterization of PBIs, especially when single-crystal XRD cannot be applied.

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OC11

TOWARDS A SIMULATION FRAMEWORK FOR MAGNETIC SUSCEPTIBILITY INDUCED RELAXATION OF SPINS DIFFUSING IN POROUS MEDIA

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Many interesting materials in nature can be classified as porous media, i.e. heterogeneous materials characterized by a matrix hosting a network of pores. Battery electrodes, rock formations, bones, brain matter, cellular scaffolding, etc, fall in this category. NMR, especially because it is not destructive, quantitative, and informative, is a technique of choice to extract structural and dynamical information from such media, However, NMR experiments in porous media are complicated by the presence of magnetic susceptibility differences between the medium and the substance filling the pores. The demagnetization field that arises and adds up to the static magnetic field is locally distorted by the porous structure. Molecules diffusing through such pores experience a randomly fluctuating magnetic field, which eventually results in a loss of spin coherence due to a magnetic susceptibility induced T_2 relaxation mechanism [1]. Many pulse sequences and experiments specifically designed for probing porous media rely on signal attenuation during molecular diffusion [2]. Hence, when the lifetimes are significantly reduced, there is insufficient time for a thorough analysis of the space before all encoded information is lost. To date, no complete analytical theory exists to describe the effects of such demagnetization fields on relaxation rates [3].

This contribution outlines the approach taken towards the production of a computational framework, built to predict relaxation rates of nuclei diffusing through porous systems. The simulation utilizes CT scans to calculate the internal field distribution and perform a random walk, depicting molecular diffusion, all within an accurate 3D replica of the structure of interest. The aim is the ability to predict spin-spin relaxation rates as a function of the path taken by the molecule as it travels through the sample. Characterization of the relaxation process will be determined by the simulated spectral density function, arising from fluctuating fields experienced during the random walk. From this, we hope to develop a greater understanding of relaxation processes resulting from diffusion within random internal field gradients. Furthermore, we hope to advance this method to study more intricate systems by implementing long-lived states such as nuclear singlet spin states. Efforts within the group have previously shown these states may persist for upwards of an hour, allowing the probing of larger and or more complex structures.

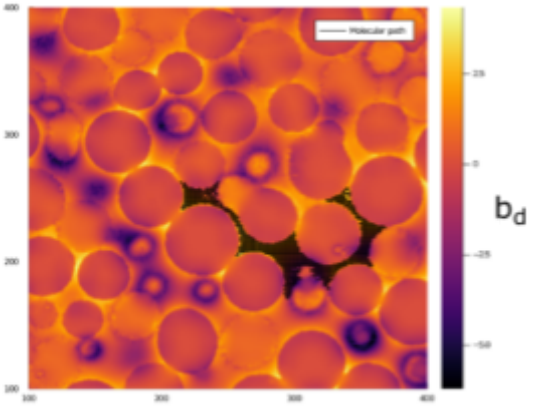

Fig. 1. Random walk through the CT data

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OC12

A METABOLIC STUDY OF CALABRIAN BERGAMOT ESSENTIAL OIL USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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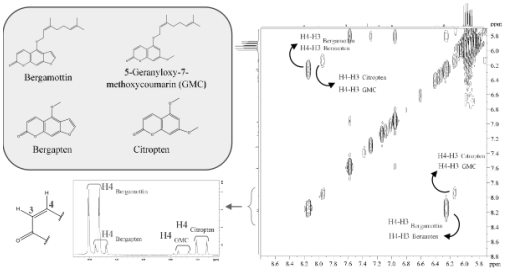
Citrus Bergamia, commonly known by the name of bergamot, is a fruit bearing tree belonging to the Rutaceae family, which grows almost exclusively in the Calabrian strip coast between Villa San Giovanni and Gioiosa Jonica (Italy). Its composition in antioxidants, vitamins and biomolecules give bergamot juice healthy characteristics ranging from anti-cholesterol to antioxidant and anti inflammatory properties. However, bergamot is mainly cultivated for the essential oil obtained from the fruit peel, that, due to its unique fragrance and thanks to its anti-seborrheic and stimulating properties, is a high commercial value extract. Indeed, this raw substance is widely used as an essential component for the international perfume and cosmetics industries but it is also employed in pharmaceutical production due to its antiseptic and antibacterial properties[1]. Evaluate the chemical profile of BEO is necessary to ensure its effectiveness in the various fields of application and, in particular, for the release of the “Protected Designation of Origin (PDO)” of "Bergamotto di Reggio Calabria – Olio essenziale ". Currently, the accepted instrumental methods used to asses the BEO quality and origin are the chromatographic techniques, but the scientific research is moving in the direction of new fast methodologies to better characterize this high values extract often subjected to fraud and sophistication[2]. In this contribution, for the first time NMR spectroscopy was used on samples provided by the “Consorzio del Bergamotto di Reggio Calabria” in order to a) explore the metabolomic profile of BEO of Reggio Calabria using 1D and 2D NMR experiments recorded on this complex mixture (fingerprinting and profiling), and b) highlight sophistication and
possible markers for the authentication combining ^{1}H-NMR spectra with statistical tools (PCA - Principal Component Analysis).

Fig. 1. Cumarines recognized in BEO sample (top); region ranging from 7.9 to 8.2 ppm of the 500 MHz ^{1}H NMR spectrum (bottom); 500 MHz COSY NMR spectrum (F1: 7.9 - 8.2 ppm, F2: 7.9 - 8.2 ppm).



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OC13

THE SYNERGIC USE OF UHPLC-HRMS AND NMR IN METABOLOMICS

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The value of combining NMR and MS, the two most commonly used techniques in metabolomics, is widely recognized [1]. So far, few studies have linked these two techniques, and often they were dedicated to developing statistical methods for weighing the two datasets [2] and for the discovery of new components of biofluids [3]. Given the high complementarity of the two techniques, it would be beneficial to combine the data obtained separately with NMR and MS to improve the ability to classify the "metabotypes" under investigation [1]. Urine is the best test bench to measure the potential value of the MS-NMR combination, as it represents one of the most challenging biofluids to characterize due to complexity and variability.
The best combination of MS and NMR data developed so far was called Statistical Heterospectroscopy (SHY) [4]. The authors showed that it is possible to correlate chemical shift and m/z data when a cohort of samples is considered. Our idea is to use the SHY concept to develop a novel strategy of MS-assisted deconvolution of NMR spectra. Instead of using the correlation with spectral regions as in the original work, we employ the metabolite concentrations determined iteratively by NMR and UHPLC-HRMS, obtaining a significant increase in the number of identified compounds. The synergistic use of both analytical methodologies allowed us to quantify 165 metabolites in the urine of nine controls, six patients affected by chronic cystitis, and thirty-one bladder cancer patients. We call this approach: *SY*nergic use of NMR and HRMS for *MET*abolomics (SYNHMET). These results can be used in classical metabolomics studies of group classification. Still, the fact that we obtain absolute concentrations opens the possibility of creating a personalized metabolic profile for each subject in the study. Its translation into clinical practice can be of great value, in line with scientific advances that follow personalized medicine.

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OC14

NMR-BASED METABOLOMICS AND MULTI-BLOCK OMIC APPROACH TO INVESTIGATE THE ROLE OF GUT MICROBIOME IN CHILDREN WITH TYPE 1 DIABETES

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Type 1 diabetes (T1D) is a complex autoimmune disease resulting from the loss of insulin-producing β-cells in the pancreas [1]. Its global incidence has heightened over the last decades [2]. The etiology of T1D is more complex than expected and includes a combination of genetic susceptibility and environmental factors that lead to heterogeneous clinical manifestations across individuals and also contribute to cognitive dysfunction [3]. It is therefore of primary importance to intervene promptly with tailored interventions and therapeutic strategies to prevent and treat this chronic disorder. It is widely recognized that the establishment of gut microbiota in the very early stage of line shape both the innate and the adaptive immune system and may play a pivotal role in influencing the development of inflammatory conditions and trigger autoimmune response such as T1D [4].

Aims of this study were to evaluate the differences in gut microbiota metabolism and composition in children affected by T1D in respect of healthy control and of the disease severity. We analyzed stools of 25 Caucasian children with newly diagnosed T1D recruited at the Endocrinology and Diabetes Unit of Children's Hospital Bambino Gesù in Rome (Italy) and compared to 18 healthy controls matched for age and gender. Furthermore, the effect of the severity of the disease was evaluated comparing the T1D patients on the basis of the blood pH levels at onset (pH<7.23 and pH>7.33).

The NMR-based metabolomics was carried out on stool samples of T1D patients and CTRLs by means of a Bruker Avance III 400 spectrometer equipped with a 9.4 T magnet operating at ^{1}H frequency of 400.13 MHz and at 298K. Signals' assignment was achieved by bidimensional experiments (COSY, TOCSY, HSQC, HMBC). Along with that, it was also assessed the gut bacterial composition by 16S rRNA-targeted metagenomics.

By fusing metagenomics and metabolomics data using a multi-block (low level fusion) approach, we obtained a multi-omic model which allowed to distinguish a gut microbiomic signatures between T1D patients and controls, as well as between patients with high and low blood pH levels.

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OC15

NMR AS A TOOL FOR THE BIOCHEMICAL CHARACTERIZATION OF MICROORGANISMS OF BIOTECHNOLOGICAL INTEREST

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NMR-spectroscopy is an efficient and highly reproducible platform for the analysis of cell cultures both in terms of their endo- and exo-metabolome. The type and abundance of metabolites detected in cell lysates and their respective growing media can be viewed as a global fingerprint that unambiguously describes the current cell phenotype [1]. In this framework, cell-metabolomics can be used for the biochemical characterization of microorganisms of biotechnological interest, such as for the production of recombinant proteins or secondary metabolites [2,3]. In this regard, the metabolism of *Pseudoalteromonas haloplanktis*, TAC125 (PhTAC125), a heterotrophic marine bacterium isolated from Antarctica, has recently gained a certain attention due to its potential biotechnological exploitation.
Here, NMR-based metabolomics was used to investigate the metabolism of PhTAC125 during growth in complex environments and its metabolic switches according to the available carbon sources. One-dimensional ^{1}H NMR spectra of PhTAC125 growing media were used to characterize the uptake of different amino acids as carbon sources and to determine the kinetics of their usage during growth. Next, we investigated the fate of different amino acids once internalized by PhTAC125 cells. To this aim, we used uniformly labelled ^{13}C amino acids and followed the path of their labelled carbon atoms inside the cells by time-resolved bi-dimensional ^{1}H-^{13}C HSQC experiments. Each amino acid showed a specific path of labelled carbon atoms that reflects their different and complementary roles within the cell. The use of different carbon sources depending on the cell growth phase and with distinct metabolic fates suggest that plastic strategies for carbon assimilation might be evolved in response to nutritionally poor and highly variable conditions [2]. Moreover, the results of ^{1}H NMR metabolomics were used to feed computational modelling with the final aim to set up efficient culture conditions for the heterologous production of the CDKL5 protein in PhTAC125 [3].

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OC16

RADIOMIC ANALYSIS ON MR IMAGES OF THALAMUS OF PATIENTS WITH ESSENTIAL TREMORS

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Radiomics is a new field of imaging analysis, which can be described as the process of extraction of quantitative features that cannot be qualitatively appreciated by human eyes from images, and the subsequent mining of these data for improved clinical decision making. These data are mined along with patient information to answer to paramount clinical questions, such as diagnosis, prognosis and response to therapy [1]. In this work we aim at applying radiomics approach to MR images of patients with essential tremor that is the most common movement disorder. In particular, a 1.5T MR scanner (GE Signa HDxt) was used to acquire morphological (T1- and T2-weighted) and ultrastructural morphological sequences (Diffusion Tensor Imaging) of about 30 patients suffering from essential tremor. A pipeline was previously developed in order to parcel thalamus through identifying the fibers that connect the primary and supplementary motor areas and the premotor cortex to the thalamus by means of probabilistic tractography [2]. This allows to find regions of interest (ROI) inside the thalamus (which otherwise would be hardly subdivided in parts if 1.5T MR images are used). Size- and shape-based features as well as first and higher order statistics features are extracted from the the ROIs previously recognized inside the thalamus.

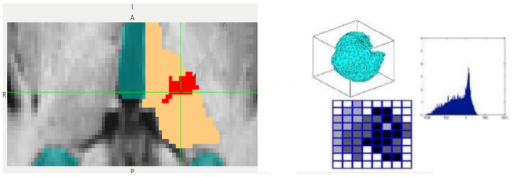

Fig. 1. Regions identified inside the thalamus through probabilistic tractography and feature extraction from them.

These features are analyzed along with clinical neurological data (which are related to tremor severity, tremor side, quality of life, etc) in order to find correlations between radiomic features and clinical parameters. The results are justified on the neurological and neuroradiological basis. These preliminary results allow for deeper insights on the correlations between thalamus regions and clinical symptoms of patients with essential tremor. Such information could be valuable for tremor treatment with various techniques among which trans-cranial MR-guided Focused UltraSound (tcMRgFUS) that is an innovative and effective procedure for this movement disorder.

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OC17

NAVIGATOR-BASED RETROSPECTIVE MOTION CORRECTION AND RESIDUAL LEARNING FOR 3D-MRF: APPLICATION TO ADULT AND PEDIATRIC PATIENTS

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A 3D Magnetic Resonance Fingerprinting (MRF) acquisition scheme has recently been proposed to enable retrospective motion correction for improving quantitative T1, T2 and proton density (PD) mapping by using its inherent navigator information [1]. In this study we evaluate the further improvement provided by a convolutional neural network (CNN) in order to learn and correct residual motion artifacts [2]. 3D MRF acquisitions [1] obtained on 1.5T and 3T systems from 26 patients (13 children and 13 adults) with negative radiological report were included in this study. Navigator-corrected k-space data [1] were transformed to image space and matched with a pre-computed dictionary of MR signal evolutions to obtain quantitative maps of T1, T2 and PD [3]. Maps went through a multi-scale 3D patch-based CNN to correct for residual motion artifacts [2]: using artificially motion-corrupted data of healthy adult volunteers, the CNN was trained to output residual maps, i.e. the difference between motion-corrupted and motion-free maps, which were then used to produce corrected maps. Synthetic images mimicking conventional MRI contrasts were generated from the quantitative maps, both before and after CNN-based motion correction, by using the signal intensity equations of FSPGR, MP2RAGE, T2-FLAIR and T2-FSE sequences. WM-GM contrast and SNR were computed [4] on quantitative maps and synthetic images before and after the use of the CNN, by using automated tissue segmentations of conventional T1w images co-registered to the MRF data. CNN-based motion correction determined a significant increase in WM-GM contrast and SNR in most cases, paralleled by a clear improvement in subjective image quality observed by radiological evaluation (Fig. 1).

Funding: grant GR-2016-02361693 (Italian Ministry of Health).

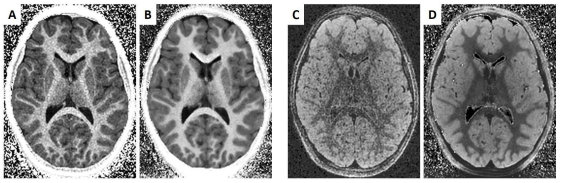

Fig. 1. Representative synthetic FSPGR (A, B) and T2-FLAIR (C, D) images obtained from MRF maps of a pediatric patient, before (A, C) and after (B, D) CNN-based motion correction

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OC18

GPU FITTING OF MULTI-COMPARTMENT MODELS ON BRAIN DIFFUSION MRI: A TEST-RETEST RELIABILITY STUDY

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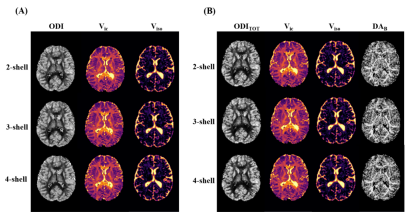
Neurite Orientation Dispersion and Density Imaging (NODDI) [1] and Bingham-NODDI [2] diffusion MRI models are very well-known models in the field of diffusion MRI as they represent powerful tools for the estimation of brain microstructure [3]. In order to efficiently translate NODDI imaging findings into the diagnostic clinical practice, a test-retest approach would be useful to assess reproducibility and reliability of NODDI biomarkers, thus providing validation on precision of different fitting toolboxes [4]. In this context, we conducted a test-retest study with the aim to assess the effects of different factors (i.e. fitting algorithms, multiband acceleration, shell configuration, age of subject and hemispheric side) on diffusion models reliability, assessed in terms of Intra-class Correlation Coefficient (ICC) and Variation Factor (VF). To this purpose, data from pediatric and adult subjects were acquired with Simultaneous-MultiSlice (SMS) imaging method with two different acceleration factor (AF) and four b-values, subsequently combined in seven shell configurations. Data were then fitted with different GPU-based algorithms to speed up the analysis. Diffusion parameter maps were computed for each shell configuration for both NODDI (Fig.1A) and Bingham-NODDI (Fig. 1B) models.

