GIDRM
GRUPPO ITALIANO
DISCUSSIONE RISONANZE MAGNETICHE

Università degli Studi della Campania *Luigi Vanvitelli*

Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche

GIDRM
9th–11th September

53rd National Congress on Magnetic Resonance

CASERTA 2026

Invited speakers

Marina Carravetta - Simonetta Geninatti Crich - Iola Duarte - Roberto Gobetto - Vincenzo Venditti

Keynote speakers

Silvia Borsacchi - Fabio Carniato - Marco Fragai - Carla Isernia - Salvo Mamone - Michele Mauri - Noemi Proietti - Marina Veronesi

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Roberto Fattorusso - *University of Campania*
Carla Isernia - *University of Campania*
Gaetano Malgieri - *University of Campania*
Luigi Russo - *University of Campania*
Giovanna Valentino - *University of Campania*
Gianluca D'Abrosca - *Link Campus University*
Maria della Valle - *CNR-IC*
Martina Dragone - *University of Campania*
Clementina Acconcia - *University of Naples Federico II*

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Laura Ragona - *CNR-SCITEC Milano*
Antonio Randazzo - *University of Naples Federico II*
Valeria Righi - *University of Bologna*
Luigi Russo - *University of Campania*

Abstract submission: **June 10th 2026**
Attendance grants application: **June 10th 2026**
Early registration: **July 10th 2026**

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Venue

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

Marina Carravetta (University of Southampton)

Iola Duarte (University of Aveiro)

Simonetta Geninatti Crich (University of Turin)

Roberto Gobetto (University of Turin)

Vincenzo Venditti (Iowa State University)

Winner of the GIDRM/GIRM Gold Medal 2026 (to be awarded)

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

Silvia Borsacchi, CNR-ICCOM-Pisa

Fabio Carniato, University of Piemonte Orientale

Marco Fragai, University of Firenze

Carla Isernia, University of Campania

Salvo Mamone, University of L'Aquila

Michele Mauri, University of Milano Bicocca

Noemi Proietti, CNR Roma

Marina Veronesi, IIT-Genova

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DISCUSSIONE RISONANZE MAGNETICHE

53RD NATIONAL CONGRESS ON MAGNETIC RESONANCE

CASERTA 9-11 SEPTEMBER 2026

SCIENTIFIC PROGRAM

Wednesday September 9th

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

14:00-14:30	**Opening (Room A2)**
	Plenary session (Room A2) **Chair: A. Mucci & M. R. Chierotti**
14:30-15:20	**Plenary Lecture 1** **GIDRM/GTRM gold medal award** **Giovanna Musco (Biomolecular NMR Unit, IRCCS Ospedale San Raffaele)** ***Do we still need N(avigating) M(ysterious) R(elationships)?***
	Plenary Lecture 2 **Chair: V. Righi**
15:20-16:05	**Iola Duarte (University of Aveiro)** ***Metabolic phenotyping of biomimetic 3D cell models using NMR-based metabolomics***

16:05-17:00	**Coffee break + Poster session (ODD abstract numbers)**

	Parallel session A (Room A2) **Chair: V. Righi**	**Parallel session B (Room B2)** **Chair: R. Fattorusso**
17:00-17:30	**Veronesi M.** NMR in drug discovery: from hit identification to Exo-metabolomics	**Mauri M.** Time-Domain NMR: mobility and interfacial phenomena across multiple scales
17:30-17:45	**Marino C.** Untargeted ^{1}H NMR-based metabolomics and high-pressure liquid chromatography analyses reveal sexual dimorphism in the serum metabolic profiles of patients with different genetic forms of Parkinson's disease	**Petrella G.** An HMBC-based J-modulation sequence for the measurement of nJHC coupling constants: application to CORROLE systems
17:45-18:00	**Krid M.A.R.** Advancing Food Analysis: Amino Acid Quantification by NMR and Deep Learning	**Petrone M.** Suppressing proton competition to reveal intrinsic Zn(II) chelation in L-carnosine mimics by solution NMR
18:00-18:15	**Gallo A.** Exploring the urinary metabolomic fingerprint of human cytomegalovirus: a ^{1}H-NMR study on congenitally infected newborns	**Schiavina M.** The Synergy of Multiple Receivers and Heteronuclear Detection to Draw a Complete Picture of Intrinsically Disordered Proteins in Action
18:15-18:30	**Vigni G.** Integrated chemometric analyses of NMR, HPLC-MS and IRMS data for geographical origin authentication	**Yang Zhou A.** Next-Generation EPR: Combining High-Performance Q Band Instrumentation with Artificial Intelligence Enhanced Spectral Processing

	Plenary session (Room A2) **Chair: S. Borsacchi**
18:30-18:45	**ABCS/Nanalysis Sponsorship Lecture: V. Vanoli** ***Quantitative Characterization of Lignin by Benchtop ^{31}P NMR***
18:45-19:30	**Plenary Lecture 3** **Marina Carravetta (University of Southampton)** ***Solid state NMR on supported palladium nanoparticles***

Thursday September 10th

	Plenary session (Room A2) **Chair: L. Russo**
8:45-9:30	**Plenary Lecture 4** **Vincenzo Venditti (Iowa State University)** ***Beyond Structures: Solution NMR as a method for resolving dynamic mechanisms across biological and heterogeneous catalysts***
9:30-9:45	**Jeol Sponsorship Lecture: M.J. Smith** ***Improving the detection limits***
	Under 35 GIDRM Award (Room A2) **Chair: M. Chierotti**
9:45-10:15	**Gaia Meoni (University of Florence)** ***NMR-based metabolomics: a journey between health and food***
10:15-10:30	**Bruker Sponsorship Lecture: M. Mayzel** ***Spatially resolved NMR spectroscopy: principles and applications***

10:30-11:20	**Coffee break + Poster session (EVEN abstract numbers)**

	Parallel session A (Room A2) **Chair: D. Lalli**	**Parallel session B (Room B2)** **Chair: G. Musco**
11:20-11:50	**Carniato F.** Investigation of transition metal-based MRI probes: a combined ^{1}H NMR relaxometry, EPR and computational study	**Isernia C.** A glimpse in the metal ion selectivity rules: Zn(II), Cd(II) and Co(II) interplay with different protein coordination spheres in determining variable thermal stability and folding scenarios
11:50-12:05	**Scarciglia A.** A Rare Earth Elements recovery strategy using natural dioptase and ^{1}H-NMR relaxometry	**Ciabini C.** Monitoring disordered protein-ligand interactions through NMR
12:05-12:20	**Micocci S.** Visualize & Destabilize: boronated carboranyl-curcuminoids as theranostic candidates in Alzheimer's MRI-guided Neutron Capture Therapy	**Santoro F.** Integrating NMR structure determination and ligand optimization of the CIAP2-BIR1 protein
12:20-12:35	**Caporale A. S.** The association between grey matter microstructural characteristics and cerebral oxidative metabolism is altered in Multiple Sclerosis	**Macchia L.** NMR mapping of Cyclophilin D pockets using ultra-low molecular weight fragments
12:35-12:50	**Piña Marcos J. N.** Targeted liposomes for combined boron neutron capture therapy and MRI visualization in non-small cell lung cancer	**Lippi C.** NMR-based solvent paramagnetic enhancement experiments on IDPs

12:50-14:10	**Lunch + Poster session (ODD abstract numbers)**

	Plenary session (Room A2) **Chair: L. Russo**
14:10-14:55	**Plenary Lecture 5** **Simonetta Geninatti Crich (University of Torino)** ***Fast Field-Cycling Relaxometry as a Tool to Investigate Pathological States through Water Dynamics in Tissues***
14:55-15:10	**Stelar Sponsorship Lecture: I. Sarwara** ***Molecular Dynamics of Animal- and Plant-Based Cheese Matrices via Temperature-Dependent FFC-NMR Relaxometry***
15:10-15:25	**Magritek Sponsorship Lecture: L. Bottalico** ***Unlocking New Capabilities in Benchtop NMR: High-Resolution Analysis at 100 MHz***
	Segre-Capitani Fellowship **Chair: L. Ragona**
15:25-15:40	**Simone Fabbian (University of Padova)** ***Oncogenic non-coding RNA targeted by small molecules: a NMR-based approach***

