52nd National Congress on Magnetic Resonance

September 10-12, 2025 | Verona

Cà Vignal 3, University of Verona, Strada le Grazie 15, Verona

GIDRM
Gruppo Italiano
Discussione Risonanze Magnetiche

Università degli Studi di Verona
UNIVERSITÀ di VERONA

52nd *NATIONAL CONFERENCE on MAGNETIC RESONANCE*

10-12 September 2025, Verona

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Antonio Randazzo (*University of Naples Federico II*)

Luigi Russo (*University of Campania*)

GENERAL INFORMATION

VENUE:

University of Verona (Dept. Biotechnology)

Ca' Vignal 3, Strada le Grazie 15, 37134 Verona

INVITED SPEAKERS:

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

- Mauro Botta, University of Eastern Piedmont
- Anna De Angelis, University of California
- Danielle Laurencin, Institut Charles Gerhardt Montpellier
- Flaminia Cesare Marincola, University of Cagliari
- Michael Assfalg, University of Verona
- Daniel O. Cicero, University of Rome "Tor Vergata": Winner of the GIDRM/GIRM Gold Medal 2025

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

- Cristina Airoldi, University of Milan Bicocca
- Ilaria Boscolo Galazzo, University of Verona
- Elisa Carignani, CNR-ICCOM-Pisa
- Luca Fusaro, University of Namur
- Vito Gallo, Polytechnic University of Bari
- Nasrollah Rezaei-Ghaleh, University of Pavia
- Giacomo Saielli, ITM-CNR, Padova
- Alba Silipo, University of Naples Federico II

POSTER SESSIONS:

- **Poster session 1**

Wednesday, September 10th, 16:05-17:00, ODD abstract numbers

- **Poster session 2**

Thursday, September 11th, 10:45-11:35, EVEN abstract numbers

- **Poster session 3**

Thursday, September 11th, 12:35-13:55, ODD abstract numbers

- **Poster session 4**

Thursday, September 11th, 15:10-16:00, EVEN abstract numbers

WITH THE ENDORSEMENT OF:

GIDRM GRATEFULLY ACKNOWLEDGES ITS PARTNERS FOR FINANCIAL SUPPORT TO THE CONFERENCE:

GIDRM
GRUPPO ITALIANO
DISCUSSIONE RISONANZE MAGNETICHE

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Scientific Program

Wednesday September 10th

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: M.R. Chierotti**
14:30-15:20	**Plenary Lecture 1** **GIDRM/GIRM gold medal award** **Daniel O. Cicero (University of Rome "Tor Vergata")** Exploring more than explaining: a journey through GIDRM meetings and schools and the people who shaped it
15:20-16:05	**Plenary Lecture 2** **Chair: S. Borsacchi** **Danielle Laurencin (University of Montpellier)** ^{17}O isotopic labeling using mechanochemistry: recent advances and applications in high-resolution NMR
16:05-17:00	**Coffee break + Poster session (ODD abstract numbers)**

	Parallel session A **Chair: L. Russo**	**Parallel session B** **Chair: D. Laurencin**
17:00-17:30	**Rezaei-Ghaleh N.** An NMR perspective on biomolecular dynamics in dilute and condensed phases	**Carignani E.** Bulk and surface properties of lead halide perovskites by solid state NMR
17:30-17:45	**Diana D.** Structural determinants driving the binding between pdz2 domain of zo1 protein and viral slim sequences	**Rosso C.** "When SSNMR met pair distribution function…" a novel structure determination method of organic compounds
17:45-18:00	**Santoro F.** Targeting the tpp1-telomerase complex: ligand discovery, experimental validation, and therapeutic implications in cancer	**Mauri M.** Compatibilization process in clay/SEBS nanocomposites explored by TD-NMR
18:00-18:15	**Fabbian S.** Dissecting the structural complexity of the ATP synthase OSCP subunit through integrative NMR, SAXS and native MS	**Calucci L.** Unravelling CO_2 dynamics and CO_2-host interactions in metal-organic frameworks by solid state NMR

18:15-18:30	**Turchi F.** High-Resolution NMR Study of Ligand and Ca^{2+} binding to the C-Terminal Region of α-Synuclein	**Nardelli F.** Dissecting the glass transition temperature in anion exchange membranes using time-domain NMR spectroscopy

	Plenary session **Chair: A. Randazzo**
18:30-18:45	**Sponsorship Lecture (ABCS/nanalysis):** **A. F. G. Köring (Nanalysis Scientific Corp)** Benchtop NMR Applications in Forensic Analysis
18:45-19:30	**Plenary Lecture 3** **Anna De Angelis (Salk Institute for Biological Studies)** Fifty Shades of Suberin

Thursday September 11th

	Plenary session **Chair: G. Parigi**
8:45-9:30	**Plenary Lecture 4** **Michael Assfalg (University of Verona)** Investigating influences on the conformational states of the amyloid-forming tau protein
9:30-9:45	**Sponsorship Lecture (Bruker):** **Benevelli F. (Bruker Italia)** MAGNETIC RESONANCE Applied to Battery Research
9:45-10:15	**Under 35 GIDRM** **Chair: M.R. Chierotti** **Andrea Cesari (University of Pisa)** Solution state NMR toolbox: analytical approaches for small to large molecular systems
10:15-10:30	**Sponsorship Lecture (Jeol):** **Nishiyama Y. (JEOL Ltd.)** Recent advances in fast MAS from technical progress to applications
10:30-10:45	**Sponsorship Lecture (Magritek/FKV):**

10:45-11:35	**Coffee break + Poster session (EVEN abstract numbers)**

	Parallel session A **Chair: M. Botta**	**Parallel session B** **Chair: M. D'Onofrio**
11:35-12:05	**Boscolo Galazzo I.** Integrating magnetic resonance imaging and artificial intelligence: the future of diagnostics between innovation and precision	**Fusaro L.** Study of the crystallization of simple organic salts using multinuclear NMR in the liquid state
12:05-12:20	**Romiti C.** Orange-derived extracellular vesicles for drug delivery: isolation and labelling with MRI contrast agents for in vivo tracking	**Ceccon A**. Probing Self-Association of (+)-Catechin Coupled with Hydrogen-Deuterium Exchange by Solution NMR Spectroscopy

12:20-12:35	**Tamanti A.** Layer-wise analysis of cortical and periventricular alterations in multiple sclerosis using multiparametric MRI	**Petrone M.** NMR assisted biometal chelation study of new L-carnosine mimics: promising neuroprotective agents

12:35-13:55	**Lunch + Poster session (ODD abstract numbers)**

	Plenary session **Chair: S. Geninatti Crich**
13:55-14:40	**Plenary Lecture 5** **Mauro Botta (University of the Eastern Piedmont)** Multinuclear and multifrequency NMR techniques for probing water exchange in paramagnetic complexes
14:40-14:55	**Sponsorship Lecture (Stelar):** **Anne-Laure Rollet (Sorbonne-University Paris)** Fast field cycling NMR relaxometry to shed light on interfacial systems

	Chair: L. Ragona **Segre-Capitani Fellowship**
14:55-15:10	**Eleonora Truzzi (University of Modena and Reggio Emilia)** Application of NMR spectrometry on the study of lipid oxidation and antioxidant capacity of essential oils and extracts from agri-food wastes on
15:10-16:00	**Coffee break + Poster session (EVEN abstract numbers)**
16:00-18.30	**GIDRM assembly + announcement of poster competition winner**
19:30	**social dinner**

Friday September 12th

	Plenary session **Chair: D.O. Cicero**
8:45-9:30	**Plenary Lecture 6** **Flaminia Cesare Marincola (University of Cagliari)** NMR spectroscopy for food analysis: perspectives and applications in dairy products

	Parallel session A **Chair: F. Cesare Marincola**	**Parallel session B** **Chair: T. Vosegaard**
9:30-10:00	**Gallo V.** Validated NMR methodologies: a decade of harmonization towards collaborative diagnostic applications	**Saielli G.** Computational NMR spectroscopy: from heavy atoms to bulk liquid phases
10:00-10:15	**Grasso D.** Monitoring tyrosine metabolism in alkaptonuria: an NMR-based approach to evaluate nitisinone efficacy and off-target effects	**Lelli M.** Efficient polarizing agents for high field and fast mas DNP
10:15-10:30	**Molteni I.** Combining NMR spectroscopy and mass spectrometry for the metabolic profiling of solid matrices	**Kubrak A.** Insight into internal motions and interactions of biomolecules in blood serum using high-resolution relaxometry

10:30-10:45	**Marino C.** NMR metabolomic characterisation of lipedema tissues: a multi-omics study	**Napolitano E.** NMR metabolomics analysis of mouse brain extracts with differential DDO expression using parahydrogen-induced hyperpolarization
10:45-11:00	**Scioli G.** Metabolic diversity of different broccoli-rabe landraces (Brassica rapa l. subsp. sylvestris) from different area of Puglia	**Mascitti B.** Solution state Overhauser DNP enabled by functionalized gold nanoparticles

11:00-11:30	**Coffee break**

	Parallel session A **Chair: A. De Angelis**	**Parallel session B** **Chair: M. Assfalg**
11:30-12:00	**Airoldi C.** An NMR toolkit for the identification and development of biomolecular tools with diagnostic and therapeutic applications	**Silipo A.** Carbohydrates as keywords in the molecular dialogue
12:00-12:15	**Cortese F.** Urinary NMR fingerprinting of muscle invasive bladder cancer: a step toward safer prognosis	**Trivellato D.** A multi-technique approach illuminates the conformational changes of the repeat domain of tau protein upon ubiquitination
12:15-12:30	**Mimmi M.** Experience report on ASICS: automated metabolite quantification in 1D-^{1}H NMR of tissue extracts	**Parafioriti M.** Deciphering heparan sulfate recognition by SARS-COV-2 spike protein variants through NMR and molecular dynamics simulations
12:30-12:45	**Petrella G** Merging NMR fingerprinting and profiling to uncover the metabolic impact of synthetic opioids	**Bolognesi T.** Probing the role of structural disorder in SARS-COV-2 N by NMR: insights into polyanion binding mechanisms
	Plenary session **Chair: L. Ragona and M.R. Chierotti**	
12:45-13:25	**Poster competition winner lectures**	
13:25-13:30	**Closing**	
13:30-14:30	**Lunch**	

Wednesday 10th

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EXPLORING MORE THAN EXPLAINING: A JOURNEY THROUGH GIDRM MEETINGS AND SCHOOLS AND THE PEOPLE WHO SHAPED IT

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Keywords: solution NMR, small molecules, biomolecules, metabolomics

When I began to reflect on how best to recount my career in NMR, I quickly realized that much of it had unfolded through posters and oral presentations at the National Congresses of the GIDRM. Looking back on those moments is like revisiting decades filled with passion, numerous frustrations, and the occasional surprising result, always in the company of mentors and remarkable colleagues who helped shape my 40-year journey in NMR.

This lecture will retrace those experiences, shared in conference rooms throughout Italy since 1992, as a way to relive the thrill of presenting experiments, proposing interpretations, and engaging with the questions and critiques that deepened our understanding.

I've had the privilege of studying molecules of all types and sizes, from early work on sugars to proteins and eventually back to small metabolites. Through NMR, we uncovered some of the secrets encoded in their structures, interactions, and dynamic behavior in solution. More often than not, the questions left unanswered outnumbered the mysteries we managed to solve. But that, I've come to believe, is the essence of scientific research: it is more the art of asking than the ability to answer.

Teaching NMR has been equally fulfilling and has inspired me just as much as discovery. Since my initial invitation to the National GIDRM School in 2003, I have rarely missed a chance to assist new researchers in understanding the technique, its power, its potential, and its limitations.

My deepest hope is that the curiosity, dedication, and sense of wonder that guided my own path will live on in the next generation of scientists—and that they, too, will be captivated by the extraordinary discipline of NMR.

^{17}O ISOTOPIC LABELING USING MECHANOCHEMISTRY: RECENT ADVANCES AND APPLICATIONS IN HIGH-RESOLUTION NMR

D. Laurencin,[a] A. Peach,[a] N. Fabregue,[a] C. Erre,[a] Ph. Noël,[a] T.-X. Métro,[a] D. Gajan,[b] J. Trébosc,[c] F. Mentink-Vigier,[d] F. Scott,[d] C. Gervais[e]

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Keywords: solid state NMR, materials

Oxygen is the most abundant element at the surface of our planet, where it is found in both living organisms and inert matter. It is thus no surprise that since the early stages of NMR, efforts have been made to apply instrumental and methodological developments to the study of this nucleus. Yet, the only NMR-active isotope, oxygen-17, suffers from a very low natural abundance (0.04%), making it extremely challenging to investigate.

In 2017, our group in Montpellier proposed a new approach for ^{17}O isotopic labeling using ball-milling [1]. Over the years, we have expanded the number and diversity of molecules and materials labeled by this technique, in order to perform high-resolution ssNMR analyses [2]. In this presentation, after an overview of our current state of the art in terms of ^{17}O mechanochemical labeling and ssNMR analyses (Fig. 1), we will present some of our most recent results, which concern the enrichment of metal carbonates, in view of studying materials developed for CO_2 capture [3].

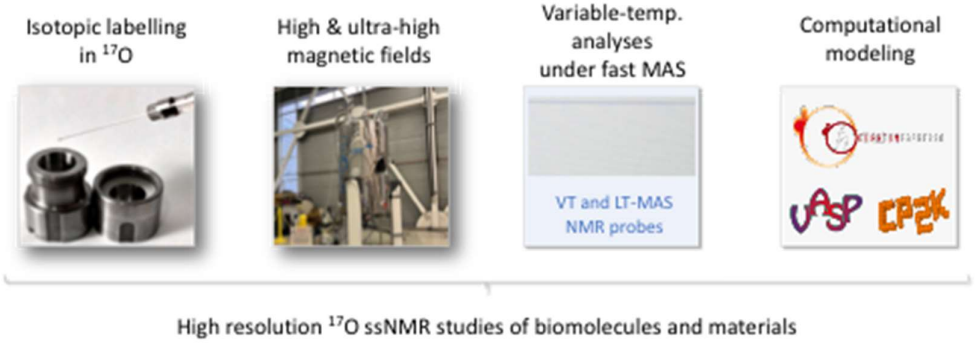

Fig. 1. Current experimental approach to high-resolution ^{17}O ssNMR analyses.

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AN NMR PERSPECTIVE ON BIOMOLECAR DYNAMICS IN DILUTE AND CONDENSED PHASES

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Keywords: solution NMR

NMR spectroscopy continues opening new routes to access the structure and dynamics of biomolecules within different contexts. These developments are particularly needed in the area of intrinsically disordered proteins, whose heterogeneous structures and complex multiscale dynamics often pose formidable challenges to elucidating their mechanistic roles in cellular processes. Here, I will present our latest research in the following areas: (i) phase separation of an intrinsically disordered peptide involved in the formation of Cajal bodies and RNA splicing, where we employ a glycine-based singlet-filtered relaxation method and demonstrate that the large nanoseconds dynamics of glycine residues are preserved within the crowded interior of the formed droplets [1], (ii) binding of the intrinsically disordered tau peptide with microtubules (MTs), where we develop a fluorine NMR-based approach and demonstrate how a frontotemporal dementia-related mutation in tau protein affects its conformation and MT binding, and (iii) the intrinsically disordered amyloid-β peptide, where the use of high pressure and fluorine NMR methods in combination with molecular dynamics simulation and density functional theory calculations elucidates some new aspects of its Alzheimer's disease-related misfolding and aggregation. Finally, I will present a quadrupolar NMR-based method allowing quantitative analysis of heterogeneous sodium ion dynamics within bimolecular condensates [2].

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[2] E. Nimerovsky, D. Sieme, N. Rezaei-Ghaleh *Methods* **228**, 54-60 (2024)

STRUCTURAL DETERMINANTS DRIVING THE BINDING BETWEEN PDZ2 DOMAIN OF ZO1 PROTEIN AND VIRAL SLiM SEQUENCES

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Keywords: solution NMR, biomolecules

The Envelope (E) protein is one of the main structural proteins that participates in many aspects of the viral life cycle such as virus maturation, assembly, and virulence mechanisms. [1] The E protein possesses a PDZ-binding motif (PBM) at its C-terminus that allows it to interact with the host PDZ domain containing proteins. One of the main binding partners of the E protein is ZO1, a protein with a crucial role in the formation of epithelial and endothelial tight junctions (TJs). [2,3] Particularly, E protein is directly involved in the mechanism of virus infection due to the presence in its primary sequence of Short Linear Motif (also known SLiM) that are responsible of protein-protein interaction with the PDZ2 domain of ZO1. Since the molecular details of such interaction have not been established, we performed a multidisciplinary study made up of experimental NMR techniques and computational docking to provide the structural determinants that drive the binding process between E-SLiM peptides and PDZ2-ZO1. This work could provide novel insights for the elucidation of the molecular mechanisms involved in the insurgence of the viral infections.

We acknowledge financial support under the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.1, Call for tender No. 104 published on 2.2.2022 by the Italian Ministry of University and Research (MUR), funded by the European Union – NextGenerationEU- Project Title Understanding molecular and structural determinants of the recognition mechanisms of the H. pylori CagA protein - Prot. 2022JY3PMB - CUP B53D23016290006 – Grant Assignment Decree No. 1017 adopted on 07/07/2023 by the Italian Ministry of University and Research (MUR).

References

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Targeting the Tpp1-Telomerase Complex: Ligand Discovery, Experimental Validation, and Therapeutic Implications in Cancer

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Keywords: solution NMR, small molecules, biomolecules.

The lifespan of a cell is intrinsically linked to the shortening of its telomeric DNA via the telomere maintenance mechanism [1]. Many cancer cells exploit this mechanism by employing the telomerase enzyme, assisted by RNA and the Shelterin protein TPP1, to elongate telomeric DNA strands [2,3]. This process enables the evasion of cellular senescence, thereby conferring cellular immortality [4,5]. Inhibiting the formation of the telomerase–TPP1 complex represents an attractive yet underexplored strategy to induce senescence in cancer cells. Through a targeted virtual screening of the "Oligonucleotide/Oligosaccharide-Binding" (OB) domain of TPP1, which anchors telomerase to telomeric DNA, we identified two promising small-molecule ligands. Their interaction with TPP1 was initially evaluated using ligand-based NMR techniques, specifically STD [6] and WaterLOGSY [7], which confirmed binding to the TPP1 OB domain. To obtain residue-specific through the assignment of the TPP1 OB domain in its free and bound forms and to gain insights into binding interfaces and structural rearrangements, we acquired a series of high-resolution 2D and 3D heteronuclear NMR spectra on uniformly ^{15}N,^{13}C-labeled TPP1 samples, including HNCO, HNCA, HNCACB, and CBCA(CO)NH [8–10]. To explore the effect of ligand binding on protein flexibility and internal motions, we also conducted NMR relaxation experiments (T_1, T_2, and heteronuclear NOE) [11]. These relaxation experiments shed light on the dynamical behavior of the protein. In parallel, secondary structure features were analyzed using chemical shift-based methods such as TALOS-N and secondary chemical shift indexing [12], to also assess possible conformational variations. These experiments provided a comprehensive framework to investigate both structural and dynamic properties of the protein in the presence and absence of ligands.

The pharmacological activity of the ligands was tested through anti-proliferative, pro-senescence, and telomeric DNA shortening experiments. Therefore, by integrating computational predictions with experimental methodologies, we can affirm the inhibitory potential of our compounds in disrupting telomere maintenance mechanisms. This integrated approach underscores the significance of TPP1 as a therapeutic target for various cancers, providing a promising foundation for future drug design campaigns focused on developing effective anticancer agents.

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DISSECTING THE STRUCTURAL COMPLEXITY OF THE ATP SYNTHASE OSCP SUBUNIT THROUGH INTEGRATIVE NMR, SAXS AND NATIVE MS

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Keywords:
solution NMR, biomolecules.

Defects in the regulation of mitochondrial Permeability Transition (PT) are involved in a wide range of human disorders, including cancer, neurodegenerative diseases, heart diseases, and aging [1]. To date, the molecular details behind PT remain elusive, severely limiting the therapeutic treatment of the pathologies associated with this phenomenon. The ATP Synthase subunit Oligomycin Sensitivity Conferral Protein (OSCP) is indicated as the key site for the PT regulation, emerging as a putative target for pharmacological strategies [2]. However, the study of OSCP as a potential PT-related therapeutic target has been so far hindered by the poor properties of the isolated protein in solution [3]. Here, we use an integrated approach based on Nuclear Magnetic Resonance (NMR), Small Angle X-ray Scattering (SAXS) and Mass Spectrometry under native conditions (nMS) to demonstrate that the isolated OSCP subunit is not affected by nonspecific aggregation, as previously reported in literature, but it is involved in a dimerization equilibrium. By the analysis of the anchoring region between the OSCP subunit and the b subunit of the ATP synthase, we identified a peptide mimicking the sequence of the C-terminal helix of the b subunit (b-CT) and capable to interfere with the OSCP dimerization equilibrium, stabilizing the monomeric state of the protein. Our characterization of the OSCP subunit paves the way for future therapeutic strategies against PT-related disorders, possibly improving our understanding of the mechanisms behind PT dysregulation in human diseases.

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High-Resolution NMR Study of Ligand and Ca^{2+} binding to the C-Terminal Region of α-Synuclein

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Keywords: solution NMR, small molecules and biomolecules.

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for characterizing the interaction between intrinsically disordered proteins (IDPs) and various binding partners at atomic resolution even in conditions approaching the physiological ones. Human α-synuclein (α-syn), an IDP implicated in several neurodegenerative diseases including Parkinson's Disease, is composed of 140 amino acids organized into three regions: a positively charged N-terminal tail, a hydrophobic central region, and a negatively charged C-terminal region. The latter has been shown to interact with both small molecules and metal ions.[1–4]

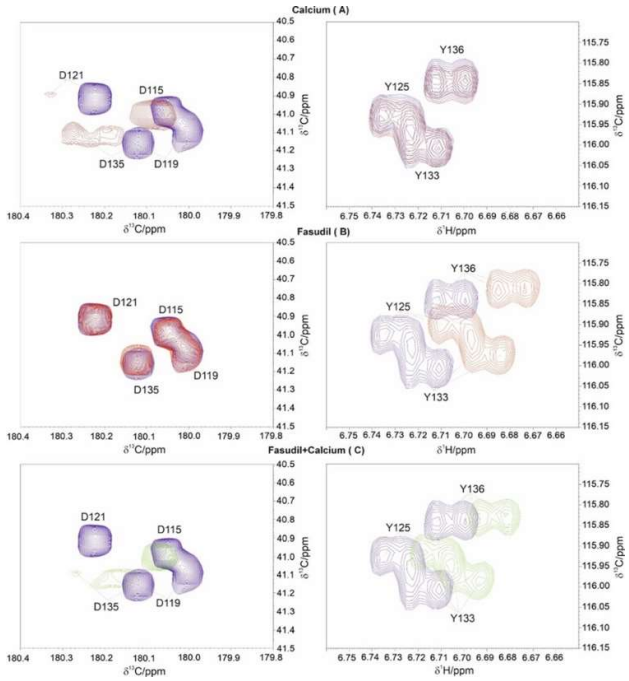

Fig. 1. Zoomed-in CACO spectra (left) highlight aspartate side chain resonances (C^{γ}-C^{β}); HC-TROSY spectra (right) show the H^{ε}–C^{ε} region. The free form of C-α-syn is always shown in blue. Panel A, B and C show the spectra upon addition of calcium, Fasudil and Fasudil + calcium in red,

In this study, we investigate the interaction between α-syn and Fasudil, a compound known to interact with the C-terminal part of α-syn and to delay the formation of its toxic aggregates.[1] We also examine the modulatory role of calcium ions in this interaction. For our study we use a construct comprising α-syn residues 112–140 (C-α-syn). High-resolution NMR spectroscopy exploring ^{13}C and ^{1}H detection, supported by molecular dynamics simulations, enables us to characterize these interactions at the side chains level. Our study focuses on the side chains of aspartic acid, glutamic acid, and tyrosine residues, which play a key role in Fasudil and calcium ions binding. We analyze their behavior in the presence and absence of calcium ions to elucidate calcium's influence in this process (Figure 1)

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Acknowledgements

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BULK AND SURFACE PROPERTIES OF LEAD HALIDE PEROVSKITES BY SOLID STATE NMR

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Keywords: solid state NMR, materials

Lead Halide Perovskites (LHPs) achieved a great success as semiconductor materials for high-performance photovoltaics, but also for many other optoelectronic applications. Impressive efforts have been dedicated to the development of the three-dimensional (3D) LHPs, but great attention has also been raised by their 2D analogs (Figure 1), and nanocrystals. In recent years Solid State Nuclear Magnetic Resonance (SSNMR) has demonstrated to be an excellent tool for the characterization of LHPs for its ability to study ion dynamics, compositional variations and ion incorporation, chemical interactions, and degradation mechanisms [1].

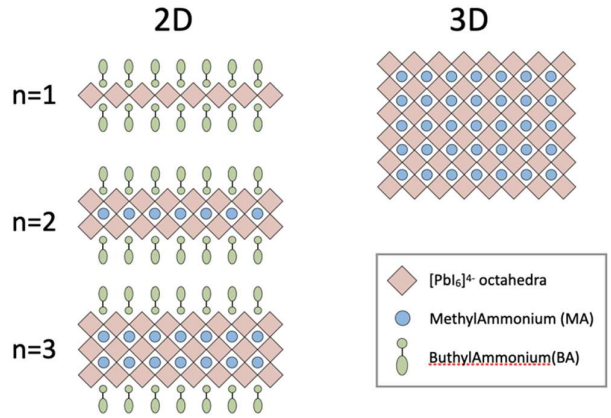

Figure 1: General structure of 2D Ruddlesden-Popper ($S_2A_{n-1}Pb_nX_{3n+1}$, with n=1,2,3) and 3D Lead Halide Perovskites ($APbX_3$).

In this contribution, recent studies on multiple-cation lead mixed-halide perovskite $Cs_{0.05}FA_{0.81}MA_{0.14}PbI_{2.55}Br_{0.45}$ [2], mono and multi layers 2D Ruddlesden-Popper (RP) phases containing butylammonium as spacer ($BA_2MA_{n-1}Pb_nI_{3n+1}$ with n=1-4) [3], and $CsPbBr_3$ nanocrystals [4] will be presented and discussed, showing how multinuclear SSNMR (^{133}Cs, ^{207}Pb, ^{13}C, ^{1}H) can provide detailed information on structural and compositional disorder, organic cation dynamics and surface properties of nanocrystals. Finally new developments on how isotopic enrichment can be used to study surface properties of powder and single crystal 3D LHPs will be shown.

PRIN 2022 **ISoTOPe** CUP: B53D23015330006 funded by the European Union - Next Generation EU, mission 4, component1, is acknowledged for financial support.

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"WHEN SSNMR MET PAIR DISTRIBUTION FUNCTION…" A NOVEL STRUCTURE DETERMINATION METHOD OF ORGANIC COMPOUNDS

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Keywords: solid state NMR, small molecules, theory and methods.

In recent years, there has been a growing interest in understanding the local structure of molecular solids, especially in systems where an average crystal structure cannot be determined, such as in poorly crystalline (*e.g.* nanocrystalline solids) or amorphous materials. In these cases, traditional methods, such as the gold-standard single-crystal X-ray diffraction (SCXRD) and the structure determination from powder diffraction (SDPD) approach, fail [1].
The Pair Distribuntion Function (PDF) has therefore emerged as a reliable approach for investigating short-range order, *i.e.* the local structure, of inorganic materials, liquids, glasses, and, to a lesser extent, of organic compounds [2]. Regarding the latter, Schlesinger et al. introduced the PDF-Global-Fit method with the aim of solving the local structure of organic substances from scratch by a fit to PDF data, without prior knowledge of lattice parameters and space group [1,3].
In this context, also solid-state NMR (SSNMR) provides insights into the local structure of solid organic compounds, due to its site-specific nature which enables probing the local chemical environment of individual sites. Through tailored 1D and 2D SSNMR experiments, critical information about the structure of the systems can be obtained, such as the number of independent molecules in the unit cell, the tautomeric and protonation states, hydrogen-bond networks, and interatomic proximities [4].
Herein, we propose a synergistic approach that combines the PDF-Global-Fit with the SSNMR data. The NMR information can be used both as restraints for the generation of structures and the PDF fit as well as in the structure selection steps, speeding up the overall process and increasing the likelihood of reaching a unique solution. We present the results obtained so far for crystalline organic compounds of pharmaceutical relevance, demonstrating the strength of this integrated approach in structure determination.

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Compatibilization process in clay/SEBS nanocomposites explored by TD-NMR

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Keywords: low field NMR, materials, polymers, theory and methods.

Nanocomposites between polymers and organophilic clays display unique properties stemming from the interaction between polymeric, molecular, and inorganic components.[1] Time Domain NMR (TD-NMR) has the potential to elucidate structural and dynamic characteristics of such complex systems [2], assisting the development of even more tailored systems.
Here we incorporate functionalized bentonite into SEBS, a thermoplastic elastomer with rigid nanophase separated polystyrene blocks acting as physical crosslinkers at room temperature, and an aliphatic ethylene-butene block that imparts rubber-like properties. Depending on the amount of butene copolymer, this can be amorphous or display partial ordering, possibly a rotator phase.[3]

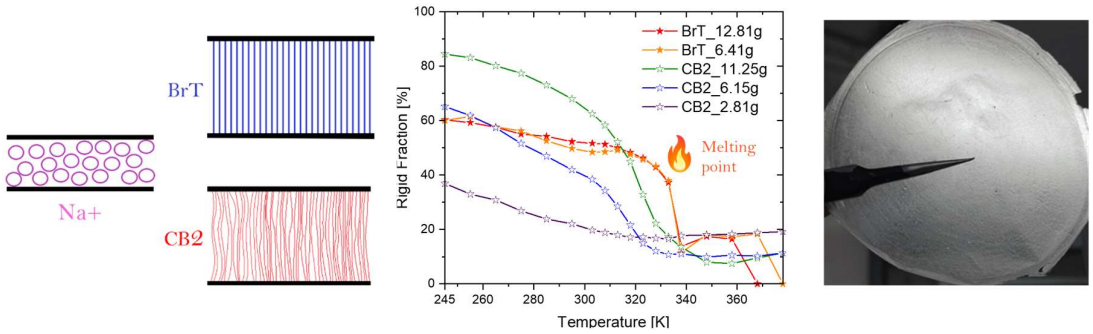

Fig. 1. Left: schematic representation of the substitution of Na+ cations with quaternary ammonium ions with aliphatic (BrT) and aromatic (CB2) substituents, the former resulting in a more structured system. Middle: rigid fraction measured using MSE refocusing block, highlighting the sharp phase transition of the aliphatic chains in the BrT clays. Right: a 40% loaded solvent cast nanocomposite

We synthesized several organo-clays by substituting the inorganic Ca^{2+} or Na^{+} cations of the mineral with quaternary ammonium ions bearing various combinations of aliphatic and aromatic groups, and characterized the mobility of the organic substituents with TD-NMR. Alkyl chains within the galleries of the clay form locally ordered phases that display an abrupt decrease of the rigid fraction corresponding to melting, as revealed by Magic Sandwich Echo in Fig. 1. The presence of aromatic substituents produces locally disordered domains that display a glass transition that is further modulated by the loading relative to theoretical capacity. The order within the substituted layer tunes the compatibility with the polymer. Both clays can be easily dispersed into a low butene SEBS up to 100% wt, but the ones with aromatic substituents present significantly improved mixing likely because the more disordered gallery environment facilitates chain diffusion
Initial measurements indicate the CB2/SEBS composite is retains most elastomeric properties up at least to 40% loading, paving the way for applications as radiation resistant rubbers.

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UNRAVELLING CO_2 DYNAMICS AND CO_2-HOST INTERACTIONS IN METAL-ORGANIC FRAMEWORKS BY SOLID STATE NMR

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Keywords: solid state NMR, materials.