Fig. 1. Diffusion parameter maps obtained for different shell configurations. Maps within mid-axial slice for NODDI (A) and Bingham-NODDI (B) computed on adult dataset.



Results show that each factor investigated had a significant effect on reliability of several diffusion parameters.Particularly, both datasets (pediatrics and adults) reveal very good ICC values for higher AF, suggesting that faster acquisitions do not jeopardize the reliability and are useful to decrease motion artifacts. Although very small reliability differences appear when comparing shell configurations, more extensive diffusion parameters variability results when considering shell configuration with lower b-values, especially for simple model like NODDI. Also fitting tools have a significant effect on reliability, but their difference occurs in both datasets and AF, so it appears to be independent from either misalignment and motion artifacts, or noise and SNR. The main achievement of the present study is to show how 10 minutes multi-shell diffusion MRI acquisition for NODDI acquisition can have reliable results in WM. More complex models do not appear to be more prone to less data acquisition as well as noisier data thus stressing the idea of Bingham-NODDI having greater sensitivity to true subject variability.

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OC19

NODDI MODEL IN HIV INFECTION

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Diffusion tensor imaging (DTI) [1] has been used to explore changes in the brain of subjects with human immunodeficiency virus (HIV) infection. However, DTI notoriously suffers from low specificity. Neurite orientation dispersion and density imaging (NODDI) [2] is a compartmental model able to provide specific microstructural information with additional sensitivity/specificity. In this study we use both the NODDI and the DTI models to evaluate microstructural differences between 35 HIV-positive patients and 20 healthy controls. Diffusion-weighted imaging was acquired using three b-values (0, 1000 and 2500 s/mm^2). Both DTI and NODDI models were fitted to the data, obtaining estimates for fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), axial diffusivity (AD), neurite density index (NDI) and orientation dispersion index (ODI), after which we performed group comparisons using Tract-based spatial statistics (TBSS). While significant group effects were found in in FA, MD, RD, AD and NDI, NDI analysis uncovered a much wider involvement of brain tissue in HIV infection as compared to DTI. In region-of interest (ROI)-based analysis, NDI estimates from the right corticospinal tract produced excellent performance in discriminating the two groups (AUC = 0.974, sensitivity = 90%; specificity =97%). When comparing HIV-infected patients to healthy controls, the NODDI model showed more widespread brain involvement as compared to DTI model, also yielding a better overall discrimination performance. The brain structures in which NODDI-related indices were significantly different are related to multimodal associative brain areas whose functions are known to be often compromised in HIV positive patients, hence offering a mechanistic explanation for these impairments. In addition, the NODDI model highlighted the involvement of the infratentorial structures, possibly related to the neurological impairments which can occur in HIV patients [3]. Finally, the superior discrimination accuracy of NODDI derived indices within a clinically feasible scan time makes it a possible candidate, neuroimaging-related biomarker for HIV studies where monitoring of brain involvement is desired.

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OC20

HYDROPHOBIC INTERACTIONS BETWEEN MACROCYCLIC GD-COMPLEXES AND POLYAROMATIC SYSTEMS AS ROUTE TO ENHANCE THE LONGITUDINAL WATER RELAXIVITY IN MAGNETIC RESONANCE IMAGING.

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Magnetic Resonance Imaging (MRI) is the election imaging technique for the diagnosis of numerous diseases. Ca. 40% of MRI scans are performed using Gadolinium based contrast agents (GBCAs). The recent findings related to Nephrogenic Systemic Fibrosis (NSF) and Gd-retention strongly required caution in the use of GBCAs [1]. Hence, chemistry becomes central in looking for i) more stable and ii) more efficient GBCAs (*i.e.* enhanced relaxivity). Different routes to enhance relaxivity were exploited so far, as i) the set-up of non-covalent binding interactions with macromolecules present in solution (*e.g.* albumin), ii) the increase of the number of coordinated or second sphere water molecules, iii) the increasing of prototropic exchange rates [2,3].

Herein, we describe the increase of relaxivity attainable through reversible binding interactions between the hydrophobic region of macrocyclic GBCAs and pyrene derivatives. Macrocyclic (ProHance, Gadovist, Dotarem) and linear (Magnevist, Omniscan, MultiHance) GBCAs were tested. The increase of relaxivity upon the addition of pyrene derivatives was assessed by ^{1}H-relaxometry and ^{1}H-/^{17}O-NMR. The binding parameters K_a (association constant) and R_b (relaxivity of the adduct) between GBCAs and the pyrene derivatives were calculated by using the Proton Relaxation Enhanced technique. ^{1}H-NMRD profiles were measured *w.* or *w/o* pyrene-derivatives. Insights into the formation of the adduct were obtained by i) ^{1}H-NMR of YbHPDO3A complex *w.* or *w/o* pyrene derivatives, ii) CEST-MRI and iii) x-ray crystallography. The *in vivo proof of concept* of contrast enhancement was obtained by MRI of tumor-bearing mice *pre* and *post* injection of Gd-HPDO3A or Gd-HPDO3A/HPTS adduct. A high binding affinity of macrocyclic GBCAs toward pyrene derivatives was observed. The supramolecular adducts display a significant increase of relaxivity. This is due to the increase of the molecular reorientation time (τ_R) and second sphere water molecules (for the presence of SO_3^- and OH). NMR spectra of the Yb-HPDO3A/ pyrene mixture and x-ray crystallography of Gd-HPDO3A/pyrene mixture fully support the formation of the supramolecular adduct. When HPTS/Gd-HPDO3A ratio is 3:1 (*m/m*), >90% of Gd-HPDO3A is in the associated adduct and there is a 40% relaxation enhancement in respect to the value observed for Gd-HPDO3A alone (*i.e.* 6.5 $mM^{-1}s^{-1}$ *vs.* 9.2 $mM^{-1}s^{-1}$ in blood serum). In T_{1w}-MRI of tumor-bearing mice there is the increase of signal enhancement from 53% (*i.v.* of only Gd-HPDO3A) to 125% (*i.v.* of Gd-HPDO3A/HPTS adduct).

By concluding, a novel tool to enhance the relaxivity of macrocyclic GBCAs is shown, occurring through reversible hydrophobic interaction, already avaiable at clinical doses.

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OC21

SPHERICAL DIELECTRIC RESONATORS FOR UHF MRI

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Metamaterial (MM) science has allowed designing of novel RF coils that improve clinical and preclinical MRI [1-3]. On a separate track high-performance dielectric resonators (DRs) and RF shimming pads have been widely used in the context of EPR, NMR, and MRI [4-5]. Only recently the two paths converged with the DRs described as an add-on RF hardware tool, capable to mimic a negative permeability MM and enhance the use of single or multiple channel RF surface coils [6-9].
In this work, we report full-wave EM simulations at 3T (128 MHz) showing that a low-losses [$\tan\delta=\mathrm{Im}(\varepsilon_{DR})/\mathrm{Re}(\varepsilon_{DR})=5\times10^{-3}$] high-permittivity ($\varepsilon_{DR}\approx1200$, $\mu_{DR}=1$) homogeneous DR sphere (diameter of 6.8 cm; tuned at the first resonant mode, L=1) can couple to a standard circular RF coil (8.4 cm diameter) and a large cylindrical sample (diameter and length 25 cm; $\varepsilon_{SAMPLE}\approx64+i100$; $\mu_{SAMPLE}=1$), such as to enhance the SNR of about 2.7 and to reduce the peak SAR_{10g} of about 22 % (2 mm within the sample surface). Higher-order modes of the DR sphere, having the same ε_{DR}, will give a better improvement (L=5: SNR=3.1, diameter DR sphere 17.6 cm), although with a reduced penetration depth within the sample.

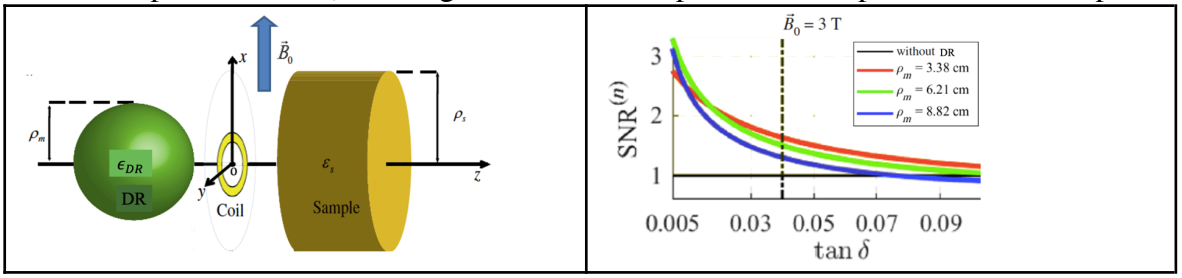

Fig. 1. Left panel: the DR sphere, circular RF Coil and cylindrical sample disposition considered in our full-wave electromagnetic model. Right panel: Normalised SNR calculated at 2 mm inside the first surface of the cylindrical sample along the *z* axis; values are normalized to the case without DR sphere. Different curves refer to DR spheres with $\mathrm{Re}(\varepsilon_{DR})=1200$ and DR radii 3.38, 6.21, and 8.82 cm, supporting the L = 1, 3, 5 resonant modes.

Our 3T results confirm some analytical predictions [7-8] and seem to anticipate useful and novel applications of DRs also for UHF MRI applications. We plan to use the available technology of lead zirconate titanate ceramics [5] to implement the current conceptual spherical DRs framework suitable for UHF MRI applications.

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OC22

A SURPRISING COMPLEXITY OF THE GD(III)AAZTA CHELATE REVEALED BY NMR IN THE FREQUENCY AND TIME DOMAINS

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Typically, lanthanide complexes are isostructural along the series, which enables studying one particular metal-chelate to derive the structural features of the others. This is not the case for the Ln(AAZTA)$^-$ systems, where structural variations along the series cause changes in the hydration number of the different metal complexes, and in particular the loss of one of the two metal-coordinated water molecules between Ho and Er [1,2]. NMR allows determining the number of water molecules coordinated to the metal, along with their exchange rates, by using the Chemical Exchange Saturation Transfer (CEST) experiments [2]. In addition, ^{1}H field-cycling relaxometry and ^{17}O NMR measurements enable accessing the different exchange dynamics processes involving the two water molecules bound to the paramagnetic center in the [Gd(AAZTA)(H2O)2]- complex. The resulting picture shows one Gd-bound water molecule with an exchange rate ~ 8 times faster than the other, due to a longer metal-water distance, as suggested by DFT calculations. Finally, by substituting the more labile water molecule with a fluoride anion in a diamagnetic-isostructural analogue [Y(AAZTA)(H_2O)$_2$]$^-$ of the Gd-complex, it is possible to follow the chemical exchange process by high-resolution NMR and to describe its thermodynamic behavior.
In conclusion NMR offers a wide variety of tools (including CEST, high resolution ^{1}H, ^{19}F NMR as a function of temperature, ^{1}H longitudinal relaxation rates v.s. B_0 and ^{17}O transverse relaxation rates v.s. T) that allow a complete description of the structure and exchange dynamics of the Ln-complexes along the series.

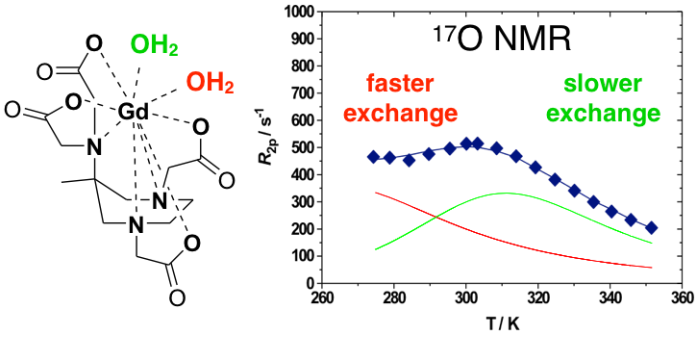

Fig.1. Different water exchange rates of the metal-bound water molecules in the Gd(AAZTA)(H_2O)$_2$ complex.

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ORAL POSTERS

OP1

RAPID SOLID-STATE NMR-DRIVEN CRYSTAL STRUCTURE DETERMINATION OF TAUTOMERIC SYSTEMS

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The coupling of Crystal Structure Prediction (CSP) methods with solid-state NMR (SSNMR) information has recently led to the development of a powerful tool for crystal structure determination, especially for molecular crystals (see Fig. 1 for a schematic representation). The initial purpose was to exploit SSNMR during the final steps of the prediction procedure, comparing *ab initio* computed and experimental ^{13}C and ^{1}H chemical shifts to select the correct structure among all predicted ones [1]. Even though this use of SSNMR significantly increased the reliability of the predictive procedure, this was nonetheless expensive in terms of required time and computational resources. Although, SSNMR can provide worthwhile information that can be exploited as constraints also in the input phase of the CSP [2], dramatically reducing the search space of the predictive algorithm and thus the number of needed calculations. We successfully applied this combined method on mebendazole, which crystallizes in three phases characterized by different tautomeric forms. 1D (^{1}H MAS, ^{13}C and ^{15}N CPMAS) and 2D (^{1}H DQ MAS and ^{13}C-^{1}H HETCOR) SSNMR experiments were used to assess the right tautomer [3] and to determine the number of independent molecules in the unit cell, leading to a considerably faster CSP process. Moreover, we managed to predict the correct crystal packing of the three phases of mebendazole, in particular that of phase B which was unknown until now.

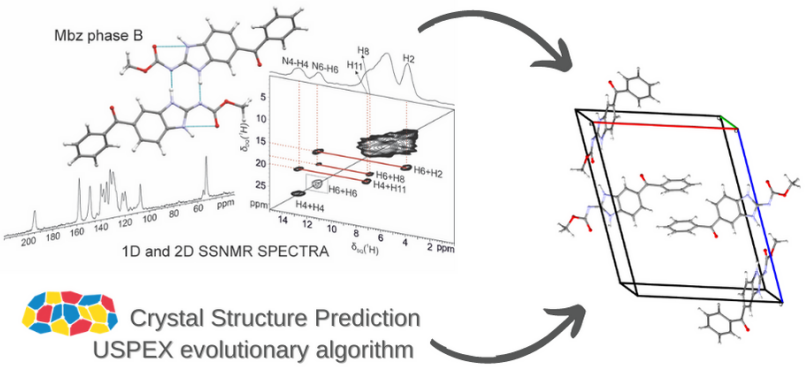

Fig. 1. Schematic description of the combined CSP-SSNMR method.

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OP2

STRUCTURE AND DYNAMICS OF CRYSTALLINE CARBIMAZOLE BY NMR CRYSTALLOGRAPHY AND RELAXOMETRY

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Molecular dynamics and structural characteristics of a solid drug strongly affect its pharmaceutical properties and release profiles. Solid State NMR (SSNMR) has been proved to be a very important technique in the study of pharmaceuticals, allowing many different experiments to be performed in order to obtain important information on dynamic and structural properties on a broad space and time range [1, 2].
In the last two decades, the combination of solid state NMR, diffractometric techniques and computational methods has been recognized as a powerful tool in the investigation of the structure of crystalline solids, and "NMR Crystallography" is seen as a rapidly maturing subject area in the crystallographic community [3,4].
In this work, the dynamic and structural properties of the crystalline form of carbimazole, a prodrug used in the treatment of hyperthyroidism, have been investigated in detail. The combination of DFT calculation with ^{13}C CP MAS, ^{1}H CRAMPS, ^{1}H-^{13}C HETCOR and ^{1}H-^{1}H DQSQ experiments has allowed the elucidation of the drug crystal structure, resolving ambiguities in diffraction-derived structures present in literature. The measurement of spin-lattice relaxation times of ^{1}H and ^{13}C nuclei at variable temperatures enabled the detailed characterization of the dynamic processes that the carbimazole molecule undergoes in the crystal lattice.

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OP3

MONITORING THE SPECIATION OF METAL COMPLEXES WITH CHLOROQUINE LIGAND BY NMR SPECTROSCOPY

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Chloroquine (CQ) is a first choice drug against malaria and autoimmune diseases. Its mechanism of action involves endosomal targeting [1] and alkalinization, through which it inhibits cellular invasion by pathogens [2]. CQ cytotoxicity is enhanced by zinc (Zn) ions, which inhibit RNA synthesis *in vitro* [3]. CQ may also act as a Zn ionophore, capable of blocking pH-dependent viral replication [4]. Hence, we decided to investigate the coordination chemistry of CQ in order to evaluate the mechanistic impact of CQ on Zn binding and intracellular distribution. Based on previous findings [5][6], we studied the effect of zinc salts on both purified CQ and its diphosphate form. The Zn complexes were characterized mainly by Nuclear Magnetic Resonance (NMR) spectroscopy in solution. The results showed that CQ can bind Zn at different pH and solution conditions. Moreover, NMR experiments with paramagnetic cobalt(II) further confirmed the preferred CQ coordination site (Fig. 1).

