GIDRM
Gruppo Italiano Discussione Risonanze Magnetiche

UNIVERSITÀ DEGLI STUDI DELL'AQUILA

XLVIII National Congress on Magnetic Resonance

September 11-13, 2019
UNIVERSITY OF L'AQUILA

XLVIII National Congress on Magnetic Resonance

L'Aquila, 11-13 September 2019

University of L'Aquila

BOOK OF ABSTRACTS

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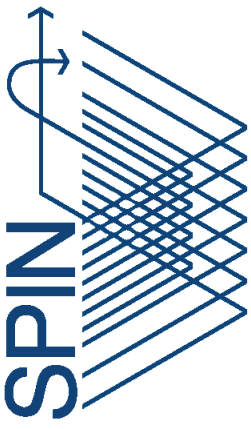

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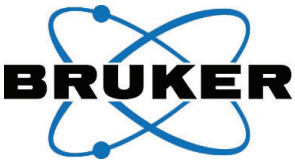
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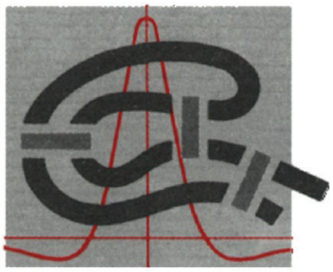
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GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XLVII National Congress on Magnetic Resonance

L'Aquila 11-13 September 2019

University of L'Aquila, Alan Turing Building

Scientific Program

Wednesday September 11th

10:00-13:00	**Registration**
10:30-12:30	**Bruker satellite meeting**
12:45-14:00	**Bruker Lunch**
14:00-14:30	**Opening**
	Plenary session **Chair: M. Geppi**
14:30-15:30	**GIDRM/GIRM gold medal award** **M. Piccioli -** Fast Nuclear Relaxation, Slow Protein Dynamics and Other Zen Time Scales
15:30-16:15	**Plenary Lecture 1** **H. Schwalbe –** New NMR Methods to Study RNA Structure and Dynamics
16:15-17:20	**Coffee break + Poster session 1**

	Parallel session A **Chair: M. Piccioli**	**Parallel session B** **Chair: L. Calucci**
17:20-17:50	**D. Cicero -** Plasma Protein Binding of Drug Candidates by NMR	
17:50-18:10	**V. Ghini -** NMR Approaches in Metabolomics: from Cells to Biofluids	**F. Gabriele –** ^{1}H-NMR-Relaxation as a Powerful Tool for Assessing Conservation Materials
18:10-18:30	**S. Todisco -** Importance of Sample Preparation in Non-Targeted NMR Analysis for the Traceability of Italian Tomatoes	**V. Di Tullio –** Single-Sided NMR to Study the Effects of Humidity and Water-Based Cleaning Systems in Oil Paintings

	Plenary session **Chair: M. Alecci**
18:30-19:00	**Stelar Lecture** **J. Ward-Williams –** Probing Interaction Strength and Liquid Dynamics in Nanoporous Oxides Using Fast Field Cycling NMR
19:00-19:45	**Plenary Lecture 2** **N. J. Shah –** Multimodal Simultaneous Imaging: Advances in MR-PET-EEG 3T, 7T and 9.4T in Humans

Thursday September 12th

	Plenary session **Chair: P. Turano**
8:45-9:30	**Plenary Lecture 3** **J. Titman –** Dynamic Nuclear Polarization Enhanced Solid-State NMR Studies of Catalysts and Catalyst Supports
9:30-10:00	**Jeol Lecture** **M. Malon -** Extracting Accurate Information from NMR Data
10:00-10:30	**Under 35 GIDRM award** **A. Conti –** Focused Ultrasound-Mediated Drug Delivery Through the Blood-Brain and Blood-Tumor Barrier
10:30-11:20	**Coffee break + Poster session 2**

	Parallel session A **Chair: P. Jezzard**	**Parallel session B** **Chair: M. Chierotti**
11:20-11:50	**R. Wise –** Quantitative Mapping of Human Brain Cerebrovascular and Metabolic Function	**G. Mollica -** New Sensitivity-Enhanced Solid-State NMR Approaches to Investigate Crystallization and Polymorphism
11:50-12:10	**Bracco lecture – A. Fringuello Mingo -** An improved blood pool MRI agent with dinuclear structure: *in vitro* and *in vivo* characterization and diagnostic capability in a model of rat cerebral ischemia.	**S. Borsacchi –** Phosphorene and Black Phosphorus: the ^{31}P NMR View
12:10-12:30	**M. Tripepi –** Paramagnetic Giant Liposomes as Highly Sensitive Versatile MRI Contrast Agents	**E. M. Vasini -** Technical Details of an FFC-NMR Study of Water-Cyclohexane-Lecithin Micelles Systems
12:30-13:50	**Lunch 1**	

	Plenary session **Chair: G. Pileio**
13:50-14:35	**Plenary Lecture 4** **P. Jezzard –** New Methods for the Study of Cerebrovascular Disease Using Magnetic Resonance Imaging
14:35-14:55	**Segre Fellowships 2018** **E. Amadio –** Applications of 1D and 2D Solid-State NMR Experiments for Studying Desmotropes of Active Pharmaceutical Ingredients and Quantifying Them
14:55-15:25	**Bruker Lecture** **F. Benevelli –** NMR Applied to the Analysis of Biologics
15:25-16:40	**Coffee break + Poster session 3**
16:10-16.40	**GIRM assembly**
16:40-19.30	**GIDRM assembly + announcement of poster competition winners**
19:30	**Departure for the social dinner**

Friday September 13th

	Parallel session A Chair: A. Galante	Parallel session B Chair: I. Kuprov
8:45-9:15	**F. Castiglione** – Diffusion NMR in Hydrogel Systems: Evidence of Lévy Flights Superdiffusive Motion	**D. Remondini** - Machine Learning Tools for Advanced Processing and Analysis of MR Data
9:15-9:35	**G. Profeta** - Water Dynamics and NMR Relaxation Properties of Nanoconfined Water	**C. Rizza** - Boosting Magnetic Resonance Imaging Signal-to-Noise Ratio Using Magnetic Surface Plasmons
9:35-9:55	**G. Melchiorre** - SAD TI: Singlet Assisted Diffusion Tensor Imaging for Porous Media Investigations	**R. Francischello** - Low Rank Approximation: a Flexible Framework for Noise Reduction in Multiple NMR Experiments
9:55-10:15	**M.E. Di Pietro** - NMR as a Tool to Probe Structure and Dynamics of Deep Eutectic Solvents and Their Mixtures	**A. Rotondo** - Mining ^{13}C-NMR Experiments Through the MARA-NMR Strategy and Other NMR Issues

	Plenary session Chair: J. Titman
10:15-10:45	**Poster competition winners lectures**

10:45-11:15	**Coffee break**

	Parallel session A Chair: M. D'Onofrio	Parallel session B Chair: N. J. Shah
11:15-11:45	**L. Ragona** - Molecular Mechanism of Antiangiogenic Agents Targeting Fibroblast Growth Factor-2/Tyrosine-Kinase Receptor Interactions	**N. Toschi** - Advanced Diffusion MRI for Whole Brain in Vivo Axonal Diameter Mapping in Multiple Sclerosis
11:45-12:05	**L. Russo** - Tuning of Micro-Millisecond Conformational Dynamics Controls the Formation of Prion Protein Intermediate States Involved in Amyloid Fibrils Assembly	**S. Della Penna** - Multivariate MEG Correlates of Bold Modulation Induced by Visuospatial Attention
12:05-12:25	**G. Parigi** - Local and Global Protein Dynamics by FFC Relaxometry	**E. Biondetti** - Assessing Volumetric Changes in the Substantia Nigra Pars Compacta (SNc) of Patients with R.E.M. Sleep Behaviour Disorder (RBD) and Parkinson's Disease (PD)
12:25-12:45	**M. Schiavina** - Taking Simultaneous Snapshots of Intrinsically Disordered Proteins in Action Exploiting Multiple Receivers and Heteronuclear Direct Detection	**M. Marrale** - Thalamic Parcellation for Target Identification in Trans-Cranial MR-Guided Focused Ultrasound (TCMRGFUS) Thalamotomies: a Preliminary Probabilistic Tractography Study

	Plenary session Chair: M. Geppi
12:45-13:30	**Plenary Lecture 5** **I. Kuprov** - Quantum Mechanical MRI Simulations: Solving the Matrix Dimension Problem

13:30-13:40	**Closing**

13:40-15:00	**Lunch**

Contents

GIDRM/GIRM GOLD MEDAL AWARD

GMA

FAST NUCLEAR RELAXATION, SLOW PROTEIN DYNAMICS AND OTHER ZEN TIME SCALES

M. Piccioli

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The effects of the hyperfine interaction between electron and nuclear spins on metalloproteins have been the path that, since late 80'es, has allowed the slow (reversible?) transition of a number of scientists from Inorganic Chemistry to Structural Biology. During this *parkour*, the first high field NMR spectrometer was installed in Florence, this lead us to solve, 25 years ago, the first-ever NMR solution structure of a paramagnetic protein. Since then (but also before, using low field spectrometers and 1D NOEs), I spent my time by exploring many roots to "turn limitations into advantages", *i.e.* to design experiments, develop approaches, explore phenomena that could contribute to exploit paramagnetism, obtain more structural information, understand the reactivity of metal centers in proteins. When signals escaped detection, seeking for them (and for their relaxation, cross correlation, dipolar and scalar couplings, ...) has been amazing and, most of all, extremely useful, once we realized that metalloproteins are about 30% of the entire proteome. The Magnetic Resonance Center of the University of Florence flourished as an exciting environment where PhD students, post docs and distinguished scientists joined, essentially from everywhere, for their research and training activities.
The golden age of NMR solution structures of biomolecules has left a legacy of established sets of experiments and automated and semi-automated approaches for resonance assignments and structure calculation. However, while "new" methodologies become rapidly old, it may happen that "old" problems re-appear and the challenge repeat itself. Like the ouroboros symbol, iron sulfur clusters extensively studied within small, stable and soluble electron transfer proteins, re-appear to take part in transient interactions involving large and unstable proteins belonging to the Iron Sulfur Cluster Assembly machinery. Manual assignments and relaxation based restraints replace, once again, automated assignments and crowded NOEs spectra. This is a simple story of passion, encounters, enjoyment, little fights and, most of all, fun. All these ingredients, I am sure, make also the personal history of You, distinguished Reader. It is the reason why we, the scientists, should consider ourselves as lucky persons; Let's take some time to engage others into this and to educate them to the scientific culture.

UNDER 35 GIDRM AWARD 2019

U35GP

FOCUSED ULTRASOUND-MEDIATED DRUG DELIVERY THROUGH THE BLOOD-BRAIN AND BLOOD-TUMOR BARRIER

A. Conti[1,2]

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Drug delivery in both healthy and pathological brain tissues is limited by the presence of the blood-brain barrier (BBB). Indeed, the BBB not only prevents neurotoxic substances from entering the brain, but also limits the passage of therapeutic products to the brain. Many studies have demonstrated that pulsed focused ultrasound (FUS) combined with circulating microbubbles can permeate the BBB in a reversible manner. Here we present a theoretical and empirical approach able to characterize MRI Contrast Agents (CA) distribution within the brain, after FUS-induced BBB permeabilization. T_1-CAs of different sizes have been delivered into rat brains thanks to a motorized FUS system installed in a 7T MRI scanner (Bruker). Here we present a model depicting both the diffusion and the amount of particles delivered into healthy brain after FUS-induced BBB permeabilization [2] (Fig(a)). In order to augment drug concentration within a brain tumor, FUS may be used to permeabilize the BBB still intact in the infiltrative areas (Fig(b)). Here, we compare different acoustic strategies to enhance molecular concentrations within Glioblastoma tumors. The efficacy of the sonication methods is evaluated on the basis of absolute concentrations of delivered MR- CA and on their rates of uptake/clearance by the tumors.

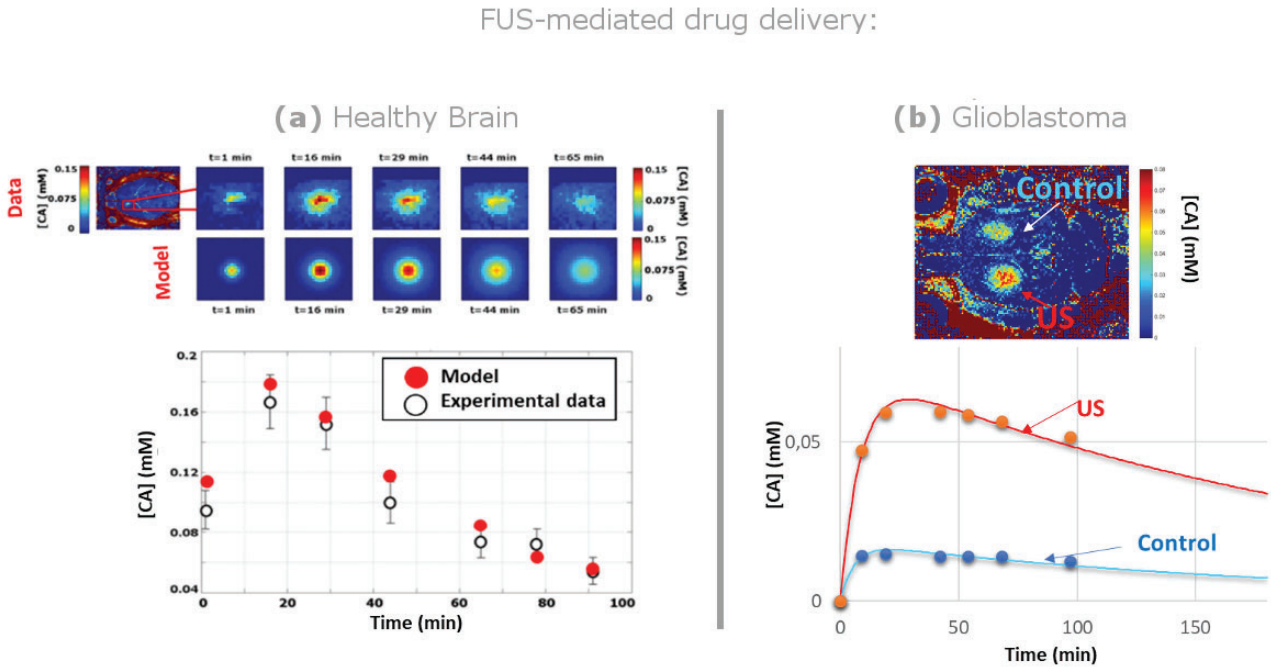

Figure 1: (a): Experimental and Simulating data of MR-Contrast Agents delivered within the healthy brain tissue. (b): Examples of higher Particles concentrations delivered in the sonicated tumor (US) respect to a control tumor.

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[1] A. Conti et al, *Physics in Medicine and Biology*,doi: 10.1088/1361-6560/ab259d, (2019).
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ANNALAURA SEGRE FELLOWSHIP 2018

ASF1

APPLICATIONS OF 1D AND 2D SOLID-STATE NMR EXPERIMENTS FOR STUDYING DESMOTROPES OF ACTIVE PHARMACEUTICAL INGREDIENTS AND QUANTIFYING THEM

E. Amadio, G. Castaldo, M. R. Chierotti, S. Bordignon, F. Rossi, R. Gobetto

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Mebendazole (MBZ) is a frequently used antihelmintic active pharmaceutical ingredient (API), present in the marketed drug VERMOX®. MBZ exists in 3 polymorphic forms: A, B and C, which correspond to 3 different tautomers of the molecule, i.e. it displays a desmotropic behavior.[1] As for many APIs, distinct MBZ polymorphs show different pharmaceutical activities.[2] Thus, for pharmaceutical companies it is extremely important both to identify the various crystal forms and to quantify them in heterogeneous mixtures. In this work, we explored the potentiality of solid-state NMR (SSNMR) in solving these two issues. By means of 2D SSNMR (^{13}C-^{1}H HETCOR and ^{1}H DQ MAS) we exploited characteristic spatial proximities in the crystal structures of MBZ A and C to unambiguously identify the tautomeric form (Fig. 1) which by X-ray diffraction resulted unclear. Moreover, we developed a protocol to quantify crystalline form C in crystalline form A and amorphous MBZ in crystalline form C. For both protocols, we established the LOD and LOQ values of our approach.

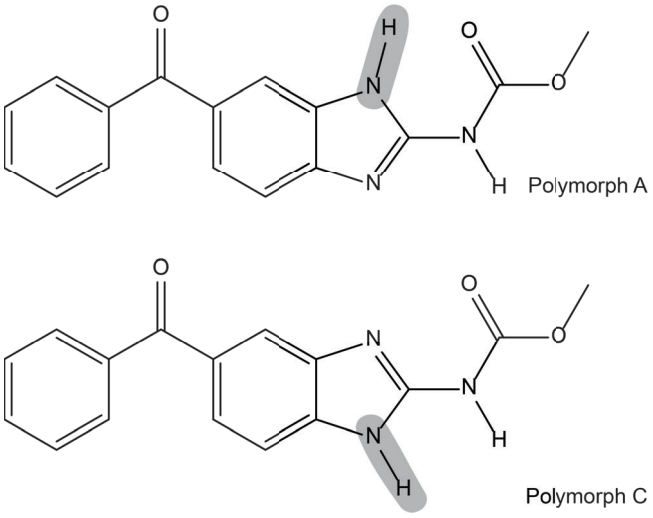

Figure 1: Molecular structures of tautomer 1 (polymorph A) and tautomer 2 (polymorph C).

References

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[2] R. Censi, P. Di Martino *Molecules* **20**, 18759-18776, (2015).

BRUKER LECTURE

SP1

NMR APPLIED TO THE ANALYSIS OF BIOLOGICS

F. Benevelli[1], C. Fischer[2], D. Baldisseri[3], D. Mathieu[2]

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Biologics and Biosimilars are therapeutic agents of natural origin. They include monoclonal antibodies, vaccines, hormones, viral agents, etc. They are characterized by a typically large size and heterogeneity in the composition, as well as by the complexity of their biosynthesis, isolation and purification. Therefore, Biologics and Biosimilars require techniques very sensitive to the structure at an atomic level. High-resolution nuclear magnetic resonance (NMR) is a key technology that provides critical information on protein conformation, aggregation, stability, and modifications such as glycosylation. Resolution and ease of use in the study of the higher order structure of proteins make this technique a uniquely valuable tool. Recently, new developments in acquisition and data analysis have emerged as a powerful tool for HOS characterization of the intact molecule utilizing both 1D and 2D NMR methods. Fast 1D methods, based on Profile1D developed by AMGEN, will give a quick answer if the biologic is similar to the reference material. To identify changes at amino acid level 2D NMR methods are required. We show recently developed and optimized acquisition techniques including excipient removal. An interlaboratory comparison coordinated by NIST (24 labs involved, different magnetic fields, worldwide) demonstrated both high precision and high reproducibility of the NMR technique. For an optimal workflow for batch to batch analysis we will introduce a new developed software at this conference. It provides established and recently developed data evaluation methods, namely: CCSD (Combined Chemical Shift Deviation), ECHOS (Easy Comparability of Higher Order Structure), Profile1D (developed by AMGEN) and PCA (Principal Component Analysis) using either the entire spectrum or peaks. We will demonstrate the workflow, show pros and cons of the different methods.

BRACCO LECTURE

SP2

AN IMPROVED BLOOD POOL MRI AGENT WITH DINUCLEAR STRUCTURE: *IN VITRO* AND *IN VIVO* CHARACTERIZATION AND DIAGNOSTIC CAPABILITY IN A MODEL OF RAT CEREBRAL ISCHEMIA

F. La Cava[1], A. Fringuello Mingo[2], S. Colombo Serra[2], I. Pietro[1], L. Miragoli[2], E. Terreno[1], L. Lattuada[2], L. Poggi[2], C. Cabella[2], F. Tedoldi[2]

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Gadolinium based contrast agents (GBCAs) have been widely used in clinic to boost the visibility of pathology and delineation of lesions in Magnetic Resonance Imaging (MRI) acquisitions. Approximately, 40 million administrations are made worldwide every year and search for new candidates is still active[1,2,3]. $(GdDTPA)_2$-Chol, a dinuclear gadolinium chelate containing two moieties of diethylenetriaminepentaacetic acid, covalently conjugated to an analogue of deoxycholic acid is proposed. The increment of molecular size assigns to the molecule useful pharmacokinetic properties, lengthening elimination half time and promoting a macromolecular behavior. In this work, $(GdDTPA)_2$-Chol was tested in vitro for its relaxometric properties [4] and then *in vivo* on healthy rats [4] and on a pathological model, in order to evaluate its efficacy, pharmacokinetic and diagnostic capability. The full *in vitro* relaxometric characterization consisted in: (1) acquisition of nuclear magnetic resonance dispersion (NMRD) in different media; (2) binding affinity to human serum albumin (HSA); (3) transmetallation assay. On the *in vivo* side, a MRI bio-distribution (carried out at 1 T) and blood pharmacokinetic were evaluated on healthy rats. Moreover, the diagnostic capability of (GdDTPA)2-Chol was tested in a pathological model of permanent cerebral ischemia in rats with a 3 T scanner. (GdDTPA)2-Chol relaxivity at 20 MHz, 37 °C in saline is 7.7 $mM^{-1}s^{-1}$; the presence of physiological ions or medium viscosity did not affect significantly r1. Conversely, the addition of HSA to saline or the use of plasma medium increased r1 up to approx. 20 $mM^{-1}s^{-1}$ [4]. NMRD profiles showed the typical peak observed in presence of binding; dedicated titration experiments allowed to estimate the affinity constant and the number of binding sites [4]. $(GdDTPA)_2$-Chol demonstrated a really slow blood kinetic in vivo, also confirmed by the pharmacokinetic study [4]. Even if the molecular rotational motion contribution to r1 is valued at low-to-intermediate magnetic field strength (0.5-1.5 T), $(GdDTPA)_2$-Chol enhancement at 3 T was comparable to a reference commercial macrocyclic agent injected at double the dose (0.1 mmol Gd/kg). Moreover, a difference in distribution and accumulation of the compound into the ischemic lesion can be observed: $(GdDTPA)_2$-Chol accumulated slowly into the lesion as a result of the increased molecular size after binding with serum albumin, and reached a plateau after about 20 min, according to the durable availability of the compound, characterized by a long elimination rate. In conclusion, the good binding affinity with HSA, the relatively high number of binding sites, the dinuclear structure together with a reduced hepatic elimination translates in an extremely long elimination half time. The observed in vivo behavior is very similar to a macromolecule, opening the field of application of the complex beyond angiography, since macromolecular CAs are known to preferentially accumulate in tumor and pathological tissues.

References

[1] V. M. Runge et al. *Invest. Radiol.* **52**, 317-323, (2017).
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[4] F. La Cava et al. *Chem. Med. Chem.* **13**, 824-834, (2018).

JEOL LECTURE

SP3

EXTRACTING ACCURATE INFORMATION FROM NMR DATA

M. Malon[1], T. Komatsu[2], R. Crouch[3]

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CRAFT (Complete Reduction to Amplitude Frequency Table) is a relatively new concept of high-resolution NMR data processing based on a Bayesian statistical approach [1]. The pioneering work for direct time-domain analysis of 1D NMR data was extended to 2D NMR in 2016 [2]. CRAFT provides a direct spectrum to spreadsheet conversion by utilizing the Bayesian analysis of the time-domain data thereby bypassing many issues brought about by the Fourier transform to a visual frequency dimension. The direct analysis of time domain completely bypasses the issues of baseline and phase correction. In addition, the concept of spectral overlap is redefined. Two peaks in the frequency domain which are too close to effectively quantify by traditional integration are completely resolved sinusoids in the time domain with separate information of frequency, amplitude, phase, and decay rate available as numbers in a table. For the purpose of comfort the resulting table is used to simulate a complete spectrum and the simulated FID (Free Induction Decay) and actual experimental FID are subtracted to provide a quick visual means to evaluate the quality of the analysis. In other words, CRAFT uses the visual frequency domain simply as guidance to make easy for a human to choose what they wish to analyze. It was shown that CRAFT can be used to quantitatively analyze components in complex mixtures [3]. Some examples of targeted and non-targeted analysis will be demonstrated in this contribution. CRAFT is robust, accurate and suitable for high-throughput enviroments.

References

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STELAR LECTURE

PROBING INTERACTION STRENGTH AND LIQUID DYNAMICS IN NANOPOROUS OXIDES USING FAST FIELD CYCLING NMR

J. Ward-Williams[1], J-P. Korb[2], L. F. Gladden[1]

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Fast field cycling relaxometry has been shown to be a powerful tool for the analysis of adsorbate dynamics and liquid-surface interactions for a wide range of porous media [1-3]. In this study the behaviour of a simple, yet catalytically important, γ-alumina surface was studied. Significantly different nuclear magnetic relaxation dispersion (NMRD) profiles were obtained for six liquids, which represented many of the key chemical functionalities used in catalysis, imbibed within the alumina (Fig 1a). The NMRD profiles could be rationalized in terms of the type and strength of surface bonding, allowing a ranking of the adsorbate-surface interaction strengths. Further insights into the molecular dynamics were obtained by transforming the data into the T_1 domain. This transformation revealed minor components in the T_1 distributions of methanol and acetone (Fig 1b), the physical origins of which were shown to be functionality-specific relaxation and competitive adsorption due to stable reaction intermediates [4]. This work has led to an improved understanding of the physiochemical processes occuring within the pore space and a more robust characterisation of interaction strength. The approaches used for single component systems were applied to binary liquid mixtues imbibed within α-alumina in order to explore the competitive adsorption process and the liquid structuring within the pore space. For both non-polar:polar and polar:polar mixtures an extreme microphase separation was observed [5].

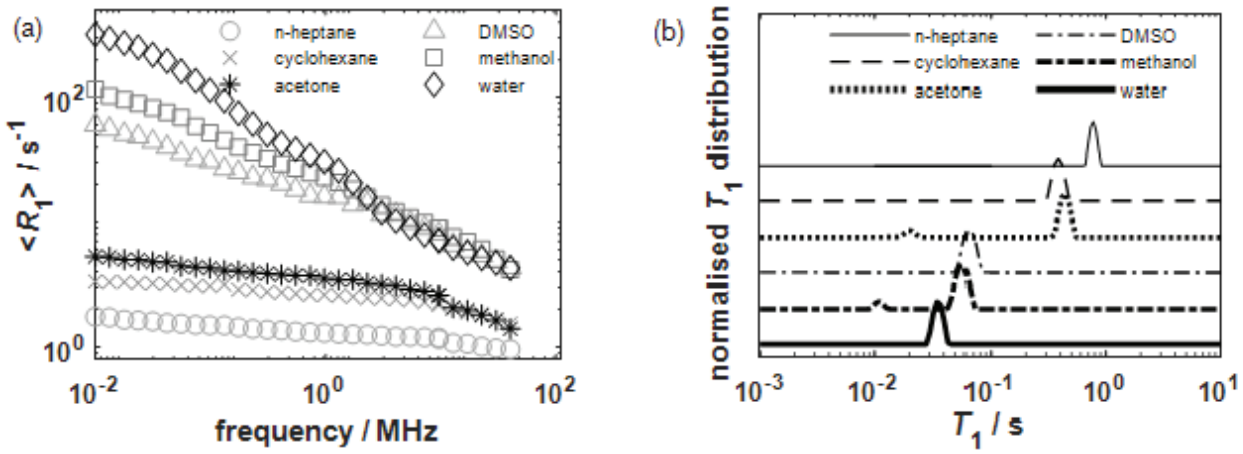

Figure 1: The (a) NMRD profiles of six different liquids imbibed within γ-alumina and (b) the corresponding T_1 distributions obtained at 1 MHz for each liquid.

