

XLIV National Congress on Magnetic Resonance

28-30 September 2015

**Aula dei Convegni - CNR
Rome**

BOOK OF ABSTRACTS



GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

UNDER THE AUSPICES OF



Consiglio Nazionale delle Ricerche

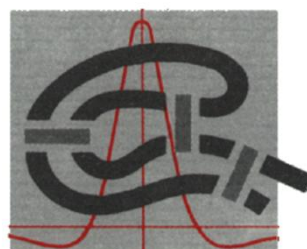


FACOLTÀ DI FARMACIA
E MEDICINA

SAPIENZA
UNIVERSITÀ DI ROMA

DIPARTIMENTO DI CHIMICA
E TECNOLOGIE DEL FARMACO

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SCIENTIFIC PROGRAM

Monday September 28th

10:00-13:00	Registration	
11:00-12:00	JEOL users meeting	
12:00-13:00	Bruker users meeting	
13:00-14:00	Lunch	
14:00-14:30	Opening	
14:30-15:30	GIDRM/GIRM gold medal award	
	M. Geppi: My complex love story with NMR: scientific and human aspects	
15:30-15:50	Annalaura Segre fellowship 2014	
	E. Cappelletto: Solid State NMR investigation of thermally treated clay sediments (geopolymer source material)	
15:50-16:10	Annalaura Segre fellowship 2014	
	C. Nesti: NMR analysis of inorganic pigments and protein binders interaction in paint layers	
16:10-17:15	Coffee break + Poster session	
	Parallel session A, dedicated to A. Bagno Chair: S.Mammi	Parallel session B Chair: P. Turano
17:15-17:45	G. Saielli: Computational NMR spectroscopy: reversing the information flow	R. Fattorusso: Exploring protein folding pathways by NMR spectroscopy
17:45-18:15	B. Mennucci: Towards the simulation of complexity: limits and potentials of multi-scale methods based on Quantum Chemistry	G. Pintacuda: Biomolecular solid-state NMR at ultra-fast magic-angle spinning: a revolution through faster revolutions
18:15-18:35	L. Di Bari: Pseudocontact shifts and structure determination of lanthanide complexes	G. Musco: The double life of PHD fingers: epigenet readers or hubs for multiple interactions? The strange case of Sp140 and NSD1
18:35-18:55	A. Borgogno: Prediction of ¹ H NMR parameters in paramagnetic species	M. D'Onofrio: Ubiquitin-nanoparticle interactions probed by NMR
18:55-19:40	Plenary Session Chair: F. Arnesano	
	C. Dobson: The nature and consequences of protein folding and misfolding	

Tuesday September 29th

Plenary Session Chair: L. Calucci																																																	
8:45-9:30	D. Capitani: Nuclear Magnetic Resonance in cultural heritage																																																
9:30-10:00	Bruker Sponsor Lecture W. Bermel: Homonuclear Decoupling																																																
10:00-11:20	Coffee break + Poster session																																																
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Wednesday September 30th

8:45-9:30	Lectures of poster competition winners Chair: H. Molinari	
9:30-10:15	Plenary Session Chair: I. Felli M. Lerche: Visualizing biochemical activities in living cells with ¹³ C hyperpolarization	
10:15-11:00	M. J. Potrzebowski: Mesoporous silica nanoparticles as drug delivery systems - Solid State NMR studies	
11:00-11:20	Coffee break	
	Parallel session A Chair: V. Righi	Parallel session B Chair: D. Capitani
11:20-11:50	A. Miccheli: NMR-based metabolomics to investigate the gut microbiota activity. What are we learning?	A. Lascialfari: Magnetic nanoparticles in biomedicine
11:50-12:20	E. Schievano: NMR fingerprinting to determine honey authenticity	G. Digilio: Microenvironment-responsive MRI probes for application in cell therapy follow-up
12:20-12:40	V. Mannella: Stable isotope-resolved metabolomics (SIRM): a method to study metabolic flux in cellular or murine models	F. Carniato: Paramagnetic mesoporous silica nanoparticles as potential MRI and theranostic probes
12:40-13:25	Plenary Session Chair: G. Musco C. Luchinat: Metabolic phenotyping: a revolution in diagnosis?	
13:25-15:00	Closure and lunch	

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GIDRM/GIRM GOLD MEDAL AWARD

MY COMPLEX LOVE STORY WITH NMR: SCIENTIFIC (AND HUMAN) ASPECTS

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About 25 years ago, with NMR it was love at first sight, but suddenly our relationship revealed very complex. I could not understand most aspects of herself and she was hiding many of her secrets to me. Moreover, I stubbornly wanted to explore her tougher, solid side: even obtaining a simple resolved spectrum from her was difficult and required at the same time much power and something magic. Even relaxation with her was uncommon, or too fast or too slow. She has always been scarcely and tremendously sensitive at the same time.

In this lecture I will present (also seriously) some of the fruits of this long-lasting relationship... consisting in the application of solid-state NMR to a variety of anisotropic systems, ranging from liquid crystals to biological soft phases, from synthetic macromolecules to organic-inorganic hybrids, from pharmaceuticals to inorganic complexes. In particular, the aspects concerning the obtainment of structural and dynamic properties over broad space and time scales will be treated through examples taken from works realized in my group. Some of the approaches developed by us will be described in detail, including the "global" analysis of the dynamic features of both organic solids and liquid crystals, and the study of heterogeneous hybrid materials through the combined analysis of multinuclear low-, high-resolution and relaxometric data.

Other than science, I wish to reserve a small fraction of this lecture to the wonderful pieces of "humanity" met along these years: friends encountered at meetings and schools (of course, mostly at those organised by GIDRM!) or involved into collaborations and, in particular, who contributed to create or grow my group in Pisa.

After 25 years, I've understood a little bit more of her, but at the same time I've realized that what remains understood is orders of magnitudes larger than I originally thought. She often makes me angry, she will never reveal all of her secrets, but I love her more than at the beginning, in spite, or maybe because, of her complexity. And, notwithstanding our bad economic situation, I still have many projects for our future together.

ANNA LAURA SEGRE FELLOWSHIP 2014

SOLID STATE NMR INVESTIGATION OF THERMALLY TREATED CLAY SEDIMENTS (GEOPOLYMER SOURCE MATERIAL)E. Cappelletto^{‡†}, R. Di Maggio[†], M. Geppi[‡][‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Moruzzi, 13, 56124 Pisa[†]Dipartimento di Ingegneria Civile Ambientale Meccanica, Università di Trento, Via Mesiano 77, 38123 TrentoE-mail: elisa.cappelletto@unitn.it

Geopolymers are polymeric alumino-silicate materials obtained by alkaline activation of alumino-silicates. They are currently of large interest, due to their good thermal, chemical and mechanical properties and their potential as “green” cementitious binder [1,2]. Reservoir clay sediments seem to be attractive precursors for the manufacture of geopolymer-based materials, due to their low cost and easy availability.

In the four months of the scholarship “GIDRM/Borse Annalaura Segre”, assigned to Elisa Cappelletto in 2014, samples from two different clay sediments, coming from reservoirs located in Southern Italy, have been investigated by Solid State NMR (SSNMR) spectroscopy. In particular, SSNMR has allowed us to obtain a detailed characterization of the products obtained after specific thermal treatments and application of different alkali activators. ²⁷Al MAS spectra have shown that the calcination process at 750° is able to transform a large amount of Al(VI) sites of the clay octahedral layers into hydroxylated aluminum tetrahedral sites, Al(IV), fundamental condition for the formation of a geopolymer material [3]. The analysis of the ²⁹Si MAS spectra has demonstrated that the addition of a slag or a sodium hydroxide solution is necessary to obtain a more ordered structure and the desired decrease of the amount of quartz into the geopolymeric material. Concluding, the SSNMR investigation has indicated that these clay sediments can be employed as precursor for the synthesis of geopolymers after suitable heat and activation processes. Furthermore, SSNMR spectroscopy proved to be a very useful tool to investigate the effect of the different alkali activators on the structure of geopolymeric gels.

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NMR ANALYSIS OF INORGANIC PIGMENTS AND PROTEIN BINDERS INTERACTION IN PAINT LAYERS

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Several organic materials, for example, oils, gums, proteins and waxes have been commonly used as binders in various painting techniques [1, 2]. Egg (egg white, yolk and mixtures of albumen and yolk), glue (obtained from animal skins) and milk were traditionally used since ancient times in pictorial artifacts as binders [1, 2]. The large use of these media is due both to the intrinsic adhesive properties and to their easy availability. For this reason, descriptions of the use of these binders are documented since ancient times (from Plinio il Vecchio to Cennino Cennini) [3, 4]. The identification of organic materials is still a topic problematic due to the large variety of compounds used historically in the artistic production. Moreover these substances were not usually used as well, but in the form of mixtures. Furthermore, aging and degradation make their recognition even more difficult [5]. Studies conducted thanks to the AnnaLauraSegre2014 Grant were focused on the NMR analysis of binders of different nature, allowing their characterization. The collected data were analyzed by PCA. PCA studies allowed the analysis of these high variable samples and the comprehension of different binders behaviour in presence of inorganic pigments. The mixtures of organic binders and inorganic pigments were prepared as per Cennino Cennini "Libro dell'Arte" in order to simulate the correct formulation [3].

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PLENARY LECTURES

THE NATURE AND CONSEQUENCES OF PROTEIN FOLDING AND MISFOLDING

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Natural proteins are a highly select group of molecules, and their properties have a number of very special characteristics when compared to random sequences of amino acids, including an ability to adopt unique and often highly intricate structures that can remain functional within the complex milieu of living systems. Such characteristics have enabled biological systems to generate a vast range of functions and an astonishing degree of specificity in their chemical processes. Because proteins are involved in virtually every chemical process taking place within living systems, however, the failure of proteins to remain within their correctly folded states can give rise to serious cellular malfunctions that frequently lead to disease. One particularly important group of such diseases is associated with the aggregation of misfolded proteins into structures known as amyloid fibrils, and includes disorders ranging from Alzheimer's disease to type 2 diabetes, conditions that are becoming increasingly common in the modern world. This talk will focus on the manner in which NMR spectroscopy, in conjunction with other biophysical and biochemical methods, has contributed to our current understanding of both folding and misfolding, and on the potential that such studies have for the development of potential therapeutic strategies targeted against misfolding diseases.

NUCLEAR MAGNETIC RESONANCE IN CULTURAL HERITAGE

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The characterization of materials, the knowledge of the causes of degradation of materials, the development of new methods and materials aimed at lengthening the life time of artifacts, are mandatory in the correct safeguard of cultural heritage.

NMR application can be extended to a wide number of different issues regarding Cultural Heritage [1-3]. Laboratory NMR instrumentation allows the investigation of the structure of organic, inorganic and hybrid materials. The availability of portable NMR instrumentation allows the study of large objects fully preserving the integrity and the dimension of the object under investigation. NMR parameters are important to establish the state of degradation of objects, to evaluate performances of consolidation and water repellent treatments carried out on porous materials, to monitor the detachment of the painted layer from the plaster, to quantitatively map the dampness in wall paintings. A further development of portable NMR devices is the availability of sensors to produce NMR stratigraphy with microscopic spatial resolution for investigating the layer structure of artifacts [4]. The study and the optimization of cleaning methods applied on materials of interest for cultural heritage is another new application. Cases of application of NMR techniques in the field of cultural heritage will be shown to illustrate the potentialities of NMR in this field of research.

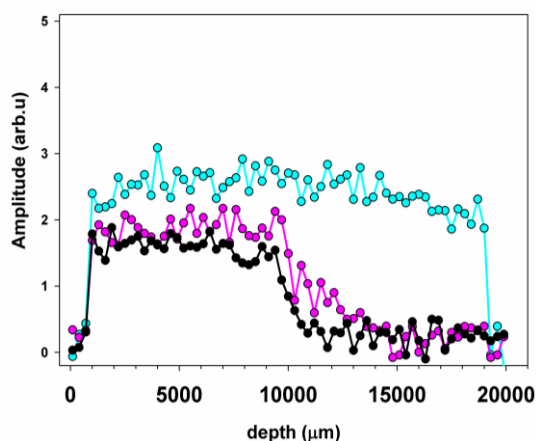


Fig. 1. NMR depth profiles of a porous stone after 30 minutes, 1 and 4 hours of water absorption from hydrogel

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STRUCTURAL INVESTIGATION OF ORGANIC MATERIALS BY SOLID-STATE DYNAMIC NUCLEAR POLARISATION (DNP) NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

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Solid-state nuclear magnetic resonance (SSNMR) is a versatile and purely non-destructive technique that can provide high-resolution molecular structural information on a large variety of materials, either directly by acquiring the NMR experiments at high magnetic fields or indirectly by taking advantage of multidimensional correlation schemes (or both). Contrary to scattering techniques, SSNMR is perfectly suited for the analysis of powdered samples (*i.e.* single crystals are not required), and it can access supramolecular structural information without the need of long-range translational order. The Achilles' heel of NMR, however, remains its low sensitivity that usually precludes analysis of structural details, which are intrinsically associated with NMR signals of low intensity. One of the most promising methods for boosting the SSNMR sensitivity is dynamic nuclear polarisation (DNP), which enhances nuclear magnetisation through the microwave-driven transfer (usually at cryogenic temperatures) of electron spin polarisation to nuclei *via* exogenous paramagnetic centres. DNP is nowadays attracting renewed attention owing to recent spectacular technological and theoretical developments. This communication will describe recent advances in the field of DNP SSNMR for the characterisation of materials in the solid-state by focusing on organic materials, including organic polymers and pharmaceutical compounds.

VISUALIZING BIOCHEMICAL ACTIVITIES IN LIVING CELLS WITH ^{13}C HYPERPOLARIZATION

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During the last decade, the development of nuclear spin polarization enhanced (hyperpolarized) molecular probes has opened up new opportunities for studying the inner workings of living cells. The hyperpolarized probes are produced *ex situ*, introduced into biological systems and detected with high sensitivity and contrast against background signals using high resolution NMR spectroscopy. A variety of natural, derivatized and designed hyperpolarized probes has emerged for diverse biological studies including assays of intracellular reaction progression, pathway kinetics, probe uptake and export, pH, redox state, reactive oxygen species, ion concentrations, drug efficacy or oncogenic signaling. These probes are readily used directly under natural conditions in biofluids and are often directly developed and optimized for cellular assays. The talk will focus on strategies used for the selection, design and use of hyperpolarized NMR probes in biological assays, and describe current developments of the technology for cellular applications.

MESOPOROUS SILICA NANOPARTICLES AS A DRUG DELIVERY SYSTEMS - SOLID STATE NMR STUDIES

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Developing the innovative methods for drug transportation in the body and increasing the therapeutic efficiency of new and old generation drugs with different physicochemical properties is one of the biggest challenges for pharmaceutical sciences. Both subjects (transportation and efficiency) can be greatly improved by introducing of strategy based on application of modern Drug Delivery Systems (DDSs). Today, the field of DDSs is growing very fast and became one of the most profitable approaches in pharmaceutical industry. The majority of DDSs are based on biological or inorganic/organic components.[1] To the latter group belong Mesoporous Silica Nanoparticles (MSNs) which were recently approved as drug carriers by Food and Drug Administration (FDA). The attractiveness and usefulness of MSNs as DDSs is due to their unique geometrical features, high surface area, large pore size and large pore volume that can be adapted to the specific needs.

In this talk we present our recent achievements related with study of ibuprofen embedded into MCM-41 (Mobil Crystalline Material 41). [2] We compared two methods (incipient wetness and melting) for the encapsulation of ibuprofen in the pores of MCM-41 through NMR (nuclear magnetic resonance) spectroscopy. We employed advanced NMR techniques for evaluation of the encapsulation methods included an analysis of the filling factor of the drug into the pores. The stability of Ibu/MCM in an environment of ethanol or water vapor was tested. Our study showed that melting a mixture of Ibu and MCM is a much more efficient method of confining the drug in the pores compared to incipient wetness. The optimal experiments for the former method achieved a filling factor of approximately 60%.

In the second part of the talk we present the applicability of melting method for confining of more complex systems into MSNs. As a model guest sample we employed cocrystal formed by benzoic acid (BA) and is penta-fluorinated analog (FBA). BA/FBA cocrystal and BA/FBA:MSN assemblies are fully characterized by ¹H, ¹⁹F and ¹³C NMR experiments. [3]. Finally, we show how to trap naproxen into MSN employing solvent free approach [4].

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METABOLIC PHENOTYPING: A REVOLUTION IN DIAGNOSIS?

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Metabolomic profiling of body fluids by NMR can be obtained in minutes, has unsurpassed reproducibility, and low costs (a few tens of Euro when done in high-throughput mode). Even on a small scale, and including statistical data analysis and comparison with databases, metabolomic profiling can be performed at less than 200 Euro, i.e. significantly less than other profiling techniques.

We and others have shown that individual metabolic profiles exist, that they are stable over periods of many years, insensitive to alterations of lifestyles or mild disease states, but sensitive to the onset of major diseases from a very early stage.

Examples of successful metabolomics profiling from our laboratory are for the diagnosis of potential celiac disease, the prediction of relapse for breast cancer, the prediction of survival of metastatic CRC, and the early diagnosis of heart failure.

Typical diagnostic accuracies range between 80-90%, which is remarkable considering that they can be obtained in the absence of clinical symptoms, and that they can be obtained from the same NMR profile by comparing it with the databases of a number of different diseases. Diagnostic accuracies improve dramatically if the profile of an individual is compared with earlier profiles of the same individual. These evidences suggest that metabolomics by NMR can become a first-line, population-wide screening method.

ORAL COMMUNICATIONS

COMPUTATIONAL NMR SPECTROSCOPY: REVERSING THE INFORMATION FLOW

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In this lecture I will go through some of the work I have been doing over the last few years with prof. Alessandro Bagno, with the aim of highlighting the power of computational chemistry for the prediction of the NMR properties of simple organic molecules, natural substances and compounds containing heavy atoms [1,2].

Notwithstanding the potential of the NMR techniques to provide plenty of information to elucidate the structure of unknown natural substances, or to characterize newly synthesized products, it still happens in some cases that the originally proposed structures need to be revised after total synthesis [3]. In this area, the prediction of the ¹H and ¹³C spectra, by DFT methods, of organic molecules composed only of light atoms (typically C, H, N, O) has now reached a stage which complements the experiments in a fruitful, and in some cases crucial, way [4-6].

On the other hand, walking down the periodic table, when the going gets heavy relativistic DFT gets going. In the field of organometallic and inorganic chemistry, heteronuclear NMR also plays a dominant role for the determination of the structure and geometrical parameters of, for example, heavy-metal complexes [7,8] or even ill-defined (from the geometrical point of view) non-covalent supra-molecular systems such as xenon dissolved in liquids [9], ionic liquids [10] or encapsulated in cryptophanes [11]. In such cases relativistic effects dominate the NMR spectra and the comparison of the results of relativistic DFT calculations of the NMR properties of the heavy atom (e.g. ¹²⁹Xe) with experimental data becomes necessary in order to rationalize and understand the experimental findings.

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TOWARDS THE SIMULATION OF COMPLEXITY: LIMITS AND POTENTIALS OF MULTISCALE METHODS BASED ON QUANTUM CHEMISTRY

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The simulation of processes and phenomena of increasing complexity is a challenge for the computational modeling as both accuracy and completeness are required in order to reach a reliable and physically sounded description.

Obviously, these two requirements cannot be achieved using a homogeneous approach but instead a multiscale strategy has to be introduced. By combining different methods suited to describe different length scales, multiscale methods can achieve a detailed molecular-level description of the process of interest still correctly accounting for the effects that the embedding environment (going from the nanoscale to the bulk) have on that process. In this talk some examples of the application of multiscale methods which combines a quantum-mechanical (QM) and a classical description will be presented and discussed.