15:40-17:00	**Coffee break + Poster session (EVEN abstract numbers)**
16:15-17:00	**GTRM assembly (Room A2)**
17:00-19.00	**GIDRM assembly + announcement of poster competition winners**

19:30	**social dinner**

Friday September 11th

	Plenary session (Room A2) Chair: E. Carignani
8:45-9:30	Plenary Lecture 6 Roberto Gobetto (University of Torino) *Solid State NMR tools in the evaluation of weak interactions*

	Parallel session A (Room A2) Chair: A. Randazzo	Parallel session B (Room B2) Chair: D. Lalli
9:30-10:00	**Mamone S.** Rational optimization of [1-^{13}C] pyruvate sabre hyperpolarization through catalyst design, kinetic modelling, and temperature control	**Proietti N.** Single-sided NMR: strengths and weaknesses
10:00-10:15	**Mosca F.** Structural investigation of Braun protein transpeptidation inhibitors in Gram-negative bacteria	**Pierigè M.** Accessing dynamics of sbr compounds by variable temperature ^{1}H FC NMR and DFMSE experiments
10:15-10:30	**Gado I.** RNA mimic in HUR-ligand studies: insights from STD-NMR and protein-based NMR techniques	**Conti L.** In-Situ Single-Sided NMR profiling to assess cleaning treatments on XI-Century wall paintings
10:30-10:45	**Cesaroni S.** Exploring ^{1}H-NMR data extraction potential in metabolomics: unveiling an opioid signature	**Ceccon A.** Reactive adduct intermediates and solvent-cage effects in azo-initiated oxidation of cinnamic acid derivatives revealed by solution NMR spectroscopy
10:45-11:00	**Parafioriti M.** Modulating heparanase activity with heparan sulfate mimetics: insights from NMR and molecular dynamics into structure–function relationships	**Napolitano E.** Metabolomic insights into the protective mechanisms of the NGF mutant CHF6467 in an Alzheimer's disease cell model

11:00-11:30	Coffee break

	Parallel session A (Room A2) Chair: G. Malgieri	Parallel session B (Room B2) Chair: E. Carignani
11:30-12:00	**Fragai M.** NMR-based characterization for the design, stability studies, and delivery control of biopharmaceuticals	**Borsacchi S.** Probing structure, dynamics and light-induced effects in halide perovskites via Solid State NMR and NQR
12:00-12:15	**Dragone M.** A ^{19}F NMR structural characterization of selumetinib and trametinib β-cyclodextrin inclusion complexes	**Scarperi A.** Solid-State NMR insights into hydration, framework flexibility, and guest dynamics in calf-20
12:15-12:30	**Barbero M. T.** Structural and Dynamic features of diastereomeric (Deep) Eutectic Systems (DIADES)	**Sabena C.** ^{14}N Solid-State NMR quadrupolar products as quantitative probes of hydrogen-bond environments in pyridinic solids
12:30-12:45	**Bolognesi T.** NMR strategies for multidomain intrinsically disordered proteins: application to the SARS-CoV-2 nucleocapsid reveals structure, dynamics, and binding mechanisms	**Ghelardi A.** Structural insights into a new dual-drug co-crystal characterised by NMR crystallography and electron diffraction

	Plenary session (Room A2) Chair: L. Ragona & M. Chierotti
12:45-13:25	Poster competition winner lectures
13:25-13:30	Closing

13:30-14:30	Lunch

Do we still need N(*avigating*) M(*ysterious*) R(*elationships*)?

Giovanna Musco[a]

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In the era of Artificial Intelligence and the cryo-EM revolution, the biomolecular NMR spectroscopist might feel an uncomfortable sense of displacement (an identity crisis?). In a world where every question is expected to yield an immediate, simple, and predictive answer, one may wonder: is Nuclear Magnetic Resonance (that, we must admit, is slow, complicate, and demanding) still at home in this rapidly evolving landscape?

Receiving this award has offered me the unvaluable opportunity to reflect on the privilege of working in NMR. It has been more than a scientific path; it has been a school of patience and humility, but also a school of fight, a place where one learns both the limits of knowledge and the persistent desire to move beyond them (despite all the obstacles, not only the scientific ones). Above all, it has fostered an appreciation for the unexpected, the freedom (because it is a freedom) of not always knowing and of making mistakes, and the beauty of the surprise of discovery when prediction fails.

Perhaps precisely in this era, we need a plurality of approaches, techniques that do not simply provide easy answers, but open new questions. Ways of exploring that allow us to move through the mystery of nature rather than resolve it too quickly, reminding us that reality often escapes even our most refined models, human or artificial.

I think that NMR is still one of these approaches. At its heart lies communication (this mysterious dialogue between spins!) making it uniquely suited to explore interactions, especially those that are *fuzzy*, dynamic and difficult to define [1,2]. Much like our own relationships, these interactions resist simplification, thus revealing their uniqueness and richness.

This talk is dedicated to all those who have shared this journey with me. Every research project feels like a path through an unexplored forest: uncertain, sometimes disorienting, yet full of possibility. I will share some of these journeys, where NMR and the people working with me have been faithful companions, helping to illuminate the delicate and often invisible threads of molecular (and human) interactions.

References

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METABOLIC PHENOTYPING OF BIOMIMETIC 3D CELL MODELS USING NMR-BASED METABOLOMICS

Iola F. Duarte

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Keywords: solution NMR, small molecules, metabolomics

Advanced three-dimensional cell models, including spheroids, organoids and biofabricated constructs, are increasingly used to reproduce key features of native tissues and disease microenvironments. Compared with conventional monolayer cultures, these systems better capture cell–cell interactions, diffusion gradients and spatial heterogeneity, but their increasing biological complexity also creates new analytical challenges. In this context, metabolomics offers a powerful functional readout, capable of revealing whether a model is not only structurally viable, but also metabolically active and biologically meaningful. Nuclear magnetic resonance spectroscopy is particularly well suited to this purpose. Its reproducibility, quantitative nature and minimal sample preparation make it an attractive platform for monitoring metabolic activity in complex *in vitro* systems. In particular, NMR analysis of culture medium provides a non-destructive window into extracellular metabolic exchanges, allowing the simultaneous assessment of nutrient consumption and metabolite secretion over time (Fig. 1). Changes in glucose, lactate, amino acids, organic acids and choline-related metabolites can provide valuable information on energy metabolism, biosynthetic demands, stress responses and treatment-induced metabolic reprogramming. This lecture will discuss how NMR-based metabolomics can be used to characterise and validate 3D cell models, with examples from studies involving spheroids, organoids and cryobioprinted models [1,2]. Current limitations and future opportunities will also be highlighted, including integration with imaging, molecular biology and other omics approaches.

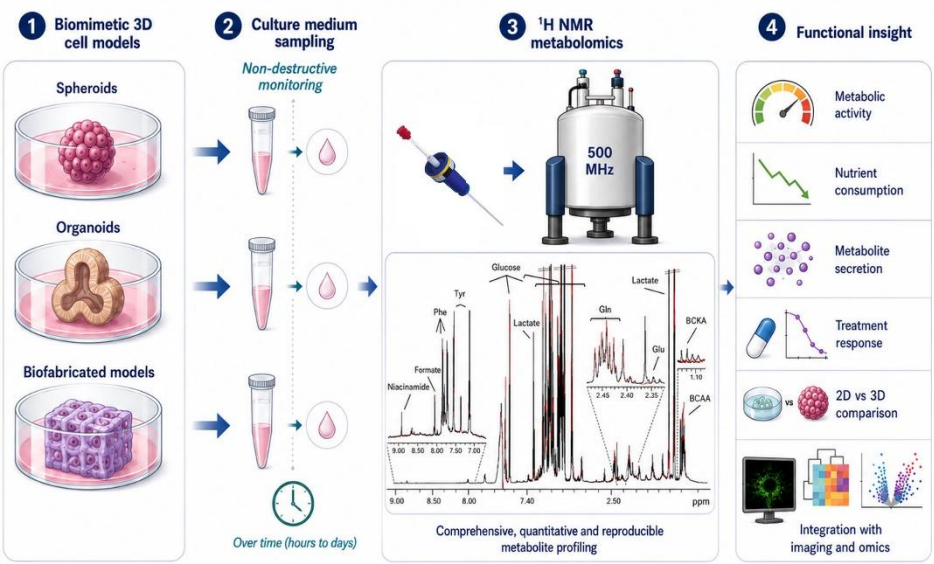

Fig. 1. NMR-based metabolomics for functional phenotyping of complex 3D *in vitro* models.