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PLENARY LECTURES

PL1

NEW METHODS FOR THE STUDY OF CEREBROVASCULAR DISEASE USING MAGNETIC RESONANCE IMAGING

P. Jezzard

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Cerebrovascular disease is a major area of healthcare burden and active research, spanning diseases such as stroke, transient ischaemic attack, vascular atherosclerosis and vascular dementia. Magnetic resonance imaging offers a unique tool to assess a number of manifestations of cerebrovascular disease, including vessel wall pathology, lumen status, tissue perfusion, collateral flow status and tissue metabolism. The talk will introduce new areas of active methodological research. These include developments in arterial spin labelling that provide information on lumen flow and collateral flow, as well as quantitative tissue perfusion; techniques to assess the stability of atherosclerotic plaque; and methods to assess tissue metabolism, including insights into oxygen metabolism via venous blood oxygenation. By use of pseudo-continuous arterial spin labelling (ASL) it is possible to gain valuable information about collateral flow by encoding signal from specific feeding arteries and then tracking the destination of signal as it moves from the large feeding arteries towards the tissue bed [1]. Hybrid sequences can obtain information in both these phases via tailored post-hoc image reconstruction. Similar pulse sequence preparation modules, but designed instead to crush signal from lumen spins, are able to yield black-blood images that allow the vessel wall to be imaged [2]. When combined with a quantitative T_2 readout the image data is able to map unstable lipid-rich plaques that are at risk of rupture.
MRI measures of metabolic stress may also be useful, including measures oxygen extraction fraction via regional measurement of venous blood T_2 [3].

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PL2

QUANTUM MECHANICAL MRI SIMULATIONS: SOLVING THE MATRIX DIMENSION PROBLEM

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We propose a solution to the matrix dimension problem in quantum mechanical simulations of MRI (magnetic resonance imaging) experiments on complex molecules. This problem is very old; it arises when Kronecker products of spin operators and spatial dynamics generators are taken - the resulting matrices are far too large for any current or future computer. However, spin and spatial operators individually have manageable dimensions, and we note here that the action by their Kronecker products on any vector may be computed without opening those products. This eliminates large matrices from the simulation process.
MRI simulations for coupled spin systems of complex metabolites in three dimensions with diffusion, flow, chemical kinetics, and quantum mechanical treatment of spin relaxation, are now possible, as well as simulations of spatially distributed ultrafast, pure shift, diffusion, and flow driven NMR experiments, including optimal control and advanced relaxation theories. This level of generality hinges on:

1. The ability to treat classical degrees of freedom (diffusion, hydrodynamics, radiofrequency and microwave phases, stochastic tumbling, etc.) at the same conceptual level as spin degrees of freedom [1].

2. The ability to survive enormous Kronecker products. In realistic systems (ten spins in three spatial dimensions), the direct products of spin and spatial dynamics generators would then have the dimension in excess of 1012 even before chemical kinetics is considered [2].

3. Code parallelisation over cluster architectures, including the possibility of using a GPU on each node of the cluster.

This report is about solving all of this, and on where the dark art of simulating a time-domain magnetic resonance experiment stands at the moment. Two recent innovations are the abandonment of Liouville equation in favour of Fokker-Planck equation [1] as the core formalism, and the use of tensor structured objects that never open Kronecker products [2].

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PL3

NEW NMR METHODS TO STUDY RNA STRUCTURE AND DYNAMICS

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The functional importance of RNAs in cells is increasingly recognized. The rapidly evolving field of RNA biology calls for NMR-structural studies. However, compared to proteins, the NMR spectroscopy of RNAs is considerably more challenging: the chemical shift dispersion is limited, the exchange of labile protons is ubiquitous for all nucleotides, and particularly strong for nucleotides not involved in base pairing, and dynamics is more pronounced.
In this contribution, we will present new NMR methods to study RNA base pairing, structure and folding. Methods include new NMR pulse sequences with detection of low-gamma nuclei, double-quantum detection of signals broadened beyond detectability and high field NMR. Further methods for advanced sample preparation will be presented.

PL4

MULTIMODAL SIMULTANEOUS IMAGING: ADVANCES IN MR-PET-EEG 3T, 7T AND 9.4T IN HUMANS

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The aim of my presentation is twofold: Firstly, to explore the potential of simultaneously acquiring multimodal MR-PET-EEG data in a human 9.4T scanner to provide a platform for metabolic brain imaging; here, data from a 3T MR-PET as well as the 9.4T will be presented. Secondly, to demonstrate that the three modalities are complementary, with MRI having the potential to provide excellent structural and functional imaging, PET providing quantitative molecular imaging, and EEG providing superior temporal resolution.
A 9.4T MRI scanner equipped with a PET insert and a commercially available EEG device were used to acquire *in vivo* proton-based images, spectra, and sodium- and oxygen-based images with MRI; EEG signals from a human subject in a static 9.4T magnetic field; and demonstrate hybrid MR-PET capability in a rat model. Furthermore, the latest results from a state-of-the-art 7T scanner will be presented and a path toward hybrid MR-PET at 7T will be outlined.

PL5

DYNAMIC NUCLEAR POLARIZATION ENHANCED SOLID-STATE NMR STUDIES OF CATALYSTS AND CATALYST SUPPORTS

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Solid-state nuclear magnetic resonance (NMR) is a powerful method for studying the molecular structure and dynamics of a broad range of systems from heterogeneous materials to biological molecules. In some situations, solid-state NMR can suffer from low sensitivity, because of the small nuclear spin polarizations involved, so that long acquisition times or large sample volumes are required. However, weak NMR signals can be dramatically enhanced by dynamic nuclear polarization (DNP), which involves transfer of electron spin polarization from radicals implanted in the sample to nearby nuclei. The substantial enhancements (up to 300-fold) obtained with DNP make NMR studies of dilute species feasible for the first time and have already prompted exciting new NMR applications to surfaces and to materials which are porous or particulate on the micro- to nanoscale.
We are currently applying DNP-enhanced solid-state NMR to structural studies of catalysts supported on alumina and silica surfaces. Catalysts are usually challenging systems to study by solid-state NMR because of the low concentration of active sites and adsorbate molecules on the support surface. However, the substantial signal enhancements obtained with DNP have allowed investigations of surface sites on catalyst supports and organic molecules supported on catalytic surfaces. In this talk I will describe our recent research on surface modified alumina and silica supported ionic liquids using DNP-enhanced solid-state NMR.

ORAL COMMUNICATIONS

ASSESSING VOLUMETRIC CHANGES IN THE SUBSTANTIA NIGRA PARS COMPACTA (SNc) OF PATIENTS WITH R.E.M. SLEEP BEHAVIOUR DISORDER (RBD) AND PARKINSON'S DISEASE (PD)

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PD is often characterised by early disease-associated features, e.g. RBD, but only diagnosed after ∼30% of dopaminergic neurons in the SNc have died [1]. Such a loss is a nonuniform spatiotemporal process predominant in the posterior and lateral SNc and poorly characterised. Here, by exploiting the bright appearance on T1-weighted (T_1-w) turbo spin echo (TSE) MRI of the neuromelanin-iron complex in the SNc [1], we studied the spatiotemporal changes in SNc neuromelanin in RBD and PD patients. Longitudinal (2 visits) T_1-w MPRAGE [2] (TR/ TE/ TI/ FA/ resolution: 5, 2.3 s/ 3, 4.2 ms/ 2.5, 0.9 s/ 5°, 9°/ 1x1x1 mm^3) and T_1-w TSE MRI (TR/ TE/ FA/ resolution: 900 ms/13, 15 ms/ 180°/ 0.4x0.4x3 mm^3) were performed in 36 subjects with RBD, 104/30 with early/advanced PD and 70 healthy volunteers (HVs) on Siemens 3T systems (Trio/Prisma, 12/32-channel head coils). A template of the average brain was calculated [3, 4] using the MPRAGE images of 36 HVs, 36 RBDs and 36 early PDs at baseline. For all subjects, the SNc was manually segmented on the TSE image, affinely aligned to the MPRAGE image and then to the template [3, 4]. For each group and visit, the average SNc segmentation (*i.e.* the probability of each voxel belonging to the SNc) was calculated, thresholded at ⩾0.3 and binarised to enable calculating SNc volumes. In patients, the probability

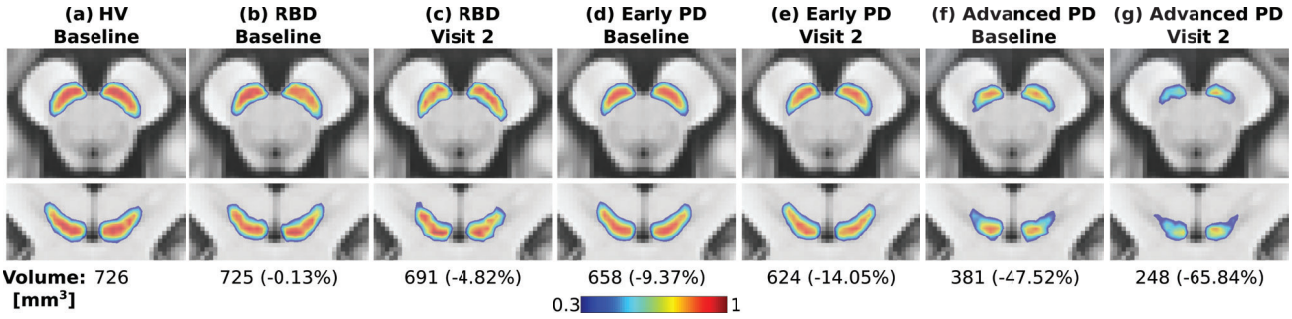

Figure 1: SNc probability maps, total volumes and differences in volume relative to the HVs at baseline.

range of a voxel belonging to the SNc was smaller than in HVs; clusters of low probability were observed in the dorsolateral SNc (Fig. 1). In patients, the SNc always had a smaller volume than in the HVs (Fig. 1). This shows quantitatively the spatial pattern of neuron loss, which has been qualitatively observed to progress from the dorsolateral to the ventral and medial SNc with PD progression [1]. The spatiotemporal pattern of SNc neuron loss in RBD and PD patients vs. HVs shown here could help develop biomarkers of disease progression/modification.

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OC2

PHOSPHORENE AND BLACK PHOSPHORUS: THE ^{31}P NMR VIEW

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The isolation of graphene in 2004 opened the way to the new fascinating world of 2D materials: from bi-dimensionality peculiar physical and chemical properties can arise, which make 2D materials extremely attractive for many different application fields, including electronics, photonics, spintronics, mechanics and medicine. In this scenario black phosphorus (bP), the most stable allotrope of elemental phosphorus, attracted in the last few years a great interest, since it is characterized by a layered crystal structure that can be exfoliated (Fig.1). Due to their promising properties for different applications, the few-layer and the single layer (phosphorene) forms of bP are at present the object of many studies [1-3]. In spite of this, so far the use of NMR for investigating bP and its exfoliated forms has been very limited, while it can add a great value to the structural knowledge and control of these materials. In this work we present the first characterization of the ^{31}P spin interactions responsible for the NMR properties of solid bP and of its exfoliated forms. In particular, by combining Solid State NMR spectroscopy and DFT calculations it has been possible to characterize in detail the ^{31}P homonuclear dipolar and chemical shift interactions. Highlights from ^{31}P NMR applications to innovative materials based on exfoliated bP will also be presented [4-6].

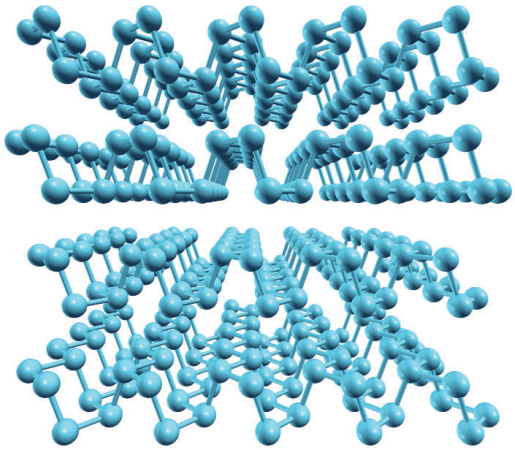

Figure 1: Crystal structure of bP.

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OC3

DIFFUSION NMR IN HYDROGEL SYSTEMS: EVIDENCE OF LÈVY FLIGHTS SUPERDIFFUSIVE MOTION

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In the last few decades, NMR diffusion measurements of small molecules in liquid phase and/or confined systems have been a topic of extensive research, due to their broad applications ranging from material science to biophysics and medicine. Translational diffusion in liquid systems and soft matter can be measured using 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]. On the other hand, in the field of drug delivery a deep understanding of the diffusive mechanisms governing solute transport at the nanoscale, and its impact on release kinetics is important as it helps to exploit the potentiality of the delivery systems. To this end, we investigated the translational diffusion of ibuprofen (as a model drug) loaded in anionic agarose-carbomer (AC) hydrogels by 1H high resolution magic angle spinning (HR-MAS) NMR spectroscopy, and compared it to its macroscopic release kinetics. In particular, we consider the effect of drug concentration and hydrogel nanoscopic mesh size on ibuprofen motion. The analysis of the experimental NMR data, performed using a specially designed procedure [2], provides the first evidence of superdiffusion motion for the majority of the samples. Indeed, superdiffusive transport is observed for samples with the smallest mesh size (7 nm) and highest ibuprofen concentrations (90-120 mg/mL). This outcome is rationalized in terms of heavy-tailed distributions of spatial displacements (Lèvy flights) and of waiting times, which depend on the nanoscopic structural heterogeneity of the gels and the strong but reversible association between ibuprofen and the agarose matrix (see Fig.1).

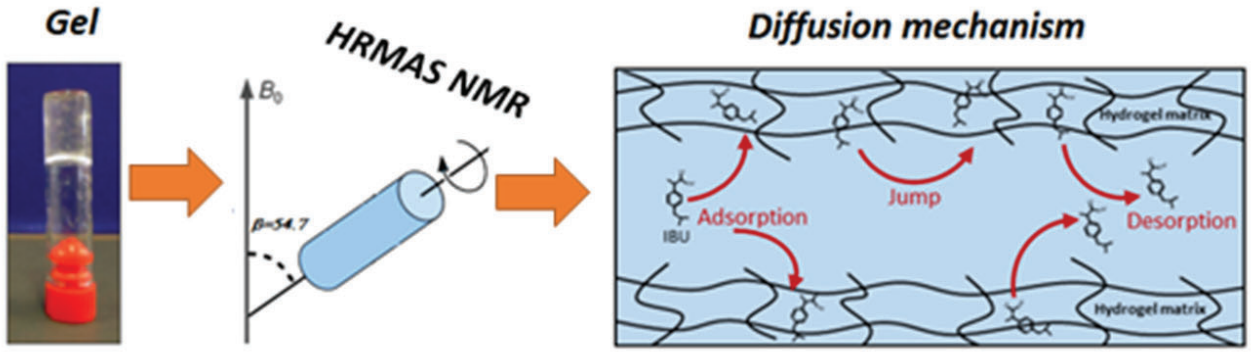

Figure 1: Diffusion mechanism of ibuprofen in hydrogel matrix.

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OC4

PLASMA PROTEIN BINDING OF DRUG CANDIDATES BY NMR

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Plasma protein binding (PPB) role in modulating the effective drug concentration at the pharmacological target has been extensively discussed in the literature [1-3]. Knowledge of the unbound drug concentration across species (preclinical and human) in a drug discovery program supports interpretation of PK/PD relationships and extrapolation of efficacy and safety margins from preclinical species to the clinical setting. Recognizing the utility of NMR as a very sensitive method for detecting binding, we have focused on developing an approach particularly fast and low cost, alternative to those currently used for determining PPB like ultrafiltration, ultracentrifugation and rapid equilibrium dialysis (RED). Signals of small molecules (MW $<$ 500 Da), as normally potential drug candidates are, in 1D ^{1}H NMR experiments are usually very sharp due to fast tumbling in solution and a consequent slow T_2 relaxation. Binding to high MW molecules, such as plasma proteins, induces peak broadening and the corresponding decrease of the ligand's NMR signal intensity because the bound ligand experiences the shorter relaxation time of the protein. This happens if the exchange regime is fast enough in NMR time scale. The observed increase in ligand linewidth in such an experiment will depend on several factors that include the dissociation equilibrium constant for the protein-ligand interaction, the fraction of bound ligand, the free ligand NMR line width, and the linewidth for the bound state of the ligand [4]. Taking into account that plasma is a complex matrix system, we decided for an empirical approach. For twenty-five drugs we observed a strong correlation between the attenuation of NMR signals in the presence of different plasma dilutions and their f_b reported in the literature. Based on these results, a protocol for a rapid and low cost calculation of f_b of small molecules was established. In this presentation, we describe this novel validated method to determine plasma protein binding (PPB) of drug candidates in drug discovery programs. Furthermore, the advantage of using plasma instead of purified recombinant proteins, as some other methods do, and the possibility of incorporating pool analysis to increase throughput are discussed. As a plus, this method contemporarily provides a quality check and solubility of the compound.

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OC5

MULTIVARIATE MEG CORRELATES OF BOLD MODULATIONS INDUCED BY VISUOSPATIAL ATTENTION

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The integration between fMRI and MEG connectivity patterns is still debated, due to the different spatial and temporal resolutions. To address this question, we compared modulations of fMRI connectivity induced by a controlled visuospatial attention task, as compared to rest [1], with modulations of MEG connectivity in the same group of subjects. At rest, the best electrophysiological correlate of BOLD-FC is the temporal correlation of band limited power (BLP) signals in alpha and beta frequencies (BLP-FC); hence, we hypothesized that task-related BOLF-FC modulations would correspond to s specific BLP frequencies. In contrast to our hypothesis, we did not find a clear relationship between

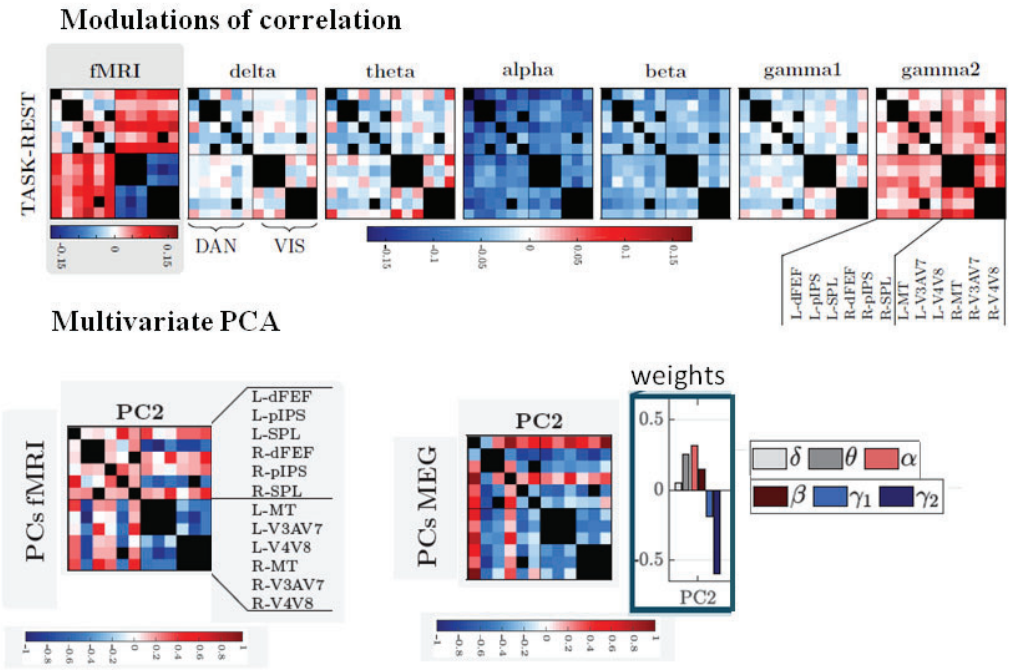

Figure 1: Upper: Task vs rest correlation matrices obtained from BOLD signal (left) and MEG Band Limited Power. It is hard to associate any single BLP correlation matrix to the BOLD one. Lower: Example of 2 matching PCs obtained from PCA over BOLD and BLP modulation matrices.

BOLD-FC changes and frequency specific BLP-FC changes. Rather, BOLD-FC changes in component space matched multi-frequency BLP-FC components involving theta, alpha and gamma bands. Hence the neurophysiological correlates of task-evoked BOLD connectivity modulations must be searched in multivariate BLP correlation patterns.

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OC6

NMR AS A TOOL TO PROBE STRUCTURE AND DYNAMICS OF DEEP EUTECTIC SOLVENTS AND THEIR MIXTURES

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Deep Eutectic Solvents (DESs) are economically and environmentally sustainable materials of great interest in green chemistry and with enormous potential in several technological and industrial applications [1]. These low transition temperature mixtures consist of a hydrogen bond donor salt and a hydrogen bond acceptor, which self-associate to form a eutectic phase characterized by an unusual low melting point. The properties of DESs may be enhanced by adding a suitable third component. Water deserves particular attention, since most DES are highly hygroscopic and it can be added to modify DES's properties such as polarity and viscosity [2]. Also cyclodextrins (CDs) are likewise of interest, in order to get new hybrid materials with improved extracting and/or sequestrating properties [3]. Both these additives act as competitors for hydrogen bond within the DES network, thus modulating the physico-chemical properties of the system. It is then imperative to investigate structural and dynamic properties of DES, and how they change in complex mixtures. Here we use ^{1}H and ^{13}C NMR relaxation and diffusion measurements to study choline chloride-based DESs under progressive addition of water, and in the presence or absence of βCD. Looking at potential applications, we demonstrate that βCD retains its encapsulation capacity within the DES/βCD/H_2O. Structural and dynamic information on the inclusion complexes with different guest molecules are collected through intermolecular host-guest NOEs measurements in the rotating frame (ROESY), diffusion ordered spectroscopy (DOSY) and nonselective and selective spin-lattice relaxation experiments (see Fig. 1).

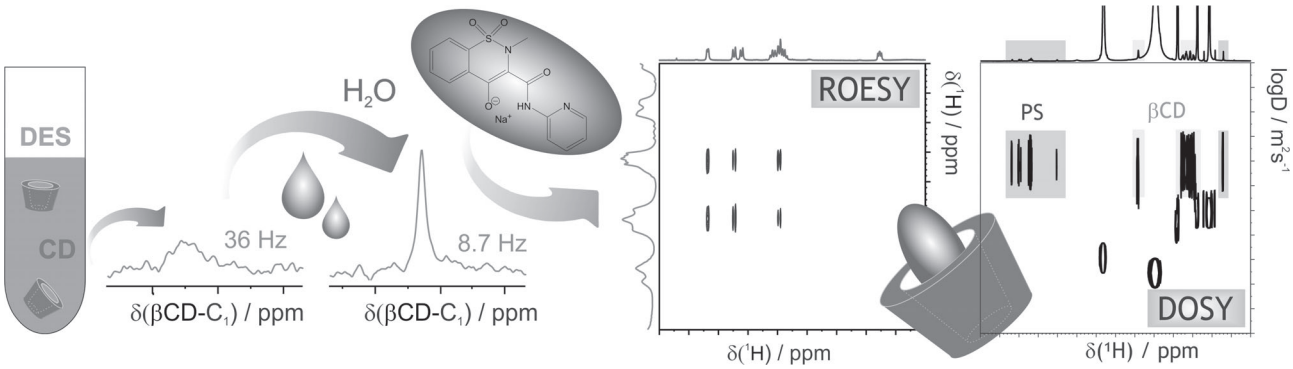

Figure 1: Sketched steps of the NMR study carried out on mixtures of DES and βCD upon dilution and addition of piroxicam sodium as guest molecule.

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OC7

SINGLE-SIDED NMR TO STUDY THE EFFECTS OF HUMIDITY AND WATER-BASED CLEANING SYSTEMS IN OIL PAINTINGS

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Water and humidity are important factors affecting physical and chemical changes in artwork. Their action has been studied for years to figure out how preserve paper, wood, and lapideous materials. To preserve oil paintings, extensive work has been conducted on studying the deterioration mechanisms (e.g. saponification of saturated fatty acids [1-3], hydrolysis of triglycerides [4], consumption of pigments [5], etc.), but there has been fewer reports of the effects of water and humidity on deterioration of oil paintings. NMR spectroscopy is a powerful tool to study transport phenomena and dynamic of water molecules in polymers and porous materials. This talk explores two recent studies performed by single-sided NMR [6-7] to evaluate the effect of humidity and water-based cleaning systems on an oil paint layer.

The first study was performed on model paint samples made of basic lead carbonate mixed with linseed oil. To study the molecular dynamic of water and oil, we measured transverse relaxation times T_{2eff} of hydrogen nuclei by portable NMR, as a function of humidity exposure. The results showed that the mobility of hydrogen domains changes as the water uptake increases. This study is part of a wider project carried out by the Metropolitan Museum of Art in collaboration with the University of Delaware funded by the US National Science Foundation (DMR-1608366 and DMR 1608594).

The second study was carried out to investigate some modern oil paintings by Capogrossi, exhibited at the National Gallery of Modern Art in Rome. The stratigraphy of the paintings, made of carbon black, zinc white and linseed oil, was analyzed by single-sided NMR through collection of ^{1}H NMR profiles from different areas of the painting. After the application of a cleaning treatment, ^{1}H NMR profiles and transverse relaxation times were collected to follow the changes in mobility of hydrogen domains in the pictorial layer and to evaluate the effectiveness of cleaning treatments based on use of various hydrogels (IPERION_CH, E-RIHS European Research Infrastructure for Heritage Science).

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OC8

LOW RANK APPROXIMATION: A FLEXIBLE FRAMEWORK FOR NOISE REDUCTION IN MULTIPLE NMR EXPERIMENT

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MR is a flexible technique to investigate several kinds of materials, exploring their physical properties even with spatial resolution. One of its limitations lies in the low Signal-to-Noise Ratio which is usually overcome by signal accumulation. Unfortunately, there are two settings in which this strategy could be troublesome: multi-dimensional experiments and analysis of evolving systems. In the case of multi-dimensional experiments, we incur in the "curse of dimensionality": due to the high dimension of the parameter space the number of experiments needed to fit them is very high. This makes accumulating multiple acquisitions particularly time consuming.
For the study of evolving system, the signal accumulation framework is limited by the evolving rate of the system. In fact, the state of the system can significantly change during the time required for multiple acquisitions.
In this work we present the preliminary results for the low rank approximation of complex matrices and tensor and we investigate its performance in different settings of NMR (e.g. low field relaxometry, d-DNP MRS experiment, qMRI).
Our preliminary results show that the low rank approximation of complex dataset is a useful noise reduction method for accelerating multidimensional experiments, and to preserve time resolution in the monitoring of evolving system in different settings from spectroscopy to quantitative imaging.

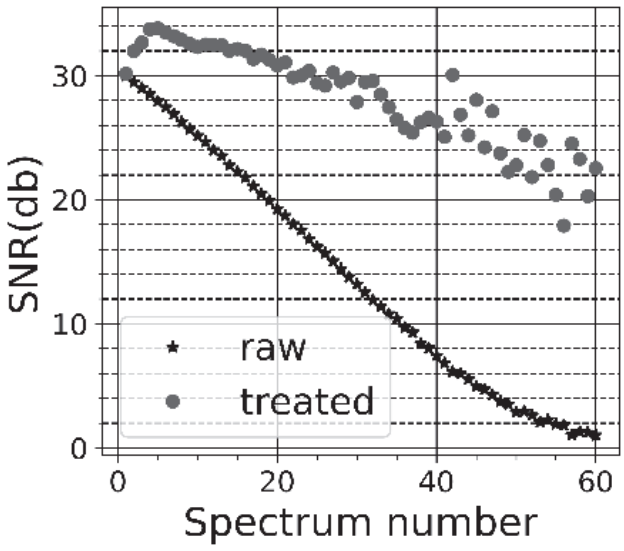

Figure 1: SNR for hyperpolarized ^{13}C-Pyruvate. In black the original data, in gray the denoised data.