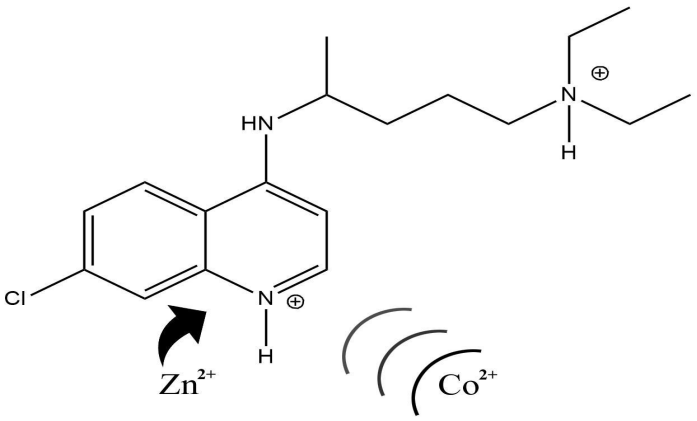

Fig.1 Use of diamagnetic (Zn^{2+}) and paramagnetic (Co^{2+}) species to monitor complex formation by NMR.

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OP4

NMR: A POWERFUL TOOL TO CHARACTERIZE PROTIC IONIC LIQUIDS

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The high emission of CO_2, global warming, and air pollution are some consequences of the excessive use of fossil fuels. To overcome environmental problems, lithium-ion batteries (LIB) and supercapacitors have become extensively explored as energy storage devices. However, some electrolyte components still must be replaced to improve the safety of these electrochemical devices. Volatile organic solvents currently used in electrolyte components cause safety risks to electronic devices due to their high vapor pressure and flammability. Thus, developing alternative solvents for electrochemical applications is crucial to improve the safety of the LIB and expand their application. In this context, ionic liquids (ILs) have been proposed as a promising alternative to replace conventional solvents.

Protic ionic liquids (PILs) are a subclass of ILs which have distinguished characteristics such as low flammability, low vapor pressure, high ionic conductivity, and high thermal stability. Besides, they are easier to synthesize than conventional ILs because they are formed by the transference of a proton from a Brønsted acid to a Brønsted base. These features make PILs a promising class of solvents for electrochemical applications. However, the transport properties and molecular interactions governing PILs still must be understood to allow their application in industry. Thus, the present research aimed to investigate and characterize a set of protic ionic liquids (PILs) based on the super-strong base 1,8-diazabicyclo-[5,4,0]-undec-7-ene (DBU).

^{1}H and ^{15}N NMR were used to gain structural information at the molecular level about the degree of protonation of the DBU base in the PILs. Also, ^{15}N spectra without ^{1}H decoupling were employed to observe the spin-spin coupling constants ($J_{N\text{-}H}$) between the acid proton and the imino nitrogen of the base. The self-diffusion coefficients (D_i) of ionic species in PILs were measured by Pulsed-Field-Gradient (PFG) NMR to describe the dynamical behavior and the transport properties of the PILs.

These results demonstrate that ^{15}N NMR is a powerful tool to identify the status of the protonation through the difference between the ^{15}N chemical shift of the protonated complex and the free base. Also, the self-diffusion results highlighted the mechanism of charge transport in these systems. Further investigations are currently performed in our laboratories to clarify the contribution of structure and acidity of the PILs constituents in governing their features.

This work is part of a joint ENEA-Regione Lombardia PhD programme on sustainable chemistry.

OP5

MONOLAYER-PROTECTED GOLD NANOPARTICLES AS TAILORABLE RECEPTOR FOR NMR CHEMOSENSING

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The majority of sensing methodologies for the detection of target compounds in complex mixtures exploit the feedback of a sensor to indirectly detect the analytes of interest. The response is then processed using standards, if available, or ensured by the robust selectivity of the sensor itself. On the other hand, NMR chemosensing aims to obtain signals directly from the analytes, in the form of an NMR spectrum, to unequivocally identified the target molecules. This is done by sensing protocols based on diffusion-ordered spectroscopy (DOSY) and magnetization transfer sequences (STD and water-assisted STD) [1]. In this context, "nanoparticle-assisted NMR chemosensing" combines these techniques with the recognition abilities of monolayer-protected gold nanoparticles (AuNPs) to push the detection of relevant diagnostic analytes in the micromolar concentration range. From an NMR point of view, the reduced molecular motion of bulky nanoparticles offers a way to transfer magnetization to the interacting analytes and promotes efficient spin diffusion, useful in saturation transfer experiments [2]. In addition to that, AuNP can be easily capped with a variety of thiols to provide tailored binding sites in term of both selectivity and strength, for virtually any class of substrates. In this sense, is crucial to rationalize the different ligand-to-receptor recognition in terms of nanoparticles size, ligand design (i.e., chain length, nature of the thiol hydrophilic groups) and the nature of chemical interaction involved (e.g., hydrophobic forces, ion pairing, metal-ligand coordination). In this communication our last results will be reported, aimed at deeply investigate the structure-affinity relationships between analytes/AuNP and their influences on the sensing performances.

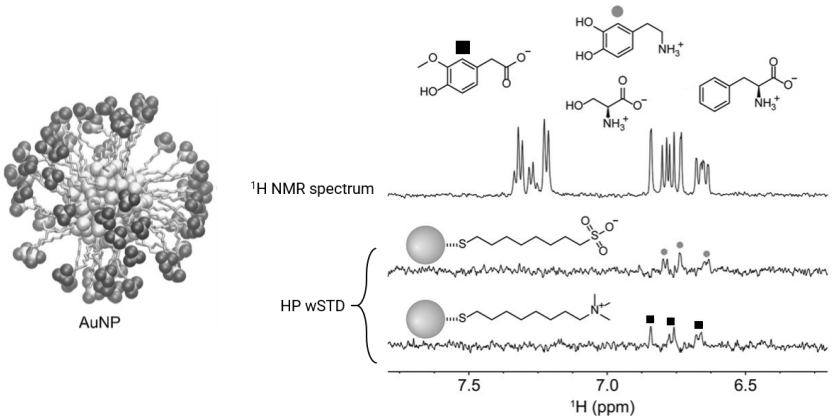

Fig. 1. Left: gold nanoparticle with sulfonated thiol ligand. Right: ^{1}H NMR spectrum of a mixture containing dopamine, homovanillic acid, L-phenylalanine and L-serine with the corresponding high-power water-assisted saturation transfer difference spectra (HP wSTD).

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OP6

SOLID-STATE NMR STUDY OF A MULTIPLE-CATION LEAD MIXED-HALIDE PEROVSKITE WITH HIGH EFFICIENCY

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Hybrid metal-halide perovskites have emerged as highly interesting materials for various applications, such as thin-film photovoltaics or light-emitting devices, due to their outstanding optoelectronics properties. A main advantage of these materials is the fact that their macroscopic properties are intrinsically related to their microscopic features (*i.e.* atomic and molecular organization and dynamics), so mixtures of different cations and anions can be used to tune the optoelectronic properties and to enhance efficiencies and stabilities.

Several variations to the perovskite structure have been tested. In particular, the use of mixed-ion structures, in which the compositional complexity is increased by introducing dopants into the perovskite structure, has resulted in a remarkable improvement in perovskite solar cell performance since their first use as sensitizers for solar cells in 2009 [1]. Some of the latest top efficiencies have been reached by multiple-cation lead mixed-halide perovskites (Cs,FA,MA)Pb(I,Br)$_3$ [2][3][4].

However, the role of the dopants and additives in the high performance of the perovskite solar cells has not been fully understood yet, and its transferability to other perovskites is not straightforward. For this reason, it is important to be able to gain atomic-level understanding of these materials.

Solid-State NMR (SSNMR) spectroscopy is strongly sensitive to the local chemical environment, and as such, it proved to be a perfectly suited technique to investigate mixed perovskites.

In this study, we focused on the perovskite with formula $Cs_{0.05}FA_{0.81}MA_{0.14}PbI_{2.55}Br_{0.45}$ because of its high performance. We investigated it by means of SSNMR for the first time, using $MAPbI_3$ as a reference compound. ^{207}Pb, ^{13}C and ^{1}H high-resolution SSNMR experiments allowed us to characterize the structure and composition of the samples, highlighting phase homogeneity and/or segregation, and to investigate ion dynamics by exploiting both spectral and relaxation properties.

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OP7

THE USE OF DEEP LEARNING FOR PWRA ESTIMATION, PERFORMANCE ANALYSIS AND COMPARISON WITH OTHER TECHNIQUE

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The spin-lattice relaxation (either in the laboratory, T_1, or the rotating frame, $T_{1\rho}$) of abundant nuclei, such as ^{1}H, is heavily affected by the spin diffusion process, often generating a non-exponential relaxation decay, which is often phenomenologically described as multi-exponential relaxation. Of course, the measured relaxation times do not reflect the intrinsic relaxation times, which could be interpreted in terms of molecular motions. Nonetheless, the Population Weighted Rate Average (PWRA) is not affected by spin diffusion [1] and therefore its correct measurement could be exploited to obtain reliable dynamic information [2]. Many different methods can be used to this aim: the scope of this work is trying to apply and compare the different methods in various experimental circumstances, in the attempt of finding the best approach case by case.
The standard estimation of the PWRA data starts with a multi-exponential fitting of the experimental data followed by the calculation of the PWRA from the estimated exponential. Unfortunately, multi-exponential modeling is an ill-posed problem.
It is also possible estimate the PWRA directly from the gradient of the decay at t=0, so representation of the decay in terms of a defined analytical function, such as a multi-exponential decay, is not necessary as long as the gradient at t=0 can be accurately estimated e.g. using polynomial fitting.
Here we propose to use deep learning for a direct estimation of the PWRA from the data without specifying and fitting a multi-exponential model on the experimental data.
I'll describe how we obtained a higher precision than the standard multi-exponential fitting estimation, using a simulated dataset.
To conclude, I'll also present a comparison between Neural Network and the direct estimation of the PWRA using polynomial fittings. Both methods provide excellent performance with a mean error on the PWRA estimation, over a simulated test set with medium SNR, in the range of +/- 5%. Neural Network requires a higher set-up cost but are faster and do not require human intervention after the training, while polynomial fitting does not require a training phase but require human intervention for the selection of the polynomial grade.

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OP8

DRUG MOTION IN HYDROGEL SYSTEMS

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The study of the diffusion of small molecules, both in confined systems and liquid phase, using NMR techniques has become increasingly relevant because of the wide range of possible applications: from medicine, to materials science and biophysics.
In particular, translational diffusion can be measured using Pulse Gradient Spin Echo (PGSE) techniques and the analysis of the experimental data based on Gaussian diffusion approximation, which yield the apparent diffusion coefficient, have been the most popular and commonly accepted procedure to investigate heterogeneous materials and polymers [1].
These approaches can be applied to the drug delivery studies, helping to understand how the diffusive mechanisms governing solute transport at the nanoscale work. Nevertheless, physical barriers, confinement or hydrogen bonding may be experienced by the traveling drug molecules with the consequent non-Gaussian diffusion regime. From previous studies, [1] the analysis of the experimental NMR data, performed using a specially designed procedure [2], provided the first evidence of superdiffusion motion of small molecules in hydrogels.
In this context, we investigated the translational diffusion of ethosuximide, an anticonvulsant drug, loaded in anionic agarose-carbomer (AC) hydrogels by ^{1}H high resolution magic angle spinning (HR-MAS) NMR spectroscopy and compared it to its macroscopic release kinetics.
Different samples were prepared considering the effect of drug concentration (40-80 mg/ml) and the hydrogel nanoscopic mesh size on ethosuximide motion. Four different mesh sizes in the range 90- 3000 nm, obtained by replacing different percentages of carbomer with hyaluronic acid, were analyzed. Superdiffusive behavior was observed in all the samples. Moreover, co-delivery with a second drug was also investigated.

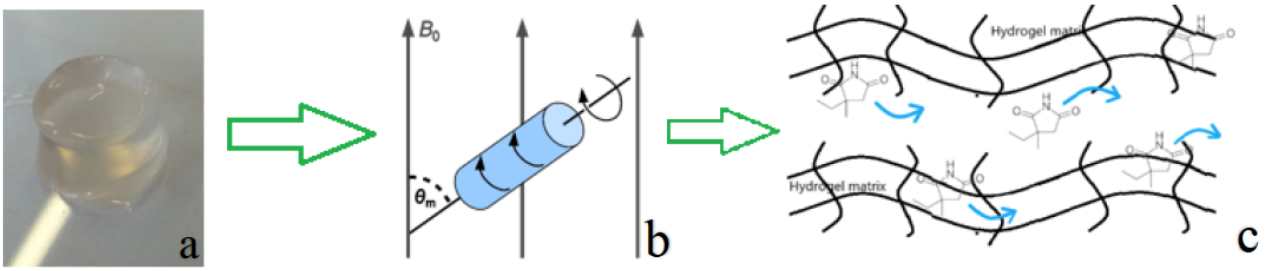

Fig.1 a) agarose-carbomer hydrogel with hyaluronic acid, b) HR MAS schematic principle, c) diffusion of small ethosuximide in hydrogelmatrix.

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OP9

MONITORING THE INTERACTION OF α-SYNUCLEIN WITH CALCIUM IONS THROUGH EXCLUSIVELY HETERONUCLEAR NUCLEAR MAGNETIC RESONANCE EXPERIMENTS

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Amino acid side chains are known to be one of the main players in modulating several properties of intrinsically disordered proteins (IDPs), such as the interaction of these macromolecules with other partners [1]. Despite the importance of IDP side chains, they are seldom assigned and characterized, due to the extensive signal overlap that affects their NMR spectra. Here, a set of *exclusively heteronuclear* NMR experiments tailored for IDP side chains' is presented. The proposed approach, suitable for many IDPs, can be applied to study how their properties are modulated by different, physiologically relevant, conditions. As an example, the experiments are used to investigate how α-synuclein senses Ca^{2+} concentration jumps associated with the transmission of nerve signals [2]. Peculiar sequence motifs optimized for calcium sensing in highly flexible, disordered protein segments are identified [3].

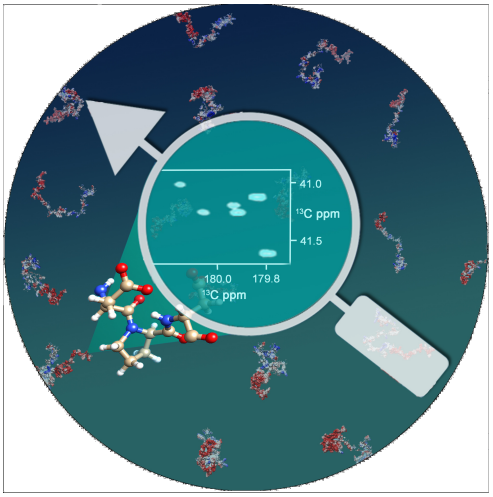

Fig. 1. The ^{13}C direct detection approach is used to investigate negatively charged sidechains of IDPs to characterize the interaction between these macromolecules and other partners.

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OP10

NMR PLASMA METABOLOMICS AND LIPIDOMICS CAN PREDICT CARDIAC ISCHEMIC RISK

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Cardiovascular diseases (CVD) are primary causes of mortality worldwide [1]. Among CVD, myocardial ischemia is a severe pathological condition characterized by a reduced oxygen flow to the heart that could lead to myocardial infarction. Classical diagnostic methods such as treadmill stress test and myocardial scintigraphy are affected by low sensitivity or involve the use of high dose of radiation. On the other hand, the gold standard test coronary angiography is an extremely invasive procedure [2].
The aim of this ongoing study is to evaluate whether the human plasma metabolic profile can be used to predict the likelihood of experiencing an ischemic event. The identification of an "ischemic fingerprint", coupled with the traditional procedures, could be extremely helpful to assess the ischemic risk more accurately.
Venous plasma samples of 208 patients from Policlinico Umberto I Cardiology Unit were collected before and after the traditional treadmill stress test and both polar metabolites and lipid extracts have been analyzed using NMR spectroscopy. The preliminary results indicate that a marked metabolism imbalance is occurring in patients who are more likely to experience an ischemic event, even before the heart is forced to work under pressure (i.e., baseline levels). Moreover, the lipid profile results to be fundamental for the identification of patients sub-groups, providing a better classification.
These findings suggest that metabolism is anticipating the outcome of the cardiac stress test and can help in determining a risk factor useful for physicians to diagnose cardiac conditions as well as stratifying the subjects.