In the search for solid sorbent materials for CO_2 capture, metal-organic frameworks (MOFs), crystalline porous materials prepared by coupling metal ions or clusters with organic linkers to form a 3D coordination network, have recently attracted much interest in both the academic and industrial communities. Indeed, thanks to their high thermal stability, high porosity, and high surface area, joined with the possibility to finely tune pore size and shape by a rational choice of metal and linker, MOFs are ideal candidates for gas separation and storage. Among the various MOFs, some bear open metal sites, which have a prominent role in CO_2 adsorption, separation, and storage; in the absence of open metal sites, pore size and shape and weak MOF-CO_2 interactions govern the gas adsorption.
Considering the promising future of MOFs as CO_2 adsorbents, a deeper, more comprehensive understanding of the CO_2 adsorption mechanism and dynamics within a variety of MOF systems and topologies is crucial for the rational design and development of MOFs with improved performance in CO_2 capture.
Solid-state Nuclear Magnetic Resonance (SSNMR) spectroscopy is considered one of the most informative techniques in getting insight into molecular-level structural and dynamic properties of solid sorbent materials and adsorbed gases through a combination of high-resolution and static experiments.
In this work, multinuclear SSNMR techniques are employed to investigate CO_2 dynamics and CO_2-framework interactions in MOFs with different chemical composition and topology developed as sorbent for CO_2 separation in the framework of the EIC Pathfinder project DAM4CO2.

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DISSECTING THE GLASS TRANSITION TEMPERATURE IN ANION EXCHANGE MEMBRANES USING TIME-DOMAIN NMR SPECTROSCOPY

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

Green hydrogen has emerged as a highly promising technology for sustainable energy production, with applications spanning domestic, industrial, and transportation sectors. One of the most efficient and environmentally friendly approaches for its production is water electrolysis using anion exchange membranes (AEMs). These membranes are typically composed of polymer backbones functionalized with charged groups, whose chemical structure can be tailored to enhance membrane performance, efficiency, and durability. However, despite significant progress in recent years, further research is needed to fully optimize AEM performance and long-term stability under real operating conditions [1,2].
To this aim, it is essential to investigate how the chemical structure and dynamics of the polymer influence the functional properties of the membrane. Among these properties, the glass transition temperature (T_g) is a critical parameter, as it governs the onset of segmental polymer motion and is closely related to the operational stability and ion transport efficiency of the membrane. However, conventional techniques for T_g determination are typically performed in dry conditions and do not reflect the actual working environment of AEMs, which operate under fully hydrated conditions.
In the present work, we employed variable-temperature time-domain NMR experiments to probe the polymer chain dynamics associated with the glass transition in AEMs for water electrolyzers based on low molecular weight polyketone (PK). This approach enabled the identification of an NMR-derived T_g [3] not only under ambient conditions, but also when the membranes are fully immersed in D_2O, thereby closely simulating the operational environment of water electrolyzers.

Acknowledgments: PRIN PNRR 2022 "In MoTion-Influence of dynaMics on the adsorption and Transport properties in polymeric materials for membrane technologies" funded by the European Union - Next Generation EU is acknowledged for financial support.

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Benchtop NMR Applications in Forensic Analysis

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Keywords: low field NMR, solution NMR, small molecules, instrumentation.

The forensic analysis of illicit drugs demands rapid, reliable methods that can keep pace with an ever-changing drug market. Traditionally, forensic laboratories have primarily relied on chemical tests, such as Nik Wipes, optical spectroscopy, handheld Raman spectrometers, and gas chromatography-mass spectrometry (GC-MS) for drug identification. While less commonly used in this context, NMR spectroscopy offers valuable capabilities for both identification and purity determination. It enables non-destructive, rapid analysis across a wide dynamic range, without requiring large volumes of flammable solvents or calibration curves. The adoption of high-field, superconducting NMR technology in forensic work has historically been limited by high capital costs, large footprint, and the need for highly trained staff.

The emergence of benchtop NMR spectrometers based on permanent magnets addresses these limitations by offering an affordable, low-maintenance, and user-friendly alternative. These compact instruments enable the identification, differentiation,[1] and quantification[2] of drug substances in forensic laboratories, as well as directly at crime scenes, customs checkpoints, and in harm reduction settings. In this talk, we highlight the expanding role of benchtop NMR in forensic drug analysis, showcasing selected applications that illustrate how this accessible technique is modernizing forensic workflows and paving the way for future advances in illicit drug detection.

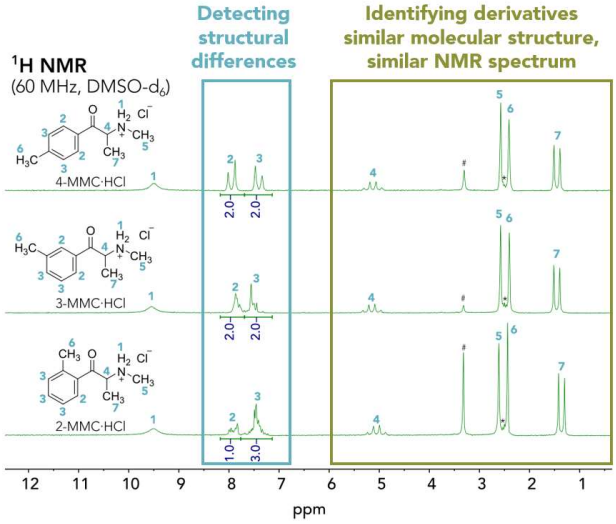

Fig. 1. Benchtop NMR enables the detection of structural similarities and differences in designer drug derivatives.

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Fifty Shades of Suberin

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

Suberin, the main component of cork, is a plant biopolymer found in the outer bark of trees, roots, seeds, abscission zones (wounds), fruit skins, leaf bundle sheaths and more. Suberin has unique physicochemical properties: it is highly impermeable to gas and water, chemically recalcitrant and exhibits very slow biodegradation. Different models for suberin in the literature include acyl and aromatic glycerol esters, linear alkyl and aromatic esters, and polyphenolic ethers. We present a new model of the lamellar structure of the plant biopolymer suberin, which integrates solid state NMR with Transmission Electron Microscopy, Mass Spectrometry, EPR and other chemical methods. The suberin lamellae are described as extended, alternating layers of crystalline, glycerol-esterified long-chain fatty acids, alternating with amorphous domains comprised of glycerol esters of fatty acids and phenolic compounds. These amorphous layers also contain the majority of suberin's mono-and polyunsaturated fatty acids. Suberin is characterized as a family of polymers that are structurally similar but exhibit variable composition and properties depending on the plant species and specific tissue. The role of suberin structures as an adaptive response is demonstrated with results from *Tidestromia oblongifolia*, also known as the "Death Valley plant".

Thursday 11th

	Plenary session **Chair: G. Parigi**
8:45-9:30	**Plenary Lecture 4** **Michael Assfalg (University of Verona)** Investigating influences on the conformational states of the amyloid-forming tau protein
9:30-9:45	**Sponsorship Lecture (Bruker):** **Benevelli F. (Bruker Italia)** MAGNETIC RESONANCE Applied to Battery Research
9:45-10:15	**Under 35 GIDRM** **Chair: M.R. Chierotti** **Andrea Cesari (University of Pisa)** Solution state NMR toolbox: analytical approaches for small to large molecular systems
10:15-10:30	**Sponsorship Lecture (Jeol):** **Nishiyama Y. (JEOL Ltd.)** Recent advances in fast MAS from technical progress to applications
10:30-10:45	**Sponsorship Lecture (Magritek/FKV):**
10:45-11:35	**Coffee break + Poster session (EVEN abstract numbers)**

	Parallel session A **Chair: M. Botta**	**Parallel session B** **Chair: M. D'Onofrio**
11:35-12:05	**Boscolo Galazzo I.** Integrating magnetic resonance imaging and artificial intelligence: the future of diagnostics between innovation and precision	**Fusaro L.** Study of the crystallization of simple organic salts using multinuclear NMR in the liquid state
12:05-12:20	**Romiti C.** Orange-derived extracellular vesicles for drug delivery: isolation and labelling with MRI contrast agents for in vivo tracking	**Ceccon** A. Probing Self-Association of (+)-Catechin Coupled with Hydrogen-Deuterium Exchange by Solution NMR Spectroscopy
12:20-12:35	**Tamanti A.** Layer-wise analysis of cortical and periventricular alterations in multiple sclerosis using multiparametric MRI	**Petrone M.** NMR assisted biometal chelation study of new L-carnosine mimics: promising neuroprotective agents
12:35-13:55	**Lunch + Poster session (ODD abstract numbers)**	

	Plenary session **Chair: S. Geninatti Crich**
13:55-14:40	**Plenary Lecture 5** **Mauro Botta (University of the Eastern Piedmont)** Multinuclear and multifrequency NMR techniques for probing water exchange in paramagnetic complexes
14:40-14:55	**Sponsorship Lecture (Stelar):** **Anne-Laure Rollet (Sorbonne-University Paris)** Fast field cycling NMR relaxometry to shed light on interfacial systems
	Chair: L. Ragona **Segre-Capitani Fellowship**
14:55-15:10	**Eleonora Truzzi (University of Modena and Reggio Emilia)** Application of NMR spectrometry on the study of lipid oxidation and antioxidant capacity of essential oils and extracts from agri-food wastes on
15:10-16:00	**Coffee break + Poster session (EVEN abstract numbers)**
16:00-18.30	**GIDRM assembly + announcement of poster competition winner**
19:30	**social dinner**

INVESTIGATING INFLUENCES ON THE CONFORMATIONAL STATES OF THE AMYLOID-FORMING TAU PROTEIN

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Keywords: solution NMR, small molecules, biomolecules

The microtubule-associated protein tau is a prototypical intrinsically disordered protein, characterized by a dynamic conformational ensemble in solution. Its conformational landscape is sensitive to perturbations from small molecules, solution conditions, concentration changes, and post-translational modifications. Tau plays a vital role in neuronal function but can undergo pathological aggregation into insoluble filaments, as seen in tauopathies like Alzheimer's disease. Understanding the triggers of these aberrant transitions requires inspecting the system from multiple standpoints.
In our lab, we investigate tau's structural responses to small molecules, nanoparticles, and post-translational modifications, combining solution-state NMR with complementary spectroscopic, calorimetric, and microscopic techniques.
We investigated the effects of unsaturated fatty acids and found that they promote tau self-aggregation and induce conformational changes [1]. Conversely, certain food-derived bioactive compounds—namely caffeine and genistein—were shown to inhibit tau aggregation in vitro, and to bind to preformed fibrils [2]. Using residue-resolved NMR and relaxation experiments, we mapped tau's interactions with ultrasmall gold nanoparticles, revealing dynamic nano–bio assemblies that could serve diagnostic or therapeutic purposes [3].
We elucidated the structural determinants underlying the molecular recognition between tau and a cognate ligase, responsible for its ubiquitination [4]. We also probed the effects of site-specific ubiquitination, showing that defined modifications promote compaction while retaining disorder, and in some cases suppress aggregation, pointing to a regulatory role in disease-relevant transitions [5].
Altogether, our findings underscore tau's sensitivity to diverse molecular cues and contribute to a mechanistic framework for targeting its pathological transitions in neurodegenerative disease.

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Thursday 11th | Sponsorship Lecture

MAGNETIC RESONANCE Applied to Battery Research

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

Magnetic resonance techniques have emerged as powerful tools in the investigation of battery materials and electrochemical processes. These non-destructive, atomically sensitive methods enable real-time, in situ, and operando studies of ion transport, phase transitions, and degradation mechanisms within electrodes and electrolytes. NMR spectroscopy provides detailed insights into lithium-ion dynamics, solid-electrolyte interphase (SEI) formation, and structural evolution during cycling. In this presentation, recent advancements of Magnetic Resonance in the field of Battery Research will be illustrated ranging from quality control and quantification of electrolytes, both at high field and on benchtop systems, to diffusion measurements and in operando studies. A focus on new hardware dedicated to this area of study is also included. This abstract highlights the transformative role of magnetic resonance in guiding the design of next-generation energy storage devices with improved performance, safety, and longevity.

SOLUTION STATE NMR TOOLBOX: ANALYTICAL APPROACHES FOR SMALL TO LARGE MOLECULAR SYSTEMS

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Keywords: solution NMR, materials, small molecules, polymers.

The present contribution focuses on the application of liquid-state Nuclear Magnetic Resonance (NMR) spectroscopy for an in-depth and comprehensive investigation of the stereochemical, dynamic, and thermodynamic properties of a wide range of chemical systems spanning from low to high molecular weights (Fig. 1). NMR spectroscopy stands as a fundamental analytical tool, capable of providing atomic-level insights into the structural and functional characteristics of materials across multiple disciplines, including biomedicine, materials science, and nanotechnology. Three major topics will be discussed: drug delivery systems, including nanoparticles based on cyclodextrins and biocompatible polymers, for controlled release and targeted therapy [1]; nanoparticles employed as NMR chemosensors for the detection of cancer biomarkers [2]; hybrid organic/inorganic optoelectronic materials such as perovskites, where understanding molecular interactions is essential for optimizing their production and performance [3]. The methodological framework and multi-experimental approach will be highlighted, providing examples that demonstrate the remarkable versatility and flexibility of NMR spectroscopy in addressing complex chemical challenges.

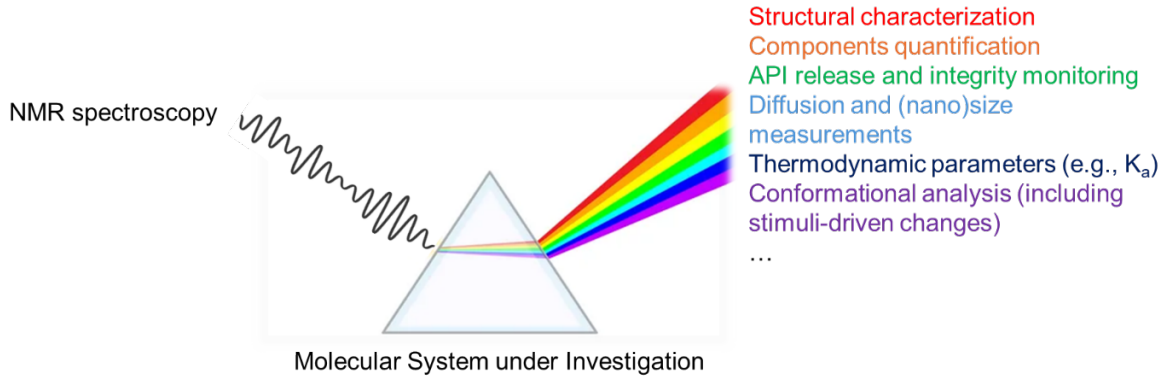

Fig. 1. Key information revealed by NMR spectroscopy.

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RECENT ADVANCES IN FAST MAS FROM TECHNICAL PROGRESS TO APPLICATIONS

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Magic Angle Spinning (MAS) is a fundamental technique in solid-state NMR (ssNMR). While most MAS ssNMR experiments are performed at moderate spinning rates of 10–20 kHz using rotors with diameters of 3.2–4 mm, fast MAS—exceeding 70 kHz—is now readily accessible in many laboratories.
In this presentation, we will highlight recent methodological advances in fast MAS and showcase its practical applications.

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Y. Nishiyama†, G. Hou†, V. Agarwal*, Y. Su*, A. Ramamoorthy*, Ultrafast Magic Angle Spinning Solid-State NMR Spectroscopy: Advances in Methodology and Applications, Chem. Rev. 123 (2023) 918–988. DOI: 10.1021/acs.chemrev.2c00197

ROUTINE REACTION MONITORING USING NOD-NMR: FROM MANUAL SAMPLING TO INLINE ANALYSIS

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Keywords:
solution NMR, low field NMR, NoD-NMR, Reaction Monitoring NMR.

NoD-NMR (NMR spectroscopy using non-deuterated solvents) has traditionally seen limited use, largely due to the reliance on high-field NMR systems located in centralized facilities. With the growing availability of benchtop NMR instruments, which can be placed directly in synthetic laboratories, NoD-NMR has become more accessible and is now increasingly applied in routine reaction monitoring.
This contribution presents examples illustrating how NoD-NMR can be used to follow chemical reactions either through manual sampling—common in research and development workflows—or through integration with flow reactors for online, real-time monitoring. In both cases, the use of standard solvents without deuteration simplifies sample handling and supports more direct analysis of reaction mixtures. Moreover, the detection of several NMR nuclei can be very useful to characterize reaction mixtures and the flexibility of modern NMR spectrometers in respect to measuring multiple nuclei is explained.
We discuss several case studies: discrete monitoring of Schiff base formation, online analysis of batch reactions and inline hydrogenation reaction monitoring using solvent suppression techniques. These examples highlight the method's applicability to a range of reaction types and conditions, and its potential to support both fundamental studies and process optimization.

INTEGRATING MAGNETIC RESONANCE IMAGING AND ARTIFICIAL INTELLIGENCE: THE FUTURE OF DIAGNOSTICS BETWEEN INNOVATION AND PRECISION

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Keywords: MRI, theory and methods, instrumentation

Magnetic Resonance Imaging (MRI) represents a crucial component of both research and clinical protocols, especially in fields such as neurology and cardiology, thanks to its ability to provide detailed morphological, metabolic, and functional information of the underlying structures. Several imaging modalities are nowadays available and continue to evolve rapidly, holding a central role in guiding diagnosis, monitoring and enhanced treatment of different conditions including neurological and neuropsychiatric disorders, tumours and cardiovascular diseases [1-2]. Such methodological advances have led to a substantial increase in imaging data and their complexity, calling for novel ways to extract relevant information as well as to enhance the operational efficiency in the medical field. Moreover, the availability of novel multimodal and multiscale data, not only from imaging but from diverse sources (e.g., healthcare records, genetics, proteomics, and wearable sensors), needs a shift in the way the data are integrated and interpreted, in order to provide a comprehensive view and enable precision medicine, with a possible multi-organ perspective [3-4].

In this framework, artificial intelligence (AI) has become a determining factor and its application on medical imaging can help improving processes as image acquisition, analysis, segmentation, feature extraction, and can serve as a component of clinical decision support systems [5-6]. While the advantages of AI are undeniable, the increased technical complexity and the lack of interpretability in many AI models, often criticized as "black boxes", raise doubts about the reliability, transparency and robustness of the final outcomes. These issues can possibly limit trust in the systems and their validity, especially in clinically-oriented settings [6-7]. Accordingly, eXplainable AI (XAI) has been proposed as a possible solution to interpret the AI results, increase trust, and uncover potential risks associated with complex approaches [8-9]. XAI has experienced significant growth over the last decades with several methods being proposed to deal with the different AI models and the data at hand, especially when considering high dimensional medical imaging data [9].

In this presentation, an overview of how AI and XAI can be combined with medical imaging will be illustrated, starting from the current state of research in the field and then selecting a series of case studies.

Moreover, their advantages, pitfalls, and limitations will be deepened, aiming to highlight some AI-based strategies currently adopted for MRI data as well as some possible future perspectives.

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ORANGE-DERIVED EXTRACELLULAR VESICLES FOR DRUG DELIVERY: ISOLATION AND LABELLING WITH MRI CONTRAST AGENTS FOR *IN VIVO* TRACKING

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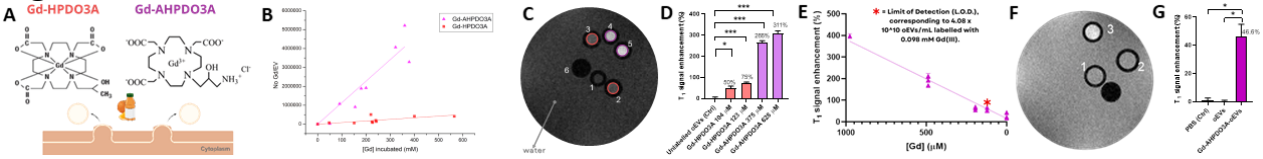
Keywords: MRI, contrast agents.

Extracellular vesicles (EVs) are nanosized membranous structures released by all cell types for intracellular cargo transport. By virtue of their natural origin, cell-specific tropism, and ability to cross physiological barriers they have been lately considered for drug delivery purposes. Unlike other EVs, edible plants-derived EVs are also abundant, easy to isolate, and stable at stomach pH, making them ideal to explore oral administration strategies [1]. However, their targeting efficiency and biodistribution are underexplored. EVs labelling with MRI contrast agents (CAs) could enable tracking their localization and tissue interactions. Orange-derived extracellular vesicles (oEVs) were isolated using ultracentrifugation [2] and characterized by Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS) to determine size, surface ζ potential, and concentration. oEVs (60-90 nm) were incubated with MRI CAs via osmotic stress, washed through studied dialysis cycles, precipitated through ultracentrifugation, and collected in 300 µL phosphate buffer. Gadoteridol (Gd-HPDO3A), and the amino-substituted Gd-AHPDO3A (Fig. 1A) were compared based on Gd concentration inside oEVs vs. incubated concentration, assessed through ICP-MS, measures of Relaxivity at 21 MHz, and T_1 contrast enhancement *in vitro* at 300 MHz. oEV isolation yielded stock samples containing 1.5×10^{12} oEVs/mL on average, and 5×10^{11} oEVs were used for each labelling condition. TEM and NTA analysis revealed no morphological changes pre- or post-labelling, besides no difference was found in the membranes of EVs exposed to both the CAs vs. water, indicating that our labelling procedure preserves membranes' integrity. oEVs labelled with Gd-AHPDO3A showed higher Gd incorporation (Fig. 1B) and a stronger longitudinal relaxation effect measured as T_1 signal enhancement at 300 MHz (Fig. 1C, D), making oEVs MRI-detectable up to a minimum concentration of 4.08[Equation]10^{10} oEVs/mL labelled with 0.098 mM Gd-AHPDO3A (Fig. 1E).This difference is likely due to the positive charge of Gd-AHPDO3A in water and its interaction with the negatively charged oEV surface (average ζ potential=-5 mV), enhancing Gd encapsulation. After having assessed oEVs nontoxicity on gastrointestinal cell models through MTT, oEVs uptake by Caco-2 cells has been investigated, resulting in a oEVs-driven Gd-AHPDO3A internalization and 47% T_1 contrast enhancement *in cellulo* (Fig. 1F, G). In conclusion, our isolation and labelling protocols efficiently enables a MRI-based investigation of oEVs as novel delivery nanocarriers that we envisage to further study also in mice.

Figure 1.



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LAYER-WISE ANALYSIS OF CORTICAL AND PERIVENTRICULAR ALTERATIONS IN MULTIPLE SCLEROSIS USING MULTIPARAMETRIC MRI

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Keywords: MRI

Introduction: Multiple sclerosis (MS) shows surface-in tissue damage patterns, with demyelination and neurodegeneration more pronounced near the cerebrospinal fluid (CSF) interfaces. This study assessed periventricular and cortical surface-in profiles using MRI estimations: magnetization transfer ratio (MTR), magnetization transfer saturation (MTS), R2* relaxation rate, and quantitative susceptibility mapping (QSM) in relapsing MS (RMS) relative to healthy controls (HC).

Methods: Forty HC (45.8±14.2 years) and 103 RMS (40.4±11.7y) underwent 3T MRI [1-3]. For the periventricular analysis, geodesic distance maps were created in the white matter excluding the dilated lesions masks. MRI metrics were averaged within each band equidistant from the ventricles. Cortical analysis sampled qMRI values at 25%, 50%, and 75% depths using intermediate surfaces used to fit slopes and intercepts to obtain vertex-wise surface-in profiles. Both the periventricular and the cortical metrics were normalized for the HC profiles. Statistical analysis employed linear mixed-effects models for periventricular data and permutation-based t-tests [4] for cortical vertex-wise comparisons. See Fig. 1 for a summary of the methods.

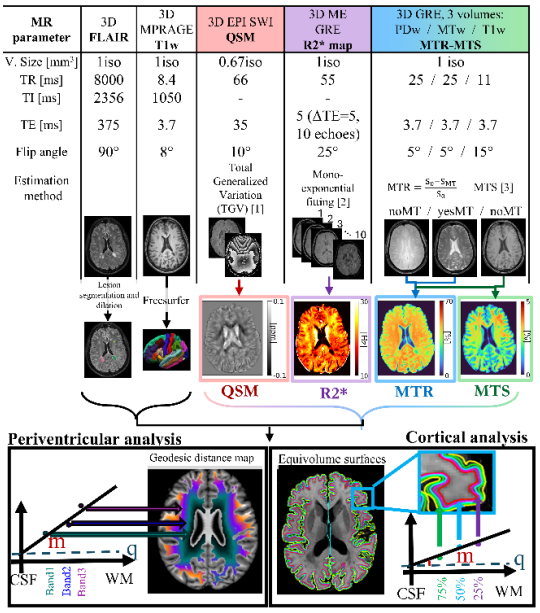

Fig. 1. Acquisition and processing steps

Results: Periventricular analysis showed significantly reduced MTR, MTS, QSM, and R2* in RMS patients vs HC ($p<0.01$), with greater alterations closer to the ventricles ($p<0.01$ for the band*group interaction). Cortical analysis revealed extensive clusters where RMS patients demonstrated higher MTR and MTS slopes and lower intercepts compared to HC ($p<0.05$, Fig. 2).

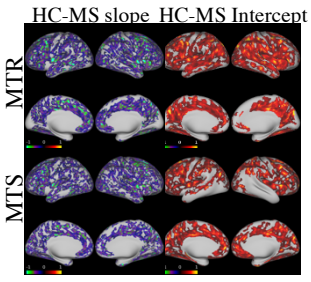

Fig. 2. Between-group clusters of significant differences in slope and intercept for HC vs MS

Conclusion: Layer-wise qMRI analyses demonstrated modest tissue alterations in MS periventricular and cortical regions, most pronounced near CSF surfaces, supporting CSF-mediated inflammatory damage mechanisms. This approach provides sensitive detection of MS alteration's patterns enhancing the understanding of pathological mechanisms.

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STUDY OF THE CRYSTALLIZATION OF SIMPLE ORGANIC SALTS USING MULTINUCLEAR NMR IN THE LIQUID STATE

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Keywords: solution NMR, small molecules.

Small organic molecules typically form simple supramolecular assemblies, resulting in crystal structures with relatively small unit cells. However, we have recently isolated four novel crystalline phases (**1–4**) of fampridine hydrochloride (4-APH$^+$Cl$^-$), a simple organic compound whose crystalline forms exhibit unexpectedly complex self-assembly behavior.[1,2] Remarkably, phases **1** and **2** represent the first reported occurrence of Frank-Kasper (FK) phases in small organic systems – FK are a class of highly ordered crystalline structures previously observed only in metal alloys [3] and soft matter. These two FK structures crystallized from a dense liquid phase (DLP) formed after liquid-liquid phase separation.

To further investigate the crystallisation behavior of simple salts capable of forming complex phases, we studied systems in which the cation was replaced with imidazolium or the anion with F^- or Br^-. Solution-state NMR experiments were performed at varying concentrations until crystallisation occurred. The cations were probed using ^{1}H, ^{13}C, and ^{14}N NMR, while ^{19}F, $^{35/37}$Cl, and $^{79/81}$Br experiments were employed to investigate the corresponding anions. Selected NOESY and ROESY spectra were compared with classical molecular dynamics simulations of the DLP to gain insight into solute–solvent interactions and pre-nucleation structuring. These results provide new perspectives on the role of specific interactions needed to obtain complex crystal architectures from simple molecular precursors.

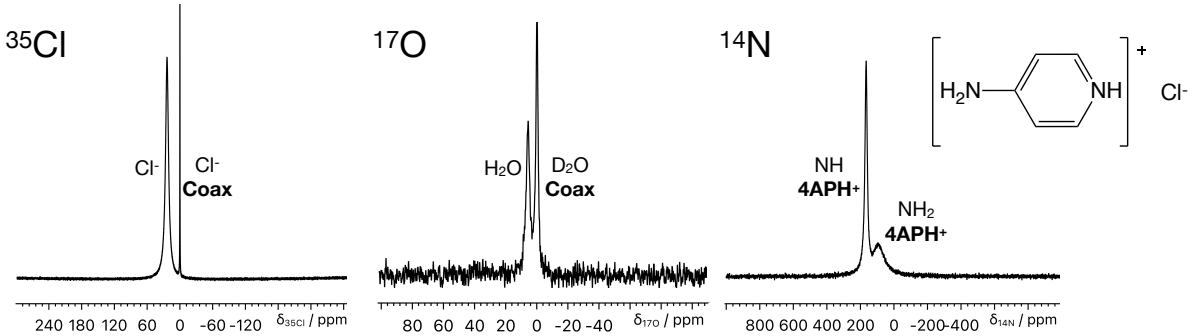

Fig. 1. ^{35}Cl, ^{17}O, and ^{14}N NMR spectra of DLP samples of fampridine-HCl, collected immediately after the LLPS, adapted from [2].

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PROBING SELF-ASSOCIATION OF (+)-CATECHIN COUPLED WITH HYDROGEN-DEUTERIUM EXCHANGE BY SOLUTION NMR SPECTROSCOPY

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

Flavan-3-ols, a subclass of flavonoids found in tea, wine, and other plant-derived foods, exhibit potent antioxidant activity and contribute to various health benefits.[1] Their ability to self-associate into supramolecular structures influences their stability, bioavailability, and function in complex matrices.[2],[3] In this study, we investigated the hydrogen–deuterium (H/D) exchange kinetics at the C6 and C8 positions on the A-ring of (+)-catechin, a widely occurring flavan-3-ol, using ^{1}H NMR spectroscopy. At low concentrations, the exchange follows a two-step pseudo-first-order mechanism, with slightly faster deuteration at C6 than at C8 under physiological conditions (298 K, pD 6).[4] Unexpectedly, higher catechin concentrations led to accelerated exchange rates, not attributable to pD variation but rather to reversible self-association. Through analysis of exchange-induced chemical shift changes (δ_{ex}), Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion data, and peak intensity time courses, we characterized a weak, transient monomer–dimer equilibrium (lifetime ~ milliseconds) (Fig. 1). Importantly, the deuteration rate within the dimer was up to 170-fold faster than in the monomer. These findings uncover a previously unrecognized role of transient self-assembly in modulating the reactivity of polyphenols in solution and underscore the relevance of H/D exchange at carbon centers as a sensitive probe for supramolecular dynamics in polyphenolic systems.[5] Furthermore, complementary LC-MS data provide insights into how reversible self-association influences irreversible oligomerization, leading to changes in dimeric procyanidins profiles, in terms of conversion rate and stereochemical preference.

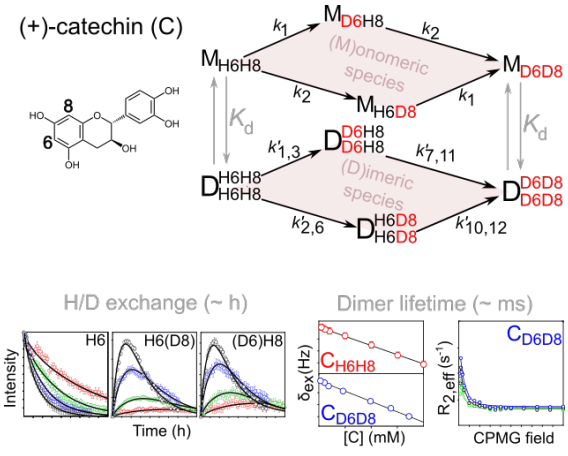

Figure 1. Kinetic model and NMR analysis of catechin H/D exchange and dimerization.

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NMR-ASSISTED BIOMETAL CHELATION STUDY OF NEW L-CARNOSINE MIMICS: PROMISING NEUROPROTECTIVE AGENTS

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

Alzheimer's Disease (AD) is a neurodegenerative syndrome that slowly destroys memory and thinking skills, resulting from the loss of brain cells and their connections. [1] It has a multifactorial etiology stemming from both accumulation and aggregation of misfolded proteins, and an **imbalance of metal ions** that promotes aggregation and neuroinflammation.
L-Car is a dipeptide (Figure 1) widely distributed in mammalian tissues and serves as a potential drug candidate [2] for neurodegenerative syndromes due to its radical scavenger, anti-inflammatory, antiaggregant, antiglycation, and **well-known zinc-chelating activities**. [3]
Since the therapeutic potential of L-Car is limited by its low bioavailability, we synthesized new L-Car mimics characterized by *phosphate isosteres* of the carboxylic group (Figure 1).
Therefore, here we illustrate the capability of the novel mimics to chelate the Zn(II) ion through **titration experiments** carried out by using the **NMR presaturation sequence ZGESGP** and to compare it with that of L-Car. The NMR analyses were performed at pH 7 and T 310 K to simulate physiological conditions, revealing two main consistent events in the spectra: a variation in chemical shift, common to both L-Car and the mimics, and broadening until complete flattening of the signals, occurring only for **Carol*p***. Based on the regions of shift and/or broadening, we anticipated the structures of the complexes, which will be confirmed by further experiments. Replacing the carboxylic group with the phosphate group enhances interaction with Zn(II), and this finding provides a basis for further experiments involving co-titration and diffusion and for designing and synthesizing novel isosteres with improved chelating profiles.