OC9

^{1}H-NMR RELAXATION AS A POWERFUL TOOL FOR ASSESSING CONSERVATION MATERIALS

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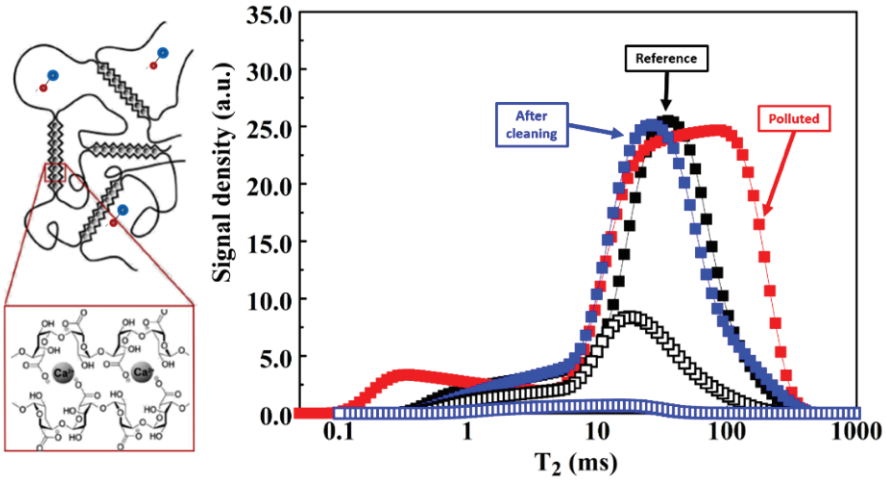
^{1}H-NMR relaxation measurements have become important tools in studying a wide class of porous materials, ranging from model systems to building materials [1,2]. Longitudinal (T_1) and transverse (T_2) relaxation of water in confined geometry scale up to three orders of magnitude using protons as NMR probe, compared with bulk water. The anisotropy of the molecular mobility induced by the wall modifies the NMR relaxation parameters, as it changes the range of the magnetic interaction fluctuations involved in the NMR processes. While investigating porous matrix-water systems through ^{1}H-NMR relaxation it is possible to get information on chemical and geometrical properties of the surface confinement, on the hydration degree of the matrix itself, and on porosity and pore-size distribution when the porous media is water saturated [3]. Among NMR relaxation equipment, mq-ProFiler (Bruker, Italy) found application in Cultural Heritage diagnostics, as it allows non-destructive and non-invasive investigations. In our research, we employ unilateral NMR to evaluate the effect of new cleaning materials for stone artifacts restoration through transverse relaxation times measurements. In this presentation, in particular, we will show examples of the effective applications of an innovative hydrogel, based on sodium alginate as inert matrix, for the removal of bio-contaminants from calcarenite samples. We carried out T_2 measurements before and after different degree of artificial bio-contamination and after the surface cleaning with the biocide hydrogel. Analyses were performed during moisture and water capillary absorption up to saturation conditions, pointing out almost no differences in samples' T_2 distributions after treatments with respect to reference.



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OC10

NMR APPROACHES IN METABOLOMICS: FROM CELLS TO BIOFLUIDS

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Applications of metabolomics are the most diverse; its use in biomedicine covers broad areas such as disease diagnosis and prognosis, monitoring the outcomes of medical intervention and unraveling the biochemical mechanisms of diseases. In terms of samples, NMR can be applied to the analysis of any type of biological matrix. The NMR spectra of biological samples are crowded and full of chemical information: NMR offers a unique view of the entire set of most abundant, free small molecules in a sample that can be measured in a single spectrum, independently on their chemical nature and with minimal sample preparation [1,2].
Solution ^{1}H-NMR is the most common approach in metabolomics. One-dimensional ^{1}H pulse sequences are routinely used at 600 MHz for the analysis of biofluids like serum, saliva or breath condensate [3]. Bi-dimensional experiments can be acquired to help signal assignment. High-field ^{1}H NMR-spectroscopy (900 MHz) can be instead used as an efficient platform for the analysis of cell cultures both in terms of their endo- and exo- metabolome [4,5]. In this framework, stable isotope-resolved metabolomics provides a more dynamic assessment of specific metabolic pathways: ^{13}C enriched molecules can be used as probes to trace their biotransformation for flux analysis. High Resolution Magic Angle Spinning (HR-MAS), instead, provides access to well-resolved liquid-like ^{1}H NMR spectra of semi-solid samples such as intact tissues.

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OC11

THALAMIC PARCELLATION FOR TARGET IDENTIFICATION IN TRANS-CRANIAL MR-GUIDED FOCUSED ULTRASOUND (TCMRGFUS) THALAMOTOMIES: A PRELIMINARY PROBABILISTC TRACTOGRAPHY STUDY

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Trans-cranial MR-guided Focused UltraSound (tcMRgFUS) is an innovative and effective procedure for treatment of some functional neurological disorders such as essential tremor that is the most common movement disorder. Nowadays, the choice of the target is based on stereotactic coordinates that do not consider the anatomical variability of each single patient. Thus, the optimization of the treatment target is based on the patient's feedback achieved through lower power sonications during the verification treatment stage.

The aim of this work is to retrospectively evaluate the possible role of thalamic parcellation for the identification of the intermediate ventral nucleus (VIM) in patients underwent a tcMRgFUS thalamotomy. A 1.5T MR scanner (GE Signa HDxt) was used to acquire morphological (3D-BRAVO T_1w) and ultrastructural morphological sequences (Diffusion Tensor Imaging). Brain eloquent areas were first extracted trough an automatic segmentation process (FreeSurfer) and then used as seed for a probabilistic tractography algorithm (FSL) aimed at representing the cortical projections to and from the thalamus with particular reference to the primary and supplementary motor areas and the premotor cortex. The thalamic parcellation maps obtained were then matched with the segmented volume of tcMRgFUS placed lesions (from high resolution 2D-FRFSE T_2w). In all cases it was possible to represent the major groups of thalamic nuclei. A good overlap between the thalamic parcellation maps (with particular reference to the VIM nucleus) and the lesions induced by tcMRgFUS was identified in patients with good clinical outcomes. These preliminary results are very encouraging: even if the requested pre- and post-processing pipeline behind such an innovative approach are still extremely complex and time consuming, the use of such a technique could result very helpful during tcMRgFUS treatment target optimization, especially in cases where the optimal treatment target deviates from atlas-based stereotactic coordinates.

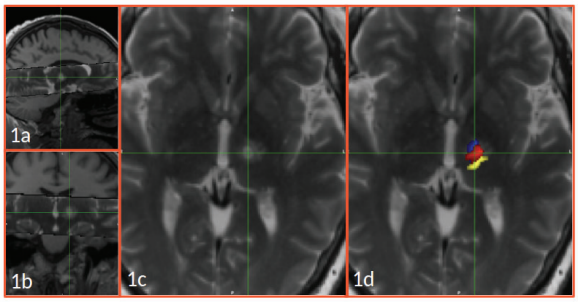

Figure 1: Example of a patient with a good clinical outcome (1y post-treatment follow-up). Sagittal (a) and coronal (b) multiplanar reconstructions from 3D T_1 FSPGR sequence with co-registered hi-resolution 2D (2mm, no gap) T_2w FRFSE of the basal ganglia region acquired 48h after the treatment (a-d). The thalamic pacellation (d) map is superimposed on the T_2w FRFSE showing a perfect match between the lesion and the probabilistic tractography representation of the motor cortex (BA4 in red). The thalamic projections of the somatosensory cortex (BA1-2-3 in yellow) and of the premotor cortex (PMA) and supplementary motor area (SMA) (BA6 in blu).

OC12

SAD TI: SINGLET ASSISTED DIFFUSION TENSOR IMAGING FOR POROUS MEDIA INVESTIGATIONS

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Translational dynamics of molecules diffusing in porous media can be characterised by NMR and MRI techniques, exploiting the positional information encoded by pulsed field gradients. The study of self-diffusion in confined and restricted environments reports on the structural features of the system in which diffusion occurs, such as tortuosity, pore length and pore orientation. The condition to make diffusion a source of such information is to track the displacement of molecules long enough for those to experience the boundaries. Since diffusion Magnetic Resonance techniques rely on the acquisition of a signal whose decay constant depends on the spin order that was created, methods based on longitudinal magnetisation have an upper limit to the scope of the technique (cavity size < 100 μm) given the fact that the relaxation times T_1 and T_2 associated with longitudinal and transverse magnetisation are typically of the order of a few seconds at best.
Our group has recently demonstrated the advantages of using long-lived spin order in diffusion experiments. By exploiting the exceptionally long relaxation time TS of singlet order, it was possible to derive tortuosity [1] and geometrical features [2] of materials on a length scale of millimetres, currently not achievable with traditional approaches.
In this work we aim to extend the use of singlet order to Diffusion Tensor Imaging (DTI) techniques. By deriving all six components of the diffusion tensor, DTI allows to investigate and depict the three-dimensional architecture of cavities and channels where a molecular probe is diffusing.
DTI's main field of application is medical MRI where the diffusion of water in brain fibres enables to explore their orientation and connectivity. However, DTI might be usefully applied to a broader class of systems where structural details are required to evaluate the quality and performance of materials, including rock samples, anisotropic battery electrodes and cells cultured on 3D-printed scaffoldings for the regeneration of biological tissue, all systems in which current DTI techniques fails to perform because of the short relaxation time of longitudinal spin order.
In this contribution we describe the newly developed singlet-assisted DTI technique and demonstrate its potential to access structural information in 3D phantoms with 1 mm cylindrical channels oriented in different directions of space. These phantoms are used as models of the more interesting structures cited above to which we aim to address our future efforts.

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OC13

NEW SENSITIVITY-ENHANCED SOLID-STATE NMR APPROACHES TO INVESTIGATE CRYSTALLIZATION AND POLYMORPHISM

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Polymorphism - *i.e.* the ability of a chemical compound to crystallize in different forms - affects almost 50% of all the organic compounds referenced in the Cambridge Structural Database. It can have huge economic and practical consequences for industrial applications in pharmacy and energy because different polymorphs display different physicochemical properties. If, on the one hand, it offers great opportunities for tuning the performance of the organic material, on the other hand, manufacture or storage-induced, unexpected, polymorph transitions can compromise the end-use of the solid product. These transformations often imply the formation of metastable forms, which are receiving growing attention because they can offer new crystal forms with improved properties. Today, detection and accurate structural analysis of these - generally transient - forms remain challenging, essentially because of the present limitations in temporal and spatial resolution of the analysis, which prevents rationalization (and hence control) of crystallization processes. In our laboratory, we develop dynamic nuclear polarization (DNP) solid-state NMR approaches to overcome these limitations. In this contribution, I will present some of our latest results showing that cryogenic MAS NMR [1] combined with the sensitivity enhancement provided by DNP [2] can be an efficient way of monitoring the structural evolution of crystallizing solutions with atomic-scale resolution on a time scale of a few minutes. This work opens up the prospect of studying the very early stages of crystallization, such as nucleation and pre-nucleation phenomena, at which the amount of solid phase present is intrinsically low.

Acknowledgements
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OC14

LOCAL AND GLOBAL PROTEIN DYNAMICS BY FFC RELAXOMETRY

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FFC relaxometry allows for the direct measurement of protein proton spectral density functions by dissolving proteins in D_2O at millimolar concentrations. This permits to collectively monitor the relaxation rates of non-exchanging protein protons as a function of the magnetic field [1]. The analysis of these profiles provides direct information on the protein reorientation time, and thus on its aggregation state, and on the collective order parameter of protein protons, and thus on internal mobility. Such analysis takes advantage of a complete relaxation matrix analysis, in order to model the distribution of the proton relaxation rates in proteins [2].
The profiles of well folded proteins are characterized by a large relaxation rate at low fields (from 100 to thousands s^{-1}), and by a Lorentzian dispersion reporting on the correlation time modulating the dipole-dipole interactions between protein protons. For a protein of about 150 amino acids, correlation times around 10 ns are expected and measured at 298 K. The low field relaxation rate is however somewhat smaller than calculated, due to the presence of local mobility, and the observed discrepancy provides an estimate of the extent of these fast motions, in the form of a model-free order parameter S_C.
For intrinsically disordered proteins (IDPs), no dispersion should be observed in the 0.01-50 MHz range of proton Larmor frequency. Instead, relaxometry measurements performed at 298 K for IDPs of 100-150 amino acids in D_2O solutions show dispersions corresponding to correlation times of about 5-8 ns, thus indicating the presence of motions occurring on a timescale much longer than that of IDP segmental motions, which are not related to specific long-range interactions between the protein residues, and thus represent an intrinsic feature of the mobility of IDPs [3]. The low field relaxation rates are however much smaller in IDPs (10-50 s^{-1}) than for well folded proteins, as a result of an S_C^2 of about 0.1.
Molecular dynamics (MD) simulations represent an ideal tool for elucidating the mobility of IDPs at an atomic level of detail. Using a very long trajectory of 34 μs recently generated for the IDP α-synuclein, we have shown that the presence of motions with correlation times of several nanoseconds is also supported by MD simulations [4]. In fact, the calculated MD trajectory shows that, although most of the conformational dynamics occurs locally, fast local motions are unable of averaging dipolar interactions completely, indicating the presence of correlation times of 6-9 ns, with S_C^2 of 0.04-0.10, in full agreement with the experimental relaxometry data.

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OC15

WATER DYNAMICS AND NMR RELAXATION PROPERTIES OF NANOCONFINED WATER

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The dynamics of fluids in confined geometries is of interest for multiples perspectives, in particular medicine and biophysics, in which water is ubiquitous. It is well demonstrated that the dynamics of water is strongly determined by confining matrix at the nanoscale. The Nuclear Magnetic Resonance represents the principal experimental tools to investigate the diffusivity of water at the nanoscale and how the confinement changes the spin relaxation properties of water's protons, determining a contrasting activity.
In this talk, we present computational results of molecular dynamics on water confined in nanometric environments formed by two-dimensional graphene-based material, like graphene oxide (GO). We calculated the diffusion constant as a function of the GO concentration and the longitudinal spin relaxation time, T_1, within the Purcell, Pound, and Bloembergen theory [1]. We show that GO affects water's diffusivity and spin-lattice relaxation properties, with respect to the bulk, due to the peculiar interaction of water with the oxide surface. In addition, we show that bilayer systems (Fig.1), formed by the ordered stacking of two-dimensional system, strongly alter water dynamics, inducing an anomalous two-dimensional diffusion mechanism.
We propose that, due these peculiar confinement effects, GO can show promising contrast activity in NMR and be used as a possible contrast agent.

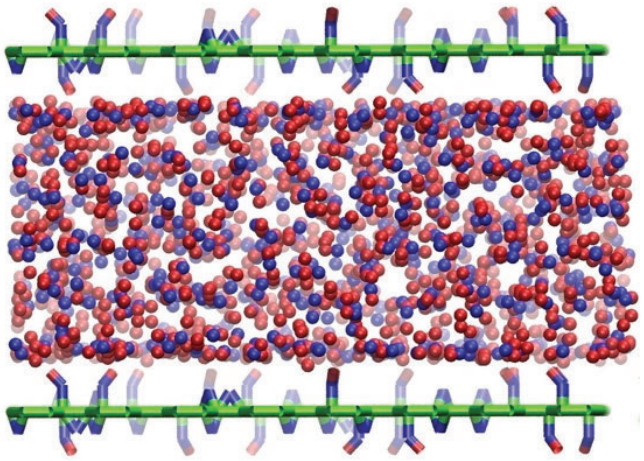

Figure 1: Structure of water confined in a bilayer graphene oxide. Carbon atoms are in green, while oxygen and hydrogen atoms in blue and red, respectively.

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OC16

MOLECULAR MECHANISM OF ANTIANGIOGENIC AGENTS TARGETING FIBROBLAST GROWTH FACTOR-2/TYROSINE-KINASE RECEPTOR INTERACTIONS

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Fibroblast growth factor-2 (FGF2) plays a major role in angiogenesis, the process of generating new capillary blood vessels from pre-existing ones, an important natural phenomenon used for healing and reproduction. In healthy tissues the body controls angiogenesis by producing a precise balance of growth and inhibitory factors. Pathological angiogenesis underlies a wide range of diseases, including cancer, and FGF2 thus represents a target for anti-angiogenic therapies. FGF2 needs to set up a productive ternary complex with the tyrosine-kinase receptors (FGFRs) and the heparan sulphate proteoglycans (HSPG) to exert its pro-angiogenic activity. Natural and synthetic molecules, able to interfere with HSPG/FGF2/FGFR interaction, have been designed starting from endogenous inhibitors of angiogenesis, such as Long Pentraxin-3 and Thrombospondin-1. We demonstrated, by a combination of NMR and MD approaches, that they regulate angiogenesis through different mechanisms, including binding and perturbing the dynamics of the complex, through direct and allosteric mechanisms. Their effective action was evaluated through in vitro and in vivo studies [1-4]. We recently investigated the role of rosmarinic acid, a natural polyphenolic compound, capable of inhibiting different angiogenic pathways in vitro [5]. RA ability to bind and modulate the dynamic features of FGF2/FGFR complex has been widely characterized by NMR to identify the hot spots of allosteric modulation, shared by antiangiogenic molecules targeting this system.

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OC17

MACHINE LEARNING TOOLS FOR ADVANCED PROCESSING AND ANALYSIS OF MR DATA

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The recent advancements in Machine Learning (particularly Deep Learning and Artificial Intelligence tools) have brought improvements in data analysis and processing in several fields, such as imaging data. These achievements have the possibility to be translated to physical fields related to bioimaging (such as Medical Physics), and more in general to MR-based diagnostic and investigation techniques (e.g. quantitative techniques).
In this talk we describe some recent results on these topics derived from our research activity (MR fingerprinting, Quantitative Susceptibility Mapping, super-resolution) and the possibility to extend these approaches on other problems related to MR data processing and analysis.

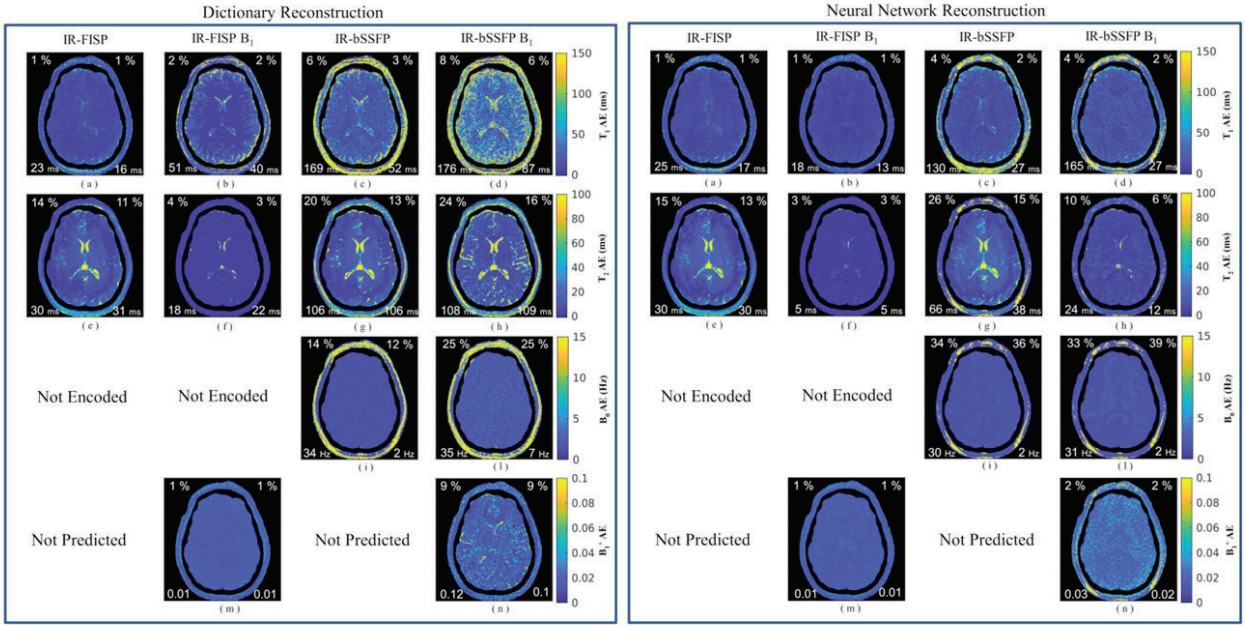

Figure 1: Comparison between MR fingerprinting image reconstruction: based on a reference dictionary (left) and based on our Feedforward Neural Network method.

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BOOSTING MAGNETIC RESONANCE IMAGING SIGNAL-TO-NOISE RATIO USING MAGNETIC SURFACE PLASMONS

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In the last decade, several research groups have introduced metamaterials concepts for designed high efficiency MRI scanners (see *e.g.* Ref.[1]). In Freire's configuration [1], a $Re(\mu_m) = -1$ metamaterial (where μ_m is the metamaterial permeability) acts as *"a poor-man's superlens"* [2] able to suitable refocus the RF magnetic field, such as to enhance both MRI signal and signal-to-noise ratio. On the other hand, it is worth noting that a $Re(\mu_m) = -1$ metamaterial can also support magnetic surface plasmonic excitations. Here, we propose to exploit this phenomenon to improve MRI efficiency. Performing full-wave simulations, we simulate the configuration displayed in Fig.1 and we show that a $Re(\mu_m) = -1$ metamaterial, coupled to a standard RF surface coil, allows to boost the magnetic resonance imaging signal to noise ratio with respect to the standard setup. In Fig.1 (*right panel*), we report

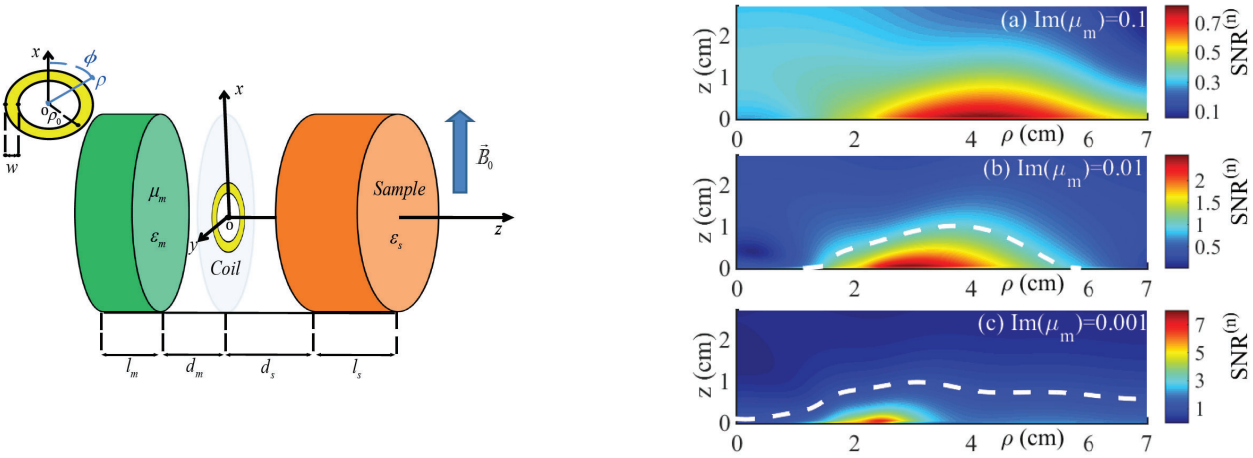

Figure 1: (*left panel*) Sketch of the considered setup. A standard surface radio-frequency (RF) coil at $z = 0$ is positioned between the metamaterial slab. (*left panel*) $\mathrm{SNR}^{(n)}$ maps with $Re(\mu_m) = -1$ with $Im(\mu_m) = 10^{-1}$ (a), $Im(\mu_m) = 10^{-2}$ (b), $Im(\mu_m) = 10^{-3}$ (c). Dashed white lines are $\mathrm{SNR}^{(n)} = 1$ contour lines.

the spatial distribution of the normalized signal-to-noise ratio $\mathrm{SNR}^{(n)}$ (viz., $\mathrm{SNR}^{(n)}$ =$\mathrm{SNR}_m/\mathrm{SNR}_v$ where SNR_m , SNR_v are the values calculated with and without the $Re(\mu_m) = -1$ metamaterial slab, respectively). In the situation very close to the surface plasmon resonance condition $(\mu_m) = -1$ [(b) and (c) sub-panels], we obtain a significant enhancement of RF coil $\mathrm{SNR}^{(n)}$. Our predictions indicate that the considered configuration holds great potential to enhance the MRI signal to noise ratio with respect to the standard setups.

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OC19

MINING ^{13}C-NMR EXPERIMENTS THROUGH THE MARA-NMR STRATEGY AND OTHER NMR ISSUES

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The Multi-Assignment Recovered Analysis by Nuclear Magnetic Resonance experiments (MARA-NMR) is an algorithm able to readily provide the thorough quantification of complex mixtures (labelling). Provided that, it is available the peak assignment of specific components in a mixture; MARA-NMR [1] is successfully employed to quantify olive oil species [2], almond and pistacho compounds [3] and investigate amino-acidic mixtures coming from food waste [4]. Beyond these issues MARA-NMR urged a deep study of the ^{13}C-NMR of vegetable oils. The scientific goal is to highlight the presence of neglected fatty esters and take the chance for a thorough quantification also endowed with the detail concerning the esterification positions (1,2 or 3) of the fatty ester of tri-acylglycerols in vegetable oils. For the ^{13}C assignment and prediction of "exotic" fatty residues we will use standard references (where available), odd crossed data concerning other oils, and theoretical chemistry knowledge. Probably NMR (through MARA) is the easiest way to reach such specific knowledge about oils and the challenge is to push NMR limits further.

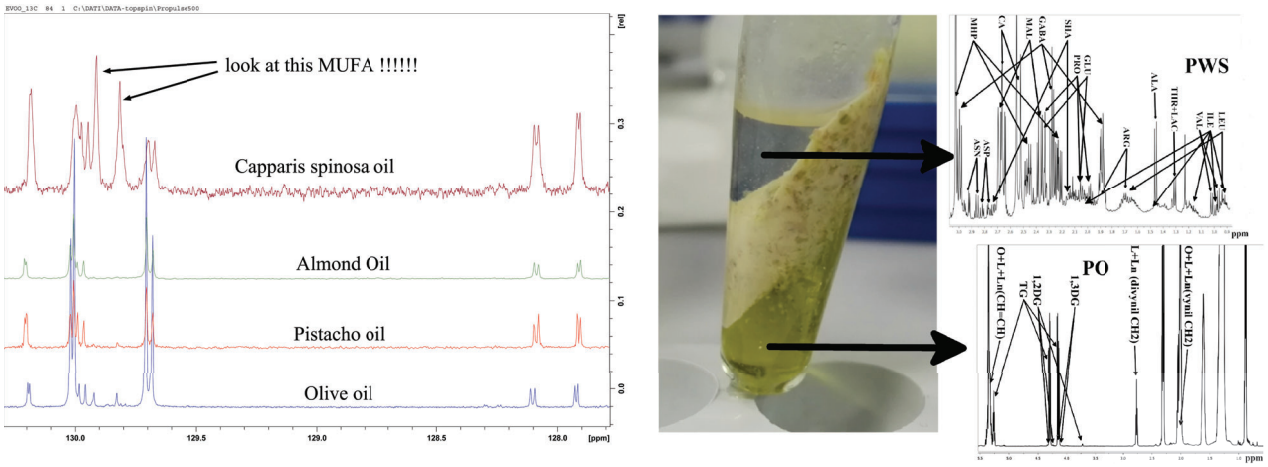

Figure 1: Left, ^{13}C-NMR profile of the unsaturated CH region for several vegetable oils; Right, ^{1}H-NMR profile of different hydrosoluble and liposoluble phases of pistachio seeds powder.

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OC20

TUNING OF MICRO-MILLISECOND CONFORMATIONAL DYNAMICS CONTROLS THE FORMATION OF PRION PROTEIN INTERMEDIATE STATES INVOLVED IN AMYLOID FIBRILS ASSEMBLY

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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 an exhaustive NMR-Based investigation of conformational equilibria and folding mechanisms for full length and 90-231 prion proteins.

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OC21

TAKING SIMULTANEOUS SNAPSHOTS OF INTRINSICALLY DISORDERED PROTEINS IN ACTION EXPLOITING MULTIPLE RECEIVERS AND HETERONUCLEAR DIRECT DETECTION

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Intrinsically disordered proteins (IDPs) as well as intrinsically disordered regions (IDRs) of complex protein machineries have recently been recognized as key players in many cellular functions. NMR represents a unique tool to access atomic resolution structural and dynamic information on highly flexible IDPs/IDRs [1]. Improvements in instrumental sensitivity made heteronuclear direct detection possible for biomolecular NMR applications. The CON experiment has become one of the most useful NMR experiments to get a snapshot of an IDP/IDR in conditions approaching physiological ones [2]. The availability of NMR spectrometers equipped with multiple receivers now enables the acquisition of several experiments simultaneously instead of one after the other [3]. Here we propose several variants of the CON experiment in which, during the recovery delay, a second 2D experiment is acquired, either based on ^{1}H detection (CON//HN) or on ^{15}N detection (CON//btNH, CON//(H)CAN). The possibility to collect simultaneous snapshots of an IDP/IDR through different 2D spectra provides a novel tool to follow chemical reactions, such as the occurrence of post-translational modifications, as well as to study samples of limited lifetime such as cell lysates or whole cells.