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OP11

A NOVEL INTEGRATED NMR-BASED APPROACH FOR STUDYING RECEPTOR-LIGAND INTERACTIONS ON LIVING CELLS SURFACE

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Nuclear Magnetic Resonance Spectroscopy represents a powerful technique for studying, at atomic resolution, protein-protein and protein-ligand interactions, directly in the intracellular environment or on the membrane surface of living cells. In- and on- cell NMR methods as transfer NOESY (trNOESY) and Saturation Transfer Difference (STD), are widely used to characterize binding of ligands to membrane receptors on the cell surface. However, the application of both NMR methodologies is limited by the short lifetime of cells in an NMR sample tube, which often prevents the acquisition of experiments longer than few hours [1,2]. Therefore, to overcome these drawbacks, we developed an alternative approach based on the application of Non-Uniform Sampling (NUS) and 1D T1ρ NMR techniques to collect structural and dynamics information on the receptor-ligand interactions with living cells, that in turn can be used as conformational constraints in computational studies. In details, our strategy relies on the combination of high-resolution NMR data with Molecular Dynamics simulations and Molecular Docking methodologies. We tested our approach to explore the recognition mechanism of αvβ5-integrin by RGDechi15D [3,4]. This peptide is a selective cyclic molecule able to interfere with tumor proliferation and progression and to regulate angiogenesis in endothelial cells. Our data demonstrate that the developed strategy represents an alternative on-cell NMR tool for studying, at atomic resolution, receptor-ligand recognition mechanism on living cells surface.

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OP12

NEW INSIGHTS ON A DIVALENT CATION CHANNEL BY >100 kHz MAGIC-ANGLE SPINNING NMR

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In recent years, faster and faster magic-angle spinning (MAS) rates have paved the way for proton-detection in the solid state, enabling the acquisition of resolved protonresonances in fully protonated samples of proteins of diverse molecular sizes and aggregation states, from microcrystalline to membrane proteins [1]. This technical progress revolutionizes the atomic-level investigation of proteins, expanding the range of information exploitable for the determination of protein structures and opening new horizons for the investigation of protein dynamics.
Here we demonstrate that MAS rates of 100 kHz and above, coupled to ultra-high magnetic fields, permit the site-specific measurement of observables connected to local and global dynamics in the bacterial divalent cation channel CorA [2] reconstituted in lipid bilayers. CorA is a 5x42 kDa pentamer comprised of two transmembrane helices and a large cytoplasmic domain hosting a metal binding site (usually Mg^{2+} or Co^{2+}). The combination of high magnetic fields and fast MAS rates allows the acquisition of wellresolved spectra for backbone and side-chains resonance assignment [3] which give information on structural elements. The measurement of residue-specific dynamic
parameters provides then insights on the transport mechanism of cations through the CorA channel, challenging models previously formulated on the basis of cryo-EM structures. This progress represents a concrete step forward in the study of challenging biological systems by solid-state NMR.

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OP13

NMR-BASED METABOLOMICS FOR THE IDENTIFICATION OF PREDICTIVE URINARY METABOLIC BIOMARKERS OF WORKERS EXPOSED TO WELDING FUMES

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Biological monitoring in occupational setting is a useful tool for assessing the health conditions of individuals exposed to chemical or biological agents. Recently, nuclear magnetic resonance (NMR) spectroscopy was introduced as a complementary technique in the field of occupational exposure in order to identify predictive biological biomarkers [1-3]. In particular, NMR-based metabolomics is a valid approach to characterize the response of a metabolic phenotype of a living organism to toxic effects originating from various chemical sources. In this context, the knowledge of the subclinical variations related to the exposure could help to better understand the complex mechanisms leading to the organism damage. Therefore, the identification of predictive biomarkers could be a further tool for the health surveillance in occupational exposure.
This work is aimed at defining and comparing the urinary metabolic profiles of 53 workers of the same company exposed to two different chemical agents: welding fumes and volatile organic compounds (VOC). Urine samples were collected at the beginning and at the end of the work-shift and the qualitative and quantitative determination of metabolites were carried out by mono- and bi-dimensional NMR experiments. All the spectra were acquired on a JEOL JNM-ECZ 600R spectrometer, equipped with a 14.09 T magnet (600 MHz for ^{1}H resonance frequency) and an autosampler. The NMR dataset, composed by 35 variables, was integrated with 5 urinary oxidative stress biomarkers (detected by LC-MS) and 16 urinary metals (detected by ICP-MS), in order to associate the NMR variables to the standard biomarkers of an oxidative stress condition as well as metal exposure. The entire dataset obtained from this integration was analyzed by multivariate partial least squares-discriminant analysis (PLS-DA) to define the metabolic changes induced by the two different conditions. Workers exposed to welding fumes showed higher levels of urinary metabolites, such as pseudouridine, methylguanidine and 8-oxo-7,8-dihydroguanosine compared to VOC exposed workers, suggesting a higher RNA catabolism and a higher protein turnover. This study represents one of the few applications of NMR in occupational medicine and constitutes an important starting point for future investigations on the metabolic changes occurring in occupational exposure.

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OP14

EARLY-DETECTION OF XYLELLA-INFECTED ASYMPTOMATIC LEAVES BY HYPERSPECTRAL REFLECTANCE AND NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Xylella fastidiosa (Xf) subsp. pauca sequence type ST53, also known as the "De Donno" strain [1], impacted severely the olive groves of Apulia region in Southern Italy, and about 11 million olive trees were infected over an area of approximately 50,000 ha. This severe impact was projected to be more than 5 billion euros for the next 50 years [2]. The disease caused by Xf is known as the olive quick decline syndrome (OQDS). Hitherto, its main control strategy is to destroy the infected plants [3]. Hence, efficient early detection of Xf-infection would offer a competitive advantage against the spread of the disease by allowing the implementation of preventive actions in advance. Over the manifestation of Xf-infection, from infection until declination, olive trees experience phenotypic and metabolic fluctuations. Nevertheless, at early stages of infection trees could remain asymptomatic for years. Hyperspectral reflectance (HSR) and nuclear magnetic resonance spectroscopy (NMR) offer a non-destructive method to detect such phenotypic and metabolic fluctuations [4,5]. In this study, asymptomatic leaves of Xf-infected olive plants were analysed using ^{1}H NMR, HSR, and chemometrics aiming at selecting diagnostic signals and wavelengths for Xf-infected trees. Artificially Xf-infected young trees of the susceptible variety "Cellina di Nardò" were grown in a thermally-controlled environment and co-inoculated with additional xylem-inhabiting fungi. Asymptomatic leaves were subjected to HSR acquisition while their extracts were subjected to a non-targeted metabolomics study. Then, the acquired spectra were further analysed using chemometrics techniques. Systematically, the covariance matrices between NMR and HSR were also investigated for linking HSR features to diagnostic NMR signals. This linking revealed different wavelength-regions with diverse association to the corresponding metabolites. The determination of diagnostic wavelengths regions associated to specific metabolites is a keystone to developing sensors capable of early-detection of Xf-infected trees.

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OP15

SARS-COV-2 M^{PRO} INHIBITION BY ZINC ION: STRUCTURAL FEATURES AND HINTS FOR DRUG DESIGN

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SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}) is a cysteine protease that hydrolyses the viral polyproteins at several sites with a preference for the Leu-Gln(Ser, Ala, Gly) sequences [1]. The enzyme represents one of the main drug-target candidates for covid-19 syndrome because the large and deep pocket at the active site and its crucial activity for viral replication [2-4].
Here, we provide X-ray structural data on SARS-CoV-2 main protease in complex with the isolated Zn^{2+} ion. The comparison with the apo SARS-CoV-2 M^{pro} shows that residues involved in zinc binding are not affected by significant structural rearrangement upon zinc binding supporting the idea that the binding site is ready to accommodate the metal.
The interaction of SARS-CoV-2 M^{pro} with Zn^{2+} ion was also investigated by NMR. Moreover, zinc binding is able to inhibit protein activity, demonstrating that the zinc ion is capable of an efficient binding also in solution. These findings provide a solid ground for designing potent and selective inhibitors of SARS-CoV-2 M^{pro} suggesting that a zinc ion incorporated into suitable ligands interacting with additional sites at the protein surface can modulate the binding energy.

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OP16

ANALYSIS OF THE MAJOR NON-PSYCHOACTIVE CANNABINOIDS IN HEMP *via* ^{1}H AND ^{13}C qNMR

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Cannabis Sativa is a fast-growing annual dioecious weed belonging to the *Cannabaceae* family and an exceptional multicomponent natural resource even in ancient times used for seed oil (3: 1 ratio ω-6/ω-3), intoxicant resin, textile fiber and religious rituals. Cannabis is also a base ingredient in the traditional medicine of different populations thanks to the analgesic, anticonvulsant, antispasmodic, anaesthetic or anti-inflammatory activity of Phytocannabinoids. The renewed and recent interest in *C. Sativa* derives from the identification, in addition to the well-known psychotropic agent Δ^9-trans-tetrahydrocannabinol (Δ^9-THC), of other metabolites such as Cannabidiol (CBD) and its precursor Cannabidiolic Acid (CBDA) having therapeutic benefits without euphoric or dysphoric effects. The Italian legislation on Cannabis Sativa cultivation and production is somewhat ambiguous about the legal and illegal uses of the plant (THC < 0.2 % and in any case, not exceed 0.6 %) and to date exist a single official procedure for the quantitative determination of the only THC content [1] using Gas Cromatography. However, the characterization of the extracts, their quantification and the extraction procedures optimization are essential steps in the development of new pharmaceutical products and functional foods. Within this framework, NMR spectroscopy was used in order to perform a fingerprinting and a profiling of different cultivar of fiber-type *Cannabis Sativa* (Tiborszallasi and Kompolti), commonly called hemp (THC/CBD ratio <<1)[2], recording in a common solvent 1D (^{1}H-NMR, ^{13}C-NMR) and 2D (1H-1H COSY, ^{1}H-^{13}C HMQC, *J*-RES) experiments. Moreover, a quantitative analysis of the main cannabinoids in the flower extracts, obtained following the official extraction procedure, was also performed using ^{1}H and ^{13}C qNMR spectra and different internal standards. In this contest, another goal is also the evaluation of sensitivity, specificity, linearity range, precision, accuracy, LOD and LOQ of the method. With the purpose of identifying correlations between metabolites, factors of discrimination and maximize the useful informations proven experimentally, the dataset of the NMR spectra was treated with the multivariate statistical analysis model PCA (Principal Component Analysis) [3].

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OP17

NMR METHODOLOGY IN THE STUDY OF ITALIAN LOCAL PRODUCTS

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In many Italian regions, local products are cultivated and used only for the local consumption. Therefore many varieties and biodiversity tend to be lost due to the agronomic and commercial selection of only a few varieties. Another factor that contribute to the loss of crop biodiversity is the replacement of local varieties with high-yielding species. Therefore, a limited number of varieties are cultivated and products coming from extensive productions, sometimes deceptively labelled as "Made in Italy", are imported to satisfy the high food request. In this context, high field NMR spectroscopy has been applied to characterize and therefore valorize local Italian products namely tomatoes of Lazio region, apple typical cultivars of the Piedmont region and potatoes from Liguria and Piedmont region. For this purpose Blight-Dyer extraction has been performed obtaining organic and hydroalcoholic fractions then analyzed by the 1D and 2D NMR methodologies.
Two variety of tomatoes cultivated in the south Lazio area, namely the recently introduced Torpedino di Fondi variety and the traditional San Marzano variety, were studied monitoring the metabolites concentration at a both pink and red ripening stages by the NMR methodologies [1]. Ten variety of traditional apple cultivars of the Piedmont region (Italy) were characterize by the NMR analysis in order to discriminate their differences/similarities and to compare their composition with the commercial cultivars [2]. Twenty potato cultivars from Liguria and Piedmont region were analyzed with the NMR spectroscopy in order to promote their valorization and use as high quality raw material in food industries [3].

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OP18

RELAXOMETRIC CHARACTERIZATION OF FUNCTIONALIZED DERIVATIVES OF $[Gd(AAZTA)(H_2O)_2]^-$ WITH AMINOACIDS

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The $[Gd(AAZTA)(H_2O)_2]^-$ complex (AAZTA = 6-amino-6-methylperhydro-1,4-diazepine tetra acetic acid) is a platform of great interest for the design of new innovative MRI probes, due to its remarkable magnetic properties, thermodynamic stability, kinetic inertness, and high chemical versatility [1,2]. We developed some derivatives functionalized with amino acid residues (AAZTA-AA) with different molecular weight and charge. Three main reasons led to the choice of including amino acid residues in the ligand structure: (i) to evaluate the increase in efficacy (relaxivity) at the imaging fields (> 1 T) associated with the increase in the rotational correlation time; (ii) to promote non-covalent interactions with protein structures in biological environments, hence forming supramolecular adducts with high relaxivity; (iii) to study the properties of model compounds prior to synthesis of derivatives containing polypeptide residues for molecular imaging applications. The relaxometric properties of these chelates were analysed in order to determine their molecular parameters, which describe the paramagnetic relaxation mechanism. These were accurately assessed by simultaneous fitting of the ^1H NMRD profiles (in the 0.01-120 MHz range) and the ^{17}O transverse relaxation rates (R_2) and shift ($\Delta\omega$) measured at 11.7 T and at different temperatures [3]. The relaxivity value of these chelates at 0.5 T is higher than that of the parent $[Gd(AAZTA)(H_2O)_2]^-$, as a consequence of the increase of their reorientational correlation time (τ_R). Furthermore, tests in reconstituted human serum indicate an interaction with the proteins. Finally, the corresponding Eu(III) chelates were synthesized and characterized by ^1H NMR and time-resolved photoluminescence, in order to obtain structural information and determine the hydration state of metal ion.

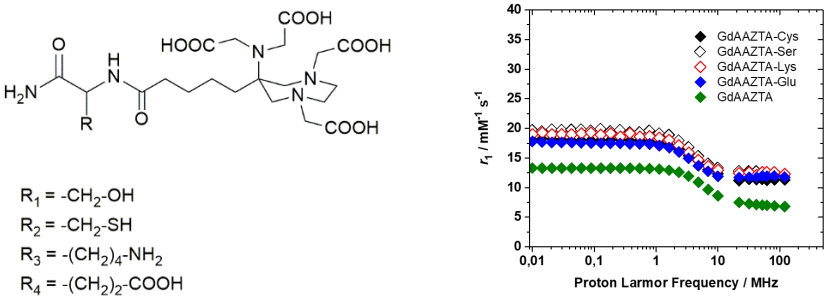

Fig. 1 and 2. From left to right, structure of the different AAZTA-AA ligands and ^1H NMRD profiles of $[Gd(AAZTA)(H_2O)_2]^-$ and its derivatives at 298K.

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OP19

SUPER RESOLUTION OF T1W AND T2W MRI USING DEEP NEURAL NETWORKS: BRAIN IMAGES FROM CAMCAN DATASET

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Background: Super-resolution models are deep learning algorithms which enhance image spatial resolution. Their use on biomedical images has been explored, through *ad hoc* training stages [1]. EDSR [2] and WDSR [3] are convolutional neural networks, trained on general purpose images, which perform 2x- and 4x-upsampling. In this work, their application to MR brain images has been studied.
Methods: Data used in this work were provided by the Cambridge Centre for Ageing and Neuroscience (CamCAN) [4]. 3D sagittal high-resolution T1w and T2w images of 70 subjects were convolved with a Gaussian filter and then down-sampled. EDSR, WDSR and bicubic interpolation were used to up-sample low-resolution images. After performing the brain extraction, pixel-wise and whole-brain average analysis was performed, using the original high-resolution images as ground truth. Sagittal, coronal and axial directions were analyzed separately.
Results: EDSR generally shows better performance. In all the T1w reconstructions and in T2w axial and coronal reconstructions there is a significant difference (p-value < .05) in favour of EDSR for all the considered criteria (quantitative similarity parameters: RMSE, pSNR, SSIM, HFEN), and on two out of four criteria in the T2w sagittal reconstructions. In Tab.1, the parameters for T1w and T2w images, averaged over the three directions, are shown.
No correlations were found between similarity parameters and subjects attributes.
WDSR was not found to be suitable, since it enhances and creates line-like artifacts.
Conclusions: EDSR, that performs 2x-upsampling, outperforms the bicubic interpolation without needing fine-tuning, showing its ability of transfer learning. It is flexible with respect the analyzed MR sequence and subject characteristics.

		RMSE	pSNR	SSIM	HFEN
T1w	EDSR	41.3 ± 20.7	28.6 ± 1.4	0.985 ± 0.001	0.16 ± 0.03
	BC	102.7 ± 43.8	24.7 ± 2.1	0.978 ± 0.002	0.26 ± 0.02
T2w	EDSR	41.3 ± 6.6	29.4 ± 0.7	0.978 ± 0.002	0.18 ± 0.02
	BC	39.9 ± 6.2	29.7 ± 0.7	0.971 ± 0.002	0.23 ± 0.01

Tab. 1. Similarity parameters, averaged over the 3 directions, for EDSR and bicubic (BC) upsampling.