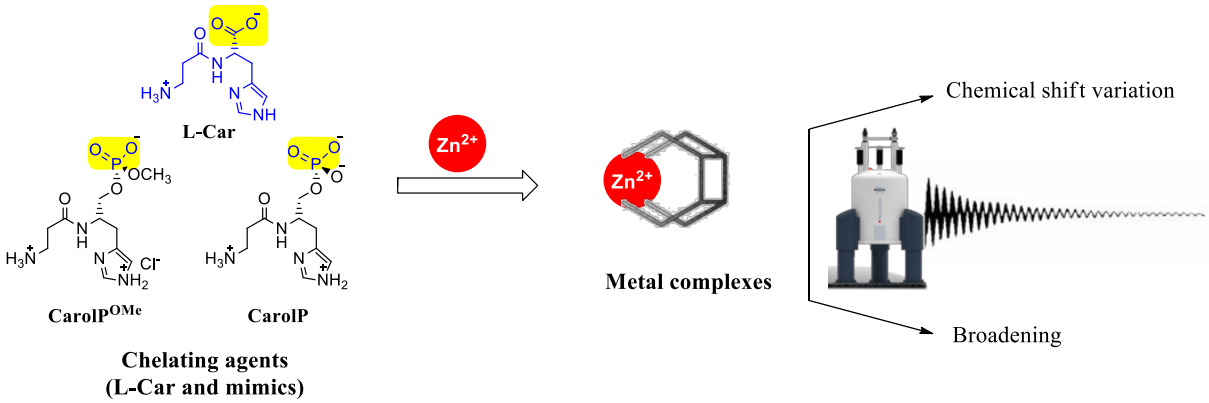

Fig. 1. L-Car and mimics Zn(II) titration via NMR strategy.

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MULTINUCLEAR AND MULTIFREQUENCY NMR TECHNIQUES FOR PROBING WATER EXCHANGE IN PARAMAGNETIC COMPLEXES

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

In clinical MRI, contrast agents (CAs) commonly comprise complexes involving the Gd(III) ion with multidentate poly-aminocarboxylic ligands, both acyclic and macrocyclic [1]. They possess inner-sphere water molecules in rapid exchange with the bulk, capable of catalysing the longitudinal and transverse relaxation times (T_1 and T_2, respectively) of bulk water, thereby modifying its NMR signal intensity [2]. The rate of water exchange (k_{ex}) stands out as a pivotal parameter significantly influencing the effectiveness of CAs in MRI. In Gd complexes, k_{ex} is affected by several key factors:

1. **Coordination Chemistry:** The chemical structure of the Gd complex and the nature of ligands coordinating with Gd ions play a significant role.
2. **Steric Effects:** The size and shape of the ligands surrounding the Gd ion affect the accessibility of water molecules. Bulky ligands can hinder water molecules from accessing the coordination sites, leading to slower water exchange rates.
3. **Electronic Effects:** The electronic properties of ligands and the Gd ion influence the rate of water exchange. Electron-donating ligands can enhance water exchange rates, while electron-withdrawing ligands might slow down the exchange process.
4. **Overall electrical charge**: Generally, the exchange rate tends to decrease when passing from anionic to cationic complexes
5. **Hydration Number:** Complexes exhibiting higher hydration numbers tend to demonstrate faster water exchange rates, attributed to the presence of more labile water molecules.

Understanding and controlling these factors allows chemists to design Gd(III) complexes with finely tuned water exchange rates, thereby enhancing the efficiency and diagnostic performance of MRI contrast agents. These effects will be illustrated through representative examples investigated in our laboratory. In addition, we will briefly highlight recent findings on Mn(II) and Fe(III) complexes, an emerging class of diagnostic probes [3].

We will also discuss cases involving bis-hydrated complexes, where the two coordinated water molecules exhibit distinct exchange rates, as well as ternary complexes containing fluoride ions, which offer unique insights into coordination dynamics and relaxometric behaviour [4].

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FAST FIELD CYCLING NMR RELAXOMETRY TO SHED LIGHT ON INTERFACIAL SYSTEMS

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Keywords:
solution NMR, low field NMR, materials, small molecules, biomolecules, food, polymers, theory and methods, instrumentation.

Fast Field Cycling (FFC) NMR relaxometry makes it possible to measure the relaxation rate over almost four decades of magnetic field. It means that it allows us to study the dynamics over four decades of timescale. Furthermore, the frequency range corresponds to the mesoscopic dynamics, that is not achievable by other techniques. Hence, FFC NMR relaxometry gives the unique possibility to the study the dynamics in the upmost relevant time domain for all complex systems: protein dispersions, colloid suspensions, porous materials, food, polymer, biological samples, etc [1]. We will illustrate this by presenting some of the most recent achievements on egg yolk based tempera paints.

Tempera paint is a fast-drying paint composed of pigment, water and binder. The interactions between the three components built during the formulation of the paint have crucial impacts on the paint drying and aging. FFC NMR relaxometry explores this problem by following the dynamics of water inside this complex mixture (Fig. 1).

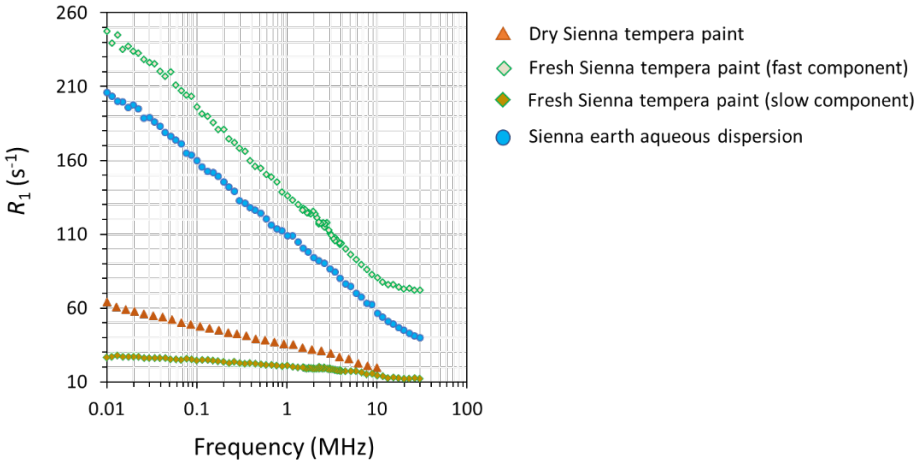

Fig. 1. NMR dispersion profile of tempera paint at the fresh and dry states and the comparison with the aqueous dispersion of the pigment (here Sienna earth).

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APPLICATION OF NMR SPECTROMETRY ON THE STUDY OF LIPID OXIDATION AND ANTIOXIDANT CAPACITY OF ESSENTIAL OILS AND EXTRACTS FROM AGRI-FOOD WASTES ON

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Keywords:
solution NMR, food.

Lipid oxidation (LO) is considered one of the major processes involved in food degradation that naturally occurs to mono- and polyunsaturated fatty acids. Oxidation reactions impair the nutritional value of foods due to the degradation and loss of essential fatty acids and vitamins. Also, the degradation of lipids plays a central role in determining the quality and shelf-life of animal- and plant-derived foods since it negatively affects the sensory properties of the product. As a consequence, oxidized foods are discarded. The LO is slowed down by the addition of synthetic or natural antioxidant additives in processed foods. However, consumer concerns about synthetic additives have increased the demand for natural solutions for food preservation in the last few years. Essential oils (EOs) are commonly used as flavorings in the food industry, and countless studies demonstrated their efficacy as antimicrobial and antioxidant agents for food preservation. Also, natural extracts from agri-food waste were proposed as food additives being rich in polyphenols, well-known antioxidant compounds. The recovery of these latter compounds leads to the valorization of agricultural products, reducing food wastage and promoting the circular economy and recycling, a central issue for the "European Green Deal". The LO is commonly studied via spectroscopic, fluorometric, or chromatographic methods to quantify the primary and secondary oxidation compounds involved in the process. In most cases, the first two methods are not accurate, reliable, and sensitive and are focused only on one type of oxidation product at a time. On the other hand, chromatographic methods are time-consuming and require calibration curves. Nuclear magnetic resonance (NMR) spectroscopy can represent a valid alternative method for quantifying LO in fats. NMR spectroscopy does not require extensive manipulation of the samples and simultaneously enables the identification, quantification, and differentiation of several compounds.
The present research aimed at the application of NMR spectroscopy for the study of LO and antioxidant mechanisms of natural extracts on animal- and plant-derived fats.
The LO was firstly induced on soybean oil under different accelerated conditions to identify the main degradation products by NMR via mono- and bi-dimensional experiments. Band-selective experiments were used to increase the receiver gain, resulting in improved digitization and signal-to-noise ratio [1].
Secondly, the LO was monitored on an O/W emulsion model prepared in buffer at pH 3 over weeks.
Finally, oregano EO and polyphenol-rich extracts of by-products obtained from the steam distillation of aromatic plants were tested as natural antioxidant at different concentrations.
The experiments are still ongoing and the preliminary results revealed that the natural extracts are capable of slow down the generation of hydroperoxides and secondary oxidation products.

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Friday 12th

	Plenary session **Chair: D.O. Cicero**
8:45-9:30	**Plenary Lecture 6** **Flaminia Cesare Marincola (University of Cagliari)** NMR spectroscopy for food analysis: perspectives and applications in dairy products

	Parallel session A **Chair: F. Cesare Marincola**	**Parallel session B** **Chair: T. Vosegaard**
9:30-10:00	**Gallo V.** Validated NMR methodologies: a decade of harmonization towards collaborative diagnostic applications	**Saielli G.** Computational NMR spectroscopy: from heavy atoms to bulk liquid phases
10:00-10:15	**Grasso D.** Monitoring tyrosine metabolism in alkaptonuria: an NMR-based approach to evaluate nitisinone efficacy and off-target effects	**Lelli M.** Efficient polarizing agents for high field and fast mas DNP
10:15-10:30	**Molteni I.** Combining NMR spectroscopy and mass spectrometry for the metabolic profiling of solid matrices	**Kubrak A.** Insight into internal motions and interactions of biomolecules in blood serum using high-resolution relaxometry
10:30-10:45	**Marino C.** NMR metabolomic characterisation of lipedema tissues: a multi-omics study	**Napolitano E.** NMR metabolomics analysis of mouse brain extracts with differential DDO expression using parahydrogen-induced hyperpolarization
10:45-11:00	**Scioli G.** Metabolic diversity of different broccoli-rabe landraces (Brassica rapa l. subsp. sylvestris) from different area of Puglia	**Mascitti B.** Solution state Overhauser DNP enabled by functionalized gold nanoparticles

11:00-11:30	**Coffee break**

	Parallel session A **Chair: A. De Angelis**	**Parallel session B** **Chair: M. Assfalg**
11:30-12:00	**Airoldi C.** An NMR toolkit for the identification and development of biomolecular tools with diagnostic and therapeutic applications	**Silipo A.** Carbohydrates as keywords in the molecular dialogue
12:00-12:15	**Cortese F.** Urinary NMR fingerprinting of muscle invasive bladder cancer: a step toward safer prognosis	**Trivellato D.** A multi-technique approach illuminates the conformational changes of the repeat domain of tau protein upon ubiquitination
12:15-12:30	**Mimmi M.** Experience report on ASICS: automated metabolite quantification in 1D-^{1}H NMR of tissue extracts	**Parafioriti M.** Deciphering heparan sulfate recognition by SARS-COV-2 spike protein variants through NMR and molecular dynamics simulations
12:30-12:45	**Petrella G** Merging NMR fingerprinting and profiling to uncover the metabolic impact of synthetic opioids	**Bolognesi T.** Probing the role of structural disorder in SARS-COV-2 N by NMR: insights into polyanion binding mechanisms
	Plenary session **Chair: L. Ragona and M.R. Chierotti**	
12:45-13:25	**Poster competition winner lectures**	
13:25-13:30	**Closing**	
13:30-14:30	**Lunch**	

NMR SPECTROSCOPY FOR FOOD ANALYSIS: PERSPECTIVES AND APPLICATIONS IN DAIRY PRODUCTS

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Keywords: solution NMR, food, biomolecules, metabolomics.

Nuclear magnetic resonance (NMR) spectroscopy is increasingly recognized as a powerful, non-destructive, and highly reproducible technique for the molecular characterization of complex food matrices. Among the various systems studied, dairy products have long represented a central application area due to their structural complexity and nutritional relevance.

Over the decades, the application of NMR in dairy science has evolved significantly. Early studies focused on the structural and dynamic characterization of macronutrients, such as proteins, lipids, and carbohydrates, shedding light on processes like thermal denaturation, fat emulsification, and protein gelation. Later, the technique was increasingly employed for advanced compositional profiling, supporting authentication, species and geographical traceability, and the assessment of technological processes.

More recently, the emergence of NMR-based metabolomics has enabled the detection and quantification of low-molecular-weight metabolites, offering novel insights into the biochemical effects of farming practices, microbial activity, and processing conditions. In parallel, its integration into nutrimetabolomics is contributing to a deeper understanding of the relationship between food components, metabolism, and human health.

This presentation aims to provide a concise overview of these developments, emphasizing the role of NMR in the compositional and functional analysis of dairy products within the broader context of food quality, traceability, and nutritional science.

VALIDATED NMR METHODOLOGIES: A DECADE OF HARMONIZATION TOWARDS COLLABORATIVE DIAGNOSTIC APPLICATIONS

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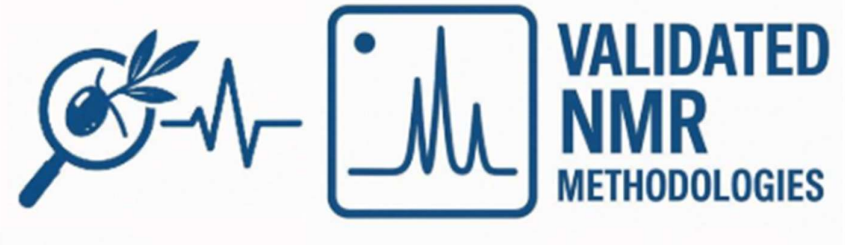
Keywords: solution NMR, metabolomics, food, theory and methods

The reliability of NMR-based analytical methods, especially when used for collective applications, critically depends on harmonized validation protocols. Over the last decade, a coordinated series of interlaboratory studies has progressively investigated the conditions for reproducible, transferable, and statistically equivalent NMR data generation across instruments, laboratories, and sample types. The journey began in 2012 with the first large-scale interlaboratory comparison (ILC) designed to assess quantitative NMR (qNMR) performance on multicomponent mixtures, introducing a performance index for calibration line consistency.[1] Following, studies expanded into non-targeted applications demonstrating the feasibility of community-built databases based on validated acquisition and processing protocols. These efforts culminated in the standardization of criteria for spectral equivalence across over 60 spectrometers, as documented in subsequent publications.[2-5] This decade-long validation framework enables a new generation of diagnostic systems. As a promising application, the early detection of *Xylella fastidiosa* infections in olive trees[6] exemplifies how harmonized NMR methods, originally developed for food authentication, can be adapted for detection of plant pathogen infections, supporting rapid and reliable decision support in agriculture.



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MONITORING TYROSINE METABOLISM IN ALKAPTONURIA: AN NMR-BASED APPROACH TO EVALUATE NITISINONE EFFICACY AND OFF-TARGET EFFECTS

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Keywords: solution NMR, small molecules, metabolomics.

INTRODUCTION: In the early 19th century, Dr. Archibald Garrod coined the term "inborn error of metabolism" to describe Alkaptonuria (AKU), an ultra-rare condition with an incidence of 1 in 1'000'000 also known as "black bone disease." Garrod rightly linked the discolouration of connective tissue (ochronotic pigment) to a phenolic compound (alkapton) buildup due to a missing enzyme. Nowadays, we know that alkaptonuria (AKU) is an autosomal recessive disorder caused by mutations in the HGD gene, leading to a deficiency of homogentisate 1,2-dioxygenase. This results in the accumulation of homogentisic acid (HGA), which is excreted in the urine and deposited in tissues, causing ochronosis, joint degeneration, valvular heart disease, and the rupture of ligaments, muscles, and tendons. The metabolic defect occurs in the tyrosine catabolic pathway, where HGA accumulates due to a block in the enzyme-mediated breakdown. nitisinone, a drug used to treat hereditary tyrosinemia, has emerged as a treatment for AKU, leading to the approval of Orfadin® for adult AKU patients. By inhibiting 4-hydroxyphenylpyruvate dioxygenase (HPPD), nitisinone reduces the production of HGA, thereby improving symptoms and preventing further metabolic damage. However, nitisinone treatment elevates plasma tyrosine levels, mirroring tyrosinemia type 2 and potentially causing eye damage. This highlights an urgent need for alternative or adjuvant treatments that can reduce HGA without triggering tyrosinemia.
AIM: Assess the impact of nitisinone on the tyrosine metabolism before and after treatment, analysing serum and urine samples of alkaptonuria patients via NMR spectroscopy.
METHOD: Serum and urine samples of 14 AKU patients were collected before and after nitisinone treatment. The samples were prepared and analysed through NMR spectroscopy; 15 principal metabolites of the tyrosine pathway were identified and quantified for both serum and urine samples.
RESULTS: As predicted, 4-hydroxyphenylpyruvate and tyrosine concentrations increase upon nitisinone administration. Additionally, 4-hydroxyphenillactate, 4-hydroxyphenilacetate and, partially, tyramine also increase in urine samples, suggesting a good metabolic clearance for these compounds. NMR has emerged as a valuable tool for monitoring the metabolic impact of nitisinone administration, with potential applications in other disorders related to tyrosine catabolism.

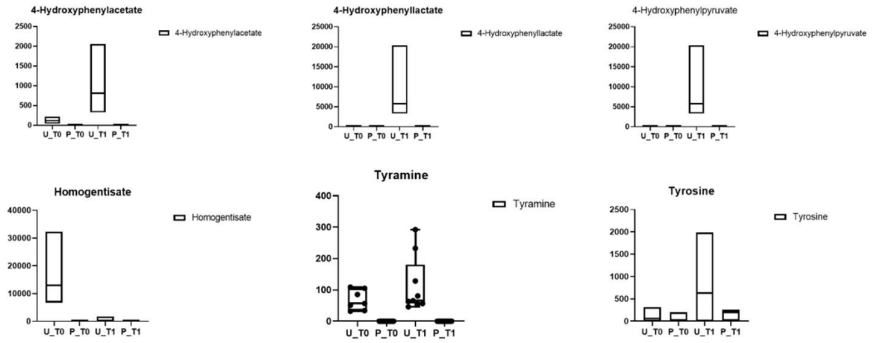

Fig. 1. Most affected metabolites after the nitisinone intake in plasma (P) and urine (U) samples.

COMBINING NMR SPECTROSCOPY AND MASS SPECTROMETRY FOR THE METABOLIC PROFILING OF SOLID MATRICES

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Keywords: solid state NMR, solution NMR, small molecules, biomolecules, metabolomics, food

The metabolic profiling of biological samples provides crucial insights into several physiological and pathological processes. Due to highly dynamic concentration ranges, extensive chemical diversity and different physical properties, the comprehensive characterization of complex samples may represent a true analytical challenge [1]. In this scenario, advanced analytical techniques, such as Nuclear Magnetic Resonance (NMR) spectroscopy, as well as Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS), represent the "gold standard" for the in-depth characterization of sample mixtures. While both techniques show advantages and disadvantages, their combination represents a powerful option to maximize metabolic coverage and facilitate compound identification and quantification [1-3].
NMR- and LC-MS-based metabolic profiling of complex matrices, such as whole cells, biopsies, or food flours, is usually carried out at the liquid state after metabolite extraction. Such approaches involve time-consuming sample manipulation; also, since extraction efficiencies are strictly metabolite-dependent, both relative and absolute quantification may be affected. To tackle this problem, the application of NMR analysis under High-Resolution Magic Angle Spinning (HR-MAS) conditions, as well as HR-MS employing the Atmospheric Solids Analysis Probe (ASAP), may be considered [4,5]; these techniques allow indeed the direct analysis of semi-solid and solid samples with minimal to no sample preparation.
Here, we report the application of both techniques to the analysis of metabolites contained in five different food flour samples (rice, quinoa, chickpeas, faba beans, and lentils). Data collected by extraction and analysis at the liquid state were compared with data provided under HR-MAS NMR and ASAP HR-MS conditions from both a qualitative and quantitative point of view by employing both targeted and untargeted multivariate analysis.
Once validated, this approach will be useful for rapid anti-fraud checks of foodstuffs [6]; also, it will increase the versatility and speed of NMR and MS analysis in resolving complex mixtures of organic compounds in a wide range of applications, minimizing solvent usage and environmental impact.

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NMR METABOLOMIC CHARACTERISATION OF LIPEDEMA TISSUES: A MULTI-OMICS STUDY.

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Keywords: solution NMR, biomolecules, metabolomics.

Lipedema is a persistent condition characterised by an asymmetrical and disproportionate increase in fat tissue, primarily affecting women's lower limbs. It is often accompanied by pain, tenderness, and a tendency to bruise easily, which significantly impacts the quality of life of affected individuals. The pathogenesis of lipedema remains poorly understood, and its aetiology and pathophysiological mechanisms are far from being clarified. [2,3] As a result, effective diagnostic and therapeutic strategies are limited. In this scenario, our study aimed to apply an NMR metabolomic approach to characterise the adipose tissue profile of patients with lipedema, comparing it with that of healthy controls following liposuction surgery. The metabolomic data obtained were further investigated using a multi-omics transcriptomic approach based on RNA-seq and epigenetic methods to identify the methylation levels of CpG islands. The integration of multi-omics data enabled us to confirm oestrogen deregulations, comprehend the role of mitochondrial dysfunction, and identify potential analytes for consideration in the treatment of lipedema.

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METABOLIC DIVERSITY OF DIFFERENT BROCCOLI-RABE LANDRACES (*Brassica rapa L. subsp. sylvestris)* FROM DIFFERENT AREA OF PUGLIA

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Keyword: Solution NMR, food, metabolomics, small molecules

Brassica rapa subsp. *sylvestris* (L.) Janch, commonly kwon as “friarielli”, “broccoletti” or “cime di rapa”, is a traditional leafy vegetable widely consumed in central and southern Italy. The edible part consists of inflorescences and the surround tender leaves, typically prepared according to traditional local recipes.
Despite its cultural and nutritional relevance, the molecular basis of its sensory and compositional diversity remains poorly investigated. This study aimed to investigate the metabolic variability among ten populations cultivated in Apulia by profiling water-soluble metabolites involved in taste and nutritional quality. NMR spectroscopy was used for metabolite identification, and multivariate statistical analyses were applied to assess metabolic variation and explore biodiversity at the molecular level.
Dry samples were extracted using a 1:1 (v/v) methanol:water solvent mixture and dried under a nitrogen flux. Each landrace was extracted three times for replication. All extracts were dissolved in a 1:1 (v/v) mixture of deuterium oxide (D_2O) and deuterated methanol (CD_3OD) containing 150 mM phosphate buffer (pH 7.0). ^{1}H-NMR spectra were recorded using a 600 MHz Bruker Avance III spectrometer, employing presaturation to water signal suppression. Metabolites quantification was carried out using trimethylsylil propionic acid sodium salt (TSP) as an internal standard for both quantification and spectral calibration.
A total of twenty-four metabolites were identified, including glucosinolates, amino acids, organic acids, sugars, and amines. PCA revealed clear population separation based on metabolite composition, while ANOVA highlighted significant genotype-specific differences, particularly in amino acids (isoleucine, valine, histidine, glutamine, and phenylalanine) and glucosinolates (gluconapin, and glucobrassicanapin) contents. Notably, the gluconapin/glucobrassicanapin ratio effectively discriminated Lecce-type landraces. These findings provide new insights into the metabolic diversity of *B. rapa* subsp. *sylvestris*, offering a biochemical framework for future breeding programs and valorisation of local germplasm.

Acknowledgements
The Agritech National Research Center, European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)- MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4–D.D. 1032 17/06/2022, CN00000022) - SPOKE 1, Task 1.2.2.

COMPUTATIONAL NMR SPECTROSCOPY: FROM HEAVY ATOMS TO BULK LIQUID PHASES

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Keywords: solution NMR, hyperpolarization, materials, small molecules, theory and methods

Computational NMR spectroscopy is now a well-established field of research in connection with the structural elucidation of natural substances. DFT calculated ^{1}H and ^{13}C chemical shifts and *J*-couplings of putative structures allow to discard or retain a given structural proposal of an unknown compound after comparison with the experimental parameters [1-3]. In this context, the complexity which is tackled with the calculations lies entirely within the complex and unusual carbon skeletons often found in natural products, leading to crowded NMR spectra arduous to interpret.
On the other hand, organometallic compounds, among which we can find for example catalysts and metallodrugs containing NMR active nuclei like ^{103}Rh, ^{113}Cd, ^{119}Sn, ^{125}Te, ^{187}Os, ^{195}Pt, may not exhibit very unusual molecular architectures, however the interpretation of their NMR spectra, either the organic part, as well as the resonances of the metal itself, is again far from trivial. This different kind of complexity stems almost entirely from relativistic effects related with the spin-orbit coupling in heavy atoms; in such cases most of the empirical rules commonly used to interpret and rationalize ^{1}H and ^{13}C spectra of organic molecules cannot be safely applied.
Finally, another kind of structural complexity in chemistry is the average structure of bulk fluids, like for example ionic liquids, where strong electrostatic interactions result in a nano-segregation between the ionic parts and the alkyl moieties. The microscopic details of the average bulk structure are hardly accessible from experiments, and MD simulations based on classical Force Fields are, in practice, the only tool to obtain a close view of the average local arrangement of the ions.
In this presentation I will go through some examples of the last two types of complexity mentioned above, showing the application of relativistic DFT computational protocols to the structural elucidation of organometallic systems containing heavy atoms and nano-structured bulk fluids. I will discuss recent results concerning iridium hydrides involved in SABRE experiments with hyperpolarized hydrogen [4] and osmium organometallic complexes. Then I will discuss bulk ionic liquids [5], as well as halomethanes probed by xenon [6], and highlight how the comparison of calculated and experimental chemical shifts averaged over the MD trajectory can shed light on the details of the Force Fields used for the MD simulations.
Finally, I will conclude with a perspective concerning through-space *J*-couplings in non-bonded systems and how the detection of this property might open new avenues for the structural investigation, by ^{129}Xe NMR, of liquid phases and porous materials [7].

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EFFICIENT POLARIZING AGENTS FOR HIGH FIELD AND FAST MAS DNP

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

MAS Dynamic Nuclear Polarization (DNP) has proved to be a valuable technique to enhance the sensitivity of more than two orders of magnitude (250-330 at 100 K at 9.4 T) in solid-state NMR. Nevertheless, the performances at 9.4 T are strongly reduced at hight field like 18.8 T (800 MHz of ^{1}H), 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, and is soluble only in organic solvents.[1,2] In 2020 we introduced novel, water soluble, dinitroxides biradicals, named TinyPol,[3] with an increased electron coupling that shows sensitivity enhancements comparable to AsymPol-POK.[4]

Here we present our further major improvements, introduced last year, in the radical design: optimizing a molecular geometry around the nitroxides and introducing protonated "antenna" side arms to promote the interaction with the bulk solvent, we significantly improved the DNP efficiency. In particular, M-TinyPol(OH)$_4$ showed enhancements up to about 200 even at 65 kHz of MAS frequency and 18.8 T (Fig. 1), overcoming previous bis-nitroxide DNP sensitivity performances.[5]

We also present a detailed analysis of the DNP efficiency of these improved TinyPol biradicals, comparing the experimental enhancements with DNP computational simulations that support the experimental data. DNP simulations, performed with a hybrid QM DNP model[6] and Molecular Dynamics, makes it possible to follow the propagation of the polarization within the solvent matrix unravelling the roles of the antenna sidechains in promoting the hyperpolarization spin-diffusion. The detailed comprehension of these processes opens the way to further progress in polarizing agent design.

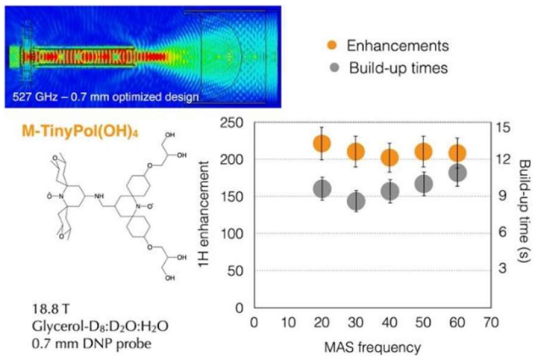

Fig. 1. Fast MAS DNP performances of M-TinyPol(OH)$_4$.

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INSIGHT INTO INTERNAL MOTIONS AND INTERACTIONS OF BIOMOLECULES IN BLOOD SERUM USING HIGH-RESOLUTION RELAXOMETRY

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

NMR Relaxometry provides tools to probe dynamic processes in a wide range of time scales by revealing the longitudinal relaxation rate R1 for magnetic fields of various orders. Nowadays, High-Resolution-Relaxometry (HRR) allows one to sense site-specific R_1 values of individual ^{1}H nuclei by taking advantage of the high sensitivity and resolution of high-field NMR spectrometers. Thus, insight into the local mobility of specific chemical groups and interactions between macromolecules and metabolites in complex biological mixtures can be obtained [1]. The detection of the relaxation rates over a large range of magnetic fields enables measurement of the spectral density function over a wide range of frequencies and therefore provides accessibility to mobility occurring from the pico to the nanosecond time scale [2]. In our studies, a conventional Bruker NMR spectrometer has been equipped with a prototypical Fast-Shuttle-System (FSS) and a Field-cycling-coil (FCC) [3] to effectively transfer the sample between low fields (down to ~ 100 μT) and ~ 14 T with temperature fixed to 298 K.
Here, we investigate the molecular dynamics inside blood serum. This complex mixture is frequently the subject of metabolomic analyses due to the many metabolites and macromolecules that compose it, which allow one to monitor the responses to diseases and drug intake [4]. During the experiments, high-resolved NMR spectra have been acquired at several magnetic fields and after the peak assignment nuclear magnetic relaxation dispersion (NMRD) profiles of single biomolecules, like lactate, creatinine or succinate were obtained. For that purpose, a home-built Python [5] script to process pseudo-2D NMR spectra has been used. NMRD profiles demonstrated the binding or its absence for each analyzed metabolite, comparing them with the profiles of the isolated molecules in solution. The changes in the rotation correlation time τ_c in fact indicate changes in binding. The results of our studies prove the applicability of HRR for metabolomics and biomedical studies.

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NMR METABOLOMICS ANALYSIS OF MOUSE BRAIN EXTRACTS WITH DIFFERENTIAL DDO EXPRESSION USING PARAHYDROGEN-INDUCED HYPERPOLARIZATION

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Keywords: solution NMR, hyperpolarization, biomolecules, metabolomics.

D-amino acids, historically thought to be restricted to bacteria and invertebrates, have been recently identified in mammalian systems. Among them, D-serine (D-Ser) and D-aspartate (D-Asp) are particularly abundant in the mammalian brain. While the physiological role of D-Ser is well characterized, the function of D-Asp within the central nervous system remains only partially understood. D-Asp level is extremely high during embryogenesis, but gradually declines after birth, due to the postnatal upregulation of D-aspartate oxidase (DDO), an enzyme that catalyses its oxidative deamination to oxaloacetate [1, 2].
To elucidate the functional implications of D-Asp in the mature brain, we performed a Nuclear Magnetic Resonance (NMR)-based metabolomics analysis on brain extracts from three groups of adult mice: i) wild-type controls ii) mice genetically engineered to lack the DDO gene (DDO knockout), iii) mice in which DDO expression is initiated from the zygotic stage. This comparison was performed to explore the neurochemical consequences of prenatal D-Asp depletion and sustained postnatal D-Asp accumulation.
For this study, we employed a parahydrogen-induced hyperpolarization (PHIP) NMR technique, which selectively enhances the signals of amino acid metabolites even within complex biological mixtures and allows for the determination of their stereochemical configuration [3, 4].
This technique was found to be particularly useful since several amino acids are directly or indirectly involved in neurotransmission. Furthermore, in contrast to classical NMR methods and mass spectrometry, it enabled the assessment of the chirality of amino acid metabolites – an important aspect for the present work. This application could represent an important example emphasising the potentiality of NMR hyperpolarisation techniques for targeted metabolomics research on amino acid alterations.

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SOLUTION STATE OVERHAUSER DNP ENABLED BY FUNCTIONALIZED GOLD NANOPARTICLES

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Keywords: solution NMR, hyperpolarization, small molecules.