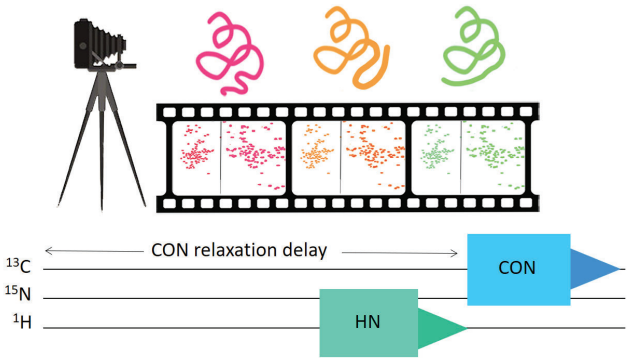

Figure 1: Pictorial representation of the usage of multiple receivers to take simultaneous snapshots of intrinsically disordered proteins. Through multiple receivers it is thus possible to acquire different FIDs during the relaxation delay needed to recover the carbonyl magnetization.

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OC22

IMPORTANCE OF SAMPLE PREPARATION IN NON-TARGETED NMR ANALYSIS FOR THE TRACEABILITY OF ITALIAN TOMATOES

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In the last years the application of nuclear magnetic resonance spectroscopy (NMR) to the metabolomics has being experienced an increasing number of applications [1], due to the development of new mathematical algorithms for the statistical analysis, and more sensitive and highly reproducible spectrometers. In metabolomic analysis the sample stability is a crucial aspect, being the prerequisite for assuring high reproducibility of the results obtained by different laboratories and, thus, a valuable inter-laboratory comparison. The stability of a sample is largely affected by its preparation protocol, which should include all the operations aimed at ensuring an efficient extraction of the metabolites and a lasting stability of the metabolic profile over the time [2].
As part of a collaborative research project (Istituto Poligrafico Zecca dello Stato - Politecnico di Bari) aimed at discriminating the geographical origin of Italian tomatoes, we disclosed the crucial aspects of the sample preparation affecting the metabolic profiles of the tomato extracts, and, importantly, we shed light on the practical procedures able to reveal the presence of some unknown metabolites and to control their transformations over the time. Moreover, we demonstrated that depending on the sample preparation the metabolic profile can suffer a significant variation. The results reported in this communication should be of general interest for the scientific community involved in food analysis.

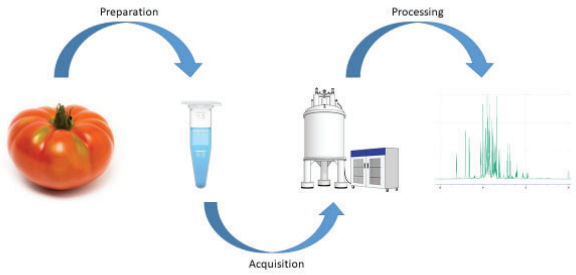

Figure 1: ^{1}H-NMR metabolomic workflow

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OC23

ADVANCED DIFFUSION MRI FOR WHOLE BRAIN IN VIVO AXONAL DIAMETER MAPPING IN MULTIPLE SCLEROSIS

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Traditional techniques based on diffusion MR imaging suffer from extremely low specificity in separating disease-related alterations in white matter microstructure, which can involve multiple phenomena including axonal loss, demyelination and changes in axonal size. Multi-shell diffusion MRI is able to greatly increase specificity by concomitantly exploring multiple diffusion timescales. If multi-shell acquisition is combined with an exploration of different diffusion times, diffusion data allows the estimation of sophisticated compartmental models, which provide greatly enhanced specificity to the presence of different tissue sub-compartments, as well as estimates of intra-voxel axonal diameter distributions. We apply a multiple-b-value, high angular resolution multi-shell diffusion MRI protocol with varying diffusion times in a cohort of multiple sclerosis (MS) patients and compare them to a population of healthy controls. Data was acquired on a Siemens 3T Connectom scanner, a customized 3T Siemens MAGNETOM Skyra system housed at the MGH/HST Athinoula A. Martinos Center for Biomedical Imaging. From the AxCaliber model, we are able to extract indices for axonal diameter (α) across the whole brain. From the diffusion tensor imaging (DTI) model, we computed voxels-wise fractional anisotropy (FA) and mean diffusivity (MD) maps. We employed a voxel-wise, permutation-based statistical framework to test for statistical differences in all indices within normal-appearing white matter (NAWM) of MS patients compared to HC, while excluding lesion masks and controlling for multiple comparisons across clusters. We show that MS is associated with widespread increases of axonal diameter and that our axonal diameter estimation provides the highest discrimination power for local alterations in in MS compared to controls. AxCaliber has the potential to disentangle microstructural alterations in MS invisible to DTI and could be a sensitive and specific non-invasive biomarker of irreversible disease progression.

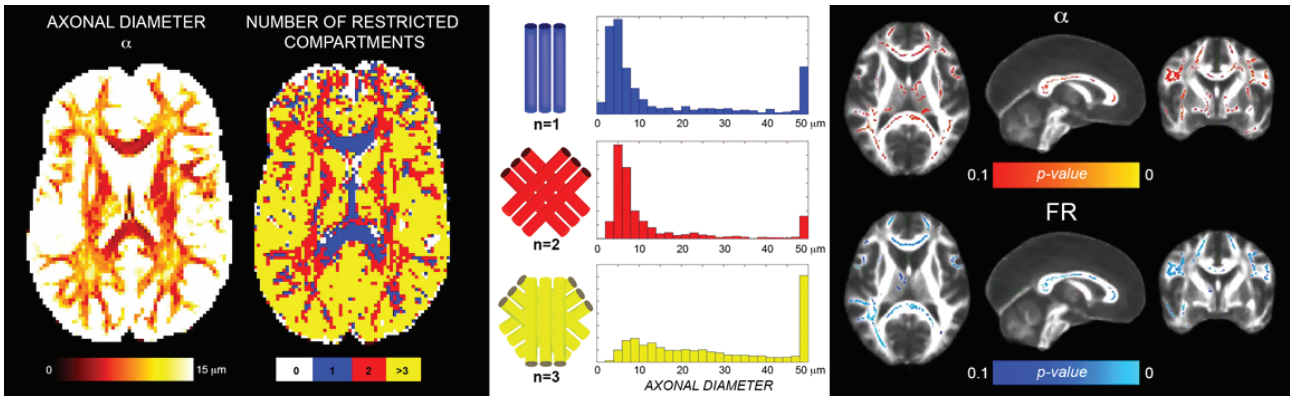

Figure 1: Left: Fitted axonal radius (α) for one subject (left on left figure) separated according to the number of restricted compartments (right on left figure) in the voxel. Center: Distribution of voxels according to the number of compartments. Right: TBSS results for α (upper pane MS>HC) and FR (lower pane MS<CON) when testing the MS vs HC condition. Opposite contrasts were not statistically significant.

OC24

PARAMAGNETIC GIANT LIPOSOMES AS HIGHLY SENSITIVE VERSATILE MRI CONTRAST AGENTS

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The last ten years have witnessed the development of a large variety of nanosystems for different applications in MRI. This trend has been spread from the need to overcome the intrinsic low sensitivity of the MRI technique in Molecular Imaging protocols. Pushing further the same reasoning, in this work we have explored the potential of Giant Liposomes (GUVs) as T_1, T_2 and CEST MRI agents. Giant Unilamellar Vesicles (GUVs), have already been described in literature as cell mimicking systems since 90s [1] but never used as therapeutic or diagnostic carriers. GUVs have been prepared according to an optimization of the "gentle swelling" method reported elsewhere [2], in order to design the most suitable probe for Molecular Imaging purposes. GdHPDO3A or TmHPDO3A paramagnetic complexes were trapped inside in order to explore paramagnetic GUVs as T_1/T_2 and CEST agents, respectively. The use of micrometric vesicles endowed with a mean diameter ten times greater (ca. 1.5 μm) than nanosized ones (ca. 150 nm) lead to increase the inner water volume of one thousand times. Consequently, T_1/T_2 and CEST sensitivity per particle has been increased of three order of magnitude. In vitro and in vivo toxicity has been evaluated and the same very low toxicity as nanosized liposomes has been found. When functionalized with targeting moieties on the external surface, fluorescent Giant Liposomes are not internalized into cells even if they can bind cells membranes and moreover the macrophagic uptake of GUVs seems to be negligible with respect to nanosized liposomes. Different MRI contrast modalities (T_1, T_2 and CEST) were tested on cancer cells targeted with paramagnetic GUVs and because of a low number of GUVs bound per cell, only T_2 contrast was significantly appreciated. Moreover, following a recent work by Delli Castelli et al. [3], Magnetization Transfer Contrast (MTC) was also exploited to indirectly detect Gadolinium. Paramagnetic GUVs targeting integrines on cancer cells were imaged and they resulted in a significant difference in terms of contrast proving MTC to be, in this context, a more sensitive way to detect paramagnetic GUVs with respect to classical T_1 contrast. In vivo experiments with integrins targeting GUVs are currently being performed. To the best of our knowledge this is the first time GUVs have been developed as MRI agents.

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OC25

TECHNICAL DETAILS OF AN FFC-NMR STUDY OF WATER-CYCLOHEXANE-LECITHIN MICELLES SYSTEMS

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In certain proportions, ternary systems composed of water, cyclohexane and lecithin form micelles of various shapes, including giant wormlike ones, which have been extensively studied by NMR diffusometry, dielectric spectroscopy and other relaxometry methods [1, 2].
In this study we have explored the applicability of fast-field cycling (FFC) NMR relaxometry to this kind of systems. Our focus was not so much on the studied system as such, as on the technical issues implicit in measuring FFC NMRD profiles of samples exhibiting the presence of several components relaxing at different rates.
The post-acquisition data evaluation includes a number of distinct steps, such as automatic FID phasing, detection and removal of distorted first FID points, noise filtering, window averaging and/or signal extrapolation to time zero, multi-exponential fitting with confidence intervals estimate of all fitted parameters, correction of the weights of the individual decay components for finite FFC switching times, and fitting of their NMRD profiles to several molecular motion models.
The evaluation was carried out using Td-Relax [4], a new general-purpose dynamic link library interfaceable with most common programming languages, as well as with the Mnova NMR data evaluation package.

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[4] Td-Relax project development is supported by Mestrelab Research SL, Santiago de Compostela, Spain.

OC26

QUANTITATIVE MAPPING OF HUMAN BRAIN CEREBROVASCULAR AND METABOLIC FUNCTION

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Blood oxygenation level dependent (BOLD) functional MRI (fMRI) has taught us a great deal about the systems level organization of the human brain over the last 25 years. However, traditional BOLD fMRI suffers from uncertainties over the interpretation of signal changes observed which hamper its use for the evaluation of the physiological state of brain tissue. These uncertainties derive from the mixed nature of the changes that contribute to the BOLD signal, including cerebral blood flow (CBF), cerebral blood volume (CBV) and the rate of cerebral metabolic oxygen consumption ($CMRO_2$), along with the need to assume a stable coupling between neural activity and the haemodynamic response (neurovascular coupling). More quantitative versions of fMRI are becoming available [1] based on CBF, using arterial spin labelling techniques, as well as relative changes in, and absolute levels of, $CMRO_2$, (Fig. 1).

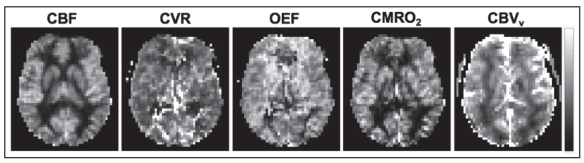

Figure 1: Multiparametric haemodynamic and metabolic imaging (healthy volunteer) using a dual-calibrated approach [1]. CBF - cerebral blood flow (from pCASL, range 0-100 ml/100g/min), CVR - cerebrovascular reactivity (range 0-5 %ΔCBF/mmHg CO_2), OEF - oxygen extraction fraction (range 0-0.6), $CMRO_2$ - rate of cerebral metabolic oxygen consumption (range 0-300 μmol/100g/min) and CBVv - venous cerebral blood volume (arb. units).

These techniques show promise in the assessment of pharmacological effects, in neuroscientific studies of brain plasticity and as biomarkers for drug [2] and disease effects as a possible replacement for radiotracer based methods (Fig. 2). I will discuss the newer MRI-based mapping techniques for assessment of cerebrovascular and oxygen metabolic function, how they work, their advantages and disadvantages, and their potential for clinical application and improving our understanding of brain tissue energetics. While our development of these methods has focused on 3T MRI, we and other groups are now beginning to extend them to ultra-high field MRI (7T) where there are opportunities to exploit the stronger NMR signal but also the challenges of B_0 and B_1 field non-uniformity to address.

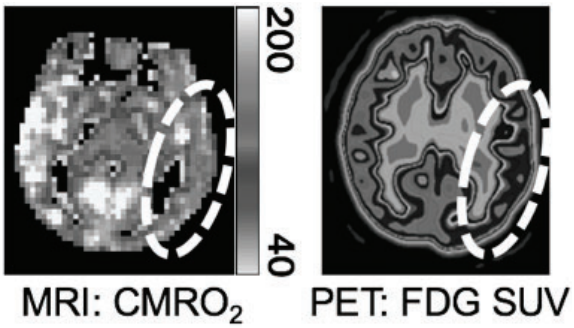

Figure 2: Pre-surgical epilepsy: hypometablism revealed by dual calibrated $CMRO_2$ maps (μmol/100g/min), matching PET FDG clinical standard.

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POSTERS

P1

NMR-BASED IDENTIFICATION OF NEUROPROTECTIVE COMPOUNDS IN COCOA EXTRACTS

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In recent years, the research of foods and natural products to be employed as nutraceuticals has largely increased, becoming of great importance in the field of prevention against diseases lacking of effective therapies, as Alzheimer's disease. In this context, we developed an experimental protocol that combines NMR spectroscopy and atomic force microscopy, in vitro biochemical and ex vivo cell assays to detect anti-Aβ molecules in natural edible matrices (Salvia sclareoides, green tea, green and roasted coffee, Peucedanum ostruthium and hop) [1], in order to identify food and beverages able to provide the regular intake of natural compounds capable of interfering with toxic amyloidogenic aggregates.
Here we present the preliminary results of the study on cocoa extracts [2]. Cocoa (Theobroma cacao) is a rich source of polyphenols and its beneficial effects for human health are well known, including anti-oxidant, anti-inflammatory and neuroprotective activities. However, the molecular mechanisms through which these biological activities are carried out have not been elucidated yet. Cocoa extracts were prepared from three types of cocoa beans (not fermented, fermented and industrially processed), according to different procedures, and their metabolic profiles were characterized and compared by NMR spectroscopy. Then, the screening protocol was applied to identify possible ligands of Aβ1-42 oligomers in cocoa extracts and to verify the potential neuroprotective activity of cocoa.

Acknowledgments
We thank GIDRM for the Post-doc fellowship "Borsa Annalaura Segre - GIDRM" to C. Ciaramelli. Financial support from the Italian Ministry of University and Research (MIUR) through grant "Dipartimenti di Eccellenza - 2017" to University of Milano - Bicocca, Department of Biotechnology and Biosciences is acknowledged.

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[2] Manuscript in preparation.

P2

CHARACTERIZATION OF GRAPHENE OXIDE WATER DISPERSIONS BY MEANS OF 2.35 T MRI AND X-BAND EPR SPECTROSCOPY

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Magnetic Resonance Imaging (MRI) is a powerful clinical diagnostic tool and its domain is currently developing towards molecular imaging suitable for animal models of disease [1]. Graphene oxide (GO), obtained by the Hummers method, seems an interesting structure to be used as MRI contrast agent to improve specificity and/or sensitivity [2-4]. In this 2.35T MRI study we characterized the relaxation times of GO water dispersions at various concentrations. X-band EPR spectroscopy was used to study the paramagnetic properties of the GO contrast agent. MRI experiments were carried out with

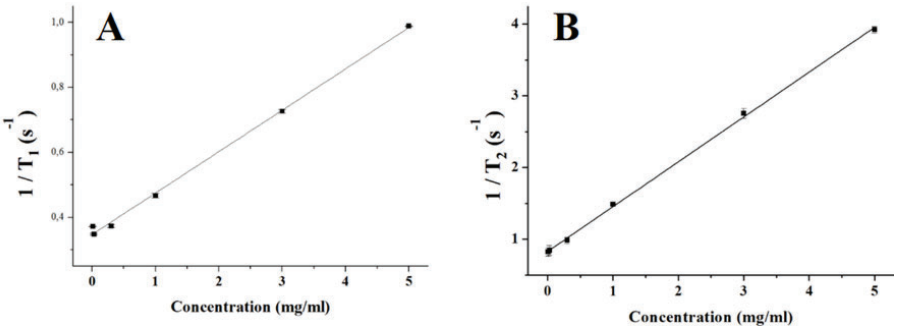

Figure 1: Relaxation rates $1/T_1$ (A) and $1/T_2$ (B) versus GO concentration. Shown are mean values in each region-of-interest, standard deviations and linear regression slopes.

a 2.35 T Biospec scanner (Bruker, Germany). The GO samples were prepared in aqueous solution, at different concentrations, in 2 ml PTFE/silicone septa glass vials (diameter 12 mm, height 31 mm), by sonicating for 10 min (Ultrasound Bath LBS 2; Frequency: 59 KHz; Peak power: 450 W). RAREVTR images were acquired to map the T_1 values (TR=[35-4500] ms; TE=18 ms; 0.78 mm/pixel). The MSME sequence was used to acquire T_2-W images (TR=4500 ms; TE=k×16 ms (k=1, 13); 0.51 mm/pixel). The GO dispersions showed, respectively, longitudinal and transverse relaxivity values of r_1=0.13 and r_2=0.62 $s^{-1}\times ml\times mg^{-1}$, to be compared with the much higher values of standard $MnCl_2$ water solutions (r_1=95 and r_2=1111 $s^{-1}\times ml\times mg^{-1}$). EPR spectra at X band allowed to confirm the presence of Mn^{2+} ions anchored to the GO planes and also the presence of radical-like carbon-inherited defects, both contributing to the NMR water relaxation mechanisms. These results should be of interest in the design of GO-based contrast agents for bio-medical applications.

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P3

FRAGMENT BASED DRUG DISCOVERY BY ^{19}F NMR FOR FINDING NEW INHIBITORS OF KEAP1-NRF2 INTERACTION

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One of the possible strategies for the discovery of new inhibitors of protein-protein interaction is the fragment-based drug discovery (FBDD) [1]. Nuclear magnetic resonance spectroscopy (NMR) plays a vital role for implementing FBDD, due to the weak interaction typical of fragments [2,3]. Several approaches have been proposed using 2D NMR as well as 1D 1H and 19F NMR for screening mixtures of compounds against a target of interest [4,5]. In this context, our purpose is the development and optimization of a FBDD methodology using competitive experiments and ^{19}F-NMR. We have chosen the Kelch Domain of Keap1 (KK1) as a target. The aim of setting the FBDD approach is to find new inhibitors of the Keap1-Nrf2 interaction. The Keap1-Nrf2 system senses and regulates the cellular response to environmental stresses. Inhibiting this protein-protein interaction may provide cytoprotection against numerous pathologies including chronic diseases of the lung and liver; autoimmune, neurodegenerative and metabolic disorders; and cancer initiation. The first step of this study involved the search of a suitable fluorinated spy molecule interacting with the KK1 binding site with an adequate affinity. Diverse possible fluorinated peptides based on the upon the high affinity Nrf2 ETGE sequence were designed. The better compound was then selected and its interaction with KK1 characterized by ^{1}H-^{15}N HSQC spectra. Once the spy molecule was found, an optimization of all the experimental parameters was performed to obtain the highest sensitivity, in the shortest experimental time. Finally, the screening of a ∼1600 fragments library started, and several active compounds were found.

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P4

HYPERPOLARIZATION OF PYRUVATE DERIVATIVES USING PARAHYDROGEN INDUCED POLARIZATION

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Hyperpolarization is the technology, which (by temporarily redistributing the populations of nuclear spin energy levels) may improve the sensitivity of various magnetic resonance (MR) techniques by a factor of several thousand at high (1-10 T) magnetic fields, and even millions at low (1-50 mT) fields. [1] The use of hyperpolarized (HP) molecules for diagnostic purposes portend a new era for in vivo magnetic resonance imaging (MRI) because it allows the detection of metabolites in low concentrations and, most importantly, the visualization of their transformations in vivo. Para-Hydrogen Induced Polarization (PHIP) is a cheap and easy-to-handle hyperpolarization method that can provide 20% hyperpolarization of ^{13}C MR signals in several substrates in a few seconds. PHIP relies on the catalytic hydrogenation, using hydrogen enriched in the para-spin isomer, of unsaturated precursors of the target molecule. Among the hyperpolarized substrates that can be used for metabolic studies, [1-^{13}C]pyruvate gained much attention because its fast metabolic transformation into lactate can give relevant diagnostic information about pathologies.[2] Hyperpolarization of [1-^{13}C]pyruvate by means of PHIP has been obtained by means of Side-Arm Hydrogenation strategy,[3][4] in which an ester derivative of pyruvate (namely, propargyl-pyruvate, A figure 1) is hydrogenated in an organic, hydrophobic solvent by means of an organometallic catalyst. The parahydrogenated allyl-pyruvate (B) thus obtained undergoes a magnetic field cycling (MFC) procedure that allows hyperpolarization transfer from the parahydrogen protons to the ^{13}C carboxylate spin. The hyperpolarization level obtained on the ^{13}C carboxylate signal depends on the hydrogenation step and on the MFC. In this work we have investigated the effect of different solvent used for hydrogenation on the hyperpolarization level. We observed that the addition of ethanol as hydrogenation co-solvent and the use of toluene instead of chloroform can considerably increase polarization level. This can be related to the catalytical hydrogenation mechanism. We also investigated different MFC profiles in order to improve the hyperpolarization transfer efficiency. These observations provide important clues as to how improve our method may be achieved and the polarization level on PHIP-SAH products increased.

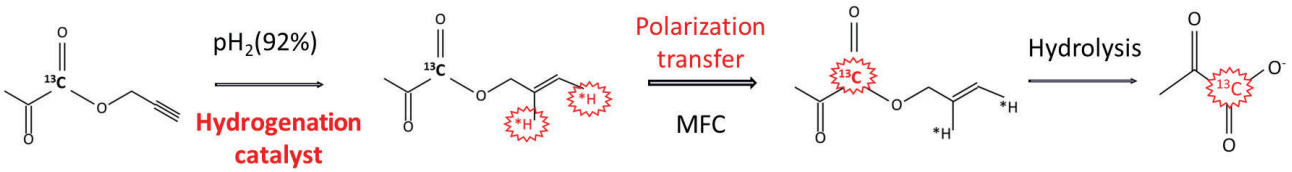

Figure 1: PHIP-SAH method.

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P5

SOLID-STATE NMR QUANTITATIVE APPROACHES FOR INORGANIC MATERIALS

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Solid-state NMR (SSNMR) has been given more and more attention as a powerful tool for quantitative applications in solid-state materials. In this sense, it has been catching on as an alternative to diffraction techniques for systems affected by low degrees of crystallinity (e.g. polymers, glasses, amorphous pharmaceutical forms, etc),[1] as well as for crystalline materials.[2] Indeed, besides the typical quantitative analysis of heterogeneous physical mixtures,[2] SSNMR proves useful to explore related matters, such as the inclusion degree of active pharmaceutical ingredients in host-guest systems,[3] degrees of functionalization of polymers and biopolymers,[4] etc.
In this work, we show how quantitative SSNMR was instrumental in two different instances. In the first case, the solid solubility limit of dimethylammonium cations in hybrid PbBr3 perovskites was evaluated;[5] in the other one, the ligands composition in mixed Zn(II) bis(pyrazolate) metal organic frameworks (MOFs) was accurately determined.[6]

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RELAXOMETRIC CHARACTERIZATION OF NOVEL Mn(II) PICOLINATE DERIVATIVES FOR MRI APPLICATIONS

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In recent years, high-spin Mn^{II}-chelates have attracted growing interest as potential MRI probes [1], alternative to the commercially available Gd^{III}-complexes, due to their intrinsically lower toxicity [2]. In particular, acyclic polydentate ligands containing picolinate groups have been extensively studied for Mn_{2+} complexation [3]. In this work, we extend this family of ligands with a new pentadentate $PAADA^{3-}$ chelating agent [4]. ^{1}H and ^{17}O NMR relaxometric characterization was carried out to determine the molecular parameters that influence the relaxing efficiency (relaxivity, r_1) of Mn^{II}-PAADA. A lipophilic derivative of $PAADA^{3-}$, bearing in the structure a dodecyloxo group attached to the pyridyl unit, ($H_3C_{12}OPAADA$) was also synthesized and compared to the previous one. The complex exhibits the ability to self-aggregate in aqueous solution (above the cmc) and to interact strongly with bovine serum albumin (BSA), leading to supramolecular structures with enhanced $r1$ values at high magnetic fields.

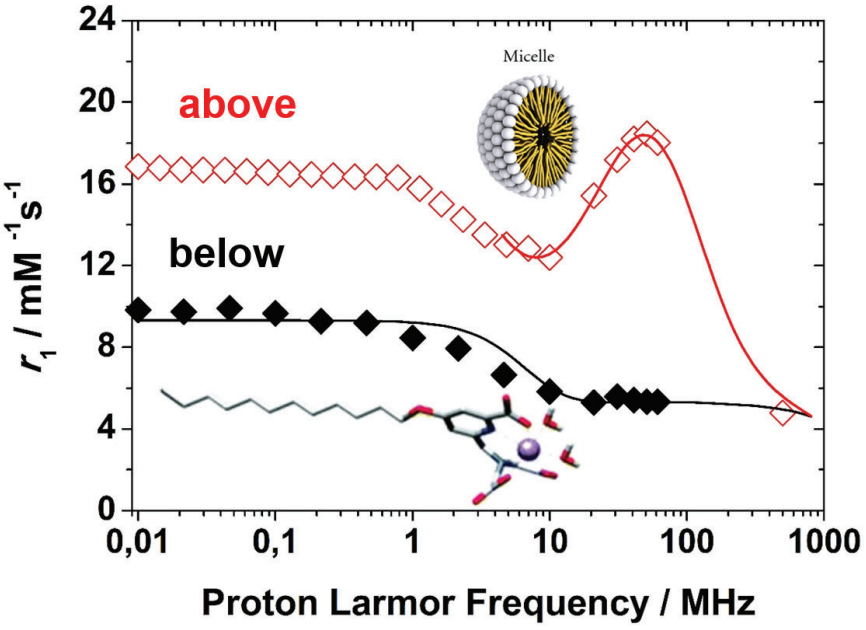

Figure 1: ^{1}H NMRD profile of the lipophilic complex in aqueous solution.

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P7

SPECTROSCOPIC EVALUATION OF GRAPHENE OXIDE FUNCTIONALIZATION WITH TRIALKOXYSILANES

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The peculiar properties of graphene oxide (GO) [1] have been recently exploited in a wide range of applications. Among them, the use of GO as nanofiller is of particular interest [2, 3, 4]. Improved dispersability and compatibility in polymeric matrices are gained generally through GO functionalization. In this regard, the organoalkoxysilanes have been widely used [5] due to the ease of manipulation and the possibility to select *ad hoc* the end chain functionality suitable for a wide range of applications. Here, the structural features of model systems obtained by functionalization of a commercial product (Graphenea) by reaction with three different organotriethoxysilanes are evaluated through solid state NMR and EPR spectroscopies. Accordingly, ^{13}C and ^{29}Si MAS and CPMAS experiments allow to determine: i) structural features of the pristine graphene oxide; ii) preferable GO reacting sites and extent of silane grafting; iii) influence on the grafting ability of the ending functional group beard by the silane, in relation with its reactivity and steric hindrance. The process of functionalization results to have impact also on the structural defects of graphene oxide sheets as confirmed by EPR analysis.