PSEUDOCONTACT SHIFTS AND STRUCTURE DETERMINATION OF LANTHANIDE COMPLEXES

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Small-size lanthanide complexes are interesting for their manifold applications as luminescent probes, as magnetic resonance imaging contrast agent, in the fabrication of OLEDs, or as catalysts in organic synthesis.

The hyperfine shift induced by Ln³⁺ ions on NMR-active nuclei offers an extraordinary means to accurate geometry determination and provides experimental evidence for computational models of electronic distribution.^{1,2}

The separation of pseudocontact and Fermi contact shifts (PCS and FC, respectively) is a preliminary operation to be conducted on raw experimental data (usually, NMR shifts of a set of isostructural complexes of Ln³⁺, Ln1 and Ln2) and we have proposed a method which avoids several limitations and drawbacks of classical Reilly's procedure.^{3,4}

In a first step, one can neglect FC (of course, excluding Gd³⁺) which results in a linear correlation of PCS of all the other Ln³⁺ compounds, one vs. the other, according to⁵

$$\delta_{i,Ln}^{para} = F_i \langle S_z \rangle_{Ln} + D_{Ln}^1 G_i + D_{Ln}^2 H_i \approx D_{Ln} [G_i + rH_i]$$

$$\delta_{i,Ln1}^{para} \approx D_{Ln1} [G_i + rH_i] = m_{Ln1,Ln2} D_{Ln2} [G_i + rH_i] \approx m_{Ln1,Ln2} \delta_{i,Ln2}^{para}$$

The proportionality constant $m_{Ln1,Ln2}$ can be used to achieve FC-PCS separation according to

$$\frac{\delta_{i,Ln1}^{para}}{\langle S_z \rangle_{Ln1}} = F_i + m_{Ln1,Ln2} \cdot D_{Ln2} [G_i + rH_i]$$

We shall discuss the principles and advantages, and show the results in a set of practical cases.

Further we shall demonstrate how this protocol lends itself to analyze variable temperature data from one complex.

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PREDICTION OF ^1H NMR PARAMETERS IN PARAMAGNETIC SPECIES

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Molecular magnets are substances that behave like a magnet on a molecular or atomic scale. Over the past two decades, the field has expanded from the study of manganese-containing materials to include other transition metals as well as lanthanides. It is clearly desirable to be able to predict and probe the spin state of such complexes, since this capability would allow designing complexes endowed with desired characteristics and monitoring their properties. While Mössbauer and EPR spectroscopies may often help to understand many such properties, these techniques, however powerful, are subject to limitations (the former is essentially limited to Fe, and in the case of fast electronic relaxation EPR spectra are difficult to obtain).[1]

On the other hand, NMR spectra of many paramagnetics are fairly easy to obtain. However, in such cases line broadening and major paramagnetic shifts, which lead to “unpredictable” resonance frequencies, complicate assignment of such spectra and limit the information that can be extracted. In most cases assignments are doubtful, purely empirical or even impossible.[1]

Such difficulties can be overcome through the calculation of nuclear shieldings, hyperfine coupling constants and g -tensors by density-functional theory (DFT) methods.[2] In this communication we will outline how DFT methods can deliver good predictions of NMR parameters for simple organic free radicals [3] and some transition metal complexes, including species with $S > 1/2$ such as Fe(IV)-oxo molecules of biochemical importance. [4-5]

Many important areas of current interest will benefit from deploying the proposed approach, such as materials science (spin-crossover systems), catalysis and organic electronics (transition metal complexes), biochemistry (metalloproteins) and imaging in medicine (lanthanide complexes).

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EXPLORING PROTEIN FOLDING PATHWAYS BY NMR SPECTROSCOPY

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Protein folding represents one of the most intensively studied phenomena of recent times in biology, but nonetheless the molecular mechanisms by which a peptide chain reaches its native structure have not been yet fully understood [1,2]. Importantly, understanding protein folding pathways plays an essential role in the comprehension of many diseases rooted in the protein misfolding processes, also considering that every functional protein is permanently in equilibrium with its unfolded state [3]. As a matter of fact, evolutionary selection favoured protein structures characterized by folding pathways preventing the formation of uncontrolled protein misfolded states, which in some peculiar conditions, either pathological or physiological, may anyhow take place. Here, the investigation, by means of NMR methodologies combined with CS, DSC and computational analysis, of protein folding mechanisms, which may help in the comprehension of misfolding molecular processes, will be described.

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BIOMOLECULAR SOLID-STATE NMR AT ULTRA-FAST MAGIC-ANGLE SPINNING: A REVOLUTION THROUGH FASTER REVOLUTIONS

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Ongoing methodological developments in nuclear magnetic resonance (NMR) have paved the way for the determination of structure and dynamics of solid biological samples at atomic resolution. Solid-state NMR is particularly useful to investigate systems that for their nature or their size are not amenable to solution NMR. Despite the recent progress, however, these studies are not routine, require large amounts of protein sample and often prohibitively long times for data acquisition and analysis.

The availability of probes capable of faster and faster magic-angle spinning has revolutionized biomolecular NMR, notably allowing the efficient detection of ¹H resonances in solid samples. We describe here measurements on a new Bruker 0.7 mm magic-angle spinning (MAS) probe where samples spin at 111 kHz at magnetic field of 1 GHz. This new probe requires less than 0.5 mg of protein and the new MAS regime halves the homogeneous contribution to the ¹H line-widths in fully-protonated protein samples. We show that triple-resonance experiments can be efficiently acquired on fully protonated microcrystalline GB1 (6.5 kDa), yielding HCN correlations in minutes [e.g. 2D (H)NH or (H)CH] to hours [e.g. 3D (H)CANH]. ¹H, ¹⁵N and ¹³C coherence lifetimes are significantly lengthened, allowing the design of improved schemes for rapid unambiguous backbone and side-chain ¹H, ¹³C and ¹⁵N assignment, yielding rapid access to important structural parameters, such as ¹H-¹H distances between side-chains. Spectra are of sufficient quality to allow unsupervised backbone resonance and distance restraints assignment, and automated structure calculation using as inputs two 3D spectra (NHH and CHH) acquired in less than 24 hours, yielding a bundle of structures with an all-atom RMSD of 0.7 Å and a bias to the X-ray structure of 1.7 Å.

These findings increase the impact of solid-state NMR to samples that cannot easily be deuterated, and for samples that can only be produced in sub milligram quantity, and applications of the method are shown on the the light-responsive membrane protein proteorhodopsin reconstituted in lipid bilayers.

THE DOUBLE LIFE OF PHD FINGERS: EPIGENET READERS OR HUBS FOR MULTIPLE INTERACTIONS? THE STRANGE CASE OF SP140 AND NSD1

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In recent years the PHD finger domain, one of the most recurrent domains in nuclear proteins has been extensively studied both from the structural and functional point of view. This small Zn²⁺ binding domain has emerged as a robust structural scaffold for diversified activities: it can work not only as epigenetic reader sensing the modification status of histones, but also as general protein-protein interaction motif, thereby expanding its role in diverse cellular processes including transcriptional regulation and/or signal transduction. Its high functional versatility relies on the low secondary structure content and on subtle but significant changes in aminoacids compositions contributing to the domain functional and structural plasticity.

Herein I present two paradigmatic examples regarding the PHD fingers of Sp140-PHD (1) and NSD1, two transcriptional regulators involved in developmental diseases (cancer and Sotos Syndrome). Functional and structural studies performed on these domains highlight their considerable structural and functional versatility.

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UBIQUITIN-NANOPARTICLE INTERACTIONS PROBED BY NMR

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The use of nanoparticles (NPs) offers outstanding potential for future biomedical applications. It is well known that a material is always covered by proteins immediately upon contact with a physiological environment [1], therefore NPs associating with biomacromolecules may interfere with protein–protein interactions and affect cellular communication pathways. Thus, understanding the interaction of nanomaterials with biological systems becomes key for their safe and efficient application. Currently very little is understood of how protein molecules interact with NPs surfaces.

In this respect, particularly relevant is the study of NP-induced functional perturbations of proteins implicated in the regulation of key biochemical pathways.

In this study [2] we focussed our attention on the interactions established by either monomeric ubiquitin (Ub) or a minimal polyubiquitin chain with polyhydroxylated [60]fullerene.

Fullerenols are among the most important and promising fullerene derivatives with tunable properties by varying the number of hydroxyl groups introduced; their water solubility is an extremely important property for life science applications.

Ubiquitin (Ub) is a prototypical protein post-translational modifier playing a central role in numerous essential biological processes (i.e. DNA repair, protein degradation via proteasome, translation). The adsorption of fullerene and fulleranol on protein samples has been investigated *in vitro* at atomic resolution using a variety of techniques including NMR.

To identify Ub sites involved in NPs recognition, changes in the NMR spectra of ¹⁵N-Ub and segmentally ¹⁵N-labeled lysine48-linked Ub2 were followed. The specific interaction epitopes were identified, coincident with functional recognition sites. Fulleranol appeared to target the open state of the dynamic structure of a dimeric Ub according to a conformational selection mechanism. Importantly, the biomolecule–NP association prevented the enzyme-catalyzed synthesis of polyubiquitin chains. Our findings provide an experiment-based insight into protein/fullerenol recognition, with possible nanotoxic consequences on cell homeostasis. Moreover, the specific inhibition of critical Ub/proteasome interactions represents a novel potential opportunity of therapeutic intervention in Ub-dependent cellular pathways.

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HOMONUCLEAR DECOUPLING

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The unambiguous identification of chemical shifts by eliminating the overlap created by the multiplet structure has been of interest ever since the early days of NMR. The first experiment proposed for this purpose was the J-resolved 2D. Over the years different approaches have been suggested to provide alternative routes. Most of them rely on the selective manipulation of a given spin within the network of coupled spins of a molecule. There are two means of doing this. The first is a spatial discrimination; applying a selective pulse (with a bandwidth of about 50Hz) in the presence of a weak gradient as first proposed by Zangger and Sterk [1]. The second way is to rely on the fact, that due to the natural abundance of ^{13}C spins, there is only one proton attached to ^{13}C in a given molecule (two or three respectively for methylene or methyl groups). Using a $\text{BIRD}^{\text{r,x}}$ element hence allows to manipulate this one spin differently from the rest by inverting all spins but the directly attached proton [2, 3]. In either case this goes along with a significant reduction in sensitivity (about two orders of magnitude). Experiments of this type are mostly referred to as Zangger-Sterk, reset or pure shift experiments.

The elements described above (Zangger-Sterk and $\text{BIRD}^{\text{r,x}}$) can be incorporated into 2D experiments in two ways, either by adding a third dimension or, as published more recently, by applying them directly in the acquisition dimension [4, 5], in order to obtain broadband homonuclear decoupled 2D spectra.

In particular the combination of instant decoupling using a $\text{BIRD}^{\text{r,x}}$ element with HSQC type experiments has proven very successful [4-7]. The concept has also been extended to band selective decoupling [8-12]. Alternative building blocks for spatial discrimination like the PSYCHE experiment have been designed to improve the sensitivity of the experiment [13].

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SOLID STATE NMR STUDY OF THE PROCESSING-DEPENDENCE OF SILK FIBROIN SECONDARY CONFORMATION

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Silk fibroin is a natural polymer characterized by complex hierarchical structures, assembled from the bottom-up processes. The different silk fibroin conformations affect both the specific biological functions and the physical properties. Thanks to this peculiarity, silk fibroin-based materials have attracted a great attention as bioactive substrates for biomedical applications, such as tissue engineering, drug storage and delivery, biosensors and nanomedicine [1][2][3]. Several in vivo and in vitro studies had shown the effectiveness of fibroin scaffolds for tissue repair or regeneration [4][5][6].

The control of the hierarchical assembling of silk fibroin is the key for finely tuning the biological functions and physico-chemical properties of the final materials for applications in biomedical fields. Accordingly, the solid state NMR analysis is here proposed together with infrared spectroscopy, with the aim of getting insight into the

protein conformation changes induced by the processing conditions, by using silk fibroin fibers and different stabilized cast films as a model. The results allow to establishing the dependence of silk fibroin configurations on processing conditions, quantifying the presence of well-known silk I and silk II structures, and experimentally assessing presence and percentage of the asymmetric three-fold helical conformation, i.e. the proposed Silk III structure [7].

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HALOGEN BONDING: A SOLID-STATE NMR STUDY

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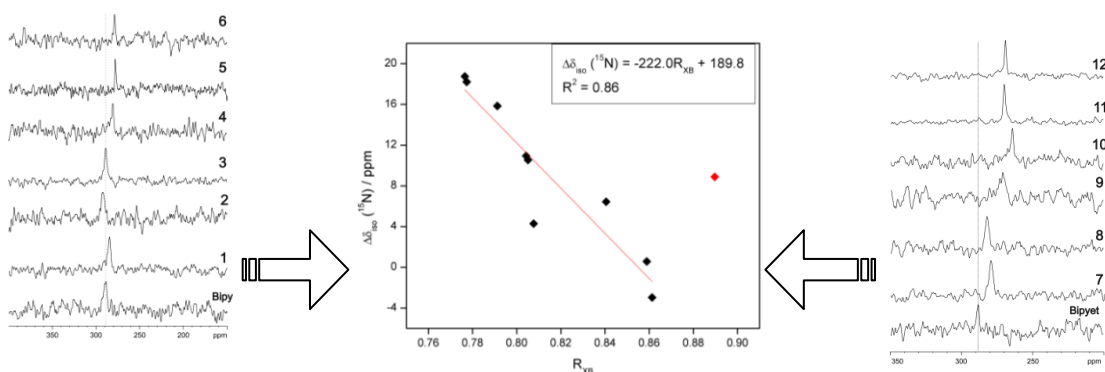
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Halogen bond (XB), R–X···Y, is a highly directional, non-covalent interaction between a covalently-bonded halogen atom X and a nucleophilic region on a neutral molecule or an anion.¹ This interaction today finds uses in many different fields, such as crystal engineering, biochemistry, and materials chemistry.² However, although some articles on the relationships between NMR parameters and XB features are already present,^{3,4} clear correlations have not been found yet.

This work is the result of a systematic study of two series of halogen-bonded supramolecular structures between dipyrindyl derivatives and either halobenzenes or haloalkanes. A combined solid-state NMR (SSNMR), X-ray diffraction and computational analysis approach has allowed for a ranking of strength and structural influence of different XB donors. The isotropic chemical shifts were obtained by ¹⁹F-¹³C NQS and ¹⁵N CPMAS experiments. The relationships between the changes in chemical shift of atoms involved in halogen bonding and strength or geometry of the interaction have been underlined for both ¹³C and ¹⁵N nuclei. The most interesting correlation has been found between the ¹⁵N chemical shift and the strength of the interaction which strictly correlated to XB donor.



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DRUG-INORGANIC MATRIX COMPOSITES STUDIED BY SOLID STATE NMR

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Many active pharmaceutical ingredients (APIs) show a limited and variable bioavailability mainly associated to inadequate biopharmaceutical properties such as aqueous solubility and dissolution rate. The latter is the main factor responsible for the limited efficacy of many biopharmaceutics classification system (BCS) class II and class IV orally administered drugs.

In this context, the employment of inorganic matrices, such as mesoporous materials and lamellar anionic clays, for the preparation of host-guest composites is a suitable strategy for improving biopharmaceutical properties [1]. Indeed, some inorganic matrices are able to host drugs into nanometric galleries or pores in non-crystalline form. In this way rapid dissolution can occur after the contact with the dissolution medium, without the need of a previous modification of the API chemical structure. The characterization of the solid form and chemical environment of the API in this kind of formulations therefore appears particularly important.

In this work Solid State NMR (SS NMR) techniques have been applied in order to investigate two different composites, containing ibuprofen as BCS class II guest API and Hallosites NanoTubes (HNT) [2] and Hydrotalcite (HTlc) anionic clays [3] as inorganic hosts. On one hand, experiments on ¹³C nuclei allowed us to obtain information on ibuprofen in the formulations. On the other hand, experiments on ²⁹Si and ²⁷Al nuclei gave insights into the properties of the inorganic matrices. Moreover, experiments on ¹H nuclei allowed both the organic and inorganic components to be inspected. The comparison of spectral and relaxation properties of the composites with respect to those of the pure components allowed interesting differences, especially on the dynamics of ibuprofen, to be highlighted and discussed.

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PERFORMANCE ASSESSMENT IN FINGERPRINTING AND MULTI COMPONENT QUANTITATIVE NMR ANALYSES

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The goal of this work was to set up a new quality control parameter suitable for performance assessment in multi component quantitative NMR analysis and NMR fingerprinting methods. In order to achieve the goal, an inter-laboratory comparison (ILC) was organized. 36 NMR data sets (corresponding to 1260 NMR spectra) were produced by 30 participants using 34 NMR spectrometers.

A model mixture made up of five compounds [Aldicarb, Methamidophos, Oxadixyl, Pirimicarb and 3-(trimethylsilyl)-2,2,3,3-tetradeutero-propionic acid sodium salt (TSP)] dissolved in deuterated water was submitted to NMR analyses. The analytical target of the comparison was the quantification of analytes by the calibration line method. Such a method was chosen as it allows for identification of a theoretical line to be taken as reference in performance assessment. The NMR experiment considered for the inter-laboratory comparison consisted of a single 90° excitation pulse preceded by a selective pre-saturation step. Nine NMR signals were selected for this study: three for Aldicarb, one for Methamidophos, two for Oxadixyl, two for Pirimicarb and the singlet of TSP which was taken as reference.

Results show that quantitative NMR is a robust quantification tool. Performance assessment was carried out on single component quantification, by the popular and traditional z-score, and on multi-component analyses by means of a new performance index (named Q_p -score) which is related to the difference between the experimental and the consensus values of the slope of the calibration lines. By an analogous reasoning followed for z-score, performance assessment by Q_p -score is considered satisfactory when $|Q_p| \leq 2.0$, questionable when $2.0 < |Q_p| < 3.0$ and unsatisfactory when $|Q_p| \geq 3.0$.

This study introduces a new quality control parameter, Q_p -score, suitable for harmonization of fingerprinting protocols and quantitative multi component analysis. Such parameter, that was designed considering consolidated internationally agreed statistics, represents an unbiased evaluation tools for NMR method validations. Q_p -score accounts for laboratory performance in terms of both instrumental adequacy and operator skill and enables laboratories to pooling of NMR data in suitable databanks. Moreover, Q_p can be valuable for the development of multi-laboratory metabolomic platforms.

THE NMR BASED APPROACH IN QUALITY ASSESSMENT OF SAFFRON

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Saffron is obtained from dried red stigmas of *Crocus sativus* L. Due to the limited production and the manual harvest, saffron represents the most expensive spice in the world. Among the different producers, Iran is the biggest followed in Asia by India while in Africa and Europe, the major producers are Morocco, and Greece, Spain and Italy respectively. Appreciated for its health benefits saffron is widely used as food ingredient. The typical color, taste, aroma and flavor are due to three secondary metabolites that are crocins, picrocrocin and safranal respectively. The spectrophotometric quantification of these compounds, together with other physicochemical parameters, are used to define the quality and consequently the commercial value of saffron.

In this work NMR spectroscopy has been employed to analyze the metabolic content of saffron. Multivariate statistical analysis protocols applied on ¹H NMR data let to evaluate different quality aspects of saffron, such as the possibility to differentiate Italian PDO saffron from the commercial ones [1], to define the period of storage during which saffron can be considered still fresh [2], and to identify possible adulterants [3]. Part of these results have been obtained within the frame of COST ACTION FA1101 "SaffronOmics".

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HOW TO PUT NMR ON A MAP: AN ALTERNATIVE APPROACH TO GEOGRAPHIC ORIGIN

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The traditional approach to the identification of territorial origin of food samples by NMR profiling consists in the use of classification algorithms based on categorical variables defined by parameters other than chemical content.

