50th National Congress on Magnetic Resonance

6-8 September 2023

Sapienza Università di Roma
Dip. di Chimica e Tecnologie del Farmaco
Città Universitaria, Building CU019

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Facoltà di Farmacia e Medicina
Sapienza Università di Roma
Dipartimento di Chimica e Tecnologie del Farmaco

GIDRM
Gruppo Italiano Discussione Risonanze Magnetiche

In Rome

50th *NATIONAL CONGRESS on MAGNETIC RESONANCE*

6-8 September 2023, Roma

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GENERAL INFORMATION

VENUE

Sapienza University of Rome
Department of Chemistry and Technology of Drugs, Building: CU019
Piazzale Aldo Moro 5, 00185 Roma (RM)

INVITED SPEAKERS

The following speakers have agreed to give plenary lectures at the meeting:

Søren Balling Engelsen (University of Copenhagen)
Marco Geppi (University of Pisa)
Mathilde Hauge Lerche (Technical University of Denmark)
Giovanna Musco (IRCCS San Raffaele Hospital, Milano)
Janez Plavec (National Institute of Chemistry, Ljubljana)
Paola Turano, Winner of the GIDRM/GIRM Gold Medal 2023 (University of Florence)

The following speakers have agreed to give lectures at the meeting:

Fabio Arnesano (University of Bari)
Lucia Calucci (ICCOM-CNR, Pisa)
Daniela Delli Castelli (University of Torino)
Valeria Di Tullio (ISPC-CNR, Roma)
Mariapina D'Onofrio (University of Verona)
Moreno Lelli (University of Firenze)
Giuseppe Pileio (University of Southampton)
Valeria Righi (University of Bologna)

POSTER SESSIONS

Poster session 1

Wednesday 6th, 16:05-17:00, ODD abstract numbers

Poster session 2

Thursday 7th, 10:30-11:20, EVEN abstract numbers

Poster session 3

Thursday 7th, 12:50-14:10, ODD abstract numbers

Poster session 4

Thursday 7th, 16:15-17:30, EVEN abstract numbers

UNDER THE AUSPICES OF

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50TH NATIONAL CONGRESS ON MAGNETIC RESONANCE

ROMA 6-8 SEPTEMBER 2023

SCIENTIFIC PROGRAM

Wednesday September 6th

9:00-14:00	**Registration**
10:00-11:30	**Bruker satellite meeting**
11:30-13:00	**Jeol satellite meeting**
13:00-14:00	**Bruker/Jeol Lunch**
14:00-14:30	**Opening**

	Plenary session **Chair: C. Airoldi and M.R. Chierotti**
14:30-15:20	**GIDRM/GIRM gold medal award** **P. Turano (University of Florence)** SPANNING THE PERIODIC TABLE BY NMR: A BIOINORGANIC VIEW
15:20-16:05	**Plenary Lecture 2** **M. Lerche (University of Denmark)** APPLYING ^{13}C-HYPERPOLARIZATION TO BREAK SENSITIVITY BARRIERS OF NMR FOR ANALYSIS OF COMPLEX MIXTURES
16:05-17:00	**Coffee break + Poster session (ODD abstract numbers)**

	Parallel session A **Chair: M. Lerche**	**Parallel session B** **Chair: P. Turano**
17:00-17:30	**D. Delli Castelli -** ^{31}P-PARACEST: ^{31}P MR-CEST IMAGING BASED ON THE FORMATION OF A TERNARY ADDUCT BETWEEN INORGANIC PHOSPHATE AND Eu-DO3A	**V. Di Tullio -** PROBING WATER DYNAMICS IN POROUS CULTURAL HERITAGE MATERIALS USING UNILATERAL NMR
17:30-17:50	**M. Inglese -** MULTIMODAL TEMPORAL MRI AND PET DATA ANALYSIS FOR THE CLASSIFICATION OF LOW- AND HIGH-GRADE GLIOMAS	**C. Mengucci -** INSIGHTS ON NUTRITIONAL QUALITY AND SHELF LIFE ESTIMATION OF FISH PRODUCTS (SPARUS AURATA) THROUGH 1H NMR METABOLOMICS AND DATA INTEGRATION
17:50-18:10	**A. Nucera -** RELAXOMETRIC INVESTIGATION OF FE(III) COMPLEXES AND THEIR SUPRAMOLEULAR ADDUCTS	**V. Gallo -** RELIABILITY AND ERROR SOURCES OF AN NMR METHOD FOR THE ANALYSIS OF WHEAT AND PASTA
18:10-18:30	**S. Elkhanoufi -** NEW CLASS OF CONTRAST AGENTS FOR OVERHAUSER MRI, BASED ON NITROXIDE RADICALS AND RESPONSIVE TO SPECIFIC ENZYMATIC ACTIVITY.	**V. Maestrello -** NMR-METABOLOMIC STUDIES ON BLUEBERRIES STORED UNDER DIFFERENT CONDITIONS

	Plenary session **Chair: L. Mannina**
18:30-18:45	**Sponsorship Lecture (Stelar): Silvio Aime** FIELD CYCLING RELAXOMETRY, A TOOL TO ASSESS TUMOUR METABOLISM
18:45-19:30	**Plenary Lecture 3** **S.B. Engelsen (University of Copenhagen)** QUANTIFICATION OF BLOOD PLASMA METABOLITES AND LIPOPROTEINS FROM ^{1}H NMR SPECTRA USING SIGNATURE MAPPING (SIGMA) APPROACH

Thursday September 7th

	Plenary session **Chair: G. Parigi and M.R. Chierotti**
8:45-9:30	**Plenary Lecture 4** **G. Musco (San Raffaele hospital, Milan)** PHARMACOLOGICAL TARGETING OF CHEMOKINE-CHEMOKINE INTERACTIONS: THE STRANGE STORY OF HMGB1-CXCL12 HETEROCOMPLEX
9:30-9:45	**Sponsorship Lecture (Bruker): F. Benevelli** BENCHTOP AND FLOOR STANDING NMR IN BIOPRODUCTION
9:45-10:15	**Under 35 GIDRM: F. Nardelli (University of Pisa)** UNVEILING MOLECULAR STRUCTURES AND DYNAMICS WITH NMR SPECTROSCOPY: APPLICATIONS FROM BIOMEDICINE TO MATERIALS
10:15-10:30	**Sponsorship Lecture (Extrabyte): G. Selva** LIVE OVERVIEW OF THE RELAXOMETRY SOFTWARE WINREX
10:30-11:20	**Coffee break + Poster session (EVEN abstract numbers)**

	Parallel session A **Chair: S.B. Engelsen**	**Parallel session B** **Chair: L Russo**
11:20-11:50	**V. Righi -** APPLICATIONS OF HIGH-RESOLUTION MAGIC ANGLE SPINNING NMR IN BIOMEDICAL STUDIES	**G. Pileio -** SINGLET-ASSISTED DIFFUSION NMR (SAD-NMR) TO PROBE TRANSLATIONAL DYNAMICS IN SPACE-AND-TIME HETEROGENEOUS MEDIA
11:50-12:10	**G. Ciufolini -** FUNCTIONAL METABOLISM IN BLADDER CANCER CELLS: NMR BASED EXO-METABOLOMICS COMBINED WITH SEAHORSE DATA	**R. Lamanna -** DEEP LEARNING APPLICATION TO COMPOUND QUANTIFICATION IN NMR SPECTRA OF MIXTURES
12:10-12:30	**G. Valentino -** HIGH-RESOLUTION NMR METABOLOMICS FOR ANTICANCER RESEARCH	**A. Rotondo** - FISHING MOLECULES: THE TALE OF HOW ART JOINED SCIENCE ON THE WAY OF STRUCTURAL ELUCIDATION
12:30-12:50	**P. Solovyev -** NMR SPECTROSCOPY IN ANALYSIS OF ORGANIC SAUERKRAUT FERMENTATION	**L. Querci -** NMR OF PARAMAGNETIC PROTEINS: NEW ROUTES FOR ^{13}C DIRECT DETECTION IN CHALLENGING IRON-SULPHUR PROTEINS
12:50-14:10	**Lunch + Poster session (ODD abstract numbers)**	

	Plenary session **Chair: A. Randazzo**
14:10-14:55	**Plenary Lecture 5** **J. Plavec (National Institute of Chemistry, Ljubljana)** NMR AS THE METHOD OF CHOICE FOR REVEALING THE POLYMORPHISM OF DNA AND ITS LIGAND INTERACTIONS
14:55-15:10	**Sponsorship Lecture (Jeol): P. Bowyer** TRIPLE RESONANCE MEASUREMENTS USING ECZ LUMINOUS SPECTROMETER
15:10-15:50	**Segre-Capitani Fellowships**
15:10-15:30	**G. Di Matteo** KOMBUCHA: AN NMR-BASED METABOLOMICS STUDY TO MONITOR ITS COMPLEX FERMENTATION PROCESS
15:30-15:50	**F. Nerli** A SOLID-STATE NMR INVESTIGATION OF THE INFLUENCE OF RESINS ON THE STRUCTURE AND DYNAMICS OF SBR ELASTOMERIC COMPOUNDS
15:50-16:05	**D. Mammoli** ERC SESSION 1: AVAILABLE SCHEMES & EVALUATION PROCESS
16:05-16:15	**Book Presentation: L. Mannina** LA RISONANZA MAGNETICA NELLA SCIENZA DEGLI ALIMENTI
16:15-17:30	**Coffee break + Poster session (EVEN abstract numbers)**
16:40-17:30	**GIRM assembly**
17:30-19.30	**GIDRM assembly + announcement of poster competition winner**
20:30	**social dinner**

Friday September 8th

	Plenary session Chair: M.R. Chierotti
8:45-9:30	**Plenary Lecture 6** **M. Geppi (University of Pisa)** A GLIMPSE ON THE MULTIFACETED WORLD OF POROUS MATERIALS BY HIGH- AND LOW-RESOLUTION SOLID STATE NMR TECHNIQUES.

	Parallel session A Chair: G. Musco	Parallel session B Chair: S. Borsacchi
9:30-10:00	**M. D'Onofrio** - UBIQUITIN PATHWAY OF TAU PROTEIN: INSIGHTS INTO FUNCTIONAL INTERACTIONS	**L. Calucci** - SOLID STATE NMR INVESTIGATION OF MATERIALS FOR GAS SEPARATION
10:00-10:20	**S. Fabbian** - A NOVEL PROTEIN-PROTEIN INTERACTION PROTECTS CANCER CELLS FROM APOPTOSIS: NMR ANALYSIS OF THE OSCP-IF1 BINDING PROCESS	**S. Bracco** - ROTOR DYNAMICS AND LIGHT-DRIVEN MOTORS IN NANOPOROUS ARCHITECTURES BY SOLID STATE NMR
10:20-10:40	**M. Della Valle** - UBIQUITIN/NANO-POLYSTYRENE INTERACTION STUDY BY USING AN INTEGRATED IN VITRO APPROACH FOR PREDICTING IN VIVO BEHAVIOUR	**M. Boventi** - NMR STUDY OF MORPHOLOGY AND POROUS STRUCTURE OF MOLECULARLY IMPRINTED POLYMERS

10:40-11:10	**Coffee break**
11:10-11:25	**D. Mammoli** ERC SESSION 2: PRACTICAL ADVICE & Q/A SESSION

	Parallel session A Chair: J. Plavec	Parallel session B Chair: M. Geppi
11:25-11:55	**F. Arnesano** - INTERFERENCE OF PLATINUM DRUGS AND ZINC IONS IN COPPER TRAFFICKING HIGHLIGHTED BY NMR SPECTROSCOPY	**M. Lelli** - DESIGN BIRADICALS FOR HIGH-FIELD AND HIGH-TEMPERATURE DNP
11:55-12:15	**A. Tino** - NMR-BASED INVESTIGATION OF INTRINSICALLY DISORDERED REGIONS OF MODULAR PROTEINS FOR TAILORED DESIGN OF INTERACTING	**M. Spano** - APPLICATION OF NMR ANALYSIS FOR MONITORING THE MALTING EFEFCT ON LEGUME SEEDS
12:15-12:35	**C. Zucchelli** - A TETRACATIONIC PORPHYRIN WITH DUAL ANTI-PRION ACTIVITY	**A. Ceccon** - IMPROVED DETECTION AND QUANTIFICATION OF CYCLOPROPANE FATTY ACIDS (CPFAS) BY ^{1}H NMR SPECTROSCOPY USING A COMBINATION OF HOMONUCLEAR DECOUPLING WITH DOUBLE IRRADIATION METHODS

	Plenary session Chair: L. Ragona and M.R. Chierotti
12:35-13:10	**Poster competition winner lectures**
13:10-13:25	**Closing**

13:25-14:30	**Lunch**

Wednesday 6th

9:00-14:00	**Registration**
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SPANNING THE PERIODIC TABLE BY NMR: A BIOINORGANIC VIEW

P. Turano

CERM & Department of Chemistry, Scientific Campus, University of Florence, 50019 Sesto Fiorentino, Italy
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Keywords: solid state NMR, solution NMR, biomolecules, metabolomics, theory and methods.

Bioinorganic chemistry is a field that encompasses the intersection between inorganic chemistry and biology. Homonuclear, heteronuclear and multinuclear NMR approaches have been applied to gain insights into the structure-function relationships in different types of protein-based systems of inorganic relevance, that fall under three main categories: *i*) metalloproteins, where the metal cofactor is an integral part of the protein by playing a key functional or structural role; *ii*) proteins involved in metal trafficking pathways; *iii*) enzymes, having metal ions as substrates. To this purpose, the properties of key paramagnetic metal ions have been exploited to expand the panel of restraints for NMR-based structural biology.
More recently, NMR-based metabolomics has been applied to get insights into the mechanism of action of metallodrugs. These activities were based on a continuous cross-talk between NMR-active nuclei of the 1st and 2nd period, taken as spectroscopic probes, and transition metal ions, as the central object of the research.

APPLYING ^{13}C-HYPERPOLARIZATION TO BREAK SENSITIVITY BARRIERS OF NMR FOR ANALYSIS OF COMPLEX MIXTURES

Mathilde Hauge Lerche[1], Pernille Rose Jensen[1], Anne B. Frahm[1], Magnus Karlsson[1], Jan Henrik Ardenkjær-Larsen[1], Andrea Capozzi[1], Sotirios Katzikis[1]

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E-mail: mhauler@dtu.dk

Keywords: solution NMR, hyperpolarization, small molecules.

Advancements in hardware development and intelligent acquisition design have overcome the sensitivity limitations of NMR for specific applications. However, the potential of quantitative NMR remains largely untapped, particularly in cases where the analysis of compounds in complex mixtures is required with limited sample amounts.
To address this, dissolution dynamic nuclear polarization (dDNP) has emerged as a technique to enhance the sensitivity and resolution of NMR for detecting compounds in complex mixtures [1][2]. Our research focuses on the development of a stable isotope tracer-based hyperpolarized NMR method [3]. The goal is to quantitatively measure metabolic flux with high sensitivity and contrast, enabling the mapping of metabolic pathways and networks. By enhancing the quantitative capabilities of NMR, this methodology opens new application perspectives in challenging fields characterized by sample complexity and low analyte concentrations.
In this study, we utilized the stable isotope tracer-based signal enhanced NMR method to investigate the metabolic signature of aggressive prostate cancer cells under hypotonic stress, comparing it to a similar treatment of early-stage prostate cancer cells. The cellular enlargement caused by hypotonicity leads to diluted concentrations not only of metabolites but also enzymes, potentially resulting in enzymatic inhibition or activation. This, in turn, could reveal a metabolic survival strategy adopted by the cells. By examining this phenomenon and other selected studies, we discuss the advantages and disadvantages of the hyperpolarization method and provide insights into future developments.
Overall, the combination of advanced hardware, smart acquisition design, and hyperpolarization techniques in quantitative NMR holds promise for analyzing complex mixtures and can greatly contribute to our understanding of metabolic processes in various fields.

References

[1] M.H. Lerche, D. Yigit, A. B. Frahm, J-H. Ardenkjær-Larsen, R. Malinowski and P.R. Jensen, *Analytical Chemistry* **90**, 674-678 (2018)
[2] V. Ribay, C. Praud, P. M. M. Letertre, J-N. Dumez, P. Giraudeau, *Curr. Opin. Chem. Biol* **74**, 102307 (2023)
[3] A.B. Frahm, D. Hill, S. Katsikis, T. Andreassen, J-H. Ardenkjær-Larsen, T. F. Bathen, S. A. Moestue, P. R. Jensen and M. H. Lerche, *Talanta* **235**, 122812 (2021)

^{31}P-PARACEST: ^{31}P MR-CEST IMAGING BASED ON THE FORMATION OF A TERNARY ADDUCT BETWEEN INORGANIC PHOSPHATE AND Eu-DO3A

D. Delli Castelli[1], G. Vassallo[1], E. Terreno[1], F.Garello[1], S. Aime[1]

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Keywords: MRI, contrast agents.

The MRI-CEST (Chemical Exchange Saturation Transfer) technique was proposed and designed to generate frequency-encoded contrast on the "bulk" water resonance by exploiting molecules with exchangeable protons. This indirect contrast amplifies the proton MRI detection threshold of these molecules by utilizing their effect on the much more intense signal of bulk water. The experiment involves selectively saturating the NMR signal of protons in chemical exchange and acquiring the water signal to measure the extent to which it is affected by this perturbation (Saturation transfer amount). The development of the field of MRI CEST contrast agents is hindered by the limited sensitivity of the technique. In water, the high proton concentration allows for a significant amplification of the exchanging proton pool, but at the same time, it limits the detectability of the mobile pool. This is because the saturation transfer efficiency depends, among other parameters, on the ratio of the number of saturated exchanging nuclei belonging to the contrast agent to the number of detected spins. The very high concentration of bulk water protons (111.2 M) implies that the number of nuclear spins of the CEST generating species has to be in the mM range. Changing the paradigm that CEST contrast can be performed only on water signal would bring advantages in terms of detectability threshold. The use of nuclei other than protons would allow exploiting signals different from water, thus lowering the concentration of the bulk pool and, in turn, the detectability of the irradiated pool. Some examples in the literature with ^{19}F and HP ^{129}Xe have been reported, both of them sharing the same limitations due to the absence of an endogenous exchanging bulk.
In this work, we report on the detection of ^{31}P signal from endogenous inorganic phosphate (Pi_{free}) as the source of CEST contrast by promoting its exchange with the Pi bound to the exogenous complex Eu-DO3A (Pi_{bound}). The results presented herein demonstrate that this approach can improve the detectability threshold by three orders of magnitude compared to the conventional 1H CEST detection (considered per single proton). This achievement reflects the decrease in the bulk concentration of the detected signal from 111.2 M (water) to 10 mM (Pi). This method paves the way for a number of biological studies and clinically translatable applications, as addressed here with a proof-of-concept in the field of cellular imaging.

References

[1] A. Bar-Shir and coworkers *Angewandte Chemie)* **60**, Issue 28,15405–15411. (2021**)**
[2] L. Schroder and coworkers *Molecules.* **25***,* Issue 20, 4227 (2020*)*

MULTIMODAL TEMPORAL MRI AND PET DATA ANALYSIS FOR THE CLASSIFICATION OF LOW- AND HIGH-GRADE GLIOMAS

Inglese Marianna[1,2], Ferrante Matteo[1], Boccato Tommaso[1], Islam Shah[2], Williams Matthew[3,4], Waldman Adam D[5], O'Neil Kevin[6], Aboagye O Eric[2], Toschi Nicola[1,7]

[1]Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy; [2]Department of Surgery and Cancer, Imperial College London, London, United Kingdom;[3]Computational Oncology Group, Department of Surgery and Cancer, Imperial College London, London, UK;[4]Institute for Global Health Innovation, Imperial College London, London, UK;[5]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK;[6]Imperial College Healthcare NHS Trust, London, UK;[7]Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, USA
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Keywords: MRI, contrast agents, theory and methods.

Gliomas are the most common primary brain tumours, and there is wide interest in imaging techniques able to stratify lesions [1]. This pilot study aimed to evaluate the potential of temporal features extracted from multimodal (i.e. dynamic positron emission tomography (PET) and magnetic resonance imaging (MRI) images) time activity curves (TACs) in discriminating high-grade gliomas (HGGs) from low-grade gliomas (LGGs). For each patient (4 LGG, 6 HGG), an average of 25202 (±14337) TACs were extracted voxelwise from the lesion mask overlaid onto the dynamic [^{18}F]FPIA PET [2], dynamic contrast-enhanced (DCE)-MRI and dynamic susceptibility contrast (DSC)-MRI images and used in a deep learning classification model that employed three 1D convolutional layers for the extraction of temporal features and fully connected layers for classification. Model performance was tested on an unseen test set using both single and multimodality TACs as input. Comparable performances were obtained from both single and multimodal TAC classification, except for DCE-TACs which delivered 88% accuracy (Fig.1 A). Interestingly, DCE-derived perfusion parameters (K^{trans}, K_{ep}, v_e) [3] averaged over the whole lesion were not statistically different between LGG and HGG, highlighting the usefulness of retaining temporal data (Fig.1 B). This pilot study introduces a promising approach for the classification of tumor grade based on time series processing[4], overcoming the challenge of pharmacokinetic model fitting[5].

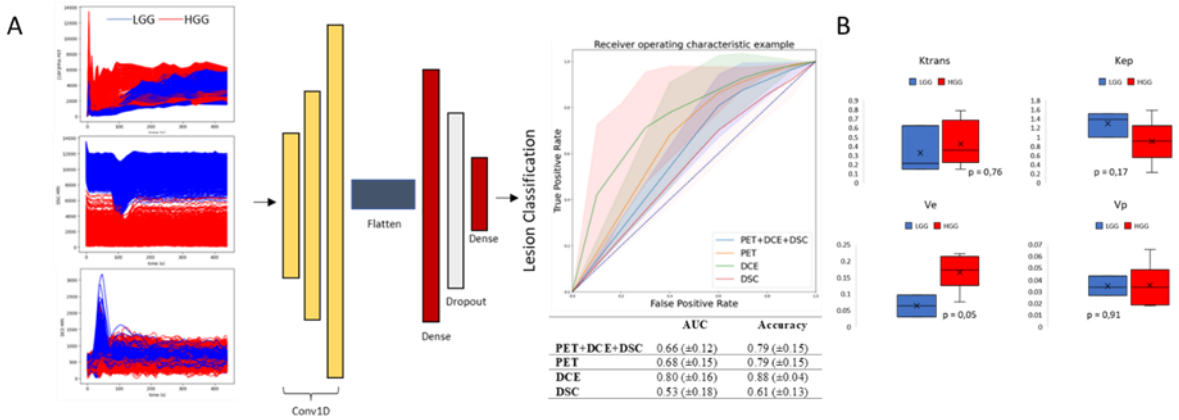

Fig. 1. Study workflow and results.

References

[1] H. Gao and X. Jiang, *Cancer Imaging*, 2013, doi: 10.1102/1470-7330.2013.0039.
[2] S. R. Dubash. *Eur J Nucl Med Mol Imaging*, 2020, doi: 10.1007/s00259-020-04724-y.
[3] M. Inglese *et al.*, *Neuroradiology*, 2019, doi: 10.1007/s00234-019-02265-2.
[4] M. Inglese, *Annu Int Conf IEEE Eng Med Biol Soc*, 2022, doi: 10.1109/EMBC48229.2022.9871033.
[5] Duan C, *Magn Reson Med*. 2017 doi: 10.1002/mrm.26189.

RELAXOMETRIC INVESTIGATION OF Fe(III) COMPLEXES AND THEIR SUPRAMOLEULAR ADDUCTS

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Keywords: solution NMR, low field NMR, small molecules, contrast agents.

Contrast agents (CAs) are used in 40% of the tens of millions of MRI scans performed each year. Currently, all FDA-approved CAs are Gd(III)-based complexes (GBCAs). Clinical and environmental concerns have renewed the interest in replacing Gd(III) with endogenous ions such as Fe(III). Some recent results indicate that Fe(III) chelates administered at higher doses than GBCAs have achieved similar results in typical clinical applications. Despite these important initial contributions, the effects and mechanisms responsible for the water proton relaxation enhancement induced by Fe(III) complexes and the relationships between the molecular parameters governing their efficacy (relaxivity) and chemical structure are still in their infancy.[1,2] For example, structural modifications that may facilitate interactions with macromolecules (e.g., human serum albumin, HSA) have been explored in a limited way, which is an intriguing aspect that deserves further investigation. For this reason, we present the ^{1}H and ^{17}O NMR relaxometric characterization of two new Fe(III)-based complexes of ethylenediaminetetraacetic acid (EDTA) derivatives carrying one or two benzyloxymethyl (BOM) functionalities, namely EDTA-BOM and EDTA-BOM_2. The presence of the BOM substituents is expected to have a dual effect. Firstly, according to the paramagnetic relaxation theory, an increase in the molecular weight of the complex is associated with increased relaxation at high magnetic fields (≥ 20 MHz, 0.47 T). Secondly, the lipophilic nature of the BOM functionalities may promote a noncovalent interaction with HSA and other substrates, which will be further explored. The formation of these macromolecular adducts leads to a strong increase in relaxivity (Fig. 1). In addition, to investigate the behavior of these complexes in supramolecular adducts, the interaction with β-cyclodextrin and poly-β-cyclodextrin is also explored. These complexes might represent an initial platform for the future design of complexes able to combine high efficacy, enhanced stability and inertness and non-covalent binding capability.

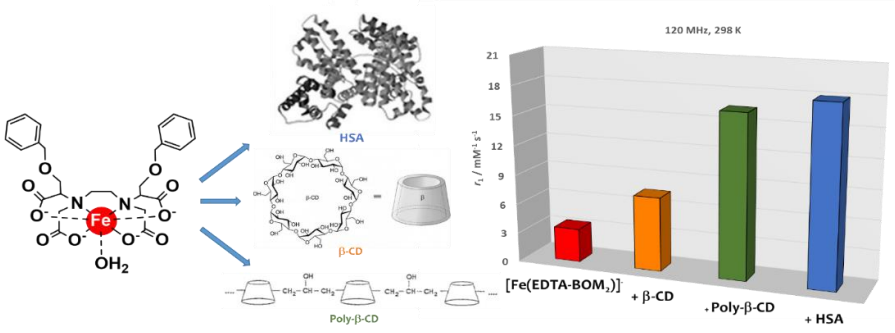

Fig. 1. Schematic representation of some of the investigated systems and their r_1 values (120 MHz, 298 K).

References

[1] Z. Baranyai, F. Carniato, A. Nucera, D. Horváth, L. Tei, C. Platas-Iglesias, M. Botta *Chem. Sci*. **12,** 11138–11145 (2021)
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NEW CLASS OF CONTRAST AGENTS FOR OVERHAUSER MRI, BASED ON NITROXIDE RADICALS AND RESPONSIVE TO SPECIFIC ENZYMATIC ACTIVITY

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Keywords: MRI, biomolecules, contrast agents.

The assessment of unregulated level of enzyme activity is a crucial parameter for early diagnoses in a wide range of pathologies and is commonly detected only "in vitro" with different techniques including spectrophotometric and spectrofluorimetric assays. Optical methods show a good sensitivity and selectivity but suffer from the interference from biological samples and low *in vivo* applicability. In this study, we propose the use of the Overhauser MRI (OMRI) as an alternative diagnostic approach allowing *in vivo* detection of enzymatic activity. The OMRI is a double resonance imaging technique that use stable radicals as contrast agents to couple the advantages of MRI with the sensitivity of EPR, by making use of the Overhauser effect. For this purpose, we synthesized two different classes of EPR probes. The first class was responsive to the activity of the carboxylesterases, which hydrolyze many endogenous and exogenous ester-containing substrates. Therefore, a nitroxide acyl esters containing a C_{12} aliphatic chain, namely Tempo-2-C_{12} ($T2C_{12}$) was synthesized (Fig. 1). In water, $T2C_{12}$ aggregate to form stable micelles that are EPR silent but following the hydrolyzation of the ester bond an intense EPR signal is generated obtaining a "off/on" EPR probes.[1] These probes were tested with different enzymes and OMRI images were acquired at 0.2T. A new formulation with Tween80 was also investigated and its responsiveness to tumor cells showing different esterases expression. In the case of the second probe, the target enzyme was the Fibroblast activation protein (FAP), proteases highly expressed in inflammation and tumors.[2] The probe, called KPAQ-DSPE (Fig.1), present an amino acid sequence recognized by FAP linked to the 4-Carboxy-Proxyl and a quinoline derivative to enhance the interaction with FAP. The probe was linked to a DSPE-PEG (2000) to obtain a silent probe in a micellar form with a low EPR signal. The micelles ware also incubated with FAP and the results show a complete hydrolyzation of the probe.