References

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[2] M. V. Monteiro, F. Moreira-Silva, M. Lagarto, L. P. Ferreira, C. Ramalhinho, I. F. Duarte, C. Jerónimo, V. M. Gaspar, and J. F. Mano Biotechnol. Bioeng. 122, 1541-1553 (2025).

NMR IN DRUG DISCOVERY: FROM HIT IDENTIFICATION TO EXO-METABOLOMICS

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

NMR spectroscopy is an indispensable tool in modern drug discovery, combining the ability to identify active compounds, elucidate molecular mechanisms of action, and perform comprehensive metabolomic analyses [1]. In fragment-based drug discovery (FBDD), ^{1}H and ^{19}F NMR functional and ligand-based screening methods play a pivotal role in the identification of weak ligands (typically in the micromolar-millimolar affinity range) [2,3], which serve as starting points for structure-guided optimization. NMR-based approaches are highly robust and reliable, enabling accurate determination of binding affinities[4], and detailed structure-activity relationship (SAR) analyses that are essential for lead optimization and drug development.
Beyond studies on purified biomolecular targets, the high versatility of NMR extends to complex biological systems, including cell cultures, spheroids, and organoids. In particular, NMR analysis of extracellular media (exo-metabolomics) has emerged as a powerful strategy for investigating mechanisms of action, identifying novel therapeutic targets through the modulation of metabolic pathways, and assessing the toxicological effects of drugs and new materials [5,6].
This presentation will highlight the broad impact of NMR across the drug discovery pipeline, from early-stage hit identification to functional characterization in physiologically relevant biological models.

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Untargeted 1H NMR-based metabolomics and high-pressure liquid chromatography analyses reveal sexual dimorphism in the serum metabolic profiles of patients with different genetic forms of Parkinson's disease

Carmen Marino[a],*, Federica Carrillo[b],*, Marcello Serra[c],*, Tommaso Nuzzo*[d,e], Matteo Vidali[f], Sara Pietracupa[g,h], Nicola Modugno[g], Manuela Grimaldi[a], Francesco Errico[c,i], Anna Maria D'Ursi[a], Teresa Esposito[b,g],@, Alessandro Usiello[c,d],@

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Keywords: solution NMR, metabolomics.

Emerging evidence indicates that sex and genetic background substantially influence systemic metabolic alterations in Parkinson's disease (PD). In this study, we integrated untargeted ^{1}H-NMR profiling with targeted HPLC amino acid quantification to characterise serum metabolic changes in 245 PD patients—121 with idiopathic PD (iPD), 124 with familial PD (gPD), or 91 with rare variants (rvPD)—compared with 140 matched healthy controls. [1-3]
Across all PD subtypes, multivariate analyses revealed a marked sexual dimorphism in serum metabolomic profiles relative to controls, whereas no metabolic distinction emerged between iPD and genetically defined PD, suggesting shared biochemical alterations despite genetic heterogeneity. Male patients exhibited broader metabolic disruptions, including reduced L-glutamate levels and alterations in amino acid metabolism, glutathione biosynthesis, and energy-related pathways. [4]Female patients exhibited more selective alterations, primarily involving methionine, betaine, and lipid-associated pathways. High-performance liquid chromatography (HPLC) data verified a significant decrease in L-glutamate, particularly in male PD patients. Amino acid concentrations demonstrated a sex-dependent correlation with motor severity: in males, L-serine, glycine, L-aspartate, and L-glutamate were associated with MDS-UPDRS III scores, whereas in females, L-serine and L-glutamine were associated with motor impairment, particularly in rvPD. Genetic analyses identified SHMT1, SHMT2, and GCSH as potential sex-specific modifiers, thereby reinforcing the significance of glycine–serine metabolism PD.[3]
Overall, these findings demonstrate that sex is a key determinant of serum metabolic phenotypes in PD and must be considered to improve biomarker discovery and guide precision-medicine strategies.

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Advancing Food Analysis: Amino Acid Quantification by NMR and Deep Learning

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Abstract

Nuclear magnetic resonance (NMR) is a strong tool for the quantification of metabolites inside bio-systems. During last years, a new approach for NMR spectral quantification has been suggested based on convolutional neural network (CNN).[1][2]

In this work, we present a Deep Learning model able to quantify mixtures of three amino acids (Alanine, Aspartic acid and Glycine). A matrix of 64 random mixtures of these amino-acids was created by design of experiment (DoE) to maximize the concentration distribution in a selected interval to be close to bio-matrices.[3] From this 64-sample set, spectra were acquired to be used as test for the model. Two simulated data sets, made of up to 100000 spectra, were constructed, based on the deconvolution results on one selected spectrum, to be used as training and validation of the deep learning model. The model is a collection of 1D convolutional, inception and fully connected layers with 16348 inputs and 4 outputs representing the concentration of the three amino acids and TSP standard.

The model shows a good linearity between the expected and predicted values either on simulated or experimental spectra. The model is capable to predict, starting for the spectrum, the concentration of a compound in a mixture with a low error level. In addition, quantification of many spectra can be achieved simultaneously.

Keywords: NMR spectrometry, Convolutional neural network (CNN), Inception, Spectra simulation

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Exploring the urinary metabolomic fingerprint of human cytomegalovirus: a ^{1}H-NMR study on congenitally infected newborns

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Keywords: metabolomics.

Human cytomegalovirus (HCMV) is the leading cause of congenital infections resulting in severe morbidity and mortality among newborns worldwide. Currently, the most significant prognostic factor of cCMV is the time of maternal infection, with a more severe clinical phenotype if the mother's first outbreak occurs during the first trimester of pregnancy. Nonetheless, the pathogenesis of cCMV infection has still to be completely characterized. Little is known about the metabolic response triggered by HCMV in congenitally infected newborns. As such, urinary metabolic profiling by 1H-NMR might represent a promising tool to be exploited in the context of cCMV infection.
This study aims to investigate the impact of HCMV infection on the urine metabolome in a population of newborns by 1H-NMR spectroscopy combined with multivariate statistical analysis.
Thirty-three newborns diagnosed with cCMV infection were recruited at the Neonatal Unit of the University of Turin and for each of them a urine sample was collected during admission medical examination. Seventeen healthy newborns without cCMV infection were also included as healthy controls.
The 1H NMR spectra of patients (n=35) and controls (n=15) allowed the identification of an overall amount of 55 metabolites. Principal Component Analysis (PCA) and clustering correctly assigned 49 out of 50 newborns into the infected and control groups. Partial Least-Squares-Discriminant Analysis (PLS-DA) revealed that newborns with cCMV resulted in having increased betaine, citrate, 3-hydroxybutyrate, 4-hydroxybutyrate, acetoacetate, formate, glycolate, lactate, succinate, and threonine levels in the urine. On the other hand, healthy controls showed increased 4-aminohippurate, creatine, creatinine, fumarate, mannitol, taurine, and dimethylamine levels. These results showed a clear difference in metabolomic fingerprint between newborns with cCMV infection and healthy controls. Thus, metabolomics can be considered a new, promising diagnostic and prognostic tool in the clinical management of cCMV patients.

INTEGRATED CHEMOMETRIC ANALYSES OF NMR, HPLC-MS AND IRMS DATA FOR GEOGRAPHICAL ORIGIN AUTHENTICATION

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

Geographical traceability has emerged as one of the major scientific and industrial challenges within the wine production chain, both for the protection of designation of origin labels and for the prevention of food fraud. In this context, chemometrics applied to food analysis plays a key role in correlating the geographical origin of wines and olive oils with their chemical composition. This research activity is conducted as part of the Italian PNRR AGRITECH project, Spoke 9, focused on the olive-oil and wine value chain, with the aim of developing advanced analytical and chemometric approaches for food traceability and authenticity assessment [1].
The aim of this study is the geographical differentiation of wines originating from closely located regions through the integration of advanced analytical techniques, namely NMR, HPLC- MS, and IRMS. Specifically, NMR provides a comprehensive overview of the metabolite profile of wines, while HPLC-MS enables the quantification of secondary metabolites, mainly polyphenols and anthocyanins. In addition, IRMS determination of $\delta^{18}O$ provides key information related to pedoclimatic conditions.
The resulting datasets can be processed using classical chemometric approaches, including PCA (Principal Component Analysis) and PLS (Partial Least Squares) [2, 3], as well as data fusion strategies. In particular, this work investigates the application of the DIABLO (Data Integration Analysis for Biomarker discovery using Latent variable approaches for Omics studies) algorithm, which enables the simultaneous integration of datasets generated from different analytical platforms [4].
The implementation of this model leads to improved characterization and discrimination performance compared to single-technique approaches, while also highlighting correlations between variables originating from the different analytical methods.