Gold nanoparticles (AuNPs) coated with organic monolayers are used in catalysis and sensing due to their ability to interact with small molecules. Their structured surfaces enable weak, protein-like interactions in solution. In nuclear magnetic resonance (NMR), AuNPs may act as magnetization reservoirs, enabling selective magnetization transfer to interacting analytes and making them promising chemosensors [1]. While NMR typically relies on nucleus–nucleus magnetization transfer, we investigated whether electron–nucleus interactions can also enable Overhauser Dynamic Nuclear Polarization (OE-DNP) [2-4]. OE-DNP is a powerful method to enhance NMR signal intensities, provided that strong electron–nuclear coupling and molecular mobility are present.
To this aim, we designed AuNPs functionalized with a nitroxide-bearing thiol and a second thiol designed for hydrogen bonding; both thiols include alkyl spacers to allow mobility. Functionalization was achieved either during synthesis or via post-synthetic thiol exchange. NMR-DNP experiments were performed on a mixture of chloroform and methanol. ^{13}C enhancement on $CHCl_3$ aligns with OE-DNP theoretical predictions. However, it was found that methanol interactions with the monolayer likely mask it from the chloroform and prevent an effective polarization transfer. Although DNP on methanol was limited, these results confirm that AuNPs may act as polarizing agents with tunable recognition properties. The ability to combine enhancement efficiency with molecular recognition offers a strategy to refine hyperpolarization techniques and expand their use in NMR detection of specific analytes. Future improvements will focus on both monolayer design and the optimization of the OE-DNP setup.

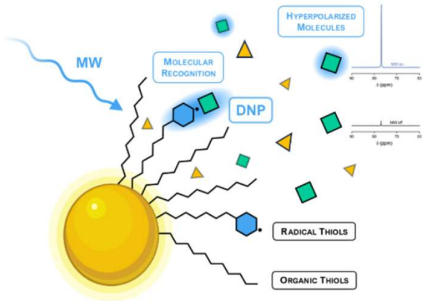

Fig. 1. Sketch of OE-DNP chemosensing experiment.

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AN NMR TOOLKIT FOR THE IDENTIFICATION AND DEVELOPMENT OF BIOMOLECULAR TOOLS WITH DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

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Keywords: solution NMR, small molecules, biomolecules

NMR-based molecular recognition studies allow the characterization of a wide variety of ligand-receptor pairs with biological and biomedical relevance [1].
We will report and discuss some selected examples covering binding events involving amyloidogenic proteins [2], oncoproteins [3], cell wall or membrane receptors [4] and the setup of methodologies for the analysis of heterogeneous systems, such as samples containing cells [1,3,4], or complex mixtures, such as natural extracts [1,2].
These methods provide structural insights for the rational design of molecular tools with diagnostic and/or therapeutic applications as well as for their rapid screening and optimization.

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URINARY NMR FINGERPRINTING OF MUSCLE INVASIVE BLADDER CANCER: A STEP TOWARD SAFER PROGNOSIS

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Keywords: metabolomics, solution NMR, small molecules

Muscle-invasive bladder cancer (MIBC) is a clinically aggressive malignancy associated with poor outcomes and high metastatic potential. Diagnosis currently depends on transurethral resection of the bladder tumor (TURBT), a procedure vital for histological confirmation and staging. However, TURBT is invasive and has been associated with complications such as tumor cell dissemination, which may adversely impact prognosis and therapeutic decisions [1,2]. These concerns emphasize the urgent need for reliable, non-invasive diagnostic tools for MIBC.
This study explored the urinary metabolic profiles of 139 bladder cancer patients from two clinical centers using NMR fingerprinting-based metabolomics to distinguish MIBC from non-muscle-invasive bladder cancer (NMIBC). Multiple linear regression on NMR binning data revealed consistent metabolic alterations, including increased lactate and decreased 3-indoxyl sulfate levels in MIBC patients. Moreover, a significant chemical shift variation was observed for creatinine between the two groups, without a corresponding change in concentration, suggesting its interactions with urinary components such as metal ions. This finding underscores the sensitivity of the NMR fingerprinting approach in capturing subtle spectral differences beyond concentration shifts.
Finally, a predictive model was developed using NMR data from patients with known tumor staging and applied to 30 urine samples from patients awaiting histological diagnosis. The model's classifications were later compared to actual outcomes, achieving 80% accuracy. This highlights the potential of urinary metabolomics for non-invasive diagnosis while providing insights into the biochemical understanding of tumor progression and systemic responses.

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EXPERIENCE REPORT ON ASICS: AUTOMATED METABOLITE QUANTIFICATION IN 1D-^{1}H NMR OF TISSUE EXTRACTS

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Keywords: solution NMR, metabolomics, theory and methods.

Introduction. Between 2017 and 2022 P.J.C Tardivel, G. Lefort *et al.* developed ASICS [1]–[3], a method for automated identification and quantification of metabolites in 1D-^{1}H NMR spectra of biofluids. ASICS builds a statistical linear model to fit a complex spectrum, using a library containing pure compound spectra. To date, only one external group has applied ASICS, and only for plasma. We present its use on tissue extracts in transplantation research.
Experimental procedures. NMR metabolomics supported a study on the effects of a drug, the PCSK9-inhibitor PEP 2-8, in a rat model of kidney transplant after circulatory death [4]. Samples consisted of 30 resections from paired kidneys (15 control, 15 treated), stored in optimal cutting temperature compound (OCT) at –80°C. The medium weight of frozen samples post-OCT removal was 50 mg.
A second study integrated tissue metabolomics to evaluate grafts and predict Primary Graft Dysfunction (PGD) in human heart transplantation [5]. Samples consisted of endomyocardial biopsies (EMBs) collected from 21 grafts in the perioperative phases of intervention. The medium weight of frozen EMBs was 10mg.
Hydrophilic metabolites were extracted and analyzed on a 700 MHz CryoProbe-equipped spectrometer using standard protocols. [6]. ASICS was used for automated metabolite identification and quantification from 1D-^{1}H spectra.
Results. In the rat model, ASICS quantified >150 of the 216 library compounds. Results aligned with histology and biochemical markers, revealing a reduction in oxidative stress as the likely mechanism of protection.
In the human EMBs, 75 metabolites spanning key metabolic classes were quantified. Despite the challenges of a pilot study on human patients, metabolic profiles correlated with clinical markers, including serum troponin and pre-retrieval medication.
Conclusions. This is the first application of ASICS to tissue extract metabolomics. Notwithstanding limitations of a quantification based on predictive models, ASICS shows strong potential: enabling automation, minimizing manual curation, and expanding metabolome coverage. It supports the use of NMR-based metabolomics as a rapid, cost-effective screening tool guiding more targeted analyses.

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MERGING NMR FINGERPRINTING AND PROFILING TO UNCOVER THE METABOLIC IMPACT OF SYNTHETIC OPIOIDS

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Keywords: metabolomics, solution NMR, small molecules

Fingerprinting and profiling approaches in NMR metabolomics are two sides of the same coin. On the one hand, fingerprinting can quickly identify overall similarities and differences in the metabolite composition of samples but locks the data into an instrument-dependent format and is limited in terms of metabolic knowledge [1,2]. On the other hand, profiling is more challenging and time-consuming but provides considerably more meaningful results from a biochemical point of view [1,2]. In recent years, a new approach to NMR profiling has been proposed. This approach, called SYNHMET (Synergic use of NMR and HRMS for METabolomics), combines data obtained separately from NMR and LC-MS, following an iterative process to increase both the number of quantified metabolites and the accuracy of their quantification [3].
We applied the fingerprint and SYNHMET approaches to analyze 63 urine samples from mice as part of a novel strategy for detecting New Psychoactive Substances (NPS), focusing on New Synthetic Opioids (NSOs). NPS are a wide range of new drugs in the illicit market that can evade standard drug tests. They have diverse structures, change quickly in the drug scene, and have largely unknown metabolic profiles, presenting evolving challenges for detection. This approach focuses on developing an indirect screening method for NSOs by measuring changes in endogenous urinary metabolites after exposure to these substances [4]. Combining fingerprinting and profiling enabled us to create a more robust and complete database with higher predictive information and meaningful biochemical insights. This matrix served as the starting point for statistical analyses aimed at identifying a common endogenous metabolic fingerprint and signature to the intake of both fentanyl and morphine, regardless of their chemical structure.

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CARBOHYDRATES AS KEYWORDS IN THE MOLECULAR DIALOGUE

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Keywords: solution NMR, small molecules, biomolecules.

The evaluation of the structure of biomolecules built up of carbohydrates is a very challenging task due to the inherent complexity of sugar chemistry, which also impairs any computerized/automated approach. Nevertheless, this is a fundamental mission devoted to understanding interaction events at atomic level, including host-guest cross-talk. The combined use of complementary, biophysical approaches, including NMR spectroscopy, computational and biophysical techniques, native MS, together with immunological experiments is essential to unravel structure, properties, functions of glycans and understanding the mechanisms at the basis of recognition of the sugar code. In this talk, I will give a special focus to the description of bacterial glycocode, either as beneficial mediator of host homeostasis and immune system development or when harmful to the host, and I will describe the chemical glyco-features located on bacterial cell surface able to tune eukaryotic immune responses.[1]

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A MULTI-TECHNIQUE APPROACH ILLUMINATES THE CONFORMATIONAL CHANGES OF THE REPEAT DOMAIN OF TAU PROTEIN UPON UBIQUITINATION

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Keywords: solution NMR, biomolecules

Intrinsically disordered proteins (IDPs) undergo numerous post-translational modifications (PTMs) that influence their conformational dynamics. This study focuses on tau, a microtubule-associated protein and prototypical IDP involved in regulating axonal microtubule dynamics in the human brain [1]. Abnormal tau aggregation into neurofibrillary tangles (NFTs) is a hallmark of tauopathies, including Alzheimer's Disease [1].
As an IDP, tau is subject to various PTMs that, when dysregulated, may promote toxic aggregation [1]. We focused on tau ubiquitination, which has been proposed to influence pathological fibril formation [2]. Using a disulfide-directed semisynthetic approach developed in our lab [3,4], we generated mono- and di-ubiquitinated tau species at disease-relevant lysines: K317, K353, and both K311 and K317.
To investigate how ubiquitination affects tau's conformational landscape, we used NMR spectroscopy, small-angle X-ray scattering, and ion-mobility mass spectrometry (IM-MS). Scaled MD simulations provided atomistic models consistent with experimental data. While secondary structure propensities and local mobility in tau's repeat domain remained largely unchanged, ubiquitination enhanced conformational compaction, depending on the site and number of ubiquitin attachments.
Our findings reveal a site-specific impact of ubiquitination on tau's solution-state conformations, advancing our understanding of how this PTM influences aggregation and phase separation, and shedding light on the molecular basis of pathological structural changes [5].

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DECIPHERING HEPARAN SULFATE RECOGNITION BY SARS-COV-2 SPIKE PROTEIN VARIANTS THROUGH NMR AND MOLECULAR DYNAMICS SIMULATIONS

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Keywords: solution NMR, biomolecules, polymers.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection begins with the recognition of cellular heparan sulfate (HS) by the receptor-binding domain of the spike protein (S1-RBD) [1]. This event facilitates the adhesion of viral particles to the cell surface and promotes the subsequent binding of S1-RBD to the angiotensin-converting enzyme 2 (ACE2) receptor, triggering the fusion of viral and host cell membranes. Heparin and other HS mimetics have been shown to inhibit viral entry in vitro [2].
This study aims to elucidate the structural basis of the interaction between HS and S1-RBD using ligand-based NMR techniques and computational methods, with a focus on how this binding is affected by the genetic evolution of the virus. Synthetic HS oligosaccharides were used as molecular probes to investigate the binding of HS to selected S1-RBD variants, including those from the Wuhan and Omicron strains. Saturation transfer difference (STD) NMR and transferred nuclear Overhauser enhancement (trNOE) experiments were employed to map the binding epitope and define the conformation of the ligands in the protein-bound state. Additionally, docking and molecular dynamics (MD) simulations revealed the contacts and intermolecular forces that drive the ligand-protein interactions, providing detailed 3D models of the complexes.
Key amino acid residues – R346, N354, R355, K356, R357, R466, K444 – were identified as principal HS-binding sites, leaving flexible loops of S1-RBD available to engage ACE2. Our data indicate that HS oligosaccharides interact with both the Wuhan and Omicron S1-RBD variants through multiple low specificity binding modes, with the ligands adopting diverse orientations across the protein surface [3]. The evidence of multiple binding modes, which enhances the initial attachment of viral particles to the cell surface, supports a highly dynamic interaction between HS and S1-RBD. This low-specificity interaction is thought to facilitate the diffusion of the virus toward the ACE2 receptor, enhancing its invasion efficiency [4]. Our results suggest that highly specific inhibitors may not be optimal against the spike protein. Instead, HS-based oligosaccharides with high affinity, including multivalent compounds, may be required to inhibit viral entry.
This research provides valuable insights into the early mechanisms of SARS-CoV-2 infection, guiding the development of novel therapeutic strategies.

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PROBING THE ROLE OF STRUCTURAL DISORDER IN SARS-COV-2 N BY NMR: INSIGHTS INTO POLYANION BINDING MECHANISMS

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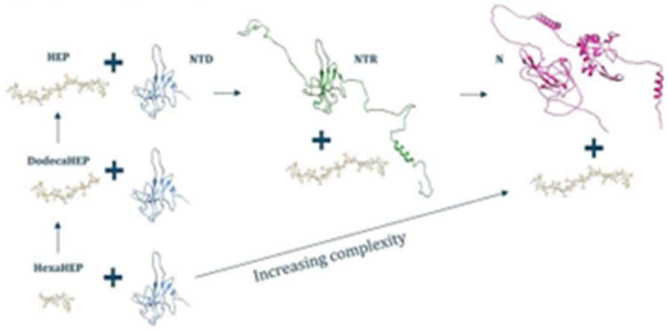
Keywords: solution NMR, small molecules, biomolecules.

Among the structural proteins of SARS-CoV-2, the nucleocapsid (N) protein stands out for its pronounced structural heterogeneity and multifunctionality throughout the viral life cycle, including RNA packaging, genome replication, and immune modulation. Comprising 419 amino acids, the N protein features two folded domains (NTD and CTD) interspersed with three extensive intrinsically disordered regions (IDR1, -2, -3). The coexistence of ordered and disordered regions raises an important question: how does this heterogeneous structural organization influence its interaction with highly charged ligands such as RNA or heparins, that is a known polyanion of biological origin?



Extensive studies have investigated individual N protein constructs, particularly the N-terminal domain (NTD, residues 44–180) and the N-terminal region (NTR, residues 1–248) [1, 2]. Building on this knowledge, we employed high-resolution NMR spectroscopy as the central analytical tool to characterize the interaction of the three N protein constructs— NTD, NTR, and N full length protein—with heparin-based ligands of increasing oligosaccharide length. Our NMR data reveal a clear correlation between ligand size and binding affinity: longer heparin oligosaccharides induce stronger and more widespread chemical shift perturbations, particularly within positively charged regions. Notably, the inclusion of intrinsically disordered segments in the NTR construct significantly enhances the interaction compared to NTD alone, underscoring the functional relevance of structural disorder. The full-length protein displayed distinct spectral behavior, potentially reflecting additional binding contributions from IDR3 and altered dynamics due to its complex and dimeric structure. These findings highlight the sensitivity and versatility of NMR spectroscopy in dissecting the dynamic and multivalent nature of protein–polyanion interactions, particularly in proteins with a complex domain architecture. Our results support a model in which disordered regions are not merely flexible linkers, but active modulators of ligand binding, capable of tuning interaction specificity and affinity. This work emphasizes the necessity of investigating full-length, multidomain proteins by NMR to fully capture the intricacies of their functional mechanisms.

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POSTERS

EXPLORING THE CONFORMATIONAL FEATURES OF PANTININ-DERIVED PEPTIDES BY NATURAL ABUNDANCE NMR SPECTROSCOPY

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Keywords: solution natural-abundance NMR, biomolecules, antiviral peptides

Pantinin peptides are bioactive molecules derived from scorpion venom Pandinus imperator exhibiting potent antimicrobial activity against a large spectrum of pathogens [1]. Their ability to act on cellular membranes with low cytotoxicity makes them favorable for different therapeutic applications. In this study, we investigated the structural properties and the antiviral activity against caprine herpesvirus 1 (CpHV-1) and bovine herpesvirus 1 (BoHV-1) [2] of two pantinin-derived antimicrobial peptides (AMPs), called pantinin-1 and pantinin-2 [3]. In detail, we characterized the structural peculiarities of the two peptides in aqueous and membrane-mimetic environments (trifluoroethanol (TFE)/H2O) by using natural abundance NMR techniques. Overall, the conformational data clearly indicate that both peptides, in aqueous solution, sample random coil conformations with pantinin-2 showing greater secondary structure propensity. In TFE/H_2O, both peptides transitioned to stable α-helical structures, which are often associated with membrane interaction and antiviral activity. Moreover, the two peptides showed antiviral effects against two important veterinary alpha herpes viruses, BoHV-1 and CpHV-1. Overall, our study offers valuable insights for the rational design of pantinin analogues with enhanced antiviral efficacy.

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[P-2]

NMR-BASED METABOLOMIC PROFILING OF *MELISSA OFFICINALIS* SUBSP. *ALTISSIMA*: CHARACTERIZATION OF POLYPHENOLIC COMPOUNDS USING DIFFUSION-ORDERED SPECTROSCOPY

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Keywords: solution NMR, small molecules, biomolecules, metabolomics

Melissa officinalis subsp. *altissima* (MOA), commonly known as wild lemon balm, is a Mediterranean plant of both pharmacological and nutraceutical interest due to its antioxidant, anti-inflammatory, and neuroprotective properties, largely attributed to its rich content of terpenes, phenolic acids, and flavonoids [1]. To elucidate the metabolomic profile of MOA collected in Corsica (France), a comprehensive analytical protocol was applied, with a focus on high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy. Hydroalcoholic and organic extracts were obtained in order to characterise primary and secondary metabolites. Advanced two-dimensional (2D) NMR experiments, including ^{1}H-^{13}C HSQC, ^{1}H-^{1}H TOCSY, and ^{1}H-^{13}C HMBC, were employed to achieve detailed resonance assignments and identify bioactive compounds within the complex mixtures. These experiments allowed structural elucidation and mapping of proton-carbon connectivity in crowded spectral regions. Furthermore, ordered scattering spectroscopy (DOSY) was used to provide information on the scattering coefficients of individual components, effectively allowing the separation of compounds based on their size and molecular interactions within the mixture. DOSY thus functioned as a chromatographic tool in the NMR domain, aiding in the discrimination of overlapping signals and the identification of classes of compounds (polyphenols) without previous separation.
The results of the NMR-based metabolomics analysis were supplemented with volatile composition data obtained by GC-MS for the essential oil fraction. In parallel, the biological activities of MOA extracts were evaluated, including antioxidant effects in hypothalamic cells and modulation of inflammation- and neurodegeneration-related gene expression (TNF-α, NOS-2, BDNF, IL-6, ACHE) in mouse brain tissue models.
This integrated methodology aims to support the identification of key phytochemicals—especially polyphenolic compounds—responsible for the observed biological activities and to contribute to the valorization of MOA as a potential functional ingredient in nutraceutical and therapeutic applications.

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[P-3]

METABOLIC PROFILING FOR CLINICAL UNDERSTANDING OF INFLAMMATORY BOWEL DISEASE (IBD)

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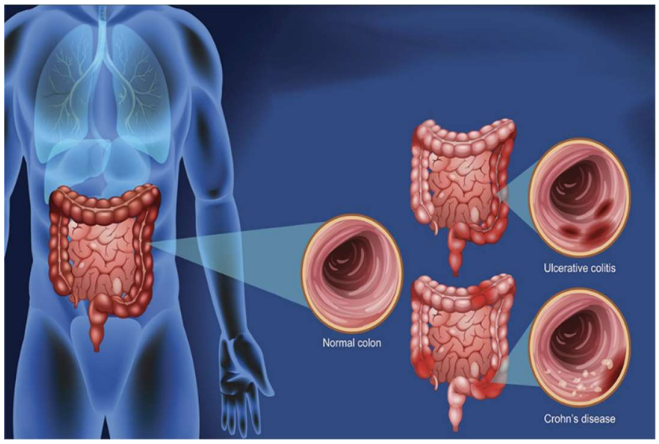
Keywords: solution NMR, small polar molecules, biomolecules, metabolomics, untargeted analysis, Inflammatory bowel disease, serum

Introduction

Inflammatory bowel disease (IBD) is a chronic gastrointestinal condition that primarily manifests as ulcerative colitis (UC) and Crohn's disease (CD). This condition is typically diagnosed early in life, often between the ages of 20 and 40, and is classified as an immune-mediated inflammatory disorder. Genetic predisposition and a dysregulated innate immune system contribute to the clinical severity of CD, making it more severe than UC. Despite these differences, IBD patients share common characteristics, including reduced activity of gut microbiomes and a compromised intestinal epithelial barrier. Although extensive research has been conducted, the pathogenesis and etiology remain complex and are not fully understood. Considering that the metabolome relies heavily on microbiome metabolism, which is affected by environmental factors, including diet, smoking, and antibiotic exposure, we propose metabolic profiling of serum as a useful tool for phenotypic characterization. Treatment and clinical responses will be integrated with metabolomic analysis for biomarker identification in IBD patients.

Results

By performing an untargeted analysis of serum samples and comparing the profiles of IBD patients with those of healthy individuals, we revealed the dysregulation of several metabolites associated with immune-mediated inflammatory disorder metabolism, such the amino acids histidine and glycine, the isobutyrate ketone body, and biomarkers related to lipid pathways, such as acetone and carnitine. On the other hand, the comparison of metabolic profiles between UC and CD does not suggest any differentiating biomarkers of interest.



Conclusions

Metabolic profiling of circulating small molecules in individuals with IBD allowed for the determination of the molecular signature of IBD patients, although not sufficient for patient stratification. Indeed, transcriptomics is ongoing, and will enable mulit-omics network analysis.

[P-4]

Solid-state NMR applied to energy storage materials: the case of a Rb- and Cs-based mixed cation amide solid solution

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Keywords:
solid state NMR, materials

When designing high-performance energy storage materials, ionic conductivity is a key parameter to be considered. In this work, a novel mixed-cation amide solid solution, namely $Rb_{0.5}Cs_{0.5}NH_2$, was obtained, characterized by an unusually high ionic conductivity of $0.4 \cdot 10^{-4}$ $S \cdot cm^{-1}$ at 373 K. The solid solution was subjected to an extensive ensemble of techniques to assess its structural features, and to investigate the origin of its peculiar conduction behavior. In particular, solid-state NMR ^1H MAS was instrumental in confirming the solid-state solution nature of the studied material. Other techniques, such as X-ray diffraction, and quasi-elastic neutron diffraction, elucidated the mechanisms that lead to ionic conduction in $Rb_{0.5}Cs_{0.5}NH_2$. Computational predictions, in agreement with the experimental results, were also crucial in corroborating the conclusions reached about the source of high conductivity. Due to the ever-growing interest in such materials for hydrogen storage, these findings provide a robust foundation for thoroughly delving into ion transport mechanisms.

[P-5]

PDF "FEAT" SOLID-STATE NMR: A COMBINED APPROACH FOR CRYSTAL STRUCTURE DETERMINATION OF POORLY CRYSTALLINE ORGANIC COMPOUNDS

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Keywords: solid state NMR, small molecules, theory and methods

The properties of solid compounds are determined by their solid-state structures. This is of particular relevance in the development of new materials, i.e., drugs and organic pigments. For nanocrystalline organic compounds (ordering lengths of 5–40 nm), the standard methods for structure determination fail. In the last decades a new technique has been established for the analysis of nanocrystalline substances: the analysis of the pair distribution function (PDF). Whilst being well established for inorganic compounds, organic compounds are rarely investigated by PDF analysis. Schmidt and co-workers developed a method called 'Global-PDF-Fit', which allows for the determination and refinement of crystal structures of organic compounds from scratch by a global fit to the pair distribution function (PDF), without prior knowledge of lattice parameters and space group [1]. Nevertheless, there are cases for which the Global-PDF-Fit method does not yield a unique solution, but several different structures, which all fit the experimental PDF data equally well [2]. Thus, we are developing a method to determine the crystal structures of organic compounds by combining a PDF fit with solid-state nuclear magnetic resonance (SSNMR), to achieve a faster and more reliable method than the existing ones to apply to such systems. 1D and 2D SSNMR experiments can provide precise information on the local structure, such as tautomeric character, hydrogen-bond network, intra- and intermolecular atom-atom proximities and precise atom-atom distances [3]. All this information can be used as restraints for the generation of the structures, for the PDF fit and as additional parameters for the selection of the correct structure. Here we are presenting the results obtained so far for crystalline organic compounds of pharmaceutical interest.

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SYNTHESIS AND CONFORMATIONAL ANALYSIS OF DIGESTION-RESISTANT ALLERGENIC EPITOPES OF ARGININE KINASE FROM THE EDIBLE INSECT HERMETIA ILLUCENS

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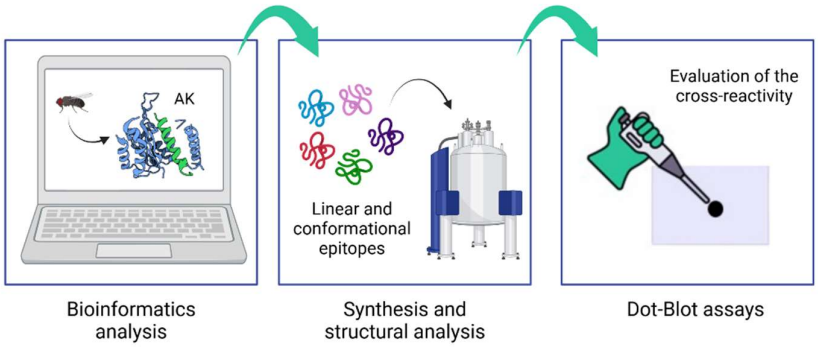
Keywords: solution NMR, biomolecules, food

Arginine kinase (AK) is an essential enzyme in the energy metabolism of invertebrates, playing a vital role in maintaining stable ATP levels. However, it is also a major allergen found in insects and crustaceans, which can trigger IgE cross-reactivity in individuals sensitized to similar proteins. In a recent study [1], our research group showed that gastrointestinal digestion reduces linear epitopes, thus decreasing allergenicity. We also observed changes in the protein secondary structure at elevated temperatures, supporting the idea that conformational epitopes might be lost or diminished.
Since *H. illucens* can be a potential allergen source for people allergic to crustaceans or mites, identifying the epitopes responsible for allergic reactions is important to evaluate the safety of novel foods containing *H. illucens* as an ingredient. Therefore, this study aims to synthesize the predicted linear and conformational epitopes based on bioinformatics analysis and to assess their allergenicity using Dot-Assays.



In particular, the sequence WPTGRGIYHNDNKTF, which has been identified as an epitope resistant to gastro-intestinal digestion conditions, was synthesized using Fmoc-based Solid Phase Peptide Synthesis (SPPS) and then purified by RP HPLC. NMR Conformational analysis was performed using JEOL 600 NMR ECZ600R NMR spectrometer, by acquiring 1D 1H, 2D 1H–1H TOCSY, 2D 1H–1H ROESY 2D 1H–1H NOESY, 2D 1H–13C HSQC, 2D 1H–13C HMBC, 2D 1H-15N HSQC. In order to confirm cross-reactivity, a complete study of the allergenicity potential of the synthesized epitopes was performed by immunoreactivity assays by using sera of patients allergic to shrimp or mites. The results of these analyses will provide valuable insights into the allergenic profile of AK when dealing with novel foods containing *H. illucens* or its derivatives.

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A SOLID-STATE NMR STUDY OF WATER AND CARBON DIOXIDE DYNAMICS IN AN ULTRAMICROPOROUS METAL-ORGANIC FRAMEWORK

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Keywords: solid state NMR, materials.

Metal-Organic Frameworks (MOFs) are crystalline porous coordination polymers composed of metal ions or clusters and organic linkers [1]. Thanks to their structural and functional tunability, MOFs are of great interest for applications in CO_2 separation [2, 3]. Among them, the ultramicroporous MOF UTSA-280 (calcium squarate, $Ca(C_4O_4)(H_2O)$, Fig. 1), exhibits high CO_2 uptake and selectivity over CH_4 in CO_2/CH_4 mixtures, due to a sieving effect [4]. Coordinated water in UTSA-280 has been found to be involved in water transport in the MOF channels through a knock-off mechanism [5], as well as in the selective uptake of ethane/ethylene mixtures [6]. To elucidate molecular transport mechanisms, the state and dynamics of water and CO_2 in the MOF were investigated using multinuclear solid-state NMR spectroscopy.

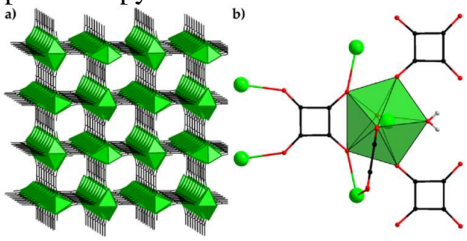

Fig. 1. a) Polyhedral representation of UTSA-280 crystal structure; b) Local coordination environment of the squaric acid dianions and calcium cations.

^1H and ^2H MAS and static NMR experiments on activated and hydrated (with either H_2O or D_2O) samples revealed that coordinated water undergoes local librations and π-flips, while hydration enables translational hopping and molecular exchange. ^{13}C NMR spectra recorded on UTSA-280 loaded with $^{13}CO_2$ showed restricted reorientational dynamics of CO_2 in the channels.

This research has received funding from the European Union's Horizon Europe research and innovation programme, European Innovation Council and SMEs Executive Agency (EISMEA), under grant agreement No 101115488, project "DAM4CO2".

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[P-8]

Ringing compensation in high-Q RF coils for NMR using a modified pulse sequence

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Keywords: RF coil, ringing, shaped pulse, NMR

Present work is dealing with the compensation of the ringing of radio-frequency (RF) coil in nuclear magnetic resonance (NMR) experiments. After the transmission (Tx) pulse any resonant Tx coil continues ringing, preventing the acquisition of the small NMR signal for some time (typically few microseconds). Generally, it is not an issue for tissues with long T2 values, but for solid-state NMR, or observation of other large molecules (proteins, polymers) each microsecond gained could be crucial. Hence, inspired by the efforts of Hoult [1], we created a pulse sequence, which retrieves 3-4 microseconds of free induction decay (FID) signal compared to regular 90-degree pulses. The sequence can be adapted from standard inversion recovery by carefully calibrating pulse's timing, amplitude and phase of each lobe. This approach is based on the destructive interference between the coil ringing and a phase-shifted secondary pulse of lesser amplitude. Experiments were carried out at 20 MHz providing evidence of ringing reduction in a high Q coil (85). A limitation of the technique is the secondary pulse length, which must be minimized (1-2 us). Another limitation is the calibration of the second pulse, which is mandatory for different loads. However, if a sustainable algorithm for pulse calibration is in place, the technique becomes highly useful. In the poster, we would like to present the theoretical model of physics behind the idea as well as practical implementation on the STELAR FFC relaxometers.

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[P-9]

PRDM2 Zinc Finger Domains 4–5: A Structural Study

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Keywords: Rb transcription factor activation, zinc finger, spectroscopic techniques, threedimensional structure, thermal stability.

Zinc Fingers represent a large group of metalloproteins, crucially involved in several cellular processes (1). In fact, their ability to interact with nucleic acids allows to regulate mechanisms such as transcription, chromatin remodeling and DNA repairment (2). The protein PRDM2, potentially involved in regulating the activation of the Rb transcription factor involved in cell proliferation and survival in human cells (3), contains eight zinc finger domains that are currently under characterization. In particular, the domains 4 and 5 (ZF 4-5) appear to be not directly involved in DNA recognition. A hypothesis is that they should be important for PRDM2 conformational stability in turn facilitating the interaction with other regulatory factors (4).
Circular dichroism data and NMR experiments on both domains ZF4 and ZF5 will be reported, together with thermal stability data, to confirm that they assume the correct folding in solution. These data will help the achievement of further information on their possible involvement in Rb regulation.