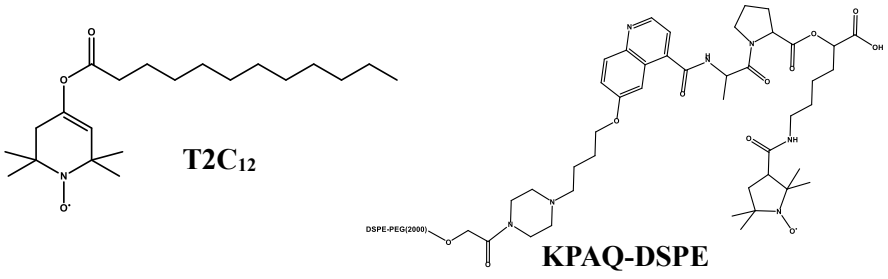

Fig. 1. Structure of $T2C_{12}$ (left) and KPAQ-DSPE (right).

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PROBING WATER DYNAMICS IN POROUS CULTURAL HERITAGE MATERIALS USING UNILATERAL NMR

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Keywords: portable NMR, porous media, NMR relaxometry, water.

Water and its interactions within porous cultural heritage (CH) materials play a crucial role in their preservation and degradation mechanisms. Understanding the behavior of water and its distribution within these materials is of paramount importance for developing effective conservation strategies. In this context, the application of unilateral portable NMR has become a consolidated technique for studying water and its interactions in cultural heritage materials [1-2].
Unilateral NMR enables non-destructive analysis of water dynamics, distribution, and interactions within porous materials. This presentation highlights recent applications of portable NMR in studying water dynamics in the paintings of Cappella Brancacci, in Roman cementitious formulations, and in carbonaceous stones consolidated with calcium oxalates.
One of the most important applications in CH is the study of water interactions in relation to humidity dynamics. Humidity is a key parameter that affects the stability and degradation of porous materials. By measuring the moisture content and monitoring changes in humidity levels, unilateral NMR, in conjunction with other techniques, offers real-time data on the moisture dynamics within cultural heritage sites and storage environments [3]. This information is essential for implementing appropriate climate control measures, preventing excessive moisture accumulation or desiccation, and maintaining optimal preservation conditions.
The study of water is also of primary importance for understanding the hydration process in ancient Roman concrete [4], known for its remarkable durability. Roman concrete, composed of lime, pozzolans, and aggregates, exhibits exceptional resistance to the test of time. By applying unilateral NMR during the hydration process, it is possible to gain insights into the water content, distribution, and mobility within the concrete structure.
Finally, NMR relaxometry is a powerful tool to obtain information about the porosity distribution, tortuosity, and open porosity in porous media. In the realm of conservation treatments, unilateral NMR plays a vital role in evaluating the impact of interventions on the porosity and water dynamics of CH materials [5]. Many conservation treatments aim to reduce the porosity of porous materials to enhance their mechanical stability and protect them from environmental factors. In this context, it is crucial to assess the long-term consequences of such treatments on the material's behavior and water movement.

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INSIGHTS ON NUTRITIONAL QUALITY AND SHELF LIFE ESTIMATION OF FISH PRODUCTS (SPARUS AURATA) THROUGH 1H NMR METABOLOMICS AND DATA INTEGRATION

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Keywords: solution NMR, metabolomics, food, theory and methods.

The definition of quality related to fish products is complex, due to its rapid deterioration. After capture and harvest, spoilage and freshness parameters change due to metabolic (autolytic) and microbiological processes, making fish products highly perishable when stored at ambient temperature and atmosphere, or without a preventive reduction in water activity and proper storage. Thus, chemical/biochemical (molecular) analyses that rely on indicators depending on various biological factors which condition post-mortem transformations, are mostly applied for freshness/spoilage determinations [1]. We propose a quality assessment of various gilthead seabream products using a metabolomic latent space trained on a large database, by linking latent components derived from complete spectral profiles to validated chemical indicators (k-index, TMA index). Furthermore, we propose a mathematical model using the integration of metabolomic profiles, microbiological, physical and chemical parameters for an enhanced estimation of the shelf-life of gilthead seabream fillets treated with innovative techniques. The proposed model will be shown to be particularly suitable for under sampled scenarios, a desirable feature for industrial application.

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RELIABILITY AND ERROR SOURCES OF AN NMR METHOD FOR THE ANALYSIS OF WHEAT AND PASTA

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Keywords: solution NMR, validation, metabolomics, wheat, pasta.

NMR spectroscopy has been undergoing an unprecedented evolution over the past decade. The striking facet of NMR is its ability to attain metrological traceability of products. The potential of NMR spectroscopy stems from its theoretical foundations – the signal ratio generated by the molecule under investigation and a reference molecule relies solely on their corresponding mole ratio. Hence, regardless of the spectrometer employed, the outcome in terms of signal ratios remains consistent, irrespective of hardware configurations.
Since 2012, we have been conducting NMR interlaboratory comparisons (ILCs) in compliance with ISO/IEC 17043:2010. The aim of this activity is to promote the use of NMR in official methods. The first ILC proved that NMR spectroscopy is apt for quantifying substances in standard calibration mixtures. This exercise utilized mixtures of four pesticides and a reference molecule, dissolved in deuterated water.[1] Second and third ILCs used complex matrices like wine grape juices,[2,3] and aqueous extracts of wheat and flours,[4] and further confirmed the statistical equivalence of NMR signal ratios and of calibration lines developed by using different spectrometers, while highlighting the impact of processing procedures.
Recently, the 4th ILC was organized by using aqueous extracts of wheat and pasta. The validation of the experiment was achieved with more than 60% of participants producing statistically equivalent NMR signal ratios. Comparison with previous work,[4] reveals consistent results. Both studies used the same NMR experimental setup and looked at aqueous extracts from wheat, flour, and pasta. Betaine signal was used as a reference point in both studies. The comparison of CV% from both studies indicates that the experimental error affecting the betaine signal remains within the range of 4.1-8.2%, regardless of the type of wheat-based matrix under investigation.
In this presentation, the results of the 4th ILC will be presented with a general view to the performance of the laboratories and to the potential error sources.

Acknowledgements

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NMR-METABOLOMIC STUDIES ON BLUEBERRIES STORED UNDER DIFFERENT CONDITIONS

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Keywords: solution NMR, metabolomics, food.

Improving the fruit quality and prolonging the storage capability are important breeding targets for blueberry fruit [1]. The focus of this study is to discover the metabolomic modifications of blueberry fruits under different storage conditions: regular atmosphere (20 kPa O_2, 0.03 kPa CO_2) and three controlled atmospheres (1 kPa O_2 and 18 kPa CO_2, 7 kPa O_2 and 18 kPa CO_2, 12 kPa O_2 and 18 kPa CO_2). NMR spectroscopy, because of its powerfulness and fastness of the analysis, was applied for this metabolomic study. Fruit of four blueberry cultivars ("Brigitta Blue", "Centurion", "Northland", "Star") were sampled and analyzed at three time points: at harvest, after 21 days and after 42 days of storage under the four aforementioned storage conditions. After the sample homogenization, the juice was firstly extracted and then analyzed with the addition of a buffer for pH and D_2O. The ^1H monodimensional experiment was carried out for the spectra acquisition and then 2D experiments helped in the confirmation of the identified compounds [2]. The spectra show a mixture of mostly amino acids and sugars. Metabolomic differences among the samples, determined with both untargeted and targeted approaches, are highlighted by multivariate analysis. Based on the PCA analysis of the fruits, the four cultivars were distinctly clustered. However, differences related to the storage conditions were found to have a lower impact on the fruit metabolic profile than genetic differences.

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FIELD CYCLING RELAXOMETRY, A TOOL TO ASSESS TUMOR METABOLISM

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Keywords: Low field NMR, theory and methods, instrumentation.

Proton T_1 acquisition over an extended range of magnetic field strengths (the so-called Nuclear Magnetic Resonance Dispersion profiles, NMRD) can provide useful insights to the detection and staging of tumors. Fist we showed that NMRD profiles can act as a high–sensitivity tool for cancer detection and staging in ex vivo murine breast tissues collected from tumor bearing murine models[1]. Next it was found that this information can be obtained also on live mice through a proper modification of the sample holder in the Stelar FFC relaxometer [2]. This work showed that, at low magnetic fields, tumor cells display proton T_1 values that are markedly longer than those shown by healthy tissue. Moreover, it has been found that the elongation of T_1 parallels the aggressiveness of the investigated tumor. T_1 lengthening is associated with an enhanced water exchange rate across the transcytolemmal membrane through an overexpression/upregulation of GLUT1 and Na + /K + ATPase transporters. The intracellular water lifetime represents a hallmark of tumor cells metabolism. These findings have been further exploited by applying this methodology to assess the presence of tumour cells in small tissue samples resected from the surgical specimens, at the margins of tumour resection, before the histopathological analysis. The obtained information has the potential to support the surgeon in real-time margin assessment during breast-conserving surgery[3].

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QUANTIFICATION OF BLOOD PLASMA METABOLITES AND LIPOPROTEINS FROM ^{1}H NMR SPECTRA USING THE SIGNATURE MAPPING (SIGMA) APPROACH

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Keywords: solution NMR, biomolecules, metabolomics, food.

This paper introduces and demonstrates a new method for semiautomatic identification and quantification of metabolites from plasma spectra using a signature mapping approach called *SigMa* [1]. *SigMa* relies on a metabolite library containing wide range of blood metabolites and advanced signal processing algorithms including *icoshift* [2] for spectral alignment and multivariate curve resolution (MCR) for signal quantification. The method requires highly standardized SOP for sample preparation, 1H NMR data acquisition, and data processing.
Recently the *SigMa* software has been extended with predictions of lipoprotein profiles in human blood plasma. Lipoproteins (LP) are important biomarkers for early diagnosis of cardiovascular diseases and the reference method, ultracentrifugation, is time consuming and there is thus a need to develop a rapid method for cohort screenings. Measurement of lipoproteins is possible by NMR, but they will have to be predicted from the strongly overlapped lipid regions in the spectra, which makes it fundamentally different from traditional NMR quantifications. Here we present partial least squares regression models developed using ^{1}H NMR spectra and concentrations of lipoproteins and lipoprotein subfractions as measured by ultracentrifugation on 316 healthy Danes [3]. The models are implemented in *SigMa* to be applied as a side-benefit for future cohort screenings.
Simultaneous predictions of multiple parameters from spectroscopy may be cohort dependent and suffer from the so-called *cage of covariance* problem [4]. The magnitude of this inherent problem will be scrutinized in the case of the difficult low density lipoprotein subfractions.

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Thursday 7th

	Plenary session **Chair: G. Parigi and M.R. Chierotti**
8:45-9:30	**Plenary Lecture 4** **G. Musco (San Raffaele hospital, Milan)** PHARMACOLOGICAL TARGETING OF CHEMOKINE-CHEMOKINE INTERACTIONS: THE STRANGE STORY OF HMGB1-CXCL12 HETEROCOMPLEX
9:30-9:45	**Sponsorship Lecture (Bruker): F. Benevelli** BENCHTOP AND FLOOR STANDING NMR IN BIOPRODUCTION
9:45-10:15	**Under 35 GIDRM: F. Nardelli (University of Pisa)** UNVEILING MOLECULAR STRUCTURES AND DYNAMICS WITH NMR SPECTROSCOPY: APPLICATIONS FROM BIOMEDICINE TO MATERIALS
10:15-10:30	**Sponsorship Lecture (Extrabyte): G. Selva** LIVE OVERVIEW OF THE RELAXOMETRY SOFTWARE WINREX
10:30-11:20	**Coffee break + Poster session (EVEN abstract numbers)**

	Parallel session A **Chair: S.B. Engelsen**	**Parallel session B** **Chair: L Russo**
11:20-11:50	**V. Righi** - APPLICATIONS OF HIGH-RESOLUTION MAGIC ANGLE SPINNING NMR IN BIOMEDICAL STUDIES	**G. Pileio** - SINGLET-ASSISTED DIFFUSION NMR (SAD-NMR) TO PROBE TRANSLATIONAL DYNAMICS IN SPACE-AND-TIME HETEROGENEOUS MEDIA
11:50-12:10	**G. Ciufolini** - FUNCTIONAL METABOLISM IN BLADDER CANCER CELLS: NMR BASED EXO-METABOLOMICS COMBINED WITH SEAHORSE DATA	**R. Lamanna** - DEEP LEARNING APPLICATION TO COMPOUND QUANTIFICATION IN NMR SPECTRA OF MIXTURES
12:10-12:30	**G. Valentino** - HIGH-RESOLUTION NMR METABOLOMICS FOR ANTICANCER RESEARCH	**A. Rotondo** - FISHING MOLECULES: THE TALE OF HOW ART JOINED SCIENCE ON THE WAY OF STRUCTURAL ELUCIDATION
12:30-12:50	**P. Solovyev** - NMR SPECTROSCOPY IN ANALYSIS OF ORGANIC SAUERKRAUT FERMENTATION	**L. Querci** - NMR OF PARAMAGNETIC PROTEINS: NEW ROUTES FOR ^{13}C DIRECT DETECTION IN CHALLENGING IRON-SULPHUR PROTEINS
12:50-14:10	**Lunch + Poster session (ODD abstract numbers)**	

	Plenary session **Chair: A. Randazzo**
14:10-14:55	**Plenary Lecture 5** **J. Plavec (National Institute of Chemistry, Ljubljana)** NMR AS THE METHOD OF CHOICE FOR REVEALING THE POLYMORPHISM OF DNA AND ITS LIGAND INTERACTIONS
14:55-15:10	**Sponsorship Lecture (Jeol): P. Bowyer** TRIPLE RESONANCE MEASUREMENTS USING ECZ LUMINOUS SPECTROMETER
15:10-15:50	**Segre-Capitani Fellowships 2023**
15:10-15:30	**G. Di Matteo** KOMBUCHA: AN NMR-BASED METABOLOMICS STUDY TO MONITOR ITS COMPLEX FERMENTATION PROCESS
15:30-15:50	**F. Nerli** A SOLID-STATE NMR INVESTIGATION OF THE INFLUENCE OF RESINS ON THE STRUCTURE AND DYNAMICS OF SBR ELASTOMERIC COMPOUNDS
15:50-16:05	**D. Mammoli** ERC SESSION 1: AVAILABLE SCHEMES & EVALUATION PROCESS
16:05-16:15	**Book Presentation: L. Mannina** LA RISONANZA MAGNETICA NELLA SCIENZA DEGLI ALIMENTI
16:15-17:30	**Coffee break + Poster session (EVEN abstract numbers)**
16:40-17:30	**GIRM assembly**
17:30-19.30	**GIDRM assembly + announcement of poster competition winner**
20:30	**social dinner**

PHARMACOLOGICAL TARGETING OF CHEMOKINE-CHEMOKINE INTERACTIONS: THE STRANGE STORY OF HMGB1●CXCL12 HETEROCOMPLEX

Malisa Vittoria Mantonico[1,2], Federica De Leo[1], Giacomo Quilici[1], Liam Colley[3,4], Francesco De Marchis[2,5], Massimo Crippa[5], Tim Schulte[6], Chiara Zucchelli[1], Stefano Ricagno[6,7], Gabriele Giachin[8], Michela Ghitti[1], Marco Bianchi[2,5], Giovanna Musco[1†]

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Keywords: solution NMR, small molecules, biomolecules.

Chemokines engage in heterodimeric interactions to activate or dampen their cognate receptors in inflammatory conditions. The chemokine CXCL12 forms with the alarmin HMGB1 a patho-physiologically relevant heterocomplex (HMGB1●CXCL12), whose formation synergically promotes the inflammatory response elicited by the G-protein coupled receptor CXCR4. We have previously shown that Diflunisal, an aspirin-like nonsteroidal anti-inflammatory drug, that has been in clinical use for decades, specifically inhibits in vitro and in vivo the chemotactic activity of HMGB1 at nanomolar concentrations, at least in part by binding directly to both HMGB1 and CXCL12 and disrupting their heterocomplex [1]. However, the molecular details of complex formation were still elusive. Through an integrative structural approach (NMR, AUC, ITC, MST, SAXS) we show that HMGB1●CXCL12 represents the first fuzzy chemokines heterocomplex reported so far. HMGB1 and CXCL12 form a dynamic equimolar assembly, rather than involving one HMGB1 and two CXCL12 molecules as previously assumed, with structured and unstructured HMGB1 regions recognizing the dimerization surface of CXCL12. We uncover an unexpected role of the acidic intrinsically disordered region (IDR) in heterocomplex formation and provide the first evidence that the acidic IDR facilitates the ternary HMGB1•CXCL12•CXCR4 interaction on the cell surface. Thus, the interaction of HMGB1 with CXCL12 diverges radically from the classical rigid heterophilic chemokine-chemokine dimerization [2].

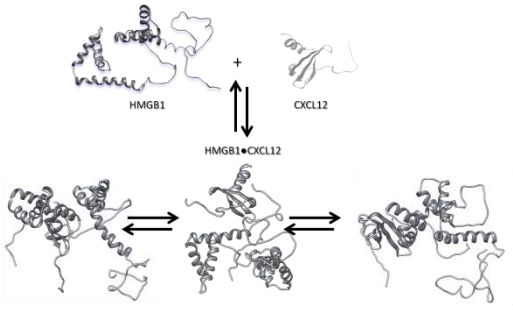

Fig. 1. HMGB1 and CXCL12 form a fuzzy heterocomplex.

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BENCHTOP AND FLOOR STANDING NMR IN BIOPRODUCTION

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Keywords: solution NMR, low field NMR.

Biologics are complex multi-attribute drugs with many features that can impact their function. High-resolution techniques, such as NMR is critical to increase product knowledge and decrease risks.
Among spectroscopic techniques, NMR has the distinct advantage of providing information on each nucleus it measures, with precise direct quantification and full structure characterization of the compounds in solution. Being a chemically and structurally specific analytical method NMR is ideal for biologics characterization.
The analysis via NMR of both the media for bioproduction as well as of the final products (e.g. monoclonal antibodies) provide extremely valuable information for monitoring and optimizing the bioproduction [3], as well as for the Quality Control of this complex drugs.

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UNVEILING MOLECULAR STRUCTURE AND DYNAMICS WITH NMR SPECTROSCOPY: APPLICATIONS FROM BIOMEDICINE TO MATERIALS

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Keywords: solid state NMR, materials, polymers.

NMR spectroscopy represents one of the most fascinating techniques to gain insights into molecular structure and dynamics in various types of systems, with relevance for different applications.
Following my research path, I will present some examples of my experience with NMR spectroscopy in various fields, including biomedicine, cultural heritage, and materials science. Concerning biomedicine, I will show the utility of ligand-based solution state NMR methods in identifying the mechanism of interaction between cell receptors overexpressed in cancerous cells and small ligands, with potential implications for tumor imaging and targeted therapy [1-2]. Then, I will move to an NMR application for the preservation of cultural heritage, presenting the results of solid-state NMR (SSNMR) experiments conducted on modern oil paints, which shed light on the chemical basis of their degradation under high relative humidity conditions [3]. Lastly, I will showcase how time-domain NMR allowed specific NMR observables to be correlated with macroscopic properties of elastomeric materials of interest to the tire industry [4]. Furthermore, a solution state NMR study for chiral molecular recognition and other applications of SSNMR and High-Resolution MAS NMR in the field of materials science will be mentioned [5-7].
These cases only scratch the surface of the remarkable potential of NMR spectroscopy in unraveling molecular structure and dynamics, offering valuable insights across diverse fields of application.

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LIVE OVERVIEW OF THE RELAXOMETRY SOFTWARE WINREX

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Extra Byte software package Rex is presented. Rex is a vendor-independent software tool dedicated to the processing of NMR Relaxometry data. It is designed either for researchers who study relaxation phenomena and molecular dynamics and for developers of specific applications of time domain NMR. The main features of the software are overviewed and a live demonstration of some example data processing is performed. The current state of the last version of Rex is summarized, along with the future advancements.

APPLICATIONS OF HIGH-RESOLUTION MAGIC ANGLE SPINNING NMR IN BIOMEDICAL STUDIES

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Keywords: metabolomics, biomolecules.

In memory of Professor Sebastian Cerdan

The field of metabolomics is influencing numerous areas of basic research and life sciences. In metabolomics, analytical methods play an important role, and the *ex-vivo* High-Resolution Magic Angle (HR-MAS) NMR spectroscopy has proven to be one of the most suitable and powerful methods.
HR-MAS NMR spectroscopy is an approach for analyzing intact biological material discovered more than 25 years ago and it has been refined by many technical developments and applied to many biomedical uses. The first applications of the HR-MAS NMR on human tissue samples date back to 1997 (1,2) and became possible due to the development of NMR probe heads capable of studying samples in rapid rotation around an axis at an angle of 54.7°.
The technique allows direct measurements of intact tissue samples and cells to provide valuable information on cellular metabolisms of physiological and pathological processes. HR-MAS produces NMR spectra with a resolution comparable to that obtained from sample extract solutions, but without complex metabolite extraction processes, and preserves the cellular structure of the tissue suitable for pathological examinations following spectroscopic analysis. The technique has been applied in a wide variety of biomedical and biochemical studies and has become an important platform for metabolomic studies. By quantifying individual metabolites, metabolite ratios or metabolic profiles in their entirety, HR-MAS NMR has been used for the diagnosis and prediction of clinical outcomes of various diseases, as well as for understanding metabolic changes resulting from drug therapies or xenobiotic interactions.
Here the results obtained using HR-MAS are reported on different diseases and present different applications of the method for understanding disease processes, the efficacy of therapies and new advances in HR-MAS methodology.

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FUNCTIONAL METABOLISM IN BLADDER CANCER CELLS: NMR BASED EXO-METABOLOMICS COMBINED WITH SEAHORSE DATA

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Keywords: solution NMR, metabolomics, cellular metabolism, bladder cancer, seahorse.

Bladder cancer (BC) is among the most common malignancies worldwide and displays high clinical and pathological heterogeneity. Identifying new markers is essential to improve patients' stratification and prognosis, avoid relapses, provide a better quality of life, and reduce disease management costs [1]. One potential source of markers to achieve this goal can arise from studying cancer cell metabolism. Reprogramming of metabolism is a hallmark of cancer, allowing cancer cells to satisfy their energy and biosynthetic needs to support increased cell growth and survival. Altered metabolic pathways represent attractive clinical targets exploitable in new therapeutic strategies.
A large amount of energy used for nutrient processing and cellular functions is essential for tumorigenesis [2]. To meet this need, total intracellular adenosine triphosphate (ATP) is mainly generated by glycolysis and mitochondrial oxidative phosphorylation (OXPHOS). We already have stressed the importance of these two main energy pathways in the growth and maintenance of different UBC cell lines and the relationship with their genomic signatures, leading to the proposal of two classes of cancer metabolism [3]. We also have shown how the continuous switching of glycolysis and OXPHOS is fruited by prostate cancer cells to cope with the consequences of cytotoxic therapy, i.e., apoptosis, senescence, and reprogramming to resume proliferation [4].
This work aims to characterize the functional metabolism of six bladder cancer cell lines at different degrees of cell differentiation, combining data obtained from exo-metabolomics and those from the Seahorse XF technology. Changes in the composition of the culture media of these cells were studied by nuclear magnetic resonance spectroscopy, evaluating the metabolite exchange rates (ERs) to provide a dynamic view of how a cell secures energy and nutrients and through which metabolic pathways.

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HIGH-RESOLUTION NMR METABOLOMICS FOR ANTICANCER RESEARCH

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Keywords: solution NMR, small molecules, metabolomics.

In the field of structure elucidation of organic molecules, solution NMR techniques are among the most useful analytical tools. As a non-invasive and non-disruptive technique, NMR methods play a significant role in the discovery of natural products and in the formulation of anticancer compounds[1].
Despite natural products being important in the treatment of cancer, their identification and isolation processes are still challenging. It often involves partial or full physical separation, which is expensive and time-consuming. In addition, these methods cannot be used to analyse intact complex mixtures, whose composition can be altered by the separation processes. To accelerate and improve the discovery and the characterization of potential anti-cancer bioactive compounds from plants, we integrated an NMR-based metabolomic approach with biological activity assays data[2].
Multivariate analysis was used to correlate ^{1}H NMR parameters (*i.e.* chemical shift and signal intensity) of the extracts with their antiproliferative activity, allowing the identification of spectral regions associated with their bioactivity. We applied modern NMR pulse sequences, such as 1D GEMSTONE-TOCSY and PSYCHE, to identify potential bioactive substances contained in complex mixtures[3,4]. Thanks to the information obtained by in-mixture NMR experiments we carried out an *ad hoc* phytochemical strategy (L-L ext., CC, HPLC) avoiding the classic bio-guided fractionation. As a result, five bioactive sesquiterpene lactones were rapidly isolated and characterized.
This study demonstrates the power of advanced NMR methods in elucidating organic compounds in complex mixtures.

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NMR SPECTROSCOPY IN ANALYSIS OF ORGANIC SAUERKRAUT FERMENTATION

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Keywords: solution NMR, small molecules, biomolecules, metabolomics, food.

Sauerkraut is a product derived from malolactic fermentation of cabbage and it is currently the most fermented product in Europe. Some scholars have linked consumption of such foods to increased immune functions, anti-inflammation activity, decreased fasting glycemia and other health beneficiary effects [1]. This multidisciplinary study was focused on characterizing the sauerkraut brine from two different producers with ^{1}H NMR-based metabolomics in combination with examination of its microbial diversity (see Fig. 1). The metabolite profiles changed significantly during the process of fermentation, and such components as lactic and acetic acid, as well as amino acids, amines, and uracil, were among the dominant metabolites [2].

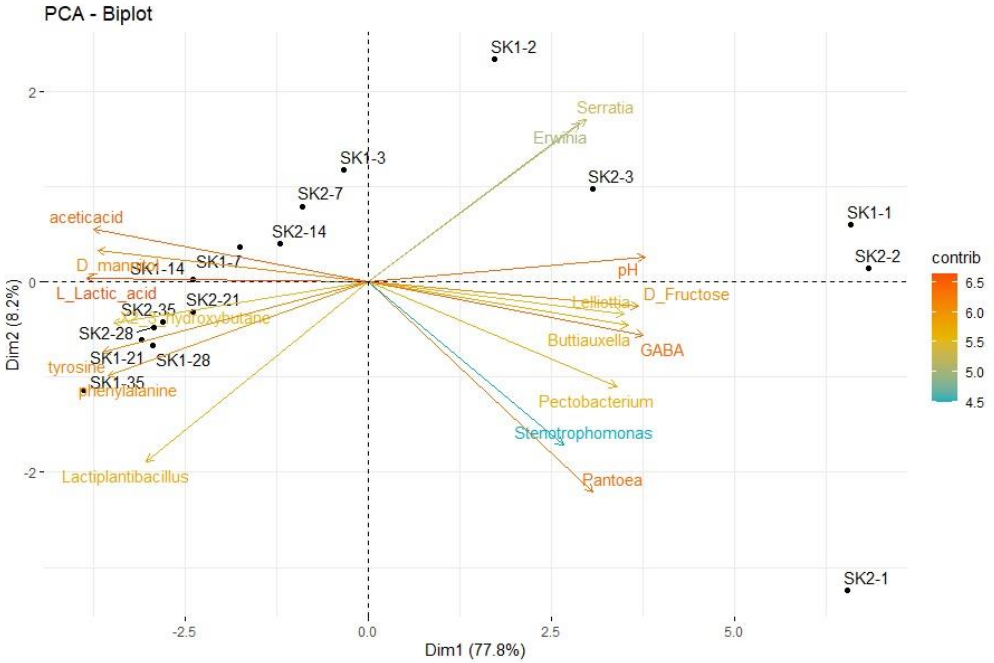

Fig. 1. PCA biplot showing correlations between bacterial genera and metabolites at different stages of sauerkraut fermentation).

From the microbiological point of view, a robust inflammatory response to endotoxin was detected which could indicate positive effect on inflammation and supporting the potential of sauerkraut brine to regulate intestinal immune function.

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SINGLET-ASSISTED DIFFUSION NMR (SAD-NMR) TO PROBE TRANSLATIONAL DYNAMICS IN SPACE-AND-TIME HETEROGENEOUS MEDIA

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Keywords: solution NMR, materials, small molecules, theory and methods, instrumentation.