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Time-Doman NMR: mobility and interfacial phenomena across multiple scales

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

Polymers span an extraordinary range of applications, from structural materials to advanced functional systems. Their behavior is governed by molecular mobility occurring over widely separated timescales, and many technologically relevant systems are further complicated by pronounced heterogeneity (e.g., Fig. 1). A substantial fraction of biological matter is itself polymeric, exhibiting hierarchical architectures and dynamic processes that closely mirror those found in synthetic systems. Furthermore, the drive toward more sustainable materials has stimulated the development of new families of bio-based and biodegradable polymers, whose complex semicrystalline and multiphase structures introduce additional layers of dynamical behavior.

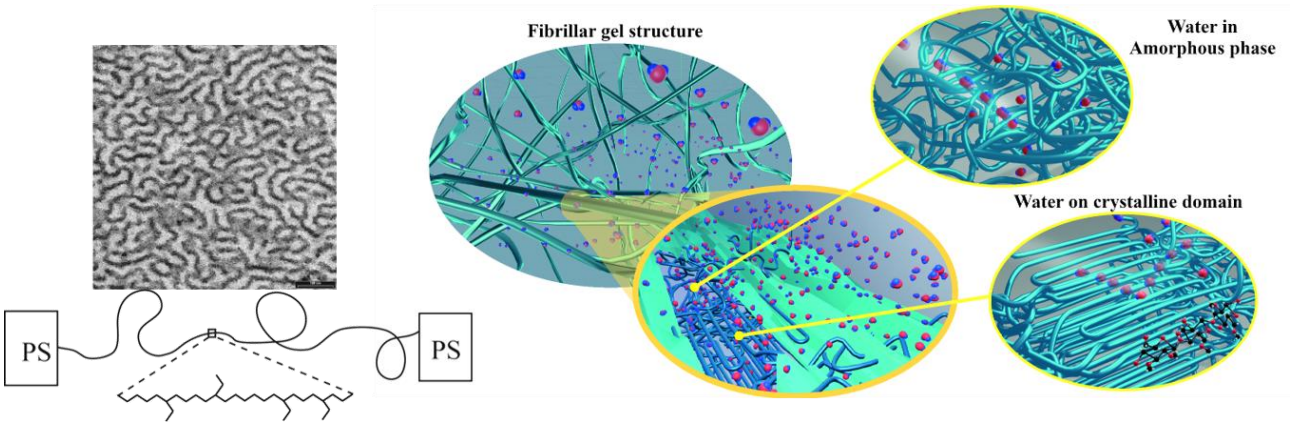

Fig. 1. *Left:* a self-assembled SEBS block copolymer exhibits peculiar motional regimes arising from its microphase-segregated morphology. *Right:* the multiscale structural organization of methylcellulose gel generates a correspondingly broad hierarchy of mobility processes.

This presentation showcases a varied range of polymer systems, investigated with a correspondingly varied palette of Time Domain (TD) NMR methods. These include classical relaxometry as well as more advanced approaches such as spin diffusion and multiple-quantum experiments [1]. Attention is devoted to polymer networks, where TD-NMR reveals motional and structural features that remain largely inaccessible to other characterization techniques. The same methodological framework extends naturally to inorganic/polymer hybrid systems, enabling the determination of nanoparticle surface energy through water relaxation and the assessment of organic-phase mobility in functionalized clays and their polymer nanocomposites. Taken together, these case studies illustrate how TD-NMR can interrogate mobility and interfacial phenomena across multiple length and time scales, providing a unified perspective on systems that range from soft matter to hybrid materials and complex interfaces.

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AN HMBC-BASED J-MODULATION SEQUENCE FOR THE MEASUREMENT OF n^JHC COUPLING CONSTANTS: APPLICATION TO CORROLE SYSTEMS

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

Corroles are contracted porphyrinoids of significant interest in catalysis, sensing, medicine, and solar energy conversion. The complete ^{13}C assignment of these systems is challenging due to the large number of quaternary carbons in the macrocyclic scaffold, which lack directly bonded protons. Such an assignment is, however, of great importance: the ^{13}C chemical shifts are directly linked to the electron density distribution within the highly conjugated π system, which in turn governs the optical, redox, and catalytic properties that underpin the practical applications of these macrocycles. While standard 2D experiments such as HSQC and HMBC allow partial assignment of ^{1}H and ^{13}C resonances, the unambiguous assignment of quaternary carbons in pyrrole rings remains problematic. HMBC crosspeaks arising from $^{2}J_{HC}$ and $^{3}J_{HC}$ couplings to the same pair of quaternary carbons cannot be discriminated on the basis of cross-peak intensities alone, unlike in phenyl systems where the two constants differ substantially.

To address this problem, we developed a novel J-modulation pulse sequence that yields accurate values of $^{n}J_{HC}$ for each cross peak, built on the sensitivity-optimized HMBC scheme previously introduced by Cicero et al. [1]. The approach was first validated on 5,10,15-tritolylcorrole (TTC) and on the β-brominated 3,17-Br_2(TTC). For both compounds, 1,1-ADEQUATE [2], which selectively detects two-bond ^{1}H–^{13}C correlations, was used to unambiguously identify which HMBC cross-peaks arise from $^{2}J_{HC}$. The coupling constants measured by J-modulation on those assigned cross-peaks establish the characteristic $^{n}J_{HC}$ ranges for pyrrolic rings. These reference values were then used to achieve the complete ^{13}C assignment of the N-substituted Vilsmeier product of TTC [3], for which the available sample concentration precluded the use of 1,1-ADEQUATE. The complete ^{13}C assignment of all three corroles enabled a systematic comparison of chemical shifts relative to TTC, revealing how peripheral β-bromination and internal N-functionalization redistribute electron density across the highly conjugated macrocycle, information that is directly relevant to the rational design of corrole-based functional materials. Since pyrrolic rings are found in a wide variety of biologically and technologically relevant molecules, the characteristic ^{2}J and ^{3}J long-range coupling constants established here can guide the assignment of quaternary carbons in other porphyrinoid systems, such as porphyrins, even when sample concentration is too low for 1,1-ADEQUATE.

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SUPPRESSING PROTON COMPETITION TO REVEAL INTRINSIC ZN(II) CHELATION IN L-CARNOSINE MIMICS BY SOLUTION NMR

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

L-Carnosine (L-Car), a histidine-containing dipeptide, has attracted interest in Alzheimer's disease research due to its antioxidant and metal-chelating properties.[1] However, its therapeutic application is limited by rapid hydrolysis mediated by serum carnosinase CN1,[2] motivating the development of more stable **L-Car mimics** able to preserve Zn(II)-binding capability.
In this work, a series of L-Car-inspired derivatives was investigated by solution-state NMR spectroscopy to evaluate their interaction with **Zn(II)** under controlled acid–base conditions. **HEPES** was employed both as an **internal buffer** and as a **reliable internal pH indicator**,[3] improving reproducibility compared to conventional direct pH adjustment methods. This strategy minimized competitive protonation effects and enabled selective observation of Zn(II) coordination phenomena. The analysis focused on the aromatic region of the spectra, since histidine-containing compounds preferentially coordinate Zn(II) through the imidazole moiety. Upon $ZnCl_2$ addition, **chemical shift perturbations** and **reductions in aromatic signal integrals** were observed, supporting ligand–metal interactions. The complete series has been characterized, and determination of the corresponding **dissociation constants** (**K_D**) is currently in progress. The aliphatic region was not systematically analyzed due to overlap with HEPES resonances. (Fig. 1)
Overall, this work presents a reproducible NMR-based approach for distinguishing protonation equilibria from genuine Zn(II) coordination processes in biologically relevant histidine-containing systems. This methodology supports the identification of stable Zn(II)-binding L-Car mimics as potential therapeutic candidates for Alzheimer's disease, with **L-CarolP** and **L-Carol$_2$P** emerging as the most promising compounds of the series.