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P8

^{1}H NMR RELAXIVITY OF NOVEL COLLOIDAL NANOSTRUCTURED Gd(III)-BASED POTENTIAL CONTRAST AGENTS

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Novel nanostructured potential contrast agents were prepared following a recently reported procedure [1-3] in which water insoluble Gd(III) complexes, with ligands based either on thiacalix[4]arene or tetrahydroxy-calix[4]arene (Figure 1), self-assemble into nanosized particles by precipitation from an organic to an aqueous phase and form stable colloids by coating with poly(sodium styrenesulfonate). ^{1}H FFC NMR relaxometry was applied to measure the longitudinal relaxation rate of water protons,

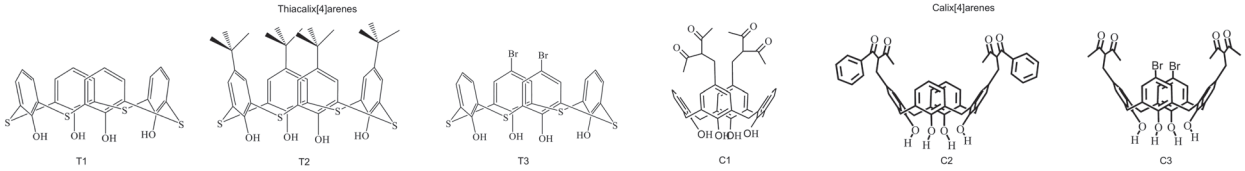

Figure 1: Ligands used to synthesize Gd(III) complexes.

R_1, as a function of the Larmor frequency in the 10 kHz to 40 MHz range. The analysis of the R_1 dispersions curves (Figure 2) allowed the mechanism at the basis of the contrast enhancement properties and the key factors affecting the contrast efficiency of the investigated systems to be highlighted.

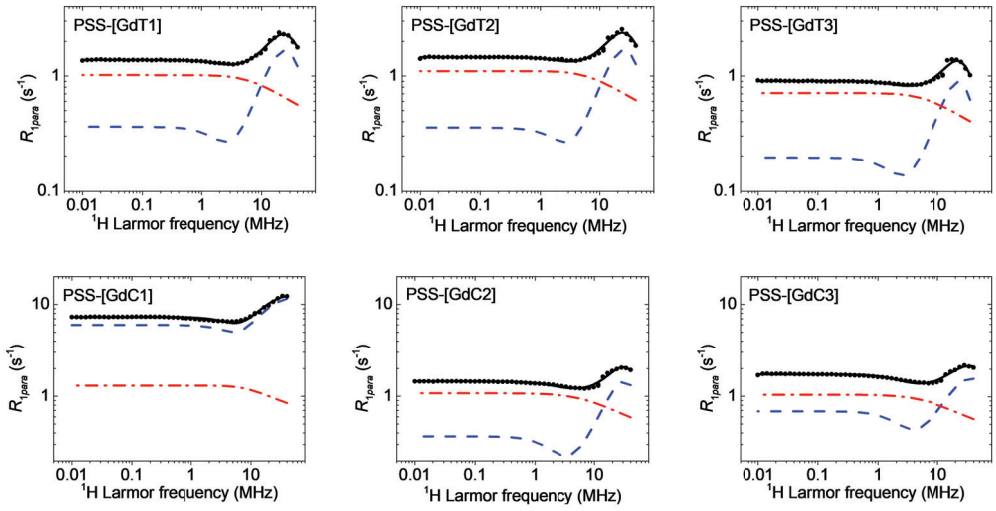

Figure 2: R_1 dispersion curves of the investigated systems at RT.

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TRAPPING OF Gd(III) IONS BY KEPLERATE NANOCAPSULES IN WATER: A ^{1}H FAST FIELD-CYCLING NMR RELAXOMETRY STUDY

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Polyoxomolybdates known as Keplerates (Kps) are polyanionic nanocapsules (diameter ∼9 nm) bearing 20 Mo_9O_9 pores connected to a central cavity by 20 channels. In solution they can entrap cations in different sites of their porous structure in equilibrium with cations free in solution. Hydrophilic colloids were prepared through the self-assembly of KpOAc or $KpHPO_4$ (*i.e.* Kp with ligand = acetate or hydrogen phosphate) macroanions and Gd(III) cations in water, further stabilized by F-127 Pluronics. The strongly enhanced water proton relaxivity observed at 20 MHz was postulated to derive from the trapping of Gd(III) aqua ions by the nanocapsules [1,2]. ^{1}H FFC NMR relaxometry allowed this hypothesis to be verified on aqueous suspensions containing either KpOAc or KpHPO4 and Gd(III) in different proportions. The analysis of the ^{1}H longitudinal relaxivity, r_1, vs the Larmor frequency

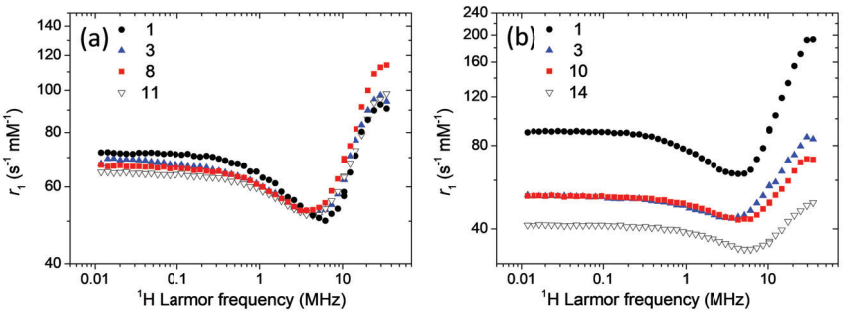

Figure 1: r_1 curves acquired at 25 °C on (a) Gdx(KpOAc) and (b) Gdx($KpHPO_4$) with the indicated x values.

curves (Fig. 1) on the basis of the theory for paramagnetic relaxation enhancement gave a detailed description of the state of $[Gd(H_2O)_8]^{3+}$ ions in dependence of the different capsule charge and Gd to Kp molar ratios [3]. $[Gd(H_2O)_8]^{3+}$ ions were found to be trapped in the KpOAc capsules, most probably located in up to 11 pores, with no evidence for free ions. On the other hand, equilibria between trapped and free $[Gd(H_2O)_8]^{3+}$ ions established in the suspensions containing $KpHPO_4$, which depended on the Gd to Kp molar ratios, and a maximum of 3-4 Gd per capsule was found. A major role of the NH_4^+ counter-ion was invoked to rationalize the different behavior of KpOAc and $KpHPO_4$.

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P10

HIGH AND LOW RESOLUTION SSNMR STUDY OF SEPIOLITE/NATURAL RUBBER COMPOSITES PREPARED BY LATEX COMPOUNDING

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Mechanical properties of rubber-based materials critically depend on addition of reinforcing fillers. Among other factors, the shape of filler particles affects the final properties and, in particular, clays can impart a larger reinforcing effect than spherical particles. Sepiolite (Sep), a natural Mg phyllosilicate, has attracted attention for its easy availability, high specific surface area, strong mechanical and chemical stability, and fiber shape.
Natural rubber latex (NRL), a colloid mainly composed by poly(1,4-cis-polyisoprene) and water, can be exploited to prepare rubber nanocomposites through the latex compounding technique (LCT), which represents an ecosustainable alternative to the well-established melt mixing technique [1]. Very recently an effective LCT procedure to produce high-loaded Sep/NR masterbatches was repoted by some of us [2]. These materials are interesting in the research for ecosustainable composites production, but it is known that metal ions, present as impurities in clays, can promote oxidation and thermo-degradation of the rubber matrix, which can also be favoured by the presence of water as solvent [3,4].The issue of controlling oxidative reactions and develop procedures able to avoid oxidation is therefore relevant.
In this context, the present work exploits a combination of high and low resolution solid state NMR experiments, with the support of DSC and FT-IR, applied to a set of Sep/NR masterbatches prepared by LTC, with the aim of investigating the effect of the Sep treatment and of the work-up procedure on the stability and dynamics of NR.
In particular, ^{1}H and ^{13}C selective MAS experiments and ^{1}H time domain experiments (MSE and CPMG sequences) were carried out on masterbatches prepared with different types of Sepiolite - either pristine or organically modified, and subjected or not to speficic acid treatmens - with the aim of understanding the effect of the sepiolite treatments on oxidation, and the relation between the oxidative processes and the dynamic properties of the rubber.

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P11

NEW FRICKE GEL WITH HIGH SENSITIVITY AND LOW DIFFUSION FOR 3D-MRI DOSIMETRY

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Fricke gel (FG) dosimeters are good candidates for 3D dose assessment in biological materials. Their effective atomic number and density are similar to those of soft tissue. In view of their chemical and morphological characteristics, FG serve as dosimeters and as phantoms at the same time. FG dosimeters are obtained by incorporating an acidic aqueous solution of ferrous ions Fe^{2+} into a gel matrix. After exposure to ionizing radiations ferrous ions are oxided into ferric ions (Fe^{3+}) which modify the relaxation times and, therefore, the 3D spatial distribution of radiation dose could be obtained through MRI. In order to address the limitations of gels based on natural matrices, we have studied FG produced with a matrix of poly-vinyl alcohol (PVA) cross-linked with glutaraldehyde (GTA). The proposed gel contains 10% w/v of PVA and GTA of 1%w/v. A common formulation agarose gel was also prepared and studied for comparison. PVA-GTA gel samples were irradiated using 6 MV x-ray clinical beams. The PVA-GTA FG was read out with magnetic resonance imaging.
MR images were recorded with a 1.5T clinical scanner in order to optimize the acquisition parameters and obtain high contrast between irradiated and non-irradiated samples. The PVA-GTA gels were found to offer good linearity in the range of 0-15 Gy and a stable signal for several hours after irradiation. The sensitivity was about 40% higher compared to gels produced with agarose as gelling agent. The analysis of the Fe^{3+} ions diffusion carried out through a 7T preclinical MRI scanner for small animals showed that the diffusion process is much slower (more than five times) for PVA-GTA gels than for agarose ones. The dosimetric accuracy of the 3D gels was investigated by comparing their response to percentage depth dose and off-axis ratio measurements made with an ionization chamber in a water phantom.

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P12

FUNCTIONAL CONNECTIVITY OF THE CEREBELLUM IN PATHOLOGICAL GAMBLER

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Our study was aimed at exploring intrinsic brain functional connectivity (FC) in a population of pathological gamblers (PGs) by using resting state functional Magnetic Resonance Imaging (RS-fMRI). Fourteen right-handed PGs and fourteen healthy controls (age 23-56, all male) participated the study. All participants underwent an extensive psychological assessment. Functional brain scan was obtained by a 1,5T scanner (GE Signa HDxt). We acquired whole brain T_2*-w gradient-echo Echo-Planar pulse sequence (33 slices; slice thickness 3 mm; TR 3000 ms; TE 60 ms). All participants were asked to quietly rest with their eyes open. A ten minutes (200 volumes) fMRI scan was performed on each participant.
The Psychological assessment showed some difference between groups: PGs showed higher levels of perceived stress ($p < 0.01$), trait anxiety ($p < 0.01$), depression ($p < 0.01$), impulsivity ($p < 0.01$) and cognitive distortions ($p < 0.01$). Using a data driven approach (Independent Component Analysis) we found an increased RS-FC in specific brain regions from PG patients compared with healthy controls ($p < 0.05$). Namely, we found a stronger connectivity in the fronto-striatal network (FSN) including the anterior cingulate, the caudate and the accumbens, as well as within the cerebellum among bilateral lobule IX, the right lobule VI and the right crus II, and the lobule X of the vermis.
The involvement of the fronto-striatal networks in reward processing and decision-making and their impairment in addiction is well established. The novelty of our findings is the interesting alteration of the cerebellar connectivity in our population of PG patients. The evidence of the cerebellar involvement opens new perspective in the comprehension of the mechanisms underlying the physiopathology of pathological gambling.

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P13

INTRA- AND INTER-SUBJECT VARIABILITY OF FUNCTIONAL CONNECTIVITY ESTIMATES IN THE HUMAN BRAIN

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A growing number of studies have focused on various methods to estimate and analyze the functional connectome of the human brain, and graph theoretical measures are commonly employed to interpret and synthesize complex network-related information. However, functional neuromonitoring data in general and resting state functional MRI in particular are known to exhibit poor reproducibility, a key factor which is commonly neglected in typical cohort studies based on connectomics-related measures used as (possibly quantitative) biomarkers. In this study we aimed to fill this gap by analyzing and comparing the inter- and intra- subject variability of both connectivity matrices and graph-theoretical measures in a large (n=1003) database of young healthy subjects which underwent four sessions of resting state functional magnetic resonance imaging each [1]. We analyzed both directed (state space Granger Causality and Transfer Entropy) [2] and undirected (Pearson Correlation and Partial Correlation) association measures and derived graph-theoretical measures. While matrix weights exhibit a higher reproducibility in undirected as opposed to directed methods (Fig. 1A), this difference disappears when looking at global graph-metrics and exhibits strong regional dependence in graphs-metrics values (Fig. 1B). Our results warrant caution in the interpretation of connectivity studies, and serve as a benchmark for future investigations by providing quantitative estimates for the inter- and intra- subject variabilities in both directed and undirected connectomic measures.

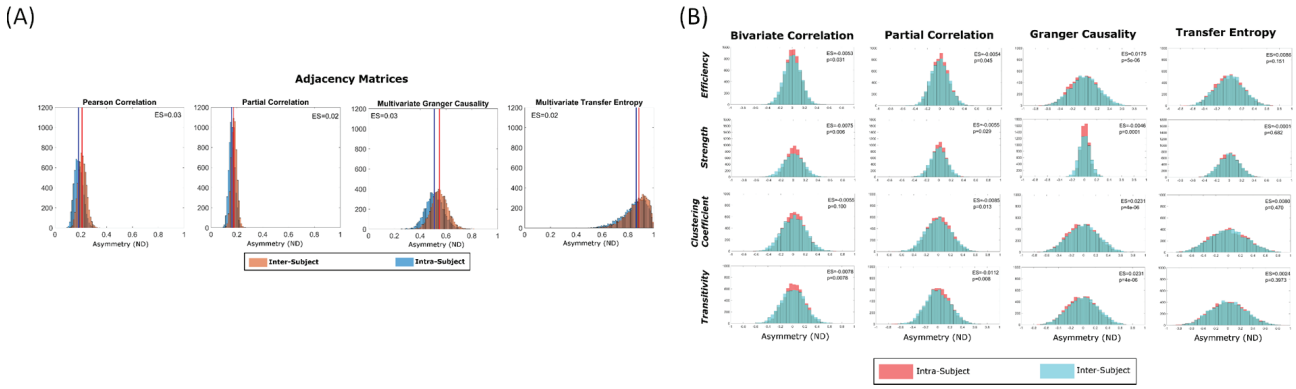

Figure 1: Intra- and Inter-subject distributions of the Normalized Differences (ND) of adjacency matrices (A) and global-graphs metrics (B) for all four connectivity estimation methods.

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P14

INVESTIGATING THE ROLE OF THE N-TERMINAL REGION IN THE OLIGOMERIZATION OF THE PROKARYOTIC ZINC-FINGER ROS AND MUCR

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The zinc fingers (ZF) were thought to belong exclusively to the Eukaryotes until the identification of the transcriptional regulator Ros protein in *Agrobacterium tumefaciens*. The C-terminal part of Ros contains a classical Cys_2His_2 ZF (Ros87) that folds in a globular domain significantly larger than its eukaryotic counterpart stabilized by a conserved, extensive, 15-residue hydrophobic core [1].
Since the discovery of this protein, many other members of the Ros/MucR family, homologous of Ros, were found in the α-proteobacteria, like MucR from *Brucella*, which regulates the pathogenicity and of these bacteria. Despite the structure and function of the C-terminal domain of these zinc-fingers have been well investigated [2], only recently the quaternary structure of the full-length proteins was studied, demonstrating their capability to form oligomers [3].
In this study, by means of Nuclear Magnetic Resonance (NMR) and Molecular Dynamics (MD) techniques, we structurally characterize the role in the oligomerization of the N-terminal region of two members of the Ros/MucR family, the homologous Ros from *A. tumefaciens* and MucR from *Brucella*.

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P15

VERY LOW MAGNETIC RESONANCE IMAGING USING SLICE SELECTION

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Since the past decade, the possibility of obtaining images at the ultra-low field (ULF) and very-low field (VLF) range has met an increasing interest in the scientific community. Despite the signal-to-noise ratio (SNR) reduction, there are several advantages to operating at lower field: an increased tissue contrast, reduced cost and weight of scanners, the potential to image patients that are not compatible with clinical scanners and the opportunity to integrate different imaging modalities such as magnetoencephalography (MEG). However, a critical issue in ULF MRI is the acquisition time, which hampers the effective operation of these systems. Increasing the field in the VLF range could allow to obtain MRIs in a shorter time. With this aim, we implemented an 8.9 mT scanner built to be compatible with a MEG environment [1] and we are currently testing the implementation of several acquisition sequence patterns on the system.
Recently we implemented a slice selection sequence with cartesian and homogeneous 2D k-space filling. The aim is to obtain 3D information through multi-slice selection keeping time acquisition lower than a simple 3D cartesian acquisition. Recent results obtained with these sequences will be presented.

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P16

CHARACTERIZATION OF A NOVEL PACKAGED HYDROGEL DRESSING BY MRI

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Biocompatible nanomaterials and hydrogels are increasingly applied in the biomedical field, including drug delivery systems, cell therapy, tissue engineering, medical devices and bandages [1-3]. We have recently developed a new process for the manufacturing of a sterile, packaged hydrogel bandage, based on an interpenetrating structure of calcium alginate, agar and polyvinylpyrrolidone [4]. This new process makes easier the treatment of patients suffering for difficult wounds, has a beneficial effect on the tissue repair process and expresses a better resistance to mechanical deformations compared to products on the market. In this work we present the 2.35T MRI characterization of the new hydrogel sterile bandage, considering a commercial hydrogel (Neoheal) as a standard reference. Axial T_1-weigthed Rapid imaging with refocused echoes with variable repetition time (RAREVTR) images were acquired to map the longitudinal relaxation time T_1 using the following parameters: TR=[16-8000] ms; TE=10 ms; tp=2.25 ms; BW=43 kHz; flip angle=90°; FOV=62.8 mm×62.8 mm; 128×128 pixels; spatial resolution 0.49 mm/pixel; slice thickness=3 mm; NEX=1; TACQ=58 min. Typical RAREVTR T_1-W (TR=370 ms, TE=10 ms) images of the hydrogel materials under study are shown in Fig. 1. Our MRI experiments at 2.35 T show that the T_1 of the novel hydrogel (MID-PVP-γ) decreases significantly (around 30%) compared to the commercial dressing (Neoheal). These results indicate that the T_1 of the water molecules within the hydrogel matrix is more efficient, due to the strong dipolar interactions with the microscopic surrounding environment. The concentration of free H_2O varies along the thickness, whereas the T_1 profile is constant. This could be seen as the water expelled because of the volume contraction.

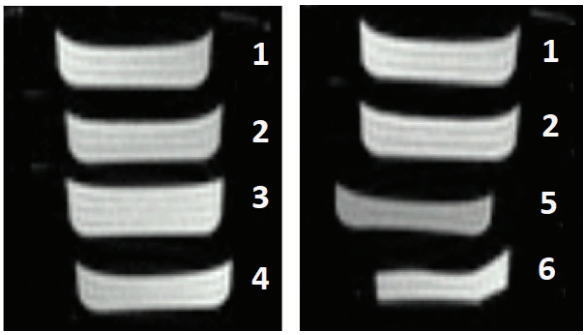

Figure 1: Axial T_1-W RARE images: FOV=47 mm for all images and ROIs labelling is: 1=MID-PVP; 2=MID-PVP-γ; 3=STD-PEG-γ; 4=STD-v; 5=MID; 6=Neoheal.

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P17

MULTIMETHODOLOGICAL NMR-DSC-IR APPROACH FOR THE CHARACTERIZATION OF TYPICAL PECORINO CHEESES

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Pecorino cheese is an Italian diary product obtained from raw or thermized ewe's whole milk. Milk is coagulated with different types of rennet and, after cutting, the curd can be cooked or not. After whey drainage and salting, cheese is ripened for a typical time in which its chemical composition and organoleptic properties evolve. During this period, indeed, enzymatic and bacterial action causes a deep transformation of milk macronutrients leading to the development of typical textures and flavors associated with mature cheeses [1]. Most varieties of Pecorino cheese produced in Italy have a strong geographical identity, because the typical cheesemaking conditions adopted in specific territories, together with the origin of milk, affect their peculiar taste and flavor. In a number of cases, the relationship between the Pecorino cheese and the production area has been officially recognized by the attribution of specific certifications, including Protected Designation of Origin (PDO) [2] and the inclusion by Slow Foundation for Biodiversity in the list of traditional food to safeguard. In this work, the potentiality of low-field unilateral ^{1}H-NMR in the characterization and dating of typical Pecorino cheeses with different ripening time is investigated. Water content was primarily analyzed by means of Differential Scanning Calorimetry (DSC) and Fourier-Transform Infrared Spectroscopy (FT-IR) techniques. These two approaches are combined with NMR measurements aimed at characterizing the water phase confined within the cheese matrix. Two-dimensional T_1-T_2 ^{1}H NMR relaxation correlation spectra in particular allow to detect water in different solid-like micro-environments, since its molecular mobility strongly influences the T_1/T_2 ratio value of protons [3]. A surface-NMR equipment (mq-ProFiler; Bruker, Italy) was applied with this aim, as non-destructive method.
Here we present preliminary NMR-DSC-IR data collected on typical Italian Pecorino cheeses. Multivariate statistical approaches are applied to handle the multimethodological experimental data in order to evaluate the potentiality of this approach in the discrimination of Pecorino samples according to the ripening time and cheesemaking techniques.

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P18

NEURAL GRANGER CAUSALITY FOR EFFECTIVE FMRI CONNECTIVITY

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While Granger Causality(GC)-based approaches have been widely employed to the estimation of information flow between brain regions (i.e. directed connectivity) they are based on linear multivariate autoregressive (MVAR) models. However, biological networks such as the brain are known to exhibit a high degree of nonlinear coupling. Therefore we reformulate the classical GC framework exploiting a specific class of Recurrent Neural Networks termed Echo-State (ES) networks [1] (Fig.1, Left).
We characterize the ability of ES-GC to capture nonlinear causal relations by simulating multivariate coupling in a network of nonlinearly coupled, noisy Duffing oscillators. The ability of detecting true causal links while rejecting false causal links is quantified as the area under the ROC curve (AUC) as a function of the threshold in causality strength. Synthetic validation shows a net advantage of the ES-GC in detecting nonlinear, causal links across different timescales. Figure 1 (centre) shows AUC differences between employing the classical MVAR conditioned GC approach (AUC=0.49, *i.e.* chance-level performance), the Kernel (K)-GC (AUC=0.73), the improved State-Spaced (SS)-GC (AUC=0.77) and our novel ES-GC (AUC=0.92) demonstrating a net advantage of ES-GC. We then explore the structure of ES-GC networks in the human brain fMRI data from 1003 healthy subjects scanned at rest at 3T (TR=0.72s) within the "HCP 1200-Subjects PTN Release" by employing the subject-specific time courses of 15 independent components (Fig.1, Right). We disclose a strong bidirectional interaction between the Default Mode Network and the Salience network, a direct modulation of the fronto-temporal network by the fronto-visual network (but not vice versa) and a direct modulation of the striate-visual network by the sensory/motor-limbic-network.

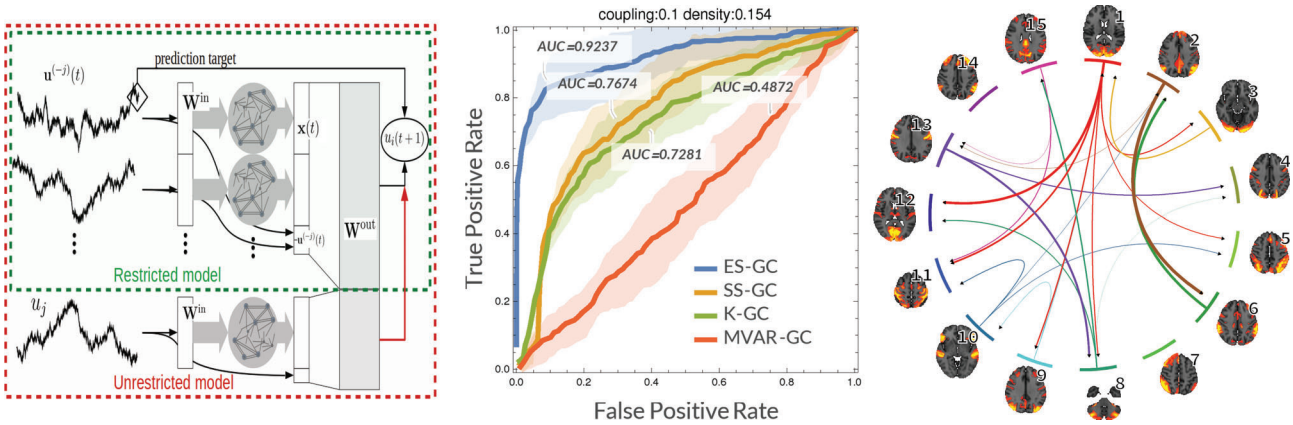

Figure 1: Left: schematic representation of ES-GC method. Centre: ES-GC performance (AUC). Right: summary of directed, between-component brain connectivity in vivo (top 1% in strength).

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P19

PRELIMINARY NMR CHARACTERIZATION OF THE OSCP SUBUNIT OF HUMAN F-ATP SYNTHASE

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The protein OSCP (Oligomycin Sensitivity Conferral Protein) is one of the mitochondrial F1-Fo (F) ATP synthase subunits and it is located in the upper part of the peripheral stalk of the enzyme. It is a highly flexible, α-helical protein that represents a biological host for different binders like cyclophilin D or Bz423 [1]. Thanks to its flexibility it can potentially influence the F-ATP synthase structure and so its enzymatic activity with important consequences on the bioenergetics of mitochondria and finally the cell life itself. OSCP is strategically located on the top of F-ATP synthase, where it is easily accessible to protein interactors and drugs. These unique features make it a potential pharmacological target for the modulation of F-ATP synthase [2]. There are several works dedicated to the bovine OSCP structure but no one regarding the human isoform [3]. Moreover, due to the difficult in obtaining the whole protein or its C-terminal domain avoiding aggregations problems, only the N-terminal domain has been characterized by NMR methods. We have developed protocols to obtain the recombinant N-terminal and C-terminal domains of human OSCP suitable for the NMR analysis and we will present here their preliminary characterization.

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P20

DECOUPLING OPTIMIZATION FOR $^{1}H/^{23}Na$ DOUBLE TUNED NESTED RF BIRDCAGE COILS CONFIGURATION FOR PRE-CLINICAL MRI APPLICATIONS

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The Double Tuned (DT) Radio Frequency birdcage coil is one of the most used configurations in Magnetic Resonance Imaging (MRI) [1] to detect both the ^{1}H and X-nuclei signals but coupling between the two channels is a critical problem [2]. For $^{1}H/^{23}Na$ at 2.35 T we studied the decoupling optimization of DT nested birdcages RF coils. We showed, using numerical and workbench tests, that a suitable geometrical selection of the two coaxial birdcages (relative angular orientation, diameters and lengths) and RF shield (diameter, length) allows a significant decoupling optimization (Fig. 1), providing valuable information about the RF B_1 field homogeneity and efficiency.