Diffusion and, more generally, translational dynamics, is perhaps one subject where NMR best reveals its advantages over other techniques. Diffusion NMR methodologies, for instance, include diffusion ordered spectroscopy, a successful tool used by communities within and outside NMR. Diffusion NMR is not only a solution state tool: it is in fact exploited to produce diffusion weighted images (DWI) and diffusion tensor imaging (DTI) in MRI, and to characterize porosity and other structural parameters in porous media applications. Crucially, all standard diffusion NMR methods are limited by the duration of *spin memory,* i.e., by how long spin order can store positional information for (typically of the order of seconds, at best).

In my laboratory, we have taken the challenge to extend the capabilities of diffusion NMR using long-lived spin order methods, an NMR topic we have long and deeply specialized in. Long-lived spin order allows information to be stored for long time, typically of the order of several minutes. An extended spin memory translates into extended diffusion NMR capabilities allowing, for example, to measure smaller diffusion coefficients or slower flows, to characterize structures with larger pores or to measure tortuosity in porous media. We are particularly interested in measuring diffusion tensors and tortuosity in the gas diffusion layer (GDL) of fuel cells and in tissue cultured on 3D-printed scaffoldings, a heterogeneous system in space and time. Both systems have pores which are too large (300-500 microns) for standard diffusion NMR, and singlet-assisted diffusion approaches are complicated by the magnetic susceptibility inhomogeneities between the porous structure and the imbibed medium.

In this talk, I shall discuss our methodological approach that merges field-cycling, long-lived spin order and pulsed field gradients. I shall present the customized hardware designed, the pulse sequences developed and a variety of experiments that highlight the capabilities offered by this methodology.

DEEP LEARNING APPLICATION TO COMPOUND QUANTIFICATION IN NMR SPECTRA OF MIXTURES

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Keywords: solution NMR, metabolomics, exotica.

Quantitative analysis of NMR spectra of mixtures is extremely time consuming and a difficult task due to several factors. Among these, signal overlapping and chemical shift variations of the same compound in different mixtures and conditions are the most significant.
Spectra quantification is usually made by signal integration, binning or deconvolution and often requires the correct assignment of the signals of the compound to be quantified. In any case significant chemical shift variations may strongly impact the quantification process.
Deep learning is a method based on complex neural network architectures able to learn from data of different kind. Actually, convolutional neural networks are particularly suitable to treat computer images and then NMR spectra of any dimension.
Deep learning requires significant computing resources during training process but is very fast in the prediction phase. It has been used in the NMR field for spectral reconstruction, denoising, peak picking, phase and baseline correction, chemical shift prediction, molecular recognition and compound identification.
A deep learning algorithm can in principle learn the composition of a mixture, in terms of compound concentration, from the structure of the NMR spectrum.
In this work the possibility to estimate the concentration of a single compound in a mixture by a convolutional neural network is explored. After training, the network is able to predict the concentration of the compound on several spectra in few seconds. The method could permit to evaluate the mixture composition by using several neural networks, one for each component of the mixture. Since the neural model can also learn chemical shift, line-width, J coupling variations and phase, baseline and line-shape distortions in the spectra, the compound quantification should be very robust against these effects.

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

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Keywords: solution NMR, small molecules, biomolecules, metabolomics, theory and methods.

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

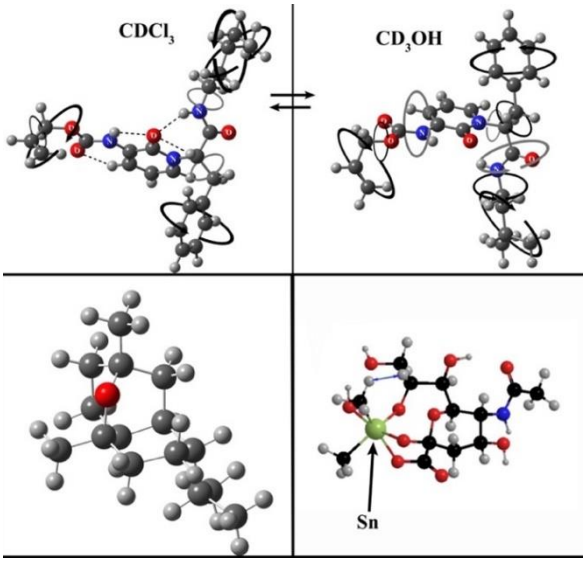

Fig. 1. Showcase of 2D and 3D Molecules characterized by Rotondo et al.

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NMR OF PARAMAGNETIC PROTEINS: NEW ROUTES FOR ^{13}C DIRECT DETECTION IN CHALLENGING IRON-SULPHUR PROTEINS

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Keywords: solution NMR, biomolecules, theory and methods, instrumentation.

For Iron-Sulfur Proteins, the robustness of NMR coherence transfer in proximity of the cluster depends on the relaxation properties of the nuclei involved, therefore recording the same experiment with different pulse schemes or different parameter sets provides often complementary results. The integrative effect of ^{1}H start and ^{13}C start CACO experiments will be discussed in practice attending a non-canonical sequence-specific assignment of revived Cisd3[1] C^{α}/C' correlations in the spectra. Additionally, in paramagnetic metalloproteins, longitudinal relaxation rates of $^{13}C'$ and $^{13}C^{\alpha}$ nuclei can be measured using ^{13}C detected experiments and converted into electron spin-nuclear spin distance restraints (Figure 1), also known as Paramagnetic Relaxation Enhancements Restraints (PRE). We will discuss the complementarity of ^{13}C PRE restraints with ^{1}H PRE restraints in the case of the High Potential Iron Sulfur Protein (HiPIP) PioC[2] and how ^{13}C R_1 values can be measured also at very short distances from the paramagnetic center. The obtained set of ^{13}C based restraints can be added to ^{1}H PREs and to other classical and paramagnetism based NMR restraints.

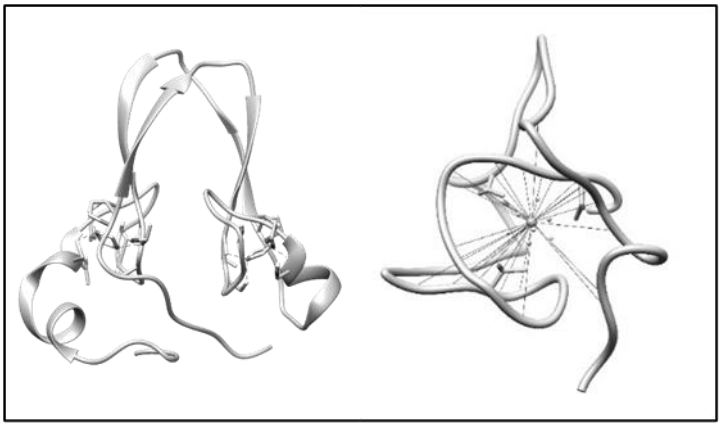

Fig. 1. 3D structure of Cisd3 (right) and PioC (left). Lines in PioC structure represent C'/C^{α}-to-metal distances calculated from longitudinal relaxation rates, R_1

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NMR AS THE METHOD OF CHOICE FOR REVEALING THE POLYMORPHISM OF DNA AND ITS LIGAND INTERACTIONS

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Keywords: solution NMR, biomolecules, small molecules.

Noncanonical DNA structures deviate from the canonical double helix held together by Watson-Crick base pairs. These structures usually form under certain conditions and are affected by changes in nucleotide sequence, environmental factors or the presence of ligands. Importantly, noncanonical DNA structures can have functional effects, such as influencing gene expression, DNA replication, recombination and repair processes. The quadruplex DNA structure is a unique arrangement formed by stacking planar guanine or other nucleobases into four-stranded motifs known as quartets. G-rich DNA sequences leading to G- and AGCGA-quadruplex structures are commonly found in telomeres as well as in certain regulatory regions of the genome.
Telomeric DNA repeats can exhibit structural polymorphism in addition to length polymorphism. A common form of structural polymorphism in telomeric DNA is the formation of different G-quadruplex structures, which may differ in their folding topologies, loop length, and stabilizing factors. To study the structure and stability of G-quadruplexes, our group uses Nuclear Magnetic Resonance (NMR) spectroscopy as the method of choice [1-8]. The G-quartet and other quartet structures of quadruplex DNA allow interaction with various small molecules through specific recognition. When ligands bind to quadruplex DNA, they can either stabilize or destabilize its structure. Interestingly, Phen-DC_3, one of the best-known G-quadruplex ligands in terms of high binding affinity and selectivity, causes d[TAGGG(TTAGGG)$_3$] to completely change its fold in KCl solution from a hybrid-1 to an antiparallel chair-type structure, wherein the ligand intercalates between two G-quartets thereby ejecting one potassium ion (pdb id: 7Z9L). This unprecedented high-resolution NMR structure that shows true ligand intercalation into an intramolecular G-quadruplex demonstrates ability of Phen-DC_3 to interact with four guanines due to its large aromatic surface area consisting of three rings and overall U-shape. It may also facilitate G-quartet assembly through a templating effect and thus promote ligand interactions even in cation depleted environment.

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TRIPLE-RESONANCE EXPERIMENTS USING ECZ LUMINOUS

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Proton (^{1}H) and carbon-13 (^{13}C) are, by some distance, the most widely studied nuclides by NMR spectroscopy. Typically, a combination of 1D and 2D spectra involving either or both ^{1}H and ^{13}C will provide sufficient information to answer the question at hand. However, in the case of molecules that contain other NMR-active heteroatoms (e.g. ^{19}F, ^{31}P), it can be useful to obtain additional structural information by collecting spectra that involve a combination of ^{1}H, ^{13}C and the heteroatom. For example, triple-resonance ^{1}H/^{19}F/^{13}C experiments are finding increasing utility in the pharmaceutical industry. Of course, in the field of biomolecular NMR, it is routine to probe the structure and dynamics of proteins by running triple-resonance experiments that involve ^{1}H, ^{13}C and ^{15}N.
Conventionally, triple-resonance experiments require not only a special NMR probe that can be simultaneously tuned to all three nuclides of interest, but also three dedicated RF channels in the spectrometer console. However, recent advances in spectrometer architecture have made it possible to perform triple-resonance experiments on a standard two-channel instrument. In this presentation, the JEOL's latest-generation of NMR spectrometers, the ECZ Luminous, will be introduced. It will be shown how, in combination with JEOL's advanced probe technologies, the unique architecture of the ECZ Luminous makes it possible to perform triple-resonance 1D, 2D and even 3D bio-NMR experiments on a standard two-channel instrument.

KOMBUCHA: AN NMR-BASED METABOLOMICS STUDY TO MONITOR ITS COMPLEX FERMENTATION PROCESS

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Keywords: solution NMR, metabolomics, food.

Kombucha is a sweetened tea fermented with a symbiotic culture of yeasts and bacteria. The beverage presents a balanced taste among sweet, due to the residual sugars, and sour, due to organic acids produced by acetic acid bacteria. Due to the complex fermentation process, the determination of its quality requires an holistic characterization. In order to evaluate the stages of kombucha fermentation objectively a metabolomics characterization was performed. Kombucha samples were collected every 5 days for 25 days of fermentation and analyzed by ^{1}H-NMR untargeted analysis and HPLC-PDA targeted analysis. By high-field NMR the levels of sugars (sucrose, glucose, fructose, trehalose), organic acids (acetic acid, lactic acid, succinic acid, malic acid, citric acid, formic acid and gluconic acid), ethanol and glycerol were quantified. By HPLC the amounts of polyphenols like (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, (+)-gallocatechin, gallic acid, caffeine and theobromine were monitored along the time. The merged results were analyzed by principal component analysis (PCA). A 60 MHz benchtop NMR instrument was also used monitoring the quantity of sucrose, lactic acid, acetic acid and succinic acid. The Benchtop NMR spectrometer can be readily used as an on-line process monitoring tool in the kombucha production. Moreover, this fast analysis method could be applied on different kombucha industrial productions around the world to expand the knowledge about the dynamics of the substrate consumption and metabolite production.

A SOLID-STATE NMR INVESTIGATION OF THE INFLUENCE OF RESINS ON THE STRUCTURE AND DYNAMICS OF SBR ELASTOMERIC COMPOUNDS

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Keywords: solid state NMR, low field NMR, materials, polymers.

The application and use of environmentally friendly components in rubber compound formulation is one of the most challenging goals of the tire industry. Specifically, tires are composed of elastomeric materials, obtained through the vulcanization of one or more polymers in the presence of curing agents and many other components, such as inorganic fillers, stabilizers, oils and resins. In particular, resins play a crucial role for the improvement of the rheological behaviour of the elastomeric compound, as well as the mechanical properties of the final product, such as rolling resistance and wet traction. However a significant issue of standard resins used for tire production is their fossil hydrocarbon source and the related environmental impact. Because of this, there is a strong interest in the development of more sustainable and renewable resins which fulfil specific requirements without altering the applicative properties of the final product. In order to achieve this goal, it is fundamental to investigate what happens at the molecular level between polymer and resin and to relate it with the macroscopic behaviour and performances of the final material [1].
In this context, we characterized both cured and uncured styrene-butadiene based compounds, containing three different resins, one of which of vegetal origin, by means of low-field time-domain and high-resolution solid-state NMR (SSNMR) techniques, which proved to be key to study resin/polymer interactions and miscibility, as well as the effect of resins on the overall dynamics of polymer chains. In particular, ^{1}H time-domain experiments allowed us to measure ^{1}H spin-lattice relaxation times in the laboratory (T_1) and in the rotating frame ($T_{1\rho}$), as well as ^{1}H spin-spin relaxation times (T_2), which are sensitive to molecular and mobility differences in heterogeneous materials, as they depend on the modulation of ^{1}H-^{1}H dipolar couplings by molecular motions. Moreover, ^{1}H T_1 and $T_{1\rho}$ relaxation times measured at different temperatures allowed us to investigate the effect of resin on polymer chain dynamics. Complementary structural information on each ingredient of the compounds were obtained by ^{13}C high-resolution SSNMR [2,3,4].
The results obtained by combining time-domain and high-resolution techniques provided useful information regarding both polymer-resin interactions and mixing degree, gaining insights into the structure-property relationship, which is helpful for the design of rubber compounds and the rationalization of their macroscopic behaviour.

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EUROPEAN RESEARCH COUNCIL SESSIONS

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The European Research Council (ERC) is Europe's leading funding agency supporting excellent and investigator-driven frontier research across all fields of science.
ERC calls are open to applicants from all over the world and provide several outstanding funding opportunities with grant-budgets ranging from 1.5 to 3.5 million euro.
All nationalities are welcome, provided that the projects are carried out at a host institution located in Europe (i.e. European Union Member States or the countries which have associated to the EU research funding programs).
Since 2007, the ERC has financed ca 600 individual research projects in the field of Physical and Analytical Chemical Sciences[1].
Two informative sessions will take place, at different times.
In the first one, the speaker will present the main features of the ERC individual grants (i.e. Starting, Consolidator, Advanced), together with general information on the application and evaluation process.
In the second session, the speaker will initially provide advice on relevant aspects of the process, such as identifying a solid idea and writing a successful proposal. After that, anybody from the audience will have the chance to ask questions.
These ERC sessions are open to all the attendees of the GIDRM congress and will be particularly helpful to early-career and mid-career participants, especially post-docs, researchers and professors who think about applying for funding to conduct breakthrough-research in Europe.

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Friday 8th

	Plenary session Chair: M.R. Chierotti
8:45-9:30	**Plenary Lecture 6** **M. Geppi (University of Pisa)** A GLIMPSE ON THE MULTIFACETED WORLD OF POROUS MATERIALS BY HIGH- AND LOW-RESOLUTION SOLID STATE NMR TECHNIQUES.

	Parallel session A Chair: G. Musco	Parallel session B Chair: S. Borsacchi
9:30-10:00	**M. D'Onofrio** - UBIQUITIN PATHWAY OF TAU PROTEIN: INSIGHTS INTO FUNCTIONAL INTERACTIONS	**L. Calucci** - SOLID STATE NMR INVESTIGATION OF MATERIALS FOR GAS SEPARATION
10:00-10:20	**S. Fabbian** - A NOVEL PROTEIN-PROTEIN INTERACTION PROTECTS CANCER CELLS FROM APOPTOSIS: NMR ANALYSIS OF THE OSCP-IF1 BINDING PROCESS	**S. Bracco** - ROTOR DYNAMICS AND LIGHT-DRIVEN MOTORS IN NANOPOROUS ARCHITECTURES BY SOLID STATE NMR
10:20-10:40	**M. Della Valle** - UBIQUITIN/NANO-POLYSTYRENE INTERACTION STUDY BY USING AN INTEGRATED IN VITRO APPROACH FOR PREDICTING IN VIVO BEHAVIOUR	**M. Boventi** - NMR STUDY OF MORPHOLOGY AND POROUS STRUCTURE OF MOLECULARLY IMPRINTED POLYMERS

10:40-11:10	**Coffee break**
11:10-11:25	**D. Mammoli** ERC SESSION 2: PRACTICAL ADVICE & Q/A SESSION

	Parallel session A Chair: J. Plavec	Parallel session B Chair: M. Geppi
11:25-11:55	**F. Arnesano** - INTERFERENCE OF PLATINUM DRUGS AND ZINC IONS IN COPPER TRAFFICKING HIGHLIGHTED BY NMR SPECTROSCOPY	**M. Lelli** - DESIGN BIRADICALS FOR HIGH-FIELD AND HIGH-TEMPERATURE DNP
11:55-12:15	**A. Tino** - NMR-BASED INVESTIGATION OF INTRINSICALLY DISORDERED REGIONS OF MODULAR PROTEINS FOR TAILORED DESIGN OF INTERACTING	**M. Spano** - APPLICATION OF NMR ANALYSIS FOR MONITORING THE MALTING EFEFCT ON LEGUME SEEDS
12:15-12:35	**C. Zucchelli** - A TETRACATIONIC PORPHYRIN WITH DUAL ANTI-PRION ACTIVITY	**A. Ceccon** - IMPROVED DETECTION AND QUANTIFICATION OF CYCLOPROPANE FATTY ACIDS (CPFAS) BY ^{1}H NMR SPECTROSCOPY USING A COMBINATION OF HOMONUCLEAR DECOUPLING WITH DOUBLE IRRADIATION METHODS

	Plenary session Chair: L. Ragona and M.R. Chierotti
12:35-13:10	**Poster competition winner lectures**
13:10-13:25	**Closing**

13:25-14:30	**Lunch**

A GLIMPSE ON THE MULTIFACETED WORLD OF POROUS MATERIALS BY HIGH- AND LOW-RESOLUTION SOLID STATE NMR TECHNIQUES

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Keywords: solid state NMR, low field NMR, materials, small molecules, polymers.

Porous materials have attracted considerable scientific and technological interest due to their critical applications in many fields, such as membrane-based gas separation, building materials, adsorption and storage, catalysis, ion exchange, nanotechnology, etc. From a chemical and structural point of view, the definition of "porous materials" encompasses a wide variety of systems, including inorganic, hybrid organic-inorganic, and polymeric materials.
Solid state NMR spectroscopy (ssNMR) has been showing its tremendous potential to clarify the often-intricate behavior of this class of materials at a molecular and nanometric level. Indeed, it provides a wide variety of tools, relying on the observation of different nuclei and the measurement of different spectral and relaxation properties, which can reveal information on structural and dynamic features on wide spatial and time scales, respectively [1,2].
This lecture will cover a selection of ssNMR studies aimed at unravelling these features, and especially the dynamic aspects, on different classes of porous materials. In particular, the case studies presented will concern microporous polymers for solid-state gas separation and ion-exchange membranes, 1D coordination polymers devised to sequester volatile organic compounds, Ce-based metal organic frameworks with potential applications in the field of CO_2 capture, and Mg- and Ca-based cement pastes. The main focus will be put on the detailed description of motional processes of polymeric chains and organic ligands and on the interactions and dynamic behavior of adsorbed water and other guest molecules. It will be shown how, depending on the type of material, on the available nuclei, on the desired detail of the information, and on the time scale of the motion, different experimental approaches can be used, combined with different analyses of the nuclear parameters measured in terms of suitable theoretical models. The experiments employed include static and Magic Angle Spinning as well as high- and low-field techniques, and in particular they rely on: ^{1}H and ^{19}F on-resonance FID analysis; ^{1}H, ^{19}F and ^{13}C spin-lattice relaxation times in the laboratory frame and of ^{1}H spin-lattice relaxation times in the rotating frame; ^{13}C chemical shift anisotropy; ^{2}H quadrupolar interaction.

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UBIQUITIN PATHWAY OF TAU PROTEIN: INSIGHTS INTO FUNCTIONAL INTERACTIONS

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Keywords: solution NMR, biomolecules.

The accumulation of aggregates of tau protein in the form of filaments is a pathological hallmark of many neurological diseases such as Alzheimer's disease. Tau filaments are highly post-translationally modified and the type and extent of modifications reflect disease progression [1].
Ubiquitination is involved in a variety of cellular functions and requires the action of a series of enzymes for the covalent attachment of one or multiple ubiquitin molecules to substrates as well as to remove ubiquitins to regulate the signaling. Many evidences suggest that ubiquitination, in addition to mediating tau signaling under physiological conditions, may also be a key player in the transition to toxic species [2]. Therefore, the tau-ubiquitin pathway has been proposed as a target for the development of novel treatments to alleviate tau pathology.
In this context, the elucidation of the structural determinants for specific protein-protein interactions at the basis of ubiquitin signaling is crucial. We employed site-resolved solution NMR spectroscopy to characterize the interaction of tau with the deubiquitinating enzyme Otub1, which has been proposed to be involved in the formation of pathological Tau forms [3]. Using enzymatic reactions we produced samples of ubiquitinated tau with segmentally isotope-labeled units and exploited chemical shift perturbation to map protein-protein interfaces, gaining significant insights into the molecular recognition of tau with Otub1, a regulator of tau ubiquitination.

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A NOVEL PROTEIN-PROTEIN INTERACTION PROTECTS CANCER CELLS FROM APOPTOSIS: NMR ANALYSIS OF THE OSCP-IF1 BINDING PROCESS

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Keywords: solution NMR, biomolecules.

IF1 is a mitochondrial protein which binds to the ATP synthase or Complex V at the catalytic domain, inhibiting the hydrolytic activity of the enzyme in anoxic conditions [1]. Noteworthy, IF1 is overexpressed in many tumors [2], acting as a pro-oncogenic protein even though its mechanism of action is still unclear. Recently, we found that the lack of IF1 sensitizes *HeLa* cells to the opening of the permeability transition pore (PTP) [3], a mitochondrial channel which promotes cellular apoptosis. A PTP dysregulated activity is directly related with aging and several human diseases as Alzheimer's and cancer. Even though the molecular details of PTP structure are still debated, the ATP synthase has been suggested as the main component of the pore and its subunit OSCP is emerging as a molecular switch for the PTP regulation [4]. Interestingly, our immunoprecipitation and proximity ligation assay showed that IF1 binds to the OSCP in *HeLa c*ells during cellular respiration [3]. The interaction between the two proteins was confirmed in vitro by NMR using the separated N-terminal (NT) and C-terminal (CT) recombinant domains of both OSCP and IF1. Chemical shift perturbation showed that the binding epitopes involve a short disordered region at the C-terminal side of IF1-NT (E29-R39) and a relatively small surface area of OSCP-NT (A53-I64) [3]. C^{α} secondary chemical shift revealed that the residues belonged to the disordered segment within IF1-NT have a high tendency to fold into alpha-helix suggesting a folding-upon-binding process with OSCP-NT.
Here we report our NMR analysis for the interaction between the recombinant OSCP and IF1 domains. Overall, our results indicate that the OSCP-IF1 interaction protects cancer cells from PTP-dependent apoptosis and help to comprehend the molecular details of the PTP modulation by protein-protein interactions for further therapeutical applications.

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UBIQUITIN/NANO-POLYSTYRENE INTERACTION STUDY BY USING AN INTEGRATED *IN VITRO* APPROACH FOR PREDICTING *IN VIVO* BEHAVIOUR

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Keywords: solution NMR, materials, biomolecules, polymers.

Nanoplastics (NPs) are ubiquitously found in the environment including water, air, food due to their many applications in daily life. [1] Humans are estimated to consume several grams per week of nanoparticles released by plastic debris. [2, 3] Nonetheless, the effects of these polymeric particles on living organisms are still mostly unknown.
It is established that NPs interact with proteins producing the so-called protein corona that covers its surface. [4] Thus, it's necessary to understand the NPs effects and their toxicity using controlled reference systems. Here, by means of Circular Dichroism (CD), Trasmission Electron Microscopy (TEM) and high-resolution Nuclear Magnetic Resonance (NMR), is described at an atomic resolution the interaction of human Ubiquitin (Ubq) with Polystyrene nanoplastics (PS-NPs), showing the formation of a *hard* protein corona, and suggesting a global rearrangement of Ubq structure. Moreover, the impact of polystyrene NPs on ubiquitin functions was tested by *in cell* ubiquitination experiments, leading to a sensible reduction of the process in human HeLa cells. Ubiquitination mis-function is at the basis of many neurodegenerative and oncogenic diseases; therefore, our findings demonstrate that the exposure of cells to PS-NPs may represent a serious harm to the human health.

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SOLID STATE NMR INVESTIGATION OF MATERIALS FOR GAS SEPARATION

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Keywords: solid state NMR, materials, polymers.

Over the last years, metal organic frameworks (MOFs), porous membranes, and MOF-based mixed matrix membranes (MMMs) have gained a lot of interest in the research community as promising materials for gas separation [1,2]. MOFs are organic/inorganic materials constituted of metal oxide clusters linked by organic ligands. Their gas separation properties can be finely tuned by combining different metals and linkers, as well as adopting post-synthetic modification procedures. MMMs, comprised of polymeric matrices and MOF fillers, are considered next-generation membranes for gas separation because they combine the benefits of the polymer processability with the enhanced separation properties of the filler. The physico-chemical properties of both the MOF and the polymer, as well as their interactions in the composite, play a key role in obtaining MMMs with enhanced separation performances. It is thus important to unravel these properties at the molecular level to understand the structure-property relationships and to guide the design of optimized materials for gas separation.
Solid-state Nuclear Magnetic Resonance (SSNMR) spectroscopy has established itself as one of the most powerful techniques to characterize structural and dynamic properties of MOFs, polymeric membranes, and MMMs at the atomic scale, as well as to gain insight into the interaction with gases [3-7]. In fact, high-resolution SSNMR spectra provide information on local structure and spatial proximity between nuclei. Moreover, other nuclear observables (e.g. nuclear relaxation times and anisotropic line shapes) give unique possibilities for the study of molecular dynamics.
In this work, SSNMR is applied to investigate structural and dynamic properties of perfluorinated MOFs with high affinity towards CO_2 [8], membranes, and MMMs. Multinuclear high-resolution SSNMR experiments are carried out to study the structural properties of each material and their changes upon gas adsorption. MAS and static ^{13}C SSNMR experiments are also applied to investigate the interaction of CO_2 with MOFs and membranes.

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ROTOR DYNAMICS AND LIGHT-DRIVEN MOTORS IN NANOPOROUS ARCHITECTURES BY SOLID STATE NMR

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Keywords: solid state NMR, hyperpolarization, materials, polymers.