In this work we suggest an alternative approach which starting from the chemical content permits to find territorial regions having metabolic similarities.

By using georeferenced samples it is actually possible to represent the spatial information contained in the NMR spectra directly on a map by appropriate geostatistical algorithms (see Fig. 1).

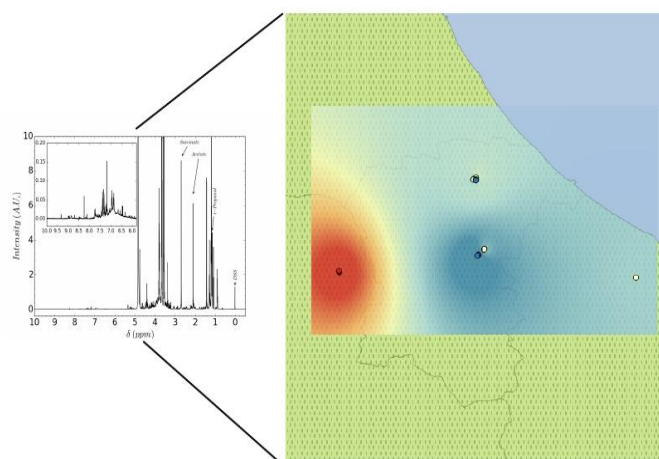


Fig. 1. GIS representation of wines NMR spectra.

From the analysis of the NMR maps, homogeneous metabolic regions can be identified and used as input for traditional classification methods.

In this work we describe the methods and the algorithms which permits to produce GIS maps from NMR profiles.

EFFECTS OF ULTRAFAST MAS

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We have recently introduced the ultrafast MAS system which has a tiny rotor less than 1mm outer diameter with a spinning speed capability of over 70 kHz.

Of course, a tiny rotor has a low sample volume, so the absolute sensitivity is less than a conventional system. However, this ultrafast MAS system can enhance the resolution, particularly for ^1H signals by reducing interactions that cause signal broadening.

This ultrafast MAS system enables us to perform various ^1H detected high resolution two dimensional experiments.

To demonstrate these possibilities, the results of various two dimensional high speed experiments will be shown.

INSIDE IONIC LIQUIDS BY NMR METHODS

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Ionic liquids (ILs) are salts generally made of an organic, bulky cation – such as N-alkyl,N-methylimidazolium, N,N-dialkylpyrrolidinium, tetralkylammonium etc. – and a variety of anions, the most popular being PF_6^- , BF_4^- and bis(trifluoromethanesulfonyl)imide (TFSI). Such structural features are largely responsible of the inability of the ions to organize in a crystal lattice, thus giving isotropic, homogeneous liquids in a wide range of temperatures. It was demonstrated that many classes of ILs do exhibit local heterogeneity with formation of polar and non-polar domains in the nanometer length-scale [1, 2]. These aspects are still object of fundamental investigation with a variety of physical methods, such as neutron and X-ray scattering, vibrational spectroscopies, NMR, and represent one of the most fascinating characteristics of these systems [3].

The NMR studies of the local structure of ILs mainly rely upon two approaches: diffusivity and intermolecular, homo- and heteronuclear NOE [4].

This communication has the main goal of introducing two novel developments in NMR of ILs.

The first is the use of ^{129}Xe NMR spectroscopy as a new component of the toolkit for the investigation of the structural features of ILs. ^{129}Xe has been successfully used as “spin spy” [5] for molecular solvents, polymers and porous materials, but only recently for uncovering structural features of ILs at atomic level [6]. Some paradigmatic examples on the most common imidazolium based ILs in terms of available void volume inside the liquid will be presented and discussed.

The second concerns a critical re-thinking of the interpretation of intermolecular NOE in ILs. Quantitative $\{^1\text{H}-^{19}\text{F}\}$ and $\{^1\text{H}-^7\text{Li}\}$ NOE data on LiTFSI doped pyrrolidinium based ILs will be presented and discussed by considering both long- and short range interactions, in line with the recent theoretical models proposed by Gabl, Steinhauser and Weingärtner [7].

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MOLECULAR SPINS: A NEW FRONTIER (?)

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The idea presented here falls halfway between “*totally crazy*” and “*how comes we have overlooked it*”. I have briefly hinted at it during the 2011 Italian GIDRM meeting [1] and later at the 2013 ENC meeting [2]. This presentation expands on it in more details.

Consider a planar *diamagnetic* molecule which has an axial sense, such as that of chloraldehyde or, in general C(XYZ), where C is a central atom and XYZ are three different atoms/groups arranged around it (see Fig.1):

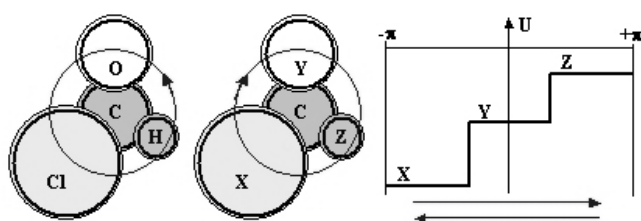


Figure 1: A bonding-shell electron shared by three different atoms XYZ is a bit like a particle constrained to a circular path and moving under the influence of a potential U (solid line) with no circular symmetry. In this case it evidently matters whether its wavefunction propagates clockwise or counter-clockwise (blue arrows) and the energies of the corresponding eigenstates are different.

The *axiality* of the arrangement of course does not give rise to distinct molecular species (such as in chiral structures). But it does define an axial reference vector, a fact compatible with the existence of a preferential direction of motion and thus a **persistent electron current loop**. An electron partially shared by the three atoms can circulate around the structure either in one sense (XYZ), or in the other one (XZY) and, when XYZ are all different, these two types of *loop orbitals* have different energies. Hence, mixing with conventional π -circular electron orbitals, they necessarily convey an axial character to the whole molecule. Obviously, this fact should be accounted for in any math model of the molecule. Yet present theories of quantum chemistry never consider such aspects. For example, they do not appear in DFT which considers only *electron densities*, but no *persistent electron current loops*.

If the insight is correct then some molecules, just like many elementary particles, and many nuclides, should possess an *intrinsic spin* (angular momentum) and an associated *permanent magnetic moment*, thus giving rise to magnetic resonance phenomena similar to those we handle in NMR. The existence of such *molecular spins* would then inevitably lead to a discipline of **Molecular Magnetic Resonance (MMR)**.

Here, starting from first principles, I attempt to estimate the magnitudes of persistent currents in circular molecules and molecular fragments, and reflect on any molecular spin phenomena that might be observable using NMR techniques.

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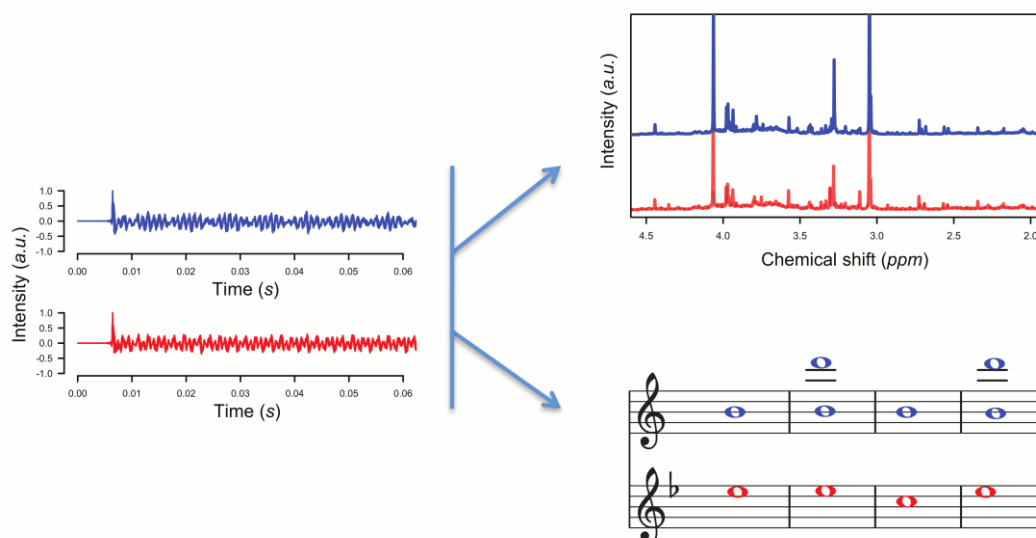
THE SOUND OF THE INDIVIDUAL METABOLIC PHENOTYPE: ACUSTIC DETECTION OF NMR EXPERIMENTS

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The *sound* of NMR signals could provide an alternative to the current representation of the individual *metabolic fingerprint* and supply equally significant information. The NMR spectra of different urine samples provided by two healthy donors were converted into audio signals that were analyzed in two audio experiments by listeners with both musical and non-musical training. The listeners were first asked to cluster the audio signals on the basis of perceived similarity and then to classify unknown samples after having listened to a set of reference signals. In the clustering experiment, the probability of obtaining the same results by pure chance was 7.04% and 0.05% for non-musicians and musicians respectively. In the classification experiment, musicians scored 84% accuracy which compared favorably with the 100% accuracy attained by sophisticated pattern recognition methods. These results support our hypothesis that the uniqueness of the metabolic phenotype is preserved even when reproduced as audio signals and warrants further consideration and testing in larger study samples.



S.Cacciatore, E. Saccenti and M. Piccioli *OMICS*, **19**, 147-156 (2015)

¹⁷O NMR STUDY OF DIAMAGNETIC AND PARAMAGNETIC LANTHANIDE(III)–DOTA COMPLEXES IN AQUEOUS SOLUTION

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The complexes between the ¹⁷O-enriched polyaminocarboxylate DOTA ligand and the whole series of stable lanthanide-(III) metal ions were studied in aqueous solution by ¹⁷O NMR:

I) The complex between DOTA and praseodymium(III) (Pr³⁺) was studied in aqueous solution by variable-temperature ¹⁷O NMR. pH effects as well as the influence of metal ions free in solution were investigated. The ¹⁷O NMR signals of both the nonchelating (O₁) and chelating (O₂) oxygen atoms could be detected. At low temperature, the signals of both the square antiprismatic (SAP) and twisted square antiprismatic (TSAP) conformational isomers were also observed. At high temperature, the spectra exhibit signal broadening that reveals the interchange of the O₁ and O₂ oxygen atoms of the carboxylate groups. The linewidths measured for O₁ were deconvolved into contributions from quadrupole relaxation and chemical exchange, allowing the corresponding activation barriers to be determined.¹

II) For all of the paramagnetic systems, except Gd³⁺, the ¹⁷O NMR signals of both O₁ and O₂ could be detected, and for some of them, the signals of both the SAP and TSAP (TSAP') conformational isomers were also observed. Line width data analysis reveals that signal broadening is not dominated by paramagnetic relaxation enhancement, as it was believed to be. The data indicate that quadrupole relaxation and, for some complexes, chemical exchange between the SAP and TSAP isomers are the major contributions to the ¹⁷O NMR line width at 25 °C. Besides, the Fermi contact and pseudocontact contributions to the observed lanthanide-induced shifts could be extracted.²

III) The ¹⁷O NMR spectrum of the non-coordinated carboxyl oxygen in the Gd(III) DOTA complex has been observed experimentally. Its line width is essentially unaffected by paramagnetic relaxation due to Gd, and due only to the quadrupole pathway. The results are supported by the relevant parameters (hyperfine and quadrupole coupling constant) calculated by relativistic DFT methods. This finding opens new avenues for investigating the structure and reactivity of paramagnetic Gd(III) complexes used as contrast agents in MRI.³

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NEW NMR METHODS BASED ON ¹³C DIRECT DETECTION TO STUDY INTRINSICALLY DISORDERED PROTEINS

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Recent progress in NMR instrumentation, in parallel to the growing interest in understanding the functional role of protein intrinsic disorder and flexibility, have stimulated the development of a variety of new NMR methods to study intrinsically disordered proteins (IDPs). The high flexibility and largely solvent exposed backbone typical of IDPs influence NMR parameters causing reduced chemical shift dispersion and extensive broadening of amide proton resonances, in particular approaching physiological conditions. These constitute general features of IDPs that need to be taken into account in the design of NMR experimental methods. ¹³C detected NMR experiments now offer a valuable tool to address these peculiar features of IDPs. The experimental variants to improve the performance of ¹³C detected NMR experiments to study IDPs will be discussed [1-5]. These open new ways to characterize IDPs of increasing size and complexity. Several examples will be presented.

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UNVEILING THE STRUCTURAL DETERMINANTS OF KIAA0323 BINDING PREFERENCE FOR NEDD8

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Protein post translational modification by covalent binding of ubiquitin and ubiquitin-like proteins (UBLs) have diverse effects including protein function, assembly, localization and lysis [1]. NEDD8 is an UBL protein having a 59% primary structure identity with ubiquitin while sharing an identical globular ubiquitin folding domain (UFD). Its main target is the ubiquitin E3 ligase family of cullins. Cullins act as scaffolding subunits of ubiquitin-protein ligases. Once cullins are neddylated, the RING-culling scaffolds, called Cullin-RING Ligases (CRLs), promote the ubiquitination of substrates. However proteins other than cullins have been reported to be also targets for Neddylation, indicating possible involvement in other biological mechanisms for this protein [2]. We used NEDD8 as a bait for the identification of potential binding partners and isolated as a candidate the C-terminal end of KIAA0323, spanning the last 52 amino acids. In this work we report for the first time both three-dimensional structures obtained by NMR spectroscopy of KIAA0323 C-terminal domain and of the NEDD8/KIAA0323 complex. Heteronuclear and homonuclear NMR spectroscopy techniques of ¹⁵N labelled and unlabeled proteins together with chemical shift perturbation (CSP), protein-protein rigid docking and molecular dynamics used during this study revealed the determinants of structure and interaction of this domain.

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**ALLOSTASIS AND RESILIENCE OF THE HUMAN INDIVIDUAL
METABOLIC PHENOTYPE**

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The urine metabotype of 12 individuals was followed over a period of 8–10 years, which provided the longest longitudinal study of metabolic phenotypes to date. More than 2000 NMR metabolic profiles were analyzed. The majority of subjects have a stable metabotype. Subjects who were exposed to important pathophysiological stressful conditions had a significant metabotype drift. When the stress conditions ceased, the original metabotypes were regained, while an irreversible stressful condition resulted in a permanent metabotype change. These results suggest that each individual occupies a well-defined region in the broad metabolic space, within which a limited degree of allostasis is permitted. The insurgence of significant stressful conditions causes a shift of the metabotype to another distinct region. The spontaneous return to the original metabolic region when the stressful conditions are removed suggests that the original metabotype has some degree of resilience. In this picture, precision medicine should aim at reinforcing the patient's metabolic resilience, that is, his or her ability to revert to his or her specific metabotype rather than to a generic healthy one [1].

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* BINGE EATING DISORDER: WHO, WHAT, WHEN, WHERE, WHY. THE ANSWERS OF NMR SPECTROSCOPY

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Episodes of binge eating (BE) in humans are characterized by compulsive, non-homeostatic consumption of an unusually large quantity of highly palatable food (HPF) in a short period of time. BE may be caused by interaction between dieting and stress, as reported in ref.1. In our model (1) BE for HPF is induced in rats by the combination of cyclic food restrictions and stress. The model employs female rats in relation to the higher prevalence of BE disorders in women. In order to demonstrate the effect of BE disorder in animal models, we faced the problem, for the first time, analyzing the metabolic profile obtained from biological fluids of BE rats, through the NMR Spectroscopy. NMR spectra are the “fingerprints” of the NMR detectable part of the whole metabolome. The metabolomic research is a consolidated area aimed to detect the pool of metabolites in biological systems (2). Female Sprague-Dawley rats were used: 1) NR+NS was normally fed and not stressed until day (d) 25, 2) R+S was exposed to 3 cycles of yo-yo dieting and stressed on d 25, 3) NR+S was normally fed and stressed until d 25 and R+NS was exposed to 3 cycles of yo-yo dieting and not stressed until d 25. All groups were fed HPF for 2 h on days 5-6 and 13-14. Stress was induced by preventing access to HPF for 15 min, while rats were able to see and smell it. After the stressful procedure, 300 μ l serum of rats were used for the NMR analyses. One- and two- dimensional NMR experiments from the 4 groups for the characterization of the metabolic profile were acquired. More than 30 metabolites were detected and assigned. To better understand the effects of the treatment, qualitative and quantitative analyses using a Mnova software to identify differences among the 4 groups were applied. Significant differences were found in small metabolites such as acetate, alanine glutamine, glycine and lactate. Another important difference is in the amount of lipids more evident in the R+S group with respect to the NR+NS ones.

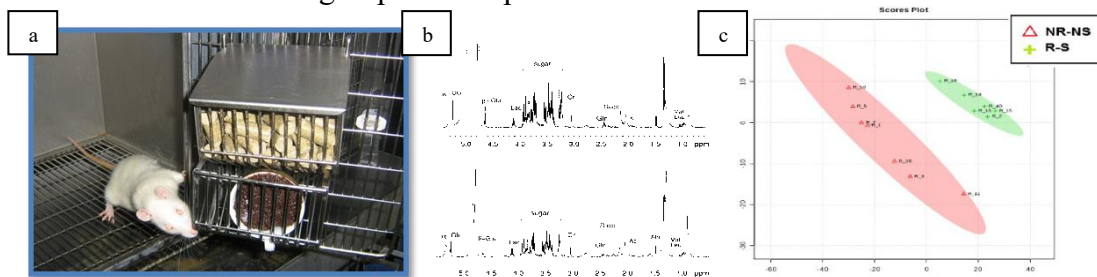


Fig. 1. a) how stress was induced by preventing access to HPF for 15' min, b) cmppg spectra of NR+NS and R+S groups and c) PCA box plot of NR+NS and R+S groups

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**NMR-BASED METABOLOMICS TO INVESTIGATE THE GUT
MICROBIOTA ACTIVITY. WHAT ARE WE LEARNING?**

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NMR-based metabolomic analyses of fecal material are gaining increasing attention because of the recent findings about gut microbial ecology and activity, that are found to have an impact on the human phenotype and host metabolism regulation. Gut microbiota contributes to the host metabolism through numerous mechanisms, including increased energy harvest from diet, modulation of lipid metabolism, altered endocrine function, and increased inflammatory response. Microbiota metabolic functions are mainly based on fermentation of available substrates which have escaped digestion in the upper gastrointestinal tract and flaking enterocytes. Thus, a major challenge is the understanding of host–microbiome metabolic interactions, notably the role of gut microbiota in early life, and how it affects the individual health programming.

NMR FINGERPRINTING TO DETERMINE HONEY AUTHENTICITYE. Schievano[‡]

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In both ancient and modern civilizations, honey represents a natural product of great importance. Far from being simply used as a sweetener, honey is known both as food with significant nutritional properties and as a natural product with valuable therapeutic applications. Honey can be obtained from *Apis mellifera*, which is present throughout the world, but also from stingless bee (Meliponinae), better known as pot-honey and mainly produced in South America, Africa and Australia [1]. Unfortunately, in the markets all over the world, the presence of honeys with incorrect declared origin in the label is increasing. The phenomenon of honey adulteration is so alarming that, in 2014, an European Union commission included honey in the list of products that are most at risk of food fraud. Several adulterations are possible in honey. These include masking the true floral, geographic, entomological origin and the dilution of honey with other, less expensive sweeteners.

The traditional approach to identify honey origin relies on pollen examination and on the evaluation of organoleptic features. Melissopalynological analysis requires highly skilled personnel and does not ensure reliable identification if the honey contains little or no pollen. Therefore, more effective quality control methods are needed to detect adulteration and to protect consumers from commercial speculations. Among the analytical methods applied in honey characterization, NMR has shown to be a powerful method to assess the origin of honey, although almost all the NMR studies on honey are based on sugar composition [2-4].