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OP20

NOVEL QUADRUPOLAR PEAKS BASED CONTRAST AGENTS FOR MONITORING TISSUE IMPLANTS

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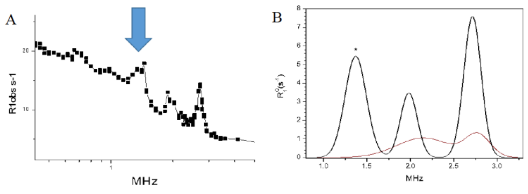
This study aims at developing an innovative class of MRI contrast agents for Fast Field Cycling-MRI applications. They represent a completely new class of MRI contrast agents that display remarkable relaxation effects on tissue water protons. Their detection requires the acquisition of images at variable magnetic field strength as provided by Fast Field Cycling MRI (FFC-MRI) scanners. FFC is an innovative technology that allows detecting the quadrupolar cross-relaxation, appearing as peaks (QPs) in the $1/T_1$ dispersion profile completely invisible to conventional (fixed-field) MRI [1]. PLGA scaffolds were prepared by dissolving PLGA conjugated with polyhistidine (n=15) in tetraglycol with glucose to create porous scaffolds. The mixture was then injected into PBS, where it precipitated by phase inversion to form a solid scaffold as the tetraglycol diffused into the PBS and allowed the porogen to be leached from the scaffold to form a porous structure. MC3T3-E1 cells (subclone 4), a pre-osteoblastic cell line derived from mouse calvaria, were seeded and cultured inside the porous scaffolds *in vitro* to investigate their ability to adhere to and proliferate on this material. NMRD profiles were acquired on a Fast-Field Cycling relaxometer (SmartTracer , Stelar S.r.l., Mede (PV)) with a microcoil (diameter 6 mm). PLGA scaffolds containing poly-Histidine of different sizes show QP at 1.35 MHz due to the 14N nuclear quadrupole resonance frequency of the imidazole groups present on the polymeric chains. This QP falls at a frequency well distinguishable from the endogenous ones and therefore it may be used as a new class af frequency-encoded specific sensor. The QPs are detectable only when the contrast agent is in a gelified or solid-like form, ie at pH>6.6, and above this value their intensity is pH dependent [2]. Thanks to this pH-dependent behaviour, the contrast agents can be used to report on tissue pH changes (that can be associated to the occurrence of a pathologic state or to cellular apoptosis/necrosis). A relaxation enhancement at 1.35 MHz was detected after 15 days cell proliferation (MC3T3-E1) on the scaffold surface.

Fig. 1. Poly-His NMRD profile A) NMRD profile acquired from 0.01 to 20 MHz of poly-His (30% w/w) in water (T=25 °C) with the expansion of the QPs region. The asterisk indicates the imidazole peak. B) Gaussian fits of the QPs, after background subtraction.



A relaxivity change proportional to the amount of adherent cells was detected thus demonstrating the potential responsiveness. In this study we exploited this technique for *in vivo* study of tissue implants. In fact, to date there is an almost complete lack of methods for the rapid, non-invasive and repeated monitoring of tissue implants and new methods are needed to monitor cell status and polymer degradation under physiological conditions (temperature, saline, pH, enzymes etc.) thus allowing the physician to control, in real time, the transplanted scaffold status.

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OP21

WHOLE-BRAIN STRUCTURAL NETWORK REORGANIZATION IN HIV

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The human immunodeficiency virus (HIV) causes an infectious disease with a high viral tropism toward CD4 T-lymphocytes and macrophage. Since the advent of combined antiretroviral therapy (CART), the number of opportunistic infectious disease has diminished, turning HIV into a chronic condition. Nevertheless, HIV-infected patients suffer from several life-long symptoms, including the HIV-associated neurocognitive disorder (HAND) [1], whose biological substrates remain unclear. HAND includes a range of cognitive impairments which have a huge impact on daily patient life. The aim of this study was to examine putative structural brain network changes in HIV-infected patient to test whether diffusion-imaging-related biomarkers, and in particular structural connectivity, could be used to discover and characterize subtle neurological alterations in HIV infection. To this end, we employed multi-shell, multi-tissue constrained spherical deconvolution in conjunction with probabilistic tractography and graph-theoretical analyses [2]. We found several statistically significant effects in both local (right postcentral gyrus (clustering coefficient p=.004 HC>HIV, local efficiency p=.003 HC>HIV), right precuneus (clustering coefficient p=.007 HC>HIV, local efficiency p=.01 HIV>HC), right inferior parietal lobule (clustering coefficient p=.004 HC>HIV, local efficiency p=.007 HIV>HC), right transverse temporal gyrus (local strength p=.005 HC>HIV), right inferior temporal gyrus (betweenness centrality p=.015 HIV>HC), right putamen (clustering coefficient p=.005 HC>HIV, local efficiency p=.007 HIV>HC) and right pallidum (clustering coefficient p=.01 HC>HIV, local efficiency p=.007 HIV>HC, local Strength p=.04 HC>HIV)) and global graph-theoretical measures (Fig. 1) (global clustering coefficient (p=.013, HC>HIV), global efficiency (p=.036, HC>HIV) and transitivity (p=.009, HC>HIV)). Our study highlights a global and local reorganization of the structural connectome in HIV patients as compared to controls. This supports the employment of neuroimaging-based brain connectivity as a sensitive biomarker for neurological alterations in HIV patients.

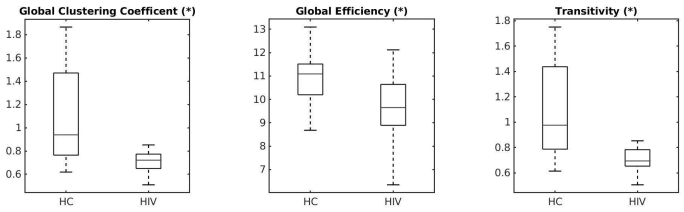

Fig. 1. Global metrics in which group-wise, statistically significant differences were found. HC= healthy controls. (*) p<0.05.

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OP22

A RETROSPECTIVE STUDY ON TREATMENT- AND PATIENT-RELATED PARAMETERS RELATED TO TRANSCRANIAL MAGNETIC RESONANCE IMAGING-GUIDED FOCUSED ULTRASOUND TREATMENTS PERFORMED AT 1.5 T

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Among the procedures for treatment of some functional neurological disorders such as essential tremor in the last years transcranial magnetic resonance imaging-guided focused ultrasound surgery (tcMRgFUS), which is an innovative, non-invasive, incision-less treatment performed by means of focused ultrasounds under the guidance of MRI has aroused an increasing interest [1].
In this work the results of a retrospective analysis of patient- and sonication-related parameters of a group of patients treated with a transcranial magnetic resonance imaging-guided focused ultrasound surgery (tcMRgFUS) system integrated with a 1.5-T MRI unit is reported.
52 patients who underwent unilateral ventral intermediate (VIM) thalamotomy tcMRgFUS procedure were considered; the data collected refers to patient-specific features such as skull density ratio (SDR) and skull area (SA) and patient-specific parameters such as sonication duration (S_d), user-defined energy (E), i.e. the energy sent to the target, but also the effective measured energy during sonications (E_m) and the maximum temperature reached during the treatment (T_{max}) [2].
The energy released onto the planned target was found to decrease with the SDR for all temperature ranges. A positive correlation was observed between the slope of T_{max} vs. E_m curve and the SDR. In addition, the T_{max} was positively correlated with SDR. The obtained results confirm the factors that significantly influence the course of a tcMRgFUS procedure even when a 1.5-T MRI scanner is used for procedure guidance. On the contrary, no significant correlation was found between SDR and SA or T_{max}.
The experience gained in this study indicates that the SDR remains one of the most significant technical parameters to be considered in a tcMRgFUS procedure. The possibility of prospectively setting the sonication energy according to the curves of energy vs SDR obtained for each treatment stage could provide a further understanding and a greater awareness of this emerging technology.
Keywords: high-intensity focused ultrasound ablation, interventional magnetic resonance imaging, stereotaxic techniques, essential tremor

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OP23

FIELD INTENSITY SHAPING PARADIGM FOR SHIMMING AND SAR CONTROL IN AN MRI SYSTEM

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Today, Clinical magnetic resonance imaging (MRI) at medium and high field strength (>=3T) is widely used because it allows to obtain high resolution images thanks to the high signal to noise ratio. On the other hand, the high frequency of the radiofrequency (RF) fields used in MRI causes electromagnetic field variations which degrade the image quality, increasing imaging artifacts. The high field also makes the procedure potentially more dangerous, as it increases the amount of specific absorption rate (SAR) deposited in the investigated region.

The issue of levelling or *shimming* the B_1^+ field has received considerable importance. The goal is to make the field B_1^+ as homogeneous as possible, to reduce artifacts, and at the same time try to contain the level of SAR and make the procedure safer [1]. Different approaches have been proposed in literature, however most of them do not involve the determination of the complex excitation currents pertaining to the RF coil of MRI systems.

In this scenario, a new active shimming procedure is introduced by relying on a completely different perspective. In particular, the proposed procedure is based on the field intensity shaping paradigm (exploited in several applications such as microwaves hyperthermia treatment [2]-[3]). More in details, this new approach to field intensity shaping set up for B_1^+ shimming, exploits one or more control points located in the region of interest and is able to take contemporaneously into account all constraints regarding polarization, strength of the B_1^+ Field and SAR levels like in [1]. The procedure can also give the possibility to shape the field inside restricted regions and delimiting the field level outside them thanks to the use of a proper designed “mask function”. Moreover, the convexity of the proposed procedure ensures to achieve the global minimum of the problem and, hence, an accurate, repeatable, and optimal solution of the shimming problem.

More details about the proposed approach, as well as some numerical examples against realistic biological scenario, will be given at the conference.

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OP24

7 TESLA PHASE RIM AND CORTICAL LESIONS IN MULTIPLE SCLEROSIS AS MARKERS OF DISEASE PROGRESSION

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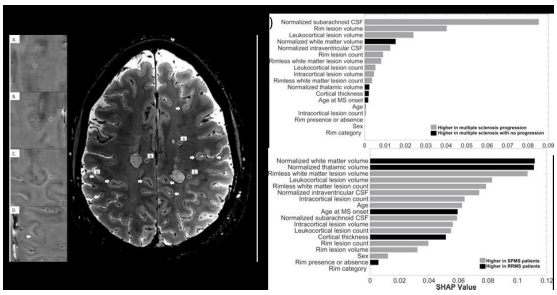
In multiple sclerosis (MS), recognizing individual lesion-type patterns on MRI may be valuable for differentially monitoring treatment effects: cortical lesions contribute to disease progression, while chronic active white matter lesions harboring a paramagnetic rim (so called “rim lesions”) are associated with an aggressive form of MS [1]. It is, however, still uncertain how these two types of lesions relate to each other, or which one plays a greater role in disability progression (quantified through the EDSS score). In this longitudinal Ultra High Field (7T) MR study we examine 100 MS patients – relapsing remitting (RRMS) N=74, secondary progressive (SPMS) N=26, to assess how lesions influence disease progression. T2* susceptibility imaging (Fig.(A)) was used to characterize cortical and rim lesion presence and evolution, while a machine learning algorithm based on extreme boosting techniques was used to assess the cumulative power of cortical and rim lesion types in predicting disease stage and disability progression, alongside with more traditional imaging markers. The unique contribution of each feature to the final prediction performance of the models was evaluated by computing the SHAP values. The models built for predicting disease stage / neurological disability progression achieved an AUC of 0.82 / 0.69, a sensitivity of 0.78 / 0.71, an accuracy of 0.77/ 0.68 and a specificity of 0.73 / 0.58. Fig.2 shows the importance ranking for these models, where larger values indicate a larger contribution to the final prediction. We found that cortical and rim lesion types were main predictors of EDSS progression (see Fig.(B)). However, their importance was lower in discriminating between RRMS/SPMS patients (Fig.2(C)). The highest ranked predictors of the disease stage identified were the traditional MRI metrics of white matter and thalamic atrophy and the rimless white matter lesion volume.



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POSTERS

P1

NMR NON-TARGETED METHOD TO PRESERVE THE BIODIVERSITY OF AUTOCHTHONOUS LENTILS CULTIVATION

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Over the last 60 years, the cultivation of lentils (*Lens culinaris* Medik.) has drastically declined in Italy. As a result, the country has become one of the biggest importers of this food product, mainly from Canada, the USA, Turkey, and China [1]. The loss of biodiversity is becoming an urgent problem and many efforts are needed to preserve the cultivation of local varieties and their authenticity. Nowadays, lentils are cultivated in restricted geographical areas of central and Southern Italy [2]. As a consequence, national trademarks have been introduced to ensure their high-quality value. *Altamura Lentil* has received recently the denomination of Protected Geographical Indication (IGP). This product is cultivated in a distinctive land between Apulia and Basilicata and its particular qualitative attributes are correlated to the environmental conditions typical of the original land. Therefore, there is increasing attention towards the development of rapid and sustainable analytical methods able to certify the geographical origin of these products. In this context, non-targeted Nuclear Magnetic Resonance (NMR) methods are attracting growing interest as a powerful analytical tool providing a fully comprehensive overview of the chemical composition of the food product under investigation, without *a priori* knowledge of the metabolites contained in. The metabolic profile resulting from the application of an NMR-based non-targeted approach on foodstuff enables the assessment of their authenticity, variety, and geographical origin [3].

In the present work, a non-targeted NMR method was successfully applied to assess the authenticity of *Altamura lentils* and establish the geographical origin, in a way to discriminate this class of product from the other Italian and/or imported lentils.The results demonstrate the advantageous application of Nuclear Magnetic Resonance as an efficient analytical approach to assess and preserve the authenticity of local food products.

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P2

^{19}F NMR FOR THE STEREOCHEMISTRY ASSIGNMENT OF NEW β-FLUORINATED g-BUTYROLACTONE DERIVATIVES

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The introduction of a fluorine atom in existing molecules is a current strategy in the quest for new drugs. Paraconic acids are an important class of functionalized γ-lactones, characterized by the presence of a carboxylic acid group at C-β, which display a wide spectrum of biological activities. We devised a simple route for the synthesis of a new class of b-fluorinated paraconic acid derivatives. The outcome is a diastereomeric pair (Fig. 1), separable by column chromatography, whose ^{19}F NMR spectrum shows two signals, 20 ppm apart (Fig. 1). Thus, the ^{19}F chemical shift can be exploited as an immediate diagnostic mean of the stereochemistry, provided the signals are unambiguously assigned. This was accomplished by means of ^{19}F,^{1}H HOESY spectra.

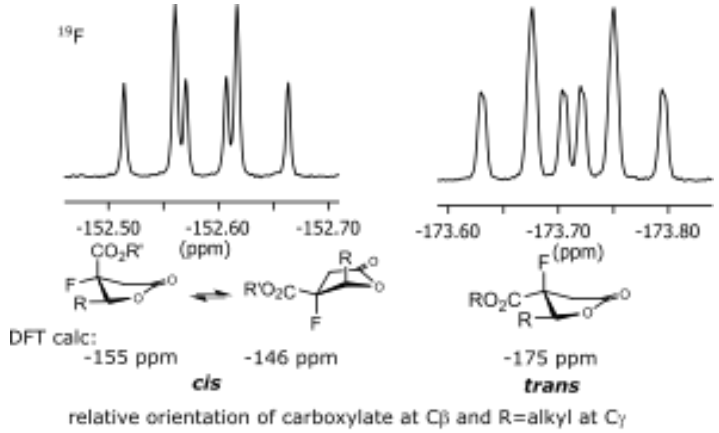

Fig. 1. ^{19}F NMR signals for the two diastereomers and relevant ring conformations.

The experimental ^{19}F chemical shift values were faithfully reproduced by two-component relativistic DFT calculations. The natural chemical shift analysis allowed to explain the sizable chemical shift difference in terms of the differential contributions of the natural localized molecular orbitals in the two isomers.
The γ-butyrolactone ring can exist in two envelope conformations, i.e., with the C-β either above or below the ring plane. The molecular flexibility was accounted for by a preliminary quick conformational screening by molecular mechanics in the RDKit environment. The geometries of two different envelope conformers for each stereoisomer were then optimized by DFT at the scalar ZORA TZ2P/BLYP level revealing that in the case of the *trans* isomer there is only one low-energy stable ring conformation, whereas for the *cis* both ring conformations are coexisting (Fig. 1), in analogy with what previously found for the parent not fluorinated molecules.

P3

LOW FIELD NMR RELAXOMETRY FOR INTRAOPERATIVE TUMOUR MARGIN ASSESSMENT IN BREAST-CONSERVING SURGERY

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As breast-conserving surgery is routinely applied for treatment of breast cancer (the most common form of cancer for women), the need for new technology to improve intraoperative margin assessment has become increasingly important [1]. In this study, the potential of Fast Field Cycling Relaxometry as new diagnostic tool was evaluated. Small freshly excised tissue samples (n=104) from 41 patients undergoing breast cancer surgery were collected and subjected to proton longitudinal relaxation rate (R_1) measurements at very low magnetic field strengths, then sent to gold standard histopathological evaluation (H&E staining). It was found that a good accuracy in margin assessment, *i.e.,* a sensitivity of 92% and a specificity of 85%, could be achieved by using two quantifiers namely *i)* the slope of the line joining the R_1 values measured at 0.02 and 1 MHz (the Ratio criterion) and *ii)* the sum of the R_1 values measured at 0.39 and 1 MHz (the $2R_1$ criterion) (Fig. 1). The discriminating ability relies mainly on the difference of fat/protein/water content and water mobility between healthy and malignant tissue [2]. The relaxometric method is low cost, fast and it does not rely on the expertise of a pathologist or cytologist as it can be highly automatized.