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Molecular rotors, motors and photo-switches in the solid state find a favourable playground in nanoporous materials, such as MOFs and POPs, thanks to their large free volume. In this field a particularly prominent application of solid state NMR is the study of dynamic processes in solids and in the gas phase. We have realized a ultra-fast molecular rotor based on bicyclo[1.1.1]pentane–dicarboxylate moiety, assembled in a cubic Zn-MOF structure. The rotors experience fast molecular reorientation even at temperature below 2 K, with a negligible activation barrier as low as 6 cal mol^{-1}, resulting from the symmetry mismatch between the tri-fold geometry of the rotor and the four-fold symmetry imposed by the frameworks [1]. Two distinct ultrafast and interacting molecular rotors can be arranged in pillar-and-layer 3D arrays MOF, wherein the rotors undergo sequential motional behaviour activated at distinct temperatures, as supported by ^{2}H solid-echo, $T_1(^{1}H)$ relaxation NMR and DFT modelling [2]. Attractive functional properties, such as dielectric switchable property, can be activated by incorporating fast-reorientable dipoles onto molecular rotors. Fluorinated Al-MOFs, comprising a wheel-shaped ligand with geminal rotating fluorine atoms, produced a benchmark dynamics of correlated dipolar rotors. Gas accessibility, shown by hyperpolarized ^{129}Xe NMR, allowed for chemical stimuli intervention: CO_2 triggered dipole reorientation, reducing their collective dynamics and stimulating a dipole configuration change in the crystal [3]. CO_2 diffusion in a porous crystalline material, in which the channels are decorated by double helices of electrostatic charges, has been described by 2D ^{1}H-^{13}C HETCOR MAS NMR experiments and by anisotropic line-shape analysis of ^{13}C, providing peculiar details about the role of electrostatic interactions in gas transport phenomena [4]. Moreover, the insertion of photo-responsive molecular motors with active dynamical behavior allowed the generation of POPs with on command responsive properties. Upon light irradiation, the quantitative molecular photoisomerization of an overcrowded alkene photoswitch has been demonstrated by ^{13}C solid state NMR [5]. The sophisticated engineering of chromophores integrated in MOF architectures enabled the fine-tuning of absorption-emission properties. Specifically, we achieved the formation of mixed-ligand photonic nanocrystals, which integrate diphenyl-anthracene moieties, thus realizing fast scintillation and large Stokes shift materials [6]. The accessibility of the pores to the gas phase under a flow of a noble gas such as Xe was demonstrated by hyperpolarized ^{129}Xe NMR and exploited to obtain visible emission stimulated by radioactive gas selective adsorption [7, 8].

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NMR STUDY OF MORPHOLOGY AND POROUS STRUCTURE OF MOLECULARLY IMPRINTED POLYMERS

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Keywords: solid state NMR, low field NMR, materials, polymers.

Molecularly Imprinted Polymers (MIP) are synthetic materials with molecular recognition properties. They have a wide variety of applications in sensing, separation, catalysis, and drug delivery. MIPs can be obtained via a non-covalent strategy by using a porogen solvent, responsible for the development of the porous structure, and by inserting a template capable of interacting with the monomers via intermolecular forces in the pre-polymerization mixture. This template-monomer complex is fixed through polymerization, and, in the end, the template is removed, leaving pores that are complementary to the template in shape, size, and chemistry.
The mechanism underlying template-monomer interactions and their role on the final recognition properties of MIPs have been widely studied in the literature. On the other hand, the influence of the degree of crosslinking on the morphology of MIPs, and the influence of morphology on the recognition properties remain relatively unexplored. To shed light on the relationship between crosslinking, morphology, and recognition properties of molecularly imprinted polymers, we studied a series of bupivacaine-templated MIPs. The employed techniques were ^{129}Xe NMR, which is an ideal technique to characterize the porous structure of complex materials [1], and ^{1}H time-domain NMR (TD-NMR) at low field, used to study the polymer chain dynamics.
In this work, we studied a series of bupivacaine-templated MIPs with variable degree of crosslinking, synthesized using toluene as a porogen. Reference, non-templated polymers with the same compositions but obtained in the absence of bupivacaine were also studied [2]. Variable temperature ^{129}Xe NMR experiments provided highly detailed information about the porous structure of the polymers. The highly crosslinked MIPs showed stable, hierarchical pore structures with a distribution of nonuniform and interconnected mesopores. On the other hand, in polymers with a low degree of crosslinking very broad distributions of pores of widely different sizes were detected. These results, coupled with ^{1}H TD-NMR, indicated that the porous structure collapsed after the removal of the template, creating a wide distribution of pores with unsatisfying recognition properties.
In this work we demonstrate the potential of ^{129}Xe NMR in studying complex hierarchical systems even in the case of soft matter. In addition to that, this work suggests the possibility of studying other soft systems, such as porous core-shell particles, polymer colloids, foams, and even biological receptors using ^{129}Xe NMR.

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INTERFERENCE OF PLATINUM DRUGS AND ZINC IONS IN COPPER TRAFFICKING HIGHLIGHTED BY NMR SPECTROSCOPY

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Keywords: solution NMR, small molecules, biomolecules. Enlightened

The ultrahigh thermodynamic stability of Cu(I)-thiolate bonds in Cu transport proteins secures the redox-active and potentially toxic Cu(I) ion, while their kinetic lability allows facile Cu(I) transfer. Dithiol motifs can also interact with Pt anticancer drugs, which are among the most used chemotherapeutics [1-3]. Cisplatin and oxaliplatin are able to interfere with the mechanism of rapid Cu(I) exchange between the CxxC motifs of metallochaperone Atox1 and the first domain of Menkes ATPase (Mnk1). The heterodimeric complex formed by the two proteins, interacting with both Cu(I) and Pt(II), was characterized by NMR spectroscopy and X-ray crystallography, thus providing the structural basis for inhibition of Cu trafficking (Fig. 1) [4]. Unlike Pt drugs, Zn(II) ions disrupt the Atox1-Cu(I)-Mnk1 heterodimer and promote the transfer of Cu(I) to Mnk1 by selectively binding to Atox1, in accordance with free energy predictions (Fig. 1) [5,6]. The interactions of Pt drugs and Zn ions with Cu transporters are also relevant, at the cellular level, for the trafficking of the Menkes ATPase between the Golgi organelle and the plasma membrane.

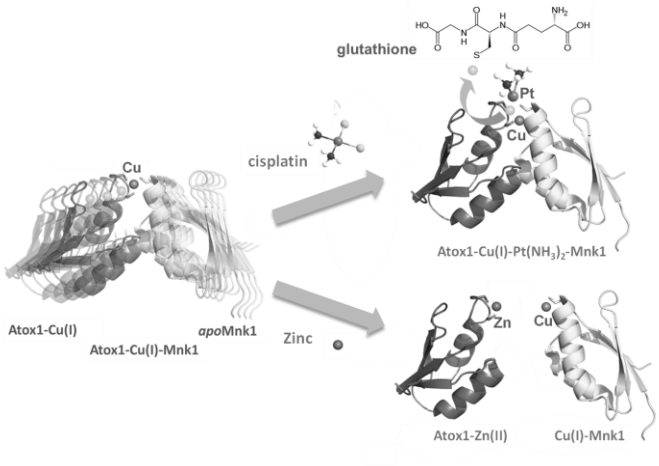

Fig. 1. Proposed mechanisms of interference of cisplatin and zinc ions in copper trafficking. (*Top right*) Inhibition of Cu(I) exchange between Atox1 and the first domain of Menkes ATPase (Mnk1) by cisplatin, which crosslinks the two proteins; the Cu(I) ion can ultimately be released to glutathione (a physiological thiol). (*Bottom right*) Selective binding to Zn(II) to Atox1 and vectorial transfer of Cu(I) to Mnk1.

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NMR-BASED INVESTIGATION OF INTRINSICALLY DISORDERED REGIONS OF MODULAR PROTEINS FOR TAILORED DESIGN OF INTERACTING PEPTIDES

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Keywords: solution NMR, biomolecules, biopolymers.

Several RNA-binding proteins (RBPs) are characterized by modular structures encompassing folded domains and intrinsically disordered regions (IDRs). Investigating the role of these domains and their possible crosstalk is crucial for understanding protein function and possible strategies to interfere with it.
The Nucleocapsid protein (N) of SARS-CoV-2 is a pivotal example of RBP. It is one of the most important proteins encoded by the virus involved in viral genome packaging and facilitating its replication inside the host cell. Interestingly, the N protein has recently been proposed as an alternative drug target because it is characterized by a low rate of mutations. Its complex structure encompasses two folded domains and three IDRs. In particular, the globular N-terminal domain (NTD) is responsible for the viral RNA interaction and the two flanking IDRs play an important synergic role [1]. The aim is the design and synthesis of peptides able to interfere with its function, monitoring the interaction through solution NMR titrations.
Five rationally designed peptide sequences have been synthesized to understand the residues and/or conformational motifs essential for the interaction with the protein target. This collection of peptides has been tested by NMR titrations (^{1}H-^{15}N HSQC experiments) to identify the sequence displaying the highest affinity with NTD. The most promising one has been finally used to monitor the interaction with the NTR protein construct, which includes two IDRs flanking the globular NTD, and to evaluate a possible enhanced interaction. The final aim is to develop de novo designed antiviral peptides able to displace the nucleic acid from the protein, to be further stabilised *in vivo* by innovative stapling synthetic strategies [2].

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A TETRACATIONIC PORPHYRIN WITH DUAL ANTI-PRION ACTIVITY

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Keywords: solution NMR, small molecules, biomolecules

Prion protein (PrP^C) is a mammalian, GPI anchored glycoprotein, expressed predominantly in the central nervous system. It has a C-terminal globular domain and a long, unstructured N-terminal region responsible for copper/zinc binding. Prion diseases (hereditary, sporadic or acquired) are deadly brain disorders for which there is not yet any cure. They arise from the conversion of PrP^C structure into a β-sheet rich, pathogenic and infectious form (PrP^{Sc}), which propagates by inducing misfolding of native PrP^C [1]. PrP^{Sc} rapidly accumulates in the brain causing neuronal dysfunction and degeneration [2]. Porphyrins are organic compounds coordinating metal cations and consisting of four pyrrole rings linked by methine groups. We discovered a tetracationic porphyrin (VA01) eliciting a unprecedented dual anti-prion effect by binding to two PrP^C regions [3]: VA01 interaction with the globular domain destabilizes it and inhibits conversion to PrP^{Sc}, while binding to the N-terminal region disrupts the intramolecular interactions occurring between the two domains and it triggers PrP^C endocytosis and lysosomal degradation, thus reducing the substrate for PrP^{Sc} generation. Elucidation of VA01 mechanism of action was possible thanks to the combined use of different biophysical techniques (NMR spectroscopy, ITC, circular dichroism, SAXS) to analyze VA01 affinity, interaction surfaces and binding effects on PrP^C structure. These data were integrated with assays *in vitro* and in prion-infected cells to evaluate VA01 anti-prion activity. Pharmacokinetic studies in VA01 treated mice indicated a ~20% PrP^C reduction in brain, despite a poor crossing of the blood-brain barrier (BBB). We are currently studying which PrP^C residues and VA01 chemical moieties are essential for VA01 binding and activity. This will allow to rationally modify VA01 to improve its pharmacokinetic properties and BBB passage, and/or to design more drug-like small molecules with the same dual anti-prion activity.

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DESIGN OF BIRADICALS FOR HIGH-FIELD AND HIGH-TEMPERATURE DNP

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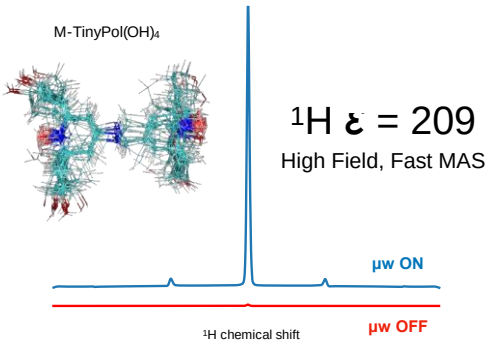
Keywords: solid state NMR, hyperpolarization, materials, biomolecules, theory and methods, instrumentation.

Dynamic Nuclear Polarization (DNP) applied to MAS solid-state NMR has proved to be a valuable technique to enhance the sensitivity of more than two orders of magnitude. The nature of the polarizing agent plays an important role in determining the mechanism and the efficiency of the hyperpolarization process. At 9.4 T, polarizing agents (PA) such as the di-nitroxides AMUPol[1] and TEKPol[2] lead to enhancement factors of up to about 250 at 100 K. Recently, the introduction stereo-controlled conformation around the nitroxide radical (like HydroPOLs [3]) that promote solvent accessibility, have shown enhancements up to about a factor 330.
Nevertheless, these excellent performances at 9.4T are strongly reduced at 18.8 T (800 MHz of ^{1}H Larmor frequency), where the design of optimal PAs is still an open problem. In 2018 we introduced hybrid hetero-biradical, like HyTEK2, which shows enhancements up to 180-200 at 18.8 and 21.3 T, respectively, with almost negligible depolarization effects, but soluble only in organic solvents.[4] In 2020 we introduced novel, water soluble, dinitroxides biradicals, named TinyPols[5]. That are expressly suited for DNP MAS NMR at high magnetic field (>14 T) and fast MAS frequencies (even up to 40 kHz and above).
Here we present a further major improvement in the radical design, optimizing a molecular geometry promoting high electron-electron (e-e) magnetic interaction, and incorporating also recent concepts such as the stereo-controlled conformation and an efficient radical-solvent interaction, showing a new family of highly performant PAs.
In particular, M-TinyPol(OH)$_4$ showed enhancements up to about 200 even at 65 kHz of MAS frequency and 18.8 T. The role of the structural improvements will be discussed, also with the help of simulations. Currently, these systems are the most efficient PAs in aqueous media at 18.8 T [6]. We also show that these biradicals, dissolved in optimized formulations, make it possible to obtain sizeable DNP enhancement even above 200 K up to room temperature [7,8]. This opens the way to new perspectives for DNP NMR.



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APPLICATION OF NMR ANALYSIS FOR MONITORING THE MALTING EFEFCT ON LEGUME SEEDS

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Keywords: solution NMR, metabolomics, food.

Malting is a steeping, germination, and kilning technique used to produce barley malt, that is used in brewing technology. However, basic aspects of malting can be applied to a wide range of plant seeds, such as legumes. Legume seeds are one of the world's most important sources of protein, fat, and energy [1]. However, legume seeds such as chickpea, lentil, beans, peas, and soybeans are difficult to process because they require a long boiling time, they are resistant to mechanical damage, and high levels of anti-nutritional factors such as phytic acid, tannins, oligosaccharides, and enzyme inhibitors are contained. Among the several nutritional and technological advantages of malting process, the reduction of anti-nutritional compounds in favor of nutritional ones has been demonstrated [2].
In the present work, 1D and 2D NMR experiments were applied to chemically characterize four legume varieties produced in Lazio region (one chickpea species, one bean species, two lentil species) and treated with malting process. Several metabolites belonging to different chemical classes in both hydroalcoholic and organic Bligh-Dyer extracts were identified. Moreover, the qualitative and quantitative changes that occurred with malting treatment allowed to observe the reduction of anti-nutritional compounds and the improvement of the legumes nutritional value.

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IMPROVED DETECTION AND QUANTIFICATION OF CYCLOPROPANE FATTY ACIDS (CPFAS) BY ^{1}H NMR SPECTROSCOPY USING A COMBINATION OF HOMONUCLEAR DECOUPLING WITH DOUBLE IRRADIATION METHODS

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Keywords: solution NMR, small molecules, metabolomics, food, theory and methods.

Cyclopropane fatty acids (CPFAs) are a class of secondary fatty acids that includes dihydrosterculic acid (DHSA), the most abundant CPFAs recently detected in milk and dairy products obtained from cows fed with maize silage. Given its importance as molecular marker for authentication of high-value dairy products (i.e., Hay Milk, PDO-labelled cheeses), implementation of more accurate and precise approaches for the detection and quantification of DHSA is required. Although NMR spectroscopy consists of an important tool for monitoring food authenticity through the identification of specific markers, this methodology applied under standard conditions suffers from low sensitivity. Here we have shown how the introduction of a simple and straight-forward homonuclear decoupling scheme on ^{1}H NMR experiments, where a single radiofrequency, RF_1, field is applied during acquisition, can enhance both SNR and the detection sensitivity of DHSA.
Among all observable signals of DHSA, the upfield-shifted resonance of the *cis*-methylene proton in the cyclopropane ring (H^C, labelled n Fig. 1) is generally picked as the target signal for quantification of DHSA, given its distinct chemical shift far from any other resonances even in complex mixtures. However, giving the peculiar chemical shift of $^1H^c$ signal (up field to the TMS signal), addition of a second "dummy" RF field (RF_2) is required to correctly quantify DHSA (Fig.1, top-right).[1] A quantitative description of how the proposed NMR scheme allows sensitivity enhancement yet accurate quantification of DHSA is provided. Previous NMR studies on CPFAs reports LOD values of ~ 0.003 mg mL^{-1} (SNR = 4) for DHSA standard solution.[2] Under similar experimental condition and by employing homonuclear decoupling we were able detect DHSA signals down to a concentration of ~ 0.001 mg mL^{-1} (SNR = 8), indeed increasing the sensitivity by 3-fold (Fig.1, bottom-right).Thus, the gain in the detection sensitivity and SNR described in this study clearly shows the effectiveness of the proposed methodology in the detection and quantification of such specific low-concentrated molecular markers found in dairy products.

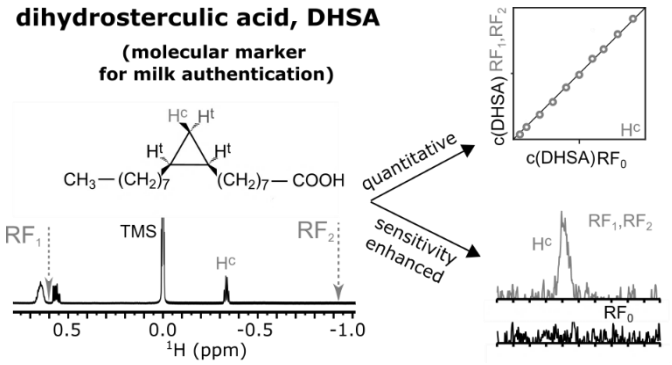

Fig. 1. Proposed NMR method for quantification and signal-enhancement of molecular marker DHSA.

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POSTERS

[P-1]

NMR-BASED COMBINATED APPROACH TO CONFORMATIONAL ANALYSIS OF TWO RGDechi-DERIVED PEPTIDES

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Keywords: solution NMR, biomolecules, Molecular Dynamics Simulations.

Integrins are a family of heterodimeric membrane receptors, composed of non-covalently associated α and β subunits [1]; their crucial role in tumor progression and metastasis formation has sparked interest to design novel pharmaceutical agents able to modulate integrins activity in order to deliver new therapeutic tools [2]. Integrins capable of binding the RGD motif such us $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$ play an important role in several tumor processes [3]. In this contest, we described the structural features of two peptides, called RGDechi1-14 and ψRGDechi, derived from RGDechi. The latter is a bi-functional flexible peptide containing a cyclic RGD penta-peptide for integrin binding that is covalently linked by a spacer to a C-term echistatin fragment conferring a high selectivity for the β3 integrin subunit [4]. To describe the molecular determinants involved in the integrin interaction mechanism, we investigated the impact of chemical modifications on the structural and dynamic characteristics of the two derived peptides. In particular, we explored the conformational space of the two derived peptides by an integrated approach combining natural-abundance NMR techniques with MD simulations data. NMR structural parameters (i.e. chemical shifts, HN temperature coefficients, coupling constants and ROEs) demonstrate that both peptides adopt a high conformational flexibility in solution without any secondary structure elements with the RGD cycle that is slightly more rigid that the C-term tail. Interestingly, in the RGDechi1-14 peptide, removal of the last five residues generates an increase in the conformational flexibility of the RGD cycle respect to the wild-type RGDechi. Overall, our data demonstrate that the flexibility of the RGD loop is driven by the C-term region of the RGDechi peptides through a coupling mechanism between the N- and C-term regions that in turns play a fundamental role in the fine tuning of the integrin binding selectivity [5].

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[P-2]

NMR AND MS-BASED METABOLOMICS OF ANTARCTIC SOILS

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Keywords: Antarctica; NMR spectroscopy; mass spectrometry; metabolites.

In Antarctica, ice-free areas can be found along the coast, on mountain peaks, and in the McMurdo Dry Valleys, where microorganisms well-adapted to harsh conditions can survive and reproduce. Metabolic analyses can shed light on the survival mechanisms of Antarctic soil communities from both coastal sites, under different plant coverage stages, and inner sites where slow-growing or dormant microorganisms, low water availability, salt accumulation, and a limited number of primary producers make metabolomic profiling difficult. Here, we report, for the first time, an efficient protocol for the extraction and the metabolic profiling of Antarctic soils based on the combination of NMR spectroscopy and mass spectrometry (MS). This approach was set up on samples harvested along different localities of Victoria Land, in continental Antarctica, devoid of or covered by differently developed biological crusts. NMR allowed for the identification of thirty metabolites (mainly sugars, amino acids, and organic acids) and the quantification of just over twenty of them. UPLC-MS analysis identified more than twenty other metabolites, in particular flavonoids, medium- and long-chain fatty acids, benzoic acid derivatives, anthracenes, and quinones. Our results highlighted the complementarity of the two analytical techniques. Moreover, we demonstrated that their combined use represents the "gold standard" for the qualitative and quantitative analysis of little-explored samples, such as those collected from Antarctic soils.

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[P-3]

BIODIVERSITY WITHIN *MELISSA OFFICINALIS* BY APPLYING NMR BASED METABOLOMICS

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Keywords: solution NMR, metabolomics, food.

Melissa officinalis is a medicinal plant belonging to the Lamiaceae family, native in the Mediterranean region. In popular tradition it is well known for its sedative, spasmolytic and carminative action [2]. The chemical composition of *M. officinalis* consists of high amounts of essential oils, triterpenes, caffeic acid-derived polyphenols such as rosmarinic acid and flavonoids [3].
Due to the variability in the production of secondary metabolites caused by multiple factors (genetics, light, temperature, humidity, altitude, nature of the soil, and cultivation), it is necessary to assess the difference in quantity of the metabolomic profile between ecotypes of the same species. The current study was aimed at characterizing the chemical profile of three different ecotypes of *M. officinalis* (*Land Spontaneous Ecotype, LSE; Mountain Spontaneous Ecotype, MSE; Organic Ecotype, OE)* by applying Nuclear Magnetic Resonance (NMR) spectroscopy in order to detect possible differences between the ecotypes of the same cultivar. Hence, the main goal has been to study the incidence of the pedoclimatic effect on the composition of primary and secondary metabolites.
The lyophilized aerial part of each ecotype was subjected to the Bligh-Dyer extraction protocol [4] to obtain hydroalcoholic extracts. Metabolomic analysis was performed through the combined interpretation of one-dimensional (^{1}H) and two-dimensional (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) NMR experiments. The ^{1}H NMR spectral assignments of the Bligh-Dyer hydroalcoholic extracts were carried out using 2D experiments and literature data of other plant matrices analyzed under the same experimental conditions [4].
Different classes of compounds, such as sugars, organic acids, free amino acids, polyphenols, and other compounds were identified and quantified. The results obtained showed the qualitative-quantitative differences of the metabolites produced between the three different ecotypes analyzed. The ecotypes were characterized by the presence of the same metabolites, albeit in different concentrations, except for *OE* lacking some metabolites detected in *MSE* and *LSE*. The rich phytochemical profile confirmed that *M. officinalis* is a medicinal plant with a considerable potential for bioactivity, as the molecules identified are well known for their antioxidant, and antimicrobial actions, along with modulating activity of metabolic biochemical processes in the body.
These results could be useful not only to delineate differences between cultivars of the same species but also to obtain a product with a chemical composition suitable for specific pharmaceutical/nutraceutical uses.

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[P-4]

RARE EARTH - BASED SINGLE ION MAGNETS: MAGNETIC PROPERTIES AND SPIN DYNAMICS

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Keywords: solid state NMR, materials, small molecules.

Single Molecule Magnets (SMMs) [1] and Single Ion Magnets (SIMs) [2], characterized by a strong uniaxial anisotropy and progressive slowing down of the magnetization, have been extensively studied in the last twenty years for their fundamental quantum properties [3] and for their possible technological applications [4]. Most of the efforts in this research field are devoted to increase the anisotropy barrier ΔE correlated to the magnetization flipping and minimize the additional relaxation mechanisms dominating at low temperature [5], for improving the performance of these molecular nanomagnets (MNMs). In this framework we are systematically investigating the spin dynamics, not yet clarified in this kind of systems [6], and the magnetic properties of three, TbTrp, DySQ and TbSQ, lanthanide - based SIMs ((Ln(Trp)$(HBPz_3)_2$ (Ln = Tb); Ln(DTBSQ)$(HBPz_3)_2$ (Ln =Tb, Dy)) [7] as a function of temperature and magnetic field applied. The used experimental techniques are DC and AC susceptibility measurements, ^{1}H NMR measurements (absorption spectra, spin – lattice (T_1) and spin-spin (T_2) nuclear relaxation times) and muon spin resonance μ^+SR measurements.
Our investigation is allowing us to point out how the substitution of the magnetic centre ((Tb^{3+} and Dy^{3+}) bound to a paramagnetic (semiquinone) or diamagnetic (tropolone) radical ligand could influence their properties due to different magnetic anisotropy, crystal field and Ln - radical ligands interaction.

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[P-5]

ISOTOPE EFFECTS IN SITE SELECTIVELY DEUTERATED FATTY ACIDS DERIVATIVES

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Keywords: solution NMR, small molecules, biomolecules.

The palmitic acid ester of (*R*)-9-hydroxy stearic acid ((*R*)-9-PAHSA) is one of the most abundant fatty acid ester of hydroxy fatty acids (FAHFA) [1], a new class of lipids discovered in 2014, in adipose tissue. Isotopologue molecules, which can be easily singled out from the natural pool by mass spectrometry, can be very useful to gain insight into the mechanism of action of PAHSA. We prepared the 7,8-d_2-9-PAHSA starting from the parent unsaturated ester, obtained through olefin metathesis, by reductive deuteration in a flow system, which greatly simplifies the experimental work, allowing to use cheap D_2O for the generation of D_2 gas [2].
The selectivity of the reaction was confirmed by means of ^1H and ^{13}C NMR.

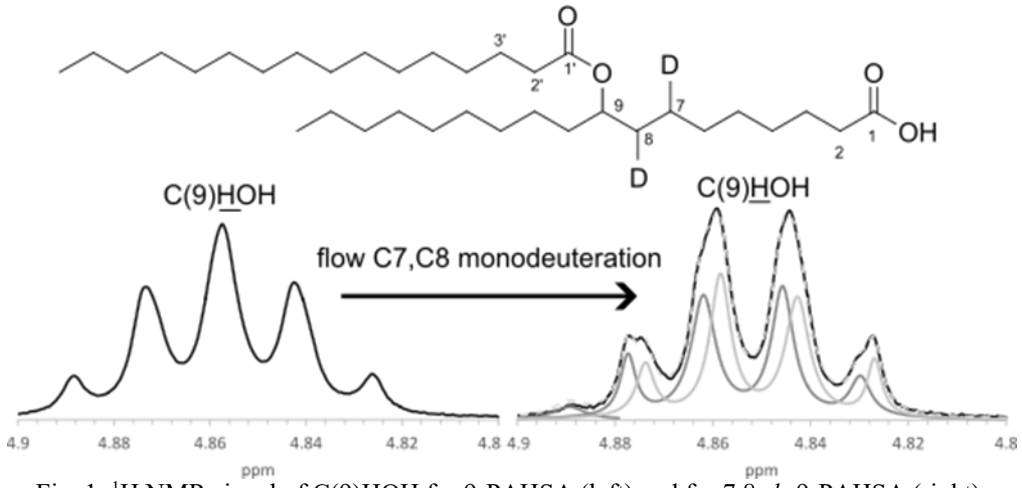

Fig. 1. ^1H NMR signal of C(9)HOH for 9-PAHSA (left) and for 7,8-d_2-9-PAHSA (right).

The most evident effect in the ^1H NMR spectrum is the reduction of the multiplicity of the signal of C(9)H, which changes from a quintet into a quartet, owing to the vicinal monodeuterated C(8). Actually, the signal of the C(9)H for 7,8-d_2-9-PAHSA is the sum of two quartets, a few ppb apart, relevant to different diastereomers, possessing the molecule three consecutive chiral centers, namely C(9), C(8) and C(7).
In the ^{13}C NMR spectrum the C(7) and C(8) singlets disappear, while at about 500 ppb higher field, in line with the expected isotope shift [3], two triplets can be detected, which, coherently, give rise to CH cross-peaks in multiplicity edited HSQC 2D maps.

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[P-6]

MONITORING OF COPPER CHAPERONE ATOX1 INHIBITION BY DC_AC50 USING NMR SPECTROSCOPY AND MOLECULAR DOCKING STUDIES

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Keywords: solution NMR, small molecules, biomolecules.

The interaction between the metallochaperone Atox1 and the ion pump Atp7a (related to Menkes syndrome) is essential for copper transport in human cells and for its detoxification, but some anticancer drugs are able to interfere with this process [1]. In particular, the chemotherapeutic drug cisplatin is able to stabilize the Atox1-Cu(I)-Mnk1 heterodimeric complex by forming interprotein cross-links between Atox1 and Mnk1 (the N-terminal domain of Atp7a), which underlie a possible mechanism of drug resistance [2]. In this work, a molecular study was performed of the effect on copper transport caused by the Atox1 inhibitor DC_AC50 [3] (Fig. 1), whose activity is aimed at preventing the interaction and exchange of copper between Atox1 and Mnk1. The combined effect of cisplatin and DC_AC50 was also investigated, which makes it possible to resensitize cancer cells to cisplatin treatment by promoting the intracellular accumulation of the drug [4].