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[P-10]

^{1}H NMR analysis of urine for a preliminary metabolomic study of Meniere's disease

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

Meniere's disease (MD) is a chronic inner ear syndrome characterized by cochlear and vestibular dysfunction [1]. The main symptoms correlated to this disease are sensorineural hearing loss, vertigo, tinnitus, and aural fullness. This condition has been estimated between 17 and 513 out of 100,000 individuals depending on gender and geographical localization [2,3]. Today, MD is considered a complex disease, whose pathophysiological mechanisms and therapeutic options haven't been yet clarified. In this perspective, the application of new strategies for metabolomic study of potential pathways involved in the development of the disease may help to investigate MD's pathophysiology. Thus, urinary metabolic profiling through ^{1}H nuclear magnetic resonance (NMR) might represent a promising tool in this field. This multicenter study evaluated the metabolomic influence of MD through the combined approach of ^{1}H NMR spectroscopy with multivariate statistical analysis. The population included in this work is a cohort of 50 individuals composed of a group of patients and one of controls. Overall, the ^{1}H NMR spectra of patients (n = 30) and controls (n = 20) allowed the identification of 109 metabolites, with an endogenous or exogenous source. The Principal Component Analysis (PCA) conducted on the multicenter two groups of individuals confirmed the absence of a center-based clusterization. Additionally, the univariate analysis through the Volcano Plot outlined the significant features downregulated in MD patients, corresponding to threonine/lactate sum, 2-hydroxyisobutyrate, dimethyl sulfone/ethanolamine sum, an unknown metabolite, and tyrosine/tyramine sum, all presenting higher levels in controls samples. These potential biomarkers showed a significant metabolomic difference between MD patients and healthy controls, confirming the importance of metabolomics study as a promising diagnostic and prognostic tool that may elucidate Meniere disease's pathophysiology.

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NMR-BASED STRUCTURAL CHARACTERIZATION OF cIAP2-BIR1 FOR THE RATIONAL DESIGN OF ANTICANCER INHIBITORS

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Keywords: solution NMR, small molecules, biomolecules.

Resistance to cell death is a hallmark of cancer [1], frequently associated with the overexpression of Inhibitor of Apoptosis Proteins (IAPs) [2-3]. These proteins, through their conserved Baculoviral IAP Repeat (BIR) domains, inhibit apoptotic pathways and contribute to the sustained survival of tumor cells [4]. Among IAP family members, cIAP2 plays a central role in regulating both apoptotic resistance and inflammatory signaling [5]. Notably, the BIR1 domain of cIAP2 has recently emerged as a promising therapeutic target, particularly in tumor settings where the loss of cIAP1 triggers a compensatory upregulation of cIAP2 [6-7]. In this context, selective targeting of the cIAP2-BIR1 domain may represent a strategic approach to overcome apoptosis resistance and improve therapeutic outcomes in cancer. To explore the molecular basis of ligand recognition and define the interaction surface at atomic resolution, we performed an extensive NMR characterization of uniformly ^{15}N, ^{13}C-labeled cIAP2-BIR1. A suite of high-resolution 2D and 3D heteronuclear NMR experiments—including HNCA, HNCOCA, HNCO, HNCACO, HNCACB, HN(CO)CACB, HBHACONH, and HCCH-TOCSY [8-10]—enabled near-complete assignment of backbone resonances (89.5%) and a substantial number of side-chain resonances (60.5%). Secondary structure determination based on chemical shift data, performed using TALOS+, showed excellent agreement with the known crystal structure (PDB ID: 7NK0), confirming correct folding of the protein in solution.
In parallel, NMR relaxation measurements (T_1, T_2, and heteronuclear NOE) [11] were acquired to assess backbone dynamics and conformational flexibility, providing further insights into the biophysical behavior of the BIR1 domain under native-like conditions. Overall, this NMR-based structural study lays the foundation for rational drug design efforts aimed at selectively targeting cIAP2-BIR1. Such an approach holds strong potential to restore apoptotic signaling in resistant tumor cells and to enhance the effectiveness of current anti-cancer therapies.

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[P-12]

BEYOND CHEMICAL STRUCTURE: A COMMON METABOLIC SIGNATURE OF NEW PSYCHOACTIVE SUBSTANCES REVEALED BY ^{1}H-NMR

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Keywords: solution NMR, metabolomics.

New Psychoactive Substances (NPS) represent a complex and diverse group of drugs of abuse, often designed as analogs of already regulated compounds or synthesized de novo to mimic the pharmacological and toxicological effects of traditional illicit drugs [1,2]. Their continuous emergence on illegal markets, frequently in chemically uncharacterized forms, poses an increasing threat to public health and presents a significant challenge to conventional identification methods, which rely heavily on prior knowledge of molecular structures [3].
This study investigates the impact of four opioids - morphine, brorphine, etonitazene, and fentanyl - on the urinary metabolic profile of CD-1 mice using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The aim is to propose a metabolomics-based approach focused on understanding systemic effects induced by this class of drugs, regardless of their specific chemical structure. Urine samples collected from mice treated with equi-effective doses of the four opioids were analyzed using an NMR fingerprinting strategy combined with multivariate statistical analysis. The results reveal that all compounds induce significant alterations in the urinary metabolic profile, with the most pronounced effects observed within the first 12 hours post-administration, although still detectable up to 24 hours. Brorphine exhibited the strongest metabolic impact, consistent with its pharmacokinetic profile and longer half-life. A particularly relevant finding is identifying a common metabolic perturbation pattern across chemically distinct structures, suggesting the potential use of such metabolic signatures as functional biomarkers of exposure to the opioid class.
Despite certain limitations - including the small number of samples, the use of an animal model, the lack of complete spectral profiling complicating biochemical interpretation, and the absence of evidence confirming the class-specificity of the observed effects - these findings offer promising insights into future forensic applications. Ultimately, this approach could help reduce reliance on structure-specific analyses and accelerate regulatory responses to emerging substances.

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[P-13]

Self-regulating magnetic nanoparticles for hyperthermic tumor treatment: evaluation of the therapeutic threshold dose.

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Keywords: MRI, contrast agents.

Cancer remains one of the most challenging global health issues [1]. Despite extensive research, clinical outcomes for patients have seen limited improvement [2]. In recent years, iron oxide nanoparticles (IONPs) have gained attention for their magnetic properties, low toxicity, and biodegradability [3], with wide application in biomedical fields such as magnetic fluid hyperthermia (MFH). MFH is a non-invasive cancer therapy where IONPs generate heat upon exposure to an alternating magnetic field (AMF), inducing tumor cell death [4]. To avoid damage to surrounding healthy tissue, self-regulating IONPs with a defined Curie temperature (TC) have been developed; they cease heating beyond TC, preventing overheating [5].
This study investigates the dose-dependent therapeutic efficacy of glucose-coated, self-regulating M55 IONPs (GM55, MBN Nanomaterialia S.p.A) in preclinical intratumoral applications.
Human breast carcinoma MDA-MB-231 cells were subcutaneously injected in female Balb/c nu/nu mice. Once tumors reached ~200 mm^3, mice were divided into five groups: CTRL (saline) and four GM55 dose groups (15, 30, 60, and 120 µgFe), administered intratumorally. Mice underwent four magnetic fluid hyperthermia (MFH) sessions at 0, 24, 48, and 96 hours post-injection, with 20-minute exposures to an alternating magnetic field. Tumor volume was monitored by MRI on days 1, 5, 7, and 10. Finally, viable tumor area (VTA), partially necrotic tumor area (PNTA), or necrotic tumor area (NTA) were investigated with H&E excided-tumor staining.
Higher doses of GM55 (60 and 120 µgFe) significantly reduced tumor volume by day 3, with sustained effects through day 9. The 30 µgFe dose showed delayed efficacy by day 6, while 15 µgFe had no significant impact. Histological analysis revealed that 120 µgFe induced the highest tumor necrosis and lowest viable tumor area. These findings indicate a clear dose-dependent response to MFH, with 120 µgFe achieving the greatest therapeutic effect and delaying tumor regrowth. Lower doses resulted in limited cellular damage, highlighting the importance of dose optimization to enhance MFH efficacy.

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[P-14]

NMR Assessment of the High Order Structure of Biological Therapeutics in Erythrocytes Provides a New Tool for Drug Delivery Design

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Keywords: (please select in this list the keywords suitable for your contribution and delete the unselected ones): solution NMR, biomolecules, theory and methods.

Abstract: The application of erythrocytes and their derivatives in the delivery of therapeutics presents promising solutions for the challenges associated with enzyme replacement therapy and biological drug delivery. They protect drugs from the immune system and plasma proteases, ensuring biocompatibility and prolonged circulation time [1,2]. L-Asparaginase, used to treat acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma, has been evaluated in its erythrocyte-encapsulated form (GRASPA) in clinical trials for ALL and pancreatic cancer [3-5].
However, the development of erythrocyte-based delivery systems is complicated by difficulties in characterizing the structural integrity of the encapsulated proteins, which is crucial for ensuring their biological activity and therapeutic efficacy. The preservation of the higher-order structure (HOS) of proteins within these systems is essential because it defines the active conformation necessary for proper binding and function. Traditional methods for assessing enzyme activity after cell lysis are insufficient to address these complexities [6].
This study demonstrates the use of NMR experiments to assess functionality, encapsulation efficiency, and structural stability of various proteins, Carbonic Anhydrase II (CAII), used to refine the protocol for its stability, human Transthyretin (TTR), serving as a model drug carrier and the L-Asparaginase. Through comparative analysis with free protein samples, substantial retention of native structural features was confirmed, even in the crowded cellular environment of erythrocytes.
Successful application of NMR techniques showed effective protein encapsulation, with nearly complete residue reassignment indicating maintained HOS: 98% for CAII, 76% for ANSII, and 90% for TTR (Figure 1). Despite signal line broadening, primarily due to the intrinsic cellular environment, these results proved pivotal in validating the structural integrity of therapeutic proteins post-encapsulation.

[P-15]

IDENTIFICATION OF DISTINCT MULTIPLE SCLEROSIS WHITE MATTER LESION PROFILES USING UNSUPERVISED ANALYSIS OF QUANTITATIVE MRI METRICS

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Keywords: MRI, theory and methods.

Introduction. This study uses unsupervised machine learning on quantitative and semi-quantitative (qMRI) data to identify distinct white matter lesion (WML) profiles in multiple sclerosis (MS).
Methods. 91 MS patients underwent 3T MRI at the University Hospital of Verona. WMLs were manually segmented, and qMRI (Fig. 1) [1-8] values extracted from lesions' core. Features were standardized to each subject's NAWM and z-scored. PCA reduced dimensionality, followed by clustering. Group differences were tested with Kruskal-Wallis and Dunn's post-hoc tests. Cluster distributions were also stratified by age quantiles (Q1 to Q4).
Results. A total of 720 WMLs were analyzed. Four PCs (explaining >80% variance) were retained, and hierarchical clustering identified 3 clusters (CLs). CL1 included lesions with the most altered MRI values with respect to NAWM (i.e., T1T2r, R2, TWC, T2IE, MTS, MTR, FWF, R2*; all $p < 0.001$), strongly contributing to PC1 ($R^2 > 0.7$). CL2 showed highest χ_{pos} ($r_{es} = 0.42$) and QSM ($r_{es} = 0.45$), key drivers of PC2 ($R^2 = 0.95$ and 0.84, $p < 0.05$). CL3 included lesions with the least altered features ($p < 0.001$). CL2 lesions number decreased with age (Q1 47.0%, Q4 7.5%), while CL1 and CL3 lesions number increased (Q1 21.9% vs Q4 36.2%; Q1 31.1% vs Q4 56.3%, respectively).
Conclusion. Clustering revealed 3 WML profiles: severe damage (CL1), iron-rich active WMLs (CL2), and milder, likely inactive WMLs (CL3), demonstrating that multiparametric qMRI clustering captures MS WMLs heterogeneity and reveals distinct tissue patterns.

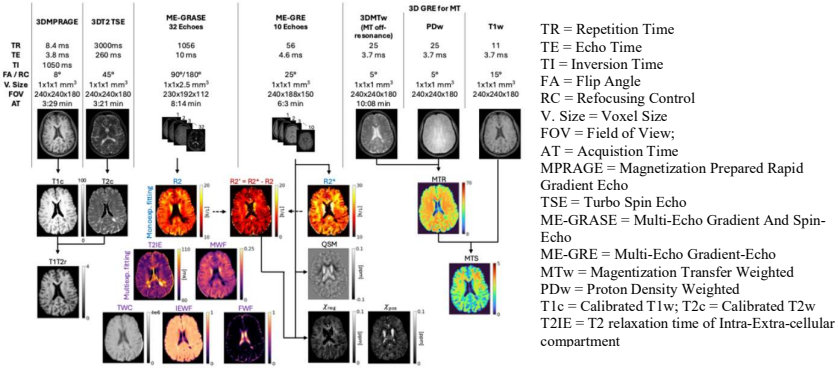

Fig. 1. Image acquisition protocol and qMRI parameters extraction.

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[P-16]

NMR-Based Metabolic Fingerprinting as a Tool for Early Detection of Adalimumab Resistance in IBD

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

NMR-based metabolomics enables detailed metabolic profiling and the detection of subtle biochemical changes linked to disease, often undetectable through conventional blood analyses [1]. This pilot study performed a metabolic analysis of serum samples from 36 Crohn's disease patients undergoing standard Adalimumab therapy, focusing on those who experienced a pharmacological resistance after the second dose of the BNT162b2 SARS-CoV-2 vaccine. To investigate the relationship between vaccination and therapeutic response, we compared the metabolomic profiles of responders (R, *n*=20) and non-responders (NR, *n*=16) patients, highlighting several key discriminant metabolites. Multivariate analysis (OPLS-DA) of NMR spectra revealed a significant metabolic shift from pre- (T0) to post-vaccination (T2). An integrated comparison of discriminant metabolites across time points and between response groups revealed substantial overlap (Fig. 1).

Urea emerged as the most prominent metabolite distinguishing R from NR patients, prompting further investigation into its potential role in modulating intestinal permeability, a key factor in the pathophysiology of Crohn's disease. Functional studies in HCEC-1CT colon epithelial cells showed that elevated urea levels influence the expression of AQP8 and tight junction proteins (ZO-1, OCLN, CLDN-1, CLDN-2, E-CAD), suggesting a role in modulating barrier integrity [2].

These findings suggest that NMR-based metabolomics represents a valuable, non-invasive tool for monitoring pharmacological treatment response, offering a comprehensive overview of the patient's metabolic state. Owing to its sensitivity, NMR should be more broadly integrated into clinical workflows supporting personalized treatment strategies, enabling early detection of therapy resistance, and ultimately enhancing patient outcomes.

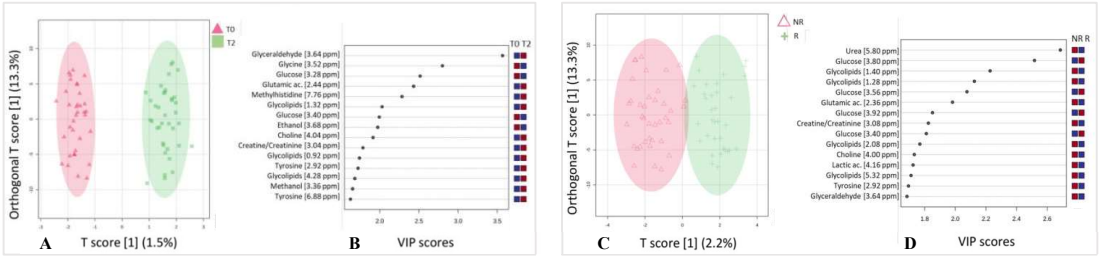

Fig. 1. **A.** OPLS-DA on 72 spectra by using UV-scaled 0.04 ppm-sized bucketing. The ellipse (T0 "▲" *vs* T2 "■") shows the 95% confidence interval with Hotelling T-square test. **B.** Analysis of the Variable Importance in Projection (VIP): metabolites decreased (blue) and increased (red) at T0 *vs* T2. **C.** OPLS-DA on 72 spectra by using UV-scaled 0.04 ppm-sized bucketing. The ellipse (NR "Δ" *vs* R "+") shows the 95% confidence interval with Hotelling T-square test. **D.** Analysis of the Variable Importance in Projection (VIP): metabolites decreased (blue) and increased (red) in NR *vs* R patients.

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[P-17]

Development of Solid-State NMR Tools for Investigating Lead Halide Perovskite Surfaces

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Keywords: solid state NMR, materials.

Lead Halide Perovskites (LHPs) with general formula $APbX_3$ (A^+ = methylammonium (MA^+), formamidinium (FA^+) or Cs^+ and X^- = Br^-, Cl^- and I^-) are promising materials for optoelectronic devices, such as solar cells and light emitting diodes (LEDs), thanks to their outstanding optoelectronic properties, low cost of materials and ease of processability [1]. However, 3D perovskites suffer from poor stability when exposed to moisture, temperature and/or oxygen [2,3]. A promising strategy to address these issues involves using hybrid 2D/3D perovskites, which combine the stability of 2D systems, with the high performances of the 3D counterparts [2]. Polycrystalline thin films, commonly used in perovskite-based devices for their simple fabrication, exhibit poor stability in humid conditions and are susceptible to grain boundary defects and structural disorder. These issues promote non-radiative recombination, ultimately reducing device efficiency. A promising alternative to mitigate these drawbacks is the use of perovskite single crystals (SCs). Understanding atomic-level structure and dynamics is essential for a deeper insight into perovskite material properties. In this context, solid-state NMR (SSNMR) has proven to be a powerful tool for probing perovskites, offering information on dopant incorporation, phase segregation, halide mixing, and local dynamics [4]. Preliminary studies have also shown its potential for surface and interface investigation [5]. This work focuses on developing tools for the characterization of the surface/interface properties in 2D/3D structures, by combining crystal engineering for isotopic enrichment of surfaces and SSNMR methods. Specifically, a surface modification with ^{2}H-enriched methylammonium ($CD_3NH_3^+$) in $MAPbI_3$ and $MAPbBr_3$ powder and single crystal perovskites was performed using two different synthetic approaches. The surface modification was probed by the direct observation of ^{2}H NMR static spectra on both SCs and powders. Furthermore, surface properties were investigated through ^{2}H spin-lattice relaxation time measurements, providing deeper insights into the structural and dynamic behavior of the modified perovskite surfaces.

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[P-18]

NMR-GUIDED CHARACTERIZATION OF BIOMIMETIC PEPTIDES TARGETING THE HUMAN 20S PROTEASOME

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Keywords: solution NMR, small peptides, biomolecules, theory and methods

A broad spectrum of disorders known as protein conformational diseases (PCDs) - Alzheimer's, Parkinson's and prion diseases - share a common pathological feature: the aberrant accumulation of misfolded and toxic protein species [1]. Central to the onset and progression of these diseases is the impairment of the proteostasis network (PN), the complicated system governing protein synthesis, folding, and degradation. Within this framework, the proteasome—a multicatalytic protease complex responsible for controlled protein degradation—has emerged as a key therapeutic target. The activation of proteolytic activity in the core particle (CP) of the proteasome is seriously dependent on conformational transitions triggered by specific regulatory particles (RPs) [2]. Recent findings have explored the rational design of nature-inspired molecular scaffolds that mimic the activating motifs of human 20S regulatory proteins (h20S-RPs), representing a novel strategy to modulate protein targets deemed inaccessible to pharmacological intervention [3, 4].
In the present study, we employed a multidisciplinary approach integrating advanced in silico techniques with high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy to investigate the molecular interactions between newly synthesized ligands and h20S, and to assess their functional implications.

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[P-19]

STRUCTURAL INSIGHTS INTO *H. PYLORI* ONCOPROTEIN CAGA INTERACTION WITH SHP2-SH2

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Keywords: solution NMR, biomolecules

Chronic infection with *H. Pylori* CagA-positive strains is causally associated with the development of gastric diseases, most notably gastric cancer [1]. CagA possesses a unique tyrosine phosphorylation sequence motif termed the EPIYA motif, comprising the 5- amino-acid sequence Glu-Pro-Ile-Tyr-Ala, in variable numbers in its C-terminal polymorphic region [2]. The EPIYA motif-containing region of individual CagA consists of various combinations of 4 distinct segments (named EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D), each of which is defined by differences in the amino acid sequences spanning the EPIYA motif [2, 3]. After CagA inject into gastric epithelial cells, several EPIYA motifs at the CagA C-terminal undergo tyrosine phosphorylation and interact with a series of proteins containing the SH2 domain to induce cell carcinogenesis and resist immune surveillance.
Most notably, CagA forms a complex with the SHP2 tyrosine phosphatase, which contains two SH2 domains in its N-terminal region, through tyrosine-phosphorylated EPIYA-C or EPIYA-D segments [3]. The CagA-SHP2 interaction leads to deregulation of the SHP2 phosphatase activity, which is essential for full activation of the RAS-ERK signaling pathway [3, 4]. In this process, the CagA-SHP2 interaction plays a crucial role in inducing cell tumorigenesis, but there was a lack of studies, from a structural point of view, on the interaction mechanism thus far.
Here, the binding of SHP2-SH2 with Tyr-phosphorylated peptides from EPIYA motifs, in particular EPIYA-C and EPIYA-D, in CagA was studied by NMR spectroscopy. Therefore, this study could provide help for design of polypeptide inhibitor drugs against *H. Pylori*.

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[P-20]

Integrating NMR Metabolomics with the INFOGEST Protocol: Insights from Zucchini Female Inflorescences

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Keywords: solution NMR, small molecules, metabolomics, food

The aim of this project is to investigate food matrices in depth, characterizing not only the raw products but also their chemical profiles throughout gastrointestinal transit. As a case study, the female inflorescences of zucchini (*Cucurbita pepo* L.) were selected due to their nutritional richness. In this study, both raw and digested samples were analyzed using proton nuclear magnetic resonance (^{1}H-NMR) as a metabolomic tool to characterize the overall chemical composition and identify bioactive compounds. The Bligh-Dyer method was applied to extract both hydroalcoholic and organic fractions [1], while the INFOGEST protocol was used to simulate gastrointestinal conditions and study the release of food components during digestion [2]. Metabolomic analysis was carried out using a Bruker AVANCE 600 spectrometer operating at 600.13 MHz, employing both one-dimensional and two-dimensional NMR experiments for the metabolites assignment.
Then, three different NMR sequences were used for the one-dimensional experiments: zgpr, a basic proton 1D experiment; cpmgpr1d, which applies a filter to suppress signals from macromolecules; and ledbpgppr2s1d, a diffusion experiment with two gradient intensities. The zgpr experiment was primarily used for characterizing the low-field region of the spectrum. In contrast, the high-field region required the use of cpmgpr1d and ledbpgppr2s1d to attenuate macromolecular signals and quantify the underlying metabolite resonances more accurately.

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[P-21]

From high-field NMR to benchtop FT-NMR to characterize the balanced taste and chemical profile of kombucha beverage: the results of a comparative study

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Keywords: solution NMR, low field NMR, small molecules, metabolomics, food

Kombucha is a sweetened tea fermented by a symbiotic culture of yeasts and bacteria (SCOBY), resulting in a beverage characterized by a balanced flavor profile—sweet from residual sugars and sour due to organic acids produced primarily by acetic acid bacteria. To assess the quality and production reproducibility of kombucha obtained through natural fermentation, an NMR-based metabolomics approach was employed. Samples were collected at 5-day intervals over a 25-day fermentation period and analyzed using untargeted ^{1}H-NMR spectroscopy. Quantitative analysis revealed the presence and evolution of various metabolites, including sugars (sucrose, glucose, fructose, trehalose, and 2-O-β-L-arabinopyranosyl-myo-inositol), organic acids (acetic, lactic, succinic, malic, citric, formic, quinic, fumaric, and gluconic acids), amino acids (alanine, isoleucine, leucine, valine, glutamate, pyroglutamate, aspartate, and GABA), as well as ethanol, glycerol, caffeine, theanine, and gallic acid. A benchtop FT-NMR spectrometer was also employed to monitor key fermentation metabolites—sucrose, lactic acid, acetic acid, and succinic acid—over time. Partial least squares regression (PLS-R) applied to the benchtop NMR data yielded accurate predictions of the fermentation stage, offering insights into both the quality and reproducibility of kombucha production. Overall, the benchtop NMR system proved to be a valuable on-line monitoring tool, offering a rapid, non-destructive, and effective method for quality control in kombucha fermentation. This approach holds promise for broader application across industrial kombucha production worldwide, contributing to a deeper understanding of substrate consumption and metabolite formation dynamics.

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[P-22]

MULTIDIMENSIONAL NMR STUDY OF SULFATED POLYSACCARIDES: STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES

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

The analysis of heterogeneous natural and chemically modified biologically active polymers, which vary in size and substitution pattern, presents a challenge for their characterization.
NMR spectroscopy has proven to be a powerful tool for evaluating the critical quality attributes (CQAs) of these compounds, such as glycosaminoglycans [1,2] (heparin [3], chondroitin sulfate and hyaluronic acid) and pentosan polysulfate [4,5]. In particular, the integration of multidimensional NMR techniques with diffusion-ordered spectroscopy (DOSY) enables not only the elucidation of fine structural details (e.g., sulfation pattern, monosaccharide composition), but also the assessment of molecular size and polydispersity.
This combined approach provides a comprehensive analytical framework for characterizing both the chemical structure and physicochemical behavior of complex polysaccharides, supporting quality control, pharmacological studies, and the development of biosimilar or synthetic analogs.
In the present work, these methodological approaches were applied to the study of pentosan polysulfate and chondroitin sulfate, demonstrating how combined NMR techniques, can provide a detailed and reliable characterization of these complex sulfated polysaccharides. Importantly, these advanced NMR methodologies have also been recognized and adopted by regulatory authorities as essential tools for ensuring the quality, safety, and consistency of polysaccharide-based pharmaceuticals.

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[P-23]

A REVIEW OF BENCHTOP FT-NMR APPLICATIONS IN FOOD SCIENCE

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

Benchtop NMR spectroscopy, known for its speed and ease of use, is increasingly valuable in food production and quality control. This review examines its applications in food and natural product analysis over the last two decades, highlighting adulteration detection and quality control as major areas: adulteration detection with eighteen works available and quality control applications in both static or continuous mode with fifteen works available. For this poster presentation, eight key articles were selected for highlighting the various analytical methods employed in food product assessment. Among these methods—targeted, metabolomic analysis, and metabolite fingerprinting—with operating frequencies between 43 MHz and 80 MHz, targeted analysis emerges as the primary method for quality control and authentication, as seen in studies identifying meat adulteration between beef and horse[1], quantifying cannabinoids in forensic applications [2], quality control of milk [3], and investigating safety concerns related to polyunsaturated fatty acid-rich frying oils [4]. While metabolomics, exemplified by discrimination of perilla oil samples from samples adulterated with soybean oils [5] and detection of extra virgin olive adulteration [6], offers comprehensive spectral analysis to identify and quantify as many metabolites as possible without relying on a predefined hypothesis, fingerprinting, used in detecting adulteration in saffron [7] and honey analysis [8], bypasses metabolite identification. Research in this field underscores the increasingly significant role of Benchtop FT NMR in food science and various industrial applications.

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[P-24]

What is hidden between amide-type solvents and acyl chlorides? Let's unveil it by NMR

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Keywords: solution NMR, small molecules.

N,N-dimethylacetamide (DMAc) and *N,N*-dimethylformamide (DMF) are the most common used solvents in amidation reactions due to their exceptional solubilising properties and their compatibility with polar reactants [1,2]. Moreover, the amide-type solvent appears to be involved in the reaction mechanism. The aim of this study is to improve the understanding of this catalytic function by performing kinetic and NMR experiments. The kinetic study was performed using an HPLC system (Waters 1525EF pump, Waters 2996 diode array detector and Waters 717 plus autosampler) equipped with a Thermo Scientific Fluophase PFP column, 5 µm, 250 x 4.6 mm. The mobile phase was a mixture of Water/CH_3OH/CH_3CN (15/12.75/72.25). The NMR samples were prepared by dissolving the starting materials in $CDCl_3$ in different molar ratios and the experiments were acquired with a Jeol ECZR 600 instrument, operating at 14 T (1H=600MHz; 1H 90° = 8.15 µs).
The amidation reaction taking place between sterically hindered anilines and an acyl chloride was conducted measuring kinetic constants (k) using DMAc, DMF and other non-amidic polar solvents. The results show that the presence of the amide (either as a solvent or additive) drastically increases the reaction rate. **Figure 1** illustrates a possible interaction between the amide solvent and acyl chloride. To support this hypothesis, we acquired proton NMR spectra of the mixture acyl chloride-DMAc (or DMF) and bi-dimensional NMR spectra (COSY, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC, ^{1}H-^{15}N HMBC) at different temperatures and molar ratios. The analysis of the NMR spectra indicates the presence of an intermediate species, whose structure can be represented by intermediate B (Fig. 1). Specifically, the chemical shift of C5 is more consistent with the structure of intermediate B than an iminium ion.

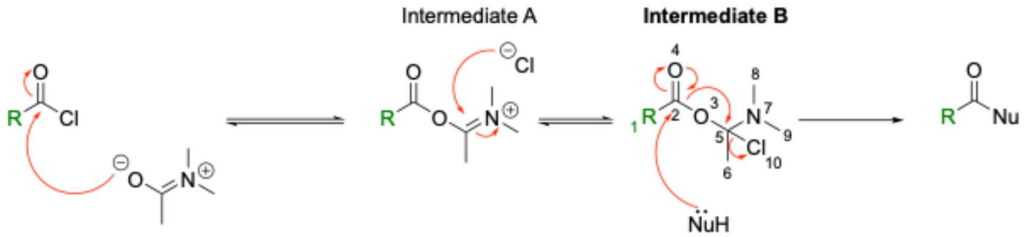

Fig. 1. Hypothesized mechanism between acyl chloride and N,N-dimethylacetamide

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[P-25]

IMPACT OF POLYSTYRENE NANO-PLASTICS EFFECTS ON THE PRION AGGREGATION PROCESS

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Keywords: solution NMR, biomolecules.

Widespread pollution of nano-plastics (NPs) poses significant threats to human health. NPs can enter the human body through inhalation, ingestion or skin contact and they are able to cross impermeable barriers, such as the blood–brain barrier, inducing toxic effects and damage to cells, tissues and organs [1]. Because NPs are present in the environment as heterogeneous mixture in terms of chemical composition and surface functional groups, they are able to interact with biological systems and biomolecules through different molecular processes [1]. In particular, macromolecules such as proteins upon interaction with NPs surface form a complex structure called protein corona, which plays a key role in defining in vivo effects [2]. The protein corona is composed by two layers, the 'hard' and 'soft' corona. The former contains protein molecules tightly bound to the surface of NPs; whereas the latter, surrounding the hard corona, is formed of weakly bounded molecules which are in slow dynamic equilibrium with the free proteins [2].
In the present work, we investigated the interaction between anionic polystyrene NPs with the N-Terminal Domain (NTD, residues 23-89) of Human Prion Protein (HuPrP), whose protein misfolding and, subsequently, aggregation to form fibrils, cause rare and fatal neurodegenerative disorders, called prion diseases [3]. In particular, we performed dynamic light scattering (DLS) measurements and thioflavin T (ThT) fluorescence assay, to describe the variation of hydrodynamic features of NPs in presence of the NTD and to detect the formation of NTD-derived amyloid oligomers induced by the presence of NPs. In addition, we applied several high resolution NMR methodologies for describing the molecular determinants involved in the formation of NTD-NP complex.

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[P-26]

Head and neck cancer metabolomic biomarkers research through ^{1}H NMR analysis of different biological samples: serum, saliva and tissue

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Keywords:
solution NMR, metabolomics

Head and neck cancer (HNC) was ranked in 2020 as the third most common type of tumor worldwide by the Global Cancer Statistics. These diseases, especially oral cancer, are still challenging in otolaryngology due to difficulties in diagnosis and surgery. Metabolomics, combining Nuclear Magnetic Resonance (NMR) and chemometrics analysis, represents a useful field to explore medical and biological aspects of those pathologies, and eventually how metabolome evolves during different tumor stages. The aim of this study is to identify possible differences, in terms of metabolic pathways, between healthy and oncological patients. Moreover, this clinical research wants to investigate metabolic biomarkers that could help to diagnose HNC at an early stage.
To outline a wider approach, different biological samples, more specifically serum and saliva, have been analyzed through ^{1}H NMR, and tumoral tissues ^{1}H spectrum were obtained using HR-MAS NMR spectroscopy. The metabolic profile was investigated for a cohort of 32 HNC patients and 18 healthy subjects, corresponding to 49 serum samples, 36 saliva, and 22 tissues. The NMR datasets were processed by multivariate analysis such as Principal Component Analysis (PCA), Partial Least Square Discriminant Analysis (PLS-DA), with the variant of sparse (sPLS-DA) and orthogonal (oPLS-DA) ones.
As a result, 10 metabolic biomarkers were detected in serum, differentiating healthy and patient samples, but also earlier and later stages of cancer. In addition, through the analysis of saliva 6 biomarkers of the two clusters were found.