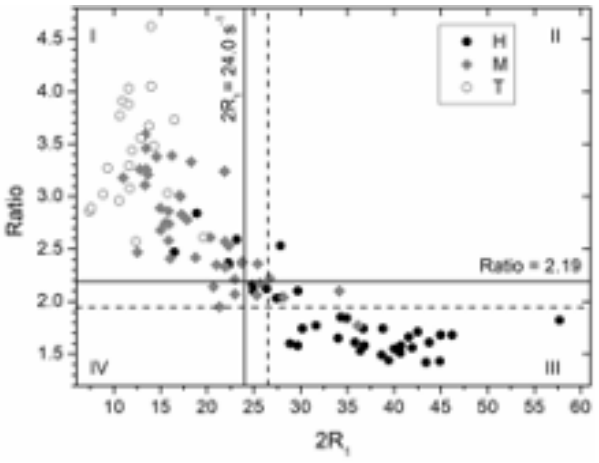

Fig. 1. The Ratio value as a function of the $2R_1$ value for all the tissue samples, classified as H (Healthy), T (Tumour) and M (Mix) by histological analysis. The two horizontal and vertical thick lines correspond to the identified cut-off values for margin assessment.

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P4

STRUCTURAL ELUCIDATION OF METAL COMPLEXES FOR CVD APPLICATIONS

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The preparation of metal oxides thin films or nanomaterials for a wide range of applications by chemical vapour deposition (CVD) presents several advantages, such as ease of scaling up of the production and automation, control of film thickness and conformational coverage [1]. However, the success of the process strongly relies on the availability of molecular precursors that must be volatile, easy to manipulate and decompose to the desired metal oxides through non-toxic and environmental friendly routes. With the aim to fulfil these features, different metal organic complexes have been prepared through molecular engineering of the metal coordination sphere [2]. Besides the synthetic route optimization, the characterization of this class of compounds represents an interesting challenge, especially for the NMR spectroscopists due to the unusually wide spectral width range (Fig. 1). According to the features of the metal center, both solid and liquid state NMR, solvent selection and variable temperature experiments should be carefully considered. Herein, the liquid and solid state NMR results metal complexes bearing different metal centers obtained from the reaction among metal chlorides, fluorinated diketones and organic amines will be presented [3].

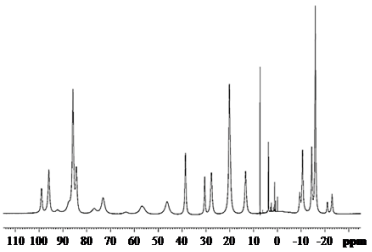

Fig. 1. 1H NMR spectrum of the cobalt complex in CDCl3

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P5

NMR-BASED METABOLIC PROFILING OF EXTRACTS OF CINNAMON BUDS AND BARK

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Cinnamon is one of the most popular spices used worldwide not only for cooking, but also in traditional and modern medicines for its beneficial properties for human health. *Cinnamomum zeylanicum*, also known as 'true cinnamon' or Ceylon cinnamon, and *Cinnamomum cassia*, also referred to as Chinese cinnamon, are the two most important cinnamon species. The most used part of the cinnamon tree is the bark. However, even if quite uncommon, also the Cinnamon buds (the unopened flowers dried in the sun) are used in the oriental culture.

Here, the metabolic profile of aqueous/alcoholic extracts of cinnamon buds was characterized for the first time by NMR spectroscopy and compared with those of bark extracts from different cinnamon species. For this purpose, an NMR-based protocol for the rapid and semi-automatic identification and quantification of metabolites has been developed, using the Simple Mixture Analysis (SMA) tool of MestReNova software. This strategy was similar to that used for the chemical characterization of extracts from other food matrices, such as cocoa, beer and coffee [1]. The SMA libraries built for cinnamon extracts are available for the scientific community [2].

This approach allowed the NMR-based metabolic profiling of cinnamon buds and bark extracts from different species (*Cinnamomum cassia* and *Cinnamomum zeyalanicum*), obtained with different extraction solvents and acquired both in CD_3OD and D_2O. The extraction procedures, with ethanol and water as the solvents, were chosen to mimic as much as possible those used in cooking and traditional medicine.

Finally, the content of bioactive compounds and the antioxidant activity of cinnamon was evaluated, depending on starting material (bud or bark) and extraction procedure (alcoholic, hydroalcoholic or aqueous).

The data collected provide useful insights for the selection of cinnamon raw material to be used in the preparation of cinnamon-based dietary supplements and nutraceuticals or for their use as functional foods.

Acknowledgments

We acknowledge the Italian Ministry of University and Research (MUR) for the grant "Fondo per il finanziamento delle attività base di ricerca (FABBR)-MIUR 2018" to Cristina Airoldi.

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P6

EXO-METABOLOMICS FINGERPRINT OF BLADDER CANCER PROGRESSION USING 1H-NMR

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Urothelial bladder cancer (UBC) is the most common tumor of the urinary system [1]. One of the biggest problems related to this disease is the lack of markers that can anticipate the progression of cancer [2]. Genomics and transcriptomics have greatly improved the prediction of risk of recurrence and progression. Further progress can be expected, including information from other omics sciences such as metabolomics. This study used 1H-NMR to characterize the intake of nutrients and the excretion of products in the extracellular medium of three UBC cell lines, representing low-grade tumors, RT4, high-grade, 5637, and a cell line, RT112, that shares genotypic features with both. We have observed that RT4 cells show activated oxidative phosphorylation, 5637 cells depend mainly on glycolysis to grow, while RT112 cells show a mixed metabolic state. Our results reveal the relative importance of glycolysis and oxidative phosphorylation in the growth and maintenance of different UBC cell lines and the relationship with their genomic signatures. They suggest that cell lines associated with a low risk of progression present an activated oxidative metabolic state, while those associated with a high risk present a non-oxidative state and high glycolytic activity.

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P7

RS-FMRI ANALYSIS ON PATIENTS TREATED WITH TRANS-CRANIAL MAGNETIC RESONANCE GUIDED FOCUSED ULTRASOUND SURGERY (tcMRgFUS): PRELIMINARY RESULTS

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Trans-cranial MR-guided Focused UltraSound (tcMRgFUS) is an innovative procedure for treatment of some functional neurological disorders such as essential tremor [1].
In this work, an Rs-fMRI analysis was performed before and after tcMRgFUS treatment investigating low frequency fluctuations in the BOLD signal in order to evaluate synchronous activations between spatially independent brain regions (FC).
All subjects underwent brain scan using a 1,5T MRI scanner. An eight-channel brain phased array coil was used. Structural images were obtained via a T1-weighted sagittal three-dimensional (3D) 1 mm thick Fast Spoiled GRadient-echo (FSPGR) prepped inversion recovery pulse sequence [2]. RS-fMRI data were acquired with a two-dimensional (2D) axial T2*-weighted gradient-echo Echo-Planar (EP) pulse sequence parallel to the anterior commissure–posterior commissure (AC–PC) line over the entire brain. A ten-minute (200 volumes) fMRI scan was performed on each participant.
All the preprocessing was performed using FSL(version 5.0.9). Preprocessing included motion correction, removal of non-brain structures, high pass temporal filtering, pre-whitening and global spatial smoothing.
FSL's MELODIC software was used for probabilistic independent component analysis. Multi-session temporal concatenated ICA (Concat-ICA) approach was chosen. 30 independent components (IC maps) were extracted.
FSL dual regression, that allows for a voxel-wise comparison of RS-fMRI, was carried out to perform the analysis for the differences between groups. Non-parametric permutation-based inference analysis was performed. For each analysis, we ran 5000 randomized permutations, while threshold-free cluster enhancement (TFCE) was used for statistical inference to validate the likelihood of extended areas of signal, which also considers information from neighboring voxels.
Preliminary results show that the functional networks belonging to the extra-pyramidal circuits responsible for controlling the voluntary movements were found to have post-thalamotomy increased functional connectivity.

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P8

A MACHINE LEARNING MODEL TO PREDICT PROGRESSION OF CORTICAL THICKNESS IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is one of the most common causes of neurological disability in young adults in the Western world. Several MRI studies have demonstrated the presence of cortical grey matter atrophy, which strongly correlates with the progression of neurological impairment in MS patients [1]. The substrate of this atrophy, is, however, still largely unknown. It is not clear whether cortical atrophy is mainly the result of local pathological processes or disconnection from distant white matter (WM) lesions. In addition, MS lesions have different degrees of destructiveness, that appears greater when a susceptibility hypointense rim is present. Here, we tackle this question combining lesion characterization at ultra high-field (7T) with a novel machine learning (ML) framework, which includes explainability, i.e. the ability to rank neuroradiological lesion signatures according to their performance in determining and predicting cortical tissue loss. 14 predictors (cortical and WM lesion counts and volumes, along with demographics and conventional MR markers) were used to predict cortical thinning in 100 MS patients. 2D- T2*-weighted scans yielding magnitude and phase images were acquired at 7T to evaluate WM lesions with or without the presence of paramagnetic rims as well as intra- and leuko-cortical lesions. Anatomical 3T 3D T1-weighted MR images were also obtained for Freesurfer reconstruction, cortical and subcortical segmentation and regional cortical thickness extraction from 150 brain regions (using Desikan atlas). In order to develop a predictive model for both local (i.e. parcellated) and globally averaged cortical thickness, we employed an XGBoost classifier as follow: the original dataset was split randomly 1000 times into training (70%) and test (30%) sets. After training, performance for each test set was assessed by calculating the mean (across 1000 repetitions) Pearson correlation (r) as well as related p-values between the real and predicted values of the held out, 30%-sized test sets, retaining only the model + region combinations which resulted in a p-value <0.05. The contribution of each feature to the final prediction performance of the model was evaluated and compared by computing the Shapley Additive explanations (SHAP) values [2]. Our ML algorithm has successfully (average $p<0.02$ and $r>0.4$) predicted globally averaged cortical thickness from lesion features only. The most important features for the prediction were, WM lesion volume, age, WM lesion count, normalized WM volume and intracortical lesion volume. Similar performances (average $p<0.02$ and $r>0.4$) were obtained in 15 brain regions, mostly belonging to frontal and temporal cortices of both brain hemispheres. When computing average ranks from the single, region-wise SHAP values, the same set of features appeared to contribute especially for predicting local cortical thickness overall. Our results suggest that lesions, mainly in the WM but also within the cortex, are fundamental determinants to cortical thinning. These results further highlight the need for both statistic and artificial intelligence approaches in understanding the complex mechanism of multiple sclerosis pathology.

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P9

STUDY OF ITO NANOPARTICLES BY SOLID STATE NMR SPECTROSCOPY

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Recently nanoscience and nanotechnology have sparked a lot of interest in many fields of science and engineering. In the field of plasmonics, noble metal nanoparticles (NPs) exhibit optical properties that differ significantly from those of the bulk. Indeed, they show a strong absorption band in the UV-Vis region due to coherent oscillation of conduction electrons, driven by the electric field of light. This phenomenon is called "Localized Surface Plasmon Resonance" (LSPR) and the position of the resonant peak depends on the size of the metal nanoparticle [1]. Plasmonic NPs are currently object of considerable interest thanks to their wide range of potential applications in the fields of nano-optics, chemical and biological sensing, spectroscopy and molecular imaging. Lately, doped semiconductors, including tin-doped indium oxide (ITO), have been proposed as new cheaper nanomaterials with plasmonic properties. Unlike noble metal NPs, ITO is a near-infrared plasmonic system and the resonant peak can be easily tuned by changing the dopant concentration [2]. Due to its properties, it has been employed in electrochromic windows [3], solar cells, flat-panel displays, sensors and architectural glasses [4]. Investigating their structural features at an atomic/molecular level can improve our understanding of the final properties of these materials. Recently [5] ^{119}Sn solid state NMR (SSNMR) has proved to be of particular interest for the study of ITO NPs. In this work we investigated the structural properties of ITO nanoparticles, stabilized with oleylamine, with different doping concentration by using high resolution SSNMR. ^{119}Sn DE/MAS (Direct Excitation/Magic Angle Spinning) measurement were performed to investigate the effect of doping on the structure and electronic properties. Moreover, ^{13}C and ^{1}H MAS experiments were carried out in order to get insights into the arrangement and dynamics of the stabilizer around the nanoparticles.

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P10

PROBING POLYSTYRENE NANOPARTICLES INTERACTIONS WITH BIOLOGICAL MACROMOLECULES BY AN INTEGRATED NMR-BASED APPROACH

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The presence of micro- and nano-plastics in the water, air and food is becoming a significant concern [1]. Most of the works reported in the literature are mainly focused on the micro/nano-plastic accumulation in marine organisms. Moreover, it has been demonstrated that human exposure occurs largely through ingestion, but also in a less well-defined manner through inhalation [2]. Despite, these numerous studies the consequences of micro- and nano-plastics explosion on human health are yet unclear. Therefore, it is extremely important to understand if and how nano-plastics (i.e. polystyrene) interact with biological macromolecules, inducing conformational changes and inhibiting their main functions.
In the frame of this project, we performed a structural and dynamical characterization of the human ubiquitin in the presence of polystyrene nanoparticles by using a multidisciplinary approach in which TEM (Trasmission Electron Microscopy) and CD (Circular Dichroism) data were integrated with high-resolution NMR (Nuclear Magnetic Resonance) methodologies. Overall, our data strongly indicate that upon addition of nano-polystyrene the ubiquitin undergoes local conformational rearrangements, which in turn activate aggregation processes.

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P11

LOWER WHITE MATTER FIBER CROSS SECTION AND GLOBAL STRUCTURAL BRAIN NETOWRK REORGANIZATION IN CHRONIC LOW BACK PAIN DISORDER

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Chronic low back pain (CLBP) is a widespread public health issue, and its prevalence is enormous. Importantly, in most chronic pain conditions the correlation between pain and physical pathology at the site of referred pain (e.g., radiographs, physical examination, etc.) is typically weak. The decoupling between pain, disability levels and "peripheral" findings strongly suggests that alterations in the central nervous system may contribute to the establishment and/or maintenance of chronic pain. Using positron emission tomography (PET), we have previously shown that [^{11}C]-PBR28 signal is elevated in the brain of CLBP patients [1]. Because [^{11}C]-PBR28 binds to the 18 kDa translocator protein (TSPO), a marker of glial activation, our results implicate neuroinflammation in the pathophysiology of CLBP patients. However, while PET imaging requires the injection of a radioactive ligand, the proximity of a cyclotron, diffusion weighted MRI (dMRI) can explore neuroinflammation-related microstructural alterations in a highly specific way. In this study, we employed constrained spherical deconvolution [2] in high angular resolution DWI datasets to compare apparent fiber density (FD), fiber-bundle cross-section (FC) and fiber density and cross-section (FDC) as well as structural connectivity matrices and related graph-theoretic metrics in a population of 14 CLBP patients and 17 healthy controls. We found statistically significant group differences in FC in the right Anterior Thalamic Radiation (ATR) ($p=0.04$, effect size=0.941, HC>CLBP, Fig 1). Moreover, we found group-wise differences in global network disruption indices related to betweenness centrality ($k=-0.187$, $p<0.001$, Fig 1) and to local strength ($k=0.183$, $p<0.001$, Fig 1). Also, the right thalamus appeared to be network hub for HC ($p=0.045$) but not in the CLBP, while the right frontal pole ($p=0.015$) and the right caudal middle frontal gyrus ($p=0.038$) are hubs in CLBP but not in HC. Overall, our findings seem to imply a global, CLBP-related reorganization of the structural connectome, and specifically support a direct involvement of the thalamus in CLBP.

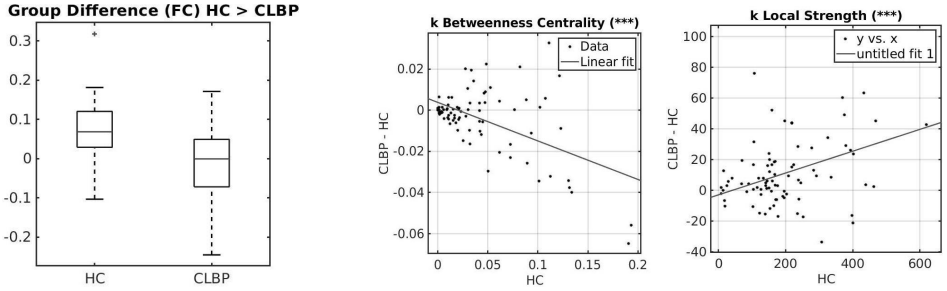

Fig. 1. Left: group difference in FC between HC and CLBP. Right: Group-wise disruption indices

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P12

IN VIVO STUDY IN HEALTHY BALB/C MICE OF THE EXCRETION TIME OF A MACROCYCLIC GADOLINIUM BASED CONTRAST AGENT AND THE SPECIFIC RETENTION IN BLADDER, SPLEEN AND BONE.