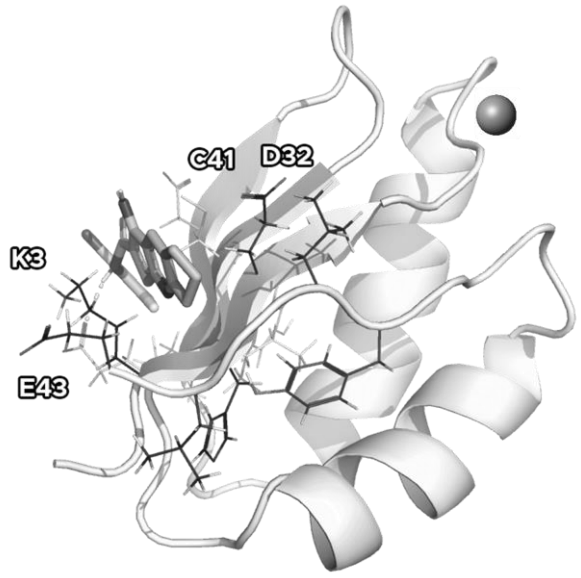

Fig. 1. Docking model of DC_AC 50 bound to Atox1 obtained with the program AutoDock based on NMR data. The Cu(I) ion is shown as a gray sphere. The protein residues in contact with the inhibitor are indicated.

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[P-7]

EXPRESSION OF NUCLEOCAPSID PROTEIN (N) FROM SARS-CoV 2 AND ITS CHARACTERIZATION THROUGH HIGH-FIELD NMR SPECTROSCOPY

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Keywords: solution NMR, small molecules, biomolecules.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) is responsible for one of the most significant global public health crises of our century. One of the structural proteins of coronavirus 2, the N protein, appears to be genetically stable, making it an excellent candidate for the development of antiviral drugs. N is the most highly expressed of the four structural proteins of the virus and its main role is to organize the ribonucleo-protein (RNP) complex formed by the interaction of N with the genomic RNA. N is a multi-domain protein composed by 419 amino acids. It is organized into an N-terminal RNA-binding domain (NTD), a C-terminal dimerization domain (CTD) and three intrinsically disordered regions (IDR1, IDR2 and IDR3) that comprise almost 40 per cent of the protein's primary sequence [1]. Our central goal is to characterize the Full Length (FL) N protein and to study its interaction with RNA in its entirety. We want to elucidate how the interaction with RNA changes as the complexity of the system increases, moving from single RNA binding domain (44-180) to the FL (1-419) also considering a construct that comprises the NTD and the flanking IDRs (NTR 1-248). Indeed, studies conducted on NTR revealed that intrinsically disordered regions play an important role in the interaction of N with RNA [2]. Nuclear magnetic resonance spectroscopy (NMR), with the support of other biophysical techniques, can provide the information needed to study the disordered components of the protein.
In particular, to study modular proteins with intrinsically disordered regions, ^{13}C detection is a key technique. ^{13}C-NMR provides a wide chemical shift dispersion, which is crucial for obtaining highly resolved spectra [3]. Furthermore, this technique overcomes the solvent exchange problem for amide proton signals when approaching physiological conditions. To study systems with very different structural and dynamic properties, composed of globular domains and disordered regions, it is necessary to filter out the resonance of the globular domains. Indeed, the flexibility of IDRs allows us to visualize them by NMR even in the context of a large protein complex. Thanks to these experiments, it was possible to analyze the differences in the IDRs between the entire N protein and the previously studied constructs (NTR, NTD).

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[P-8]

THE KETOPROFEN-LYSINE PHARMACEUTICAL SYSTEM: SSNMR APPLIED TO THE CRYSTAL-FORM LANDSCAPE OF A COMMON NON-STEROIDAL ANTI-INFLAMMATORY DRUG

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Keywords: solid state NMR, materials, small molecules.

When the impossibility of obtaining suitable crystals for X-ray structure determination arises, solid-state NMR (SSNMR) proves a valuable ally in exploring the structural features of molecular crystalline materials. In this work, the technique was applied to some crystal forms of ketoprofen. In particular, in the world of nonsteroidal anti-inflammatory drugs (NSAIDs), the ketoprofen-lysine (KLS) system is well-known and widely used [1,2].
The solid-state characteristics of KLS were deeply investigated to possibly identify new polymorphs and new multicomponent crystal forms of KLS. By means of 1D and 2D SSNMR it was possible to highlight how the commercial form of KLS, commonly referred to as a molecular salt, is indeed a cocrystal (KET-LYS P1), and to recognize a newly discovered polymorph of KLS (KET-LYS P2) as a proper salt, revealing the existence of a rare case of salt/cocrystal polymorphism [3].
The exploration of the crystal-form landscape of the KLS pharmaceutical system continued with the obtainment of a novel promising adduct, comprising of ketoprofen, lysine and gabapentin (KLS-GABA) [4]. Once again, SSNMR proved a powerful tool for shedding light on the structural features of the adduct. The main results of the SSNMR approach consisted in the assessment of the salt cocrystal nature of the adduct, as well as the identification of a peculiar supramolecular fragment involving lysine and gabapentin, which turned out to recur in similar ternary systems. Later, the crystal structure of KLS-GABA was solved starting from powder data, which confirmed what was observed through 1D and 2D SSNMR experiments.
Further pharmacological characterization revealed astounding results for KLS-GABA in terms of therapeutic efficacy against pain and highly reduced side-effects, related to common NSAID-induced gastrolesivity.

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[P-9]

INSIDE INNOVATIVE MATERIALS FOR OSTEOSARCOMA TREATMENT THROUGH SOLID STATE NMR

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Keywords: solid state NMR, materials.

Bone cancer and bone metastases are usually associated with severe bone pain and osteolysis, the latter being also accompanied by increased bone fragility and susceptibility to fracture. Nowadays, chemotherapy and/or radiotherapy represent the main treatments able to block the metastases progression. However, these treatments inhibit cell division without distinction between healthy and cancer cells, thus inducing many side effects in patients. Hence the need for developing innovative treatments able to inhibit metastases progression and, at the same time, to promote the formation of new tissue. In this contribution we present innovative organic-inorganic hybrid injectable materials designed to have the dual function of inhibiting cancer cells proliferation and inducing new bone tissue formation/mineralization [1, 2]. In particular we will show the results of a multinuclear Solid State Nuclear Magnetic Resonance investigation [3, 4] that allowed the phase behaviour and the structural features of the hybrid materials, before and after mineralization, to be characterized in detail.

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[P-10]

STRUCTURE AND FREE CARRIER PARAMETERS IN ITO NANOCRYSTALS PROBED BY SOLID STATE NMR AND OPTICAL SPECTROSCOPY

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Keywords: solid state NMR, materials.

In the last decades, plasmonic nanocrystals (NCs) have been subject of intense research due to their strong optical response. Indeed, the electric field of light induces a coherent oscillation of conduction electrons, known as "Local Surface Plasmon Resonance" (LSPR), which leads to a strong absorption peak typically in the UV-Vis – near IR (NIR) regions [1,2]. Among plasmonic NCs, degenerately doped *semiconductors* are particularly interesting for infrared plasmonics. In these NCs conduction electrons are generated by introducing aliovalent dopants. Among this class of materials, Tin-doped Indium Oxide (ITO) is an n-type semiconductor in which the aliovalent doping consists in the partial substitution of In^{3+} cations in the bixbyite In_2O_3 crystal structure with Sn^{4+}. ITO NCs show a resonant peak in the NIR region, tunable by varying the dopant content (Sn%) [3]. Thanks to their infrared plasmonic properties, ITO NCs have been proposed in many different fields such as magnetoplasmonics and smart materials activated by NIR light [4,5]. The correlation between free electron parameters and the presence of dopant-related structural defects is an important task in the rationalization of the optical properties of these materials, and it is still far from being completely understood. Solid State NMR (SSNMR) appears particularly attractive for this scope [6-8], as the presence of free electrons affects several NMR properties of the nuclei. In this work, we present a SSNMR investigation on ITO NCs stabilized with oleic acid, containing an increasing amount of Sn. ^{119}Sn SSNMR spectra and spin-counting experiments allowed us to identify different Sn species, correlated with different electronic properties. Further information was obtained by measuring ^{119}Sn spin-lattice relaxation times (T_1) at different temperatures. Optical and magneto-optical spectroscopies were also employed to extract free electrons parameters and correlate the parameter obtained with the structural information provided by SSNMR.

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[P-11]

LEUCOPTERIN, A SMALL BUT COMPLEX MOLECULE: CRYSTAL STRUCTURE AND TAUTOMERIC STATE ELUCIDATION BY X-RAY DIFFRACTION AND SOLID-STATE NMR

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Keywords: solid state NMR, small molecules.

Leucopterin (LCPT), $C_6H_5N_5O_3$, a member of the class of pteridines, is the white pigment in the wings of *Pieris brassicae* butterflies (Fig. 1) [1]. At ambient conditions, LCPT crystallises as a hemihydrate. The crystal structure of the hemihydrate was solved by single-crystal XRD, but hydrogen atoms positions were still uncertain. Furthermore, LCPT is characterised by tautomerism: indeed, at least 17 possible tautomers can exist. Through the combination of diffraction data with solid-state NMR (SSNMR) and DFT-D calculations, the correct tautomeric state was elucidated. In particular, multinuclear high-resolution SSNMR experiments were performed.
Variable contact time ^{15}N CPMAS spectra proved the presence of one NH_2 and three NH groups, and one unprotonated N atom, which agreed with the ^{1}H MAS and ^{13}C CPMAS spectra. This information reduced the number of possible tautomers to only 2. The final tautomeric state was assessed analysing ^{1}H-^{1}H atom proximities in the ^{1}H DQ MAS 2D spectrum [2]. The results of lattice energy minimisations with DFT-D, performed independently of SSNMR, on the 17 most chemically reasonable tautomeric forms, were in agreement.
This work [3] represents an elegant way to prove the pivotal role of SSNMR in the elucidation of structural features that intimately define a material, with possible repercussions on its macroscopic physicochemical properties.

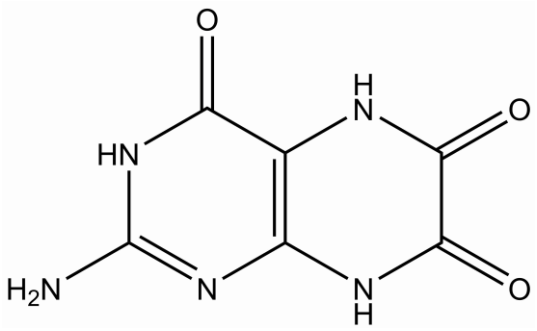

Fig. 1. Molecular structure of leucopterin.

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[P-12]

STUDY OF THE REGIOISOMERIC DISTRIBUTION OF FATTY ACIDS IN COCOA BUTTER AND ITS DERIVATIVES USING NMR SPECTROSCOPY

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Keywords: solution NMR, low field NMR, metabolomics, food.

Food industry, especially in the last few years, has become increasingly involved in researching vegetable fats and oils with appropriate mechanical properties (ease of transport, processing, and storage) and a specific Fatty Acids (FAs) composition to ensure healthy products for consumers. However, the chemical composition of these matrices is not the only responsible for their particular chemical-physical behavior and not enough to ensure a specific metabolic digestion and absorption in the human body [1,2]. These properties, as demonstrated here, are largely determined not only by the compositions in terms FAs but are also by their regiosomerism within the TriAcylGlycerols (TAG) moieties (*sn*-1,2,3 positions). The main purpose of this work is to investigate natural Cocoa Butter (CB) and three modified vegetable fats obtained directly from this one using an unconventional process that manipulates only the distribution of FAs but not their nature. To this end, NMR spectroscopy proved to be a useful tool to understand at a molecular level which factors could be responsible for an improved chemical–physical behavior of modified sample and in particular: i) ^{1}H and ^{13}C NMR (1D and 2D) spectroscopy to analyze the regioisomeric distribution of FAs on the glycerol (using ^{13}C NMR) as an alternative to the GC-Mass techniques routinely employed for the this type of investigation [3]; ii) TD-NMR experiments to measure nuclear-spin transverse (T_2) relaxation times [4,5] that could give some structural informations about the modified samples as the complexity of the system increases. In addition, other techniques (DSC, rheological testing, and Powder X-Ray Diffraction (PXRD)) were used for a complete investigation of the chemical-physical properties of CB and its derivatives as support for NMR experimental data.

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[P-13]

CHARACTERIZATION *via* NMR AND REUSE OF LEAVES AND WASTEWATERS (OMWs) FROM OLIVE OIL PRODUCTION CHAIN

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Keywords: solution NMR, small molecules, metabolomics.

Every year, food industrial activities generate millions of tons of wastes or by-products during food processing and today destined only for disposal on agricultural land. Therefore, correct management of waste is necessary to protect the ecosystem. Olive mill wastewaters (OMWs), essentially consisting of process waters and the aqueous fraction of squeezed olives, are always considered a highly polluting agri-food waste to be disposed as special. Therefore, OMWs as well as other waste materials such as leaves derived from the olive oil production process, are very abounding with bioactive natural with a high economic value as phenolic compounds and amino acids which exhibit a broad spectrum of bioactivities, including antioxidant, anti-inflammatory, antimicrobial, antihypertensive, hypoglycemic, cardioprotective activities and very interesting for the cosmetical, pharmaceutical and food industry [1,2]. Nuclear Magnetic Resonance (NMR) Spectroscopy, employed in this work for the metabolic analysis of OMWs and leaves, is an alternative technique to the traditional used in the agri-food industry to investigate the composition of these waste materials and to evaluate the recovery and the preservation of the bioactive metabolites if the original matrix is treated in different ways. In particular for the identifications of metabolites presents in OMWs and olive leaves, high resolution NMR experiments have been used including routine and also more complex 1D and 2D pulse sequences such as: ^{1}H NMR, decupled ^{13}C- {^{1}H} NMR, correlation 2D experiments ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{1}H-^{1}H *J*-Res.
Indeed, if properly managed, in the perspective of a circular economy, these matrices could be classified as secondary raw materials providing both environmental and economic advantages [3]. The enhancement of the by-products rich in bioactive compounds with high potential could have several interesting fields including their application in active packaging film including plant-based polymers (e.g., cellulose, starch) or use of bio-based additive derived from olive leaf residue as a modifier and antioxidant agent for bitumen. Indeed, as demonstrated from NMR experimental data coupled with rheological measurement, the composition in terms of phenol, chlorophyll, lignin, and cellulose content was directly correlated with the mechanical properties of the tested samples.

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[P14]

IDENTIFICATION OF METABOLITES PREDICTIVE OF OUTCOME FOR PATIENTS WITH SEVERE SARS-COV-2 INFECTION SHOWED SIMILARITY WITH CANCER

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Keywords: metabolomics.

SARS-CoV-2 infection is characterized by several clinical manifestations, ranging from the absence of symptoms to severe forms that necessitate intensive care treatment [1]. It is known that this infection induces modifications in host metabolism, including pathways related to amino acids, energy generation, and lipids, leading to metabolic reprogramming, which is closely linked to metabolic changes in cancer [2]. We evaluated untargeted plasma metabolomics profiling using a Bruker Avance 600 NMR spectrometer operated at a 599.97 MHz ^{1}H resonance frequency and equipped with a cryoprobe in a training set of patients with severe SARS-CoV-2 infection classified on the basis of their outcome: the “Exitus”, which comprised patients who died during infection, and the “Good Prognosis”, which comprised patients who recovered from COVID-19. Univariate analysis and Kaplan-Meier curves related to hospitalization time showed that lower levels of several metabolites such as 3-hydroxybutyrate ($p=0.012$), lactate ($p=0.0078$), leucine ($p=0.042$) and phenylalanine ($p=0.0022$), correlated with a good outcome in these patients and these data were confirmed in a validation set of patients with similar characteristics. However, after the multivariate analysis, only lactate and phenylalanine retained a significant prediction of survival. Finally, the combined analysis of lactate and phenylalanine levels correctly predicted the outcome of 83.3% of patients in both the training and the validation set. Finally, we highlighted that the metabolites involved in COVID-19 patients' poor outcomes are similar to those responsible for cancer development and progression, suggesting the possibility of targeting them by repurposing anticancer drugs as a therapeutic strategy against severe SARS-CoV-2 infection [3].

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[P-15]

NEW FLUORINATED LIPOSOMES FOR ^{19}F-MRI ACQUISITION

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Keywords: MRI, biomolecules, contrast agents.

^{19}F MRI is an imaging modality that relies on the NMR properties of the fluorine-19 isotope. Despite the great NMR properties [1], ^{19}F has low sensitivity and signal to noise ratio. For this study, we formulated liposomes entrapping hexafluorophosphate (PF_6^-) solutions at different concentrations using the thin-film hydration technique and we evaluated the relaxometric characteristics of the resulting systems using a Bruker Avance 7T MRI scanner. T_1 and T_2 relaxation times were determined using the IR and the CPMG sequences.
T_1 and T_2 relaxation times of free PF_6^- solutions were high and not affected by changes in concentrations, while in liposomes we observed low T_1 and T_2 relaxation times varying accordingly to the concentration of PF_6^- (Fig. 1 a, b). T_1 and T_2 measurements were also performed on liposomes before and after US-induced release of PF_6^- (Fig. 1 c, d). Compared to T_1 and T_2 of the solution before release, after the release of PF_6^- there is a great increase in the relaxation times. This opens the possibility to use the systems as reporters of drug delivery and release. Experiments to assess the feasibility of this hypothesis are now being carried out.

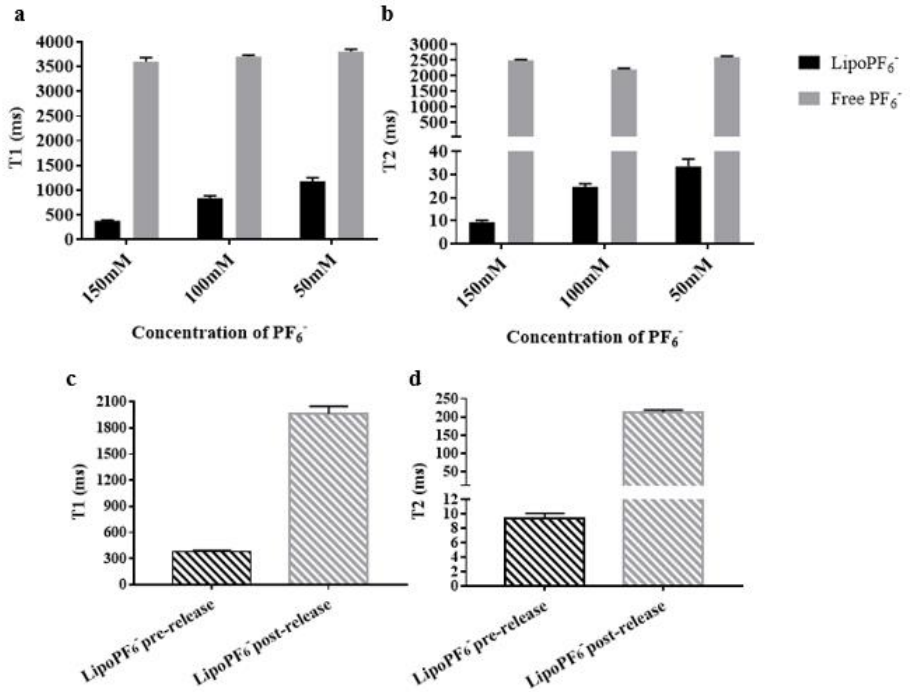

Fig. 1. T_1 and T_2 of the systems at different concentrations of PF_6^- (a, b) and pre- and after release with US (c, d)

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[P-16]

BIOCOMPATIBLE HYPERPOLARIZED [1-^{13}C]PYRUVATE FOR METABOLIC INVESTIGATION IN MAGNETIC RESONANCE IMAGING

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Keywords: MRI, hyperpolarization, contrast agents.

MRI is a powerful tool for the non-invasive detection of metabolic changes related to many diseases (such as cancer) both in cell cultures and living systems. The main limitation for the application of this technique is its intrinsic low sensitivity, which makes the metabolites almost undetectable. For this reason, in the last decades a method termed hyperpolarization has been used to obtain metabolites with more intense signals that can be injected on cells or in vivo to follow the metabolism. In particular, the PHIP-SAH method [1] allows to obtain hyperpolarized (HP) [1-^{13}C]pyruvate in a fast, simple and cheap way. This method relies on the presence of an organic phase (chloroform), in which is carried out the para-hydrogenation of a pyruvate precursor, and an aqueous phase in which, after the hydrolysis, the hyperpolarized pyruvate is extracted. Due to the presence of the chloroform during the reaction, some solvent and metal traces (deriving from the catalyst used in the hydrogenation step) can still be present in the HP water solution, affecting its biocompatibility. To overcome this issue, the final solution was filtered with a lipophilic resin to remove traces of chloroform, resulting in a fully biocompatible HP product with unchanged polarization. This final hyperpolarized [1-^{13}C]pyruvate was therefore used for in vivo metabolic studies, in which its presence and conversion in [1-^{13}C]lactate was detected.

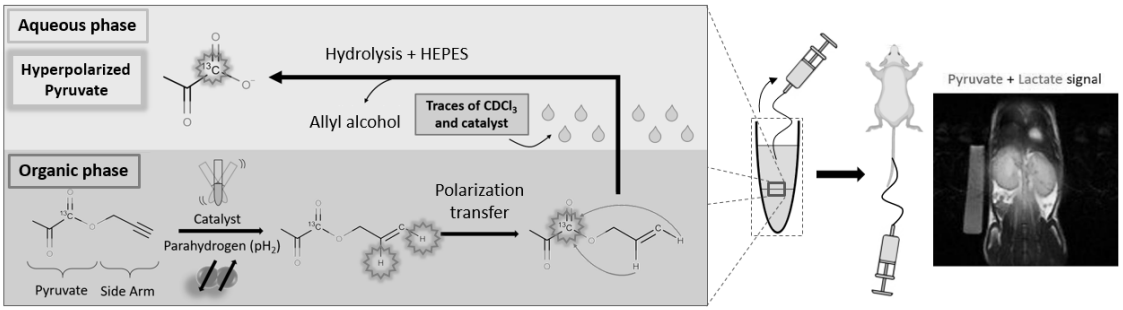

Fig. 1 Hyperpolarization process

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[P-17]

METAL BINDING AFFINITY AND SECONDARY STRUCTURE CONTENT OF ROS87-C27D

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Keywords: aspartate, zinc fingers, metal binding domain, dissociation constant, secondary structure.

In eukaryotic organisms, Cys_2His_2 zinc finger proteins coordinate metal ion with tetrahedral geometry, essential for the stabilization of the classical ββα structure. As a model of Cys_2His_2 zinc finger in prokaryotic proteins, Ros, a transcriptional regulator found in *Agrobacterium tumefaciens*, was largely used. The NMR structure of Ros87[1], the DNA binding portion composed by 87 amino acids, shows a globular domain with a βββαα structure, that appears bigger of the eukaryotic one. Also, in this case the binding of the zinc ion is fundamental for the correct fold.
Interestingly, the sequences of several prokaryotic Ros homologues have the second cysteine replaced by an aspartate rising questions about the role of this amino acid in the coordination and in the evolution of this domain from prokaryotes to eukaryotes or vice versa. In previous studies, it was demonstrated that the presence of aspartate in Ros87 mutant, named C27D, shows just a light effect on the functional structure of the protein while it influences its thermodynamical properties [2]. Furthermore, when in the wild-type protein the zinc is replaced by cadmium or cobalt, Ros87 keeps unchanged its functional structure.
Here, we explore the role of aspartic acid in zinc fingers metal coordination by replacing the native zinc ion in Ros87-C27D with cadmium and cobalt, evaluating the metal binding affinity by means of UV-Vis Spectroscopy. In particular, dissociation constants of the metal ion-protein complexes are measured and the effect of metal binding on the protein fold evaluated by means of Circular Dichroism and NMR.

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[P-18]

A NOVEL NMR-BASED METHODOLOGY TO SCREEN ULTRA-LOW MOLECULAR WEIGHT FRAGMENTS: APPLICATION TO THE ANTIAPOPTOTIC PROTEIN HUMAN BFL-1.

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Keywords: solution NMR, small molecules, biomolecules, theory and methods.

One of the approaches which have gained popularity with the flagship of improving productivity is Fragment-Based Lead Discovery (FBLD). The key concept of FBLD is the identification of one or multiple small organic molecules named fragments even with a weak affinity, but which chemical growing or combination leads to a potent binder. The combination of fragments leads to the additive sum of their molecular weights but, notably, their affinity often follows a *quasi*-multiplicative growth [1].
Recently, it has been proposed the use of a novel class of fragments characterized by an ultra-low-molecular-weight; they are formed by at most 7-8 heavy atoms and one ring [2]. Such class of fragments presents a low chemical complexity that offers the notable advantage that they could be a better probe for protein hot spots. Moreover, since multiple low-complexity fragments are expected to bind proximal protein pockets the chemical space explored in a fragment screening is remarkably large even if a small set of fragments is used. On the contrary, due to their very low chemical complexity, these fragments bind to the target with very low affinity requiring reliable biophysical methods to be detected [3]. The first successful attempt in this field was reported in 2019 using a set of 81 fragments, named MiniFrag, and screening by X-ray Crystallography [2]. This achievement has attracted a notable interest in the field opening a new paradigm in FBLD. We extended the use of this novel class of fragments to the other gold standard technique for fragment-based screening: Nuclear Magnetic Resonance. Here we reported a robust NMR protocol, not yet reported in the literature, to detect and analyze such challenging interactions for a real scenario work useful for flexible systems or targets, which are difficult to crystallize. To do that we used a library of around 100 highly soluble ultra-low-molecular-weight fragments and the antiapoptotic protein *human* Bfl-1 as a case study.

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[P-19]

DECODING BRAIN ACTIVITY INTO MEANINGFUL TEXT AND IMAGES FROM FMRI USING DEEP LEARNING

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Keywords: MRI, theory and methods, exotica.

In the field of neuroscience, machine learning algorithms have driven remarkable progress and transformative applications. One intriguing avenue is the conversion of fMRI data into interpretable formats like text or images [1, 2]. In this study, we present a novel approach using a deep learning paradigm called Generative Image Transformer (GIT) to convert fMRI activity into textual descriptions of scenes. By combining GIT with a latent diffusion model, we can reconstruct realistic images from brain activity. We utilized the Natural Scenes Dataset [3], which consists of 7T fMRI data from eight subjects exposed to approximately 9000 natural images from the COCO image dataset. The processed fMRI data underwent a General Linear Model (GLM) analysis across all diverse stimuli, which led to a selection of a Region of Interest (ROI) comprising approximately 15000 voxels. Instead of using natural images as input, we employed a pretrained image captioning model as a proxy for brain activity. Through training a linear model with regularization, we predicted the latent space of this pretrained model using fMRI data, while keeping the deep learning parameters frozen. Our GIT-based architecture was designed to accept fMRI data from a subject who viewed a specific scene and decode this data into a textual descriptor of the perceived scene. Our approach, leveraging the capabilities of the transformer architecture, can provide a paradigm shift in the comprehension and interpretation of neuroimaging data. Additionally, by incorporating a latent diffusion model, we were able to reconstruct images from brain activity, i.e. "decode" the stimulus the subject had been exposed to from physiological information only. Figure 1 provides examples of the decoded text and corresponding reconstructed images. The results of our study hold promising implications for the neuroscience community, as they expand the horizons of subjective experience decoding and offer new avenues for obtaining neuroscientific insights from fMRI studies. In this study, we introduced a pioneering strategy for converting fMRI activity into textual descriptions of scenes using a GIT-centered deep learning paradigm. By training a linear model to predict the latent space of a pretrained image captioning model from fMRI data, we demonstrated the potential of decoding subjective experiences from brain activity. The integration of a latent diffusion model further allowed us to reconstruct images from the same brain activity. Our findings not only contribute to the field of neuroscience but also highlight the transformative power of machine learning algorithms in unlocking new knowledge from fMRI data.