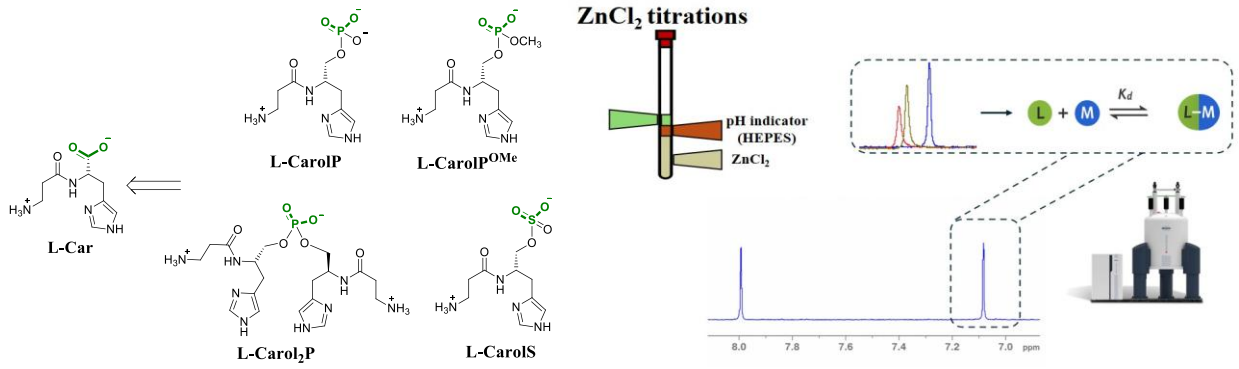

Fig. 1. Work-flow of NMR analyses of L-Car mimics.

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The Synergy of Multiple Receivers and Heteronuclear Detection to Draw a Complete Picture of Intrinsically Disordered Proteins in Action

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Keywords: solution NMR, biomolecules, theory and methods.

Most of the NMR studies performed to date on Intrinsically Disordered Proteins (IDPs) have focused on the investigation of backbone resonances through ^{1}H-^{15}N-based experiments. However, amino acid side chains are the main characters in determining IDPs' properties and in modulating their interactions. Despite their importance side chains have been seldom investigated due to the extreme resonance overlap typical of IDPs' NMR spectra. To overcome this limitation and obtain firm assignment of IDP's side chains we opt for the combination of heteronuclear direct detection and multiple receivers (mr_NMR). Combining ^{13}C-detection with mr_NMR permits saving time and obtaining complementary information from different detected correlations. ^{13}C-direct detection ensures high resolution, crucial for the characterization of flexible regions.[1] mr_NMR allows us to acquire one experiment during the recycle delay of a second one.[2-3]. Here, I will focus on three key experiments for obtaining the full assignment of α-synuclein, including the backbone resonances as well as all the resonances arising from ^{1}H and ^{13}C nuclei of aliphatic side chains. The first one combines two 3D experiments (one ^{13}C detected and one ^{1}H^{N} detected) in a single one for obtaining backbone assignments with a clear save in time. The others combine the superior resolution provided by the CON correlation (C'_i-N_{i+1}) with resonances arising from aliphatic side chains (^{13}C or ^{1}H nuclei). The obtained chemical shift assignment will eventually enable the investigation of the interactions of IDPs with biologically relevant partners from the side chain point of view. This will allow us to shed light on the complex mechanisms governing IDPs' interaction.

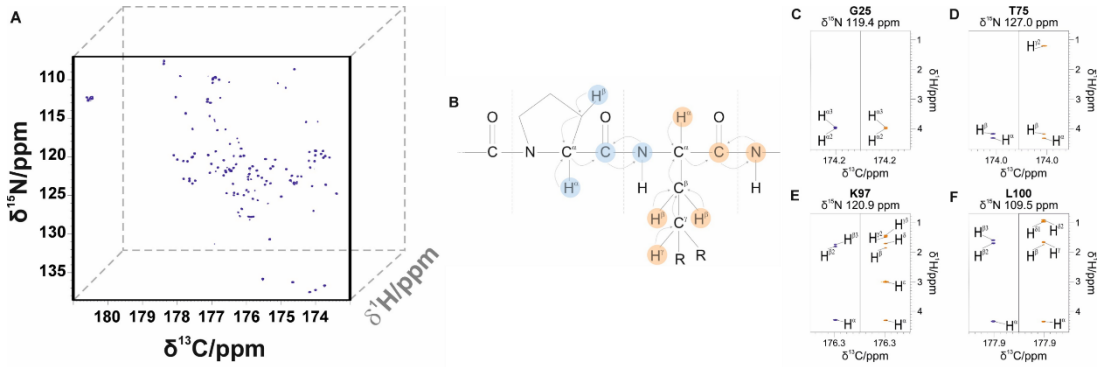

Figure 1. The figure shows the ^{13}C′-^{15}N orthogonal plane obtained through the 3D HBHA(CBCA)CON and 3D H(CC)CON experiment acquired on α-synuclein (A). The observed nuclei and schematic magnetization transfer pathways for the two experiments are shown in blue and orange respectively (B). The $^{13}C'_i$-$^{1}H^{\alpha/\beta}_i$ and $^{13}C'_i$-H^{ali}_i planes extracted at the corresponding $^{15}N_{i+1}$ chemical shifts are reported in blue and orange respectively (C – F).

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Next-Generation EPR: Combining High-Performance Q Band Instrumentation with Artificial Intelligence Enhanced Spectral Processing

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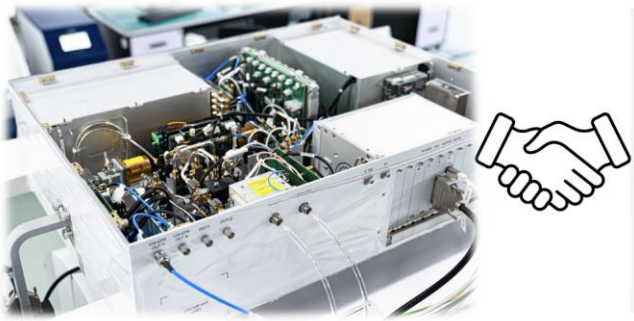
Keywords: materials, small molecules, biomolecules, theory and methods, instrumentation.

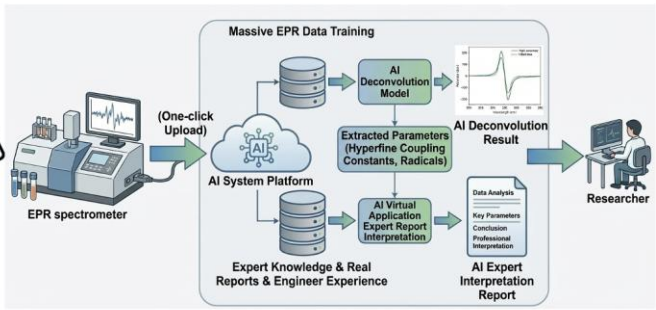
The advancement of Electron Paramagnetic Resonance spectroscopy is increasingly defined by the convergence of robust hardware and intelligent software. While EPR remains a vital tool for probing paramagnetic centers, broader adoption is limited by instrument complexity and the steep learning curve of spectral analysis. Researchers at CIQTEK address these barriers by developing high-performance hardware alongside the first dedicated AI model for EPR.

Our Q-band pulsed EPR system achieves less than 10 ns $\pi/2$ pulse wide-bandwidth excitation using a Solid-State Power Amplifier, offering superior longevity and reduced maintenance over conventional technology. This architecture maximizes sensitivity and spectral resolution, deciphering complex metal hyperfine couplings and extracting dipolar information for high-resolution DEER distance mapping.

Complementing the hardware is a three-layer AI EPR model that streamlines the workflow from raw data to publication. Trained on over 100,000 real and simulated datasets — achieving 99.9% precision for simulations and 92% for real-world samples — the model automates spectral fitting, component characterization, and experimental report generation. It further offers predictive guidance, suggesting follow-up experiments to validate findings.

This integrated approach lowers the entry barrier for researchers in chemistry, biology, and materials science, driving EPR toward a more impactful technology.