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Gadolinium based contrast agents (GBCAs) are commonly employed at clinical settings for the anatomical resolution of the magnetic resonance images.[1] In recent years, concern on the use of GBCAs has raised as it has been found that tiny amount of Gd can be retained in brain and other tissues.[2] The aim of this work is to bring the attention to tissues less considered in the past such as bladder, spleen and bones. In order to evaluate the amount of Gd retained in the tissues, mice were administered with 20 doses of 0.6 mmol Gadoteridol/kg over a period of 4 weeks. The sacrifice time was set at 4 different time points (4, 15, 30 and 90 days) after the last injection. After sacrifice, urine, tissues and organs were collected. One tibia was weighted, mineralized and Gd quantified through ICP-MS. The other tibia was handled in order to separate bone matrix and bone marrow and Gd quantified separately. Spleen was processed as well, to measure the amount of Gd in the splenocytes and in its fibrous part. In bladder, beside ICP-MS total Gd quantification, UPLC-MS was performed to study the chemical form of the retained metal. The quantification of Gd in the bladder showed the highest amount of metal retained, especially at the shortest times, among all the investigated organs. Urine samples were also analysed to determine the Gd concentration and the rate of elimination over time. Urine Gd concentration rapidly decreased over time to suggest that most of the GBCA is correctly excreted through the renal route (Figure 1A). In addition, the amount of Gd found in the spleen decreased over time and the metal found in the fibrous part was higher than that found in the splenocytes (Figure 1B). The quantitative analysis of the whole tibia showed a constant quantity of metal retained until 90 days after the last administration. The separate analysis of bone marrow and bone matrix revealed that most of Gd was retained constantly by the bone matrix (Figure 1C). Our results point out that bladder could be an extremely specific organ for the metal retention. The analysis on bones showed a very quick deposition of Gd: the excretion rate is very low, in particular in the bone matrix, where most of Gd is retained.

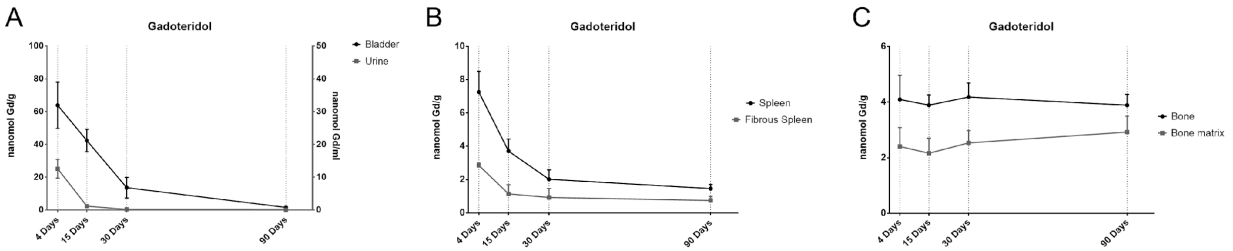

Fig. 1. Amount of Gd 4, 15, 30, and 90 days after the last administration of Gadoteridol (20 doses of 0.6 mmol Gadoteridol/kg over a period of 4 weeks) in: A) Bladder and Urine; B) Spleen and its fibrous part; C) Bone and bone matrix.

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P13

AN INTEGRATED APPROACH BASED ON NMR AND HPLC-UV-ESI-MS/MS TO CHARACTERIZE CALABRIAN APPLE JUICES AND THEIR NANOFILTRATION (NF) EXTRACTS

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In the present work, Calabrian apple juices belonging to four different cultivars, Royal Gala, Pink Lady, Golden Delicious and Fuji, were studied. One of the objectives of this work was the characterization of metabolites present in the food matrix [1,2] and at the same time the possibility of using innovative methodologies based on membrane operations, such as ultrafiltration (UF) and nanofiltration (NF), to obtain from the squeezed apple juice, extracts rich in antioxidants.[3,4] To improve the overall quality of the study and enhance the coverage of the metabolome two analytical tools were used: NMR and HPLC-UV-ESI-MS/MS.[5,6] One- and two-dimensional multinuclear NMR spectra combined with chemometric analysis (PCA) were used to identify the metabolic profile of different juice varieties and variations in metabolic composition. 12 more abundant compounds with low molecular weight in the fresh juices of the four apple varieties have been identified by NMR analysis while a clear separation between the four varieties is established by multivariate analysis.

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P14

SENSITIVITY ENHANCEMENT AND QUANTITATIVE ASPECTS OF ^{29}SI SOLID STATE NMR SPECTRA

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^{29}Si solid state NMR spectroscopy (SSNMR) has proven to be a valuable tool for the characterization of silicon-based materials. ^{29}Si SSNMR techniques have indeed been applied to the study of a wide variety of systems, such as silica and silicate surfaces [1] and cements [2]. The utility of ^{29}Si SSNMR arises from the extreme sensitivity of ^{29}Si chemical shift to the local chemical environment, and in particular to the number of bridging oxygen and OX groups (X = H, R) bonded to the observed ^{29}Si nucleus; therefore, signals of M ($R_3SiO_{0.5}$), D ($R_2Si(O_{0.5})_2$), T ($RSi(O_{0.5})_3$), and Q ($Si(O_{0.5})_4$) silicon species fall in different regions of the SSNMR spectrum. Unfortunately, ^{29}Si NMR presents some inherent downsides, mainly related to the scarce isotopic abundance (4.67%) of ^{29}Si nuclei and to their long spin-lattice relaxation times T_1. These features can extremely dilate (from several hours to days) the experimental time for acquisition of quantitative direct excitation (DE) spectra and, for this reason, non-quantitative and less time-consuming approaches are often preferred. ^{1}H-^{29}Si cross polarization (CP) is among the most employed techniques for recording ^{29}Si SSNMR spectra with enhanced sensitivity at the expense of quantitative information. In this work, different approaches based on several DE and CP techniques for recording ^{29}Si SSNMR spectra were discussed and compared in terms of sensitivity and quantitative aspects, in order to identify the most efficient experimental approach for the desired information. In some cases, these experiments were coupled with modern noise reduction techniques [3]. In particular, the different methods were applied on a sample of silica functionalized with 3-(trimethoxysilyl)propyl methacrylate (TSPM) [1], used as filler in polymeric materials. In this kind of systems the observation and quantification of T silicon species is fundamental to assess the efficiency of the functionalization reaction and to understand the properties of the final composite materials. Beside "standard" CP experiments, recently developed Multiple-Cross Polarization (MultiCP) techniques [4], combining the enhanced sensitivity of CP with the possibility of retaining quantitative information, were also tested. Notably, in the last few years, MultiCP experiments have been successfully employed to record quantitative ^{13}C spectra of organic materials with good signal-to-noise ratios, and their application to ^{29}Si is currently being explored [5].

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STRUCTURAL PROPERTIES OF THE F4_MIL-140A(CE) MOF BY SOLID-STATE NMR SPECTROSCOPY

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Metal-Organic Frameworks (MOFs) are a class of crystalline compounds whose scaffolding derives from metal clusters or ions that are interconnected by organic linkers. The high number of possible combinations of metals and ligands leads to high tunability of macroscopic properties and thus it is possible to employ MOFs in many fields of applications, including gas storage [1], gas separation [2], catalysis [3] and others [4,5]. During the design and development of a new MOF, it is extremely important to completely understand every macroscopic property of the compound and to relate it to its microscopic origin.
Many techniques can be exploited and combined to characterize the structural and dynamic molecular properties of a MOF. Among them, Solid State Nuclear Magnetic Resonance (SSNMR) spectroscopy is certainly one of the most important because it can shed light on many aspects of the compound at a molecular level, such as 3D structure [6], porosity [7], local dynamics [8], and host-guest interactions [9].
In this work, ^{1}H, ^{13}C, and ^{19}F SSNMR spectroscopy has been employed to gain an in-depth knowledge of a MOF belonging to the MIL class, precisely F4_MIL-140A(Ce), in which chain-like inorganic building units of Cerium^{IV} are interconnected by tetrafluoroterephtalates. This MOF is extremely promising for possible applications, in particular as a sorbent for gas separation, because of its water-based synthesis and its step-shaped CO_2 adsorption isotherm [10].
High-resolution SSNMR techniques and 2D correlation spectra have been used to obtain, also by comparison with powder X-ray diffraction results, a detailed characterization of the framework structure both in the presence and after removal of crystallization water, highlighting the presence of different molecular environments with different symmetry. Particular attention has been put into the investigation of dynamic processes involving the fluorinated aromatic rings through the variable-temperature analysis of ^{19}F spin-lattice relaxation times and ^{13}C chemical shift anisotropy.

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THE CHANGE OF CONDITIONS DOES NOT AFFECT ROS87 DOWNHILL FOLDING MECHANISM

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Downhill folding has been defined as a unique thermodynamic process involving a conformations ensemble that progressively loses structure with the decrease of protein stability [1]. Downhill folders are quite rare in nature because there isn't an energetically substantial folding barrier that can protect against aggregation and proteolysis [2-4]. We have previously demonstrated that the prokaryotic zinc finger protein Ros87 shows a folding/unfolding process in which a metal binding intermediate converts to the native structure through a delicate barrier-less downhill transition [5]. Significant variation in folding scenarios can be detected within protein families with high sequence identity and very similar folds and for the same sequence by varying conditions. For this reason, we here show, by means of DSC, CD and NMR, that also in different pH and ionic strength conditions Ros87 is capable to conserve its partly downhill folding mechanism demonstrating that the downhill mechanism can be found under a much wider range of conditions. We also show that mutations of Ros87 zinc coordination sphere produces a different folding scenario demonstrating that the organization of the metal ion core is determinant in the folding process of this family of proteins.

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P17

2D RUDDLESDEN-POPPER PEROVSKITES $BA_2MA_{n-1}Pb_nI_{3n+1}$ AS STUDIED BY SOLID-STATE NMR SPECTROSCOPY

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Lead Halide Perovskites are semiconductors used in different optoelectronic devices (e.g. sensitizers for solar cells, photodetectors, LEDs). 2D Ruddlesden-Popper (RP) perovskites ($L_2A_{n-1}B_nX_{3n+1}$) are layered materials made by perovskite layers separated by large monoammonium cations L^+, whose ammonium head is oriented towards the perovskite layers. The perovskite sheets have the usual perovskite structure of corner-sharing BX_6 octahedra and A^+ cations in the cavities. The number of planes of BX_6 octahedra in each perovskite sheet is specified by n.
Solid-State NMR stands out as characterization technique of Lead Halide Perovskites thanks to its ability to study ion dynamics, compositional variations and ion incorporation, chemical interactions and degradation mechanisms [1].
In the present study, we investigated the series of 2D RP perovskites $BA_2MA_{n-1}Pb_nI_{3n+1}$ for n=1, 2 and 3 and their 3D analogous $MAPbI_3$ (Figure 1) by Solid-State NMR. ^{207}Pb, ^{1}H, and ^{13}C spectra recorded under Magic Angle Spinning and/or static conditions allowed the structural characterization of these systems; the obtained results have been discussed also by comparison with very recent literature [2]. In addition, the spin-lattice relaxation times T_1 of ^{207}Pb were measured to evaluate if different Pb sites show distinct relaxation behaviors. Finally, the spatial proximity between the organic cations BA and MA was probed by 2D PMLG Spin Diffusion experiments.

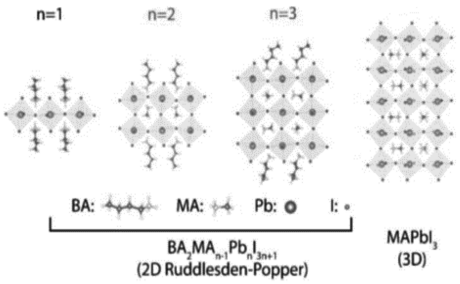

Fig. 1. Schematic structure of 2D RP perovskites $BA_2MA_{n-1}Pb_nI_{3n+1}$ for n=1, 2, 3, ~~4,~~ and of the corresponding 3D perovskites $MAPbI_3$. Here BA is butylammonium and MA is methylammonium.

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P18

DEVELOPMENT OF A CALIBRATION SYSTEM TO ASSESS THE REPRODUCIBILITY OF qNMR METABOLOMICS

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Nowadays, qNMR methods are widely applied in foodstuff control for product identification and traceability thanks to the NMR spectroscopy ability to generate statistically equivalent signals regardless of the instrumental configuration.[1,2] Nevertheless, current literature still lacks official harmonized criteria to assess the reproducibility of qNMR data produced in diverse laboratories employing differently configured spectrometers. The present study, exploiting the large amount of information deriving from an interlaboratory comparison involving 65 participants from 15 countries, aims at establishing the reproducibility of the produced spectroscopic data after passing a sequence of five chemometric tests. The well-performing calibration data were used to develop seven community-built calibration lines which acted as reference lines for the quantification of four selected metabolites (arginine, alanine, fructose, and glucose). Importantly, the newly built reference calibration lines may be queried by other laboratories which are engaged in the quantification of the same metabolites and were not originally involved in the interlaboratory comparison, upon producing calibration data under the same conditions established for the interlaboratory comparison.

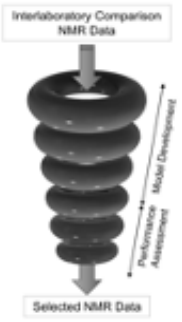

Figure 1. Schematic representation of the selection process for the evaluation of the reproducibility of the NMR spectra produced by 65 different spectrometers.

This work introduces important milestones to develop a harmonized qNMR protocol to be used in standardized analytical procedures not only for food products analysis but, more generally speaking, for quantification of analytes in unknown mixtures. The described approach, finding applications as an analytical platform for omics development, might contribute to the development of rapid methods for data assessment.

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P19

^{1}H AND ^{17}O NMR RELAXATION STUDIES OF THE Fe^{III}-TIRON SYSTEM

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Relaxometry is a very powerful technique for investigating the solution structural and dynamic properties of paramagnetic complexes. Due to their peculiar magnetic characteristics and their great success as diagnostic probes for applications in MRI, many studies have been carried out on Gd(III)- and Mn(II)-based systems. Surprisingly, very few investigations have been reported on Fe(III) complexes despite their considerable importance in various fields of chemistry, starting from bioinorganic chemistry. Fe(III), with five unpaired electrons in the *d* orbitals, a ^{6}S configuration and a high magnetic moment, is very well suited to be studied with this technique. In particular, in this work we focused on well-defined type of catecholate complexes of iron(III), Fe-Tiron (Tiron® = disodium 4,5-dihydroxy-1,3-benzenedisulfonate). The formation constants (see species distribution diagram in Fig. 1) should allow for independent study of these complexes by an appropriate choice of starting pH and Tiron concentration [1]. We report for the first time the complete characterization of the various species through a combined ^{1}H and ^{17}O NMR relaxometric study. In particular we investigated $[Fe(Tiron)(H_2O)_4]^-$ (pH=2.3), $[Fe(Tiron)_2(H_2O)_2]^{5-}$ (pH=4) and $[Fe(Tiron)_3]^{9-}$ (pH=8). Through a simultaneous fit of ^{1}H Nuclear Magnetic Resonance Dispersion profiles (from 0.01 up to 500 MHz), ^{17}O transverse relaxation rates (R_2) and shifts ($\Delta\omega$) (measured at 11.7 T as a function of temperature) we were able to obtain accurate value of the structural and dynamic parameters that adequately describe the behaviour of these paramagnetic complexes in aqueous solution [2].

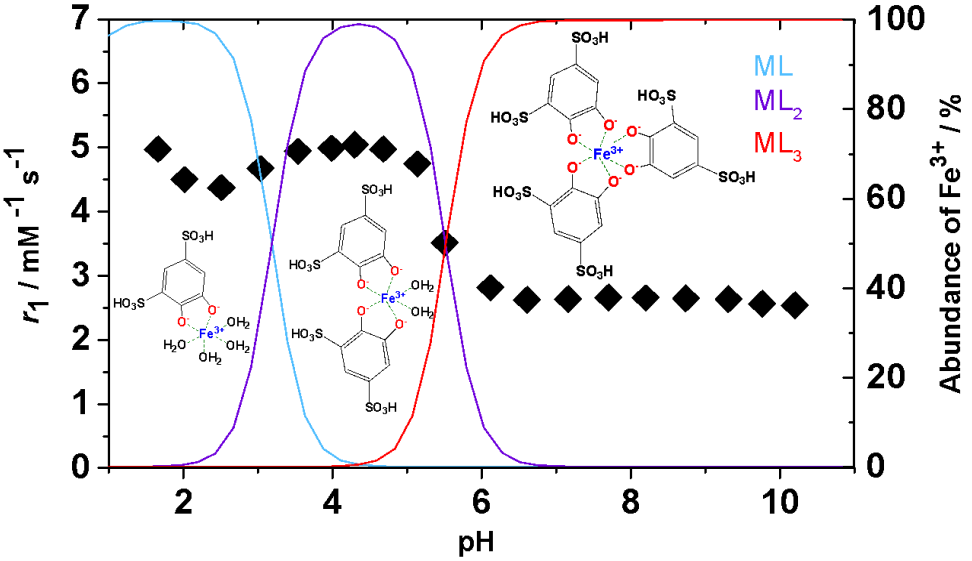

Fig. 1. pH-dependence of r_1 and relative abundances of the Fe^{III}-Tiron species (298 K, 32 MHz).