In this work, a different approach was developed [5], focused on minor components of honey with the aim to find reliable markers to ascertain its origin. An NMR-profiling approach, coupled with chemometric analysis, was applied to a data set of about 850 honey samples (700 from *Apis mellifera* and 150 from Meliponini). The NMR spectra of chloroform extracts revealed to be a good fingerprint for entomological, botanical and geographical discrimination.

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STABLE ISOTOPE-RESOLVED METABOLOMICS (SIRM): A METHOD TO STUDY METABOLIC FLUX IN CELLULAR OR MURINE MODELS

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The complexity of the metabolome in a biological system, especially the redundant use of the same metabolites in many biochemical pathways, makes impractical to obtain a mechanistic understanding of disease state and drug response based on metabolite concentrations alone. For studying the dynamism of specific metabolic pathway(s) and/or for following the fate of a specific metabolite, in the last years the use of stable isotope tracers coupled with metabolomics is gaining increasing interest. This method, called stable isotope-resolved metabolomics (SIRM), uses stable isotopes, non radioactive isotope, as a tracers, in animal models, patients, cells [1]. The most common used isotopes are ¹³C and ¹⁵N, that can be detected through the analytical tools used for metabolomics analysis: i.e. nuclear magnetic resonance (NMR) and mass spectrometry (MS), [2].

In the proton based NMR spectra it is easy to detect ¹³C labelled metabolites as they generate characteristics spectral fine structures (so called splittings due to ¹³C satellites), for the presence of NMR active isotope. By the ratio of the peaks integrals deriving from unlabelled and labelled metabolites (Integral ¹³C metabolite / (integral ¹³C metabolite+ integral ¹²C metabolite)) [3], it is possible to measure the percentage of ¹³C incorporated into the metabolite.

We applied the SIRM method in NMR-based metabolomics in a murine model of Autosomal Dominant Polycystic Kidney Disease (ADPKD) for confirming the Warburg effect *in vivo*. Uniformly labelled ¹³C glucose was injected in the peritoneal vein in *Ksp-Cre:Pkdl^{lox/+}* (wild-type, WT) and *Ksp-Cre:Pkdl^{lox/-}* (knock-out, KO) murine model, after 40 minutes mice were sacrificed and kidneys were collected. Polar metabolites were extracted from kidneys and NMR spectra were recorded. Through this method we showed that KO kidneys present more ¹³C glucose and ¹³C lactate than WT ones.

We applied the SIRM method in NMR-based metabolomics in a cellular model, the *TSC1^{-/-}* (knock-out, KO) and *TSC1^{+/+}* (wild-type, WT) murine embryonic fibroblasts (MEF) cells, for understanding if the accumulated metabolites of the Krebs cycle which we observe in these cells, derived from glucose or glutamine metabolism. Cells were fed with ¹³C glucose or with ¹³C glutamine respectively; polar metabolites were extracted from cells and NMR spectra were recorded for detecting satellites of produced ¹³C metabolites. Acquisition and analysis of the spectra is ongoing.

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MAGNETIC NANOPARTICLES IN BIOMEDICINE

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In the last two decades, a lot of attention was devoted to novel multifunctional nanostructures, based on magnetic nanoparticles (MNP), useful as agents for Magnetic Resonance Imaging, Optical Imaging and Magnetic Fluid Hyperthermia, as drug carriers and molecular targeting vectors. Many systems synthesized by different research groups, have been shown to possess high nuclear relaxivities, i.e. high efficiency in Magnetic Resonance images contrast, and Specific Absorption Rate (SAR). For some of these new compounds, the possibility to collect images of the regions where the MNP are delivered through MRI and Optical Imaging, is joint to the use of radio-frequency fields that can heat locally the tumour cells, possibly inducing their death; a theranostic agent is thus obtained. In the field of drug delivery, magnetic transport and molecular targeting, few examples of reproducible experiments using superparamagnetic nanoparticles are actually present in literature. All the above cases will be introduced and briefly discussed.

MICROENVIRONMENT-RESPONSIVE MRI PROBES FOR APPLICATION IN CELL THERAPY FOLLOW-UP

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Cell therapy can be broadly defined as the transplantation of living cells for the treatment of a wide number of medical disorders. Although cell therapy is poised to establish a new clinical paradigm, studies completed so far have produced mixed results, with overall limited clinical benefits on the long term [1]. A critical issue to understand how therapy works is the lack of clinically compliant methods to follow-up on the long term the state of transplanted cells.

A viable approach to follow-up therapeutic cells by Magnetic Resonance Imaging (MRI) is based on the integration of microenvironment-responsive MRI contrast agents within tissue mimetic hydrogels that are often used to encapsulate cells. Imaging-labelled, cell-encapsulating hydrogels respond at once to two critical aspects to improve the long term efficacy of cell therapy: *i*) to protect cells against immune-rejection and *ii*) to enable the longitudinal monitoring of the capacity of the microenvironment to sustain cell life. Here we describe gadolinium-containing microsized systems as extracellular pH or redox responsive MRI contrast agents. The pH_c responsive probe is based on microspheres (100 micron) made of poly(lactide-co-glycolide) (PLGA) surrounded by a chitosan hydrogel shell incorporating gadolinium fluoride nanoparticles as the T₁-contrast agent [2]. The size and surface properties add these microspheres with cell scaffold functionality for human mesenchymal stem cells.

The redox responsive systems is based on porous silica microspheres (SiMSs) whose surface has been decorated with a Gd-HPDO3A chelate through a disulfide bond, that provides MRI contrast responsivity to extracellular redox conditions. SiMSs have been designed with micrometer size and surface properties suitable to ensure a stable entrapment within a collagen/hyaluronic acid synthetic matrix while minimizing interactions with interspersed therapeutic cells. Responsivity is achieved by pharmacokinetic modulation of T₁-contrast enhancement.

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PARAMAGNETIC MESOPOROUS SILICA NANOPARTICLES AS POTENTIAL MRI AND THERANOSTIC PROBES

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The characteristic properties of mesoporous silica nanoparticles (MSNs), such as uniform mesopores, thermal stability and high versatility, render these solids ideal platforms for the development of efficient magnetic resonance imaging (MRI) probes. The optimization of the physico-chemical properties of both the Gd^{III}-chelates and the silica carriers is of great importance for the development of effective nanosystems for MRI applications. In recent years, we have started a project aimed at understanding the chemical role of the support on the magnetic properties of the systems. We found that the mesoporous silica itself plays a significant role in the physico-chemical properties of the final material, since the pore size directs the anchoring of the Gd^{III}-complexes inside the pores and/or on the outer surface, thus affecting the relaxometric properties [1-2]. The best performances in terms of relaxivity (r_1) have been achieved when the paramagnetic centers are accessible to water and in weak interaction with silica. The ¹H (R_1) NMR relaxometric properties of the probe were deeply investigated. In detail, the relaxivity was measured at 310 K as a function of the magnetic field strength over the frequency range 1–70 MHz. The sample showed NMRD profile typical of slowly tumbling systems with maximum of 79.1 mM⁻¹s⁻¹ at 0.5 T (per Gd), one of the highest so far reported [2]. MSN nanoparticles, functionalized on the external surface with a stable Gd³⁺-chelate (GdDOTA-monoamide derivative) and loaded inside the pores with ibuprofen

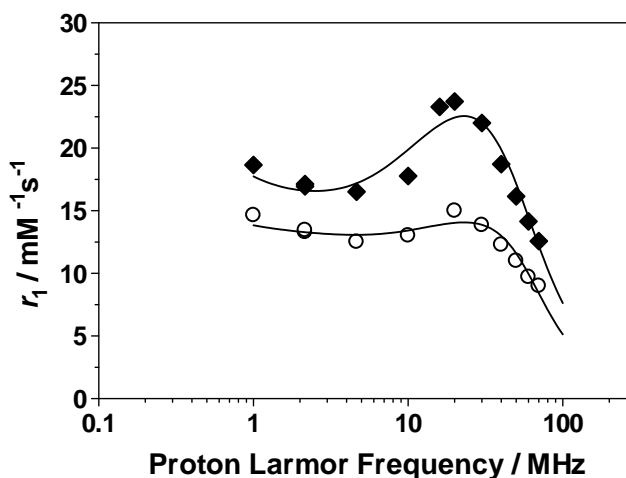


Fig. 1. $1/T_1$ NMRD profiles at 310 K for the hybrid material impregnated with ibuprofen and functionalized with Gd³⁺-chelates before (circles) and after partial release of the drug (diamonds).

were also prepared. Both the relaxivity of the Gd-based MSNs and the time-dependent release of the drug are affected by the presence of the other component in the final material. In particular, the relaxivity increases (+58%) after partial drug release as a consequence of an acceleration of the water exchange rate (Fig. 1). This result may open the way to a smart theranostic agent that allows to follow the drug release process by measuring the changes in relaxivity.

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POSTERS

**NATURAL COMPOUNDS AGAINST ALZHEIMER'S DISEASE:
NMR METABOLIC PROFILING OF DIFFERENT HOP EXTRACTS AND
THEIR EFFECTS ON A β PEPTIDE-INDUCED TOXICITY ON NEURONAL
CELL LINES**

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In recent years we have focused our interest on the identification of new ligands of A β 1-42 oligomers, involved in Alzheimer's disease[1]. Due to the severe impact of this pathology on the quality of life of the patients and their families, its massive economic burden and the lack of effective therapies and diagnostic tools, there is an urgent need for effective molecules for its treatment and diagnosis. In this context, the availability of new screening methods is strategic.

Recently, exploiting STD NMR and trNOESY experiments we were able to identify ligands of amyloid peptides and proteins in *Salvia sclareoides*[2], *Genista tenera* [3] and green tea extracts[4].

Now we are investigating the presence of molecules able to interfere with A β 1-42 peptide aggregation in hop extracts, also testing extract activity against A β -induced toxicity on neuronal cell lines

In this communication we show the results of our NMR metabolic profiling of four different hops varieties and preliminary data concerning molecular recognition studies with A β oligomers, together with a first evaluation of their neuroprotective activity.

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NMR PRELIMINAR EVALUATION OF THE EFFECTS OF AGRONOMICAL PRACTISE ON METABOLIC PROFILE OF CANNONAU WINE

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Wine is a food product of remarkable commercial interest for a lot of countries, among which Italy of course. The chemical composition of grapes and their wines is significantly influenced by the environmental conditions of the vineyard. The ¹H NMR metabolic approach can provide interesting information regarding the variety, geographical origin and behavior of fermentation [1].

By this approach we are performing a qualitative and quantitative analysis of the metabolome of pulp and skins of grapes, musts (before and after fermentation) and wines produced by different vineyards that deal with the vinification of Cannonau, a typical Sardinian wine, perhaps the oldest in the Mediterranean Basin.

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

The goal is to identifying the metabolites most representative and responsible for the differentiation during the fermentation process, the origin of the wine, as well as the sub-region and the winery.

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EXPLOITING NMR-BASED LIGAND-RECEPTOR INTERACTION STUDIES TO UNVEIL THE MECHANISM OF ACTION OF ANTI-AMYLOIDOGENIC COMPOUNDS

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In the last years, we focused our interest on the identification of new ligands of amyloidogenic peptides and proteins (A β 1-42, Ataxin-3 and HIV-1 matrix protein p17) involved in Alzheimer’s [1] Machado-Joseph’s neurodegenerative diseases [2] and HIV-associated neurocognitive disorder (HAND) [3]. Due to the severe impact of these pathologies on the quality of life of the patients and their families, their massive economic burden, and the lack of effective therapies and diagnostic tools, there is an urgent need for effective molecules for their treatment and diagnosis.

To this purpose, we have exploited STD-NMR, trNOESY and ¹⁵N-HSQC experiments to investigate the binding mode of some natural and synthetic compounds to the molecular targets previously mentioned. In addition, NMR results have been combined with data obtained through other biophysical techniques, among with AFM, CD, TEM and fluorescence microscopy, to further deepen the effect of ligand binding on amyloid aggregate morphology [4]. Here we report some of the most significant examples achieved to date.

Our data provide important information for the rational design of new compounds with higher affinity for A β 1-42, Ataxin-3 and HIV-1 matrix protein p17, to generate new anti-amyloidogenic molecules and/or molecular tools for the specific targeting of amyloid aggregates *in vivo*.

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NMR FOR MEASUREMENT OF THE AGGREGATION KINETICS OF POLYGLUTAMINE PEPTIDES AND INTERACTION WITH SMALL PEPTIDES BY NMR

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Misfolding and abnormal aggregation of proteins in the brain are implicated in the pathogenesis of various neurodegenerative diseases including Alzheimer's, Parkinson's, and the polyglutamine (polyQ) diseases. In the polyQ diseases, an abnormally expanded polyQ stretch triggers misfolding and aggregation of the disease-causing proteins, eventually resulting in neurodegeneration. We have characterized by NMR the synthetic aggregating peptide YAQ₁₂A. In addition, a protocol for measuring the aggregation kinetics by NMR was set up and the inhibition of the aggregation of YAQ₁₂A by the small 5QMe₂ (Ac-K-Q-Q(Me₂)-Q-Q(Me₂)-Q-CONH₂) was investigated [1]. Finally, the interaction in solution of 5QMe₂ with YAQ₁₂A and with a thioredoxin-polyQ₃₂ (thio-polyQ) fusion protein was also characterized. These experiments indicate that characterization of the NMR-observable soluble species may be a versatile and useful means to follow aggregation in different experimental conditions and should facilitate further investigations about the dynamics of the aggregation process of polyQ containing peptides and proteins.

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DOSE-DEPENDENT CHANGES OF RED BLOOD CELLS METABOLOME UPON γ -IRRADIATION: A NMR STUDY

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Blood transfusion is a fundamental therapy in several pathological conditions.

Many studies in the last years aimed to the identification of biomarkers of storage lesions and to evaluate the quality of red blood cells (RBCs) conserved in blood bank. In a previous study, the metabolic profile of RBCs after prestorage-leukoreduction has been determined [1] and 5-oxoproline has been proposed as candidate biomarker of RBCs protection against oxidative stress.

Leukoreduction is not the only prestorage treatment routinely performed on RBCs: γ -irradiation is a widespread practice to reduce the risk of graft-versus-host disease in immune-compromised patients [2]. Research on the consequences of blood products irradiation, so far, has focused on cellular immunity, lymphocyte-killing effects, free hemoglobin levels and changes in ions concentrations. Generally, all these studies indicate that irradiation causes damages to RBCs, that directly correlate with the irradiation dose. Although the impact on the activity and function of RBC is not massive [3], the real alteration of RBC vitality and the possible presence of by-products that may interfere with the transfusion therapy has not been comprehended, yet.

Indeed, metabolomics studies offer the possibility to provide new and precious information on those aspects, critical for the clinical practice. Aim of this work is to characterize the metabolic profile of RBCs subjected to treatment with different γ -irradiation doses, with the final aim to identify markers of γ -irradiation induced damages.

After leukoreduction, RBC aliquots have been irradiated with different nominal doses: 15, 25, 35, 45 and 60 Gy. ¹H-NMR spectroscopy is used to acquire spectra of every sample at day 0, 7 and 14, maximum storage time for γ -irradiated RBCs according to the Italian guidelines. Electrolytes quantification, hemolysis, cytofluorimetric and biochemical assays are performed in order to complement the NMR results. Biochemical data evidence a massive change dose-and time-dependent. A qualitative and chemometric analysis of the collected data will be presented. Finally, we will be able to evaluate the efficacy of this storage protocol with respect to the standard storage in conservation medium without any treatment.

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STUDY OF THE SIZE, SHAPE AND SOLVENT EFFECT ON LONGITUDINAL AND TRANSVERSE RELAXOMETRY OF FERRITE-BASED MNP

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The spin dynamics of novel superparamagnetic systems based on colloidal Iron-Oxide magnetic nanoparticles (MNPs) has been investigated by a systematic experimental comparison between dynamic magnetic susceptibility measurements and NMR relaxometry as a function of magnetic core diameter. Two differently shaped systems with similar size have also been studied. The nanostructures contain a surfactant-capped magnetite (Fe₃O₄) inorganic core, with different controlled size and shape ranging from 3.5 to 17.5 nm [1]. The as-synthesized nanostructures are passivated by hydrophobic surfactants (oleic acid) and fully dispersed both in hexane and in aqueous media by means of vesicles. These magnetic nanocrystals are potentially useful as contrast agents (CA) for magnetic resonance imaging (MRI) because of the high values of transverse relaxivity, which gives rise to a proper negative contrast in the MR images.

In order to study the fundamental physical mechanisms of nuclear relaxation, the complete NMR-D profile of r_1 (longitudinal relaxivity) and r_2 (transversal relaxivity) have been measured, until frequencies as low as 10 kHz, a not usual occurrence especially for r_2 . The NMR-D curves have been qualitatively compared with those predicted by theoretical models [2] describing the dependence of relaxivity on the size of the magnetic spheres, and confirm the hints on the nature of the involved physical mechanism: at low frequencies (<1 MHz about) the nuclear relaxation enhancement is led by the Neel correlation time while at higher frequencies the Curie relaxation mechanism dominates. Key parameters obtained from the models have been exploited to evaluate the impact of the contribution from magnetic anisotropy to relaxivity curves, and a comparison between the reversal time (τ_N) of magnetisation as seen by NMR and by AC susceptibility experiments shows a good agreement between the τ_N 's estimated with the two techniques.

To complete the magnetic investigation, a study of the energy barrier distribution and the Neel time as a function of the applied field has also been performed.

Acknowledgements

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STRUCTURE OF A NEW TRX-RELATED PROTEIN FROM *ECHINOCOCCUS GRANULOSUS* ABLE TO COORDINATE Fe/S CLUSTERS

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Echinococcus granulosus, also called Dog Tapeworm, is a cyclophyllid cestode that parasitizes the small intestine of canids as an adult, but which has important intermediate hosts such as livestock and humans, where it causes cystic echinococcosis, also known as hydatid disease. Echinococcal cysts are slow growing, but can cause clinical symptoms in humans and be life-threatening [1].

A unique aspect of flatworm metabolism is that these parasites are entirely dependent, for the control of redox homeostasis, on the enzyme thioredoxin glutathione reductase which provides electrons to thioredoxins (Trxs) and glutaredoxins (Grxs) at the expenses of NADPH [3]. This enzyme replaces the canonical thioredoxin reductase and glutathione reductase system. Parasitic tapeworm genomes have revealed an unexpected diversity of Trxs and Grxs [2], but the functions of most of these proteins have not been studied.

A new Trx-fold protein in the tapeworm *Echinococcus granulosus* has been recently characterized and designated Iron-sulfur Trx-related protein (IsTRP) [4]. The dimeric form of IsTRP coordinates Fe₂S₂ in a glutathione independent manner, relying on two adjacent cysteine residues highly conserved among Trxs. This novel binding mechanism allows holo-IsTRP to be highly resistant to oxidation.

Here, we present the high-resolution NMR structure of the apo-IsTRP and discuss it with major emphasis on the structural features required for the coordination of the cluster with the proposed binding mechanism.

Taking advantage of the high stability of the Fe/S cluster, we have also measured the circular dichroism (CD) spectrum of freshly purified IsTRP, under aerobic condition. CD spectroscopy confirmed the formation of the cluster, but the observed spectrum differs significantly from those reported for class II holo-glutaredoxins, proteins with a fold similar to IsTRP and able to coordinate Fe/S clusters [5].