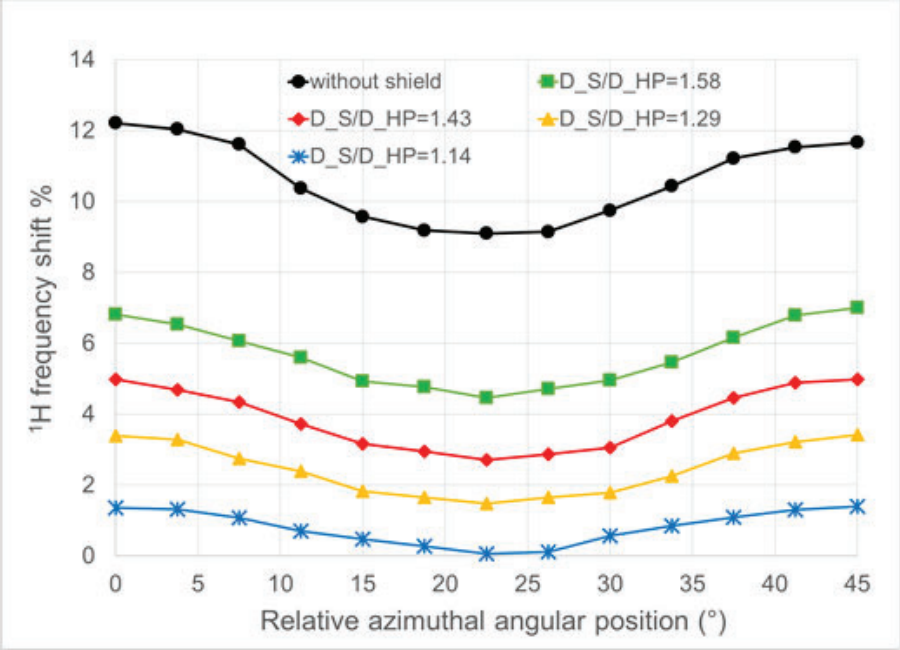

Figure 1: FEM simulations of the percent frequency shift $\Delta f/f$ for the ^{1}H useful MRI mode versus the relative azimuthal angular position for: (i) birdcages' length ratio 0.65, (ii) different shield diameters.

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P21

OPTIMIZATION OF A NMR AND *IN-SILICO* FRAGMENT BASED DRUG DISCOVERY APPROACH FOR THE BCL-2 PROTEIN FAMILY

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Bcl-XL and Bfl-1 are two members of the Bcl-2 protein family and they are involved in the regulation of apoptosis. Specifically, their physiological role is to bind proapoptotic members of the family, such as BAK and PUMA, and to prevent cellular apoptosis. Overexpression of these proteins causes cellular death avoidance and this is associated with tumorigenesis of both solid and liquid tumors. Moreover, it has emerged as a resistance mechanism against common anticancer therapies [1]. Fragment Based Drug Discovery (FBDD) is one of the most efficient methods to identify high affinity ligands. This method consists of identifying low molecular weight molecules (lower than 300 Da, typically), called "fragments", that have low affinity for the protein (μM $< K_D <$ mM) and combining them chemically to obtain stronger binders [2].
The focus of this work is the creation of a Molecular Dynamics (MD) protocol for the analysis of interactions between fragments and proteins based on NMR validation. In literature there is only one MD protocol for the analysis of fragments but it has not any experimental counterpart: this work wants to fulfil this gap.
Firstly, an NMR-based screening and a MD-based one were conducted separately on Bcl-XL and on the same set of 100 fragments. These independent results were then compared and the Molecular Dynamics parameters were more finely calibrated on the results of the experimental screening. Finally, a screening on a wider library of 300 compounds was carried out using only the validated-on-experiments MD approach. Only the ten best potential binders identified in this virtual screening between the initial 300 ones were experimentally analysed also with NMR.
A similar analysis is going to be carried out also on a 400 compound library on Bfl-1. By now, the greater bottleneck for Bfl-1 analysis had been the amount of the protein. Investigations to obtain protein yields compatible with NMR analysis through recombinant protein expression and purification were carried out.

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P22

LOW RANK APPROXIMATION FOR 1D DATASET: LOOKING FOR ALTERNATIVES TO DENOISE METHODS BASED ON HENKEL MATRIX

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The low sensitivity of NMR experiments forces to the use of long acquisition times which cause an increase of the experimental set-up complexity and a reduction of the analysis throughput. Therefore, noise reduction methods are important to obtain high signal to noise ratio spectra for low concentration samples or low giromagnetic factor nuclei.
Low rank approximation is a flexible framework for noise reduction in NMR multidimensional dataset. For 2D dataset the truncated singular value decomposition is the solution to the low rank approximation problem with Frobenius norm. To apply low rank approximation methods to 1D dataset the conversion of the vectorial signal into a matrix with low rank is needed. The classic solution to this problem is to create a square lag matrix M(i,m)=v(i+m-1) in which the i-th row is the portion of the signal starting at the i-th signal value with length N. This matrix is a Hankel one and its rank equals the number of exponential components in the signal due to the auto regressive properties of the exponential function. Thanks to this technique, it is possible to create a low-rank matrix from a 1D signal.
In this contribution I propose a different approach to create a low rank matrix from a single NMR signal: the use of transformation which eigenfunctions are the exponential functions.
If a signal S(t) is a linear combination of eigenfunctions of a transformation G(.), than S'(t)=G(S(t)) is still a linear combination of the original components. The matrix in which the i-th row is obtained applying the G(.) transformation to the i-1 th row has rank at most equal to the number of signal components.
With my work, I present the preliminary results of the use of this method on synthetic datasets representing the different application in low and high resolution NMR.

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CHARACTERIZATION OF DIFFERENT CULTIVAR OF GRAPE USING MRI TECHNIQUE

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Grape (Vitis vinifera L.), is one of the most important commercial fruit crops of temperate zone, it is widely farmed in France, Italy, America, Africa, Australia, Chile, Algeria, India, etc. This fruit can be eaten fresh as table grapes or it can be used to produce wine, jam, juice, jelly, grape seed extract, raisins, vinegar, and grape seed oil.
The sugar content or its accumulation in a berry is usually measured in °Brix units using densitometry or refractometry. High values of soluble solids (°Brix) indicate a high degree of ripeness, which normally ceases at about (25-26)°Brix in the late stages of ripening [1]. In grape berries, it is possible to follow the ripening process by the MRI estimation of the degrees Brix values of berries using T_1 relaxation, which are in good agreement with the refractometry results. Shorter T_1 values are reported for ripe grape berries, associated with the higher sugar content in the ripe stage (fig.1) [2]. This study evaluates the potential of MRI as a nondestructive technique to characterize the different cultivar of grape. Models for characterization of grape in terms of sugar content (°Brix), water content and relaxation times (T_1 and T_2) are developed.

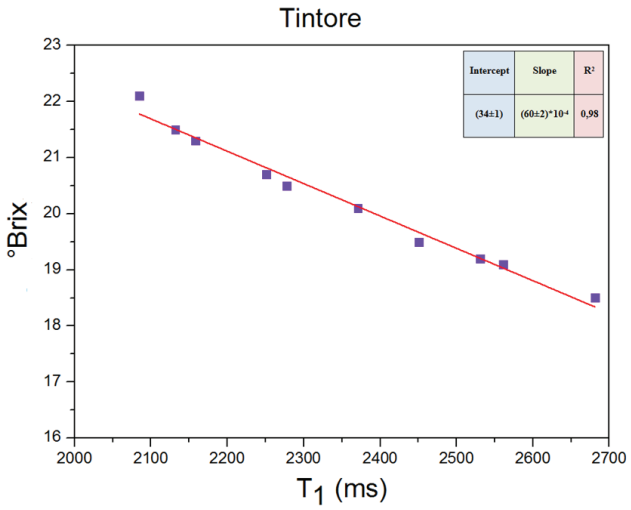

Figure 1: Correlation of °Brix and relaxation time T_1 for the Tintore grapes.

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TRUFFLES POST-HARVEST STRUCTURE CHANGES BY 1T MRI

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Magnetic Resonance Imaging (MRI) has been extensively used in food science [1], offering a non-invasive method to evaluate changes in both structure [2] and relaxometry parameters related to food quality. We evaluated the evolution of n=6 truffles, maintained at 2 °C inside absorbing paper, during a period of 17 days. An Aspect M3 scanner (1T) was used to extract Proton Density (PD), T_1, T_2, ADC maps from SE sequences (1x1x1 mm^3 spatial resolution) on samples after their thermalization at 21 °C. The above data set was complemented by weight measurements.
Results show a marked reduction of all the measured parameters with time. The weight reduction is larger than the PD one, thus suggesting that volatile compounds produced inside the sample are responsible of a consistent fraction of mass loss. The above finding is consistent with the observed T_1 and T_2 variations that suggest the ongoing of internal microbiological activity.

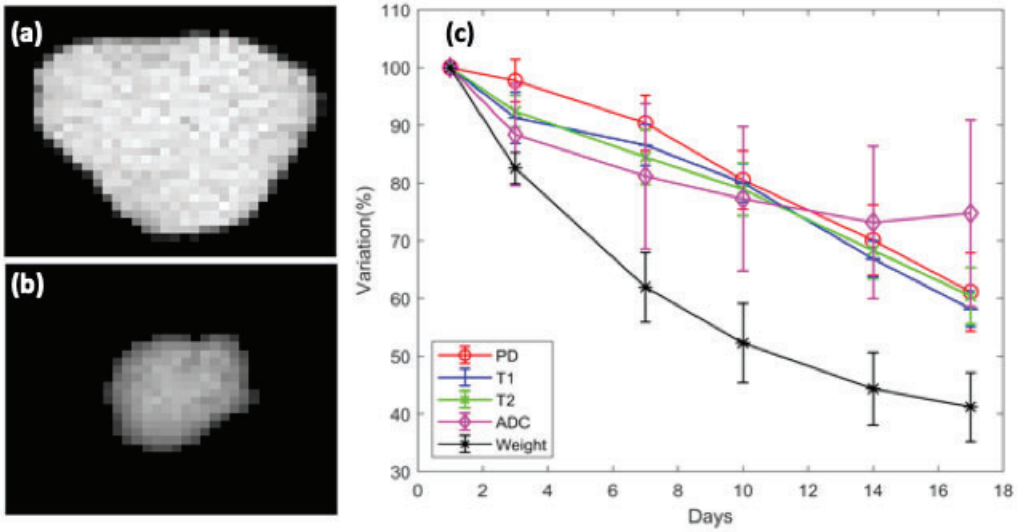

Figure 1: MRI images (Proton density weighted images) of the same truffle on first day (a) and after 17 days (b). Both volume and water content reduction are clearly seen. Panel (c) shows the time course, expressed as percentage changes relative to the first day (mean and standard deviation), for PD, T_1, T_2, ADC and mass weight.

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P25

EFFECT OF DATA PROCESSING ON NON-TARGETED NMR ANALYSIS

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Nuclear Magnetic Resonance (NMR) spectroscopy is a recognized analytical method able to furnish a huge amount of information rapidly, in a non-destructive and reproducible way. Several algorithms have been introduced to facilitate the statistical elaboration of a large number of data. A pre-processing step, involving data bucketing and bucket scaling, is always required before submitting the data to the statistical elaboration.[1,2] So far, the choice of the pre-processing approach has been based mainly on the observation of the best matches according to the initial goal, and no unique criterion has been introduced. In this communication, we describe the results observed during a chemometric study conducted on a large amount of data deriving from an interlaboratory comparison involving 65 spectrometers, which were different in manufacturer and magnetic field strength. In particular, we demonstrated that variable bucketing and scaling modes can stress the importance of different factors (nature of sample vs instrument features) influencing the observed data distribution (Fig. 1).

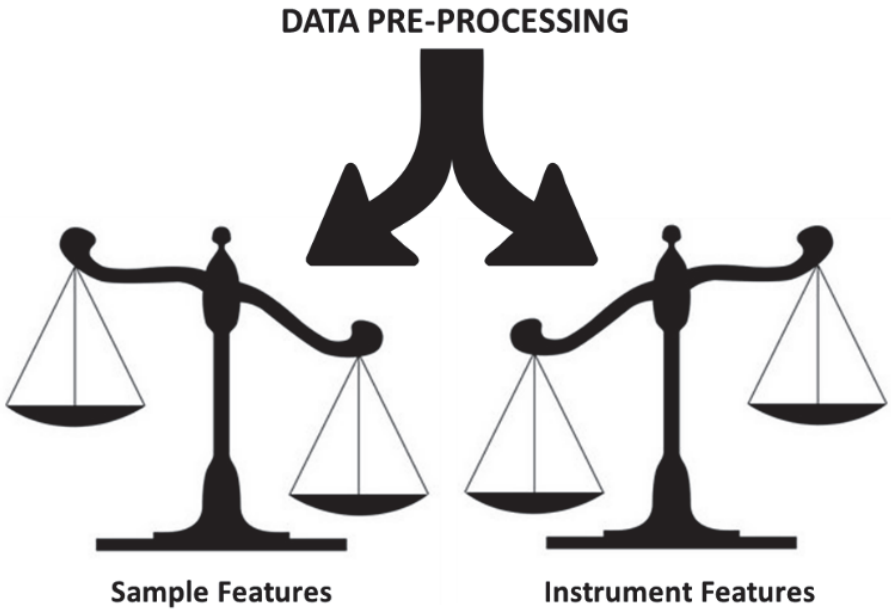

Figure 1: Schematic representation of the influence of data pre-processing mode on the factors causing the discrimination of data distribution.

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P26

NOVEL QUADRUPOLAR PEAKS BASED CONTRAST AGENTS

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The present study aims at developing an innovative class of MRI contrast agents for Fast Field Cycling-MRI applications. They represent a completely new class of 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. The peculiar property of the proposed agents relies on the generation of ^{14}N-Quadrupolar Peaks (QPs) that cause a relaxation enhancement of water protons at the proton NMR frequency corresponding to the ^{14}N quadrupolar resonance frequency.[1] The QPs from these innovative contrast agents has to fall at frequencies well distinguishable from those associated with the amidic peptide bonds from endogenous proteins. This study relies on an innovative technology, FFC-MRI, which opens new avenues for non-invasive imaging technologies with human applications. The uniqueness of this technology relies on its ability to image how the magnetic relaxation time of materials varies with the magnetic field strength. In particular, FFC allows detecting the quadrupolar cross-relaxation, appearing as peaks (QPs) in the $1/T_1$ dispersion profile completely invisible to conventional (fixed-field) MRI. 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). We expect that this technique can be exploited 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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CHARACTERIZATION AND FUNCTIONALIZATION OF SWITCHABLE CONTRAST AGENTS FOR NANOTHERANOSTICS IN MRI

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Exploiting conventional magnetic particles, the chance to use the same nanoparticles (Nps) as contrast agents and as therapeutic agent for cancer treatment is an open challenge [1]. Moreover, the hyperthermal localized treatment of cancerous tissues could be difficult due to the low control of the overheating effect. In this study we characterize a new class of nanoparticles that have the property of self-regulating heating effect. The importance of this new Nps lies in a multiple effect, due to the chemical composition when a specific phase transition occurs the material could be able to switch contrast ratio in MRI. The aim of this study is to functionalize and characterize this new class of nanoparticles in order to exploit them as switchable contrast agents in MRI and for magnetic fluid hyperthermia (MFH) [1]. In vitro relaxometry and in vivo MRI biodistribution were performed as structural and morphological characterization of the naked and coated nanoparticles [2]. TEM analysis and DLS size measurement were performed before and after the functionalization with a double shell of citrate and glucose. The stability of the double shell, evaluated by z-potential, was -50 mV [3]. Cytotoxicity assay on HeLa cells and MDA-MB-231 demonstrates low toxicity at concentrations of Nps up to 150 μg/mL.
MRI relaxivity was tested showing promising results: the relaxivity coefficient $r2$ was 32 $\mathrm{mM^{-1}s^{-1}}$. After injection via tail vein of functionalized nanoparticles (concentration of 2 mg/mL) biodistribution of the contrast agent has shown a slow accumulation in liver up to 24 hours. MRI signal has been collected and has been observed that the T_2 value of the liver decrease of 30%. MFH provided promising results with an increase of temperature of 14,0 $\pm$ 0.5 °C. Ongoing in vitro test to demonstrate switching contrast ratio property are performed in MRI. MFH will be evaluated in vitro on HeLa cells and MDA-MB-231 and then in vivo on mouse tumor.
*The research has received the financial support of Fondazione di Ricerca Nanoteranostica per la cura del cancro RNC, Treviso, Italy

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P28

UP-TO-DATE INDUSTRIAL APPLICATIONS OF TIME-DOMAIN NMR

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Avalanche-like explosion of digitalization and cloud technologies of the Internet of Things (IoT) nowadays embrace nearly every branch of human activity starting from intelligent kitchenettes, amateur DIY by micro-controllers, wireless remote car maintenance and ending up at total automation of factory mass production, logistics and artificial intelligence in companies' boards of directors. According to forecasts of experts the market of sensors that are involved in digital life flow will be increased in a geometrical progression for the upcoming decade. This fact is bringing up new requirements for every analytical technique to migrate from stationary laboratory setups to inexpensive robust miniature modular sensors that can be immersed in standardized online solutions.
There is a good chance for the Time-Domain NMR (TD-NMR) to merge into this trend involving recent progress in permanent magnets, microelectronics and embedded software technologies. The presented talk is uncovering some aspects of this trend:

1. Development of miniaturized and cost effective hardware;
2. Easiness of installation;
3. Very robust, accurate and fast measurement techniques;
4. Reliable models of Sensor+Sample (development of Digital Twins)
5. Accessibility for online performance control and data analyzing/storing via Application Programming Interface (API) of IoT systems

The following measurement techniques of TD-NMR will be discussed:

- Solid Fat Content (SFC) and Total Fat Content (TFC) - mostly in food products
- Oil content - in food, seeds, artificial tissues (Spin Finish)
- Sugar content - in food and dairy products
- Moisture - in food, gun powders, sand, fuels (calorific value)
- ^{1}H (proton) density - for fuels, polymers...
- Na, Fe, Al, P, Ca, F, N... content
- Droplet size distribution - oil and water droplet sizes in emulsions
- Specific Area - for particles dispersions and sorbents
- Particles size - for particles dispersions
- Pores volume - for adsorbent, construction materials
- Molecular weight and chain length - for medium size oligomers and fats
- Crystallinity - from sugars and celluloses to artificial polymers, their solubility and density
- Crosslinking - for elastic polymers network
- Purity of water and paramagnetic ions in trace concentrations for environmental research

P29

IMPACT OF LOW PERFUSION COMPARTMENT ON GBM PATIENTS' SURVIVAL

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Glioblastoma classified as Grade IV glial tumour carries the worst prognosis. The overall survival of patients remains low at 14.5 months, but that is different among patients. Tumours exhibit intratumoral heterogeneity in diffusion and perfusion MRI, which might have prognostic significance and influence therapy response. Our purpose was to evaluate MD and CBV maps based on patients' survival to investigate their effects on survival. They may assist to planning treatment strategies.
12 GBM patients with known survival were selected from an individual database at ITAB from 2011. All patients underwent MRI examination with a 3T Philips Achieve Scanner, and pre-operative tumour protocols consists of T_1 with GD, DTI with b-value of 800 and DSC. Data was pre-processed using FSL to eddy current correction, brain extraction and registration. All images were co-registered on DTI-B_0 images. The MD and rCBV were calculated from diffusion and perfusion imaging, respectively. The ROIs were drawn on every T_1-Gd and T_2-w tumour section by a neuroradiologist and transferred to the MD and rCBV maps to drive volume-based data of the entire tumour. By K-means clustering algorithm, low and high diffusion and low perfusion clusters were selected. Regions of lowest rCBV with the lowest MD and highest MD were identified to evaluate their effects on survival. Patients' survival was considered in 3 groups which consist less than 200, between 500-700, and 1000 to 2000 days.
First using an SVM trained model, patients' survival using all data were classified with 80.55% accuracy (permutation test, $P<0.001$). The sensitivity and specificity for each SVM classifier were 91.66, 83.33, 66.6 and 87.5, 91.66, 91.99 respectively.
The differences between MD and CBV histogram parameters and survival were assessed using pearson correlation coefficient and linear regression ($P<0.01$) as well. Among these factors, 4 were independently associated with predicting the survival. Low perfusion values in restricted diffusion region values had higher correlation coefficient, then MD values in high diffusion. Low perfusion values in high diffusion region were smaller than in low diffusion.
In this study we combined perfusion and diffusion MRI to identify two low perfusion compartments that may be responsible for treatment resistance. The method was able to predict survival significantly.
The decreased MD values are correlated with shorter survival. Low perfusion in the restricted diffusivity compartment suggests this compartment may contain other microstructures such as hypoxic which will be responsible for treatment resistant.
The negative correlation between low diffusion tumour volume and survival could indicate tumour grows. Tumours with larger low perfusion area had shorter survival which might be to prone treatment resistant due to hypoxia.

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P30

CHEMICAL ANALYSIS OF THE FOOD WASTE FOR POTENTIAL SUSTAINABLE HEALTHY PRODUCTS

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The aim of this study is to prompt the recovery of industrial by-products through the production of new functional foods; it takes advantage from new throughput technologies with low environmental impact and high economic sustainability. In the field of fish processing, in order to recover the worthy protein-rich fish waste, residues from the production of Anchovies (*Engraulis encrasicolus*), have been converted into hydrolysate through enzymatic treatment. The obtained hydrolysate product showed a promising biological and nutritional content made by differently sized peptides and free amino acids endowed with assessed benefic effects [1, 2]. The study showed the possibility to produce a dry powder with an activity water (aw) of 0.3-0.5 and an essential amino acids (EAA) fraction of 42.0% over the total amino acids (TAAs). These results pave the way to the smart recovery of commercial products featured by high nutritional value, either as stand-alone items or as components of functional foods.

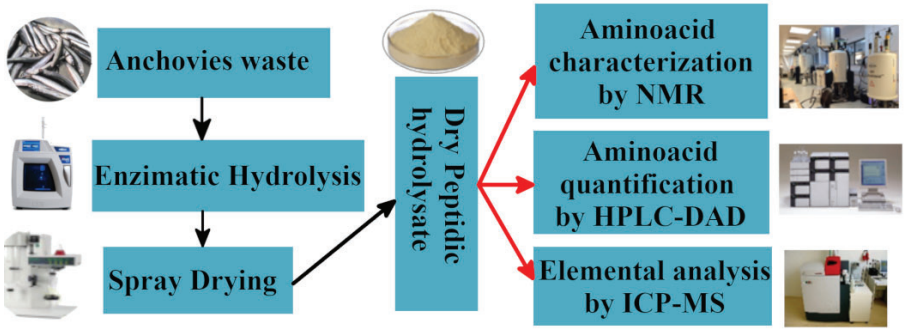

Figure 1: Scheme of the process performed on food waste.

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P31

THE IMPORTANCE OF BUCKETING PROCEDURE FOR NMR-BASED METABOLOMIC FINGERPRINTING

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NMR spectroscopy is a powerful, versatile and reproducible technique used for structure elucidation and for the analysis of complex biological matrices. However, many parameters, such as temperature, pH, concentration of salts and ionic strength can influence peak location leading to frequent misalignments [1]. Subsequently, chemometric classification models will include information from these shifts and final results will be strongly affected. "Bucketing or binning" represents a useful procedure to compensate for these misalignments and it is also used to reduce the total number of variables [2] by dividing NMR spectra into small "bins" typically spanning 0.02 - 0.04 ppm. [3] Although there are different bucketing techniques [3], the equidistant binning is the most commonly applied method and works quite well despite its simplicity. This approach is particularly useful to perform untargeted NMR-metabolomics via fingerprinting which allows the rapid evaluation of the spectrum as a whole in the way it can be considered a "fingerprint" of all (assigned or not) detectable metabolites in the biological sample under study. [2] But, how can NMR-based metabolomic fingerprinting be affected if we use different ways to perform the bucketing procedure? Can sample classification and patter recognition change? In this study, we address these questions using large cohorts of urine and serum NMR spectra to evaluate firstly, the change of the bucket width going from considering the full resolution spectra to bins of 1 ppm and secondly, the effect of shifting the starting point of the bucket integration. The results achieved suggest the best compromise to choose among bucket width values, signal-to-noise ratio and starting point of binning integration to perform NMR-based metabolomic fingerprinting. They also confirm the use of the bucketing technique as the best and the easiest method to keep all the necessary information to perform sample classification.

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P32

STRUCTURE AND DYNAMICS OF ELASTOMERIC MATERIALS BY MEANS OF ^{1}H TIME-DOMAIN NMR: EFFECT OF CROSS-LINKING

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Elastomeric materials are nowadays of central importance in many fields of application, where they need to fulfil specific mechanical requirements. The mechanical properties of an elastomeric material take their origin on the features and topology of the polymer network. In fact fixed chemical cross-links and physical entanglements among polymer chains impose notable restrictions on chain mobility and are at the basis of rubber elasticity [1]. An additional reinforcement effect can be achieved by incorporation in the rubber matrix of different nanoparticles, such as carbon black, carbon nanotubes, nanosilica, and clays [2, 3]. So far extensive research efforts have been addressed to the comprehension of the relationships between the "molecular" and mechanical properties of elastomeric materials, but a full understanding is still lacking. In this frame, NMR spectroscopy can play an important role giving access to many structural and dynamics information on wide spatial and time scales.
In this work we applied a combination of different time-domain NMR (TD-NMR) techniques to the study of elastomeric materials based on isoprene, butadiene and styrene-butadiene rubbers, with application in the tyre industry. In particular the influence of chemical cross-links on the polymer chain dynamics in a wide spectrum of motion frequencies was investigated, by studying samples obtained using different vulcanization conditions. ^{1}H Multiple Quantum (MQ) experiments [4] were used for the measurement of the residual ^{1}H-^{1}H dipolar interaction: the latter is dependent on the anisotopic character of the fast reorietations of chain segments and, therefore, it is related to the amount and distribution of the topological constraints within the polymer network. Further and complementary information on different regimes of polymer dynamics were also obtained by means of measurements of ^{1}H spin-spin relaxation times (T_2) and variable temperature ^{1}H T_1 Fast Field Cycling (FFC) [5] experiments.

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GEOGRAPHICAL ORIGIN OF ITALIAN BUFFALO MOZZARELLA CHEESE: PRELIMINARY STUDIES ON A COMPLEX FOOD SUPPLY CHAIN BY ^{1}H HR-NMR SPECTROSCOPY

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Buffalo Mozzarella Cheese is a popular Italian product and "Mozzarella di Bufala Campana" is a food product with a Protected Designation of Origin (PDO) known as one of the most popular and appreciated of Italy. In order to fight the phenomena of frauds and adulterations, it is growing the interest in developing of new analytical method to detect them. The supply chain of Buffalo Mozzarella Cheese is complex. With the purpose to approach this Food Supply Chain, an analytical method using High Resolution Proton Nuclear Resonance Spectroscopy (^{1}H HR-NMR) was developed using untargeted food fingerprinting approach coupled with multivariate statistical analysis in order to obtain, validate and evaluate classification and predictive models, fit for the discrimination of the geographical origin of samples. The workflow provided an initial first preliminary study on a very simple system, taking into account only small farms that include own dairy farm and also buffalo breeding; this study showed good predictive performance in terms of geographical origin. These first results encouraged the investigation and another more complex study was developed increasing the number of samples collected in a wider range of Italian regions and including more different dairy farms in the same place in order to obtain, finally, a more detailed study that represents the real samples in the Buffalo Mozzarella Cheese Supply Chain. The second preliminary model, much more ambitious in terms of determining geographical origin, have had different performance compared to the first one.
To understand the differences between the two built systems, only the samples with the same criteria of the first preliminary work were selected, from the second study, to build a new smaller classification model. In this case, obtained model resulted similar in the performance of the geographical origin classification of samples to the first one.These studies used more than six hundred samples collecting from more than one hundred dairy farms. The results suggest that it is necessary to implement the study with additional information of the dairies and their system of production. This is important to better understand all the variables involved in the study and to develop, in the future, new more effective models to classify and predict the geographical origin of real samples in a Complex Food Supply Chain.