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P20

ALLOSTERIC MODULATORS OF FGF/FGFR SIGNALLING AS INNOVATIVE TOOLS AGAINST CANCER AND OTHER FGFR DRIVEN PATHOLOGIES

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Fibroblast growth factor (FGF2)/fibroblast growth factor receptor (FGFR) signalling is involved in several pathologies, including cancer development, metastasis formation and resistance to therapy, angiogenesis driven pathologies, vascular diseases, and viral infections. The development of small molecules, acting extracellularly to target FGF2/FGFR interactions, has the advantage of limiting the adverse effects associated with current intracellular FGFR inhibitors. We identified a few leads, either targeting FGF2 [1, 2] (Figure 1A) or FGFR-D2 domain [3] (Figure 1B), able to destabilize the FGF2/FGFR complex and inhibiting the subsequent signalling cascade. A detailed molecular-level description of FGF2/FGFR/inhibitor system, based on 1H-15N CSP and intensity perturbations, relaxation data, temperature coefficients, highlighted an allosteric cross-talk between the growth factor and its receptor. Unrestrained docking approaches were employed to screen, among a small library of natural compounds, the potential inhibitors of FGF2/FGFR interaction and DOSY experiments were used to evaluate inhibitors efficacy in destabilizing the complex. The characterization of the mechanism underpinning the inhibitors activity, opens the way to the research of innovative allosteric modulators for precision medicine.

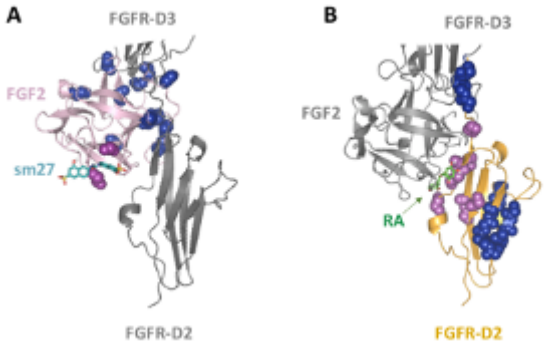

Fig. 1. Sm27 (A) and rosmarinic acid (RA) (B) binding sites (pink spheres) and residues affected by long-range dynamics perturbations (blue spheres) along FGF2/FGFR interface region.

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P21

ON-CELL SATURATION TRANSFER DIFFERENCE NMR FOR THE IDENTIFICATION OF FIMH LIGANDS AND INHIBITORS

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FimH is a mannose-binding bacterial adhesin expressed at the apical end of type 1 pili of uropathogenic bacterial strains and responsible for their D-mannose sensitive adhesion to host mammalian epithelial cells. [1] Because of these properties, FimH is a key virulence factor and an attractive therapeutic target for urinary tract infection. [2]
Here, we describe the development of an on-cell NMR method for the rapid screening of FimH ligands and the structural identification of ligand binding epitopes. [3] For this purpose, we prepared synthetic D-mannose decorated dendrimers, we tested their ability to prevent the FimH-mediated yeast agglutination, and thus we used the compounds showing the best inhibitory activity as models of FimH multivalent ligands to set up our NMR methodology.
Our experimental protocol, based on on-cell STD NMR techniques, is a suitable tool for the screening and the epitope mapping of FimH ligands aimed at the development of new antiadhesive and diagnostic tools against urinary tract infection pathogens. Notably, the study is carried out in a physiological environment, i.e. at the surface of living pathogen cells expressing FimH.

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The authors acknowledge MIUR for funding PRIN-2017 project 2017E44A9P - BacHounds: Supramolecular nanostructures for bacteria detection.

P22

MRI-GUIDED ULTRASOUND-TRIGGERED DRUG DELIVERY TO OVERCOME DRUG RESISTANCE IN HUMAN OVARIAN CANCER

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Ovarian cancer (OC) is the most fatal of all gynaecological tumors. The major cause of its lethality is the chemotherapy resistance, due to drug efflux mediated by cell membrane proteins, which prevents the drug to reach its target in the nucleus [1]. Sonoporation (SNP) is an appealing method to modify cell membrane permeability and increase the cell uptake of a target molecule. The aim of this study is to assess the potential of this biophysical approach to obtain at the same time the nuclear uptake of drug and the cell internalization of a Magnetic Resonance Imaging (MRI) agent, that could be used as imaging reporter for overcoming drug resistance. To evaluate the ability of SNP to bypass the drug efflux, *in vitro* experiments were carried out on two human OC cell lines, one drug sensitive and the other drug resistant. Both cell lines were incubated with the MRI agent gadoteridol and the fluorescent drug doxorubicin. After US treatment, T_1 weighted MR images of cell pellet were acquired at 7 T MRI. Sonoporated cells showed a superior T_1 contrast, and the presence of gadoteridol in the cytosol was confirmed through an in-depth relaxometric analysis, while the drug localization in the nuclei was assessed by confocal fluorescence microscopy. *In vivo* experiments were performed on athymic nude mice inoculated with human OC drug-resistant cells. In the US treated group, mice were locally insonated immediately after the injection of the drug to favour the cell internalization in the tumour. The T_1 contrast enhancement of this group was higher than the untreated mice, and, very important, the tumor growth was blocked (Fig.1). The increased tumour uptake of the drug was paralleled by the MRI T_1 contrast enhancement, thus demonstrating the great potential of MRI in monitoring, both *in vitro* and *in vivo*, the efficiency of the drug internalization, and consequently, of the therapeutic outcome. These results, though preliminary, clearly illustrate the potential of local SNP to overcome the mechanism of drug resistance in OC.

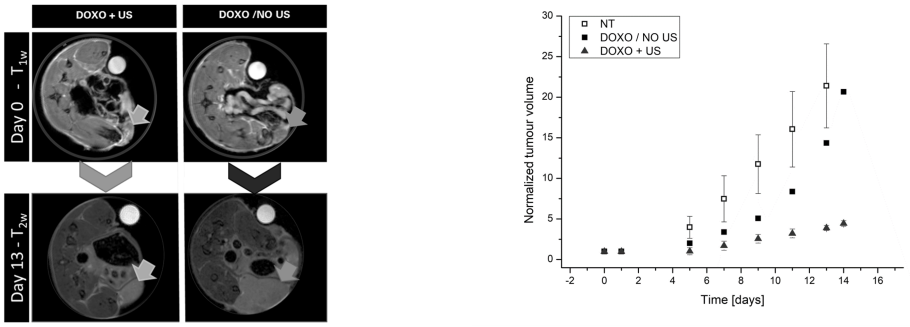

Fig. 1. Left: T_{1w} MR axial images of tumor acquired immediately after therapy with (DOXO + US) and without ultrasound (DOXO/NO US) and after 13 days
Right: Drug resistant tumor growth of xenograft mouse model: i) not treated (NT) (white squares) ii) treated with doxorubicin (DOXO/NO US) (black squares) iii) treated with ultrasound and doxorubicin (DOXO+ US) (grey triangles)

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P23

FISHING MOLECULES: THE TALE OF HOW ART JOINED SCIENCE ON THE ROAD OF STRUCTURAL ELUCIDATION

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The discovery of new molecules is a very fascinating and remarkable task. To pursue structural elucidation several 1D and 2D NMR techniques are suitable to collect pieces of information which, with a patient and clever work can eventually lead to success (molecular characterization). Science provides assessed tools aimed to collect clues about the molecular structure, however this is not right away leading to structural elucidation, often some "art" is needed together with researcher's insight (human skill). First, molecules are not all identical, this is why often we adopt different strategies to unveil secrets stored inside different molecules. A common challenge is the presence of very small amounts of unknown compounds: in these cases, the pretty insensitive nuclei (^{13}C and ^{15}N) are not directly detectable, however, it is possible to take advantage from the inverse hetero-correlated spectroscopies suitable to detect these nuclei as an echo relayed by protons (intrinsically more sensitive) directly (2D-HSQC) or long-range connected (2D-HMBC) to them. Sometimes the problem is interference of solvents or residual water, sometimes can be useful to evaporate back solvents and dissolve again with many *caveats*. In other cases molecules are tumbling very fast because of their size or their shape and other customized experiments will be necessary. Structural elucidation, just like fishing, requires patience, science but also some art to be accomplished.

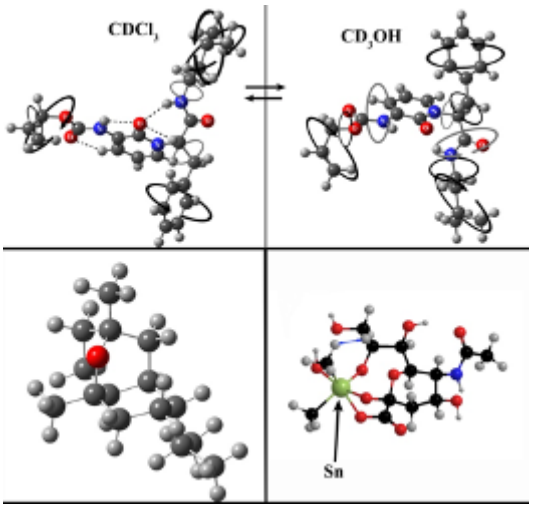

Fig. 1. 2D and 3D Molecular Drawing's of molecules characterized by Rotondo et al.

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P24

MODULATION OF TAU AGGREGATION WITH NATURAL COFFEE COMPOUNDS

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Neurodegenerative diseases (NDs) are an ever-increasing threat to human life. An early event in NDs is the misfolding, aggregation, and accumulation of specific proteins in neuronal cells, leading to cellular dysfunction, loss of synaptic connections, and brain damage. Tau protein is an unfolded protein, which transition among different conformations. Tau modifications and aggregation lead to intracellular neurofibrillary tangles (NFTs), one of pathological hallmarks in Alzheimer's disease and other tauopathies. Growing evidence suggests the possibility to perturb the dynamic interconversion of Tau among conformational states using small molecules, macromolecules, and nanoparticles to redirect the formation of neurotoxic aggregates. Coffee and coffee compounds are attracting interest in the field of neuro-inflammation and neuro-protection against oxidative-stress thanks to their bioavailability and ability to cross the Blood Brain Barrier [3]. Recent works demonstrate that brewed-coffee and some of these molecules, such as phenylindanes and other flavonoids, have the additional ability to inhibit Aβ and Tau protein aggregation [4]. Moreover, it has been suggested that coffee elements might have synergistic effects to produce the overall neuroprotective effect [5].
Relying on these promising perspectives, in our work we investigate the effects of Italian espresso coffee and a selection of coffee-derived bioactive molecules towards mitigation of Tau aggregation. We studied the kinetics of aggregation, the formation of prefibrillar aggregates and oligomers, the morphology of fibrils, and the conformational transitions of Tau in the presence of Italian espresso coffee, caffeine, trigonelline, theobromine, and genistein.
Specifically, with the aid of NMR spectroscopy we acquired different bi-dimensional spectra to better characterize the composition of our coffee mixture and to detect the characteristic fingerprints of the molecules of our interest.We performed a titration of Tau with the elected compounds to verify possible conformational changes in their presence and if there were a dose-dependent effect. Moreover, NMR was employed to elucidate the behaviour of the chosen compounds in our experimental conditions, in particular if heparin interfered with protein-coffee derived molecules association.We believe that this approach based on natural and readily available molecules could open new possibilities in the Alzheimer's disease treatment.

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P25

NMR BASED OPTIMIZATION OF BACTERICIDAL SILICON MATERIALS FOR BIOMEDICAL DEVICES

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Bacterial colonization of the surfaces of biomedical devices, such as urinary or intravenous catheters, is one of the most important causes of infections. Biomedical devices associated infections are considered a major threat to public health worldwide as they substantially impact on increased hospital stays, morbidity and mortality[1]. Considerable research efforts have focused on increasing the bactericidal activity of polymeric materials through surface modifications, coatings with bactericidal compounds, or inclusion of selected compounds in the polymeric matrix.
We report here on the feasibility of including 5-chloro-2-(2, 4-dichlorophenoxy) phenol) molecule (irgasan), a strong broad-spectrum anti-microbial agent, already in use in many consumer products, in silicon catheter tubes, through swelling approaches. The goal is to obtain a biocompatible material preventing *E. coli* and *S. aureus* growth and biofilm formation, without significant release of the toxic bactericidal molecule. NMR approaches offer a robust strategy to quantify the upload and release of the bactericidal molecule. NMR analysis of the metabolites secreted by the bacterial cells grown in the presence of the antibacterial molecule, at different concentrations, allows monitoring cell vitality. Specifically, the combined analysis of the decrease of metabolites production together with the blocking of nutrient consumption, upon IRG addition, allows for the determination of NIC, i.e. the concentration above which a negative effect on the growth starts to be observed and of MIC, i.e. the concentration above which no growth is observed. The same approach has been employed to detect any unwanted leakage effect of the bactericidal molecule from the impregnated silicon materials, even at sub-lethal concentrations. This information, easily derived from NMR analysis, is crucial for the development of medical devices, as both biocompatibility and induced bacteria resistance depend on the amounts of released antibacterial molecule.

Acknowledgements

This work was carried out with the financial support of Regione Lombardia Project “Piattaforma tecnologica per lo sviluppo di sonde innovative in ambito biomedicale” (ID 244356). Fondazione Antonio De Marco is acknowledged for financial support. SCITEC-POPLab is acknowledged for sample preparation and AFM measurements.

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P26

NMR CRYOPOROMETRY vs NMR RELAXOMETRY -A COMPARATIVE STUDY-

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Low-field Nuclear Magnetic Resonance (NMR) is a technique widely used both in industries and academia for product/material/substance characterization and quality control. It is used in food industries (fish, meat, dairy products) for the quantification of total fat and solid-to-fat ratio, building material industries (cement, wood) and in petrophysics for porous media characterization in terms of pore size distribution and porous matrix properties, such as wettability, permeability, porosity [1]. In particular, for what concern porous media and the pore size distribution, there are three main NMR related techniques:
(i) cryoporometry, which is a technique for the characterization of porous media through the evaluation of the alteration of thermodynamic properties of a confined liquid (the main observed effect is the depression of melting point), being a suitable technique for pore size distribution determination between nano and micro scale (2 nm - 1 μm) [1, 2, 3].
(ii) The determination of longitudinal and transversal relaxation times distribution by observing the magnetization relaxation once it has been perturbed. This method relies on the relation between the surface-to-volume ratio, the relaxation times and the surface relaxivity [4, 5, 6].
(iii) Pulse Field Gradient (PFG) NMR and the analysis of the decay due to Diffusion in Internal Field, which are diffusion-based methods, can be used up to nano scale pores [7, 8].
In this work the authors wanted to focus only on NMR cryoporometry method (i) in comparison with the NMR interpretation of relaxation times distribution related to surface-to-volume ratio (ii) (in brief "surface-to-volume ratio"), in order to highlight strengths and weaknesses of both the techniques. Mercury Intrusion Porosimetry have been used to determine pore size distribution and to validate the results obtained with NMR. The study is organized with the following structure: first a brief introduction to the theory of both NMR techniques is provided, then a description of the samples and experimental methods is shown. Finally, we will present the results.

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P27

APPLICATION OF ULTRA SMALL GOLD NANOPARTICLES IN THE STUDY OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (NDs) are commonly characterized by the slow and progressive disfunction of neuronal cells and brain damage [1-3]. An hallmark occurrence in NDs is the misfolding, aggregation and finally abnormal accumulation in the brain of proteins like α-synuclein (αS) or Tau [4,5]. Both αS and Tau are intrinsically disordered proteins, which may undergo different conformational transitions such as monomers, oligomers and finally fibrils, the latter are the most toxic species [6,7]. It is clear that, finding out a way to prevent these proteins from flowing into pathological forms, would be beneficial against NDs. In this scenario, nanoparticles offer an attractive perspective to interfere with the conversion of these protein into toxic species [8,9].
In this work, we synthetized ultrasmall Gold Nanoparticles (US-AuNP), capped with lipoic acid, and characterized them using biophysical techniques including NMR.
We performed titration experiments and acquired 2D ^{1}H-^{15}N HSQC spectra to gain insight into the adsorption mechanisms of αS or Tau to US-AuNPs, at single-residue resolution. The intensity variation peaks in the HSQC spectra of αS allow us to define a region of interest.

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