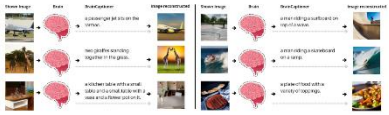

Figure 1. Representative examples of our experiment outcomes. Displayed are test images (left column), used as stimuli during the fMRI experiment. The 'BrainCaptioner' (middle column) denotes the textual description of the perceived scene, as inferred from the corresponding brain activity using our proposed method. The 'Image Reconstructed' column (right) illustrates images reconstructed solely from the associated fMRI data, utilizing the estimated textual description and additional features derived from the brain activity.

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[P-20]

STRUCTURAL CHARACTERIZATION OF THE INTERACTION OF GP36 PEPTIDE FOR ANTI-FIV TARGET

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Keywords: solution NMR, biomolecules, glycoproteins, FIV, structural characterization, molecular dynamics.

The envelope glycoproteins gp36 (in FIV) and gp41 (in HIV) play a crucial role in facilitating the fusion of the virus envelope with the host cell membranes. Both proteins share a similar framework, consisting of the fusion peptide (FP), N-terminal heptad repeat (NHR), C-terminal heptad repeat (CHR), and membrane-proximal extracellular region (MPER) [1]. The MPER region, characterized by hydrophobic and Trp-rich nature, exhibits a significant affinity for bio membrane [2]. The virus entry process involves the folding back of NHR and CHR to form a stable six-helical bundle (6HB) with low energy, allowing effective fusion between the virus and host cell membranes [3]. In a previous study, we identified C8, a peptide derived from Gp36 MPER, which displayed significant anti-FIV activity in vitro and in vivo [4]. Here we report the NMR-based conformational study of C20, a peptide corresponding to the gp36 NHR sequence [5] which inhibited C8 activity in a previous study. Our data demonstrate that C20 has a preference for adopting a regular α-helix conformation in the central part of the sequence. Molecular dynamics simulations performed in explicit water using GROMACS [6] and CHARMM 36 force field [7], revealed that C20 forms a stable structural complex with the NMR structure of CHR-MPER fragment previously solved by us [8]. CHR-MPER/C20 complex is stabilized by a high number of interactions, mainly involving the CHR portion. Notably, analysis of these interactions suggests that C20, replicates the biological conditions observed in the formation of the hairpin-like conformation in the 6HB.

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[P-21]

INSIGHTS ON THE EXTRAORDINARY SELF-ASSEMBLY OF FAMPRIDINE BY MULTINUCLEAR NMR

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Keywords: solid state NMR, solution NMR, small molecules.

Small organic molecules generally form simple supramolecular arrangements, producing crystal structures with relatively small unit cells. However, we have recently isolated four new crystalline phases (**1**-**4**) of fampridine hydrochloride (4-APH$^+$Cl$^-$), a simple organic compound whose crystalline phases can adopt an incredibly complex self-assembly.[1,2]
Interestingly, **1** and **2** represent the first observation of Frank-Kasper (FK) phases in small organic systems. FK is a remarkable class of crystalline phases previously observed only in metal alloys [3] and different types of supramolecular soft matter. The two FK structures crystallised from a dense liquid phase (DLP) obtained after liquid-liquid phase separation.
To understand how such a simple molecule may crystallise as an FK phase, we monitored the DLP precursor of complex phase **1** and the aqueous precursor of simple phase **3** by liquid-state NMR. ^{1}H, ^{13}C, ^{14}N and ^{35}Cl NMR experiments were carried out as a function of the concentration of 4-APH$^+$Cl$^-$ until the precipitation of the crystalline phases occurred. The results are compared to Molecular Dynamics simulations of DLP, investigating the potential solute/solvents interactions. In addition, these phases were studied also by ^{13}C solid-state NMR, showing the potential of NMR to explore such complex crystalline phases.[2]

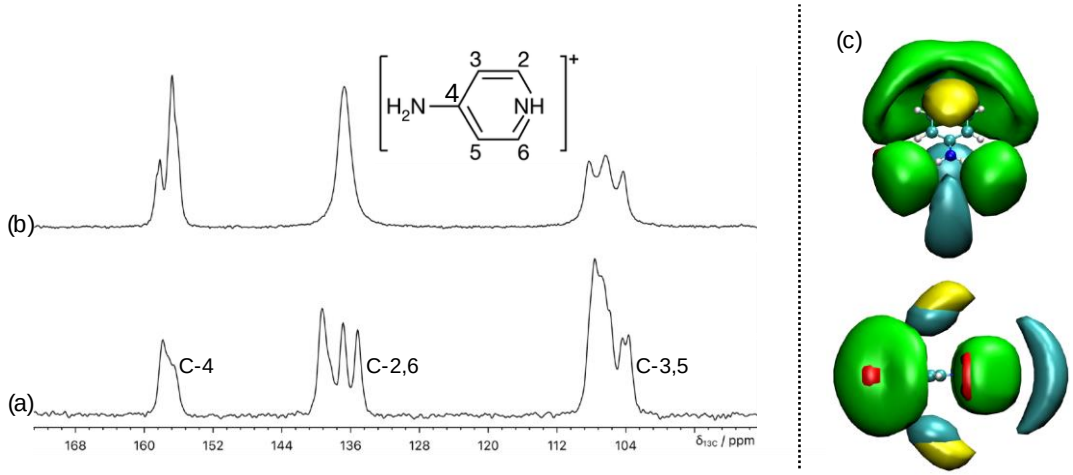

Fig. 1. ^{13}CTOSS CP-MAS NMR spectra of 4APH$^+$Cl$^-$: (a) phase **3** and (b) phase **1**. (c) Spatial distribution functions around 4APH$^+$ cation, shown for the Cl$^-$ (green), water (red), acetone (yellow), and 4APH+ (cyan).

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[P-22]

NMR METABOLOMIC STUDY OF A BREAD-MAKING PROCESS: FROM RAW MATERIAL TO BAKERY GOODS

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Keywords: solution NMR, small molecules, metabolomics, food.

Bread and bakery goods are undeniably among the most consumed food items worldwide. Consumers often opt to purchase them due to their hectic schedules, leaving them no time for cooking, and because of bread's flexibility as a side dish and primary energy source [1]. The bread-making process may seem simple as it involves just a few ingredients, but it has more chemistry than meets the eye. Raw materials and manufacturing/storing steps may affect chemical reactions, which impact the final product's quality [3]. In this work, the metabolomic pathway of baked goods was studied through High-Resolution Nuclear Magnetic Resonance to evaluate alterations in nutrient composition from breeding to processing. The focus of the research is centered on a type of bread made by an Apulian industry with innovative techniques to preserve its genuineness. Deuterium oxide (D_2O) and Ultrasound-Assisted Extraction (UAE) were adopted to capture polar metabolites from food matrices. NMR was initially used to characterize the raw material (flour), the intermediate products (doughs at different leavening times) and, in the end, the final innovative product. Multivariate statistical analysis, particularly PCA, was used to rationalize NMR spectra and follow the production process from a metabolomic point of view. Moreover, always recording NMR experiments coupled with chemometric tools, the innovative bread was compared with a fresh and a frozen product to further asses the nutritional and commercial potential of the groundbreaking product.

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[P-23]

BINDING ADAPTATION OF GS-441524 IN VARIOUS MACRO DOMAINS AND ITS ROLE IN INHIBITING SARS-COV-2 MACRO DOMAIN

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Keywords: solution NMR, small molecules, biomolecules.

Viral infection in cells triggers a cascade of molecular defense mechanisms to maintain host-cell homeostasis. One of these mechanisms is ADP-ribosylation, a fundamental post-translational modification characterized by the addition of ADP-ribose (ADPr) on both human and various viral substrates.
Some viral families contain structural motifs called macro domains (MDs) that can reverse this post-translational modification. MDs are evolutionarily conserved protein domains found in all kingdoms of life and they are generally divided into different classes with the viral MDs belonging mainly to Macro-D-type class.
Viral MDs are potential pharmaceutical targets since they are capable of counteracting the host immune response by reversing the Poly(ADP-ribose) polymerases' mediated ADP-ribosylation. The sequence and structural homology between viral and human MDs are an impediment for the development of new active compounds against their function.
Here, GS-441524, the active metabolite of remdesivir, is tested as an inhibitor for several viral MDs enzymatic activity and for its binding to human homologs found in PARPs. We also present biochemical and biophysical studies of GS-441524 interaction and de-MARylation activity with several human and viral MDs. The structural investigation of MD•GS-441524 complexes, using solution NMR and X-ray crystallography, reveals the impact of specific amino acids in the ADPr binding cavity and their adjacent residues that tune the selective binding of the inhibitor for SARS-CoV-2 MD.

[P-24]

NMR METABOLOMICS BASED STUDY ON ORAL MALIGNANT DISORDERS

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Keywords: small molecules, metabolomics.

Leukoplakia is an oral potentially malignant disorder (OPMD) whose evolution to oral squamous cell carcinoma (OSCC) is very common and unclear [1]. To date, the presence of dysplastic tissue in the biopsy is considered as an indicator for the malignant transformation of the lesion to cancer. However, to get a better understanding of the evolution of the malignancy, further research is needed [2]. We hypothesized that the NMR metabolomics approach may provide developments to understand alterations in the early stage of the precancerous lesion and OSCC. If OSCC were identified at an early stage, the survival rate would improve.
Metabolites from oral cavity mucosa from patients affected by OSCC and OPMD with different degree of dysplasia were characterized with HR-MAS NMR. The analysis of the spectra data was performed with exploratory multivariate statistical analysis (PCA, PLS-DA).
PLS-DA scores plot of OPMD and OSCC tissues and of leukoplakia with and without dysplasia are reported in Figure 1. OSCCs are characterized by positive LV1 values, and inspection of the loadings indicates that the level of choline is higher and that of creatine lower than in OPMDs. OPMD samples without dysplasia are more clustered while samples with dysplasia are more disperse and show probably the precancerous metabolomics alteration. OPMDs with dysplasia are characterized by positive LV2 values. Analysis of LV2 shows that creatine and glycerophosphocholine levels are higher in OPMDs with dysplasia, whereas glycine, alanine, choline and serine levels are higher in OPMDs without dysplasia.

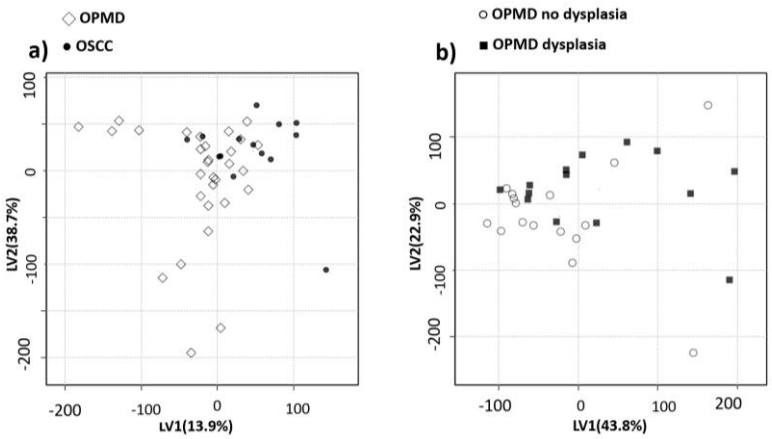

Figure 1: a) PLS-DA scores plot of OPMD and OSCC samples.; b) PLS-DA scores plot of OPMD samples with and without dysplasia.

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[P-25]

NMR ACTIVE NUCLEI IN FERRITIN

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Keywords: solution NMR, biomolecules.

Human ferritin is a 24-mer nanocage protein of about 500 kDa that directs the reversible biomineralization of iron. Due to the huge protein size, NMR analysis of this system represents a real challenge.
Our research activities have been performed on recombinant human homopolymeric ferritins [1-4]. The highly symmetric structure of homopolymeric ferritins is an obvious simplification for their NMR spectra, where the number of detectable signals equals that expected for a single subunit. Instead, the high molecular mass causes very short T_2 and large linewidths. Thus, ^{1}H resonances display linewidth beyond detection, while signals of reasonable linewidths can be retrieved for low-gyromagnetic nuclei such ^{13}C. The state-of-the-art experiment for the detection of ferritin side chains in solution is the ^{13}C-^{13}C NOESY experiment, which gains intensity due to the long tumbling time and relatively long T_1 values associated to the 12-nm cage. This approach has been used to characterize the process of iron biomineralization inside the protein [1-3].
Recently, the use of ^{19}F has been also exploited to improve the NMR detectability of the protein, when used as nanocarrier for drug targeted delivery, this being an application area of increasing importance. We recently described a method for the efficient production of 5-F-Trp human H ferritin, via the selective incorporation of ^{19}F into the side chain of W93 (the only Trp residue present in each subunit), placed in a loop exposed on the external surface of the protein nanocage [4]. This makes 5-F-Trp a probe for the study of intermolecular interactions in solution via chemical shift perturbation mapping and monitoring the uptake of ferritin by cells treated with ferritin-based drug carriers.

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[P-26]

PHASE SEPARATION AND DYNAMIC PROPERTIES OF A MODEL ANION EXCHANGE MEMBRANE

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Keywords: solid state NMR, materials, polymers

The urgent need of a green transition requires new strategies for the production and conservation of energy. Among different solutions, the so called green hydrogen is gaining extreme attention because of the high availability of water.
Anion Exchange Membranes (AEMs) are vital components of alkaline water electrolysers because they allow the transfer of hydroxyl ions from cathode to anode while acting as an electric insulator.
In recent years, Fumatech GmbH developed a new class of membranes based of FAA-3, a ionomer deriving from the functionalization of Poly-Phenylene Oxide (PPO) with an undisclosed quaternary ammonium group. Because of their improved characteristics and reduced costs, these membranes are commonly used as a benchmark material to compare the properties of new AEMs. However, these materials have been characterized only in a handful of works and some aspects are still unknown [1-3].
Solid state NMR could serve as a powerful tool for the characterization of these materials because it can allow to focus the attention on different aspects of membrane, such as structure, phase separation and dynamics.
In this work, we have characterized with solid state NMR a commercially available membrane known as FAA-3-PK-130. This material is based on FAA-3, which is responsible for the anion conductivity, and is reinforced with Poly-Ether-Ether-Ketone (PEEK) to improve mechanical properties and dimensional stability.
The phase separation between the two components has been investigated by spin diffusion by measuring proton spin-lattice relaxation times at 500 MHz through an inversion recovery-cross polarization sequence (IR-CP).
Moreover, low field NMR has been used to unravel the effect of water adsorption on side-chains dynamics and on the separation of ionic channels, which has previously been reported for FAA-3 by means of atomic force microscopy [1]. In order to investigate this aspect, the membrane has been analysed in its dry form and after hydration with normal or deuterated water by ^{1}H FID analysis, ^{2}H static spectra and ^{1}H and ^{2}H spin-lattice relaxation measurements.

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[P-27]

FLUORIDE BINDING BY LN(III)-COMPLEXES: A MULTINUCLEAR AND MULTIFREQUENCY NMR STUDY

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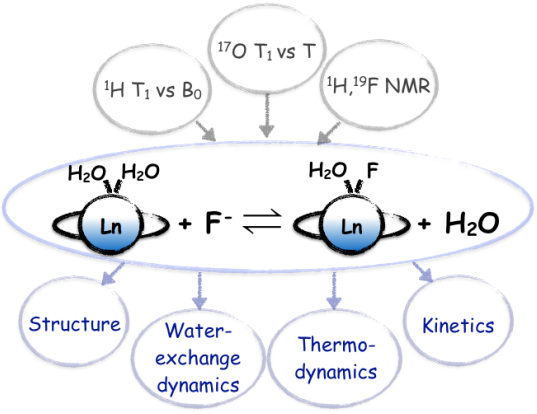
Keywords: solution NMR, low field NMR, MRI, contrast agents.

Halide recognition by coordination complexes represents a major challenge for coordination chemistry. In particular, fluoride binding to lanthanide chelates has attracted increasing interest in recent years, has proven to be very useful for studying the spectroscopic and magnetic properties of paramagnetic complexes, and essential for designing new responsive Ln(III) receptors [1, 2].
Here we investigate the interaction between fluoride and a series of mono-anionic Ln(III)-complexes characterized by different structure, coordination geometry and hydration numbers (q = 1 and q = 2), with the aim of determining how the nature of the ligand affects the halide-binding. For this purpose a combination of high- and low-resolution NMR techniques in the frequency and time domains has been employed, which allows an in depth characterization of the kinetics and thermodynamics of the fluoride binding event and provides a detailed picture of the structural, magnetic, and dynamic properties of ternary complexes (Ln(III)-ligand-F^-). In particular, the observed reduction of water protons' longitudinal relaxation rates upon halide binding indicates the substitution of one inner-sphere water molecule by F^-, and allows determining the affinity constant. Water ^{1}H longitudinal relaxation rates vs B_0, and ^{17}O transverse relaxation rates vs T enables characterizing the dynamics of the hydration phenomena of the ternary complexes and their relaxometric properties. Finally, ^{19}F NMR spectroscopy describes the kinetics and thermodynamics parameters associated to the fluoride binding in diamagnetic ternary Y(III)-complexes.
The results of this study represent a step forward to understand the structural and dynamic properties of lanthanide chelates, and their molecular interactions with halides, which is an essential prerequisite to design new receptors with increased anion affinity and selectivity.



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[P-28]

IN SITU INSONATION OF ALKALINE BUFFER CONTAINING LIPOSOMES FOR THERAPY OF TRIPLE NEGATIVE BREAST CANCER IN MURINE MODEL

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Keywords: MRI, CEST, ultrasound, liposomes, drug release, nanomedicine.

Breast cancer is characterized by metabolic alteration and poor perfusion resulting in an acidification of its micro-environment, as observed in solid tumors[1]. Acidic extracellular pH is known to give cancer cells an evolutionary advantage over healthy ones and to promote metastasis formation, for this reason neutralization of the extracellular pH has been proposed and explored as a potential therapeutic strategy[2]. In this work we developed a method based on the use of low frequency ultrasound and sono-sensitive liposomes loaded with buffers at alkaline pH (LipHUS). The aim of this study is to evaluate whether inducing a pH increase selectively in the tumor environment could affect the primary tumor growth, avoiding the side effects of systemic administration. The pH rise is induced selectively in the tumor region by ultrasound (US)-mediated in situ release of liposomes loaded with sodium bicarbonate or with sodium phosphate (PBS) at alkaline pH. A triple negative breast cancer mouse model was exploited. After the tumor onset the in situ alkalinisation protocol has been applied and the tumor growth has proven to be much slower (in some cases it was completely stopped) in treated mice rather than in control groups. Nearly all the treated mice didn't display any recurrence for 10 days after surgery whereas untreated mice did. Moreover, lung metastasis formation had been evaluated in treated and not treated groups and a significant decrease could be observed for treated mice. In order to avoid completely the metastasis insurgency, we have added a further step in the developed protocol consisting in insonation of the pulmonary artery just after the insonation of the tumor mass. By applying this double insonation protocols we observed that most of the treated mice didn't display tumor metastasis at all. Tumor pH variation in vivo was monitored by CEST-MRI using iopamidol, confirming the alkalinisation of the tumor micro-environment.

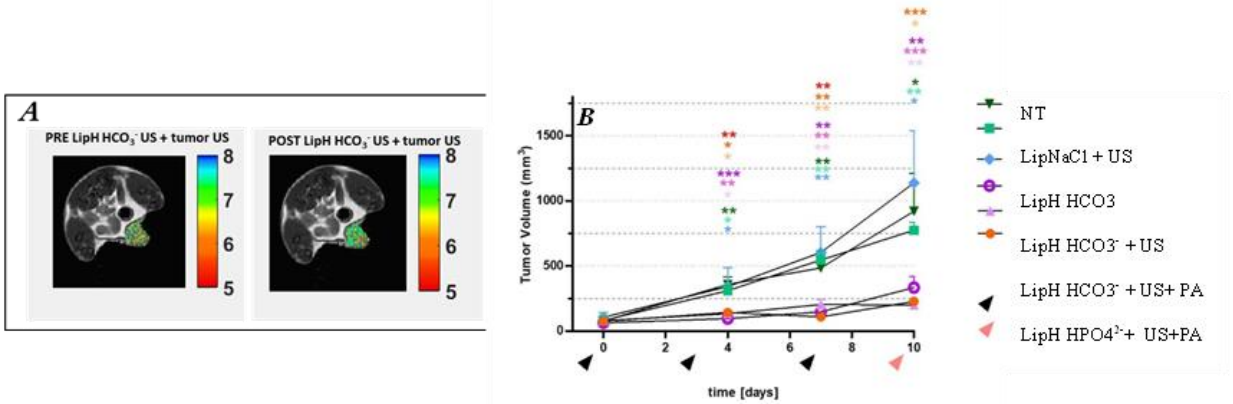

Fig. 1.A. MRI-cest maps of extracellular pH before and after the treatment. B. Primary tumor evolution in murine models

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[P-29]

FLUORINATED CURCUMIN DERIVATIVES: POTENTIAL PROBES FOR AMYLOID-BETA DETECTION BY ^{19}F-MRI

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Keywords: Magnetic Resonance Imaging (MRI), Biomolecules.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline, irreversible memory loss, disorientation and language impairment. Following the amyloid-beta (Aβ) hypothesis, Aβ accumulation in the human brain is the main responsible of the etiology of AD [1]. Estimating the level of Aβ deposition in the brain would be informative for AD early diagnosis and progression evaluation. Positron emission tomography (PET) Aβ tracers retention in the cerebral cortex is traditionally used to distinguish between AD and non-Ad forms of dementia. ^{19}F "in vivo" spectroscopy and/or ^{19}F-MRI may be good alternatives as based on the use of nonradioactive probes. ^{19}F is characterized by a relatively high sensitivity, and due to the absence of F in biological tissues, by *in vivo* background-free images. Many studies have focused in developing probes with high affinity for Aβ aggregates. Among them Curcumin a molecule of natural origin, has many advantages due to its blood-brain barrier permeability, high-affinity for Aβ and low toxicity [2]. In this study, we designed and synthetized novel curcumin derivatives containing nine atoms of ^{19}F in which the nonafluoro-tert-butyl ether is linked to curcumin by different spacers (Fig. 1). All the synthesized compounds were characterized by ^{1}H-NMR and ^{1}H,^{13}C heterocorrelated 2D were acquired with a Bruker Avance spectrometer operating at 14 T. Resonance assignment was based on the analysis of homonuclear 2D-COSY, 2D-NOESY and 2D ROESY NMR spectra. The affinity of the compounds for the Aβ aggregates has been evaluated by spectrofluorometry and compared with the commercially available curcumin. ^{19}F- T_1 and T_2 values estimated at 7T (T_1 around 500 ms and T_2 around 100 ms) were in the proper range for MRI detection and a phantom containing the three compounds (10 mg/mL) was imaged in 10 min with a good signal-to-noise ratio. Therefore, the minimum detectable ^{19}F concentration was evaluated before and after the binding with Aβ fibril aggregates and with the low molecular weight monomeric and oligomeric species.

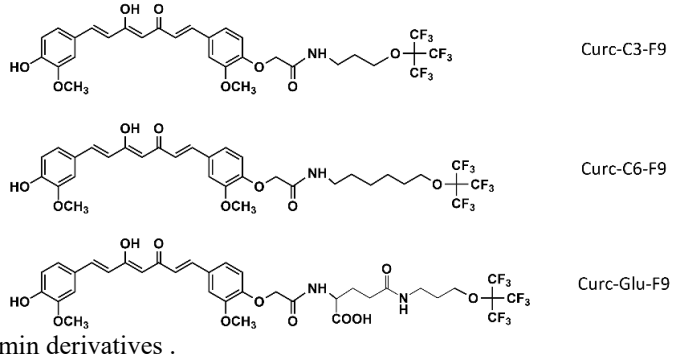

Fig. 1. The novel ^{19}F-curcumin derivatives .

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[P-30]

SOLID STATE NMR INVESTIGATION OF MIXED MATRIX MEMBRANES BASED ON FUORINATED METAL-ORGANIC FRAMEWORKS

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Keywords: solid state NMR, materials, polymers.

Mixed matrix membranes (MMMs) are emerging as a promising technology for gas separation. These materials are composites made by blending a polymer with a porous filler that exhibits exceptional adsorption properties, such as a covalent organic framework or a metal-organic framework (MOF). Due to their composition, MMMs are considered next-generation membranes, since they combine the processability advantages of the polymer with the enhanced separation properties of the filler.[1] Understanding the separation properties of the composites requires characterizing the structural and dynamic properties of both filler and membrane at the atomic level, as well as understanding how they are altered in the composite. This knowledge is crucial for the design of novel materials with improved separation properties.

Solid State NMR (SSNMR) is widely recognized as one of the most powerful techniques for characterizing the structural and dynamic properties of MOFs, composite materials, and their adsorbates at the atomic scale.[2,3] This is primarily due to the possibility to detect different nuclear observables (chemical shifts and anisotropic line shapes, dipolar couplings, nuclear relaxation times, etc.) that are highly sensitive to local structure and dynamics.

In this study, multinuclear SSNMR is used to investigate three different perfluorinated MOFs with high affinity for CO_2,[4,5,6] gas separation membranes obtained from the commercial polymer Hyflon, and their corresponding MMMs. ^{13}C, ^{19}F and ^{1}H high-resolution SSNMR spectra and longitudinal relaxation times are analyzed to unravel structural and dynamic properties of the MOFs and the membrane and how they change in the MMMs.

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[P-31]

NMR SPECTROSCOPY IN CHARACTERIZATION OF HYDROALCOHOLIC PHASE OF ASTERACEAE FLOWERS FROM ALGERIA

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Keywords: solution NMR; metabolomics; food.

Asteraceae flowers are known for their medicinal properties and traditional use in folk medicine. However, the precise chemical composition of the hydroalcoholic phase of Asteraceae flowers namely Chamomile and Daisy flower has not been fully explored. In this study, NMR spectroscopy was applied to characterize the Bligh-Dyer hydroalcoholic phase [1] of Asteraceae flowers from Algeria.

NMR mono- and two-dimensional experiments were recorded to identify and quantify the compounds present in the hydroalcoholic phase namely sugars, amino acids, organic acids, and other metabolites. The obtained preliminary results revealed a wide diversity of compounds in the hydroalcoholic phase of Asteraceae flowers. Additionally, significant variations in the chemical composition were observed among the different studied Asteraceae species. These findings suggest that the hydroalcoholic phase of Asteraceae flowers could be a valuable source of bioactive compounds.

This study provides a detailed characterization of the chemical composition of the hydroalcoholic phase of Asteraceae flowers from Algeria using NMR spectroscopy. These results contribute to a better understanding of the potential therapeutic properties of these flowers and may pave the way for future research on their utilization in traditional medicine and phytotherapy.

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[P-32]

ON-CELL NMR CHARACTERIZATION OF MOLECULAR INTERACTIONS OCCURRING AT BACTERIAL CELL SURFACE

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Keywords: solution NMR, small molecules, biomolecules.

Antimicrobial resistance is one of the major threats of the 21st century; as a consequence, novel strategies to fight bacterial infections remain an urgent need. An innovative approach, imposing less evolutionary pressure than standard antibiotics for the development of resistance, is the targeting of virulence factors [1], as molecules produced by bacteria enabling them to colonize the host and evade or inhibit the host's immune system response.
To this aim, we developed different multivalent ligands, based on calixarene or dendrimeric scaffolds, targeting specific molecular patterns of the different bacteria classes, such as the terminal part of peptidoglycan (D-ala-D-ala) and teichoic acids for Gram+ bacteria, LPS for Gram- bacteria, mycolic acid, glycolipids and trehalose transporter for mycobacteria. Moreover, for a specific pathogen targeting, the adhesin FimH located at the pili end of an uropathogenic strain of *Escherichia coli* can be targeted through the glycoside cluster effect of carbohydrate-lectin interactions.
Here we present our recent results on the developments of an advanced NMR approach for the screening of bacteria ligands working on living bacterial cells under HRMAS conditions. [2-4] On-cell STD NMR experiments have been set up and allowed the identification of promising hit compounds as selective bacteria ligands and anti-virulence factors.

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[P-33]

NEW PSYCHOACTIVE SUBSTANCES: A STUDY OF URINARY METABOLIC CHANGES OF OPIOID-TREATED MICE BY SYNHMET METHOD

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Keywords: solution NMR, LC-MS, metabolomics, new psychoactive drugs.