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STRUCTURAL INSIGHTS INTO THE INTERACTION OF *O*-ACETYLSENERINE SULFHYDRYLASE WITH PEPTIDES: LESSONS FROM STD-NMR

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The last step of cysteine biosynthesis in bacteria and plants is catalyzed by *O*-acetylserine sulfhydrylase (OASS). In bacteria, two isozymes with similar binding sites, *O*-acetylserine sulfhydrylase-A (CysK) and *O*-acetylserine sulfhydrylase-B (CysM), have been identified, whose respective specific functions are still debated. OASS plays a key role in the adaptation of bacteria to the host environment, in the defense mechanisms against oxidative stress and in antibiotic resistance. Since mammals synthesize cysteine from methionine and lack OASS, the enzyme is a potential target for antimicrobials. In this work STD-NMR was applied to study the interaction of the inhibitory pentapeptide MNYDI with CysK and CysM from *Salmonella typhimurium* (StCysK and StCysM) and with CysK from *Haemophilus influenzae* (HiCysK). The structure of HiCysK in complex with MNYDI has already been solved and served as an internal control for method optimization. On the contrary, no three dimensional structure of either StCysK or StCysM in complex with reversible ligands has been solved to date.

NMR spectra were recorded in a Varian INOVA 600AS spectrometer. The STD experiments were performed with an enzyme:peptide molar ratio of 1:300 in 5 mM phosphate buffer, 5% D₂O, pH 8 at 20°C. The Group Epitope Mapping (GEM) calculation was carried out using the STD amplification factor (ASTD) data after correction for the signal artifacts generated by the on-resonance saturation of the peptide alone. In all the complexes the highest STD signal is associated with the protons of Ile5 side chain that is thus likely to make a dominant contribution to the binding energy. In the case of StCysK, the enzyme form showing the highest affinity for MNYDI peptide, a significant saturation of aryllic protons was visible in the STD-NMR spectrum. In particular, protons 2,6 of Tyr3 contribute more than 60% to GEM. These data are in good agreement with docking studies and structure-activity relationships, that suggested a pivotal role played by an aromatic residue at position P3 for high affinity binding.

**STRUCTURAL INSIGHTS INTO THE INTERACTION BETWEEN THE
TANDEM PHD FINGER DOMAIN P5C5 OF NSD1 AND THE ZINC FINGER
MOTIF C2HR OF NIZP1**

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Point Mutations or deletions in *NSD1* gene cause human Sotos syndrome, a development overgrowth disorder characterized by facial dysmorphism, learning disability, and cerebral gigantism [1]. NSD1 is a multitasked protein with a dual activity that can act both as a repressor and coactivator [2,3]. It contains several epigenetic domains including a SET domain responsible for histone methyltransferases activity, two nuclear receptor-interaction (NID) motifs, five zinc finger domains (PHD1-5), a variant PHD finger (C5HCH), two proline-tryptophan-proline-tryptophan (PWWP1-2) domains [4], suggesting a role in chromatin regulation and gene expression. The presence of deletion and/or point mutations targeting all the NSD1 modules suggests an essential, non-redundant role for these domains in the Sotos Syndrome [5]. It was speculated that the abrogation of NSD1-mediated repression of growth-promoting genes might contribute to pathological conditions [4]. In this context, about two-thirds of these mutations target the tandem domain PHD5-C5HCH of NSD1 (NSD1-P5C5), suggesting that it has a pathophysiological role, however its biological function is still unclear. NSD1-P5C5 appears to play a multifaceted role working as both histone reader (H3K4me3 and H3K9me3, [4]) and as interaction domain with the C2HR domain of the co-repressor Nizp1 [7]. In order to get more insights into the physiological and pathological role of NSD1-P5C5, we have solved its structure and characterized its dynamics and the interaction with histone markers and a non-histone protein. Contradictory data have been published on the ability of NSD1-P5C5 to recognize epigenetic markers and our NMR titrations confirm the interaction with histone H3 tail but exclude a specific binding with H3K4me3 and H3K9me3 epigenetic markers. We next solved the structure of the zinc finger Nizp1-C2HR, an unconventional zinc-finger motif, and we characterized its interaction with NSD1-P5C5, using a combination of NMR, ITC and computational methods. This is the first molecular description of the binding of NSD1 to an interactor and these data support the role of NSD1 as transcriptional repressor. In the context of the Sotos syndrome, we characterized the structural effect on NSD1-P5C5 of nine non-cysteine pathological mutations by NMR spectroscopy. The majority of them destabilize the fold, with the exception of the substitution His2162Arg that partially destroys the protein domain fold and the mutations Arg2152Gln and His2205Arg that maintain the protein fold. Our NMR and ITC titrations indicate that the mutation Arg2152Gln does not affect the interaction with Nizp1-C2HR, while the pathological substitutions His2162Arg and His2205Arg destroy or decrease seven-fold the binding to NSD1, respectively. The abrogation or the reduced interaction with a repressor like Nizp1 could be in line with the observed overgrowth phenotype. In future, we aim to solve the structure of the NSD1-P5C5:Nizp1-C2HR complex and investigate how the NSD1-P5C5 Arg2152Gln and His2206Arg Sotos mutations affect the interactome and genomic network of NSD1.

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ECO-SUSTAINABLE CEMENTS: UNRAVELING THE STRUCTURE OF MAGNESIUM SILICATE HYDRATE

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MgO-based cements are recently attracting a noticeable interest as eco-sustainable alternative to traditional Portland cements, thanks to the much lower CO₂ emissions associated with their production process [1]. Magnesium Silicate Hydrate (MSH), the binder phase of these innovative cements, arising from the hydration of MgO and a silica source, is a complex amorphous phase, with a micro- and nano-structure still relatively unknown [2, 3].

In this work we present the results of the first extensive characterization of the structural properties of MSH, obtained by means of multinuclear Solid State NMR and ¹H relaxometry techniques. In particular, ¹H and quantitative ²⁹Si mono- and bi-dimensional Solid State NMR experiments, carried out on MSH samples freeze-dried at precise times of hydration, allowed us to obtain detailed structural information at the nanoscale, identifying, quantifying and characterizing two different kinds of phyllosilicate-like domains present. On the other hand, ¹H T₁, obtained by Fast Field Cycling technique, shed light on the the status of water in pastes at different hydration times and on the evolution of the surface to volume ratio of the material.

The NMR results, together with those obtained from thermal, X-ray diffraction and SEM analyses allowed a detailed multi-scale characterization of the structural features of MSH to be obtained, which can improve the comprehension of MgO-based cements and contribute to tailoring the macroscopic properties from the modification at the nanoscale.

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THE UBIQUITIN-UBA INTERACTION INVESTIGATED UNDER CROWDING CONDITIONS

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The interior of cells is highly crowded with macromolecules, which may have an influence on protein structure, stability, dynamics, and interactions [1]. Usually, *in vitro* protein studies are performed on samples containing a total protein concentration below 10 g/L. These dilute solutions give optimal signals, but may lack biological relevance. Macromolecules occupy up to 30% of a cell's volume and reach concentrations of 100 to 400 g/L[2]. Such high concentration of macromolecules, referred to as crowding, results in non-specific interactions between the protein of interest and the crowder components. In order to explore the effects of the complex cellular medium on protein-protein interactions we chose the ubiquitin (Ub) - UBA interaction as a test system. Ub is a highly conserved, small regulatory protein, involved in diverse cellular functions such as protein degradation, chromatin structure, and heat shock, by conjugation to substrate proteins through an isopeptide bond [3]. The UBA domain is a short sequence motif of 45 amino acid residues that occurs frequently in proteins found in all eukaryotes. UBA can bind directly to Ub and/or poly-Ub, leading to an inhibition of the degradation of target substrates through the proteasome [4]. The goal of our work is to study the impact of crowding on the Ub-UBA association. We performed an NMR study of the interaction in the presence of an inert polymeric crowding agent. In addition to chemical shift perturbations and spin relaxation rate measurements, we investigated complex formation using paramagnetic spin labels[5]. The obtained data were compared to those measured in dilute solution.

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SIMULTANEOUS REFINEMENT OF BIOMOLECULAR STRUCTURES BY LONG RANGE PARAMAGNETIC NMR DATA AND X-RAY DATA

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Integrated experimental approaches play an increasingly important role in the structural biology field, taking advantage of the complementary information provided by different techniques. In particular, the combination of NMR data with X-ray diffraction patterns may provide accurate and precise information about local conformations not available from average-resolution X-ray data alone. This also offers unique opportunities to detect structural differences between the solid and the solution states, and can reveal the presence of either static or dynamic conformational changes. Here, we present a recently developed computational tool (REFMAC-NMR) for the joint refinement of crystallographic X-ray data and either paramagnetic NMR data or diamagnetic residual dipolar couplings, [1]. Pseudo-contact shifts (PCSs) and residual dipolar couplings (RDCs) gained an increasing interest during last decades due to their property to be exploited as long range restraints, helping in solving protein structures and providing useful insights on individual unit rearrangements in case of multi-domain proteins and protein-protein complexes [2]. The addition of PCSs and RDCs data as structural restraints, providing complementary information with respect to primary X-ray data, to one of the most widely used structural refinement software (REFMAC5 [3]) permits i) to point out the differences between structures in the crystal and in solution and, ii) in case of consistency of the data, to obtain more reliable refined structures. The program was tested on several proteins producing, in some cases, a single model consistent with both sets of observations and, in other cases, indicating the presence of non negligible differences between conformations in solution and in the solid state.

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NMR STRUCTURE ELUCIDATION OF TWO NEW ALKALOIDS ISOLATED FROM *GYMNOSPERMIUM MALOI*

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Gymnospermium maloi (Tan, Shuka, Maloi) is a new endemic species from *Gymnospermium* genus identified in the south region of Albania [1]. The members of this genus are poorly studied for what it concern the secondary metabolites in general and the class of alkaloids in particular. In fact from *Gymnospermium* genus, there are only few alkaloids characterized, (namely albertramine, albertidine, albertine) isolated from *Gymnospermium albertii* [2]. Until now the chemical composition and the structure elucidation of other possible secondary metabolites, specially alkaloids, remain still largely unknown. Here we report the structure, for the first time, of two new alkaloids isolated from *G. maloi*, designated as maloine and malonine, obtained by the use of 2D homonuclear and heteronuclear NMR spectroscopy, FTIR, UV and HPLC/MS spectra. Data collected from different techniques used in this study were congruent and sufficient to determine their final structures. Maloine and malonine cannot be ascribed to any known scaffold d of quinolidizine alkaloids , for that they are to be considered as new members of this class.

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CO₂ DYNAMICS IN METAL-ORGANIC FRAMEWORKS BY SOLID-STATE NMR

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Nowadays carbon dioxide (CO₂) emissions have become one of the most serious issues facing our civilization. Post combustion separation processes traditionally use aqueous solution of alkanolamine to chemically adsorb CO₂. [1] On the other hand, Metal-organic frameworks (MOFs) are emerging as promising candidates for CO₂ capture from dry flue gas. In this context, knowledge about both MOF binding sites and adsorbed gas dynamics within microporous solids is crucial for the design of more efficient gas capture MOFs. Recently, solid-state NMR techniques have been used for investigating the absorption of CO₂ by MOFs. [2]

Here we show the use of static and MAS (Magic Angle Spinning) ¹³C solid-state NMR techniques to investigate the interaction of ¹³C-enriched CO₂ molecules with the host structure of MOFs. In particular, we focused our efforts on UTSA-16. [3] UTSA-16 is a recently synthesized Cobalt-citric acid based MOF that exhibits extraordinary high CO₂ uptake and selectivity at ambient temperature.

Defined amounts of ¹³C-enriched molecules per metal atom were adsorbed on an activated sample. ¹³C NMR spectra were acquired with single pulse experiments which are able to highlight signals from the CO₂ molecules adsorbed in the MOF. Analysis of the resulting spectra reveals details of the binding and CO₂ rotational motion within the material. Three types of CO₂ molecules were observed: adsorbed CO₂ in the MOF, free CO₂ inside the MOF and free CO₂ outside the MOF. The two formers are in fast exchange dynamic. On the other hand, a slow exchange (in the NMR time scale characterized by the shift differences) is observed between adsorbed molecules and the gas phase under equilibrium conditions, without change of the total number of molecules, i.e., only redistribution. The dynamics of the motional processes are evaluated via analysis of the NMR line shapes and relaxation times observed in a range of different temperatures.

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GREEN COFFEE BEAN EXTRACTS AS POTENTIAL NEUROPROTECTIVE AND CHEMOPROTECTIVE DIETARY SUPPLEMENTS

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Among the metabolites present in green coffee extracts, most studies have been recently focused on chlorogenic acids (CGAs), esters formed between hydroxycinnamic derivatives (mainly caffeic and ferulic acid) and quinic acid, that represent the most abundant family of polyphenols in green coffee beans and occur ubiquitously in food.[1]

A number of beneficial biological effects have been described for CGAs, including anti-inflammatory activity, anti-carcinogenic activity and protection in neurodegenerative diseases.[2] However, the molecular mechanisms through which these biological activities are carried out have not been completely elucidated yet.

Here, the preliminary results obtained on green coffee extracts tested for their neuro- and chemo-protective activities are presented. Different ground green coffee beans, belonging to the specie Arabica or Robusta and coming from several geographical origins, were extracted according to different procedures, in order to evaluate their metabolic profiles and the efficacy of the extraction methods. The quali-quantitative characterization of the compounds contained in green coffee bean extracts was performed by mean of the combined use of NMR and UPLC/HRMS and the principal metabolites were identified and quantified, with particular attention to CGAs.[3]

Both the green coffee extracts and the most abundant phenolic constituent from CGA family, the 5-CGA, found in green coffee beans were tested for their biological activities. In particular, the molecular interaction with protooncogenic human model (hRAS) and neurodegenerative amyloid oligomers model (A β 1-42 oligomers) was evaluated by means of STD-NMR spectroscopy [4] and with other *in vitro* and *ex-vivo* techniques, among which cellular and biochemical assays, to validate the correlation among the molecular targets and the biological responses.

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NMR-BASED METABOLIC PROFILING OF BRONCHO ALVEOLAR LAVAGE FLUID (BALF) IN BRONCHIOLITIS OBLITERANS SYNDROME

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Early and long-term graft and patient survival after lung transplantation is challenged by chronic lung allograft dysfunction (CLAD), whose main phenotype is represented by an irreversible obstructive graft dysfunction known as bronchiolitis obliterans syndrome (BOS). CLAD diagnosis relies on the decline in lung function compared to the best post-transplant value and on chest imaging. Tools that will help unraveling the complexity of the disease and identifying useful predictive markers are urgently needed. Being a platform capable of capturing disease-relevant metabolic profile changes, metabolomics may occupy an important role in this diagnosis and an NMR-based approach may be suitable for this scope. In order to analyze via NMR samples from patients, BALf was obtained during fiberoptic bronchoscopy from lung transplant patients, before and after BOS development, and from subjects affected by other respiratory pathologies. After short sample preparation, BALf samples were submitted to NMR analyses. Exploiting the combination of mono and bi-dimensional experiments, NMR spectroscopy allowed the unequivocal identification and quantification of around 35 polar metabolites among which aminoacids, monosaccharides, nucleotides, Krebs cycle intermediates and phospholipid precursors. [1] The resonances of about 10 additional metabolites have not been assigned, yet. The experiments were performed on 20 samples collected from patients affected by different respiratory pathologies (12 BOS, 4 sarcoidosis, 2 pneumonia, 2 pulmonary condensation). A close correlation between metabolite abundance and pathology was found, with a significant dependence on the inflammatory state (the higher the level of inflammation, the higher the total amount of metabolites in BALf). Moreover, samples corresponding to different pathologies presented variations in the concentrations of some specific metabolites. In addition, to account for possible sample degradation after storage for different times (days or weeks) and/or at different temperatures (-20 °C or -80 °C), the metabolic profiling of samples recovered from the same patients, but differently stored, were compared. The NMR data revealed the potential of this methodology for the identification of predictive biomarkers to unravel lung pathologies, including BOS. These preliminary results strongly support the possibility to afford a metabolic signature of BOS from NMR BALf analysis.

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CONFORMATIONAL ENSEMBLES OF DISORDERED PEPTIDES TARGETING CHEMOKINE RECEPTORS: NMR AND MOLECULAR DYNAMICS SIMULATION STUDIES

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Recently, literature data have reported that each intrinsically disordered protein (IDP) or its regions (IDR) can dynamically explore in solution an ensemble of extended conformations, and may assume more stable and ordered structures only after binding to specific ligands [1]. Herein, we report the solution conformational preferences of two synthetic linear peptides: YGECPCCK-OAllyl (PepK) and YGECPCE-OAllyl (PepE) designed by choosing some amino acids with disorder propensity. The conformational features of these peptides were studied in solution by 1D [¹H] and 2D [¹H, ¹H] NMR (Nuclear Magnetic Resonance) spectroscopies and Molecular Dynamics (MD) techniques. NMR solution studies revealed the absence of regular secondary structure elements, thus confirming the natively disordered nature of these peptides. MD simulations evidenced that, even in presence of high flexibility, the two peptides are dynamically stabilized by a network of transient intra-molecular H-bonds of main chain-main chain (MM) type and by interactions with water molecules. Therefore, it becomes necessary to represent them by conformational ensembles characterized by the most populated conformers present at equilibrium (Fig. 1). We have consequently performed a cluster analysis to determine the groups of structures that share similar conformational features according to their root mean squared deviation (RMSD) values. In conclusion, despite peptides high flexibility did not allow us to calculate a 3D model based on NMR parameters, the MD was very useful to understand dynamically the evolution of their structures as well as to select the most populated conformational families needed to get a clear representation [2].

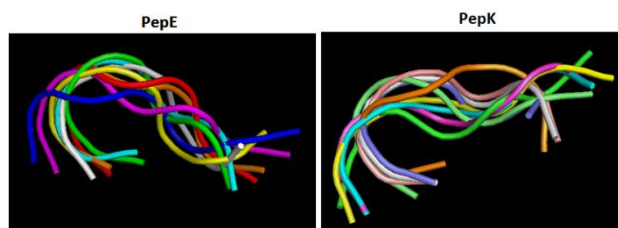


Fig. 1. Superimposition of the most populated clusters obtained for the PepE (8 clusters) and the PepK (9 clusters) during their molecular dynamics.

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ADVANCED NMR METHODOLOGIES TO CHARACTERIZE THE STRATIGRAPHY AND MATERIALS OF 14TH CENTURY TUSCANY WOODEN PAINTINGS

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In this study a multi-analytical approach was used to characterize ancient and modern constitutive materials and the stratigraphy of a Sienese wooden painting consisting of a lost Polyptych, by Andrea di Bartolo dated back to 14th century, restored and repainted in the 16th and 20th centuries. In order to distinguish original layers from the other ones, a comparison with the painting Adorazione dei Magi by Bartolo di Fredi (Andrea's father) was performed.

In situ measurements by portable NMR were performed to investigate the stratigraphy of the paintings in a non-destructive and non-invasive way [1-3]. With this technique, information about all layers constituting the paintings were obtained and used to plan the successive micro-sampling. Furthermore, solid state ¹³C and ³¹P CPMAS NMR spectroscopy and ¹H NMR spectroscopy in solution were carried out to characterize original and non-original materials. Complementary techniques such as ToF-SIMS, Infrared and Raman spectroscopy were also applied.

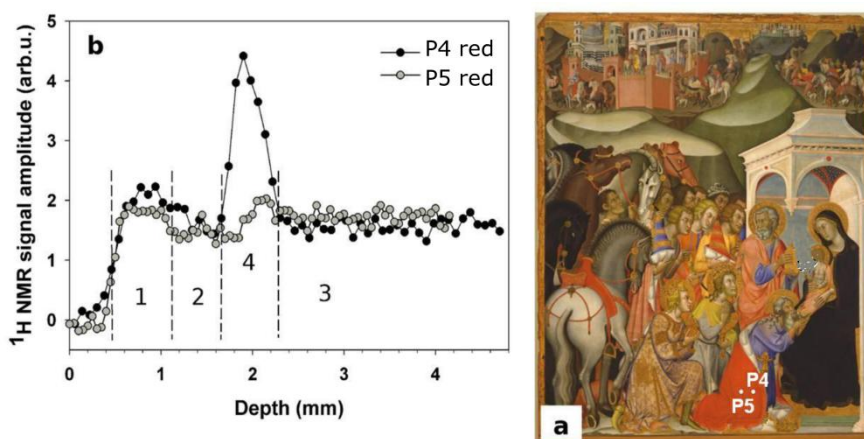


Fig. 1. a) Adorazione dei Magi. b) ¹H NMR stratigraphy of two red painted regions (P4 and P5) of Adorazione dei Magi. Layers 1, 2 and 3 correspond to the paint layer, the primer and the wooden panel, layer 4 was ascribed to canvas and glue of the *incamottatura*.