[P-27]

Metabolomic profiling of SH-SY5Y Cells exposed to PFBS reveals potential neurotoxic effects

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Keywords:
small molecules, metabolomics

Short-chain per- and polyfluoroalkyl substances (PFAS) are used as alternatives to long-chain PFAS, yet their potential health effects, especially on the nervous system, are still unclear. [1]
This study aims to investigate the impact of perfluorobutanesulfonate (PFBS), a short-chain PFAS detected in brain tissue [2], on neuronal function.
We used the human neuron-like SH-SY5Y cell line as an in vitro model. Cells were exposed to PFBS at concentrations ranging from nanomolar to millimolar for 48, 72, and 96 hours. Culture supernatants were analyzed using nuclear magnetic resonance (NMR) spectroscopy.
Metabolomic analysis revealed that prolonged exposure to high concentrations of PFBS resulted in increased levels of the neurotoxins such as 3-methyl-2-oxovalerate compared to cells treated with lower PFBS concentrations.
These findings suggest a potential neurotoxic effect of PFBS under prolonged and high-dose exposure. Further investigation into its safety profile is needed.

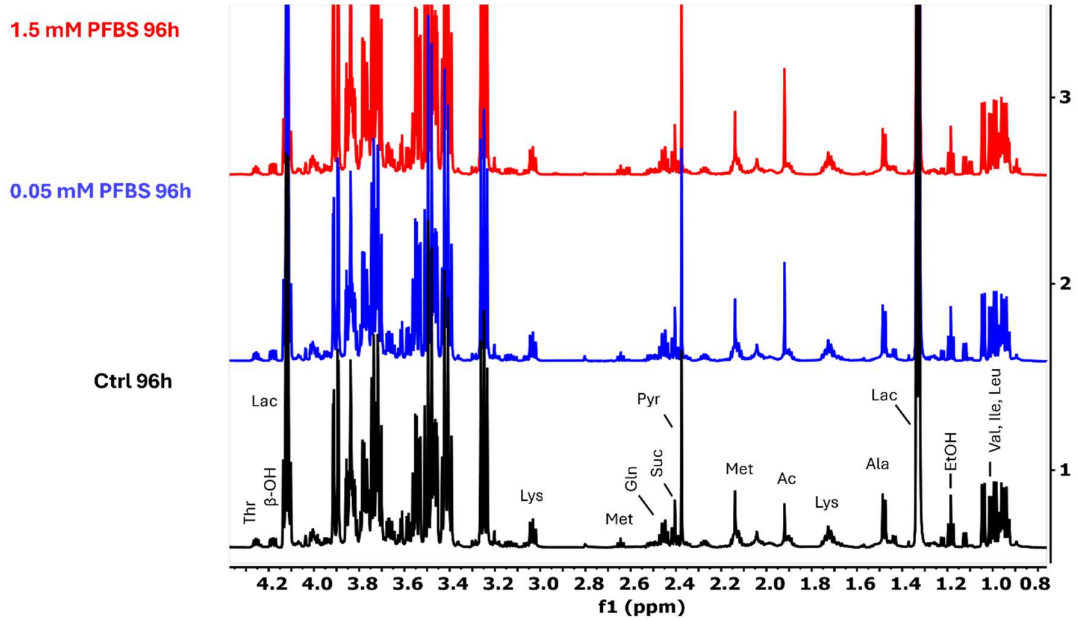

Fig. 1. Examples of ^{1}H spectra of supernatant treated for 96 hours. Black: control sample, Blue: treatment with 0.05 mM PFBS, Red: treatment with 1.5 mM PFBS.

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[P-28]

Improving the Stability of Colloidal CsPbBr3 Nanocrystals with an Alkylphosphonium Bromide as Surface Ligand Pair

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Keywords: solution NMR, materials, small molecules

Colloidal nanocrystals (NCs) of lead halide perovskites with general formula $CsPbX_3$ (X = Cl, Br, I), are very promising for optoelectronic devices, because of their optical properties [1]. However, they suffer from the drawback of being sensitive to the air humidity, which compromises their colloidal stability [1]. In this study [1] we synthesized a trimethyl(tetradecyl)phosphonium bromide (TTP-Br) ligand and employed it in a ligand exchange procedure involving Cs-oleate-capped $CsPbBr_3$ NCs (Figure 1a). The TTP-Br NCs increased the PLQY from ~60% (of Cs- oleate NCs) to more than 90%. The ligand shell composition was investigated by ^1H and ^1H–^{13}C HSQC (Heteronuclear Single Quantum Coherence) experiments reveling 92% of TTP-Br and only a residual 8% of Cs-oleate, Figure 1b. The dynamic interaction of the TTP^+ ligand with the NC's surface was evidenced by TTP peaks broadening in the ^1H NMR spectrum and a change in the sign of the NOE cross peaks in the ^1H -^1H NOESY (Nuclear Overhauser Spectroscopy) experiment, Figure 1c [1].Quantitative PULCON (PUlse Length-based CONcentration determination) NMR method [2] and elemental analysis performed via inductively coupled plasma optical emission spectroscopy (ICPOES) revealed a ligand density of 1.28 ligands/nm^2. The presence of TTP-Br ligands on $CsPbBr_3$ NCs conferred high air stability, with the NCs retaining ~90% of their PLQY after 6 weeks of air exposure, which surpasses that of didodecyldimethylammonium bromide-capped $CsPbBr_3$ NCs, i.e. the benchmark system.

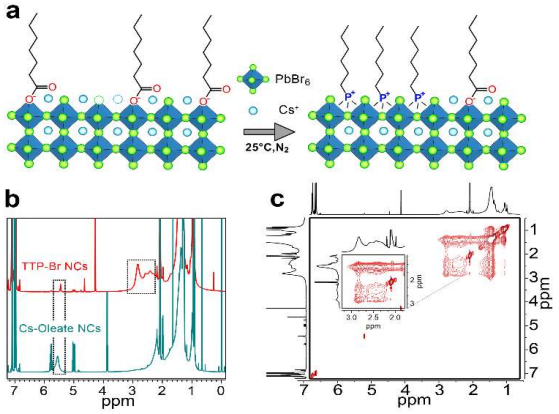

Fig. 1. a) Reaction scheme b) ^1H NMR spectra of Cs-Oleate and TTP-Br NCs in toluene-D at 298 K, c) ^1H -^1H NOESY experiment of TTP-Br NCs in toluene-D, at 313 K.

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[P-29]

Biocompatible Iron Oxide and Gold-Iron Nanoparticles for Multimodal In Vivo Imaging of Stem Cells and Extracellular Vesicles

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Keywords: *Biomolecules, Contrast agents, Multimodal Imaging, Magnetic Resonance Imaging (MRI)*

Nanoparticle-based imaging represents an increasing area of regenerative medicine, with magnetic resonance imaging (MRI) emerging as a crucial modality for non-invasive tracking of cell-based and cell-free therapies. To ensure sensitive and specific MRI detection, T2-based nanoparticles (NPs), which are recognized for their high relaxivity and ability to induce local magnetic field inhomogeneities, are extensively utilized in this context [1].

This study evaluates two commercial superparamagnetic iron oxide nanoparticles (SPIONs) (VivoTrax and VivoTrax Plus) and a gold-iron (AuFe) nanoalloy as T2 contrast agents through comparative physicochemical and biological characterization [2,3].

Nanoparticles were characterized using Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), and Transmission Electron Microscopy (TEM). Relaxation rates (1/T2) and relaxivity (r2) were assessed via MRI in different media to evaluate environmental effects. Adipose-derived stem cells (ASCs) internalize NPs, and labeled cells embedded in agarose phantoms underwent MRI to determine detection thresholds across pulse sequences.

Results showed that dispersion medium significantly affects SPIONs physicochemical and magnetic properties. VivoTrax Plus exhibited higher r_2 values than VivoTrax in water and HEPES, maintaining stability over time. However, in PBS, VivoTrax Plus showed a significant r2 decline.

AuFe NPs displayed high transverse and minimal longitudinal relaxivity, comparable to SPIONs, confirming their potential as T2 agents. MicroCT validated their CT contrast capability through a linear correlation between gold concentration and attenuation.

MRI analyses of labeled ASCs revealed that detection sensitivity varies with acquisition sequence, with the FLASH gradient echo sequence demonstrating greater sensitivity, evidenced by more pronounced signal loss, than the T2 turbo spin echo sequence. These findings underscore the potential of NPs-labeled ASCs for enhanced MRI detection.

Preliminary data also confirmed the presence of these NPs within EVs, underscoring the promise of this platform for MRI-based and multimodal tracking in cell-free therapies.

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[P-30]

PARTIAL LEAST SQUARES-DISCRIMINANT ANALYSIS TO CHARACTERIZE MRI ESTIMATES IN PARAMAGNETIC RIM LESIONS IN MULTIPLE SCLEROSIS

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Keywords: MRI, theory and methods.

Introduction: Quantitative MRI (qMRI) techniques are sensitive to microstructural tissue changes and provide insights into disability progression in neurodegenerative diseases such as Multiple Sclerosis (MS). Paramagnetic rim lesions (PRLs), marked by iron-laden microglia at lesion borders, indicate chronic active inflammation and are linked to worse outcomes. However, their complete MRI profile remains poorly characterized. This study aimed to investigate qMRI features of PRLs using Partial Least Squares Discriminant Analysis (PLS-DA), a supervised dimensionality reduction method that identifies components separating PRL+ from PRL− lesions.

Methods: Ninety-one MS patients were acquired on a 3T Philips Elition S MRI scanner using a multiparametric semi-quantitative and quantitative protocol, including: T1w and T2w sequences for T1-calibrated, T2-calibrated, and T1/T2 ratio (T1T2r) maps; Magnetization Transfer (MT) imaging for MT ratio (MTR) and saturation (MTS); multi-echo gradient echo (ME-GRE) for quantitative susceptibility mapping (QSM) and R2* relaxation rate (R2*rt); and multi-echo gradient and spin echo (GRASE) sequences processed with multi-component T2 relaxometry (MET2) to derive myelin water fraction (MWF), intra-/extra-cellular water fraction (IEWF), free water fraction (FWF), and corresponding T2 values (T2M, T2IE). ME-GRE and GRASE data were further combined to separate diamagnetic and paramagnetic susceptibility sources (χdia, χpara). Lesions were segmented on FLAIR images. Paramagnetic rim lesions (PRL+) were defined by a hyperintense rim on QSM at the lesion border. Partial least squares discriminant analysis (PLS-DA) was used to classify PRL+ vs. PRL– lesions based on mean lesion core qMRI values, normalized using each subject's normal-appearing white matter (NAWM) mean and standard deviation. Model performance was evaluated via 1000 random subject-wise train-test splits (85%/15%), ensuring each test set contained at least five subjects with 1 PRL+ lesion. Class imbalance in the training sets was addressed by random undersampling of the majority class. Classification metrics and regression coefficients were averaged across all permutations.

Results: A total of 115 PRL+ and 601 PRL- were identified. An average count of 115 lesions (of which 10% PRL+ and 90% PRL-) belonged to the test set in each permutation. The classification model demonstrated modest overall performance, with a mean accuracy of 0.77 and a weighted F1 score of 0.81. Macro-averaged metrics showed lower precision (0.62) and F1 (0.62). The strongest positive contributions to the regression model were associated with QSM and χpara (QSM=0.067, χpara=0.062), whereas the most prominent negative contributions were linked to MTR metrics, χ_{dia}, and MWF estimates (MTR=-0.065, MTS=-0.053, χ_{dia}=-0.055, MWF=-0.053).

Conclusions: Despite class imbalance, the model achieved stable performance, with high weighted F1 and ROC AUC values. The analysis revealed distinct imaging profiles, with PRL+ lesions core associated with lower magnetization transfer and myelin-related features and higher paramagnetic susceptibility markers. These findings support the potential of qMRI-based models to characterize the pathological outcome of chronic activity in MS.

[P-31]

LOW-FIELD MAGNETIC RESONANCE FINGERPRINTING

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

Magnetic Resonance Fingerprinting (MRF) is a novel imaging technique that offers a rapid quantitative approach for magnetic resonance imaging (MRI). We show the adaptation of the MRF for time-domain NMR at low field, with the objective of accelerating NMR multiparametric analysis for a wide range of applications, which at present necessitate the acquisition of multiple NMR sequences. The use of a pseudo-random RF sequence results in the generation of a unique signal, or "fingerprint", for differing NMR parameters (i.e. T_1, T_2). The matching of these fingerprints against a precomputed dictionary of synthetic signals enables MRF to identify the NMR parameters. In order to accomplish this task and ensure the robustness of the data analysis, RF pulse sequences can be customized for use with laboratory instrumentation.
The acquisition apparatus consisted of a Stelar NMR console connected to a 0.5 T permanent magnet, using 10 ml of 2 mM $MnCl_2$ as sample.
The MRF sequence implemented in this study was based on an IR-bSSFP scheme (269 RF pulses, flip angle pattern as described in [1], pulse spacing TR = 1 ms, repetition time = 4 s, 16 scans).
This new Stelar configuration enabled the use of over three times more RF pulses compared to previous implementations [3], enhancing the richness of the acquired signal.
The MRF signals were simulated with an in-house MATLAB toolbox (MARSS) [2]. A fully-connected Neural Network (NN) could be designed and trained by adapting the Deep Learning approach presented in [4]. Experimental data showed that B0 and B1 inhomogeneities strongly influenced signal modulation, and a procedure to characterize the B0-B1 correlation function was established to circumvent this issue.
The preliminary results obtained thus far suggest that low-field nuclear magnetic resonance (NMR) relaxometry is an evolving field of research. The findings indicate the potential for the development of analysis techniques for materials that are versatile and can be employed for a wide range of purposes.

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[P-32]

NMR-BASED CHARACTERIZATION OF THE CHEMICAL PROFILE, QUALITY, AND SHELF-LIFE OF KISSABEL RED FLESH APPLES IN BIODEGRADABLE PACKAGING

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Keywords: solution NMR, small molecules, food

Fruit and vegetables are highly perishable, resulting in significant post-harvest losses that impact their availability. Effective packaging is essential for reducing damage and contamination; however, traditional packaging methods contribute to global waste. Eco-sustainable bio-packaging minimizes environmental impact while keeping products fresh. Minimally processed apples are particularly susceptible to tissue softening, browning, and microbial growth, which complicates their marketing. Whithin the On Foods project [1], the present study aimed to chemically characterise red-fleshed apple slices (Kissabel) using NMR to assess the effects of coating and biofilm on storage. The study analysed hydrophilic and lipophilic metabolites over 21 days under controlled conditions, allowing for the identification and quantification of metabolite changes in response to treatment and packaging. Kissabel' apples (R201®) from Consorzio Melinda S.c.a. were treated with a bilayer coating of carboxymethylcellulose, alginate, citric, and oxalic acid. The uncoated (K) and coated (KT) apples were sliced into 100 g portions and packed in biodegradable films: PLA and ECO. Stored at 5°C for 21 days, with monitoring at 14 days, the samples were then freeze-dried, ground, and extracted using the Bligh-Dyer procedure [2]. The resulting extracts were dried and resolubilized for NMR spectroscopic analysis. The analysis of the samples was performed by combining the interpretation of 1D and 2D (^{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 extracts were performed using 2D experiments and literature data from other matrices analyzed under the same experimental conditions [3]. Sugars, organic acids, amino acids, polyphenols, fatty acids, sterols, phospholipids, and galactolipids were identified and quantified in the extracts. To assess the functionality of the two biofilms, we compared the metabolite profiles of untreated (K) and treated (KT) apples packaged in ECO and PLA at 14 and 21 days with those of the samples at time zero (t0). Analysing the quantification data across the three time points (t0, t14, t21), it was evident that both ECO and PLA biofilms effectively slowed the quality loss of Kissabel apples during storage, preserving cell structure and minimising spoilage.
Notably, the ECO biofilm demonstrated a superior ability to maintain a favourable internal storage environment, leading to reduced water loss.

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[P-33]

NMR PROFILING OF COMMERCIAL PROCESSED TOMATOES

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Keywords: solution NMR, metabolomics, food.

Tomato (Solanum lycopersicum L.) is a highly nutritious vegetable cultivated extensively worldwide. Over 80% of its production is used in processed forms. Recent studies indicate that a substantial portion of global tomato production is allocated to semi-processed products, including purée, peeled tomatoes, and concentrates, which consumers highly value. [1]
However, variability in both processing methods and raw material sources poses challenges in ensuring the final products' consistent quality, traceability, and chemical integrity.
Within the framework of the Agritech and MediHealthLab projects [2,3], this study aimed to chemically characterize processed tomato products available in large-scale retail and evaluate their quality and traceability through the use of NMR spectroscopy.
A total of 95 samples of tomato purées, peeled tomatoes, and concentrates, representative of products found in retail markets, were analyzed. Freeze-dried samples underwent biphasic extraction using deuterated solvents (D_2O/$CDCl_3$, 1:1), resulting in aqueous and organic extracts that were subsequently analyzed by NMR spectroscopy. [4,5]
Metabolite identification was performed by interpreting a combination of 1D (^1H) and 2D NMR experiments (^1H-^1H COSY, ^1H-^1H TOCSY, ^1H-^{13}C HSQC, and J-Resolved). The resulting data were statistically analyzed to explore potential correlations with variables such as product type, origin of raw materials (when declared on label), presence of added ingredients, expiration date, production site, and nutritional profile. The integrated approach combining NMR spectroscopy and statistical analysis revealed significant differences related to raw material origin and processing methods. These findings suggest that this methodology could serve as a valuable tool for quality control and traceability across the supply chain, contributing to the valorization of tomato-based products within sustainable agri-food innovation.

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[P-34]

SOLID-STATE AND SOLUTION NMR CHARACTERIZATION OF FONIO (*Digitaria exilis*).

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Keywords: :
solid state NMR, solution NMR, metabolomics, food.

Fonio (*Digitaria exilis*) is an African cultivated grass whose seed are an important food source for West African populations.. In Europe, it is marketed as Novel Food, and because of its nutritional value and sustainability, it has been listed as one of the 50 Foods of the Future by WWF For this reason, in order to better understand the characteristics of such a cereal, we analyzed, by NMR, some Fonio samples of different origin and compared the results with those obtained on wheat flour.
A cereal flour is mainly composed of polysaccharides, lipids, proteins and mineral salts coming from the different parts of the caryopsis. The polysaccharidic fraction is made of amylose, amylopectins and cellulose while the proteic fraction contains albumin, globulines, gliadins, glutenins and some enzymes mainly active on the saccharidic fraction. The protein content is one of the main factor determining the technological quality of a flour. In addition, the presence of some proteins is a source of allergies and intolerances
Moreover, in the flour are present also other low molecular weight metabolites linked to the metabolic activity of the plant which are more related to organoleptic quality and are good markers for the determination of the territorial origin of cereals [1].
To cover all the variety of this molecular landscape both solid state and solution state NMR has to be used.
In this work, we analyzed, by solid state CPMAS technique, the solid dry fraction of Fonio and of some soft and hard wheat flour samples to highlight the differences into the polysaccharide, protein and lipids contents.
In addition, we followed, by HR-MAS NMR, the enzymatic activity of amylases during the autolysis process, which is a crucial step in some baked products and give some insides into the digestibility of the cereal based products .
Finally, we analyzed the aqueous extracts of Fonio flours by high resolution liquid state NMR in order to compare the low molecular weight profiles of the different samples.

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[P-35]

THE SAM DOMAIN OF EPHA2 RECEPTOR AND ITS INTERACTOME: NMR-BASED DRUG-DISCOVERY APPROACHES

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Keywords: solution NMR, small molecules, biomolecules.

EphA2 is a receptor tyrosine kinase involved in several physiological and pathological events. Although EphA2 role in cancer is complex, depending on the cellular context, the receptor is known to contribute to tumorigenesis and cancer progression. Nevertheless, EphA2 levels are up-regulated in several solid tumors (including, but not limited to, esophageal, cervical, breast, ovary, prostate, pancreas, melanoma, and glioblastoma) and often correlate with tumor metastasis and poor patient survival. In addition, EphA2 favors the epithelial to mesenchymal transition and maintenance of cancer stem-cell-like characteristics [1].
Sam domains are small (~70 a.a.) protein binding modules: a Sam (Sterile alpha motif) domain is present at the EphA2 C-terminus (EphA2-Sam) and interacts with the Sam domains from the lipid phosphatase Ship2 (Ship2-Sam) and the adaptor protein Odin (Odin-Sam1). Both Sam-Sam complexes have been well characterized from the structural point of view by diverse techniques including solution NMR [1,2].
The binding of Ship2-Sam to EphA2-Sam has been better investigated from the functional side. Ship2 negatively regulates receptor endocytosis and degradation and modulates cell migration thus, mainly exhorting a pro-cancer effect [3]. Odin instead should increase receptor stability by acting on the ubiquitination process [4]. Hence, molecules able to inhibit Sam-Sam interactions mediated by EphA2 attract great attention for their anticancer potential. With this in mind, during the last few years, we designed and evaluated several peptide antagonists of heterotypic Sam-Sam associations involving EphA2 by employing mixed computational and experimental approaches where NMR spectroscopy plays a leading role [5,6]. Lately, we switched our interest to small molecule inhibitors of Sam-Sam complexes, due to their likely improved ADME properties with respect to peptides. Therefore, in our laboratory we are assembling a small fragment library to be implemented in NMR drug-discovery approaches. For library design, the dataset “Maybridge fragment collection” was largely inspected to select compounds respecting certain drug-like characteristics (according to the so called “Rule of Three”) and provided with high chemical diversity. A few molecules within the library were also chosen based on their possible employment in ^{19}F NMR drug discovery approaches. Herein, we will present preliminary data related to NMR screenings against the Sam domain of EphA2 and its binding partners.

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Solvent PRE as a biomolecular investigation tool: focusing on structurally heterogeneous proteins and IDPs

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Keywords: solution NMR, biomolecules, contrast agents, theory and methods.

NMR-based solvent paramagnetic relaxation enhancement (sPRE) has become a widely used tool for the study of biomolecules. Its appeal lies in the simplicity of its preparation: a soluble paramagnetic probe is added directly to the sample solution, inducing a concentration-dependent increase in relaxation rates that depends on the properties of the probe, the biomolecule, and the distance of the probe from the biomolecular surface. By measuring the relaxation rates of different nuclei at increasing concentrations of the paramagnetic agent, sPREs can be quantified along the protein sequence to map different conformations, probe solvent accessibility and weak interactions [1]. Such information is crucial to study how medically relevant proteins carry out their biological function. In this context, we aim to assess the applicability of this approach to develop a solid methodology for the characterization of structurally heterogeneous proteins. To this end, we performed a titration on α-synuclein – a well known IDP involved in Parkinson's disease – using Fe(NOTA) [2, 3] as the co-solute paramagnetic agent. For each point of the titration, a series of 2D Inversion Recovery experiments are acquired using different relaxation delays to obtain longitudinal relaxation rates of proton nuclei (R_1). R_1 rates were then fitted against Fe(NOTA) concentration to quantify the sPRE effect along the protein sequence and investigate the presence of transient conformations or weak interactions.

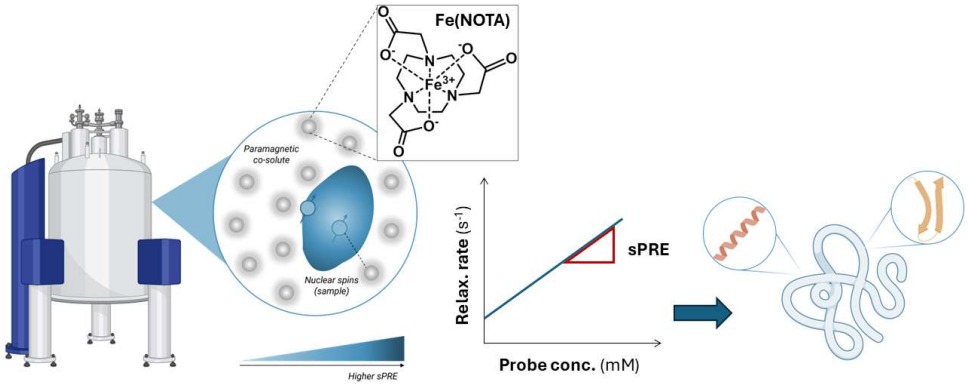

Fig. 1. Schematic representation of NMR sPRE experiments acquisition and interpretation

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SWELLABLE HYBRID MATERIALS FOR THE ADSORPTION REMOVAL OF PERFLUOROALKYL SUBSTANCES FROM WATER

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Keywords: solid state NMR, solution NMR, materials.

Perfluoroalkyl substances (PFAS) are persistent chemicals with stable carbon-fluorine bonds that lead to their bioaccumulation and biomagnification in living organisms, causing adverse health effects in humans and other organisms, including weakened immune responses, hormone disruption, and elevated cholesterol. Detected globally in water sources, often above regulatory limits, PFAS necessitate effective remediation. Adsorbent materials like zeolites, activated carbons, and polymers are actively being researched for PFAS removal from water [1]. Swellable Organically Modified Silicas (SOMS), hybrid materials synthesized by sol-gel process using bis(trimethoxysilylethyl)benzene (BTEB), are a promising option for PFAS removal, due to their unique ability to swell in organic solvents, that enhances pollutant adsorption. SOMS can also be functionalized with positive quaternary amine silanes (QA-SOMS) to impart a positive charge [2,3]. The materials were characterized by a multi-analytical approach to investigate physico-chemical properties, such as elemental analysis, SEM, XRD, N_2 physisorption, TGA, ss-NMR and Z Potential measurements. From ^{1}H, ^{13}C and ^{29}Si ss-NMR spectra it was possible to hypothesize a chemical structure (Fig. 1) and, from the latter, the degree of condensation of these materials. While HPLC is commonly used for PFAS detection, ^{19}F NMR spectroscopy presents a promising alternative, due to ^{19}F isotope's natural abundance and NMR activity, allowing nanogram-level detection of PFAS, if sample preconcentration is done [4]. SOMS and QA-SOMS materials have been tested for removing linear PFAS molecules, using ^{19}F NMR spectroscopy to measure their concentrations in water.

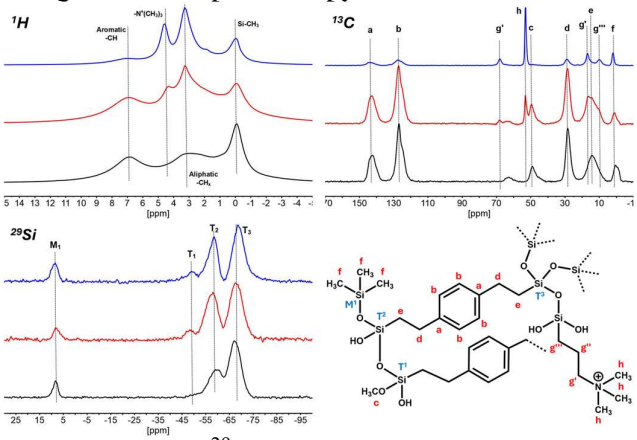

Fig. 1. ^{1}H (top-left), ^{13}C (top-right) and ^{29}Si (bottom-left) ss-NMR spectra of SOMS (black), QA-SOMS 20 (red) and QA-SOMS 200 (blue) and a hypothesized chemical structure of materials (bottom-right).

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[P-38]

RELAXOMETRIC INVESTIGATION OF AMPHIPHILIC Fe(III) COMPLEXES IN MIXED MICELLES AND HSA-BOUND ADDUCTS

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

Amphiphilic paramagnetic complexes offer significant advantages for MRI contrast agent development, particularly their prolonged plasma half-life and boosted relaxivity, often attributable to an increased rotational correlation time (τ_R). Despite this well-established design strategy, amphiphilic Fe(III)-based contrast agents have remained notably underinvestigated, with only a limited number of examples reported thus far [1]. This study investigates Fe(III) and Mn(II) amphiphilic complexes based on EDTA derivatives functionalized with a stearyl moiety, focusing on their ability to form micelles and bind to human serum albumin (HSA).

While spontaneous self-assembly was limited, stable mixed micelles with DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) were successfully formed for both metal ions and studied alongside with complexes interaction with HSA. Relaxometric analysis was performed through a global evaluation of ^{1}H NMRD and ^{17}O NMR profiles across temperatures and magnetic fields at physiological pH. Data were interpreted using Lipari-Szabo and Swift-Connick models to extract rotational and water exchange parameters [2]. Fe(III)-based micelles and HSA adducts showed a distinct relaxivity maximum shifted to higher fields compared to Gd(III) and Mn(II) [3,4], consistent with the fast electronic relaxation of Fe(III), which contributes significantly at high fields particularly in macromolecular systems, thereby limiting the accuracy of the Lipari-Szabo model [1].

These results demonstrate that optimizing the efficacy of Fe-based MRI contrast agents hinges significantly more on lengthening T_{1e} than on slowing down rotation.

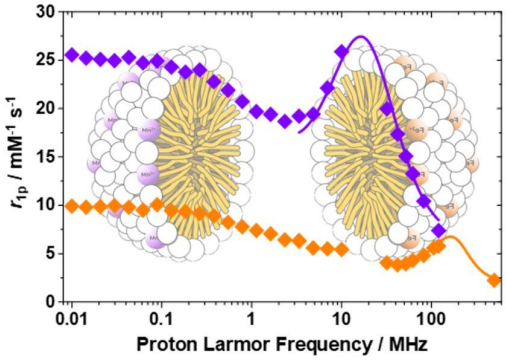

Fig 1. ^{1}H NMRD profiles of [Fe(St-EDTA)]$^{-}$ (♦) and [Mn(St-EDTA)]$^{2-}$ (♦) mixed micelles (298K).

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[P-39]

NMR SPECTROSCOPY CHARACTERIZATION OF PHOTOCROSSLINKED POLYMERS BASED ON VEGETABLE OILS

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

Nowadays, due to the increasing awareness about environmental impact, also in the polymers production sector, there is the need of adopting alternative synthetic paths. Photopolymers are a good substitute to thermally cured and solvent based polymers, thanks to high polymerization rates, low energy consumption, and no volatile solvents [1]. It has been already reported the use of vegetable oils as raw materials for the synthesis of photocrosslinked biobased polymers, by reaction of their triglyceride unsaturations or modifying them with more reactive functional groups [2]. This study aims at understanding the structure-properties relationship of photocured coatings based on vegetable oils thanks to NMR spectroscopy. Indeed, to relate properties to chemical structures it is necessary to understand the spatial heterogeneity of the polymer chains [3]. First, we characterized the vegetable oils and their derivatives by 2D liquid NMR spectroscopy (Figure 1a). Then we synthetized different polymeric networks by radical polymerization of the triglyceride double bonds or their acrylate derivatives also in the presence of difunctional thiols (thiol-ene polymerization). Photocrosslinking processes were monitored by real-time FT-IR and assured good conversions up to 90% (Figure 1b). Analyzing the final polymers through solid state NMR (Figure 1c), it was possible to assess the packing level of the polymer and to distinguish between a mobile and a rigid phase in the network.

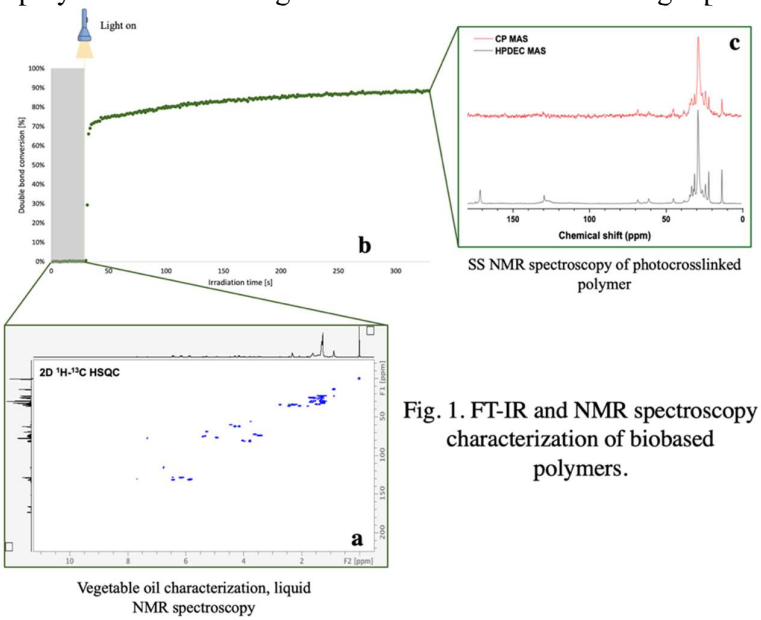

Fig. 1. FT-IR and NMR spectroscopy characterization of biobased polymers.