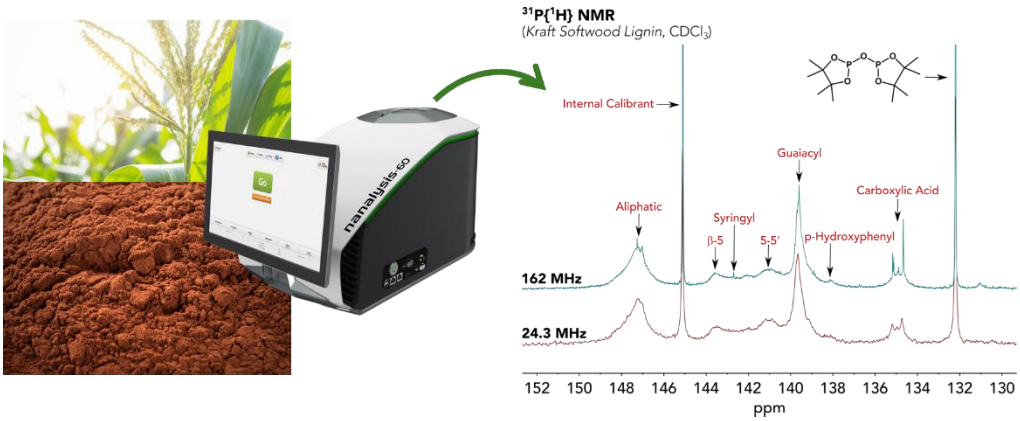




Quantitative Characterization of Lignin by Benchtop ^{31}P NMR
ABCS/Nanalysis Sponsorship Lecture

Lignin is one of the most abundant renewable sources of aromatic compounds and represents a promising feedstock for the sustainable production of high-value chemicals and materials. The quantitative characterization of hydroxyl groups is essential for understanding its structure and enabling its valorization. Quantitative ^{31}P NMR spectroscopy, following phosphitylation of hydroxyl groups with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), is widely recognized as a reference technique for lignin analysis. However, routine implementation is often limited by restricted access to high-field NMR instrumentation.
In this study, the performance of a 60 MHz benchtop NMR spectrometer for the quantitative characterization of different technical lignins was evaluated and compared with that of a 400 MHz high-field NMR instrument.

Four types of lignin (softwood kraft, hardwood kraft, organosolv, and soda lignin) were analyzed using an identical phosphitylation and quantitative acquisition protocol on both instruments. The results demonstrated excellent agreement between benchtop and high-field NMR measurements, with differences generally below 10% in the quantification of the main hydroxyl functional groups and excellent reproducibility across replicate analyses. These findings demonstrate that benchtop ^{31}P NMR is a reliable, accessible, and cost-effective alternative to high-field instrumentation for the quantitative characterization of lignin, thereby facilitating the broader adoption of NMR analysis in both research laboratories and industrial settings.



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SOLID STATE NMR ON SUPPORTED PALLADIUM NANOPARTICLES

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Keywords: Supported palladium nanoparticles (NPs) are impactful for a wide range of catalytic processes. The reactivity can be finely tuned by doping, to form Pd carbide, nitride or hydride [1,2], easily distinguished on a structural level by XANES, looking at the bulk Pd signal (Figure 1). Solid state NMR (ssNMR) can easily deal with a wide range of nuclei, whether spin ½ or quadrupolar, to provide information on the *local* environment near the modified Pd NP sites, under realistic conditions. We will demonstrate how our approach, utilizing magic-angle spinning ssNMR methods, can be utilized for the study of these supported Pd NPs. The ssNMR data are complemented by a multi-technique approach, including EPR, DRIFTS, and first principle computational methods, in order to validate our interpretation of the NMR observables.

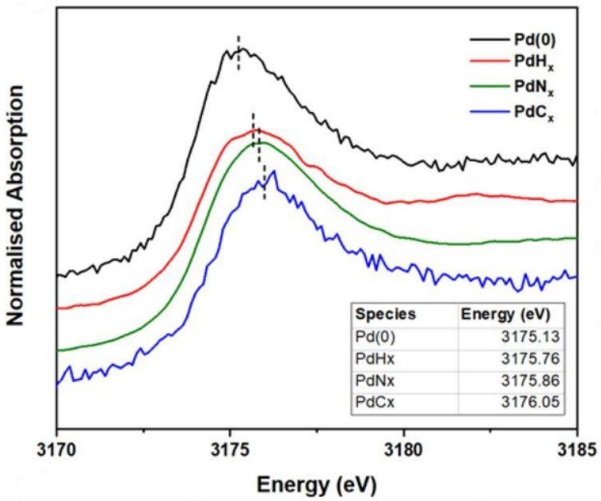

Fig. 1. Comparison of doped, supported Pd nanoparticles from XANES.

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Beyond Structures: Solution NMR as a Method for Resolving Dynamic Mechanisms Across Biological and Heterogeneous Catalysts

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Keywords: solution NMR, small molecules, biomolecules.

Structural biology is moving beyond static molecular structures toward quantitative descriptions of molecular mechanisms involving conformational heterogeneity, exchange kinetics, transient interactions, allostery, disorder, assembly, and phase behavior. These properties are often difficult to characterize fully using crystallography, cryo-electron microscopy, scattering, imaging, mass spectrometry, simulations, or structural prediction alone. Solution NMR occupies a distinctive position within this integrated landscape by providing residue- and atom-specific observables of structure, dynamics, populations, kinetics, and interactions directly in solution.

Here, we illustrate the contribution of solution NMR to mechanistic studies of two classes of catalytic systems. First, we examine the AlkB family of Fe(II)/α-ketoglutarate (αKG)-dependent nucleic acid demethylases. Despite the availability of several crystallographic structures, fundamental questions remain regarding substrate recognition, selectivity, and activation. By combining solution NMR and molecular dynamics simulations, we identified a conformational equilibrium within αKG that is not apparent from crystallographic structures but plays a central role in enzyme activation and substrate selectivity. Substrates that more effectively stabilize the catalytically competent αKG conformation are demethylated more efficiently, directly linking cosubstrate dynamics to catalytic activity [1, 2].

We then extend this approach to nanoparticle catalysts. Using surface-contrast NMR, we characterize the interactions and dynamic partitioning of phenolic substrates among distinct nanoparticle-bound states. Their distribution among these interfacial states is a major determinant of the activity and selectivity of palladium-on-ceria nanoparticles in phenol hydrogenation [3, 4].

Together, these studies demonstrate how solution NMR, integrated with molecular simulations and complementary methods, transforms structural models into dynamic, quantitative, and experimentally constrained descriptions of mechanisms in biological and heterogeneous catalysts.

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IMPROVING THE DETECTION LIMITS

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

It is routinely recommended in NMR labs to prepare samples with several milligrams of the compound to be analyzed, even for the simplest 1D acquisitions [1-6]. This is a perfectly reasonable recommendation given the wide variability of sample conditions and instrumentation available. However, the latest advances in probe technology have enabled significant improvements in the probe efficiency using magnetic couplings [7]. Here we demonstrate that this technology enables pushing the sample requirements of 5mm nitrogen cooled probes down to less than 1 mg of sample for commonly run 1D and 2D experiments, and we introduce new applications of this technology.

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NMR-BASED METABOLOMICS: A JOURNEY BETWEEN HEALTH AND FOOD

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

Metabolomics represents an important and well-established line of research within our group [1-3]. Within this framework, the NMR technique has been applied to various sectors, ranging from biomedicine and clinical research to foodomics and the agri-food chain.
Here, I present the snapshots that best characterize my individual contribution to NMR-based metabolomics research.
In the biomedical domain, I have carried out research on the metabolic and lipoproteomic alterations associated with neurodegeneration and ageing, within the framework of the H2020 PROPAG-AGEING project. Serum metabolomics highlighted a sex-related oxidative stress imbalance in de novo, drug-naïve Parkinson's disease patients [4], while NMR spectroscopy combined with machine learning enabled biological age prediction in healthy and Parkinson's disease cohorts [5]. Moreover, in the clinical setting, plasma metabolomics and lipidomics were applied to an Italian COVID-19 cohort within the COMETA project [6], linking metabolic alterations to disease severity, viral variant and individual response to tocilizumab therapy [7]. Parallel activity on saliva and faecal metabolomes contributed to the characterization of periodontitis [8] and colorectal cancer [9].
In the agri-food domain, I present the use of NMR-based metabolomics for food quality assessment and authentication, with particular focus on the olive oil sector. An NMR-based predictive model for the chemical and sensory profile of virgin olive oil, together with machine-learning tools for geographic and cultivar authentication [10], was developed and applied to Tuscan extra-virgin olive oils, work recognized with the 2026 "Antico Fattore" award of the Accademia dei Georgofili [11]. The same analytical philosophy has been extended to dairy products [12] and to the geographical traceability of coffee [13].
Finally, the ongoing BONSAI project is extending this methodology from high-field to benchtop NMR platforms, with the aim of translating laboratory-grade metabolomics into accessible, field-deployable analytical tools suited for decentralized applications.