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PUTATIVE REORGANIZATION OF MULTIVARIATE BRAIN NETWORKS IN PRIMARY OPEN ANGLE GLAUCOMA

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Resting-state functional magnetic resonance imaging (rs-fMRI) is commonly employed to study changes in functional brain connectivity. The recent hypothesis of a brain involvement in primary open angle Glaucoma has sprung interest for neuroimaging studies in this classically ophthalmological pathology. We explored a putative reorganization of functional brain networks in Glaucomatous patients, and evaluated the potential of functional network disruption indices as biomarkers of disease severity in terms of their relationship to clinical variables as well as select retinal layer thicknesses. Nineteen Glaucoma patients and sixteen healthy control subjects (age: 50-76, mean 61.0 $\pm$8.2 years) underwent rs-fMRI examination at 3T. After preprocessing, rs-fMRI time series were parcellated into 116 regions using the Automated Anatomical Labeling atlas and adjacency matrices were computed based on partial correlations. Graph-theoretical measures of integration, segregation and centrality as well as group-wise and subject-wise disruption index estimates were then generated for all subjects. All subjects also underwent Optical-Coherence Tomography (OCT) and visual field index (VFI) quantification. We then examined associations between brain network measures and VFI, as well as thickness of retinal nerve fiber layer (RNFL) and macular ganglion cell layer (MaculaGCL). In Glaucoma, group-wise disruption indices were negative for all graph theoretical metrics. Also, we found statistically significant group-wise differences in subject-wise disruption indexes in all local metrics. Two brain regions serving as hubs in healthy controls were not present in the Glaucoma group. Instead, three hub regions were present in Glaucoma patients but not in controls. We found significant associations between all disruption indices and VFI, RNFL as well as MaculaGCL. The disruption index based on the clustering coefficient yielded the best discriminative power for differentiating Glaucoma patients from healthy controls (Area Under the ROC curve (AUC) 0.91, sensitivity, 100%; specificity, 78.95%). Our findings support a possible relationship between functional brain changes and disease severity in Glaucoma, as well as alternative explanations for motor and cognitive symptoms in Glaucoma, possibly pointing towards an inclusion of this pathology in the heterogeneous group of disconnection syndromes.

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BIOPHYSICS APPROACH IN STRUCTURE-BASED AND LIGAND-BASED DRUG DISCOVERY WORKFLOW

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The research to find new more effective and selective drugs to target different diseases has always been a challenge. The process of identification, optimization and validation of biologically active molecules need the interplay of several techniques able to highlight chemical and structural information. We established a biophysical platform for structure-based and ligand-based drug discovery workflow, which provides both structural (binding mode) and energetic (binding affinity) data to select and design molecules for pre-clinical studies. The selection process starts with the screening of fragment libraries by Biolayer Interferometry technique (BLI) in order to select the best hits by binding affinity. The positive hits are then validated by NMR experiments as CSP, STD, DOSY and trNOE to elucidate the binding mode of the molecular interaction, and by Isothermal Titration Calorimetry (ITC) to obtain the thermodynamic parameters of the recognition. All the experimental data are then used by the computational unit to optimize and grow fragments for hit compounds generation and hits expansions. A robust iteration of the entire process, coupled to biochemical and cellular assays is crucial for generating promising lead compounds with a good selectivity towards the biological target.

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INSIGHTS INTO THE USE OF (TRIMETHYLSILYL)PROPIONIC ACID IN qNMR ANALYSIS OF FOOD PRODUCTS

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Quantitative nuclear magnetic resonance (qNMR) is nowadays an established metrological method widely used in several fields of chemical science [1]. Still, one critical issue faced by spectroscopists remains the judicious choice of an opportune internal reference compound, which ideally must not react with the substances under investigation.
To date, (trimethylsilyl)propionic acid (TSP) has been largely employed as chemical shift reference in NMR analysis of food products. Nevertheless, its application as internal reference compound for quantification purposes is strongly hampered by its ability to interact with components in biologic matrices [2], [3]. During an ongoing collaborative research project (Istituto Poligrafico Zecca dello Stato - Politecnico di Bari) focused on the application of NMR methods to the traceability of European oranges we disclosed the factors affecting the interaction of TSP with the metabolites contained in the mixtures under analysis. We demonstrated that the practical conditions for the sample preparation exerted a strong influence on the extent of such interactions. The most suitable protocol for ensuring the stability of the TSP signal in aqueous solution over the time was developed, enabling the use of this compound as internal reference for metabolites quantification (Fig. 1).
The results reported in this communication, helping to shade light on the nature of the TSP interactions with components in authentic matrices, should be of general interest for the scientific community involved in qNMR studies of food products or biologic fluids.

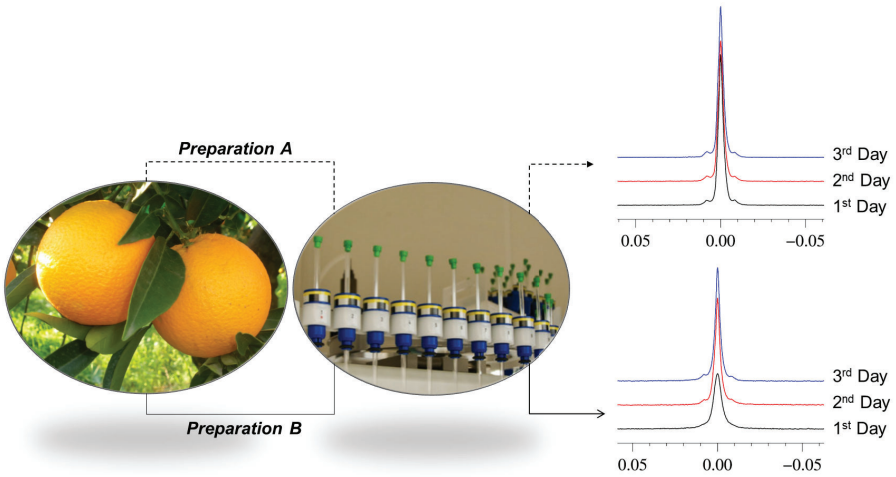

Figure 1: Dependence of the TSP signal resolution on the adopted protocol for the sample preparation.

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MONITORING THE EFFECT OF FILLER IN ELASTOMERIC MATERIALS BY TIME DOMAIN NMR SPECTROSCOPY

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In the last decades, many efforts have been dedicated to the improvement of the mechanical properties of elastomeric composite materials, as they are particularly attractive for several industrial applications. As a matter of fact, these properties are mainly related to the motional constraints of the polymer network, which are due to physical entanglements and chemical cross-linking between polymer chains, and may be influenced by the presence of different additives and reinforcement fillers (carbon black, nanosilica, clays) [1,2]. Usually, the mechanical properties of the materials are monitored by rheological measurements, which provide only macroscopic observables; however, also a description of the topology and dynamics of the polymer network at the molecular scale is needed in order to have a more complete comprehension of the factors that influence these properties, with the final aim to guide the design of optimized materials. In this context, low field ^{1}H time domain NMR (TD-NMR) can give an important contribution [3].
In this work, we studied different elastomeric materials with application in the tyre industry, by TD-NMR spectroscopy, with the aim of investigating the effect of filler particles on polymer structure and dynamics. ^{1}H Multiple Quantum (MQ) experiments [4] were used to evaluate the residual ^{1}H-^{1}H dipolar couplings, which arise from the fast anisotropic motion of the polymer chains and are thus directly related to the amount of topological constraints within the polymer network. Moreover, ^{1}H relaxation times (T_1, T_2) [5,6] were measured to probe a wide range of motional frequencies of the polymer chains. In particular, ^{1}H spin-lattice relaxation times (T_1) were evaluated by means of Fast Field Cycling [6] experiments at different temperatures, covering Larmor frequencies from 10 kHz to 35 MHz.

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^{1}H AND ^{17}O NMR RELAXATION STUDIES OF THE AQUA IONS OF Gd^{III}, Mn^{II} AND Fe^{III}

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A detailed re-examination was carried out on the relaxometric properties of the aqua ions of Gd^{III}, Mn^{II} and Fe^{III}, by measuring ^{1}H longitudinal relaxation data from 0.01 up to 500 MHz and ^{17}O transverse relaxation rates (R_2) and shift ($\Delta\omega$) at 11.7 T, as a function of temperature [1]. The availability of (i) a wider range of magnetic fields, (ii) relaxation data as a function of temperature and (iii) ^{17}O shift and relaxation data measured at high magnetic field strength enable to obtain very accurate value of the structural and dynamic parameters that adequately describe the behaviour of the paramagnetic complexes in solution [2]. A thorough and accurate knowledge is preliminary and necessary for research on contrast enhancing agents for clinical and pre-clinical MRI. The efficiency of a contrast agent is evaluated in vitro in terms of its relaxivity, which represents the relaxation rate enhancement of water proton nuclei per mM concentration of the paramagnetic ion. Relaxivity depends upon a large number of parameters that can hardly be determined by analyzing relaxivity data alone. Indeed, variable temperature ^{17}O NMR measurements of R_2 and $\Delta\omega$ constitute a valuable tool to investigate the parameters influencing relaxivity [3]. ^{17}O NMR data provide information on the water exchange kinetics of the complex, and depend on the hyperfine coupling constant $A_O/\hbar$ between the electron spin of the metal ion and the ^{17}O nuclear spin. Additionally, the Nuclear Magnetic Relaxation Dispersion (NMRD) profiles recorded over a wide range of frequencies allows obtaining an accurate description of the rotational dynamics. The occurrence in solution of hydration equilibria has also been explicitly considered.

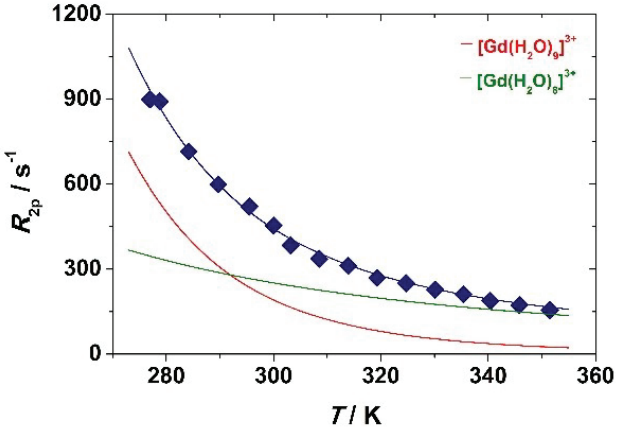

Figure 1: ^{17}O NMR (11.7 T) transverse relaxation rates as a function of temperature for a 18.9 mM solution of gadolinium(III) aqua ion. The calculated contributions of the species with *q=9* and *q=8* are shown.

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THE VALIDITY OF A STEPWISE ^{15}N CSP FOR THE STUDY OF PROTEIN-PROTEIN INTERACTION

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Recently a protein-protein interaction study was performed by the use of a protocol of ^{15}N NMR CSP revealing patterns of perturbations on both the labelled proteins in the presence of the unlabelled ones at different molar ratios. This approach indicated some validity to investigate the surface of the interaction particularly in the cases where the hydrophobic patches are involved in the first recognition and several ionic interactions are involved in a second adjustment to a final complex. This approach revealed to be valid within the interaction takes place with minor or no changes of conformation during the intermolecular interaction with slight or null conformational search of the two partners. Future collaborative help in the MD simulation to better understand the results and to formulate a more widely use of this protocol are required.

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COMBINING NMR AND UPLC-HR-MS TO IDENTIFY NATURAL BIOACTIVE COMPOUNDS: THE EXAMPLE OF PEUCEDANUM OSTRUTHIUM ANTI-Aβ ACTIVITY

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The growing interest in medicinal plants for the identification of new bioactive compounds and the formulation of new nutraceuticals and drugs prompted us to develop a powerful analytical approach allowing the detailed metabolic profiling of complex plant extracts and the identification of ligands of macromolecular targets of biomedical relevance. With this end, we selected Peucedanum ostruthium, a plant traditionally employed in Austria and Italy for its several potential therapeutic applications, as case study. We combined the use of NMR and UPLC-HR-MS for the identification of the metabolites present in its leaf and rhizome extracts [1]. Due to the significant content of polyphenols, particularly chlorogenic acids, recently identified as anti-amyloidogenic compounds [2], polyphenol-enriched fractions were prepared and tested for their ability to prevent Aβ1-42 peptide aggregation and neurotoxicity in a neuronal human cell line. STD-NMR experiments allowed the detailed identification of Aβ oligomers' ligands responsible for the anti-amyloidogenic activity.

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FFC CHARACTERIZATION OF "BETI" - A UNIQUE IONIC COMPOUND

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Herein, we discuss the use of Fast Field Cycling relaxometry (FFC) [1], combined with other analytical techniques, to reveal the dynamics present in an unusual ionic compound, $[N_{10,111}][beti]$, whose solid possesses a unique combination of properties. Above its melting point (31 °C) $[N_{10,111}][beti]$ behaves as a typical room-temperature ionic liquid [2]. Upon solidification it forms an optically clear glass-like material, yet its X-ray diffraction shows only sharp peaks and no diffuse component. After employing a number of techniques (such as X-ray diffraction, NMR high-field measurements and others) to better understand the molecular structure of this unique solid, we investigated its dynamics using FFC. FFC is a low-field NMR technique which allows measurement of the dependence of the longitudinal relaxation rate $R_1 = 1/T_1$ of the samples over a wide range of magnetic fields using just one instrument [3]. We performed FFC measurements using a Stelar SPINMASTER 1T FFC relaxometer on ^{1}H and ^{19}F nuclei from 10 kHz up to 40 MHz). Furthermore, experiments were carried out over a wide range of temperatures (Fig.1). Temperature-dependent ^{1}H and ^{19}F FFC data in the liquid are similar and both were analyzed in terms of relaxation models used for other liquid systems [2]. In the low temperature phase, both ^{1}H-cations and anion-localized ^{19}F spin-lattice relaxation dispersions take on an additional relaxation contribution with a power-law character whose interpretation is currently under investigation.

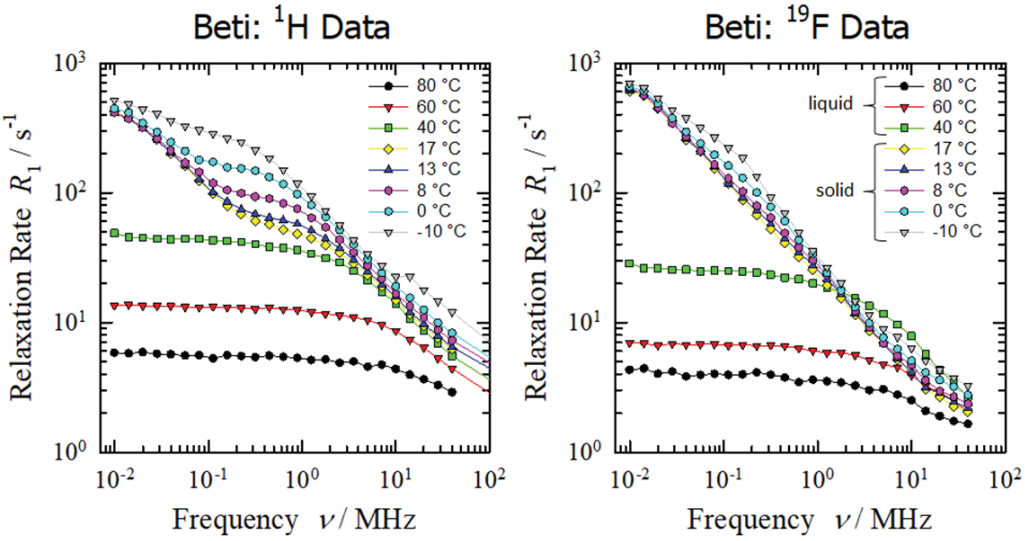

Figure 1: NMRD profiles for ^{1}H and ^{19}F nuclei at different temperatures.

References:
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FITTING RELAXATION DISPERSION PROFILES (NMRD) WITH FITTEIA - A $MnCl_2$ CASE STUDY

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Fitting relaxometry data, including NMRD profiles from Fast Field Cycling (FFC) NMR relaxometry is a problem which researchers have approached using different tools, both non-specific commercial software and self-developed software. To date there is no commercial software program for specific use with FFC relaxometry data [1]. FITTEIA is a possible solution which can be adopted and adapted specifically for FFC relaxometry data. FITTEIA is an open online model fitting platform (developed by Prof. Pedro Sebastiõ from the University of Lisbon) throughout many years of experience in fitting relaxometry data. It does not require the installation of local software or the purchase of a software license and, in addition, it can be accessed from any operating system [2]. Herein, we will go through a case study showing how FITTEIA can be used to fit the NMRD profile of a paramagnetic sample, 2mM manganese chloride ($MnCl_2$). The final purpose of the fitting is to extract the values of the dynamical parameters which characterize the sample.

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LABELLING CELLS WITH Gd-BASED MRI AGENTS USING SONOPORATION

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Sonoporation is a suitable bio-physical method to enhance the cell membrane permeability using ultrasound waves (0,5-3 MHz) with potential application both *in vitro* and *in vivo*. [1] The cellular internalization of bioactive molecules such as drugs, genes or contrast agents has great potential for diagnostic and therapeutic purposes (controlled drug delivery, gene therapy, cell tracking in regenerative medicine). Monitoring of therapeutic outcomes requires a safety imaging modality with high spatial resolution as Magnetic Resonance Imaging (MRI). In this contribution, a new sonoporation-based method for labeling cells with paramagnetic MRI contrast agents was developed and compared with other already validated methods. [2] The optimization of the internalization was obtained through the modulation of the parameters involved in the pulsed US application as the pulse repetition frequency the duty cycle, and the "acoustic pressure" in a setup composed by an amplified wave generator connected to a piezoelectric transducer. Cells (K562, J774A.1, A2780, or TS/A) were suspended in a solution containing variable concentration of the clinically approved Gd-based MRI agent ProHance®. Degassed water was used as interface between cells and the 1 MHz transducer. The amount of ProHance® internalized in the cells was assessed by relaxometry and ICP-MS. In terms of labelling efficiency, the sonoporation-based method performed well when compared with electroporation, hypotonic swelling, and simple incubation. Furthermore, the US application did not affect cell proliferation and viability that was higher than that obtained by using electroporation. The MRI acquisition of cells labelled with ProHance® by sonoporation or simple incubation (Fig.1) revealed the great potential of the former technique. Compared with the other labelling methods, sonoporation is much faster than pinocytosis, can be applied in vivo differently from the hypotonic swelling, and it appears more biocompatible than electroporation.

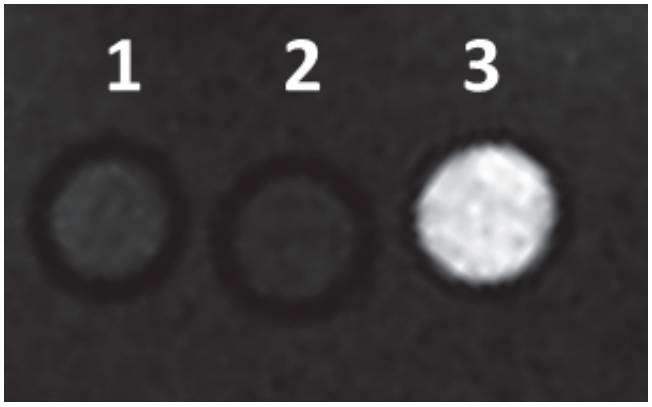

Figure 1: T_1-w image of a phantom composed by 1) K562 cells labelled by simple incubation (1 min) 2) unlabeled K562 cells 3) K562 labelled by sonoporation (US exposure 1 min).

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DRUG EFFECTS ON METABOLIC PROFILES OF SCHISTOSOMA MANSONI ADULT MALE PARASITES BY ^{1}H-NMR SPECTROSCOPY

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Schistosomiasis, caused by trematodes of the *Schistosoma genus*, is one of the most devastating and neglected tropical parasitic diseases. Eggs layed within the definitive host by mature pairs are responsible for both pathology and life cycle maintenance [1]. Schistosomiasis treatment relies on praziquantel which is a safe and very effective drug against mature worms but poorly active on larval and juvenile parasite stages. Therefore, the search for new pharmacological treatments appears mandatory [2]. The aim of this study is to evaluate the metabolic profile in adult male parasites treated with perhexiline maleate (PHX), previously shown to be very effective on Schistosoma mansoni [2]. To this purpose, a ^{1}H-NMR spectroscopy study was performed on adult male worm extracts to evaluate the metabolic perturbations due to PHX, gambogic acid (GA), and DMSO (drug vehicle). The effects of PHX and GA compound treatments were compared in order to highlight different metabolic profiles and specificity of the PHX action.

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UNTARGETED METABOLIC PROFILING OF CARDIAC ISCHEMIA FINGERPRINT IN HUMAN PLASMA BY ^{1}H-NMR SPECTROSCOPY

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Cardiac ischaemia is a severe pathological condition characterized by a reduced oxygen flow to the heart that can lead to myocardial infarction. The reduced blood flow is usually the result of a partial or complete blockage of coronary arteries [1, 2]. Classical diagnostic methods, ECG treadmill stress test and coronary scintigraphy, are affected, respectively, by low sensitivity and high dose of radiation [3]. Here we present a non-invasive metabolomics profiling of cardiac ischemia fingerprint in human plasma. In collaboration with "Umberto I" cardiology unit, plasma samples of ischaemic and healthy patients were collected before and after a cardiac stress test. The samples were analyzed by ^{1}H NMR spectroscopy and 57 metabolites were identified and quantified in each spectrum. Those metabolite concentrations constitute the dataset for the multivariate statistical analysis. Correlating our data with objective scintigraphy results, a significant discrimination between ischaemic and healthy patients metabolic fingerprint was obtained in both stress and, surprisingly, basal plasma samples. These preliminary results could underline a physiological adaptation of human metabolome to ischaemic condition opening the possibility of using an innovative non-invasive metabolomics approach for ischaemic risk assessment. Further analysis will be focused both on validation of these results and the increment of dataset size.

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INTRODUCTION OF A 400 MHZ (^{1}H) LIQUID-CRYOGEN FREE HIGH-TC SUPERCONDUCTING MAGNET FOR HIGH-RESOLUTION NMR

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High-T_c superconductors (HTS) have greatly expanded the operating envelope for superconducting magnets. In particular, their higher operating temperatures mean mechanical cryocoolers with much greater available cooling power can be used compared with those for conventional superconducting magnets and this in turn allows the realisation of industrially robust magnet systems. Here we describe the development and performance of a 400 MHz (^{1}H) HTS magnet for high-resolution NMR spectroscopy. Principal features of the magnet are compactness (Fig. 1), the absence of liquid cryogens, and the absence of elaborate vibration isolation. These advantages open the possibility of operation in industrial process settings or directly in the chemical laboratory, with a particularly attractive target being reaction monitoring. The new wire technology also means the magnet operates in driven mode with a permanently attached power supply, and with field uniformity being addressed using a mixture of ferromagnetic and electric shimming solutions. Despite these differences, the magnet is designed to operate with standard NMR hardware and accessories and performance of the NMR system has proven comparable to standard NMR solutions [1,2].

Figure 1: A mobile 400 MHz (^{1}H) HTS magnet for high resolution spectroscopy

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NON CANONICAL CYCLIC NUCLEOTIDES MONOPHOSPHATES IN *APHANIZOMENON FLOS-AQUAE*: NUCLEAR MAGNETIC RESONANCE AND MASS SPECTROMETRY

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Aphanizomenon flos-aquae (AFA) cyanobacteria from Klamath Lake (Oregon) are considered a "superfood", due to their complete nutritional profile that has proved to have health-enhancing properties. AFA metabolome is quite complex. Here, we present a study that, combining multinuclear ^{1}H, ^{31}P and ^{13}C NMR spectroscopy and high-resolution mass spectrometry, allows the detection of rather unusual phosphorylated metabolites in AFA [1,2]. In this study we focused our attention on AFA phosphorylated metabolites giving ^{31}P NMR signals at 20 ppm, a chemical shift that pointed to phosphonates. They instead revealed to be nucleoside 2',3'-cyclic monophosphates (cNMPs), that were characterized by multinuclear ^{1}H, ^{31}P and ^{13}C NMR spectroscopy and high-resolution mass spectrometry. Our data are fully consistent with the proposed structures and hence demonstrate the presence of cNMPs in AFA, for the first time. The most studied of these biomolecules is cAMP that activates a protective mechanism in the case of brain tissue injury, whereas it inhibits mitophagy of damaged mitochondria in the kidney [3]. The role of the other cNMPs there is much to be discovered.

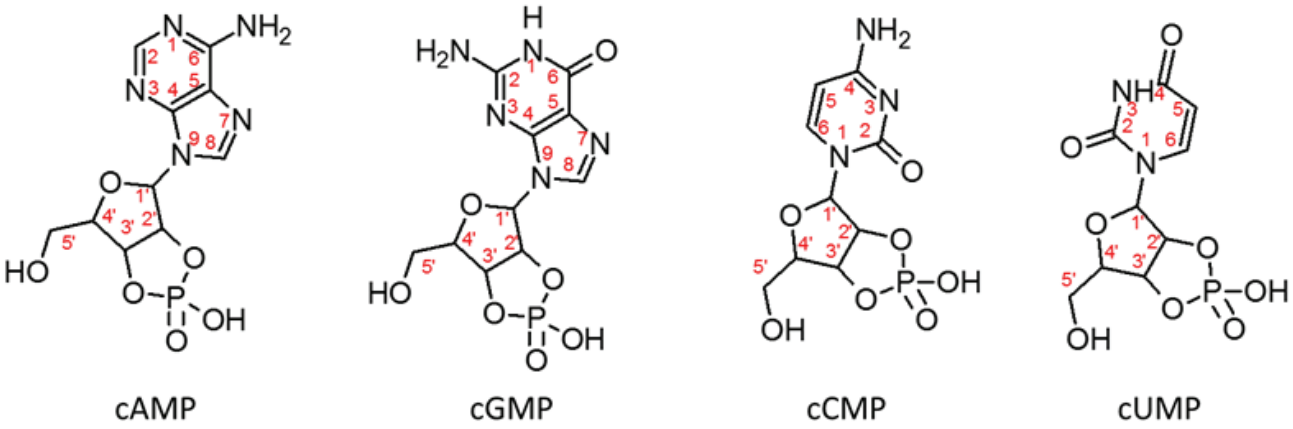

Figure 1: The four nucleoside 2',3'-cyclic monophosphates identified in this study

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THE TIME COURSE OF STRIATAL T_1 CHANGES IN THE 6-OHDA HEMIPARKINSONIAN RAT MODEL CORRELATES WITH THE TAIL SUSPENSION SWING TEST

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MRI plays an important role in characterizing the preclinical evolution of PD [1], showing that different striatal areas can influence motor symptomatology [2,3]. In this work, we investigated the correlation between the time course of striatal T_1 changes in the 6-OHDA hemiparkinsonian rat model with the Tail Suspension Swing Test (TSST) during the unilateral degeneration of the right Substantia nigra pars compacta (SNpc) induced by intranigral injection of 6-OHDA (8 μg/4 μl). *Ex vivo* MRI was carried out on 4% paraformaldehyde fixed whole brains using a 2.35T Bruker Biospec scanner. Whole brain T_1 maps were acquired over 3 weeks post lesion. Comparison of toxin-lesioned and sham-lesioned rats was aimed to detect structural changes soon after the injury (24 hours) and during the following 3 weeks. The experimental protocol comprised the TSST [4] in drug-free state and after single/multiple apomorphine administrations. The sham-lesioned brains did not show inter-hemispheric differentiation of T_1. On the contrary, we observed that the lesioned brains showed a significant increase of the T_1 values in the ipsilateral striatum (ST) with respect to the contralateral one, during the first week post lesion, significantly correlated with the SNpc degeneration. We also found a significative correlation between the apomorphine-induced TSST behavioural biased parameter and T_1 increases in the ST (Fig. 1).

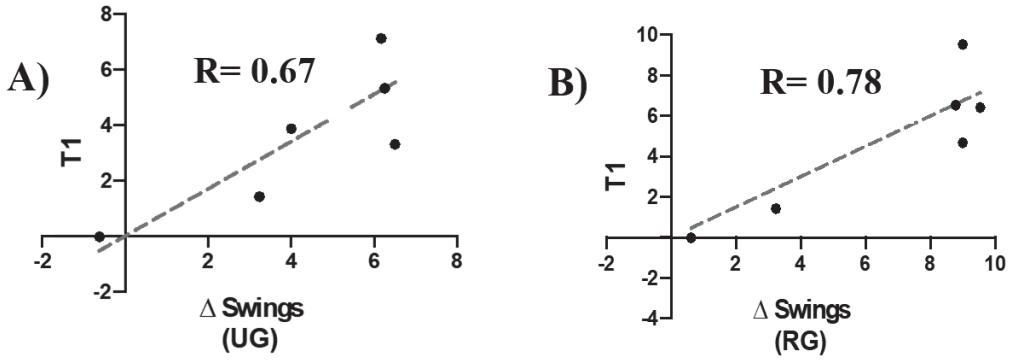

Figure 1: A,B Correlations between T_1 increases in the ST and TSST-measured ΔSwings (right - left) apomorphine induced. UG: unrepeated group (single dose); RG: repeated group (multiple doses).