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EXPLORING THE CONFORMATIONAL DYNAMICS OF HUMAN SERINE RACEMASE USING ^{31}P NMR

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Human serine racemase (hSR) is a homodimeric pyridoxal 5'-phosphate (PLP)-dependent enzyme present in neurons and astrocytes [1]. hSR catalyzes the racemization of L-serine to D-serine and the beta-elimination of both enantiomers to form pyruvate and ammonia. hSR regulates the homeostasis of D-serine, a co-agonist of the N-methyl-D-aspartate receptors for glutamate (NMDARs). Several neuropathologies, such as Alzheimer, Parkinson, and amyotrophic lateral sclerosis are associated with high levels of D-serine, while low levels are found in patients with schizophrenia. Therefore, hSR represents a potential pharmaceutical target for the modulation of glutamatergic neurotransmission. The activity of hSR is modulated by several factors, such as divalent cations and ATP. Cations such as Mg^{2+} increase the activity of the protein. ATP activates hSR through cooperative binding to two symmetric sites at the subunit interface of the homodimer. Glycine increases the affinity for ATP, upon binding to the PLP in the active site, which is about 15 Å away from the ATP site [2]. Also malonate, a competitive inhibitor, increases the affinity of hSR for ATP [3]. It was suggested that hSR undergoes an open/closed conformational transition upon binding of ATP.

In order to unveil the structural changes involved in the cross-talk between allosteric and active site, we have monitored the resonance of the phosphate group of PLP using ^{31}P NMR, under different conditions. ^{31}P NMR is sensitive to the microenvironment of the active site, as proved by studies on several PLP-dependent enzymes [4,5]. The intrinsic low sensitivity of ^{31}P NMR signals and the size of hSR (74 kDa as homodimer) required to increase the yield of production of hSR by co-expressing the protein with molecular chaperones. NMR measurements were carried out using a Bruker 400 MHz instrument. The 1D ^{31}P NMR spectrum of the internal aldimine in the presence of Mg^{2+} is composed by different peaks, centered at 3.64, 3.88 and 4.55 ppm, suggesting the presence of an ensemble of conformations. When glycine is added, in order to form the external aldimine, a sharper peak appears upshifted at 3.15 ppm. The binding of ATP causes the formation of a peak centered at 3.51 ppm. Hence, glycine and ATP cause structural rearrangements at the level of the active site which are likely to be linked to the formation of a more stable conformation of the protein.

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QUANTIFICATION OF 16-O-METHYLCAFEESTOL IN *COFFEA CANEPHORA* VAR. ROBUSTA COFFEE BEANS BY NUCLEAR MAGNETIC RESONANCE

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Typical commercial frauds in the coffee business consist in mixing *Coffea arabica* species with low value Robustas and also in varying the declared content in blends. 16-O-methylcafestol (16-OMC) is a *Coffea Canephora* var. Robusta specific marker, and its quantification is useful to monitor the authenticity of roasted coffee. A new method that provides a quantitative determination of esterified 16-OMC directly in coffee extracts by means of high-resolution proton nuclear magnetic resonance spectroscopy has been developed [1] and proved to be much faster, more sensitive, and much more reproducible than the German standard method DIN 10779 [2]. This method was applied to 39 samples to study the variability of 16-OMC and of other compounds depending on the different geographical origin, and to produce a range of reference values to find a reliable parameter to estimate the Robusta percentage in an unknown blend.

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²⁹Si NMR STUDY OF A POLYHEDRAL OLIGOMERIC SILSESQUIOXANE BASED CATALYST

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Development of green processes based on chemical fixation of carbon dioxide has attracted the attention in industrial chemistry due to the possibility to transform a waste, such as CO₂, into useful products.¹ Cyclic carbonates, synthesized through the reaction between CO₂ and epoxides, are interesting compounds that can be used for several applications, such as electrolytes for lithium batteries, polar aprotic solvents and precursor for pharmaceuticals as well as for polycarbonates. Various homogeneous and heterogeneous catalysts have been proposed for this reaction. In particular, imidazolium-based ionic liquids have become very attractive since they are one of the most efficient catalysts for CO₂ fixation to produce cyclic carbonate from epoxydes.² Here, the ²⁹Si NMR study of a novel class of imidazolium catalyst based on the functionalization of polyhedral oligomeric silsesquioxane (POSS) is presented.

While the ²⁹Si NMR spectra of the precursors (POSS-vinyl and POSS-Cl) show only one sharp resonance line, the presence of several broad lines is observed for the imidazolium-based POSS (POSS-Imi). These findings suggest a possible opening of the POSS nanocage. However, the influence of a partial functionalization or of the ionic nature and aromatic character of the ionic imidazolium moieties on the ²⁹Si chemical shift should not be underestimated.

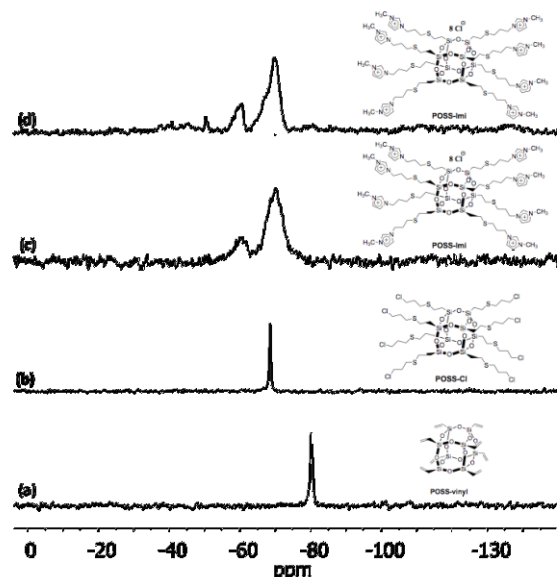


Fig. 1: Liquid state ²⁹Si NMR spectra of the POSS-vinyl (a), POSS-Cl (b) and POSS-Imi (d) and solid state ²⁹Si MAS NMR of the POSS-Imi (c).

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**KINETIC, THERMODYNAMIC, AND STRUCTURAL
CHARACTERIZATIONS OF THE INTERACTION OF NRF2 DERIVED
PEPTIDES WITH THE KELCH DOMAIN OF THE KEAP1 PROTEIN BY NMR**

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The Keap1-Nrf2-ARE pathway is the major regulator of cytoprotective responses to oxidative and electrophilic stress, which is believed to be responsible for the development of many diseases including cancer, Alzheimer's and Parkinson's diseases, and inflammatory bowel diseases. Recent research indicates that disruption of the interaction between the ubiquitination facilitator protein Keap1 and Nrf2, the bZIP transcription factor in response of oxidative stress, may be a strategy to enhance expression of antioxidant and free radical detoxification gene products regulated by Nrf2 [1].

Understanding Keap1-Nrf2 interaction provides a strong structural basis for the rational design of highly potent direct inhibitors. We have characterized the interaction of Nrf2 with the Kelch domain of the Keap1 protein by NMR. By dissecting the 9mer minimal Nrf2 sequence required for high affinity Keap1 binding [2], we determined the role of individual amino acids for Nrf2 binding to Keap1 and the hot spots of the interaction. Shorter peptides binding modes were investigated using chemical shift perturbation and molecular dynamics. We also measured the equilibrium dissociation constant and estimated association and dissociation kinetic constants for the interaction of these peptides and Keap1 Kelch domain in solution, in order to understand the contribution of the individual amino acids to the binding affinity. In addition, ¹⁵N relaxation experiments of the domain in the absence and in the presence of a ligand peptide were performed. These results provide new insights for the rational drug design of peptidomimetic inhibitors of the Keap1-Nrf2 interaction.

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LOOP ALTERATIONS AND THE CONSEQUENCES ON FOLDING, STABILITY AND STRUCTURAL DYNAMICS OF THE HUMAN FRATAXIN

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We study the structure, stability and internal motions of human frataxin (hFXN), a highly conserved mitochondrial protein involved in iron homeostasis and iron-sulphur cluster assembly, whose deficiency causes the neurodegenerative disease Friedreich’s ataxia. One of the pathological mutations that cause Friedreich’s ataxia is the early truncation of the protein producing a frataxin variant lacking of the C-terminal region (CTR). In a recent work, we found that the CTR of hFXN is crucial for consolidation of hFXN structure: deletion of the CTR destabilizes the global structure and alters the dynamics of the whole protein [1-2]. Molecular dynamics simulations and preliminary NMR experiments suggest that the mobility of the CTR and the loop-1 could be coupled. Sequence changes that alter the local dynamics of the CTR, also alter the dynamics of loop-1. The loop-1 is part of the region of frataxin involved in iron binding. In this work, we investigated the effect of loop-1 alterations on hFXN stability and dynamics. Loop-1 mutants were generated by site-directed mutagenesis, expressed and purified as the wild-type protein. Circular dichroism analysis, equilibrium unfolding experiments and light scattering measurements, indicated that loop-1 variants are well folded, stable and monomeric. This demonstrates that this structural element can be perturbed by mutations without inducing severe alterations in the tertiary structure or the aggregation state of the protein. However, a glycine-insertion variant exhibits higher sensitivity to protease action, suggesting an increase in mobility in the vicinity of loop-1. In this context, a complete NMR characterization of this variant indicates that even though fast motions are only locally increased upon loop-1 mutation, slower conformational motions of hFXN are significantly enhanced in regions that are not in direct contact with loop-1. Furthermore, protection against proton-solvent exchange is also decreased for these regions in the loop-1 mutant. These results suggest a long-range relationship between loop-1 dynamics and motions of an extended region of the protein, far from the former, which includes residues from the beta-sheet and alpha-helix 1.

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**FLAVONOIDS AS ANTI AMYLOIDOGENIC COMPOUNDS: INTERACTION
STUDIES WITH A β 1-42 PEPTIDE TO DEVELOP NEW THERAPIES
AGAINST ALZHEIMER'S DISEASE**

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To date, no effective therapies exist to prevent or treat amyloid diseases (e.g. Alzheimer's disease) that have a severe impact on patients and their families' quality of life. Flavonoids are natural polyphenolic compounds found in several edible plants and many evidences exist about their neuroprotective properties but almost nothing is known about their molecular mechanism from the structural point of view [1-4]. By NMR spectroscopy techniques [5], we have investigated the interaction between different flavonoids and A β 1-42 amyloid peptide, a main constituent of neuritic plaques of Alzheimer's disease. In particular, we have focused our study on the effects of flavonoids glycosylation that occurs in Nature and influences their solubility, chemical stability, bioavailability and pharmacokinetic. Combining TEM morphological analysis and *in vitro* biological assays, we have evaluated the ability of flavonoids to inhibit the amyloidogenic process, as well as their ability to inhibit the A β -induced neurotoxicity on neuronal cells [6]. Our work provides insights into the mechanisms of flavonoids–A β interactions that could lead to the development of new therapeutic and/or diagnostic devices for neurodegenerative diseases.

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LIGAND-BASED DRUG DESIGN AGAINST NEURODEGENERATIVE DISEASES BY TARGETING A β 1-42 PEPTIDE

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The deposition of self-assembled amyloidogenic proteins (main component of neuritic plaques) is associated with multiple diseases, including Alzheimer's and Parkinson's disease. Non-fibrillar soluble oligomer A β 1-42 is considered the most amyloidogenic one [1], hence, it represents a key target to develop potential drugs and diagnostic tools for neurodegenerative diseases.

An increasing number of reports have described a variety of natural and synthetic inhibitors, especially polyphenols, against self-assembled amyloidogenic proteins [2]. Synthetic glyco-fused benzopyran compounds have been previously identified as A β 1-42 ligands [3, 4]. In this work, we have introduced chemical modifications on the central pyran ring and investigated their influence on the conformation of the tricyclic compounds as well as on binding to A β peptides, by using STD NMR experiments [5] and molecular modelling.

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^1H NMR PROFILING OF GEOREFERENCED OLIVE OILS

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^1H NMR profiling is nowadays a consolidated technique for the identification of geographical origin of food samples. The common approach consists in correlating NMR spectra of food samples to their territorial origin by multivariate classification statistical algorithms.

In the present work we propose an alternative perspective based on the creation of GIS (Geographic Information System) maps according to the information contained in the NMR spectra. This approach permits to identify territorial regions having strong similarities in the chemical content of the produced olive oil.

To set up the method we have analyzed more than 190 georeferenced olive oil samples produced in the Abruzzo Region in Italy.

Suitable features are created from the NMR spectra which are then traduced in GIS maps (see Fig. 1).

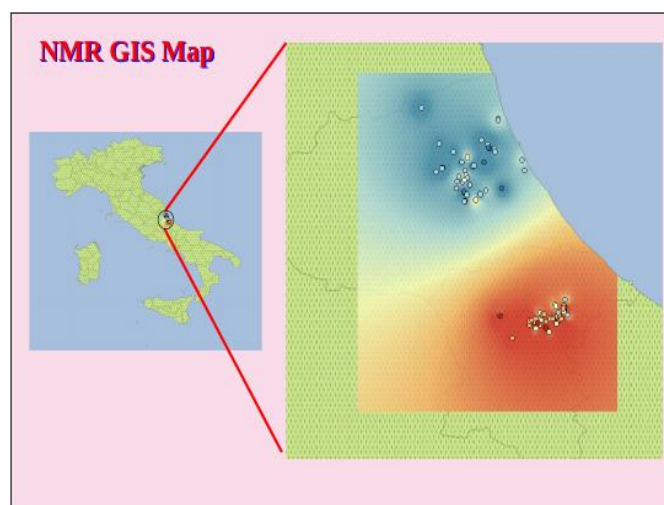


Fig. 1. GIS representation of olive oils NMR spectra.

INTERACTION OF CALMODULIN WITH PEPTIDES DERIVED FROM dINAD AND hMUPP1 M.

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Calmodulin (CaM) is a 17 kDa ubiquitous protein that binds numerous target proteins in a calcium-dependent manner. Structurally, CaM is composed of two globular domains connected by a flexible linker. To date, CaM-binding domain regions have been identified in many different types of proteins, and they all become α -helical upon binding to CaM. In the context of studying the non covalent binding between proteins containing PDZ domains and CaM, we identified putative Calmodulin Binding Peptides (CBP) from the N-terminal extension of INAD-PDZ2 domain and from MUPP1-PDZ8 domain. Inactivation-no-afterpotential D (INAD) is a photoreceptor-specific scaffold protein containing five PDZ domains that in *Drosophila melanogaster* regulates deactivation of vision [1]. Human multi-PDZ domain protein 1 (MUPP1) is a 13-PDZ domain-containing adaptor protein found in several human tissues [2]. Using Fmoc chemistry in solid phase methods, we synthesized a series of unlabeled peptides encompassing the putative CBP regions of dINAD-PDZ2 and MUPP1-PDZ8. A protocol for the production and purification of recombinant ¹³C,¹⁵N-labeled CaM was optimized and its ¹⁵N-HSQC was assigned using triple resonance spectra. The hypothetical binding between the synthesized peptides and CaM was investigated by heteronuclear NMR. Titration of CaM with the peptide derived from dINAD led to significant progressive shifts for many residues, indicative of a fast exchange regime interaction. Results suggest that a complex 1:2 is formed, involving most of the protein, with no difference in the affinity for N-terminal or C-terminal domains of CaM. Titration of CaM with the peptide from MUPP1 confirms an interaction between the two molecules. Interestingly, the peptide binds initially only to the C-terminal domain of CaM and only after the first equivalent of ligand has been added, it starts to interact with the N-terminal lobe. The high affinity binding of the peptide to the C-terminal domain is indicated by the slow exchange regime observed during the titration.

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THYMOSIN α 1 INSERTS IN MODEL MEMBRANES BY THE N-TERMINAL REGION

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Thymosin α 1 is a peptidic hormone used in the therapy of several diseases [1]. It is unstructured in water solution and interacts with negative regions of micelles and vesicles assuming two tracts of helical conformation with a structural break in between. Previous work reported that during the interaction of Thymosin α 1 with sodium dodecylsulphate micelles the peptide assumes structural elements in the binding process [2]. The study of the interaction of Thymosin α 1 with mixed dodecylphosphocholine–sodium dodecylsulphate micelles and with mixed dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylserine (the negative component of the membranes) is herewith reported. The results indicate that the preferred interactions are those where the membrane is negatively charged. These results may shed more light on the mechanism of action of Thymosin α 1 and its insertion in negative regions of membranes due to the exposure of phosphatidylserine. Once the Thymosin α 1 has inserted in the membrane it may interact with nearby proteins and/or receptors acting as effector and causing a biological signaling cascade.

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**¹H NMR STUDY OF SPIN DYNAMICS IN FULLY FRUSTRATED XY
HELIMAGNETIC NANOCHAIN Gd(hfac)₃NITeT**

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In the last decades the family of the quasi-1D molecular magnetic chains Gd(hfac)₃NITR (where hfac is hexafluoro-acetylacetonate and NITR is 2-R-4,4,5,5-tetramethyl-4,5-dihydro-1H-imidazolyl-1-oxyl 3-oxyl) has been intensively studied because of its intriguing magnetic properties. These compounds consist of Gd(hfac)₃ moieties ($S_{\text{Gd}}=7/2$) alternated to nitronyl-nitroxide organic radicals NITR (R = iPr, Et, Ph, Me) ($S_{\text{rad}}=1/2$) [1,2]: the shortest interchain distance is $\sim 10.5\text{\AA}$ leading to $J_{\text{inter}}/J_{\text{intra}} < 10^{-5}$ [1] between interchain and intrachain exchange interactions, being very weak the dipolar interchain interactions. These systems can be divided into two different classes of XY helimagnets due to the competition of nn and nnn exchange interactions. The derivative containing Ethyl is a fully frustrated system [3] fulfilling the Villain’s conjecture [4,5]: it presents a high temperature paramagnetic phase, an intermediate chiral ordered magnetic phase setting up at $T_c^{\text{h}} \sim 2.2\text{K}$ and a further phase transition to the 3D long-range ordered helimagnetic phase at $T_c^{3\text{D}} \sim 1.9\text{K}$ [6]. Here we present a NMR investigation that confirms the Villain’s two-step magnetic ordering sequence. We performed ¹H nuclear spin-lattice relaxation rate (NSLR) $1/T_1$ and absorption spectra measurements as a function of temperature at low magnetic fields $H=0.1, 0.33\text{T}$, to be far from the critical field ($H_c \cong 3\text{T}$), where a strong distortion of the spin arrangement occurs. The chiral phase transition is detected by the asymmetric broadening of the proton NMR line due to the partial freezing of the magnetic moments. The insurgence of the 3D long-range magnetic helical phase is signalled by a sharp peak in the NSLR at $T \sim 1.9\text{K}$, giving evidence of an anomaly in the two-spin correlation function and by a sudden line broadening of the absorption spectra (the FWHM passing from $\Delta\nu \sim 100\text{kHz}$ for $T > 1.9\text{K}$ to $\Delta\nu > 1\text{MHz}$ for $T < 1.9\text{K}$), due to the insurgence of a local field H_{loc} at protons sites generated by the ordered arrangement of the electronic spins.