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[P-40]

SONOSENSIBLE LIPOSOMES DEVELOPMENT FOR TRANSPORT AND RELEASE OF GD-PICLENOL

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Keywords:
contrast agents

Liposomes are among the most used drug carriers with active and passive ways to release the drugs inside their core. In this work we focus on liposomes which release their content when stimulated with ultrasounds (US) to temporarily fenestrate the phospholipid membrane. In our case the goal is the controlled release of Gd-piclenol, an innovative MRI contrast agent. Since everything, from the US setup, to the lipids choice, till the content in-side the nanoparticle, influences the amount of release[1] we decided to do an extensive study with the help of chemometrics experimental design.
Based on our previous works we found a spot in the beam that was stable enough to stimulate exactly the space we wanted and we confirmed with the design of experiments (DoE) that has a comparable stability to the main focus. We found out that, as expected, the higher the amplitude of the signal, the higher the percentage of release, same thing for the duty cycle but in a positive-quadratic way. Frequency of the US stimulation was also important with the best release at a lower frequency than the transducer-rated 1 MHz. Pulse Repetition Period seems not to be an important variable in our setup, which aims to a strictly related mechanical release, not related to thermal events.
This will be also useful to be in safe levels of heating for a future in vivo study, for which the US setup was specifically designed (Fig. 1). In the immediate future we are going to study the impact of liposome dimension, liposome concentration, incapsulated solute concentration on the US sensitivity following the same steps.

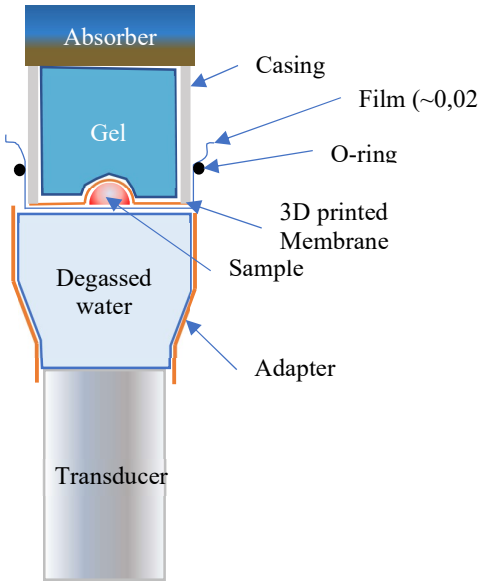

Fig. 1. The US stimulation setup, specifically designed to mimic the subcutaneous tumor model.

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[P-41]

FOODS AND NMR: A CHALLENGE FOR COMPLETE DATABASES

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

Foods are complex matrices important for satisfying nutritional needs but also because a proper diet combined to a healthy life-style offers numerous benefits, reducing the risk of diseases and promoting general well-being. A first fundamental step is in-depth knowledge of the chemical composition of the food. Current databases do not report information on bioactive components and metabolites that drive biological functions, nutritional status, and body health. Information on these bioactive components is pivotal for more precise dietary interventions against malnutrition and diseases.
The project "MeDiHealthLab", sponsored by the Italian Ministry of Health (Piano Operativo Salute, Traiettoria 5) [1], is dedicated to the creation of a research infrastructure in human nutrition that could provide help to National Network Against Malnutrition promoting the principles of the Mediterranean Diet.
One of the objectives of "MeDiHealthLab" is the development of a database including relevant data on conventional foods typical of the Mediterranean Diet. To this purpose, a systematic analysis of the available literature on the chemical composition of foods typical of the Mediterranean Diet was carried out using the main archives of biomedical and life sciences journals. In this work, some examples are reported, such as extra virgin olive oil, tomatoes and beans [1, 2].
In this perspective, the Italian Group of Magnetic Resonance in Food Science (within GIDRM) is developing a new database of NMR spectra foods. The database can be interrogated through different keys such as type of food, variety, geographic origin etc. At the end of the query, the NMR spectrum of the selected food is displayed with specific annotations if available.
Together, these initiatives lay the groundwork for an integrated approach to nutrition, supporting more targeted and effective dietary interventions.

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[P-42]

HISTIDINE OXIDATION IN AMYLOID-β FIBRILS TRIGGERED BY BORON NEUTRON CAPTURE THERAPY (BNCT) WITH BORONATED PROBES

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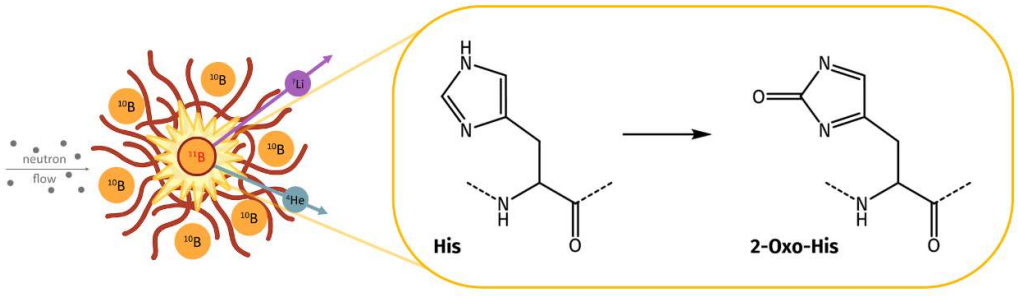
Keywords: solution NMR, biomolecules.

Alzheimer's disease (AD) is a neurodegenerative disorder marked by progressive cognitive decline. The aggregation of amyloid-beta (Aβ) peptides into oligomers and fibrils plays a central role in its pathogenesis. While oligomers are the most neurotoxic species, larger aggregates act as reservoirs, sustaining pathological Aβ levels [1]. Although anti-Aβ monoclonal antibodies have recently been approved, their therapeutic efficacy remains limited and side effects are significant. In other amyloid-related diseases, radiotherapy approaches (Targeted Alpha Therapy (TAT), External Beam Radiation Therapy (EBRT)...) have demonstrated promising results[2,3]. Building on this concept, the NECTAR project investigates Boron Neutron Capture Therapy (BNCT) to selectively destabilize Aβ aggregates and aid their clearance. This is achieved using ^{10}B-enriched monocarbonyl analogs of curcumin (BMACs) [4], which bind Aβ fibrils and permit site-specific alpha emission upon neutron irradiation. In vitro Aβ aggregates were characterized by FESEM and Thioflavin T staining, while the binding affinities of BMACs were obtained through competition assays, using the Cheng-Prusoff equation to calculate inhibition constants. Post-irradiation analysis by ^{1}H-NMR and mass spectrometry revealed a selective oxidation on histidine residues, a chemical modification that can



induce fibril destabilization and impair further amyloid aggregation (Fig. 1). This study may extend the understanding of radiation effects on proteins, particularly in the context of amyloidosis.

Fig.1: Schematic illustration of the effects of BNCT on Amyloid-β fibrils.

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[P-43]

NMR METABOLOMICS COMPLEMENTS A STUDY ON KIDNEY TRANSPLANT AFTER MARGINAL DONATION

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Keywords:
solution NMR, metabolomics, theory and methods.

Introduction. NMR metabolomics was adopted to complement a study on kidney transplant after marginal donation. The study investigated the effects of a drug, the PCSK9-inhibitor PEP 2-8, on ischaemic injury in a rat model of donation after circulatory death (DCD) [1].
Methods. Kidneys deriving from 15 rats, were perfused for 6 h, at 4°C with a standard solution for preservation of explanted grafts, with/without PEP 2-8 supplementation. After 6 h various parameters were assessed, including tubular ischaemic injury (TID) score and an array of indicators of energy metabolism and oxidative stress. In addition, metabolomics of kidney biopsies embedded in optimal cutting temperature compound (OCT) was performed.
After tissue release from OCT and homogenization/extraction of hydrophilic metabolites, the study samples were analyzed at a 700MHz spectrometer, with standard acquisition procedures [2]. The ASICS [3], [4] approach for automated metabolite identification and quantification of metabolites from 1D-^{1}H spectra was adopted.
Results. Preliminary test experiments showed that OCT embedded biopsies exhibit a globally different profile compared to plain frozen ones: a protective effect of OCT is suggested by the detected concentration of diagnostic compounds as ATP and lactate.
Over 160 of the 216 compounds included in the ASICS library of metabolites were quantified in the study samples. Metabolomics contributed to clarify the molecular mechanism by which the inhibition of PCSK9 during pre-transplant renal conditioning protects against ischaemic damage: the reduction of sarcosine, carnosine, biliverdin, dimethylamine and 2-oxybutyrate associated to PEP 2-8 treatment suggests that its main effect is reducing oxidative stress.
Conclusions. It was demonstrated that OCT-frozen tissues are usable for metabolomics. This is one of the first reported application of the ASICS approach on non-biofluid sample: the efficiency of the tool was proved.

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[P-44]

COMBINING NMR SPECTROSCOPY AND BIOPHYSICAL TECHNIQUES FOR THE CHARACTERIZATION OF XANTHOHUMOL ANTI-AMYLOIDOGENIC PROPERTIES

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Keywords: solution NMR, small molecules, biomolecules, food

In recent years, age-related disorders have gained significant attention; among these, Alzheimer's disease (AD) stands out as one of the most prevalent neurodegenerative conditions. One of the key challenges of AD is the considerable delay between the triggering of the underlying molecular processes and the onset of the first symptoms, thus making therapeutic interventions ineffective. Therefore, the development of primary prevention strategies should be of major importance [1]. In this context, diet emerges as one of the most promising preventive interventions, as it can modulate several key biochemical processes in AD pathogenesis through a multi-targeted approach [2].
Notably, prenylated flavonoids (PFs), a class of compounds found in several plant families, exhibit a broad range of beneficial properties [3]; in particular, they have been shown to inhibit Aβ1-42 peptide aggregation, one of the many hallmarks of AD, thereby preventing the formation of neurotoxic oligomers and fibrils [4]. This ability is especially relevant in the context of AD prevention, as it may interfere with the early stages of aggregation and help slow disease progression.
Here we present a comprehensive structural analysis [5] of the interaction between Aβ1–42 oligomers and xanthohumol (XN), a PF naturally occurring in hops, revealing its potential to modulate amyloid aggregation pathways. By integrating STD-NMR and ^{15}N-SOFAST-HMQC NMR experiments with other biophysical techniques, we have demonstrated that XN forms stable complexes with Aβ1–42 oligomers, effectively preventing their transition to β-sheet-rich fibrils. These findings dissect the anti-amyloidogenic mechanism of action of XN at molecular level, providing structural insights for the rational design of a novel class of Aβ inhibitors. Moreover, this compound holds promise as a nutraceutical for AD prevention; dietary supplementation with XN may modulate early molecular events linked to neurodegeneration, offering a safe and sustainable approach to neuroprotection.

Acknowledgements
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[P-45]

DEVELOPMENT OF PEPTIDE-BASED STRATEGIES FOR PRION DISEASE THERAPY

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Keywords: Neurodegenerative diseases, Nuclear Magnetic Resonance, protein misfolding.

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are degenerative disorders of the nervous system caused by transmissible particles that contain a pathogenic isoform of the prion protein, a normal constituent of cell membranes. In particular, the crucial event of prion diseases is the conformational change of the normal prion protein (PrP^{C}) into a β-sheet rich pathogenic isoform (PrP^{Sc}) that is prone for aggregation into insoluble amyloid fibrils [1]. This structural transition can be triggered either by genetic mutations or by infection through seeding PrP^{Sc} forms, proceeding through misfolded intermediate conformations. Recently, it was demonstrated the crucial role of the interaction between the N-terminal domain (NTD, residues 23-89) and the C-terminal domain (CTD, residues 90-231) in regulating the conformational dynamics that modulate the folding process of the entire prion protein. Especially, the structural and dynamic factors governing the prion misfolding mechanism were characterized, showing how the truncated HuPrP90-231 (CTD) transforms into amyloid fibrils via the formation of a β-sheet-rich intermediate state (β-PrPI) that in turn drives the initial stages of prion fibrillation. [2] Based on these findings, the present study aims to inhibit the pathogenic transformation of prions by designing peptide-based strategies able to interfere with the early stages of misfolding and aggregation process, thus avoiding dangerous misfolded intermediate states or oligomeric species critical to amyloid assembly. We show an analysis of the conformational peculiarities of a twenty-one amino acid peptide, encompassing the region from Lys23 to Ser43 of the human prion protein's N-terminal domain (NTD), named MANTRAP 1. Moreover, a description of the structural proprieties of the NTD is also reported. Ineterstngly, NMR spectroscopy reveals that MANTRAP 1 exhibits significant conformational flexibility without adopting defined secondary structures, and it can transiently bind to HuPrP(90-231).

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[P-46]

METABOLOMIC PROFILING OF MYRTLE LEAVES EXTRACTS AND EVALUATION OF THEIR ANTI-AMYLOIDOGENIC ACTIVITY AGAINST Aβ1-42 PEPTIDE

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

Neurodegenerative disorders, such as Alzheimer's disease (AD), are characterized by the accumulation of extracellular insoluble aggregates of misfolded proteins, notably the Amyloid-β (Aβ) peptide, that lead to synaptic dysfunction and nerve cell death [1]. Among the strategies aimed at counteracting Aβ aggregation, natural polyphenolic compounds, particularly flavonoids and catechins, have shown promising anti-amyloidogenic and neuroprotective properties [2-4].
Myrtle (*Myrtus communis*) is an evergreen aromatic plant, historically used in cooking and liqueur production. While the leaves are often considered a waste product, they could represent a valuable resource, as they are known to contain a variety of bioactive compounds [5].
Here we describe the characterization of hydro-alcoholic extracts of myrtle leaves using a combined NMR and LC-HRMS metabolomic approach. Both total extracts and phenolic-enriched fraction were analyzed, revealing the presence of several bioactive compounds, including flavonoids, hydrolysable tannins, myrtucommulones, organic acids, and glycosylated phenolic acids. The biological activity of myrtle leaves extracts was evaluated at first by binding assays employing STD-NMR to confirm direct binding interactions between selected extract compounds and Aβ1-42 oligomers. Furthermore, myrtle extracts anti-amyloidogenic activity against Aβ1-42 peptide was investigated by other complementary approaches, such as Fourier-transform infrared (FTIR) spectroscopy and atomic force microscopy (AFM), which provided structural and morphological evidence supporting a significant alteration in Aβ1-42 aggregation pathway after incubation with myrtle extracts.
Our data suggest that myrtle bioactive compounds could represent promising tools for the safe and sustainable prevention and treatment of neurodegenerative disorders by exploiting the combination of their antioxidant, anti-inflammatory, and anti-amyloidogenic properties [2] in the modulation of early molecular events linked to neurodegeneration.

Acknowledgements
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[P-47]

Structural Diversity and Evolutionary Expansion of Ros/MucR Proteins from Bacteria to Eukaryotes

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Keywords:
solution NMR, biomolecules.

The identification of the ROS protein in *Agrobacterium tumefaciens*, a Gram-negative α-proteobacterium, challenged the long-held view that zinc finger (ZF) domains are exclusive to eukaryotes. Subsequent genetic and biochemical studies have classified ROS homologues within the Ros/MucR family—transcriptional regulators widely distributed across bacterial phyla [1, 2]. These proteins bind AT-rich DNA through oligomeric assemblies that modulate DNA topology and repress gene expression, functioning analogously to H-NS proteins in bacteria lacking canonical H-NS systems [3].

In this study, we performed a comprehensive computational analysis, identifying 1,840 Ros/MucR homologues spanning a broad taxonomic range. The majority were detected in α-proteobacteria, followed by γ- and β-proteobacteria, with a smaller presence in δ-proteobacteria and Gram-positive bacteria. Notably, 17 homologues were also found in eukaryotic lineages including Chromista, Plantae, Fungi, and Animalia. While α-proteobacterial sequences showed high similarity to the original ROS protein, increased divergence was observed in β- and γ-proteobacteria, and moderate similarity in eukaryotic representatives.

Our analysis of zinc coordination patterns revealed striking structural diversity across taxa, with β- and γ-proteobacteria displaying the greatest variability. Eukaryotic homologues also exhibited distinct coordination motifs, highlighting the structural adaptability of the Ros/MucR family. However, experimental validation of zinc-binding functionality in these variants is still required.

Among Ros/MucR family members, we selected MucR2 from *Sinorhizobium meliloti* for detailed structural characterization due to its unusual zinc-coordinating residues—Cys, Asp, His, Lys, and Tyr (CDHKY). Using NMR spectroscopy, we confirmed that the DNA-binding domain of MucR2 adopts a monomeric state in solution and binds zinc under near-physiological conditions.

These preliminary results expand our understanding of Ros/MucR evolution and function, opening new avenues for exploring the roles of this family of zinc fingers in both prokaryotic and eukaryotic systems.

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[P-48]

FROM GRAMS TO MILLIGRAMS: AN NMR-BASED SCALABLE ANALYTICAL APPROACH FOR TOTAL GADOLINIUM CONTENT

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Keywords: solution NMR, MRI, contrast agents, theory and methods

Gadolinium-based contrast agents (GBCAs) play a crucial role in the pharmaceutical industry, particularly in the development of diagnostic tools for magnetic resonance imaging (MRI). Ensuring a precise and accurate determination of total gadolinium content is essential to guarantee product quality and regulatory compliance throughout research, development, and manufacturing phases.
In early-stage research and development, however, available sample quantities are often extremely limited. This poses a significant challenge to conventional analytical methods that typically require larger amounts of material. To address this, we evaluated an alternative NMR-based scalable methodology capable of maintaining high accuracy and precision even at reduced sample scales. Using $Gd(NO_3)_3$ and Gadopiclenol as representative compounds, we revisited and adapted a literature-reported analytical protocol to assess its robustness and suitability for small-scale applications.[1] In the case of Gadopiclenol, we achieved a significant reduction in the required sample amount compared to existing internal procedures. Moreover, this method requires a more straightforward preparation process, yet yields reliable results. In fact, it provided recoveries within the acceptance range of 97–103%, and RSD% values consistently below 1%, confirming its excellent accuracy and precision. Furthermore, considering the growing interest in emerging classes of MRI contrast agents based on Fe(III) and Mn(II),[2,3] we also extended and validated the method for the determination of their total metal content. These results highlight the versatility and reliability of this NMR-based approach across a broader range of paramagnetic agents.

Fig. 1. NMR provides accurate and precise titrations of GBCAs using lower quantities than conventional techniques.

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[P-49]

CATECHOLAMINE PROFILING AND CLASSIFICATION OF URINE SAMPLES FOR DIAGNOSIS AND PROGNOSIS OF NEUROBLASTOMA

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Keywords: (please select in this list the keywords suitable for your contribution and delete the unselected ones):
solution NMR, biomolecules, metabolomics, theory and methods.

Neuroblastoma is an extracranial solid tumor accounting for nearly 15% of cancer-related mortality in pediatric patients. Despite being among the most common tumors in neonatal age, the currently existing risk classification and treatment methodologies are frequently inadequate. Enhanced diagnostic and prognostic tools are therefore necessary to reduce both mortality rates and treatment-associated side effects.
The presence of neuroblastoma is associated with supraphysiological levels of various catecholamine derivatives in both blood and urine, suggesting that a comprehensive profiling of such metabolites could significantly improve diagnostic and prognostic evaluations. For this purpose, Nuclear Magnetic Resonance (NMR) offers several advantages over existing methods, particularly regarding sample preparation and data richness.
However, the high information content of NMR spectra for physiological samples such as urine can pose a significant challenge. To convert raw spectral data into clinically useful insights, a chemometric strategy is proposed. Multivariate data analysis techniques, such as Principal Component Analysis (PCA), allow for the dimensionality reduction of complex datasets and the extraction of meaningful patterns. This process facilitates the visualization of trends and clustering that may be linked to disease states. In clinical metabolomics, this ability to recognize subtle but consistent variations across samples is essential to developing robust diagnostic tools, especially when dealing with heterogeneous conditions like neuroblastoma.
To complement this data-driven approach, we propose the use of nanoparticle-assisted NMR chemosensing. This technique is based on magnetization transfer between a host nanoparticle and guest analytes, allowing for selective signal enhancement. Gold nanoparticles are employed as tunable supramolecular hosts, with their binding properties controlled via ligand design. Specific NMR sequences, such as High-Power water Saturation Transfer Difference (HPwSTD), are used to highlight signals of analytes rapidly exchanging with the host, effectively simplifying the spectral landscape.
Together, these two strategies converge into a promising methodology for improved data acquisition and processing. The combined use of NMR chemosensing and chemometric analysis may thus support more accurate and effective diagnostic and prognostic approaches in neuroblastoma.

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[P-50]

NMR-BASED CHARACTERIZATION OF LUCANIAN PROPOLIS EXTRACTS FOR POTENTIAL USE AS ANTIMICROBIAL AGENTS IN ELECTROSPUN SCAFFOLDS FOR SKIN DISEASE TREATMENT

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Keywords:
solution NMR, biomolecules, metabolomics, food, polymers.

This study presents the NMR-based characterization of propolis extracts collected from the Lucanian region (Basilicata, Southern Italy), aiming to investigate their complex chemical composition[1]. Propolis—a resinous substance produced by honeybees through the combination of plant resins and bee-derived enzymes—is renowned for its rich content of bioactive compounds, including flavonoids, phenolic acids, terpenoids, and aromatic esters.
However, the full valorization of propolis is hindered by its highly heterogeneous composition, which varies with geographical origin and seasonal factors. Therefore, the development of a robust method for propolis standardization is urgently needed.
This study utilizes 1D and 2D NMR techniques—such as ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC—to perform a non-destructive, high-resolution metabolic profiling of ethanol-based extracts of Lucanian propolis. The goal is to identify and structurally characterize the key constituents, compare the chemical profiles based on extraction methods, and establish a spectral fingerprint that may be used for authentication, quality control, and bioactivity correlation.
Propolis from 3 different beekeeping companies, that are practicing migratory bee keeping in different areas of Lucanian region were collected and extracted with different methods: traditional maceration, sonication and Soxhlet extraction.
The propolis extracts exhibited highly similar chemical profiles, as confirmed by NMR spectroscopy, despite variations in their geographical origin and botanical sources. Several key compounds, such as apigenin, pinocembrin, galangin, and caffeic acid derivatives, were identified and structurally confirmed [2]. The NMR profiles also reflect the botanical signature of the Lucanian region, supporting the potential use of these data for geographic authentication and quality control. Finally, the extract with the highest phenolic content (373 mg GAE/g extract) was successfully incorporated into electrospun nanostructured scaffolds composed of poly-D,L-lactide (PDLLA) and gelatin.

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[P-51]

GE11-Functionalized Liposomes for Dual Boron Neutron Capture Therapy and MRI Visualization in Non-Small Cell Lung Cancer

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Keywords: MRI, materials, contrast agents.

Cancer remains a major health challenge worldwide due to its uncontrolled cell growth and resistance to conventional therapies. Lung cancer is among the most prevalent types, and approximately 80% of all cases are non-small cell lung cancer. Among innovative treatment approaches, boron neutron capture therapy (BNCT) has been shown to be a promising therapeutic strategy. BNCT is based on the selective accumulation of boron-containing compounds in tumor cells [1]. Upon neutron irradiation, these compounds undergo nuclear reactions that release cytotoxic alpha particles, selectively destroying malignant cells while minimizing damage to surrounding healthy tissue.

However, for BNCT to be effective, a high concentration of boron atoms must be delivered to tumor cells. Currently, only two BNCT drugs are available for clinical investigation, L-para-boronophenylalanine (BPA) and sodium mercaptododecaborate (BSH). Although both drugs are used clinically, their selectivity is relatively low.
To overcome this limitation, advanced drug delivery systems are being developed to enhance tumor targeting and improve therapeutic efficacy. Additionally, incorporating a Magnetic Resonance Imaging (MRI) probe allows for the direct observation of the biodistribution of the administered compound, enabling the assessment of the amount of compound that has reached the tumor site.

Among the most promising molecular targets for treatment of non-small cell lung cancer is the epidermal growth factor receptor (EGFR), as it frequently shows a high expression in malignant lung cells. [2] For carrying both therapeutic and contrast agents, liposomes represent a versatile nanocarrier platform, capable of internalizing compounds both in the internal aqueous cavity and in the phospholipid bilayer, allowing the transport of both hydrophilic and hydrophobic compounds.

In this work, we propose the use of EGFR targeted liposomes, functionalized with GE11 peptide, to selectively target the EGFR receptor in lung cancer cells. The liposomes were prepared using the thin lipid film hydration method, followed by surface modification with GE11 peptide for active targeting. BSH, as a BNCT drug, and a Gd complex as an MRI contrast agent, were encapsulated in the aqueous core. This system aims to enhance the accumulation of boron in tumor tissues, and the quantification of its distribution in the tumor.

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[P-52]

THERMO-OXIDATIVE DEGRADATION OF COTTON TREATED WITH FLAME RETARDANTS: A JOINT SOLID-STATE NMR AND EPR INVESTIGATION

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Keywords: solid state NMR, materials, polymers.

Cotton is naturally flammable, posing a serious risk in the event of a fire, especially when used in indoor furnishings. Its fire resistance can be improved with flame-retardant treatments. Among emerging bio-based solutions, polyamidoamines (PAAs) derived from natural α-amino acids stand out as sustainable, effective, and smoke-free alternatives [1]. This study explores the degradation of cotton treated with PAAs under fire-like conditions. Since combustion is a fast and complex process, controlled thermos-oxidation was used to simulate it, allowing the characterization of intermediate products. To date, no detailed mechanism has been reported for the thermo-oxidation of cotton in the presence of PAAs. To gain insight into this process, ^{1}H and ^{13}C solid-state NMR and EPR spectroscopies were employed to analyze the char residues of cotton treated with PAAs derived from glycine and cystine, as well as the residues of the chars from pure PAAs. The combined use of these spectroscopies suggests that the PAAs form a protective layer inaccessible to oxygen which effectively shifts the thermal degradation pathway of the impregnated cotton towards a pyrolysis-like process. As an example, Fig. 1 shows that pyrolyzed cotton and cotton impregnated with the PAA and thermo-oxidized exhibit similar spectra, which are markedly different from that of thermo-oxidized pure cotton.

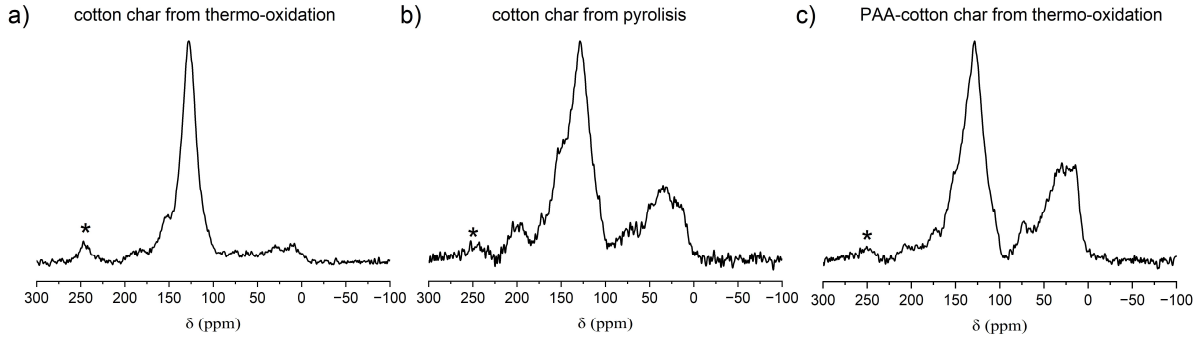

Fig. 1. ^{1}H-^{13}C cross-polarization MAS NMR spectra for: a) pure cotton and c) cotton impregnated with a cystine-based PAA, both thermo-oxidized at 350 °C, and b) char from pure cotton pyrolyzed at 350 °C. Spinning sidebands are marked with asterisks.

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Acknowledgments

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[P-53]

REAL TIME OLIGOMERS DISTRIBUTION OF AMYLOIDOGENIC GELSOLIN G2 DOMAIN BY DOSY NMR SPECTROSCOPY

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Keywords:
Solution NMR, biomolecules

Gelsolin amyloidosis (AGel) is a rare autosomal dominant disease caused by the deposition of mutated Gelsolin amyloid fibrils in various tissues. Gelsolin is a six-domain ubiquitous calcium-activated, actin-modulating protein involved in multiple biological processes. The most common disease-associated mutations of gelsolin are caused by substitutions in the second domain (G2), including D187N/Y (Finnish type) which occur at the G2 calcium binding site [1].
NMR spectroscopy offers the unique capability to characterize transient and heterogeneous systems in all-atom detail, without perturbing the self-assembly process. In this study, we employ solution NMR spectroscopy to investigate the initial steps of the aggregation process of the G2-WT and G2-D187N mutant, mapping the time evolution of oligomers populations. We combined NMR diffusion ordered spectroscopy (DOSY) with the Inverse Laplace Transform reconstruction method [2] to detect rapidly interconverting oligomers and understand the key events of the amyloidogenic process. This study paves the way for investigating the mechanism of action of peptidomimetics [3] as inhibitors of the aggregation process and evaluating their efficacy in binding oligomers and modulating their toxicity.

Acknowledgements

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[P-54]

Les Liaisons Dangereuses: NMR and Data Science

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Keywords: solid state NMR, solution NMR, theory and methods.

The only experimental technique that has complete chemical specificity and is universally quantitative is Nuclear Magnetic Resonance (NMR). However, as an old joke goes, NMR has three problems: sensitivity, sensitivity, and sensitivity. The NMR community recognized the need to apply numerical methods to solve these problems in a post-acquisition stage, improving the analysis that will follow. In this presentation, we will focus on some of the issues that degrade spectral quality – including baseline and phase distortions (Figure 1), and on the processing methods that our group proposed to deal with them, as well as on the applications to relaxometry and reaction monitoring [1-4].

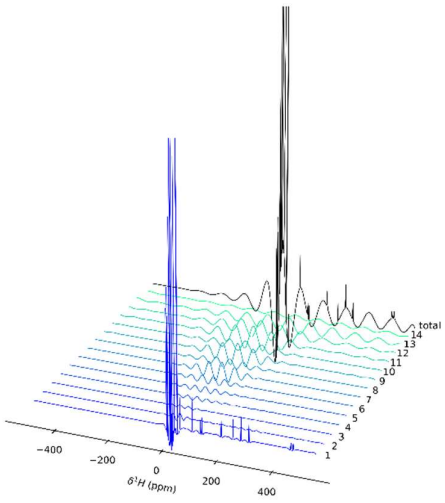

Fig. 1. Decomposition of a distorted ^{1}H NMR spectrum of a paramagnetic compound at 28 T.

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[5] L. Fiorucci, F. Bruno, M. Ricci, A. Cicone, E. Ravera, *MRC* **in press**

[P-55]

ELECTROCYCLIZATION REACTIONS OF DIPHENYL-AMINES: NMR STUDY ON THE EFFECT OF SUSTAINABLE AND CONFINED MEDIA

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Keywords: solution NMR, small molecules.

Due to its biological properties like antitumor, antibiotic, antiviral and anticonvulsant activities [1], the synthesis of relevant carbazole scaffolds (via C-C and C-N bond formation as the key-step) is an issue that is under permanent revision [2] and several approaches have been proposed.
We present herein a spectroscopic investigation on the reactivity of the diphenylamines under oxidative conditions in organic solvents (cyclohexane, acetonitrile and methanol) and in micellar solutions (built up using anionic, cationic, and neutral surfactants) [3] to afford carbazole scaffolds.
The NMR study, especially through the application of NOESY and DOSY experiments, allowed to detect the diphenylamine- and N-methyldiphenylamine-surfactant complexes and to investigate the location of the amines within the hydrophobic core of micelles [4].

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[P-56]

EVALUATION OF THE CYTOTOXIC AND REGENERATIVE EFFECTS OF SERICOSIDE ON SH-SY5Y CELLS: INSIGHTS INTO THE METABOLIC PROFILE

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Keywords: solution NMR, biomolecules, metabolomics.