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Spatially resolved NMR spectroscopy: principles and applications

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Keywords: solution NMR, theory and methods, instrumentation

Spatially resolved NMR spectroscopy provides a powerful approach to probe chemically and physically heterogeneous systems by introducing spatial encoding into conventional NMR experiments [1]. In this contribution, we present the principles and applications of slice-selective NMR methods, enabling selective observation of defined regions along the z-axis. The methodology is based on gradient echo experiments, where spatial information is encoded via position-dependent frequency shifts and selectively refocused using shaped pulses in the presence of pulsed field gradients.

We demonstrate how these principles can be implemented in 1D and 2D experiments [2–4] and discuss different selection schemes, as well as the importance of accurate gradient calibration and optimisation of pulse parameters to control slice thickness and position. Practical implementations that can be incorporated into existing pulse sequences are presented, facilitating routine use.

In addition to applications in heterogeneous samples, spatially resolved NMR provides a valuable tool for probe characterisation. Slice-selective nutation experiments enable direct assessment of B1 field homogeneity along the sample [4,5]. This provides insight into RF field distribution and pulse performance, improving experimental accuracy and reproducibility.

Overall, spatially resolved NMR complements conventional spectroscopy by enabling the selective observation of different regions within a sample. These approaches provide robust tools for analysing inhomogeneous systems and for probing instrumental performance.

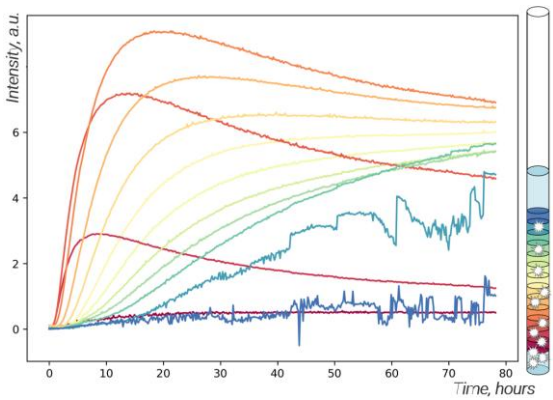

Fig. 1. Dissolution and diffusion kinetics by spatially-resolved NMR spectroscopy.

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INVESTIGATION OF TRANSITION METAL-BASED MRI PROBES: A COMBINED ^{1}H NMR RELAXOMETRY, EPR AND COMPUTATIONAL STUDY

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Keywords: low field NMR, contrast agents,

Magnetic resonance imaging (MRI) is established as a powerful, noninvasive diagnostic tool, with its sensitivity significantly boosted by efficient contrast agents. While the majority of clinically approved agents currently utilize Gd(III) complexes due to their favorable electronic and magnetic properties, growing safety concerns regarding long-term gadolinium exposure, especially in patients with kidney impairment, have sparked intensive research into gadolinium-free alternatives. Among these emerging options, Mn(II) and Fe(III) complexes have drawn significant interest, whereas broader transition-metal-based systems (TM) still remain largely underexplored. For instance, well-tolerated biological chelates with a spin S = 1/2, such as vanadyl V(IV) or Cu(II), also show promise as potential MRI contrast agents for specific applications.[1,2] These systems are already being investigated for various biomedical purposes, including therapeutic agents for diabetes, anticancer drugs, and radiotracers for PET imaging. It is evident that these last probes will never reach the paramagnetism displayed by Gd(III), however, the electron magnetic moment is just one of the relevant parameters involved. Ligand exchange rates, electronic relaxation times, metal–water distances and spin density distributions are all parameters that can be effectively tuned. In order to make these molecular entities viable alternatives to Gd(III), it is of central importance a thorough understanding of the spin dynamics of paramagnetic complexes of earth abundant and essential TM complexes, with the specific goal of designing of new and safer MRI contrast agents. To address this gap, this contribution introduces an integrated approach that combines ^{1}H NMR relaxometry, electron paramagnetic resonance (EPR) and computational chemistry to investigate the structural, magnetic, and dynamic properties of paramagnetic complexes in aqueous solution.[1] This comprehensive methodology enables the independent, experimental determination of key parameters governing relaxivity, specifically, metal-proton distances, the magnitude of metal-proton hyperfine interactions, and the rotational correlation times (τ_R) of the complexes. We apply this approach to various V(IV) and Cu(II) chelates[2,3] characterized by different hydration states, chemical structures and geometries.

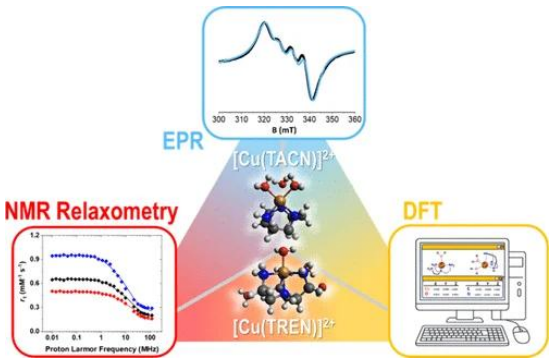

Fig. 1. The integrated methodology used to characterize Cu(II) complexes for MRI applications.

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A Rare Earth Elements Recovery Strategy Using Natural Dioptase and ^{1}H-NMR Relaxometry

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Keywords: Dioptase, Gd-based MRI contrast agents, low field NMR, materials.

The increasing demand for rare earth elements (REEs), as lanthanoid metals has intensified environmental concerns regarding their release into aquatic ecosystems. A prominent example is gadolinium (Gd^{3+}), widely employed in Gd-based contrast agents (GBCAs) for magnetic resonance imaging (MRI), where the clinical administration and the excretion through the urine represent both a source of environmental hazard and an economic loss [1]. Here we report the use of dioptase ($Cu_6Si_6O_{18}\cdot 6H_2O$), a natural copper cyclosilicate rich in surface hydroxyl groups, as a low-cost natural sorbent for the recovery of REEs from water under mildly acidic conditions (pH 5.5). The adsorption and desorption behaviour was investigated using a combined approach of low-field ^{1}H-NMR relaxometry, for real-time, non-invasive monitoring [2], and ICP-MS. The use of NMR relaxometry represents a key aspect of this work, demonstrating how magnetic resonance techniques can serve as sensitive and practical tools for tracking REE removal processes and supporting the development of sustainable remediation strategies. Morphological properties and structural integrity of dioptase before and after sorption were studied (FE-SEM, PXRD). Near-complete removal of Gd^{3+} (99.6%) from a 0.1 mM solution was achieved within 7 hours. The adsorption kinetics were best described by a parabolic diffusion model (R^2 = 0.9885), indicating a diffusion-controlled capture mechanism, with a distribution coefficient K_d = 641 mL g^{-1} after the first cycle [3]. The simultaneous tracking with multi-ion competition experiments, revealed a preferential affinity of dioptase for Lu^{3+} over Gd^{3+} and La^{3+}, consistent with the smaller ionic radius and higher charge density of Lu^{3+} favouring inner-sphere complexation with the silicate surface. Desorption experiments under acidic conditions (pH 2) allowed recovery of approximately 95% of the adsorbed Gd^{3+}, with no significant leaching of structural Cu^{2+} or alteration of the crystal structure. To the best of our knowledge, this is the first systematic study reporting dioptase as a sorbent for REE recovery, combining diagnostic-grade NMR relaxometry with ICP-MS quantification. The mineral's performance, structural robustness, and minimal processing requirements make it a promising alternative to synthetic adsorbents for REE remediation and circular economy applications.