Over the past decade, many new psychoactive substances (NSPs) have entered the drug market, posing a global problem for human health. Among the various classes of NSPs, synthetic opioids ranked third in 2020 in the number of substances entering the market annually. This rapidly growing number has made clear the need to find tools to combat the spread of these drugs [1]. Metabolomic approaches are helpful tools for simultaneously detecting endogenous metabolites influenced by drug use. With these approaches, common responses of an organism to exposure to a given class of substances can be identified, also helping to understand their mechanisms of action. In this pilot work, the urinary metabolic profile of CD-1 mice was studied following the administration of a dose of two opioids: morphine or fentanyl. The analysis was performed by nuclear magnetic resonance (NMR) and ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) techniques to describe the metabolic response following the administration of the two substances. In addition, we aimed to verify the effectiveness of the SYNHMET method, which combines data from NMR and MS, for characterizing the urinary metabolic profile compared with the single use of NMR spectroscopy [2]. The results obtained confirmed the advantages of applying SYNHMET for metabolomic analysis. Using this method, 82 metabolites were quantified in urine for 43 samples. Given the exploratory nature of the study, two fundamental parameters were evaluated for the proper design of future experiments, namely the sex of the subjects and the dose of substance to be administered, proposing to treat individuals of different genders separately and assuming that under the present experimental conditions, the dose of morphine was too low to obtain a comparable metabolic effect between the two opioids. Finally, a common response at the metabolic level following morphine or fentanyl administration was nonetheless found to be shared, finding energy and amino acid metabolisms significantly altered. The analyses conducted in this dissertation may provide the basis for further in-depth and targeted studies for endogenous biomarkers for opioid consumption.

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[P-34]

COMPLETE INVESTIGATION OF FLUORINATED MOF COMPRISING DIPOLAR MOLECULAR ROTORS BY SOLID-STATE NMR

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Keywords:
solid state NMR, hyperpolarization, materials, polymers.

The design of solid materials comprising rotors that can convert external stimuli into actions is growing exponentially. [1] In particular, the incorporation of fast-reorientating dipoles onto molecular rotors is very attractive for the fabrication of materials responsive to oscillating or static electric fields. In this work, we successfully synthetized two new isostructural aluminum-based metal-organic frameworks (MOFs), endowing bicyclopentanedicarboxylate molecular rotors as linkers, denominated frustrated trigonal rotors (FTR), in the hydrogenated and partly fluorinated derivatives (FTR-F2). [2] Multi-nuclear high-resolution ssNMR techniques were used to characterize the new crystal structures, demonstrating the purity and symmetry of the materials. The empty space that surrounds the rotors allowing them to move freely was directly probed with continuous-flow hyperpolarized (HP) ^{129}Xe NMR. The observed chemical shift anisotropy (see Fig. 1 Left) indicates that the xenon is exploring an ellipsoidal cross-section, as observed in the rhombohedral structure of the framework. ^{1}H T_1 relaxation times were collected down to 2 K (see Fig.1, Right) in order to understand the motional behavior of the molecular rotors having CF_2 groups mounted, which were proved hyper-mobile even at such low temperatures with extremely low activation energies for dipole reorientation. In particular, the T_1 revealed multiple relaxation phenomena due to correlated dipole-rotor configurations**.** The discovery paved the way for the realization of dipolar rotors unaffected by thermal noise to be used in molecular machines with low energy dissipation and controllable dynamics.

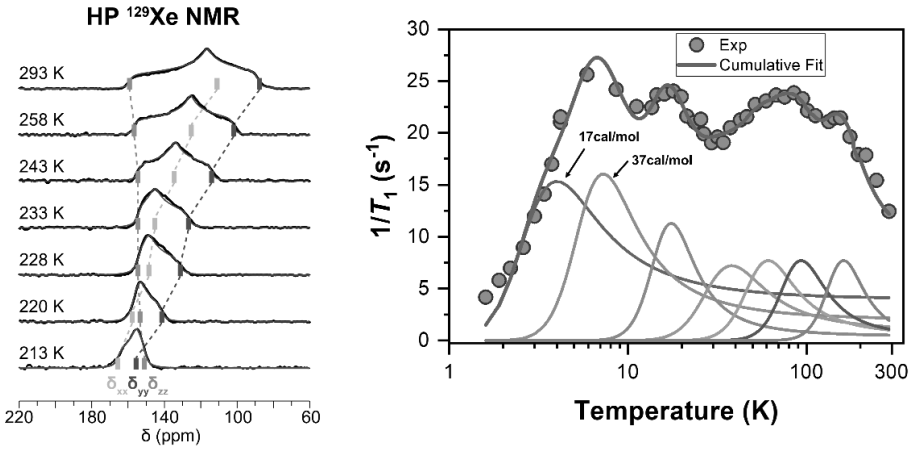

Fig. 1. Left: VT HP ^{129}Xe NMR spectra of Al-FTR-F2; the dotted lines show the movement of the three main components of the tensor. Right: ^{1}H T_1 relaxation times of Al-FTR-F2 at 22 MHz; the circles indicate the experimental data, while the lines represent the seven Kubo-Tomita functions used to fit the data.

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[P-35]

NON INVASIVE SINGLE SIDE NMR, SOLID STATE NMR SPECTROSCOPY AND MICRO-ANALYTICAL TECHNIQUES FOR STUDYING THE *CORAMI* (GILT AND PAINTED LEATHER) WALL COVERINGS FROM CHIGI PALACE, ITALY

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Keywords: solid state NMR, low field NMR, materials, small molecules.

Italian leather with a gilded or silvered background, tooled and painted in bright, transparent colors is called "*corami*" (from the Latin *corium*), "*cuoi d'oro*" or "*cuoridoro*" (gilt leather). The first gilded/silvered and painted leathers were produced in Muslim Spain, Cordoba being the center with the most flourishing production. In fact, the leather from Spain is called Cordovan leather. This type of leather then spread to Europe thanks to imports from Spain and the Middle East. In Italy, the manufacture of the so-called *corami* reached its peak in the XVIth and throughout the XVIIth century. The most important production centers were Naples, Rome, Venice, Bologna, Ferrara and Modena. The *corami* were preferred for wall covering or for decorating the front of the altars.
It is known that gilt leather rarely survives in its original state due to the inherent problems of leather as a support for the richly decorated surface. The specific layering of materials in this type of leathers (leather support, animal glue, silver leaf, oil paints, glazes and varnishes) makes their conservation and restoration a challenge. The *corami* as also exhibited forms of physical and mechanical deterioration (dust and dirt, cracks and fragility, shrinkage, erosion and loss of painted surfaces). The specific layering of materials in this type of leather (leather backing, animal glue, silver leaf, oil paints, enamels and paints) makes their preservation and restoration a real challenge.
One of the most spectacular examples of XVIth century corami is preserved in Palazzo Chigi, located in Ariccia, near Rome, This case study has been the subject of extensive in situ and laboratory analysis campaigns aimed at studying the materials used for production, evaluate the deterioration and evaluate the general conditions of conservation of wall panels. NMR single-sided measurements conducted in situ provided the thickness of the skin and its stratigraphy.
Some micro samples were analyzed by Fourier transform infrared spectroscopy (FTIR), optical microscopy, SEM-EDX, and 13C CPMAS NMR spectroscopy. The results obtained provided information for example on the type of leather (very thin goat skin), tannins (sumac and alum), used in this rare type of *corami*, as well as their forms of deterioration (presence of copper and calcium oxalates, conversion of collagen into gelatin and subsequent amorphous form).
The novelty in this study is the combination of non-invasive analysis such as single sided NMR, NMR spectroscopy and micro-analytical techniques, which allowed us to obtain significant results on such a sophisticated and fragile material and highlight potential, limitations and use of of NMR techniques for investigating gilt and painted leather.

[P-36]

MAPPING THE SMALL OLIGOMERS POPULATION ALONG THE FULL TIME COURSE OF AMYLOID AB1-40 BY NMR

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Keywords: solution NMR, biomolecules.

The aggregation of misfolded fibrils is a characteristic of human amyloid diseases, including Alzheimer's disease (AD). AD is a fatal neurodegenerative disorder caused by the pathologic aggregation of the amyloid β (Aβ) peptide into oligomeric species followed by the formation of fibrils that accumulate in patient's brain [1]. It is widely recognized that toxic oligomeric species formed in the earlier stages of the Aβ peptide aggregation are the key players in Alzheimer's disease. Little is still known about the detailed mechanism of their formation, mainly due to the transient and unstable nature of the oligomeric states explored by Aβ peptide and to the difficult preparation of homogeneous and seed-free Aβ peptide preparation [2, 3].
Here, we set an efficient optimized experimental protocol for the preparation of Aβ1-40 through the depsi-peptide technique [4], that leads to high Aβ1-40 yield and purity. The obtained seed-free starting Aβ1-40 samples ensured consistency in the sample preparation and minimized experimental variability. The first steps of Aβ1-40 aggregation kinetics were investigated by NMR. A specific DOSY reconstruction method was employed for the first time to characterize the evolution of Aβ1-40 aggregate size distributions during the aggregation process. Our data shed new light on the amyloid aggregation phenomenon and ultimately open the way to the development of novel therapeutic drugs.

Acknowledgements

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[P-37]

PARAMAGNETIC CHELATES EMBEDDED IN NANOGELS AS MRI PROBES

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Keywords: nanogels, paramagnetic chelates, contrast agents, relaxometry, MRI applications.

Nanogels (NGs) can be considered as a new route to hypersensitive MRI probes, due to their high biocompatibility, synthetic versatility and to their hydrophilic nature. As a matter of fact, the confinement of contrast agents (CAs) within NGs can lead to a strong relaxivity (r_1) enhancement due to the restriction of the rotational dynamics related to the encapsulation of the CA, the high-water content and the increased viscosity of the water molecules entrapped within the polymeric network. Most of the NGs described in literature are stabilized by noncovalent interactions (ionic, H-bonds, hydrophobic forces) between biocompatible polymers such as chitosan, hyaluronic acid, or oligo/polypeptide-based systems. In a preliminary study, we synthesized NGs cross-linked by electrostatic interactions between chitosan and hyaluronic acid, which embedded Gd^{3+}-chelates with different hydration state and charge.[1] Interesting results were obtained with the formulation containing the $[Gd(DOTP)]^{5-}$ complex, which shows a remarkable r_1 value of 78.0 mM^{-1} s^{-1} at 0.5 T and 298 K despite the absence of inner sphere water molecules.[2] However, an *in vivo* MRI study demonstrated insufficient stability of the NG, with a complete release of the complex within 24 h in solutions of high ionic strength. To overcome this limit, we propose a more stable chitosan based nanogel covalently cross-linked with a bisamide-derivative of *t*-CDTA, which is able to stably coordinate Mn^{2+} ions (Fig. 1).[3] This new nanogel shows: i) a seven-fold increase in r_1 compared to that of typical monohydrated Mn^{2+} chelates at the clinical fields, ii) high stability of the formulation over time at pH 5 and 7.4 thus excluding metal leaching or particles aggregation, iii) good extravasation and accumulation of the NPs in a subcutaneous breast cancer tumor mice model, with a maximum contrast achieved in the tumor area between 4 and 24 h post-injection

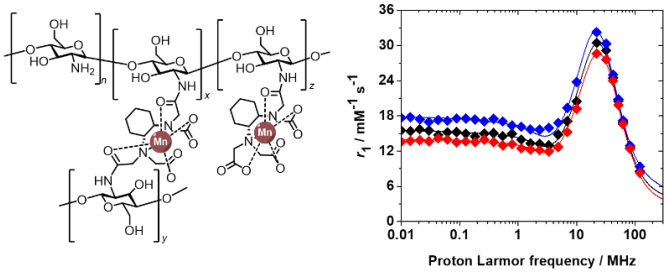

Fig. 1. left: schematic illustration of nanogel; right: ^{1}H NMRD profiles of the Mn-NG at 283 (blue), 298 (black) and 310 K (red) at neutral pH.

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[P-38]

METABOLOMIC CHARACTERIZATION OF *ARCTIUM LAPPA L.* AND *TARAXACUM OFFICINALE BY UNTARGETED* NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

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Keywords: NMR, metabolomics, nutraceutical.

The medicinal plants are a source of secondary metabolites with relevant biological properties. [1] The chemical composition depends on multiple factors such as pedoclimatic conditions (nature of the soil and cultivation light, temperature, humidity, altitude) and plant part.
The present study aimed at characterizing the metabolic profile of *Arctium lappa L*. (Burdock) and *Taraxacum officinale* (Dandelion) using the NMR-based metabolomics approach.
Specifically, three different ecotypes were analyzed for each plant: Land Spontaneous Ecotype (LSE), Mountain Spontaneous Ecotype (MSE), and Organic Ecotype (OE).
Burdock and Dandelion roots were extracted by the Bligh-Dyer [2] protocol to obtain two different fractions with different polarity compounds. The hydroalcoholic phase was prepared for the NMR analysis. Both one-dimensional proton (^{1}H NMR) and two-dimensional (^{1}H-^{1}H TOCSY, 1H-^{13}C HSQC and ^{1}H-^{13}C HMBC) experiments were performed, using previously reported experimental conditions [3], to achieve a complete peak assignment. Sugars, organic acids, amino acids, nucleoside, and other metabolites, such as choline, trigonelline and polyphenols, were quantified in all the analyzed samples.
Chemical characterization by NMR spectroscopy has made it possible to obtain the metabolomic profile of the three ecotypes of *Arctium lappa L.* and *Taraxacum officnale* to highlight useful differences for health and nutraceutical application.

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[P-39]

SURPRISING HINDERED COPPER(I) COMPLEXES: STRUCTURAL AND DYNAMIC CHARACTERIZATION BY CYCLIC VOLTAMMETRY AND VARIABLE TEMPERATURE ^{1}H NMR

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Keywords: solution NMR, materials, small molecules.

Copper complexes are currently among the best performing redox mediators in DSSCs (dye-sensitized solar cells), cheaper and more sustainable than the cobalt-based analogues.[1] The adoption of sterically hindered ligands lowers the high inner reorganization energy between copper(I) and (II) species and leads to higher photovoltaic performances.[2] For this purpose, a novel class of copper(I) complexes based on differently bulky mono- and tris-substituted imidazo[1,5-*a*]pyridine ligands (*impy*) has been developed (*Fig. 1a*).
After a complete photo- and electrochemical characterization of all the complexes, a combined CV and VT ^{1}H NMR study was carried out. This returned surprising CV curves and ^{1}H NMR spectra: the first exhibiting a splitting of the oxidation peak and the latter showing sharp signals, in contrast to the peculiar broad peaks generally observed for such loose-geometry complexes. The collected data suggest that the tetrahedral coordination, typical of copper(I) complexes, is strongly influenced by the type of *impy* ligand used, generating a series of complexation equilibria. The various systems can be classified into four different categories as shown in *Figure 1b*. Cases 1 and 3 represent two different locked structures, characterized by straightforward NMR spectra and a mono-electronic oxidation peak in CV. On the other hand, cases 2 and 4 describe two equilibria between two different coordination forms. In fact, the complexes within cases 2 and 4 show a very particular behavior in VT ^{1}H NMR: as the temperature decreases, all peaks become narrower and a second set of signals appears, proving the equilibrium condition.
Besides the structural characterization, different dynamic rates for such equilibria were evaluated both qualitatively and quantitatively. Fast equilibrium is characterized by a single oxidation peak in CV, whereas for slow equilibrium CV clearly shows two peaks with very similar redox potential. Moreover, the values of activation energy (ΔG) for such equilibria were measured by means of VT ^{1}H NMR and coalescence temperature, confirming a lower ΔG for complexes in fast equilibrium regime.

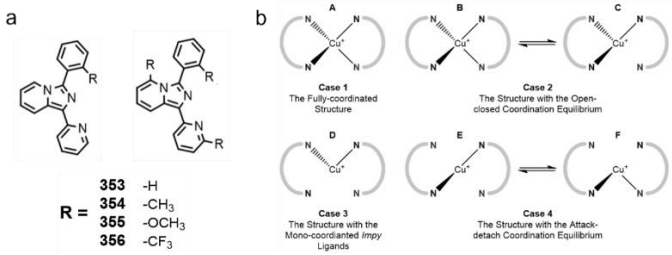

Fig. 1. a) General structure of mono- and tris-substituted *impy* ligands, and b) possible coordination modes of *impy* ligands around central metal cation in the copper(I) complexes.

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[P-40]

MULTINUCLEAR SOLID STATE NUCLEAR MAGNETIC RESONANCE FOR STUDYING $CsPbBr_3$ NANOCUBES

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Keywords: Solid State NMR, Materials.

In recent years, metal halide perovskites (MHPs) have aroused a lot of enthusiasm in the materials science community due to their unique tunability, which allows a fine regulation of the desired properties. $CsPbBr_3$ perovskite nanocrystals (Fig. 1) have shown highly attractive light emitting properties, thanks to their narrow emission bandwidths, high quantum-yield values, and the possibility to perform a fine tune of the emission wavelength by controlling the size of the nanocrystals [1]. In the last few years, Solid-State NMR spectroscopy (SSNMR) has emerged as one of the main techniques for an in-depth structural and dynamic characterization of MHPs [2-3].
In this work, a multinuclear SSNMR approach was adopted for a structural study of cubic CsPbBr3 nanoparticles stabilized with oleic acid and oleylamine. In particular, the surface ligands and their interactions with the nanocubes surface were investigated by ^{1}H and ^{13}C NMR experiments, while the structural investigation of the perovskite nanocubes was addressed by exploiting ^{207}Pb and ^{133}Cs spectral properties in comparison with bulk $CsPbBr_3$. Static ^{207}Pb NMR spectra indicated a possible contribution of chemical shift anisotropy from the ^{207}Pb nuclei of the outer layer. The ^{133}Cs NMR spectra showed signals with different chemical shifts for cesium atoms in at least three regions of the nanocubes, from the inner core to the surface, which were interpreted in terms of cubic layers with different distances from the surface using a simple geometrical model. This interpretation was also supported by ^{133}Cs longitudinal relaxation time measurements [4].

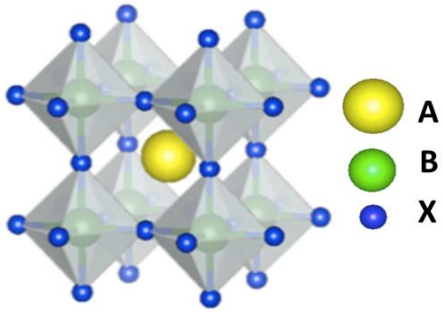

Fig. 1. Structure of $CsPbBr_3$ perovskite

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[P-41]

UNRAVELING THE LOCAL STRUCTURE IN MIXED HALIDE DOUBLE PEROVSKITES BY MEANS OF ^{125}Te SSNMR AND DFT

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Keywords: Solid State NMR, Materials, theory and methods.

Perovskite materials have aroused enormous interest in the materials science community over the past decade due to their surprising optoelectronic properties, making them promising candidates in the production of new generation solar cells and light emitting diodes. In particular, metal halide perovskites such methylammonium lead iodide ($CH_3NH_3PbI_3$) and formamidinium lead iodide ($CH(NH_2)_2PbI_3$) have shown excellent power conversion efficiencies values. Nevertheless, concerns about lead toxicity and instability issues have limited their commercial scale deployment. Alternative compositions and structure of perovskites are therefore under investigation in order to overcome these problems.

Vacancy-ordered double perovskites (A_2BX_6) are a structural defect-ordered derivate from the archetypical ABX_3 perovskite structure, characterized by an antifluorite arrangement of isolated octahedral units connected by A-site cations (Fig. 1a); when B-site cations consist of a Group 16 metal, these materials provide improved stability against air and moisture, while maintaining similar properties to the ABX_3 perovskites.

In this work, high resolution ^{125}Te SSNMR experiments were carried out to study different compositions of the mixed-halide double perovskites $MA_2Te(Br_xI_{1-x})_6$, $Cs_2Te(Br_xCl_{1-x})_6$ and $Cs_2Te(Br_xI_{1-x})_6$ (Fig. 1b). The combination of this technique with chemical shift calculations by density functional theory, along with statistical analysis, allowed for a comprehensive understanding of the systems composition. Specifically, it was possible to quantify the different octahedral coordination environments, enabling us to draw conclusions on halide mixing uniformity within the mixed-halide materials.

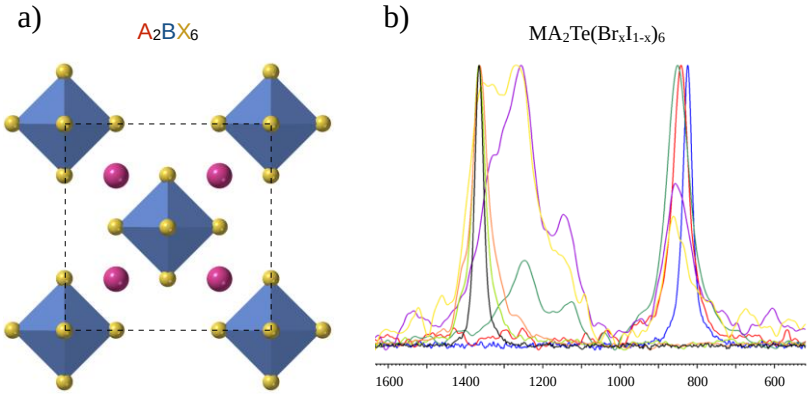

Fig. 1. a) Structure of a A_2BX_6 perovskite; b) ^{125}Te spectra of different $MA_2Te(Br_xI_{1-x})_6$ compositions

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[P-42]

A COMMUNITY SOFTWARE TOOL FOR NMR RELAXOMETRY- CASE STUDY: QUALITY CONTROL OF PECORINO SARDO

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Keywords: low field NMR, relaxometry, biomolecules, metabolomics, food, theory and methods.

Low-field NMR applications in the food sector cover many different categories, such as the distinction of sample components or phases (muscle/fat, solid/liquid) or inner states (ripe or damaged), rheological and textural properties [1], monitoring melting/freezing or diffusion processes [2], determination of particle/droplet sizes in emulsions (milk, cream), ageing of materials (stocked food, cheese ripening), or even the assessment of the authenticity of food products protected by the EU quality policy and geographical indications (PDO, GPI or GI), for example on PDO buffalo mozzarella cheese [3] .
Despite the great number of applications, there are not so many specific tools dedicated to data analysis for specific use in both academy and industries. The hardware characteristics of low-field, and particularly low-resolution NMR instruments, often differ significantly from instrument to instrument and provide very limited software support to any particular application. Moreover, the employed data formats and evaluation procedures are presently not-standard, in some cases even proprietary.
In this situation, application developers struggle to guarantee reproducibility of the results. There is therefore a great need of a uniform, vendor-agnostic software toolkit, one sufficiently sophisticated to allow an expert user, once he selects a potential application, to find out the best methodology to address the problem, optimize it, assess its precision and reproducibility, and in the end automate it, making it suitable for practical use in industrial environments.
With this fact in mind, the development of a dedicated software utility becomes a community-oriented toolkit for NMR Relaxometry.
In this study, we combine current data processing routines (for example several categories of model-based, fitting methods) with other approaches (projection on latent structures) to study cheese.
The study here presented concerns Pecorino Sardo PDO, whose specification requires the use of raw milk, i.e., not heat-treated, while the most common fraud is that of using pasteurized milk. Since no modifications at the level of the molecular composition are expected, attention is focused on the changes that may occur at the level of the supramolecular structure. For this reason, the TD-NMR approach is considered very promising, provided that the protocols for collecting samples, acquiring relaxation data and for analysis are well defined.

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[P-43]

FRAGMENT-BASED DRUG DISCOVERY WITH FLUORINE-19 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY: A PROMISING APPROACH FOR ACCELERATED DRUG DEVELOPMENT TARGETING CYCLOPHILIN D (CYP-D)

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Keywords: solution NMR, small molecules, biomolecules, theory and methods.

Fluorine ^{19}F NMR strategies have been widley adopted for evaluating ligand binding to macromolecules. This method present numeros advantages due to its highly sensitive spin ½ nucleus, 100% natural abundance and wide range of chemical shift. Additionally, fluorine is absent from biological samples and it makes easy to monitor ligand binding without any background interference. All these features make it a promising approach for screening compounds.
Here, we evaluated 175 fluorinated possible candidate compounds as CyP-D ligands by fragment-base hit discovery. Furthermore, we propose a T2-CPMG NMR acquisition method for screening. It result in a promising NMR approach in hit validation, binding mode determination, and optimization of fragment hits into lead compounds targeting Cyp-D [1]. Multiple factors were evaluated for each candidate to monitor the suitability for ^{19}F NMR screening purpose, including aquous solubility, stability and buffer compatibility. A method was used to obtain the protein with the fluorinated tryptophan residue in the active site, starting from a precursor. In this way we propose protein based ^{19}F NMR screening so it could be extended to libraries of compounds that do not have fluorine allowing for a greater search spectrum and probability of finding positives. Research using rapid screening methods allows fast development approach of specific therapeutics with the potential to fight various diseases associated with CyP-D dysregulation, including: neurodegenerative diseases [2], ischemia-reperfusion injury, and cardiovascular diseases.

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[P-44]

CHARACTERIZATION OF MARINE OILS QUALITY USING ^{1}H HR-NMR AND MULTIVARIATE ANALYSIS

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Keywords: biomolecules, metabolomics, food, ω-3 PUFA.

The assumption of long-chain omega-3 (ω-3) polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) plays an important role in the treatment of several diseases such as heart and neurological disorders, diabetes, inflammation, and depression [1, 2]. For this reason, in the last years, many efforts have been done to find new sources of these compounds, characterized by high bio-availability. Marine oils from fish like anchovies, tuna, and salmon [2], and from zooplankton such as krill and Calanus, can play a key role [3]. Another important "green" source of ω-3 PUFA comes from the recovery of the high nutritional value fractions extracted from fishery by-products. The valorization of these by-products is a main objective of the European project BIOZOOSTAIN (https://healthsciences.hi.is/biozoostain). This project aims to develop new high-quality ingredients for food supplementation, starting from valuable biomolecules, such as astaxanthin, chitin, ω-3 PUFA, wax-esters, and enzymes, from *Calanus finmarchicus* -one of the main components of zooplankton in the northern Atlantic Ocean-, recovered from fishery by-products.
The task of the present study is to define a classification system of different ω-3 PUFA sources from marine oils, including that from the BIOZOOSTAIN project, exploiting the principles of Foodomics and Metabolomics [4-5]. The adopted approach is based on the ^{1}H High-Resolution Nuclear Magnetic Resonance (^{1}H HR-NMR) spectroscopy that has been used, first, to characterize the lipid profiles of different Calanus, krill, and fish commercial oil samples. In the second part of the work, multivariate statistical analysis (MvSA) as Principal Component Analysis (PCA) has been applied on the entire lipidic data set to point out similarities and differences among the different marine oil sources.

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[P-45]

CONFORMATIONAL STUDIES OF EARLY STAGES OF PRION AMYLOID FIBRIL FORMATION

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Keywords: solution NMR, biomolecules, theory and methods.

Prion diseases, which are also called transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders characterized by neuronal loss and vacuolation. TSEs can undergo long incubation periods ranging from years to decades. These diseases are associated with the conversion of cellular prion protein (PrP^{C}) into a misfolded oligomeric or fibrillary form (PrP^{Sc}) which accumulates in the brain [1]. Intermediate conformations forming during the conversion of PrP^{C} into its scrapie PrP^{Sc} conformation are key drivers of the misfolding process. Our prior research provided a high-resolution description of a β-enriched intermediate state (β-PrPI) involved in the initial stages of PrP^{C} fibrillation [2]. Using Nuclear Magnetic Resonance (NMR) techniques we investigated the structural and dynamical features of β-PrPI showing that the intermediate state presents a substantial tendency to increase the β-sheet folding with respect to the native state with preserving the three native α-helices. We also demonstrated that in the full-length HuPrP(23-231) a self-regulated folding mechanism in which the N-terminal domain, interacting transiently with the C-terminal domain through electrostatic interactions, avoids the formation of dangerous intermediate misfolded states by tuning long-range μs-ms conformational dynamics [2]. Besides, NMR data revealed that the amyloid fibril formation of β-PrPI intermediate state occurs via a specific assembly mechanism involving transient oligomeric species [2]. However, the molecular basis of prion aggregation involved in PrP diseases remains poorly understood. Therefore, we have propelled to the forefront the investigation of the early stages of the mechanism by which β-PrPI activates the formation of prion amyloid fibrils using solution NMR which has the ability to quantitatively probe exchange dynamics between interconverting states [3]. In particular, Chemical Exchange Saturation Transfer (CEST) NMR experiments, which involve a stepwise systematic scanning of the chemical shift regions by selective saturation, were acquired on HuPrP(90-231) at low temperature (15 °C) in the absence and presence of β-PrPI-oligomers with the intention to provide hints about the structural rearrangements involved the formation of transient oligomeric species that in turn govern the amyloid assembly mechanism.