Terminalia sericea is a medicinal plant rich in bioactive compounds like saponins, flavonoids, tannins, and triterpenic acids, exhibiting antioxidant, antimicrobial, and skin-rejuvenating effects. Particularly, its saponin sericoside contributes to skin health and connective tissue support. Recent research demonstrates its promising potential in dermatology and anti-aging treatments [1,2].
The aim of the study was to evaluate both the cytotoxic activity and cell regeneration capacity of sericoside at different concentrations on the SH-SY5Y cell line. Moreover, we analyzed the metabolic profile of the cells under untreated conditions and after treatment with different concentrations of sericoside, using the HR-MAS NMR spectroscopy. This approach allowed for the comparison of cellular activity and metabolic changes. Preliminary results revealed significant variations in choline-containing compounds, key elements in membrane synthesis, which were correlated with the cell regeneration data obtained from scratch tests. These results suggest a link between membrane metabolism and the regenerative response influenced by sericoside, providing insight into its potential biological effects.

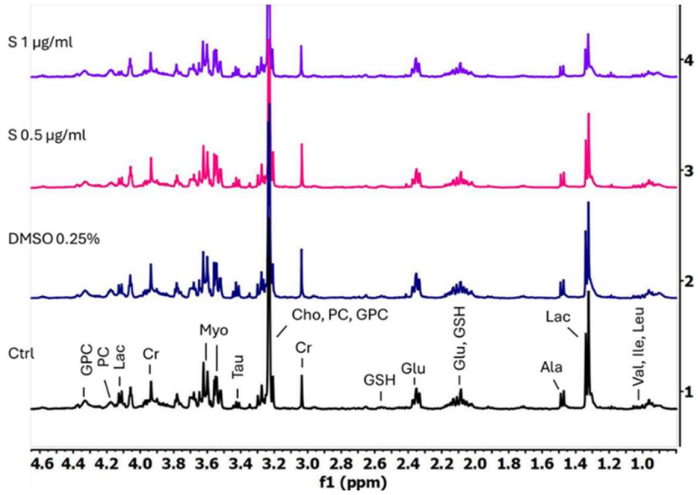

Fig. 1. Representative ^{1}H HR-MAS NMR spectrum acquired on human neuronal SH-SY5Y control cells (CCtrl) and treated with DMSO, with sericoside 0.5 µg/mL and with sericoside 1µg/mL.

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[P-57]

Characterization of brain microstructure and connectivity in patients with bipolar disorder and schizophrenia

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Keywords: MRI, theory and methods.

Advanced neuroimaging techniques, such as Diffusion-Weighted Imaging (DWI) and Diffusion Tensor Imaging (DTI), enable non-invasive examination of the white matter (WM) microstructure and structural connectivity by analyzing the diffusion properties of water in neural tissue.
This study investigates WM microstructure and structural connectivity in patients with early-stage major psychoses, specifically schizophrenia (SCZ) and bipolar disorder (BD), compared to healthy controls (HC). Although these disorders may share common disruptions, it remains uncertain whether such alterations emerge early or gradually evolve with the persistence or recurrence of illness [1].
DWI data were pre-processed to remove artifacts, and DTI was applied to extract four metrics for assessing microstructural WM alterations: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Structural brain connectivity was evaluated using tractography based on single-shell 3-tissue Constrained Spherical Deconvolution (SS3T-CSD) for estimating fiber orientation distributions. The resulting connectomes constructed using SS3T-CSD-derived streamline counts and mean values of FA, MD, AD, and RD, were represented as graphs for subsequent graph-theoretical analysis.
Two statistical comparisons were conducted. First, a region-based ANCOVA (controlling for age and sex) compared DTI-based metrics between patients and HC. After Bonferroni correction, only the left thalamus exhibited a statistically significant difference, with reduced FA values in patients, aligning with its known involvement in sensory, cognitive, and emotional processing [2]. Second, global network metrics (density, strength, clustering coefficient, efficiency, and modularity) were extracted from the five connectomes using MATLAB's Brain Connectivity Toolbox to assess potential alterations in structural connectivity. The Wilcoxon rank-sum test revealed significant group differences in global efficiency (FA, MD, RD) and mean strength (FA, RD). Patients showed reduced global efficiency and mean strength in FA-based networks, alongside increased MD and RD, reflecting possible axonal degeneration or demyelination. In conclusion, early-stage patients exhibited reduced thalamic FA and disrupted brain connectivity, supporting the presence of early pathological changes in major psychoses.

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[P-58]

STRUCTURAL ELUCIDATION OF POWDERED SAMPLES: OVERCOMING CHALLENGES BY COMBINING SSNMR AND MICRO-ED

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

Mechanochemical synthesis is an efficient and sustainable method for preparing multicomponent crystalline forms of pharmaceutical interest – such as cocrystals and salts – capable of modulating the physicochemical properties of Active Pharmaceutical Ingredients (APIs) [1]. However, the structural characterization of the resulting microcrystalline powders remains challenging due to the difficulty – or impossibility – of obtaining crystals suitable for single-crystal X-ray diffraction (SCXRD) [2]. To overcome this limitation, an integrated approach combining Micro-Electron Diffraction (MicroED) with advanced solid-state NMR (SSNMR) techniques has recently been applied to single-component systems [3]. In this work, the combined approach was extended to multi-component systems, enabling the complete structural determination of a nutraceutical–API adduct, the pyridoxine-N-acetylcysteine (PN-NAC) salt [4]. PN-NAC was synthesized exclusively by manual dry grinding and, despite numerous attempts, suitable single crystals for SCXRD analysis could not be obtained. These characteristics make it a representative and challenging case for evaluating the capabilities of the combined SSNMR and MicroED approach. Furthermore, high-resolution mass spectrometry and solution NMR were employed to preliminary determine molecular formula and composition, establishing a complete workflow for unknown compound structural determination.
A comprehensive SSNMR study – including ^{13}C and ^{15}N CPMAS, ^{1}H MAS, 2D $^{1}H/\{^{14}N\}$ T-HMQC, $^{1}H\{^{13}C\}$ short- and long-range DCP, ^{1}H DQ/^{1}H SQ, and PM-RESPDOR experiments – enabled near-complete resonance assignment and revealed the formation of a 1:1 salt with two crystallographically independent PN and NAC molecules in the asymmetric unit. Protonation of the pyridine nitrogen of PN was confirmed by a significant low-frequency shift (~86 ppm) of the ^{15}N pyridine nitrogen signal [5], as well as by a strong N–H correlation between the pyridine nitrogen and the NAC carboxylic proton in the $^{1}H/\{^{14}N\}$ T-HMQC spectrum. Moreover, the formation of the N–H bond was unambiguously demonstrated by the short ^{1}H–^{14}N distance measured *via* PM-S-RESPDOR [6].
Complementary MicroED analysis on the powder sample provided a three-dimensional structural model fully consistent with SSNMR data. This result highlights the potential of combining these techniques in modern structural chemistry, particularly in pharmaceutical development, overcoming the limitations associated with mechanochemistry. This integrated methodology provides a robust alternative for obtaining detailed structural information when conventional SCXRD is not applicable.

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[P-59]

PROBING LOCAL ENVIRONMENTS IN $CsPb(Br_xI_{1-x})_3$ PEROVSKITES VIA NQR SPECTROSCOPY AND DFT CALCULATIONS

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Keywords: NQR, materials, theory and methods.

Mixed-halide perovskites are emerging as promising candidates for optoelectronic applications due to their tunable bandgap and favorable light absorption properties. This tunability, achieved by varying the halide composition, makes them particularly attractive for high-efficiency tandem solar cells. However, such compositional flexibility also introduces significant challenges for structural characterization, as these materials exhibit a high degree of structural and dynamic disorder [1].
In this context, Nuclear Quadrupole Resonance (NQR) spectroscopy has recently gained attention as a powerful tool for probing local halide environments, regions that are difficult to investigate using Solid-State NMR when observing halide nuclei [2].
Combining experimental data with theoretical modeling is a promising strategy to better interpret NQR spectra. In particular, first-principles calculations can provide useful predictions of NQR frequencies and help assign them to specific local environments. Nonetheless, current DFT methodologies systematically overestimate absolute NQR frequencies by a few MHz, limiting their reliability in accurately associating spectral features with specific coordination environments [3,4]. As such, methodological improvements are needed before quantitative insights into local disorder can be extracted from experimental data.
In this study, we employed $^{79/81}$Br and ^{127}I NQR spectroscopy to investigate a series of mixed-halide perovskites with formula $CsPb(Br_xI_{1-x})_3$, aiming to explore the local halogen environments. Additionally, we performed a series of DFT calculations to simulate the NQR frequencies of these compounds, representing the first computational study of this kind on mixed-halide perovskites.

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[P-60]

NMR METABOLOMIC INVESTIGATION ON INFLORESCENCES FROM *CANNABIS SATIVA* L. VARIETY EARLINA CULTIVATED IN SOUTHERN ITALY

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Keywords: (solution NMR, small molecules, biomolecules, metabolomics, food)

Hemp (*Cannabis sativa* L.) is a well-known fast-growing and high-biomass crop, that has been used for centuries for multiple purposes, especially for fiber and seed oil production. Different genotypes with low levels of psychoactive cannabinoids were selected through breeding in the last decades, thus making them suitable for non-pharmaceutical uses like biofuel and textile fiber production. Earlina 8FC is a monoecious hemp variety developed in France for seed production; it has gained attention in the last decades due to various positive features like early flowering and short development period, high seed production (relatively to the entire biomass) and good pest resistance.

NMR spectroscopy is a versatile and robust analytical method with high potential for metabolomics, though its application is still being developed. The present study aimed to further develop the existing NMR methodology for characterizing hemp inflorescences through multiple-solvent analysis. This method was then applied to a comparative analysis of two hemp cultivars, the poorly characterized Earlina 8FC and the well-described Futura 75.

The metabolites were extracted from lyophilized inflorescences using simultaneous chloroform-methanol-water liquid extraction (Bligh-Dyer protocol [1]). Hydroalcoholic extracts were dried and divided into two parts, re-dissolved either in deuterated water with phosphate buffer (400 mM, pH =7) or in CD_3OD. Similarly, the chloroform fraction was evaporated and divided into two parts, dissolved either in $CDCl_3$ or in a 2:1 v/v mixture of $CDCl_3/CD_3OD$.

Amino acids, sugars and organic acids were quantified in the aqueous extracts. Flavonoids, mostly apigenin and luteolin derivatives, were identified and quantified in methanol solution. Organic fraction contained fatty acids, sterols, phospho- and galactolipids, pheophytins and cannabinoids identified and quantified either in pure chloroform or in a mixed solvent. The multiple-solvent analysis enabled the extension of the number of metabolites determined by NMR, making the characterization more comprehensive.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

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[P-61]

NMR METABOLOMICS TO STUDY THE EFFECTS OF AQUAPONIC CULTIVATION ON AROMATIC HERBS METABOLITE PROFILE

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Keywords: solution NMR, metabolomics, food

Aromatic herbs are plants traditionally used in culinary field for salads or toppings. Anyway, the interest for these plants has recently increased due to elucidation of their metabolite composition rich in bioactive compounds, making these matrices valuable not only from a nutritional point of view, but also from a biological one [1]. The increased interest and production of aromatic herbs requires cultivation processes to be more sustainable and with high yield or biomass. In this context, aquaponic cultivation represents an innovative approach that combines aquaculture (for fish raising) with hydroculture (growing plants in water) [2].
In the present study, NMR metabolomics has been applied to study the metabolite profile of three aromatic herbs namely mizuna, rocket, and red basil cultivated with both conventional and aquaponic approaches. Mono- and two-dimensional NMR experiments allowed to identify several metabolite classes namely sugars, amino acids, organic acids, glucosinolates, fatty acids, sterols, terpenoids, and other compounds. Results obtained from the analysis of the three species underlined significative differences among the two cultivation approaches, with aquaponic approach increasing most of the identified metabolites.

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[P-62]

CHARACTERIZATION OF THE BINDING BETWEEN A NOVEL E3 LIGASE, RNF32, AND ARRYTHMOGENIC CALMODULIN VARIANTS

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Keywords: solution NMR, small molecules, biomolecules, polymers

Cardiovascular diseases remain the leading cause of mortality worldwide, accounting for an estimated 17.9 million deaths annually (approximately 32% of all global deaths). Despite ongoing advances in diagnosis and treatment, the global burden of life-threatening cardiovascular conditions remains unresolved. Intracellular calcium (Ca^{2+}) plays a central role in cardiovascular physiology, particularly in the mechanism of excitation-contraction coupling [1]. A key mediator of calcium signaling is calmodulin (CaM), a ubiquitously expressed calcium-binding protein composed of two lobes (N- and C-lobe), each containing two canonical EF-hand Ca^{2+}-binding motifs. In the absence of calcium, CaM can bind to IQ motif-containing target proteins. Upon calcium binding, CaM undergoes a dramatic conformational change, enabling it to interact with and regulate over 300 different target proteins [2]. In recent years, mutations in genes encoding calmodulin have been associated with severe cardiac arrhythmias, including long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT). These conditions are often accompanied by neurological or neurodevelopmental symptoms [3]. Preliminary findings from the Guardavaccaro laboratory identified RNF32, a novel and still uncharacterized E3 ubiquitin ligase containing an IQ motif, as a CaM-interacting protein in a calcium-dependent manner. Interestingly, RNF32 is expressed in both cardiac and brain tissues, suggesting potential roles in excitable systems beyond the heart. Based on these observations, we investigated whether arrhythmogenic CaM mutations affect the CaM–RNF32 interaction in HEK293T cells. We analyzed seven CaM point mutants and, among these, four variants – namely N98S, G114R, D130G, and F142L – showed a pronounced alteration in their ability to interact with RNF32, indicating possible functional relevance and warranting further biochemical and biophysical investigation. To this end, we expressed and purified the four CaM variants and conducted circular dichroism (CD) and solution NMR spectroscopy in the presence of $CaCl_2$ and EGTA. These studies revealed substantial differences in calcium-binding properties among the mutants, consistent with the altered interactions observed in cell-based assays. To validate and further characterize these findings, we plan to perform isothermal titration calorimetry (ITC) and NMR titration experiments using a synthetic IQ motif peptide derived from RNF32. These studies will complement the calcium-dependent interaction dynamics between CaM mutants and RNF32 observed in cellular assays.

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[P-63]

SABRE of [1-^{13}C]Pyruvate: Molecular design for better hyperpolarization and filtration

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Keywords: solution NMR, hyperpolarization, theory and methods.

We present a systematic investigation of SABRE hyperpolarization of [1-^{13}C]pyruvate-h_3 using a series of Ir–NHC catalysts. In addition to the benchmark IMes ligand[1,2], we explore sterically distinct analogues such as IPr and SIPr, and more importantly we introduce novel fluorinated NHCs with single and double fluorinated chains designed to maintain hyperpolarization performance while facilitating post-polarization catalyst removal.
Carbon polarization levels exceeding 3% are achieved with 50% parahydrogen, extrapolating to over 10% under full enrichment. Experimental results indicate that polarization efficiency is primarily driven by exchange kinetics rather than spin–spin couplings. This is supported by a reduced-dimension Liouville-space kinetic model that accurately captures the observed temperature dependence. These insights enable rational catalyst design, including fluorinated variants with enhanced hydrophobicity and prospects for biocompatible applications.

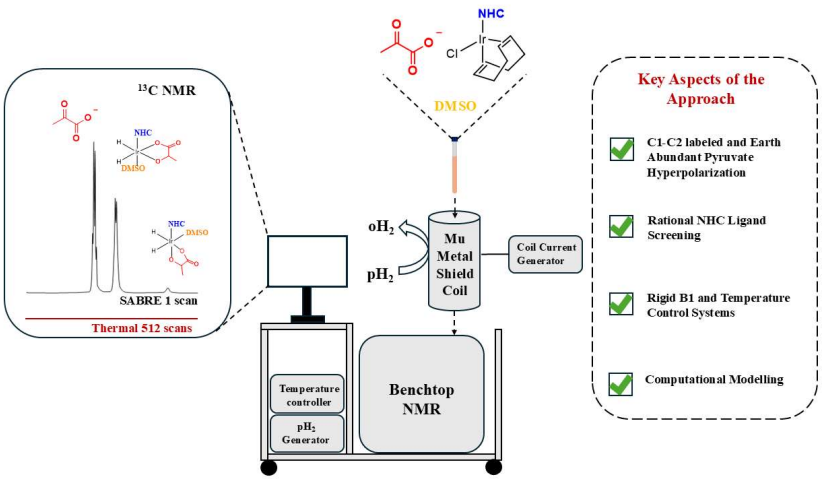

Fig. 1. SABRE-SHEATH protocol for benchtop NMR hyperpolarization experiments.

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[P-64]

NMR-BASED INVESTIGATION OF TAU-ANNEXIN A2 INTERACTION

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Keywords:
solution NMR, biomolecules, small molecules

With rising life expectancy, over 50 million people are affected by neurodegenerative diseases linked to protein aggregation (Aβ, Tau, α-synuclein). While neurons are primary sites for the aggregation, microglia and astrocytes aid in clearance, though astrocytic roles remain debated [1]. Annexin A2 (ANXA2) has been recently identified as a novel modulator of α-syn clearance in astrocytes [2]. ANXA2 is also known to functionally interact with Tau protein and regulate its neuronal localization [3]. Cytoplasmic inclusions of Tau in astrocytes have been observed in several neurodegenerative diseases [4]. These findings suggest that ANXA2 may be a promising candidate for limiting the spread of Tau fibrils among neurons in Alzheimer's disease. However, a deeper understanding of the molecular mechanisms underlying the ANXA2–Tau interaction is essential to guide the design of novel molecules capable of modulating this interaction. For this purpose, we investigated the ANXA2–Tau interaction using NMR spectroscopy. A series of ^{15}N-HSQC experiments acquired on ^{15}N-labeled Tau constructs of different length in the presence of ANXA2 were used to map the binding interface on the Tau protein. We revealed that only the N-terminal part of the Tau protein is involved in interaction with ANXA2, and that the binding is calcium dependent.

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[P-65]

CHARACTERIZATION OF NEW MATERIALS BASED ON GELLAN GUM LOADED WITH NATURAL COMPOUNDS BY SOLUTION AND SOLID STATE NMR

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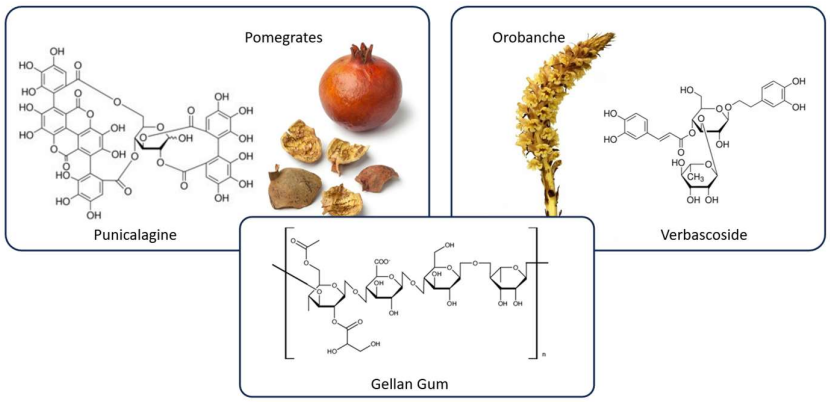
Keywords: Gellan; Natural Compounds, ^{13}C CP/MAS.

Gellan gum is a natural, unbranched polymer that consists mainly of three monosaccharides: glucose, glucuronic acid, and rhamnose. Commercially it is produced by biosynthesis through a fermentative process of the Sphingomonas elodea bacterium. This material has attracted considerable interest from the scientific community in the field of tissue engineering. The low production cost, biocompatibility, non-toxicity, biodegradability, and the ease of modulating the mechanical properties make this material an excellent candidate for the development of new scaffolds for tissue regeneration [1].
Recently, several studies have reported that the mechanical properties of gellan gum can be regulated by intercalating inorganic filler or bioactive substances within the polymer chains.
This preliminary study describes the NMR characterization of new gellan gum-based materials functionalized with aqueous extracts obtained from natural waste products such as the parasitic plant Orobanche and pomegranate peel.
Although Orobanche poses a threat to crops, it has long been used in traditional medicine thanks to its high polyphenolic content and its purifying, anti-inflammatory, calming, antispasmodic, astringent, diuretic and healing properties. Pomegranate peel is also used for its anti-proliferative, anti-inflammatory and anti-tumour action. [2].



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[P-66]

NMR based real-time analysis of exometabolites decodes the mechanism of action of antibacterial molecules, nanoparticles, and materials

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Keywords: Solution NMR, biomolecules, exometabolome, antibacterial mechanism

Understanding the mechanism of action of antimicrobial agents is critical for guiding the development of new drugs to overcome antimicrobial resistance. We present a label-free NMR-based approach to characterize the mechanism of action of antibacterial compounds and materials by the analysis of metabolites secretion kinetics. The method is demonstrated using Escherichia coli and Staphylococcus aureus as representative Gram-negative and Gram-positive model organisms. By monitoring the real-time production of key secreted metabolites (acetate, formate, lactate, ethanol, pyruvate, succinate) in response to antimicrobial treatment and analyzing the secretion kinetics, we are able to classify the agents' mechanisms of action. We validate our method using agents with well-characterized mechanism of action (kanamycin, ampicillin, Irgasan, caprylic acid, graphene-like nanoparticles and a functionalized silicon material), and we further apply it to silver nanoparticles, whose mechanism of action remains under debate. Agents that disrupt the cell wall reduce secretion rates while maintaining end-point metabolite concentrations, with only moderate lag phase extension. In contrast, agents that act on intracellular pathways drastically prolong lag phases and reduce both secretion rates and end-point concentrations. When plotted in 3D parameter space (lag time, secretion rate, end-point concentration), antibacterial agents cluster according to their mode of action, offering a mechanistically informative phenotypic readout. This platform provides a generalizable and robust analytical framework for rapid antimicrobial profiling and mechanism-based screening of novel bioactive agents.

Acknowledgements

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[P-67]

Thermal and Structural Characterization of Sulfo-PEG Composites with Silica and Sepiolite Fillers via TD-NMR and DSC

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Keywords:
low field NMR, materials, polymers, theory and methods.

This study investigates the thermal and structural behavior of sulfonated polyethylene glycol (Sulfo-PEG) composites incorporating different inorganic fillers—silica and sepiolite—at various weight ratios. Differential Scanning Calorimetry (DSC) analyses revealed a distinctive thermal phenomenon, known as the "bathtub" effect [1], occurring between 223 K and 253 K (Fig. 1). This effect, particularly pronounced in the Filler-PEG 1:3 (sepiolite) and 1:2 (silica) systems, is characterized by an endothermic region followed by a "cooling crystallization" peak, during which a molecular rearrangement of the samples occurs [2].

To complement the DSC data, time-domain nuclear magnetic resonance (TD-NMR) measurements were conducted using the Magic Sandwich Echo (MSE) sequence [3] over the 223–278 K temperature range. These measurements provided quantitative insights into the rigid fractions and polymer chain mobility. The results indicated that composites containing sepiolite retain higher rigidity throughout the temperature range, whereas those with silica exhibit greater chain mobility, attributable to the intrinsic differences between the two fillers (Fig. 2).

Further TD-NMR experiments using the Hahn echo sequence supported these findings: as the temperature increases, the transverse relaxation times (T_2) of silica-based samples increase more rapidly than those of sepiolite-based ones, confirming the higher mobility associated with silica.

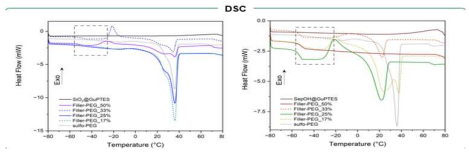

Fig. 1 DSC analysis of Sulfo-PEG with silica and sepiolite at various weight ratios.

Fig. 2.

Fig. 2. Left: Rigid fraction vs T(K) from MSE, showing higher mobility in silica-filled samples. Right: T_2 vs T(K) from Hahn Echo, with longer relaxation times reflecting silica's greater chain mobility.

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Empowering surgeons with real-time precision and efficiency with innovative instrumentation and methods for enhanced surgical outcomes in breast cancer using the FFC NMR method

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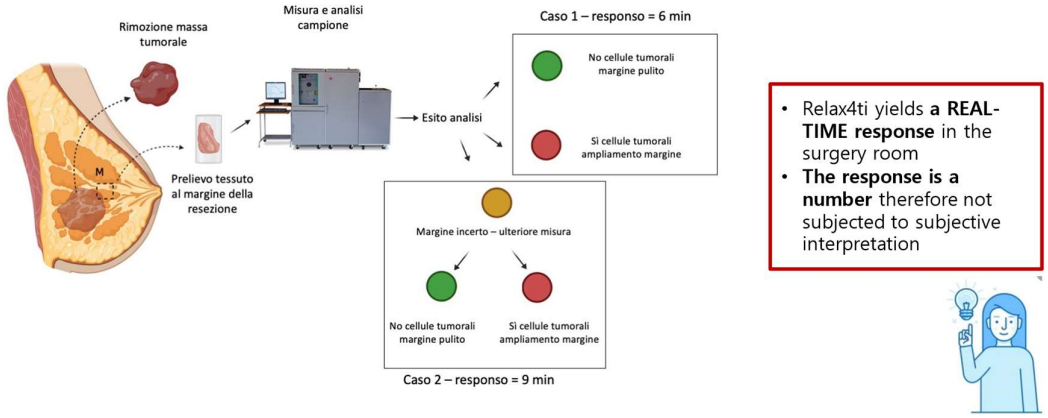
Breast cancer is the most common cancer among women, with approximately 69,900 new cases diagnosed annually in Germany alone. Additionally, over 6,000 women are diagnosed with in situ tumours each year, with about 1% of all cases affecting men. Based on current trends, one in eight women will develop breast cancer in their lifetime. Early and effective surgical intervention is critical, yet challenges remain in ensuring the complete removal of cancerous tissue during breast-conserving surgery (BCS). One of the most significant risks in BCS is the recurrence of tumours due to residual cancer cells at the surgical margins.

Currently, up to 40% of BCS procedures yield either positive or uncertain margins, leading to increased risks of locoregional recurrence. The gold standard for margin assessment—histological evaluation requires several days for results and depends on highly specialised personnel, causing delays and higher costs in treatment.

Relaxi4Ti is a cutting-edge diagnostic method developed based on Fast Field Cycling Nuclear Magnetic Resonance (FFC-NMR) technology. This innovative approach addresses the limitations of current histological assessment by providing real-time, high-precision analysis of tissue during surgery (https://www.mdpi.com/2072-6694/13/16/4141 and https://onlinelibrary.wiley.com/doi/10.1002/ange.201713318).

Relax4Ti relies on the acquisition of the relaxation time T_1 of water protons measured at low magnetic field strength (a parameter easy to acquire on NMR spectrometers), as reporter of the occurrence of tumor tissue



[P-69]

AMYLOIDOGENIC PROTEINS AS INNATE DEFENDERS: EXPLORING THE ANTIMICROBIAL ACTIVITY OF TAU PROTEIN

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Keywords:
Solid-state NMR, solution NMR, small molecules, biomolecules, polymers.

Amyloidogenic proteins such as tau and amyloid-β (Aβ) are traditionally studied in the context of neurodegenerative diseases like Alzheimer's, where their aggregation is considered pathogenic. However, emerging evidence suggests that these proteins may possess functional roles beyond neurotoxicity, including activity as innate immune effectors. [1]
This project explores the hypothesis that tau and other amyloidogenic proteins exhibit antimicrobial peptide (AMP)-like properties, contributing to host defense mechanisms. Drawing on recent findings, we examine the structural and biochemical determinants that allow them to bind microbial membranes, form oligomeric pores, and induce microbial death. Understanding tau as a potential AMP not only reframes its role in neurodegeneration but also opens new avenues for therapeutic intervention aimed at modulating its innate immune function without triggering pathological cascades. [2]
Tau is encoded by the *MAPT* (microtubule-associated protein tau) gene, which undergoes alternative splicing of exons 2, 3, and 10 to produce six distinct isoforms in the central nervous system.
While tau is primarily studied in the context of the nervous system, its expression in multiple tissue types suggests it plays broader physiological roles beyond those limited to neurobiology. [3,4]
Indeed, tau protein interacts with cellular membranes through its intrinsically disordered domains, particularly the proline-rich (PRD) and repeat domains (RD), which are rich in positively charged lysine and arginine residues. These regions facilitate electrostatic interactions with negatively charged phospholipids.
Liposomes can mimic cellular membranes, enabling in vitro modeling of tau-membrane interactions, and can also serve as delivery vehicles for drugs or genetic material aimed at modulating tau pathology. In the present work, we performed ^{2}H and ^{1}H-^{15}N HSQC NMR spectroscopy experiments to obtain detailed molecular-level information about the interaction between tau and the liposomes. We used 100% POPC liposomes to mimic a typical mammalian cell membrane and 70% POPC:30% POPG liposomes to mimic a bacterial membrane. Moreover, we assessed the capability of tau protein to penetrate SUV (small unilamellar vesicles) based on a leakage assay.
Understanding the interaction between tau protein and liposomal systems promises to advance fundamental neuroscience research and deepen our knowledge of antimicrobial peptide function.

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[P-70]

OVERCOMING AGE BIAS IN LIVER FIBROSIS STAGING: A NOVEL NMR-BASED APPROACH

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Keywords: solution NMR, small molecules, metabolomics.

Metabolically associated steatotic liver disease (MASLD) is marked by excessive hepatic fat accumulation in the absence of significant alcohol intake and often progresses to liver fibrosis, a major health concern [1]. While liver biopsy remains the gold standard for fibrosis assessment, its invasiveness limits clinical utility [2]. Non-invasive tools like FIB-4 incorporate age, which can reduce accuracy, especially in elderly patients [3]. Thus, age-independent markers are urgently needed.
This study aimed to identify such markers using metabolic, hematological, and inflammatory profiles. Serum samples from MASLD patients at Policlinico Umberto I, Rome, were analyzed by ^{1}H-NMR spectroscopy, enabling quantification of 52 metabolites. Combined with hematological and inflammatory parameters, a panel of 83 variables was evaluated.
Linear regression identified variables associated with fibrosis independent of age, while logistic regression assessed their diagnostic performance. Among six fibrosis-associated metabolites, glutamine and propionate yielded the most robust model, leading to the development of the GP Index. The GP Index is simple, non-invasive, cost-effective, and age-independent, offering a promising tool for staging liver fibrosis in MASLD and improving patient stratification and outcomes.

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[P-71]

Modeling Small-Molecule Inhibition of Huntingtin Aggregation via NMR and Kinetic Analysis

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

Huntington's disease is a fatal neurodegenerative disorder caused by a polyglutamine (poly-Q) expansion (≥35 repeats) of the huntingtin protein (htt^{ex1}), leading to fibril accumulation in neuronal inclusion bodies. Recent studies have dissected htt^{ex1} aggregation using solution NMR spectroscopy.[1,2] By analyzing both non-pathogenic $htt^{ex1}Q_7$ and pathogenic $htt^{ex1}Q_{35}$, distinct stages of the aggregation process corresponding to different kinetic regimes were characterized. Pre-nucleation events corresponding to the formation of short-lived oligomeric species (dimers, tetramers) on the microsecond timescale were studied in the $htt^{ex1}Q_7$ construct via concentration-dependent chemical shift (δ_{ex}) changes, ^{15}N $R1_\rho$ measurements and ^{15}N CPMG relaxation dispersion experiments. In contrast, nucleation and fibril formation in $htt^{ex1}Q_{35}$ were monitored using time-resolved SOFAST-HMQC allowing observation of time-dependent chemical shift changes and variations in NMR cross-peak volume/intensity. These observables were globally fitted to a kinetic model incorporating tetramerization, conversion to nuclei, elongation, and secondary nucleation. Building on this framework, we extended the model including the action of small-molecule inhibitors at different stages of either pre-nucleation, nucleation or aggregation. The extended model considers small-molecule interactions with monomeric htt^{ex1} species (m) and nuclei (P), whose lifetimes are compatible with the timescale of small-molecule binding (milliseconds to seconds). While direct binding of inhibitors to short-lived oligomers is excluded from the time-dependent kinetic model due to timescale incompatibility, relaxation-based NMR experiments with $htt^{ex1}Q_7$ can still reveal inhibitor-induced modulation of early oligomerization equilibria and exchange kinetics. This theoretical framework advances classical amyloid aggregation models by incorporating reversible binding equilibria of small-molecule inhibitors across multiple aggregation stages. In this context, flavan-3-ols represent a promising molecular class for probing the mechanisms that govern early nucleation events.[3] Ultimately, our goal is to provide a conceptual and methodological platform to guide the rational design of inhibitors targeting nucleating species in amyloid-related disorders.

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