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DYNAMICAL PROCESSES IN CRYSTALLINE SOLID SOLUTIONS OF IONIC ROTORS: A STRUCTURAL AND SPECTROSCOPIC STUDY

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Dynamic processes, such as rotation and libration of molecules within crystals, have long attracted the attention of researchers. Indeed, the understanding, control and exploitation of such movements can give access to new properties in functional materials [1-4]. We report on the synthesis and characterization of two supramolecular salts of general formula [(12-crown-4)•(DABCOH2)]X_2 (X = Cl- or Br-) and of their solid solutions [(12-crown-4)•(DABCOH_2)]$Cl_{2x}Br_{2(1-x)}$ in the whole composition range (0<x<1). The mixed crystals have been investigated by a combination of solid-state techniques including variable temperature single crystal and powder X-ray diffraction (XRD), and solid-state NMR spectroscopy. The combined use of these methods has made possible the rationalization of the correlation between the dynamical processes taking place within the crystalline materials and the solid solution composition. VT solid-state NMR measurements allowed to describe an uncommon thermally activated molecular motion, based on a precession of the DABCO unit within the cage created in the crystal structure by the crown ether and the halide packing (Figure 1). To the best of the authors' knowledge, this is the first observation of a RT precession motion (a "precession within a cage") that is frozen as the hosting cage is made tighter by decreasing the temperature.

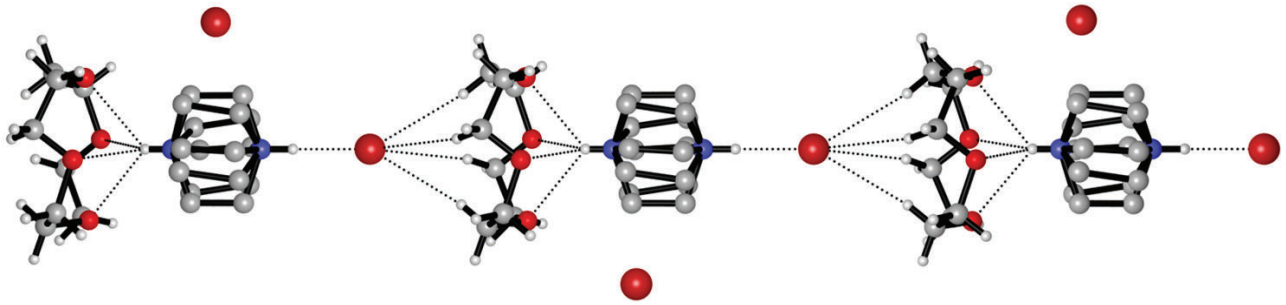

Figure 1: The infinite hydrogen bonded chain in crystalline [(12-crown-4)•(DABCOH_2)]Br_2

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INTRACELLULAR WATER LIFETIME AS A TUMOUR BIOMARKER FOR DIAGNOSIS AND THERAPY OUTCOME BY FFC-RELAXOMETRY IN BREAST CANCER

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The diagnostic power of Magnetic Resonance Imaging in tumour phenotyping could be improved observing the marked decrease of T_1 in biological tissues at low magnetic field strength. It is well known that the T_1 of a given tissue changes as a function of the applied magnetic field strength. In particular, the lower is the magnetic field the higher is the differences among tissues. Known as "T_1-dispersion", this phenomenon is a marker of disease and it is invisible to conventional, fixed-field MRI scanners. The Fast Field-Cycling (FFC)-NMR is the only practicable way of measuring it. An overall increase of water content together with an impairment in water exchange across membranes have fundamental role in this behaviour. The measurement of the intracellular water lifetime (τ_{in}) in vitro and in vivo may bring relevant information on the ongoing metabolism of the tumour cell, as report on the pathological status, grade and therapeutic outcome. The measurement of τ_{in} was performed *in vitro* and *in vivo* on murine adenocarcinoma cell line (4T1). Different doses of doxorubicin have been tested before the T_1 measurement. The data were analysed using two-site exchange (2SX) model in which the Bloch equations are modified to describe two-compartments (intra and extracellular) in which water exchange modulates the observed relaxation behaviour. The most striking result from the fitting procedure is the observation of a significant τ_{in} increase after the first treatment due to the slower tumour metabolism caused by doxorubicin, that it was not observed on the corresponding doxorubicin resistant cell line. Recently, [1] we showed that the τ_{in} represents a hallmark of tumour tissue cells status that can be easily monitored by measuring T_1 at different and relatively low magnetic field strengths. Currently, tumour responses to therapy are monitored primarily by imaging evaluating essentially the decrease of tumour size. This approach, however, lacks sensitivity and can only give a delayed indication of a positive response to treatment. In this study, we propose the use of FFC-NMR to provide relevant information about response to treatment by monitoring changes of water exchange rates through cell membranes that are directly dependent on the metabolism alterations caused by the chemo- or radio-therapy.

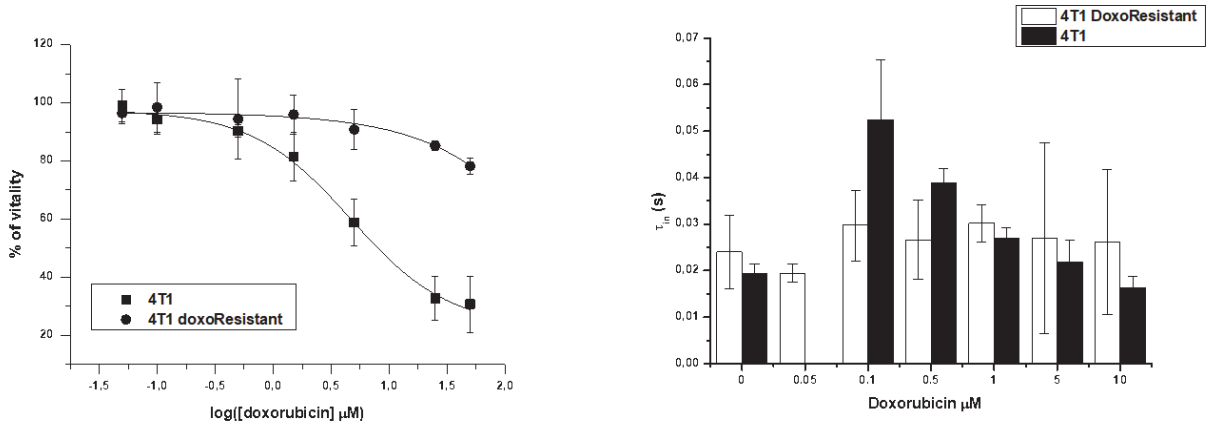

Figure 1: Left, Toxicity of Doxorubicin on 4T1 and 4T1 resistant; Right, τ_{in} values at different concentration of drug.

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QUALIFICATION OF ^{1}H-^{13}C HSQC QUANTITATIVE METHOD FOR COMPOSITIONAL ANALYSIS OF LOW MOLECULAR WEIGHT HEPARIN

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The complex problem of heparins characterization, to ensure efficacy and safety of these drugs, was addressed with use of orthogonal methods [1], one of which is quantitative ^{1}H-^{13}C HSQC. In 2D quantitative NMR the unique feature of intra- and intermolecular uniform response factor is lost due to its dependence on active parameters in polarization transfer and on T_2. Applications of quantitative ^{1}H-^{13}C HSQC for the determination of glycosaminoglycans composition get round this problem by using normalization formulae only containing structurally homogeneous signals. A full validation of this methods on heparin and low molecular weight heparins (LMWH) [2] was performed according to the principles of the lifecycle of the analytical procedures [3], starting from the analysis of all uncertainty sources; the approach took into account the need of a flexible procedure description allowing changes, adjustment to specific NMR instruments and improvements.
The present work describes the validation of the ^{1}H-^{13}C HSQC method to a specific source of LMWH.
The analytical development step consisted in optimization of experimental parameters considering characteristics of the specific material in terms of molecular weight, solubility and counterion and evolution of NMR instruments.
A formal validation of the procedure was then performed by assessing validation characteristics of all fragments. Assignments were reviewed in order to confirm specificity of the method according to the most recent papers. Measurement uncertainty was obtained in terms of intermediate precision and repeatability: this quantity is necessary to evaluate the discriminating power of different batches. Precision obviously depend on the considered fragment and is related to the signal-to-noise ratio (SNR) and of the shape of the signal, but a plot of CV values vs. content level allowed an easy interpretation of these complex data and a confirmation of the candidate LOQ value obtained by SNR. A system suitability test was proposed as a control strategy of the performance of the method ensuring the flexibility necessary to account differences in NMR instruments. In conclusion the validation approach proposed in this work results to be suitable for the regulatory and scientific qualification of the quantitative 2D NMR.

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NMR CHARACTERIZATION OF *CANNABIS SATIVA* NATURAL EXTRACTS

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Cannabis Sativa is an herbaceous fast-growing plant originating from Central Asia and currently cultivated worldwide. Its multi-purpose applications, ranging from medicine to industrial fiber production, makes this plant very intriguing in different fields of research [1,2]. From the metabolomics point of view Cannabis Sativa is composed by chemical compounds having pharmacological properties that go much beyond psychotropic effects. This kind of substances can be extract from their original matrix and used as pharmaceutical products or as additives for food enrichment. The characterization of the extracts, their quantification and the extraction procedures optimization are essential steps in the development of new drugs and functional foods. In this contribution, NMR spectroscopy was used in order to perform a fingerprinting and a profiling of Cannabis Sativa extracts. The samples studied were obtained with different extraction techniques from both flowers and seeds. The 1D (^{1}H-NMR and ^{13}C-NMR) and 2D experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and J-resolved ^{1}H-^{1}H) were recorded in a common solvent for the characterization of the compounds present in the mixtures and to appreciate the possible differences between the different samples [3]. Moreover, a quantitative proton NMR of the main cannabinoids in flowers extracts was performed using anthracene as internal standard [4]. With the purpose of identifying correlations between metabolites and factors of discrimination, the dataset of the NMR spectra was treated with the multivariate statistical analysis model PCA (Principal Component Analysis) [5].

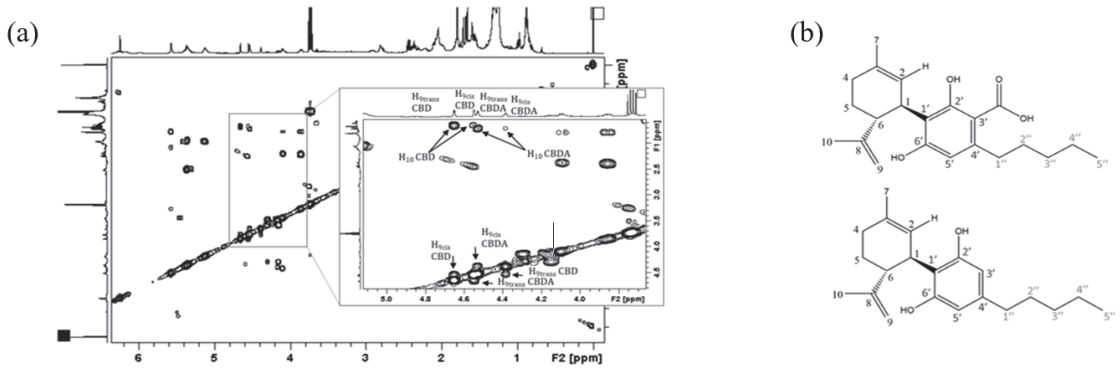

Figure 1: (a) 2D ^{1}H-^{1}H COSY spectrum of flowers extract with enlargements of correlation signals corresponding to the acyclic olefinic portion of CBD and CBDA. (b) Topological structure and proton atoms labelling of CBDA (top) and CBD (bottom).

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

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The knowledge on carbohydrates composition is of great importance to determine natural matrix properties. Due to their structural similarity and to the high number of isomers they present, carbohydrate analysis is complex. There is a growing interest in the development of new analytical methods, both for raw materials and for finished products, that do not require pre-treatment of the sample and that are, at the same time, specific and highly accurate in identification and quantification of sugars. Here, we have devised an innovative qNMR analytical procedure based on a selective TOCSY experiment [1]. The method was developed on honey samples previously dissolved in water with no other sample treatment. Twenty two main sugars present in honey were simultaneously quantified: four monosaccharides (glucose, fructose, mannose, rhamnose), eleven disaccharides (sucrose, threalose, turanose, maltose, maltulose, palatinose, melibiose, melezitose, isomaltose, gentiobiose, nigerose and kojibiosio) and seven trisaccharides (raffinose, isomaltotriose, erlose, melezitose, maltotriose, panose and 1-kestose). Satisfactory results in term of limit of quantification, precision, trueness and recovery were obtained. This methodology allows for the accurate quantification of sugars regardless of whether they are major or minor components and may be useful, for example, for the detection of honey adulteration by different sugar syrups by a targeted metabolomics approach.
While validated on honey, which is one of the most complex natural matrices in terms of saccharides composition, this innovative approach can be easily transferred to other food matrices such as fruit juices, milk, and also to biofluids or even to classes of molecules other than carbohydrates.

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

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NMR CHARACTERIZATION OF HEMP PRODUCTS FROM LAZIO

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Cannabis sativa L., better known as industrial hemp, is an important raw material in different fields such as textile, pharmaceutical, biomatherial applications and food. For hundred years industrial hemp was cultivated all over the world, gaining a great commercial importance in Europe, especially in Italy, which was the largest hemp producer after Russia. However, because of the association with narcotic Cannabis (*Cannabis indica*) [1,2] in the 20th century the cultivation of industrial hemp actually disappear, although it was still allowed. Recently, the European Union published a regulation regarding the reintroduction of selected varieties of this crop (with a THC content lower than 0.2%) in the European countries [1].
The present study, developed in the frame of the Regione Lazio project titled *Industrial Hemp: development and valorization of a sustainable new food chain* aimed at the identification of the *Cannabis sativa* L. cultivars best suited to the pedoclimatic condition present in well-defined Lazio areas (an Italian region). In this attempt, both agronomical and chemical-nutritional aspects related to the French monoic Ferimon variety of *Cannabis sativa* L. were investigated using different level of Nitrogen and Phosforous fertilization, irrigation and plant density. Moreover, samples from different dioic cultivars, cultivated in selected Northen Lazio areas were analyzed. The raw material (inflorescences) of samples from all varieties collected over the season and the corresponding processed products, such as seeds, flour and hemp seed oil were characterized though a multi-methodological protocol, involving different advanced techniques, such as NMR, HPLC, spectrophotometry. NMR analyses were carried out on each sample, using hydroalcoholic and organic extracts obtained by Bligh-Dyer procedure [3]. One dimensional (^{1}H) and 2D (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) experiments were performed in order to define the metabolic profile and monitor the trend of primary and secondary metabolites.

Acknowledgement: Progetto di Ricerca, finanziato ai sensi della L.R. 13/08 Protocol 85-2017-15069 CUP: B86C18000730002

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ELECTROCHEMICAL REDUCTION OF CO_2: LIQUID PHASE PRODUCTS QUANTIFICATION WITH NMR

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Carbon dioxide can be considered an important and sustainable carbon source for the production of valuable chemicals and fuels; of all the possible exploitation processes, electrochemical reduction of CO_2 is one of the main targets especially if powered by a renewable electricity source.
There are however several limitations that prevent the use of this technology for large-scale application; in order to overcome them a key point is the development of catalysts capable of effectively reducing CO_2 electrochemically in aqueous solutions. During the course of the reaction, a mix of products as gaseous as in aqueous phase are formed, whose identification and quantification is important, in order to evaluate the selectivity of the catalyst of interest but mainly because, through their analysis, some hypothesis regarding the reaction mechanism can be put forward.
Products detection and quantification can be obtained by means of gas chromatography, mainly for volatile products, and by NMR analysis of the electrolytic solution at the conclusion of the electrolytic cycle for all the liquid phase products, with suppression of the water signal, in order to resolve even the smallest product signals close to it.
NMR analysis has the significant advantage that it does not require the purification or the separation of the collected products, thus avoiding any possible loss. However, some additional steps must be followed for a precise quantification, such as the construction of calibration curves, over the concentration range of interest, for all the detectable products and the use of an external standard solution (1 mM phenol in DMSO-d6) in a coaxial capillary tube, in order to compare peak areas of products in different solutions. Besides, attention must be paid to use same spectral acquisition parameters for all the quantification spectra and to a relaxation delay properly calibrated as function of the products' relaxation time T_1. Here we report, as example of this methodology, a study of CO_2 reduction (CO_2RR), performed on a H-type cell in a three-electrode configuration, separated by a cation exchange membrane: in the cathodic compartment, a Cu-based working electrode in a 0.5M $KHCO_3$ is present. The solution is saturated with CO_2, that flows through the cell, and the reduction tests were performed in galvanostatic mode.
Different Cu-based catalysts were tested, and after the runs, the electrolytic solutions have been collected and analyzed. The observed products are mainly anionic products such as $HCOO^-$ and CH_3COO^- and alcohols, mainly ethanol and n-propanol, in agreement with what reported in the literarure [1].
The mechanism which leads to the formation of these products is not yet clearly understood but CO appears to be an important intermediate. Probably CO is coupled to make C2 and longer chain products, with a C-C bond formation. The presence of CO_2RR products with hydroxyl and/or carbonyl moieties suggests that the C-C coupling step may occur when at least one of the two carbon-oxygen bonds in CO_2 is still unbroken.

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RATIONAL DESIGN IN GD(III) AND BIS-NITROXIDE SYSTEMS FOR HIGH-FIELD MAS-DNP

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Since the last 15 years MAS DNP has developed into an unavoidable research tool in the structural investigation of materials and surface science. MAS DNP at high magnetic field can be achieved with bis-nitroxides [1,2] radicals (like AMUPol and TEKPol) and with high-spin complexes (like Gd(dota) [3]). Here we demonstrate that by selecting the chelating ligand we obtain with [Gd(tpatcn)] a ^{1}H enhancement of **37 at 9.4 T and 100 K** *(a factor 2 better than [Gd(dota)(H$_2$O)]$^-$)* [4]. Moreover, by using a simple theoretical model tested on different Gd(III) complexes we show that *the reduction of zero-field splitting impacts quadratically* the expected MAS-DNP signal enhancement for high-spin complexes, therefore establishing an important design parameter.
We have also identified a new parameter which could be relevant in the design of bis-nitroxide PAs.
The local conformation around the N-O$^\bullet$ region can lead to dramatic impact of the MAS-DNP performance, with **HydrOPol** returning a ^{1}H enhancement of **330 at 9.4 T and 100 K**: about 30% better than the current standard in the field. This result is possible by selecting the conformer with the nitroxide sidearms pointing away from the N-O$^\bullet$ bond.

Overall, by rational design of either the local geometry around the N-O$^\bullet$ region in bi-nitroxide systems or of the chelating ligand in Gd(III) complexes **we achieved the so far best reported enhancements at 9.4 T and 100 K for both bi-nitroxides and Gd(III) complexes**, significantly improving on the current understanding and performance of MAS-DNP PAs.

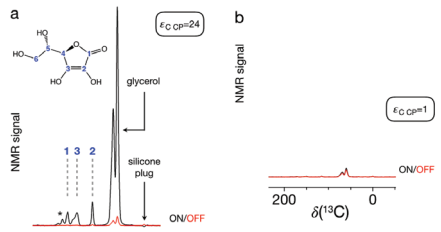

Figure 1: MAS DNP spectra at 9.4 T, ∼100 K and 12.5 kHz MAS reporting ^{1}H enhancements via ^{13}C cross polarization (CP), *i.e.* ϵ_{CCP}, of a glycerol-d$_8$:D$_2$O:H$_2$O (6:3:1 v% solution of 1.3 M ascorbic acid and 8 mM [Gd(tpatcn)] in (a) and 10 mM AMUPol in (b).

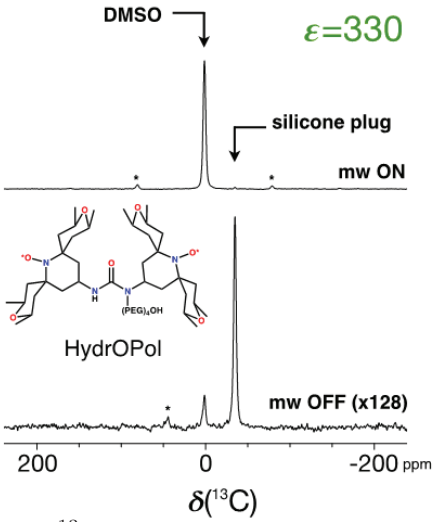

Figure 2: ^{13}C CP microwave ON and OFF signals for 10mM HydrOPol in DMSOd$_6$:D$_2$O:H$_2$O (6:3:1 v%) at 9.4T and 100K.

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QUANTITATIVE NMR ANALYSIS AT NANOMOLAR CONCENTRATIONS VIA PARA-HYDROGEN INDUCED HYPERPOLARIZATION

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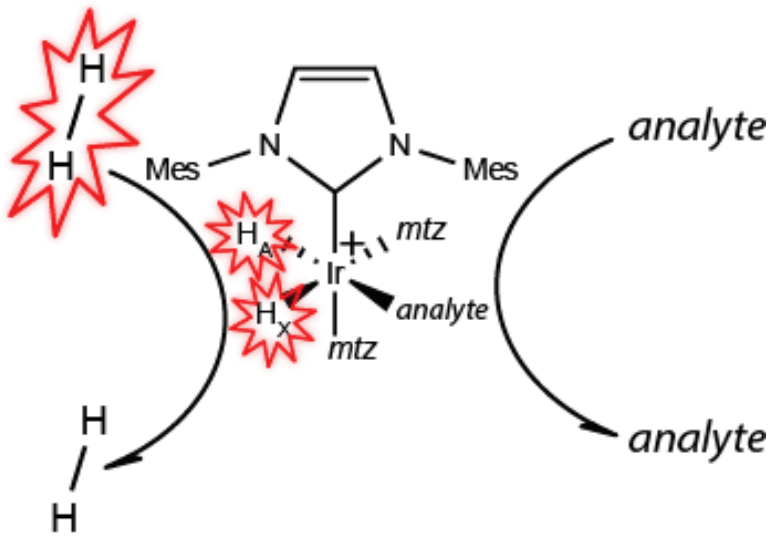
Nuclear spin hyperpolarization (e.g., Dynamic Nuclear Polarization (DNP), Para-Hydrogen Induced Polarization (PHIP), etc.) has gained a widespread interest over the last years as a tool to enhance NMR sensitivity. Particularly, SABRE [1] is a hyperpolarization technique based on the reversible association of substrate molecules and parahydrogen (p-H_2) to an iridium complex in solution. At low magnetic field, a transient scalar coupling network within this complex allows the spontaneous transfer of spin-order from p-H_2 to the nuclear spin of the substrate molecules. By rapidly shuttling the hyperpolarized sample to high magnetic field, NMR signals enhanced up to three orders of magnitude compared to thermal equilibrium conditions can be detected.
Here, I present a novel high-field PHIP approach that can be applied to highly complex mixtures, such as biofluids and natural extracts. The proposed approach is based on the reversible association of SABRE substrates to an iridium catalyst that acts as an NMR chemosensor, allowing the selective detection of target compounds (down to nanomolar concentrations) while removing the large background originating from the complex matrix [2]. We have recently applied this technique to complex mixtures, such as urine extracts, in which hundreds SABRE substrates at low- or sub-micromolar concentrations could be simultaneously detected [3].



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ALPHA-SYNUCLEIN ADSORPTION ON COLLOIDAL NANOPARTICLES

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The notion that nanoparticles can influence amyloid formation has stimulated efforts to investigate the structure and dynamics of the protein corona with fibrillogenic polypeptides [1]. Alpha-synuclein (αS) is a prototypical amyloidogenic protein whose aberrant aggregation is associated with neurodegenerative disorders [2]. We investigated the interaction of αS with silica nanoparticles using NMR spectroscopy, fluorimetry, and SDS-PAGE. We determined the binding orientation of αS to nanoparticles, at single-residue resolution and found that the organization of the αS corona is influenced by the presence of free protein molecules. Experiments with isotopic homologues and different proteins showed that cosolutes elicit molecular exchange in a protein-specific manner. Competing equilibria were found to modulate the composition of the adsorbed layer in protein-rich solution. This study reveals the dynamic nature of αS interactions with colloidal nanoparticles, an aspect that crucially impacts on our ability to control conformational preferences and pathogenic aggregation mechanisms of αS.

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NMR RELAXOMETRY FOR STUDYING ANCIENT COTTON PAPER BOOKBINDING

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Cotton paper is a random network of hydrated cellulose fibers. The degree of polymerization (DP $\sim$ 104), the relative amount of the two polymorphous (Iα/ Iβ $\sim$ 40%) and of crystalline material in cellulose (CI $\sim$ 80%) influence the mechanical and physical properties of cellulose. Based on this architecture, the amorphous regions in a microfiber along with microfibers-microfibers and fibers-fibers inter-spaces allow considering cellulose as a porous system. Although water is required to achieve the paper's flexibility, water vapor adsorption/desorption mechanisms are responsible for solubilizing and carrying atmospheric reactive compounds, soluble salts and minerals, contributing to the deterioration of cellulose. ^{1}H-NMR-R measurements allow characterizing the dynamics of water molecules confined in cellulose amorphous phase, acting as hydration water or filling pores [1]. ^{1}H-NMR longitudinal (T_1) and transverse (T_2) relaxation times correlation maps permit distinguishing water molecules populations with different dynamics in a porous material as cellulose [2]. Moreover, the acquisition of T_1-T_2 correlation maps with a surface probe provides the assessment of water molecules volume fraction within the NMR sensitive volume, a value strictly related to the material's moisture content [3]. In the present study, we present a case study aiming at surveying the state of conservation of a 19th century book, focusing on its paperboard cover ideally divided in twelve sections. We employed a single-sided ^{1}H-NMR probe to perform relaxation measurements at different RH values. T_1-T_2 correlation maps pointed out the high hydration capacity of two sections (namely L_1 and L_5) compared to others. In our opinion, the larger amount of signal can probably be the effect of biological/chemical degradation phenomena that led to an increase of the amorphous phase. NMR data were confirmed by Infrared Thermography inspections.

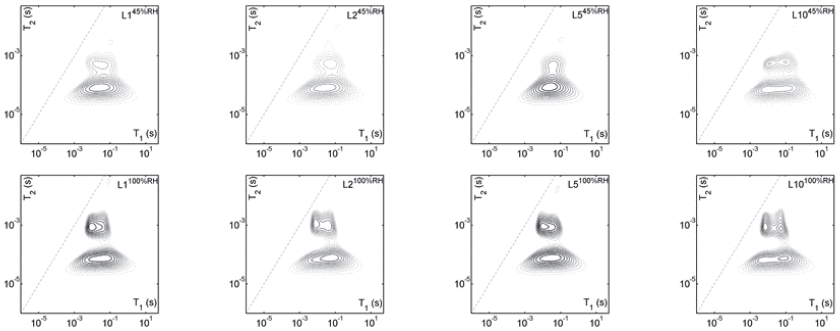

Figure 1: 2D ^{1}H-NMR T_1-T_2 maps of L_1-, L_2-, L_5- and L_{10}-sections collected at 45% (first row) and 100% (second row) RH.

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RECOMBINANT MUSSEL PROTEIN PVFP5β: A POTENTIAL TISSUE BIOADHESIVE

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In the last few decades searching for new bio-adhesive molecules able to work in wet environment is a challenge for regenerative medicine and surgery. Despite the strong need of these biomaterials, no efficient bio-adhesive capable of making tissues adhere together in an efficient way has yet been found. Mussel adhesive proteins have received increased attention in recent years for their ability of strongly adhere to their substrate in aqueous tidal environments [1].The Asian green mussel *Perna viridis* secretes several byssal plaque proteins (*Perna viridis* foot proteins, Pvfps) and each type has a unique function and contribute differently to adhesion. In particular, the Pvfp-5β explicates a primary role in the first step of adhesion [2]. In the present study we focus our attention on the structural studies of Pvfp-5β, establishing the first recombinant expression in *E. coli* of the protein. Circular dichroism, mass spectrometry and nuclear magnetic resonance results are comparatively discussed and showed that Pvfp-5β folds as a stable tandem of two EGF-domains and mainly possess beta sheet structure. Based on these outstanding results, we are very close to obtain important aspect regarding Pvfp-5β structure determination useful to understand the chemistry behind the interaction of Pvfps with marine surface.

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