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MULTI-SCALE STRUCTURAL INVESTIGATION OF INNOVATIVE MgO/CaO BASED CEMENTS

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Eco-compatible MgO-based cements represent one of the most promising alternative to the traditional CaO-based ones, allowing a strong reduction of CO₂ emissions involved in the production process. Indeed, the hydration of reactive periclase (MgO) in the presence of a source of silica gives rise to a binder phase, known as Magnesium Silicate Hydrate (MSH), analogous to the CSH (Calcium Silicate Hydrate) phase present in traditional cements. However the mechanical properties achieved so far are still inferior to those of CaO-based cements, and the research for new formulations with improved performances is necessary in order for a diffusion on industrial scale to take place. To this aim precious information can arise from the study of the multi-scale structural properties of these systems, as well as of the formation process of the binder phases, whose nature is still not well understood.

This work presents a solid-state NMR (SSNMR) and relaxometric study of eco-compatible cements obtained by hydration of mixtures of MgO, traditional Portland cement and silica fumes. Both SSNMR and relaxometry already proved to be very powerful techniques for the study of cements [1-4]. Here, the nature and the structure of the species formed at different times of hydration were investigated on freeze-dried samples by means a multinuclear SSNMR approach, based on the observation of ¹H, ²⁹Si and ²⁷Al. In particular, mono- and bi-dimensional ²⁹Si experiments allowed to identify the presence of both CSH and MSH domains, and to study their properties and relative composition also as a function of hydration time. On the other hand, the measurement of ¹H T₁'s by means of Fast Field Cycling (FFC) relaxometry was used to obtain information on the status of water in the cement pastes, as well as on the evolution of the porous structure.

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INSIGHTS INTO THE ORIGIN OF THE AGGREGACHROMIC BEHAVIOUR OF PERYLENE DYES IN THERMOCHROMIC LLDPE FILMS

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Films of semicrystalline LLDPE (Low Density PolyEthylene) doped with N,N'-bis-(1'-phenylethyl)-perylene-3,4,9,10-tetracarboxydiimide (PE-Pery), a fluorescent aggregachromic dye, showed a thermochromic response upon heating from 30 to 70 °C [1]. By increasing temperature the fluorescence of the dye as a monomer (emission bands at 525 and 565 nm) increased with respect to that of dye aggregates (emission band at 620-680 nm), with a corresponding change of color from a dull red-violet at 30 °C to a bright yellow-green at 70 °C. This behaviour makes PE-Pery-enriched LLDPE films suitable to detect temperature changes close to the physiological regime with a wide range of possible applications as plastic sensing devices [2].

In this work spectrofluorimetric, calorimetric, and solid-state NMR (SSNMR) techniques were combined to investigate the processes occurring upon heating in PE-Pery/LLDPE films, possibly driving PE-Pery aggregachromic behaviour. Understanding the origin of the thermochromic response is indeed fundamental to the research for materials with similar and improved sensing properties. Variable-temperature SSNMR experiments gave important information on the phase properties of the LLDPE domains in the presence of PE-Pery and on the transformations occurring upon heating. Interestingly, both ¹³C CP/MAS spectra and on resonance ¹H FID's showed evidences of the presence of a LLDPE interphase, intermediate between the crystalline and the amorphous phases [3] and showing a peculiar behaviour upon heating, which seems to play a crucial role in determining the thermochromic response. Finally, combining all the results obtained, we can propose a model explaining the aggregachromic behaviour of PE-Pery in LLDPE films.

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SOLID-STATE NMR STUDY OF MODIFIED STARCH/SUCROSE SPRAY DRIED AMORPHOUS BLENDS

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Biopolymer-based systems are today encountered in a wide variety of possible applications, since they are usually low-cost materials and respond to the actual eco-sustainable requirements of renewability and biodegradability. On the other hand, these systems are sometimes related to low chemical and mechanical stability. For this reason, the research for new formulations with higher performance is continuously growing. To this aim, understanding the structural and dynamic features of these systems at a nanometric and sub-nanometric level is crucial to predict their final macroscopic behaviour.

In this work, the phase, dynamic and miscibility properties of modified food starch/sucrose spray dried amorphous blends were investigated by means of solid-state NMR (SSNMR) techniques. Indeed, SSNMR offers a large variety of tools for the investigation of both structural and dynamic properties, on large spatial (0.1-100 nm) and frequency ranges, of amorphous polymeric materials [1,2]. Furthermore, its utility for the study of similar starch/sucrose systems has been already proved [3,4]. Here, ¹H and ¹³C high- and low-resolution SSNMR experiments, carried out on blends with different modified food starch/sucrose ratios, gave interesting information on the properties of the starch and sucrose domains and on the degree of interaction between the two components. ¹H FID's, recorded under on-resonance conditions, showed evidences of the presence of domains with different degree of mobility, whose properties and relative content depend on composition. In addition, the measurement of ¹H T₁ revealed information on the degree of homogeneity on a spatial scale of 10-20 nm.

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ODIN-SAM1 PEPTIDES: NMR CONFORMATIONAL AND BINDING STUDIES WITH EPHA2-SAM

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Sam domains are small modules which mediate protein-protein interactions in a wide number of physiological and pathological processes [1]. Odin is a protein belonging to the ANKS (Ankyrin repeat and Sterile alpha motif domain protein) family and contains two Sam (Sterile alpha motif) domains in tandem in its primary sequence [2]. Odin is able to modulate the endocytosis and consequent degradation of the tyrosine kinase receptor EphA2, possibly regulating its pro-oncogenic activities [3]. Odin is engaged at the receptor site by means of a heterotypic Sam-Sam interaction. In detail, the first Sam domain of Odin (Odin-Sam1) interacts with the Sam domain of EphA2 (EphA2-Sam) via the “Mid-Loop/End-Helix” topology of binding, in which Odin provides the Mid-Loop interface [4]. Here, we report on the design of peptide segments -of different lengths- encompassing the Odin-Sam1 interacting portion for EphA2-Sam. Circular dichroism (CD) and nuclear magnetic resonance (NMR) techniques, under different experimental conditions, were implemented to analyze peptide conformational properties. Moreover, interaction studies with EphA2-Sam were conducted by means of surface plasmon resonance (SPR) assays [5]. These data pave the way for the future design of molecules with enhanced binding affinity for EphA2-Sam and with potential therapeutic applications.

Acknowledgements

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**NON-TARGETED FOOD FINGERPRINTING APPROACH:
¹H-HR-NMR SPECTRA AND AUTHENTICITY OF “PECORINO DI
FARINDOLA” CHEESE**

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Food fingerprinting approaches are potent tool in authentication processes aiming at a comprehensive characterization of complex food matrices [1]. Food matrices can be investigated in terms of their geographical origin, species variety, production system, possible adulterations and authenticity by ¹H-HR-NMR spectroscopic analysis with a subsequent non-targeted multivariate statistical evaluation of acquired data [2].

“Pecorino di Farindola” cheese is produced only in Abruzzo region (Italy) with ovine milk and shows unique organoleptic properties coming from traditional manufacturing practices together with the use of pig rennet instead of calf rennet and the influence of environment.

Using ¹H-HR-NMR non-targeted fingerprinting approach, 121 “Pecorino di Farindola” and 90 different Italian Pecorino sheep cheese samples were analysed in order to obtain a model able to recognise the authenticity of the “Pecorino di Farindola” cheese. The predictive model obtained with Linear Discriminant Analysis shows an high prediction ability (98%) after internal and external validation processes.

Further investigation should be carried out in order to confirm the results obtained analysing more samples and different kind of sheep cheeses.

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FATTY ACID BINDING PROPERTIES OF HORSE SPLEEN FERRITIN

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Vertebrate ferritins, ubiquitous and well-characterized iron storage proteins [1], consist of 24 similar subunits of two types H and L. The H subunit is responsible for the oxidation of Fe²⁺ to Fe³⁺ at a catalytic ferroxidase center, whereas the L subunit appears to play a role in iron nucleation and mineralization [2]. Horse spleen ferritin (HSF) has been suggested to be a novel fatty acid (FA) binding protein, with the 2-fold symmetric inter-subunit pocket acting as FA binding site [3]. HSF is an heteropolymer composed essentially of L chains (ca 92% L and 8% H chains). The X-ray structure of HSF in the presence of arachidonate (ARA) revealed that residues in contact with ARA are the same involved in the ferrihydrite mineralization site, thus providing a rationale for the observed modulation of mineralization in the presence of ARA [3].

In order to further investigate the lipid binding capability of HSF and the modulation of iron mineralization exerted by different lipids, we report here a study of the interaction of apo HSF with a pool of lipids. FA binding capability was assessed by NMR DOSY experiments optimized for small molecule observation. The complexes formation was monitored following the resonances of the free fatty acids at different protein/FA molar ratios. Iron mineralization into apo HSF alone and in the presence of FAs was monitored by measuring the absorbance change at 350 nm, characteristic of iron species inside the protein cage. The results obtained, here reported, are relevant in view of the investigation of the physiological relationship between iron, oxidative injury, and fatty acid metabolism.

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ACCESSING DIPOLAR COUPLINGS OF NATURAL ABUNDANCE SAMPLES VIA DNP: A NEW TOOL FOR NMR CRYSTALLOGRAPHY

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Diffraction from single crystals has revolutionized our knowledge of crystalline matter by providing atomic-scale images of countless samples and leading to landmark achievements in science. However, when crystals of sufficient dimensions cannot be grown, structure can hardly be retrieved using currently available methodologies. This hampers our understanding of the physico-chemical behavior of numerous samples occurring as powders, such as pharmaceutical products, precluding the design of new materials with tailored properties and leading to possibly harmful consequences. Solid-state NMR has the potential to be the key to access the crystal structure of powders. However, the inherently low sensitivity of NMR constitutes the main barrier to retrieve valuable constraints such as interatomic distances from spin-spin couplings involving rare nuclei on organic samples at natural isotopic abundance.

We present a straightforward methodology to quantitatively relate structural constraints based on ^{13}C - ^{13}C double quantum dipolar build-up curves obtained by Dynamic Nuclear Polarization (DNP) solid-state NMR to the crystal structure of organic powders at natural isotopic abundance [1]. This methodology relies on the tremendous sensitivity enhancement provided by DNP (about 50 here, reducing the experimental times from a few years to a few days) and is sensitive to both the molecular conformation and the crystal packing of the studied powder sample. The method is demonstrated on the challenging sample of theophylline [2]. We show here how the proposed methodology allowed the correct crystal structure of anhydrous theophylline to be rapidly and effectively identified among a set of both existing and virtual theophylline polymorphs. These encouraging results suggest that this methodology could pave the way to three-dimensional structural elucidation of powders via the combination with powder X-ray diffraction, crystal structure prediction [3] and density functional theory computation of chemical shifts [4].

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NMR CHARACTERIZATION OF INTEGRIN α V β 6/RGD CYCLOPEPTIDES INTERACTIONS

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Integrins are heterodimeric cell surface receptors that mediate adhesion to the extracellular matrix and are involved in many severe pathological processes. Among all, α V β 6 integrin plays a key role in the initiation of metastasis and is up-regulated in many types of cancer, but not in normal healthy organs.[1] Therefore α V β 6 is an exciting target for both imaging and treatment, across many common cancer types. Up to date only few α V β 6 selective antagonists have been developed,[2] including long peptides which contain the RGD β 3 sequence, such as a TGF β 3 peptide (HGRGDLGRLKK).[3]

As a matter of fact, the rationalization of the effect induced by the RGD flanking amino acids in the interaction with α V β 6 is still an open question. In this context, we wanted to explore the effect of the RGD flanking residues in the binding to α V β 6.

In a recent study we have developed a multistage protocol based on metadynamics and docking to study the modulation of α V β 3-binding selectivity. We carried out a systematic enhanced MD exploration on 400 RGD cyclic head-to-tail pentapeptides (X1-RGD-X2) in order to study the effect the flanking residues X1 and X2 on the cyclopeptides conformational equilibrium. The 400 cyclopeptides were subsequently screened, based on their conformation criteria and computational affinity scoring values for α V β 3, and we ended up with a small library of 16 cyclopeptides that were tested for binding to different integrins (α V β 3, β 5, β 6, β 8, α 5 β 1 and α I**II** β 3) in ELISA assays.

Unexpectedly, within this library we identified three cyclopeptides (MRGDW, RRGDF, NRGDW) that showed nanomolar IC₅₀ towards α V β 6.

In order to get structural insights into peptide/integrin interaction, we have performed preliminary Saturation Transfer Difference (STD) and transfer-NOE (tr-NOE) experiments between the soluble extracellular domain of α V β 6 and c(MRGDW) cyclopeptide. The STD experiment evidenced the presence of an interaction between c(MRGDW) and α V β 6, and allowed us to perform an epitope mapping of the cyclopeptide protons, which highlighted the proximity of the ligand indole ring to the integrin surface. As concerns the tr-NOE, only for the sample containing c(MRGDW)/ α V β 6 mixture we detected negative intramolecular NOEs of the cyclopeptide, which confirms the presence of the peptide/integrin interaction.

In the future we will extend this study to the other two cyclopeptides. We plan also to perform NMR competition experiments with TGF β 3 peptide and to perform STD and tr-NOE using directly human cancer cells expressing α V β 6 in order to structurally characterize the interaction in a physiological context.[4]

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HYDRATION AND DEGRADATION BEHAVIORS OF NOVEL CALCIUM SULFOALUMINATE CEMENTS: A COMBINED SOLID-STATE NMR AND XRPD STUDY

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The increased attention towards the development of sustainable products in the building industry encourages the study of new low environmental impact materials: in this context, Calcium Sulphoaluminate (CSA) cements are gaining more importance, being high performance cementing systems whose production, beyond requiring lower energy, allows a significant spare in CO₂ emission. Different calcium Sulfoaluminate clinker/cements and Portland-calcium sulfoaluminate mixed cement system have been submitted to hydration study. The hydration mechanism was investigated by means of X-Ray Powder Diffraction & Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy. A suite of multinuclear multidimensional solid-state NMR experiments has been employed to investigate the hydration dynamics in CSA cement pastes. ¹H MAS NMR was used to follow the nature and dynamics of water in CSA paste as well as the formation of various hydroxyl species. ²⁹Si MAS NMR has been extensively used to characterize the silicate species and their network formation mechanism upon hydration. ²⁷Al MAS and MQMAS NMR experiments revealed the formation of different octahedral aluminate species upon cement hydration and solidification. Hydration products such as Ettringite, Monosulfate, aluminum hydroxide and calcium silicate hydrate were detected and quantified. The structural modification of various phases due to chloride and sulfate attack on the hydrated CSA cement pastes has also been probed [1].

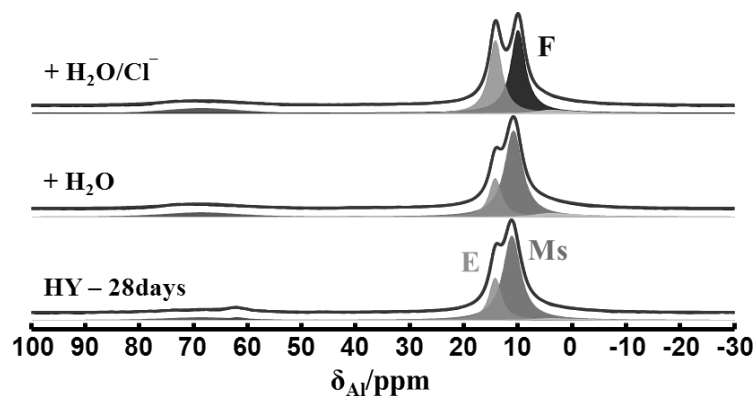


Fig. 1. ²⁷Al MAS NMR spectra of Portland-CSA mixed cement system hydrated for 28 days followed by treatment in water and NaCl solution. E = Ettringite; Ms = Monosulfate; F = Friedel's salt

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NMR SPECTROSCOPY OF FATTY ACID BINDING PROTEINS UNDER MACROMOLECULAR CROWDING

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The distinctive features of cellular environments are macromolecular crowding, local viscosity, compartmentalization, and confinement [1]. Cytoplasmic medium is deeply crowded with significant concentrations of macromolecules (50-400 g/L) which affect several protein attributes (i.e. ligand binding, protein-protein interaction, folding, etc.) [2]. Usually protein studies are carried out in solutions where the total protein concentration is less than 10 g/L [3]. These dilute solutions allow us to have good signals, but may lack biological relevance. In the biological systems the macromolecular crowding results in non-specific interactions between the protein of interest and the crowder components [4]. In order to explore the effects of the complex cellular medium on the properties of important cytosolic lipid carrier proteins, we focused on the human liver fatty acid binding protein (hLFABP) and the human ileal bile acid binding protein (IBABP), both belonging to the family of intracellular lipid binding proteins (iLBPs). iLBPs play central roles in intracellular lipid transport and systemic metabolic homeostasis, they are widely distributed among tissues and reach extraordinary high intracellular concentrations (as much as 7-11% of total cytosolic proteins) [5-7]. The current goal of this work is to study hLFABP and IBABP under crowded conditions (Fig. 1A) by NMR spectroscopy. We mimicked the crowding environment including synthetic crowding agents such as PEG, Ficoll, or biomacromolecules such as BSA or Lysozyme (concentrations in the range 50-300g/L), and explored structural, dynamic, and interaction properties using tailored experiments. Moreover, we study hLFABP within artificial cell systems, such as water-in-oil emulsions (Fig. 1B) and liposomes to mimic the restricted, lipid-bounded cytosolic milieu.

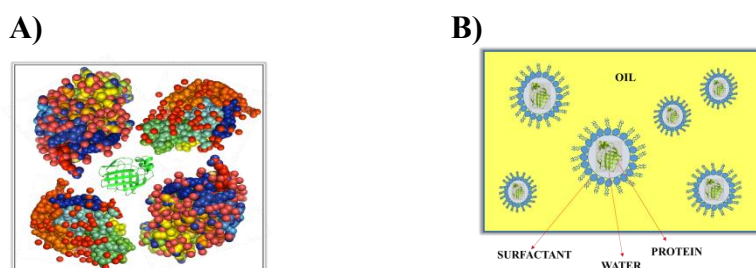


Figure 1. A) hLFABP in macromolecular crowding environment. B) hLFABP within water in oil emulsion.

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CHARACTERIZATION OF SICILIAN AMBER (*SIMETITE*) BY ^{13}C SOLID STATE NMR

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Amber is a fossilized form of terpenoid resin found in many parts of the world that has undergone geological maturation over millions of years. It is translucent, of a color that can range from yellow, to red, to brown up to green. Even in its raw form this material has an attractive appearance and it has been used by people of many different cultures for decorative objects over several millennia. The best known and diffused fossil resins in the actual gemological trade come from the Baltic region of Northern Europe and the Dominican Republic. Sicily is a source of a rare and fashionable amber, mainly found along the hydrographic basin of Simeto River, from which it takes its name *simetite*. Sicilian amber is considered by gemological studies as the most valuable in the world for the physical-chemical properties and for its rarity. The limitation of the study of *Simetite* is due both to the lack of information in literature and the lack of authentic *Simetite* samples in mineralogical collections. The knowledge of the chemical structure of amber may allow one to associate a given object with its geographical origin and to draw maps of cultural and trade networks, besides, information on authenticity of artifacts may be obtained. Chemical characteristics of amber depend on both biological origin and geological environment. The non crystalline nature of amber and its poor solubility prevent the use of many analytical techniques.

The use of ^{13}C Solid state NMR spectroscopy in characterizing amber has been demonstrated to be a powerful method to obtain information on biological origin and geological environment [1-3]. In this research ^{13}C CPMAS NMR and micro-Raman spectroscopy were applied to characterize a private collection of Sicilian amber samples. The main goal of the work is to supply a complete study of *simetite*, highlighting discriminating criteria useful to distinguish between Sicilian amber and amber from other regions such as Baltic and Dominican amber, and lay the foundations for a spectroscopic database of Sicilian fossil resins. Measurements allowed us to obtain information on structure and maturity. The Principal Component Analysis was applied to solid state NMR and Raman spectroscopic data.