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[P-46]

COMPOUNDS AGAINST SAM DOMAINS: A COMBINED NMR AND COMPUTATIONAL APPROACH

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Keywords: solution NMR, small molecules, biomolecules.

Sam (Sterile alpha motif) domains represent small helical protein binding modules playing roles in a variety of physiological and pathological conditions [1]. The EphA2 tyrosine kinase receptor contains a Sam domain at the C-terminal side that is important to engage protein regulators of receptor endocytosis. EphA2 endocytosis is induced by ligand binding and followed by receptor degradation. The process can be intended as a way to decrease pro-tumor effects by lowering receptor levels. The lipid phosphatase Ship2 can bind EphA2 through a heterotypic Sam-Sam interaction and, by inhibiting endocytosis, stimulates chiefly pro-cancer activities [2]. The goal of this project was to identify small molecules able to hamper EphA2-Sam/Ship2-Sam interaction thus enhancing receptor endocytosis and working as possible anticancer agents.
The first step of our research plan was an *in silico* screening approach through docking studies to identify potential EphA2-Sam and Ship2-Sam ligands, by employing the 3D NMR structures of EphA2-Sam (pdb code: 2E8N) and Ship2-Sam (pdb code: 2K4P [3]), a variety of databases of virtual molecules, and an array of bioinformatic tools. A few of the best docking hits were purchased and a small library of drug-like compounds was set up (about 100 compounds). Compounds' identity and purity was checked by 1D and 2D NMR techniques. Next, binding to Sam domains was evaluated by means of NMR chemical shift perturbation (CSP) studies. This approach led to identifying a small molecule targeting Ship2-Sam that is further being analyzed in our laboratory along with a few analogue compounds.

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[P-47]

SOLID-STATE NMR CHARACTERIZATION OF A NOVEL AMPHIPHILIC POLYACRYLATE DERIVATIVE

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Keywords: solid state NMR, hyperpolarization, polymers, small molecules.

Polyacrylic acid (PAA), the synthetic homopolymer of acrylic acid, plays a key role in both pharmaceutical and cosmetic fields, for its thickening properties, mucoadhesivity, and remarkable water retention. These features may be enhanced and tuned by means of chemical or modifications, leading to new derivatives such as block copolymers, cross-linked gels, and amphiphilic polymers [1]. The main goal of the project was to chemically modify the PAA in order to improve both its viscosity-enhancing properties and amphiphilic behavior, while preserving its already well-known characteristics. This would result in an even more versatile polymer, easily adaptable for a wide range of pharmaceutical and cosmetic formulations, without requiring additional excipients. PAA was modified through the introduction of hydrophobic moieties along its backbone chain (See Fig. 1): cholesterol was chosen as the lipophilic group, for its biocompatibility with human tissues and for its safety from a toxicological point of view [2]. Both the derivative and the reactants were characterized by means of ^{1}H, ^{13}C, and ^{19}F solid-state NMR analysis. ^{13}C-^{1}H cross-polarization and DNP hyperpolarization techniques [3,4] were employed in order to increase spectra sensitivity: this allowed to confirm a successful polymer functionalization, evaluate the polymer derivatization degree and the polymer chain non-covalent interactions with a model drug molecule betamethasone valerate.

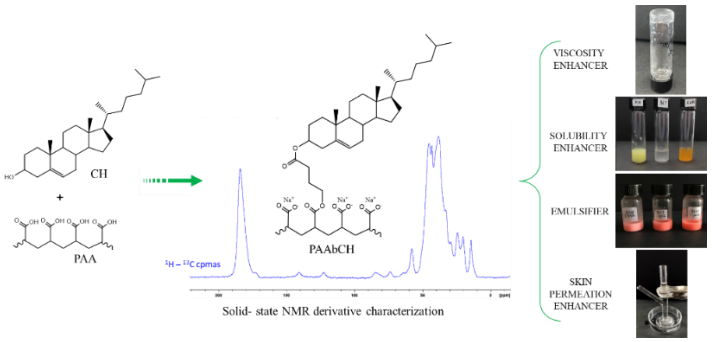

Fig. 1. Scheme of the project: functionalization, derivative characterization, and application development

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[P-48]

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AS A TOOL FOR THE EARLY-DETECTION OF OLIVE QUICK DECLINE SYNDROME

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Keywords: solution NMR, small molecules, metabolomics.

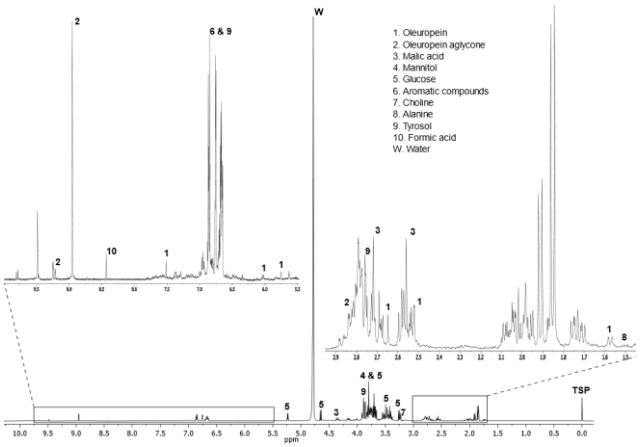

Fig. 1. NMR spectrum (1D ^{1}H NOESY) of aqueous extract of olive leaf.

Plant stress responses cause a biochemical cascade in which gene expression is altered. Such an event leads to further downstream changes that result in alterations in metabolism to reduce the effect of the stress. Stress can produce a phenotypic change that is visual (symptomatic) or pre-visual (asymptomatic). This work focused on the cultivated asymptomatic olive leaves affected by *Xylella fastidiosa*. Young olive plants grown in controlled environment were artificially infected by *Xylella fastidiosa* subsp. *pauca* ST53, which is also known as the "De Donno" strain. *Xylella fastidiosa* is the causal agent of olive quick decline syndrome (OQDS).[1] After 2 years from inoculation by *X. fastidiosa*, leaves were collected and analysed using real-time quantitative polymerase chain reaction (qPCR) for ascertaining the presence of *X. fastidiosa*, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy for determining the metabolic profile, and hyperspectral reflectance (HSR) for identifying possible diagnostic wavelengths.[2,3] As a result, OQDS-related diagnostic signals and wavelengths were identified for asymptomatic olive leaves infected by *X. fastidiosa*. These findings are key initial points for the development of early-detection tools capable of detecting OQDS at the very early stages of the disease.

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[P-49]

METABOLIC CHANGES IN MOUSE BRAIN FOLLOWING ROCKs INHIBITION BY FASUDIL: A PROTON MAGNETIC RESONANCE SPECTROSCOPY (1H-MRS) STUDY

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Keywords: MRI, biomolecules.

The Rho GTPases are key modulator proteins of the actin cytoskeleton and, due to their critical role in the regulation of neuronal plasticity [1], they have been linked with cognitive and, more recently, motor impairments in neurodegenerative and neurodevelopmental disorders [2].
Here we treated mice with fasudil, an inhibitor of the RhoA downstream effectors Rho kinases (ROCKs) [3], to evaluate the effect of RhoGTPases pathways modulation on motor behaviour, brain metabolism and structure.
CD1 five-months-old male mice were divided into 2 groups: fasudil (100 mg/Kg/d in drinking water for three months) and controls. After treatment and behavioural tests on locomotion and motor-coordination (open field task and rotarod), MRI/MRS experiments were performed on a small animal system (Pharmascan 7.0 T, Bruker) equipped with a cryo-probe. Brain metabolism was analysed by quantitative MRS in the motor cortex and cerebellum, to evaluate possible somatosensory alterations and/or deficits in motor coordination circuitries. Finally, structural alterations were studied by DTI (30 gradient directions).
MRS shows an increase of glutamate in the motor cortex of fasudil-treated mice. Also, fasudil increases total choline content in both brain regions examined and creatine/phosphocreatine ratio in the cerebellum. The decrease in phosphocreatine could be indicative of a higher brain energy consumption; these metabolic alterations, however, were not paralleled by motor coordination or locomotor activity impairments. Further studies are needed to clarify whether brain metabolism alterations induced by chronic fasudil treatment may have effects on more subtle motor functions or additional behavioural domains.

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[P-50]

SOLID-STATE NMR CHARACTERIZATION OF NEW PYRIDOXINE CRYSTAL FORMS

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Keywords: solid state NMR, small molecules, biomolecules.

In recent years, multicomponent crystal forms, such as salts and co-crystals, have gained considerable attention in the field of pharmaceutical research due to their ability to modulate pharmaceutical and physicochemical properties (*e.g.* stability, solubility, manufacturability and bioavailability) of solid Active Pharmaceutical Ingredients (APIs) [1]. A key challenge for pharmaceutical companies is to determine whether a proton transfer has occurred between the coformer and API, resulting in the formation of a salt, or if a co-crystal has been formed, where the API and coformer engage in neutral hydrogen bonding and supramolecular interactions. Several techniques can be used to characterize multicomponent solids, in particular X-ray diffraction from single crystal (SCXRD) is usually used to get an in-depth structural analysis, however, it cannot always be performed because it requires high-quality single crystals; in these cases, solid-state NMR (SSNMR) provides an excellent method to characterize multicomponent crystal adducts and to distinguish salts and co-crystals [2].
This work is focused on pyridoxine (PN), a vitamin of B group, and its new crystal forms with 2-ketoglutaric acid (2KGA), pimelic acid (PMA), cinnamic acid (CNA), gallic acid (GLA), N-acetylcysteine (NAC) and caffeic acid (CFA). PN adducts have been synthesized using mechanochemical methods, so they resulted in fine powders, and it was possible to obtain the XRD crystal structure only for PN-CFA while all the others are in powdered form. Consequently, SSNMR spectroscopy was used for the structural characterization and for detecting proton transfers. Thanks to the induced shifts of the ^{13}C and ^{15}N signals of the involved groups, it was possible to determine the ionic character of the hydrogen bonds in the new crystal forms of PN. More specifically, in our systems, a carboxylic acid group is present in each coformer and a high-frequency shift of its signal in the ^{13}C CPMAS spectra of all adducts indicated a salt formation. In addition, the formation of salts of PN was confirmed also by ^{15}N CPMAS spectra, where a low-frequency shift of the ^{15}N pyridine signal of more than 80 ppm could be observed [3]. These data overcome the uncertainty given by the pK_a rule, that is an empirical rule typically used by Food and Drug Administration (FDA), European Medicines Agency (EMA) and pharmaceutical companies for predicting the formation of co-crystals and salts [4]. In fact, the ΔpK_a value of all the new crystal forms is in a range ($-1 < \Delta pK_a < 4$) where the rule cannot define if a salt or a co-crystal will be formed.

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[P-51]

SOLID-STATE AND SOLUTION NMR FOR THE CHARACTERIZATION AND PROPERTIES ANALYSIS OF NEW CRYSTALLINE ADDUCTS

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Keywords: solid state NMR, solution NMR, small molecules, biomolecules.

NMR, both in solution and in the solid state, is an advanced analytical technique that has versatile applications in pharmaceutical research and development. For instance, its potential for the characterization and evaluation of the properties of pharmaceutical multicomponent crystalline systems, such as molecular salts and cocrystals, has been explored. Solid-state NMR (SSNMR) allows obtaining structural information and investigating the character of hydrogen bond interaction enabling the discernment between cocrystals and salts without the need of single crystal X-ray diffraction (SCXRD) analysis, *i.e.* with powdered crystalline samples [1]. Solution NMR, on the other hand, has proven to be an efficient technique for investigating the stoichiometry of crystalline adducts and the physicochemical properties of Active Pharmaceutical Ingredients (APIs), including solubility [2].
Herein, SSNMR was employed for the characterization of five new tyramine crystalline adducts of nonsteroidal anti-inflammatory drugs – ibuprofen, ketoprofen, S-naproxen, flurbiprofen, and diflunisal – obtained by mechanochemical techniques. For all the adducts 1D ^{13}C and ^{15}N CP MAS NMR experiments were acquired to have a first confirmation about the formation and the nature of the products. For those systems for which single crystal could be obtained, SCXRD analysis were performed. For the others or when XRD did not allow the determination of the ionic or neutral nature, 2D SSNMR ^{1}H-^{1}H DQ MAS and ^{14}N-^{1}H D-HMQC experiments were carried out, which allowed obtaining further information on the chemical structure and the protonation state of the investigated samples. Solution ^{1}H NMR experiments were acquired to confirm the stoichiometry deducted from SSNMR and to evaluate the solubility of the APIs in the presence of tyramine, as an alternative technique to HPLC-UV, which is typically used for that purpose. This analysis allowed observing an increase in solubility of APIs in the adducts compared to the pure form.

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[P-52]

APPLICATION OF ^{13}C CP–MAS NMR TO ASSESS THE TRACEABILITY OF APULIAN DURUM WHEAT

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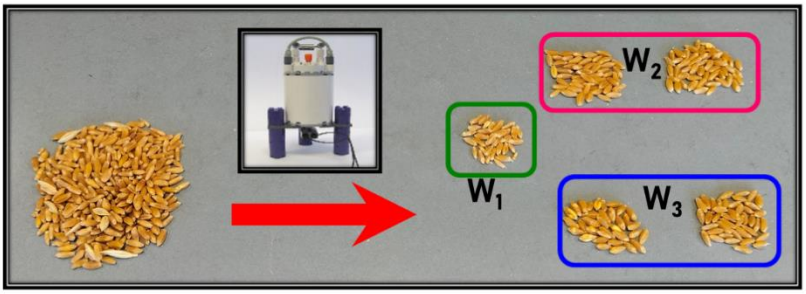
Keywords: solid state NMR, metabolomics, food.

Applications and developments of solid-state NMR methods are constantly growing in many research fields including food analysis. The development and introduction of new pulse sequences along with the improvements made to the instrumentation, allows NMR to extract more and more information related to the structure of the food samples. Numerous studies have shown that NMR in solution coupled with multivariate statistical analysis is an effective and reliable combination for metabolic studies in food chemistry. Even though less exploited of solution NMR, solid state NMR is also gaining attention and popularity [1].
In the case of foods with low water content it is necessary to extract the metabolites but, often they represent only a small portion of the organic material, such as in the case of wheat where most of the organic component is made up of insoluble polysaccharides. Removal of the insoluble component involves a significant loss of information that sometimes can compromise the result of the multivariate statistical analysis. Combining the potential of ^{13}C SS-NMR spectroscopy with multivariate statistical analysis may represent a winning combination, but at the moment, there are few works which used this approach [2-3].
As part of the Iperdurum Project, we combined ^{13}C SS-NMR spectroscopy and multivariate statistical analysis with the aim of evaluating the effects of the farming practices on the metabolic profile of durum wheat cv Saragolla. The final goal is to select the wheat lots allowing for the production of high-quality bread and pasta. In this presentation, the preliminary results will be shown.



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[P-53]

FAST FIELD CYCLING NMR RELAXOMETRY: A GENERAL OVERVIEW OF THE MAIN APPLICATIONS

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Keywords: low field NMR, MRI, materials, food, polymers, contrast agents, theory and methods.

Fast Field Cycling NMR relaxometry is a non-destructive variable-field magnetic resonance technique which measures **the field dependence** of the NMR longitudinal spin-lattice relaxation time T1, as well as the transverse spin-spin relaxation time T2, as a function of the magnetic field strength in the range of a few kHz up to around 100 MHz, depending on the instrument. The information obtained is connected to the physical and chemical properties of a substance or complex material, becoming particularly useful in revealing information on slow molecular dynamics which can only be carried out at very low magnetic field strengths. FFC NMR relaxometry allows to characterize rotational dynamics, determine thermal activation energies, as well as discriminate between different molecular dynamics (solid/liquid, fast/slow, intermolecular/intramolecular) and characterize the surface exchange effects with solvents and other small molecules. These characterizations can in principle be made on any substance in any physical form: solid, liquid and in-solution. The technique has become a standard method for investigating the molecular dynamics and structure of a variety of systems and is enjoying success in several academic laboratories, where it is looked upon as an important analytical tool for NMR research and material characterization, both in industrial and academic environments. With this work, we aim to present the potential of the method and the advantages of working at low magnetic eld strengths, within a wide range of application fields, ranging from the characterization of materials, including polymers and porous media, of hetero-nuclei, up to the pharmaceutical, biomedical science, protein and food spoilage and quality control. [1]

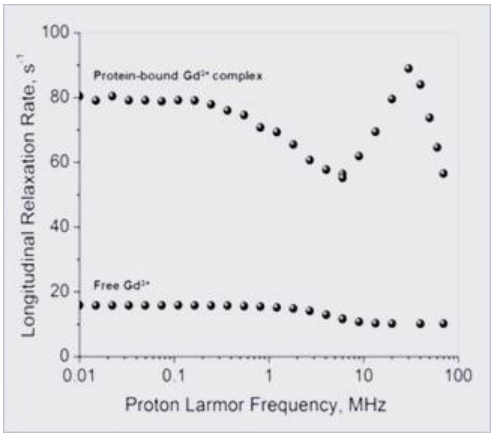

Fig. 1. NMRD profile of Gadolinium complex for MRI contrast agent.

References

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[P-54]

AN INNOVATIVE APPROACH FOR THE DESIGN OF A 20 MT, 90 CM BORE, BITTER-TYPE ELECTROMAGNET FOR EARTH FIELD MRI.

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Keywords: hyperpolarization, contrast agents, instrumentation.

In the poster we describe an innovative project for a Bitter-type electromagnet featuring 20mTesla B0 and 90cm inner bore, designed as a magnetic polarization coil for a 'pre-polarized Earth-Field MRI' system.
This novel Bitter-type electromagnet offers an excellent homogeneity over 60 cm sphere and presents a very simple mechanical assembly.
The pre-polarized earth field MRI system is developed within the PRIMOGAIA, Horizon2020 project, funded by the European community.[1]
The main objective of the whole project is to develop a suitable technology to search for new contrasts linked to molecular events for the very early diagnosis of pathologies.
Results, more details and specs of the magnet system will be shown in the poster.

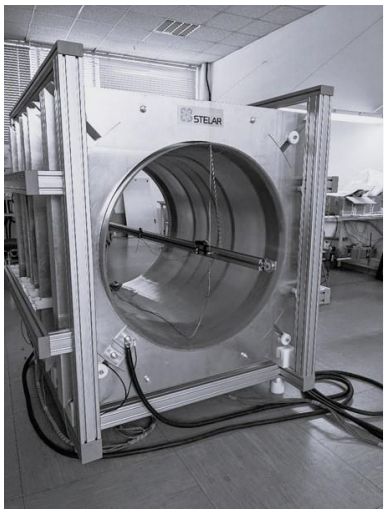

Fig. 1. Proprietary patented technology

References

[1] Project "Funded by the European Union."

[P-55]

HOW FUNCTIONAL CONNECTIVITY INFLUENCES BEHAVIOUR: A STUDY FROM THE WELSH ADVANCED NEUROIMAGING DATABASE

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Keywords: MRI.

Functional connectivity, which impacts all brain functions including cognition, can be assessed using resting-state functional magnetic resonance imaging (rsfMRI). This non-invasive technique provides high spatial resolution for evaluating brain connectivity. Loss of functional connectivity often occurs in neurological diseases and can precede measurable brain atrophy. We present preliminary results from the Welsh Advanced Neuroimaging Database (WAND), which aims to generate a comprehensive description of brain coupling across multiple domains. WAND includes data on macro- and micro-structural, functional, metabolic, chemical, and behavioural measures from a large population of healthy participants aged 18-65 years.

In this study, we analyze rsfMRI data from a subset of 103 participants in the WAND study. Eyes-open rsfMRI data were acquired on a Siemen's 3T Prisma scanner, with repetition time=2000ms, echo time=30ms and multiband factor=4. Physiological data such as heart rate and respiration were monitored during the scan. Data processing and analysis were performed using SPM, the PhysIO package, fMRIprep and FSL. Motion and physiological noise parameters were estimated for each participant. Noise regressors were used to remove physiological noise from the data following preprocessing with fMRIprep. Group ICA was used to reconstruct the main resting-state networks, and subject-specific network maps were obtained using dual regression. The relationship between the resting-state networks and behavioural data will be assessed using FSL's randomise.

Although the analysis is still ongoing, we have successfully identified physiological noise regressors for all participants. We anticipate finding significant associations between functional connectivity and age, as well as with behavioural test results.

[P-56]

TIME DOMAIN NMR ELUCIDATES FIBRIL FORMATION IN METHYLCELLULOSE HYDROGELS

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Keywords: low field NMR, materials, food, polymers.

Methylcellulose is obtained by methylation of cellulose of plant or bacterial origin. If the degree of substitution is sufficiently high, it presents water solubility and gelation at high temperature. Applications range from food science to oil extraction industry where it can be used as thickener. In the current view, gelation proceeds through the self-assembly of MC chains into a network of polymer rich fibrils trapping a polymer-lean phase mostly consisting of solvents.[1] This architecture generates a variety of dynamically separated populations of H atoms (see Fig. 1), an inviting environment for Time Domain NMR (TD-NMR) that comprises an array of techniques that can evaluate and probe each of these populations. [2]

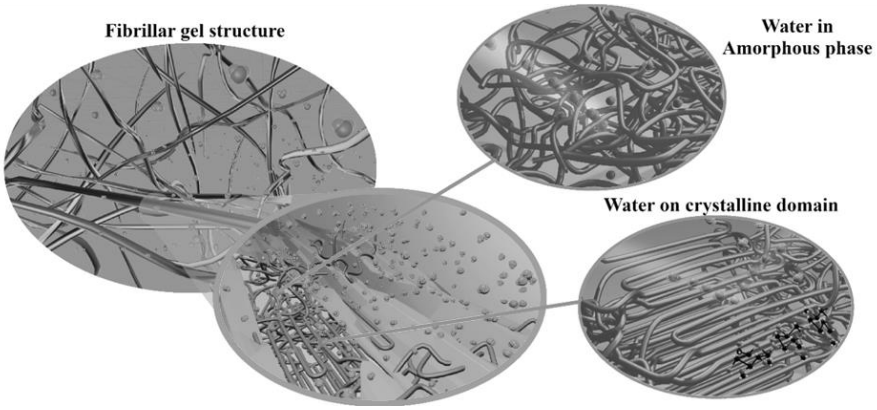

Fig. 1. When fibrils are formed, water is present in several dynamically distinct phases, including fast moving water molecules in dynamic exchange with the fibril surface, and water embedded within the fibril structure.

Low field T_1 and T_2 relaxometry at variable temperature, as well as PFGSE diffusion measurements, could characterize the motion of free water molecules and their exchange with the structures before, after, and even in real time during gelation. The use of Magic Sandwich Echo provided a quantitative measurement of the rigid fractions associated with fibril formation. All measurements were performed on a wide range of molecular weights and concentrations. The results confirm the current fibrillar model and furthermore describe the fibrils as semicrystalline where the amorphous phase is also significantly swollen with water.

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[P-57]

INTEGRATING MULTI-OMICS-BASED APPROACHES TO DISCOVER NOVEL IMMUNE-NMR-BASED METABOLIC FEATURES OF OVARIAN CANCER PATIENTS' ASCITES ASSOCIATED WITH TREATMENT RESPONSE

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Keywords: solution NMR, metabolomics.

Despite advances in the development of new therapies, epithelial ovarian cancer (EOC) remains the deadliest gynaecological cancer, mainly due to late diagnosis and the development of incurable chemo-resistant recurrences.
Residual disease, while helpful for patient management, is not accurate enough to determine treatment strategies for EOC. Therefore, there is a need to identify prognostic and predictive biomarkers of response to therapy and to develop novel approaches to assess drug response as additional biological endpoints.
The aim of this study is to develop a new multiparametric tool to discover biological features and potential prognostic and predictive biomarkers for assessing of response to EOC chemotherapy.
An integrated multi-omics approach based on NMR-based metabolomics and secretomes using Luminex multiplex technologies was applied to ascites from EOC patients with disease relapse within 6 (resistant) or 18 months (sensitive) after the end of treatment.
For each sample, three one-dimensional 1H NMR spectra with suppression of water peaks and different pulse sequences (NOESY 1Dpresat, CPMG and diffusion-processed pulse sequence) were acquired to selectively observe aqueous and lipid metabolites.
Multivariate statistical analysis revealed higher levels of alanine, isoleucine, acetoacetate and unsaturated fatty acids and lower levels of lactic acid, histidine, fumaric acid, phosphatidylcholine and its lyso derivatives and triacylglycerols in intact ascites from Pt-resistant EOC patients, as shown in the PLS-DA scores plot.
In parallel, we detected more than 40 systemic inflammatory markers such as cytokines, chemokines, matrix metalloproteinases and growth factors using Luminex technology. Secretome analyses revealed significantly higher levels of IL-1b, serpin E1, TNF-a, VCAM-1, IL-7, angiopoietin-1, UPAR and lower levels of MMP-12 ($p < 0.05$) in the resistant group.
Finally, correlation analysis identified metabolite signatures associated with immune profiles in EOC patients with different sensitivity to cisplatin.
Cluster analysis showed that PBEF/visfatin, adiponectin, resistin and INFg molecules were strongly correlated with metabolites involved in glucose and lipid metabolism, suggesting a potential immune-metabolic signature in the ascites of resistant and sensitive patients. By integrating these data with proteomics, transcriptomics and clinical parameters, we will discover new biological features to predict response to chemotherapy.

[P58]

UNTARGETED NMR METABOLOMICS FOR BIOMARKER DISCOVERY IN THE RARE MALIGNANCIES SOFT TISSUE SARCOMAS.

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Keywords: solution NMR, small molecules, biomolecules, metabolomics.

Soft tissue sarcomas are rare malignancies amounting to less than 1% of all human malignant tumors with an annual incidence of about 30/million. Even if the survival of the patients affected by these tumors has consistently increased in the last decades with an actual 65-75% 5-year survival in comparison with the past when they were almost always a fatal disease, a significant portion of the patients still die for distant metastases. Due to the rarity of the disease and its complexity (more than 50 histological types of soft tissue sarcomas were described), our comprehension of the disease is still limited, as limited are the therapeutic options available.
The investigation into biomarkers specific for soft tissue sarcomas is urgently needed. The aim of the present work is, therefore, the molecular signature characterization of primary soft tissue sarcomas, based on a combined analysis of genomic, transcriptomic, proteomic, and metabolomics studies, in search for selective local or circulating biomarkers related to different aggressiveness of the disease and possibly related also to different phases. Liquid biopsies were used for NMR metabolomic profiling of patients; in particular, a novel method for profiling intact serum was developed. Parallel to metabolomics, transcriptomics on liquid biopsies and proteomics on tissue samples was carried out.
Results
Our untargeted approach revealed the ketone bodies and serine-glycine metabolism to be dysregulated at a different level according to the progression-free survival of patients.
Conclusions
The ketone bodies (KBs) α-hydroxybutyrate and acetoacetate are important alternative energy sources for glucose during nutrient deprivation, a mechanism already observed in tumors. Such finding in soft tissue sarcomas opens the way to diagnostic and prognostic application.

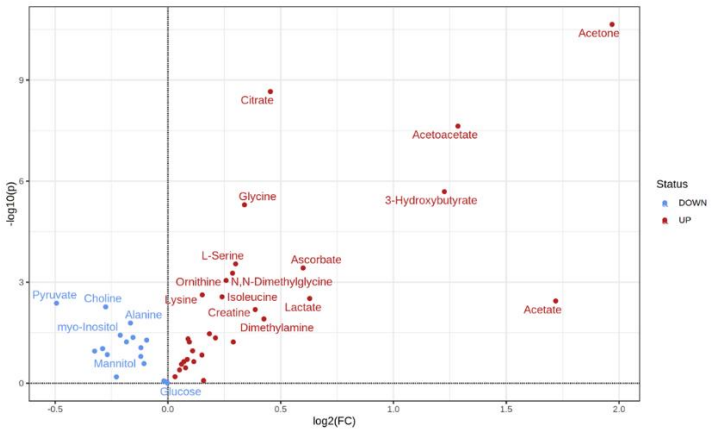

Figure 2. Volcano plot of metabolites from soft tissue sarcomas patients.