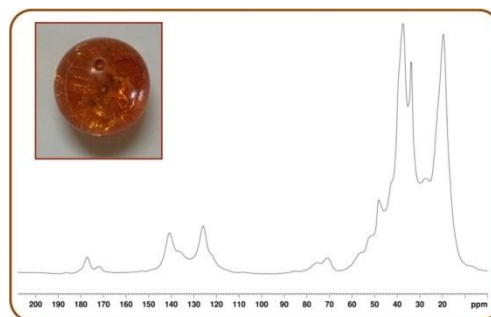


Figure 1. ^{13}C CPMAS NMR spectra of a representative *simetite* sample

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STRUCTURAL AND DYNAMICAL CHARACTERIZATION OF BPSL1445 FROM BURKHOLDERIA PSEUDOMALLEI

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

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BILE ACID BINDING PROTEIN RESPONSE TO A LIGAND LIBRARY: A COMBINED NMR AND STATISTICAL APPROACH

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Primary bile acids, differing in the hydroxylation pattern, are synthesized from cholesterol in the liver and, once formed, can undergo extensive enzyme-catalyzed glycine/taurine conjugation, giving rise to a complex mixture, the bile acid pool. Composition and concentration of the bile acid pool may be altered in diseases, posing a general question on the response of the carrier (bile acid binding protein) to the binding of ligands with different hydrophobic and steric profiles. A collection of NMR experiments (H/D exchange, Het-SOFAST, ePHOGSY-NOESY/ROESY and 15N relaxation measurements) was thus performed on apo and five different holo proteins, to monitor the binding pocket accessibility and dynamics [1]. The ensemble of obtained data could be rationalised by a statistical approach, based on chemical shift covariance analysis (CHESCA) [2], in term of residue-specific correlations and collective protein response to ligand binding. The results indicate that the same residues are influenced by diverse chemical stresses: ligand binding always induces silencing of motions at the protein portal with a concomitant conformational rearrangement of a network of residues, located at the protein anti-portal region. This network of amino acids, which do not belong to the binding site, forms a contiguous surface, sensing the presence of the bound lipids, with a signalling role in switching on and off protein/membrane interactions.

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CLASSIFICATION OF HUMAN PLASMA SAMPLES USING METABOLIC PROFILES DETERMINED BY $^1\text{H-NMR}$

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Metabolites are crucial components of a biological system and highly informative about its functional state [1]. Metabolomics is a diagnostic tool for metabolic classification of individuals. For example, plasmas rich or poor in platelets and growth factors, which may find clinical application in regenerative medicine, can be characterized on the basis of their metabolic profile by different methodologies [3], but standard analytical approaches are not adequate to achieve information precise enough for some kinds of applications. Thus, as NMR is a well-established powerful analytical method for generating metabolomics profiles, [2] we carried out a study finalized to the understanding of the potential of NMR in recognizing metabolic profile differences within samples of human plasma which have received different treatments. We performed $^1\text{H-NMR}$ based metabolomics on samples of human blood plasma (80 samples from 20 donators provided by Centro di Riferimento Oncologico di Aviano). By comparing the samples spectra with library reference data, several metabolites were identified and quantified. A Principal Component Analysis (PCA) was performed in order to resolve the smallest differences between spectra of samples from different donors and/or groups of treatments. The PCA analysis was applied both to the full spectra (see Fig.1) and to specific zones, allowing to identify and discriminate the samples belonging to the four different classes of treatment considered (plasma rich in platelets and growth factors; plasma poor in platelets and growth factors; plasma washed and cell membrane lysed; cell membrane lysed) and to assign each sample to one of these. We could therefore conclude that NMR can indeed be used to determine the metabolic profiles of plasma samples and to get information about the origin and composition of samples, and the method may be extended to the discrimination of samples from healthy and diseased patients and/or other clinical applications.

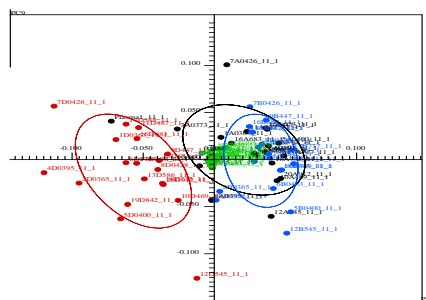


Fig. 1. PCA on full spectra Noesypr1d PC8/PC9

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NMR DETECTION OF SPECIFIC MINOR COMPONENTS SEPARATED BY COMPLEX MATRICES SUCH AS EXTRA-VIRGIN OLIVE OIL (EVOO)

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Extra-Virgin olive oil (EVOO) is one of the most studied food stuff in Italy. The NMR techniques have been shown to be very suitable for specific characterization of EVOO and/or ready detection of interesting minor component, especially because it does not require chemical pre-treatments and it's a non-destructive technique [1-3]. Herein we present data coming from the NMR monitoring of the crude EVOO (just dissolved in CDCl₃); the separation of the components present in 2% of the minority fraction unsaponifiable was obtained from a new stationary phase that we invented and patent pending, by noting spectrum well as the fraction of triglycerides is practically demolished.

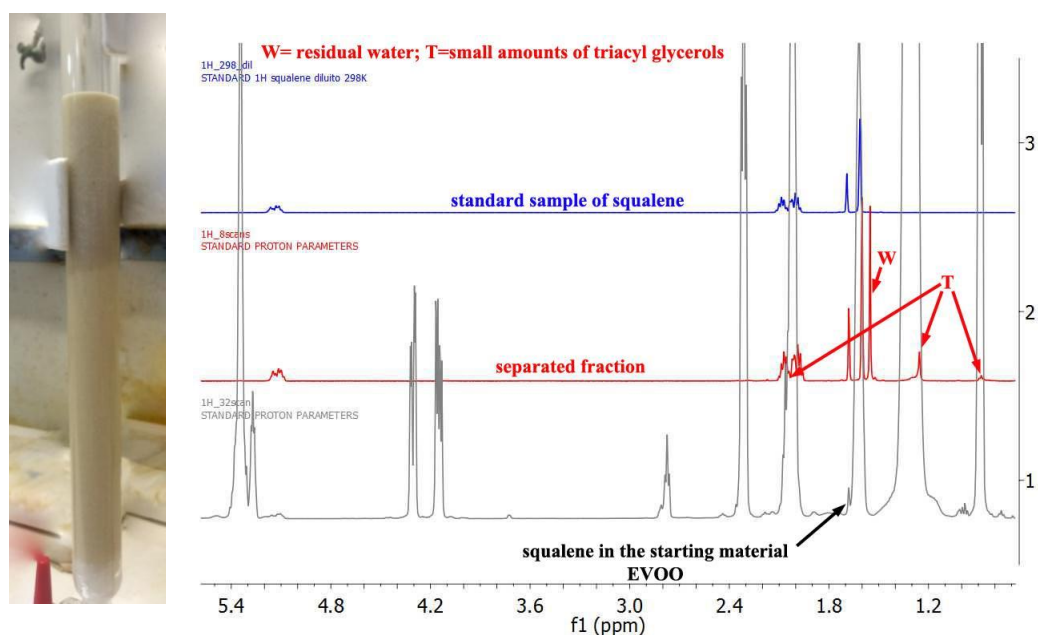


Fig. 1. Column packed with the new resin, and an NMR spectrum, where you put in comparison with an extra virgin olive oil, one of an oil passed through the resin and one with the standard squalene.

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**TIME-RESOLVED ASSEMBLY OF A NUCLEOPROTEIN COMPLEX
BETWEEN SHIGELLA FLEXNERI VIRF PROMOTER AND ITS
TRANSCRIPTIONAL REPRESSOR H-NS**

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The *virF* gene of *Shigella*, responsible for triggering the virulence cascade in this pathogenic bacterium, is transcriptionally repressed by the nucleoid-associated protein H-NS. The primary binding sites of H-NS within the promoter region of *virF* have been detected here by footprinting experiments in the presence of H-NS or its monomeric DNA-binding domain (H-NS*Sctd*), which displays the same specificity as intact H-NS. Of the 14 short DNA fragments identified, 10 overlap sequences similar to the H-NS binding motif. The ‘fast’, ‘intermediate’ and ‘slow’ H-NS binding events leading to the formation of the nucleoprotein complex responsible for transcription repression have been determined by time-resolved hydroxyl radical footprinting experiments in the presence of full-length H-NS. We demonstrate that this process is completed in ≤ 1 s and H-NS protections occur simultaneously on site I and site II of the *virF* promoter. Furthermore, all ‘fast’ protections have been identified in regions containing predicted H-NS binding motifs, in agreement with the hypothesis that H-NS nucleoprotein complex assembles from a few nucleation sites containing high-affinity binding sequences.

Structural analysis shows that the 22-bp fragment corresponding to one of the HNS binding sites deviates from canonical B-DNA structure at three TpA steps rather than the single one observed in the case of H-NS promoter.

¹H NMR STUDY OF SAFFRON SAMPLES OF DIFFERENT ORIGIN

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Saffron is a very expensive spice cultivated in few countries (mainly Iran, India, Spain, Greece, Italy and Morocco), and it is generally used as a food additive and for coloring purposes. The spice is obtained from the dried stigmas of the plant *Crocus sativus* L. (Iridaceae). It has recently gained interest as a potential source of pharmacological bioactive compounds with cardioprotective effect [1]. Among other methods, NMR spectroscopy [2] has been employed in characterization of saffron in order to discriminate its geographical origin and possible adulterations [3,4]. The present work reports an original NMR based protocol of saffron characterization using a simple and environmentally-friendly microwave-assisted extraction procedure [5]. The protocol was applied to saffron samples of different geographical origin (Greece, Spain, Hungary, Turkey and Italy). Metabolite profiles of geographically different saffron extracts were compared showing significant differences.

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SOLID STATE ANALYSIS OF MODEL MIXTURES FOR THE OPTIMIZATION OF THE HTL PROCESS TO OBTAIN BIO-OIL FROM WASTE BIOMASSES

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Hydrothermal treatment of waste biomass is a promising alternative way to produce bio-oils which are similar to fossil crude oils. Although the so obtained bio-oils are a very complex mixture of several thousands of compounds whose relative amount strictly depends on the nature and composition of the starting biomass, nevertheless it's possible to identify some common components, as all biomasses are made up mainly of the same constituents: carbohydrates, proteins, lipids and lignin in different percentages. For this reason a study was approached, where each component of an hypothetical biomass has been treated separately under HTL conditions (300°C, 90 bar) and the resulting products have been carefully analyzed. Most of the model compounds under HTL treatment produce four phases: a gas phase, the bio-oil, an aqueous phase and a solid residue. In this work, the solid residue has been the object of a detailed characterization performed with solid-state NMR spectroscopy.

In a previous study, cellulose and glucose were chosen as carbohydrate model compounds, while BSA (bovine serum albumin) was chosen as protein model. These model compounds were previously treated separately and then as binary mixture (cellulose/BSA). The ¹³C CPMAS NMR spectra of the solid residue obtained from cellulose and glucose showed a strong aromatic content due to polyfuranic networks and graphite-like domains. By increasing the reaction time, the contribution of the graphitic-like domains increased at the expense of the furan-based structures.

The HTL residues of the mixture BSA/cellulose still showed, in the NMR spectra, a strong aromatic component mainly due to graphitic-like structures while the amount of the polyfuranic network was much lower. This observation led to the hypothesis that the presence of nitrogen inhibits the polyfuranic degradation pathway: the furanic derivatives formed during the carbohydrates degradation, react with ammonia and other nitrogen containing compounds (arising from proteins degradation), forming aromatic structures. These in turn tend to further react and polymerize leading to a polycondensed material in which nitrogen is mainly incorporated in C=C-N bonds.

In order to represent a more complete biomass model mixture, a lipidic component was added (tripalmitin was chosen as model compound), and the ternary mixture cellulose/BSA/tripalmitin was tested under HTL conditions. In this case the derived solid residue showed a strong aliphatic carbons content (due to the palmitic acid long chain) and just a small amount of aromatic carbons. Signals belonging to esterified glycerol are detected that decrease as function of the reaction time, pointing out an extensive hydrolysis of the triglyceride and a continuous consumption of the reagent. Signals belonging to hydrolyzed glycerol appear, that subsequently is transferred into the aqueous phase. In the carbonyl region, the formation of amides is evidenced, increasing with the reaction time, thus confirming the formation of fatty acid amides, due to the reaction between glycerides and ammonia and/or natural amino acids.

FEASIBILITY OF NMR METABOLIC PROFILE OF NEWLY DIAGNOSED AML PATIENTS UNDERGOING CHEMOTHERAPY TREATMENT

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Acute Myeloid Leukemia (AML) is a cancer of the blood resulting in the accumulation of immature cells (blasts) with a reduction of functional blood cells in peripheral blood and bone marrow. Its outcome is poor and new prognostic markers are warranted in particular for elderly or frail patients. The present study aimed at investigating the feasibility of a nuclear magnetic resonance (NMR) metabolomic approach to study metabolism in adult patients during chemotherapy treatment.

Metabolomics investigates changes in metabolites and small molecules involved in biochemical processes offering a powerful tool for examining disease-related biochemical changes and identification of new biomarkers [1, 2]. NMR combined with multivariate statistics allow a record of global changes in metabolites associated with phenotypic changes [3, 4]. Blood samples from peripheral blood and bone marrow of 9 newly diagnosed AML patients were collected prior and after chemotherapy treatment at multiple time-points and were analyzed by NMR spectroscopy. NMR data were used for multivariate statistical analysis with Simca (Umetrics) in order to correlate metabolomic markers with AML clinical and biological features.

1D ¹H NMR spectra of the blood samples were acquired at 310K on a Bruker Avance 600 MHz spectrometer equipped with a triple-resonance TCI cryoprobe. After preprocessing, NMR data were subjected to multivariate analysis in the form of unsupervised principal components analysis (PCA) and supervised partial least squares-discriminate analysis (PLS-DA), or orthogonal PLS-DA (OPLS-DA). Results showed that the NMR metabolic profiles of peripheral blood and plasma were very similar. Besides, we were able to correlate the NMR profile at AML diagnosis with the number of blasts. Indeed, the patients with more than 50% of blasts clustered together versus the ones with 20-50% of blasts. The biggest contributions were associated with lipid metabolism according to what reported for other types of cancer [5]. The metabolic trajectory during chemotherapy treatment will be determined in the next weeks.

Our first results at diagnosis suggested that NMR metabolic profile of blood may be a unique model to track AML tumor metabolism during chemotherapy. The strength of the study relies on the direct monitoring of the metabolism at tumor origin site.

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INTERACTION STUDIES, AGGREGATION KINETICS AND MORPHOLOGY OF A β 1-42/A β py3-42 MIXTURE

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Alzheimer's disease (AD) is the most common form of dementia between the over sixties. Extracellular plaques are the main histopathological signatures of AD, they are composed of amyloid β peptide (A β). Despite the importance of plaques to AD, soluble A β oligomers (A β o) are considered to be the principal toxic forms of A β . The N-terminally truncated A β peptides show an enhanced aggregation propensity compared to full-length peptides. Among these, N-terminus pyroglutamated peptides (A β py) are prominent in AD and have been postulated to initiate amyloid plaque formation [1]. Nussbaum and coworkers demonstrated that A β py3-42, isolated from human AD brain, strongly affects cultured neuron and astrocyte survival [1]. It also cooligomerizes with excess of A β 1-42 to form metastable A β o that are structurally distinct and more toxic to cultured neuron than comparable A β o made from A β 1-42 alone. It was therefore proposed that A β py3-42 can act as templates (seeds) inducing misfolding on A β 1-42. Based on the above-mentioned hypothesis, our study is dedicated to the structural characterisation of A β 1-42/A β py3-42 mixtures in order to open a new door in the difficult comprehension of the onset of AD. Here we present an NMR structural and kinetic study of A β 1-42/A β py3-42 complex coupled with CD and fluorescence analyses. The time evolution of the ultrastructure peptide aggregates is approached by transmission electron microscopy (TEM) and Atomic Force Microscopy (AFM) [2].

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SPECIFIC UBIQUITIN ADSORPTION ONTO LANTHANIDE-DOPED UPCONVERTING SRF₂ NANOPARTICLES

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Understanding and controlling the interactions of nanoparticles (NPs) with biomolecules is essential for the successful development of novel applications in bionanoscience, such as diagnostic, sensing, and imaging and therapeutic applications.

It is now firmly established that entering in a biological environment NPs interact with a collection of biomolecules including proteins. However, interfacing NPs with proteins may significantly affect the physical (e.g. optical) properties of the NPs and/or the structural and functional properties of the interacting biomolecules, sometimes leading to biological injury, and thereby justifying the need for an improved characterization of the protein-NPs interfaces [1,2].

Here we reported the study of interactions between Ubiquitin (Ub), a small cytosolic protein playing a central role in numerous biological processes and the lanthanide-doped upconverting SrF₂ nanoparticles (UCNPs). These UCNPs show attractive applications in biomedical luminescence due to their ability to produce up-conversion emission, e.g. emission at higher energy with respect to the exciting radiation [3]. NMR spectroscopy, up-conversion luminescence measurements and isothermal titration calorimetry were used to probe and characterize the Ub-UCNPs interactions.

The obtained results support the view that the binding of UCNPs to Ub may affect fundamental interaction patterns of Ub, representing a potential opportunity of therapeutic intervention against cancer development.

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SUPPRESSING SOLVENT SIGNALS IN DYNAMIC NUCLEAR POLARIZATION (DNP) SOLID-STATE NMR *via* THE ELECTRONIC MIXING-MEDIATED ANNIHILATION (EMMA) METHOD

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A simple procedure based on the so-called Electronic Mixing-Mediated Annihilation (EMMA) methodology [1] is shown here to effectively suppress solvent signals in dynamic nuclear polarization (DNP) solid-state NMR [2,3]. These signals are present when analyzing samples prepared by glass forming or incipient wetness impregnation, two common methods used in DNP solid-state NMR for adding polarizing agents (*e.g.* biradicals) to diamagnetic compounds [2,3]. This becomes especially critical when the amount of solvent is large with respect to the sample under study, which may hamper proper analysis of the resulting CPMAS spectrum. Similarly to the ERETICTM method [4,5], which uses an electronic signal as an internal standard for quantification, EMMA is based on an electronically generated time-dependent signal that is injected into the receiver coil of the NMR probe head during signal acquisition. More specifically, the line shape, width and frequency of this electronic signal are determined by deconvoluting the solvent signal in the frequency domain. This deconvoluted signal is then converted into a time-dependent function through inverse Fourier Transform, which is used to generate the shaped pulse that is fed into the receiver coil during the acquisition of the Free Induction Decay. The power of the shaped pulse is adjusted to match the intensity of the solvent signal, and its phase is shifted by 180° with respect to the receiver reference phase. We are presenting here preliminary data illustrating the potential of the EMMA method for DNP-enhanced solid-state NMR, using polymer materials as a case study [6].

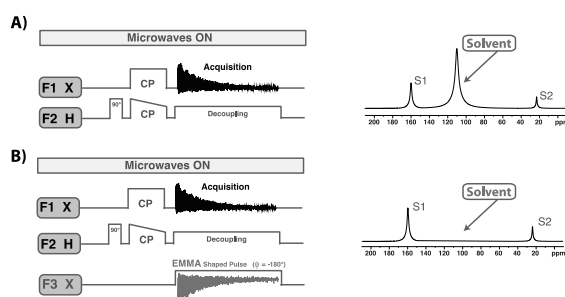


Fig. 1. Schematic representation of a DNP-NMR CPMAS

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