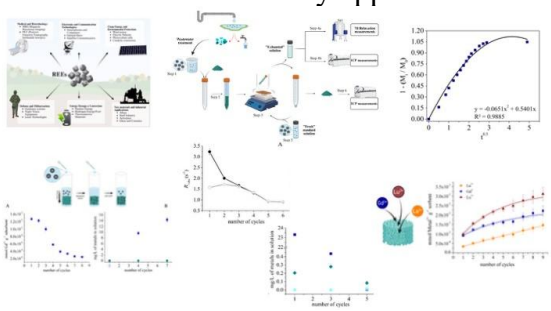

Fig. 1. Summarizing panel

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Visualize & Destabilize: Boronated Carboranyl-Curcuminoids as Theranostic candidates in Alzheimer's MRI-guided Neutron Capture Therapy

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Keywords: solution NMR, MRI, small molecules, contrast agents.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder wherein the aggregation of amyloid-beta (Aβ) peptide into neurotoxic oligomers and insoluble fibrils plays a central pathogenic role.[1] This study investigated Neutron Enhanced Amyloid Disaggregation (NEAD) as an innovative tool to selectively destabilize Aβ aggregates. This approach relies on ^{10}B-enriched monocarbonyl analogs of curcumin (BMACs),[2] which bind to Aβ fibrils to enable a localized neutron capture reaction. The resulting high linear-energy-transfer (LET) α particles and lithium ions, potentially induce chemical modifications withinin the protein fragments forming the amyloid plaques rather than merely causing disaggregation. *In vitro* post-irradiation analysis employing FESEM, ^{1}H-NMR and mass spectrometry showed a 34% disaggregation rate and the selective oxidation of histidine residues,[3] a chemical modification linked to the disruption of the aggregated protein architecture (Fig. 1). To achieve a multimodal theranostic platform, the agent was modified into a Gd-DOTA derivative (Gd-DOTA-BMAC-9) to act as an MRI probe and dual-NCT active species. In aqueous solution, this compound self-assembles into stable micelles, exhibiting a high longitudinal relaxivity (r1p) (20 mM $^{-1}$s^{-1} at 21.5 MHz, 25°C). ThT competition assay revealed that Gd-DOTA-BMAC-9 maintains a nanomolar affinity for Aβ fibrils, comparable to the parent compound. Ultimately, this system serves as an efficient carrier for the co-delivery of B and Gd atoms, facilitating MRI-guided NCT and enabling non-invasive quantification of ^{10}B concentrations via Gd(III) signal enhancement.

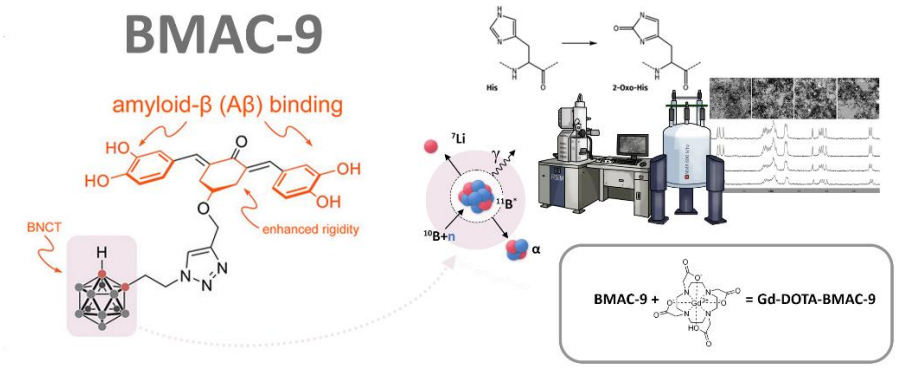

Fig. 1. BMAC-9 structure and NCT-induced histidine oxidation analysis by FESEM and NMR, alongside the schematic formulation of the multimodal Gd-DOTA-BMAC-9 derivative.

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The association between grey matter microstructural characteristics and cerebral oxidative metabolism is altered in Multiple Sclerosis

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Keywords:
MRI, biomolecules, theory and methods

Our goal was to test whether the association between grey matter (GM) microstructure and GM oxidative metabolism, found in previous studies in animals and healthy humans [1,2], was altered in people with Multiple Sclerosis (MS). We scanned 29 relapsing-remitting MS patients (age=39.5±9.3 yrs; 20F/9M) and 24 healthy controls (HC; age=36.2±5.6 yrs; 9F/15M). The MRI protocol included: T1-MP2RAGE; multi-band Diffusion-Weighted Imaging (DWI) for Soma And Neurite Density Imaging (SANDI) [3]; breath-hold-calibrated dual-excitation pCASL (DEXI) for cerebral metabolic rate of oxygen ($CMRO_2$) quantification [4]; FLAIR (in MS patients). GM microstructural features were extracted, namely the signal fraction of soma and neurites (fsoma, fneurite) and the average cellular size (Rsoma) from preprocessed DWI data. DEXI images were motion corrected and despiked, and $CMRO_2$ was quantified by inverting a modified Davis model [5]. The Harvard-Oxford atlas was used for GM parcellation [6]. Linear models were fitted between $CMRO_2$ and the SANDI parameters accounting for residual partial volume contamination of non-GM tissues (MATLAB, The MathWorks Inc., R2022b). In HC there was a significant association between $CMRO_2$ and fsoma (regression coefficient β=0.498, p<0.0001), Rsoma (β=0.428, p<0.0001) and fneurite (β=-0.380, p<0.0001). These associations were all weaker in the MS group (difference in β: p=0.051, p=0.089, p=0.038 for fsoma, Rsoma and fneurite, respectively). Excluding GM lesions did not affect the results. This microstructure–metabolism uncoupling may reflect the neurodegenerative, inflammatory, energetic, and vascular alterations underlying MS pathology [7].

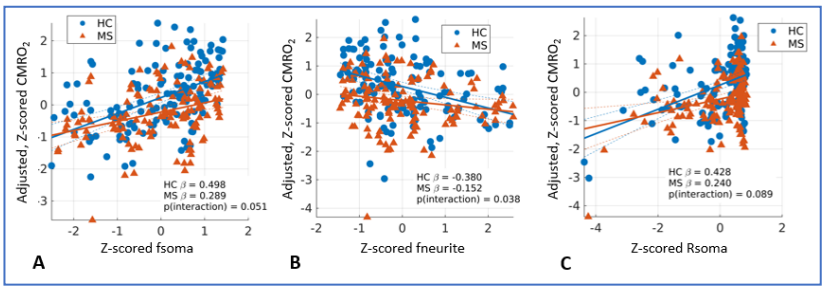

Fig. 1. Microstructure versus O_2 metabolism. $CMRO_2$ (adjusted for covariates) is plotted against fsoma, fneurite and Rsoma across GM ROIs. Regression coefficients, 95% confidence intervals and the group-interaction p-value are indicated. HC=healthy controls; MS=Multiple Sclerosis patients.

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Targeted Liposomes for Combined Boron Neutron Capture Therapy and MRI Visualization in Non-Small Cell Lung Cancer

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Keywords: MRI, materials, contrast agents.

Boron neutron capture therapy (BNCT) is a type of radiotherapy applied for cancer treatment that relies on the selective accumulation of boron-containing compounds in tumor cells followed by neutron irradiation. Upon irradiation, the ^{10}B isotope, undergoes a reaction that produces highly ionizing alpha particles and lithium nuclei, leading to the destruction of malignant cells while minimizing damage to surrounding healthy tissue. However, the clinical efficacy of BNCT is limited by the low selectivity of clinical agents such as sodium mercaptoundecahydro-closo dodecaborate (BSH) [1]. To overcome this limitation, a theragnostic liposomal nanosystem was developed, co-encapsulating BSH as a boron source for BNCT and the gadolinium-based contrast agent Prohance to allow the monitoring of the biodistribution of the system using MRI. This liposome was designed for the treatment of non-small cell lung cancer (NSCLC), one of the most aggressive and treatment resistant forms of lung cancer. To achieve selective targeting, liposomes were functionalized with Ge11 peptide, which recognizes the epidermal growth factor receptor (EGFR), overexpressed in NSCLC cancer cells. Liposomes were prepared using the thin film hydration method [2], with a formulation including POPC, cholesterol and DSPE-PEG2000. DSPE-PEG2000-maleimide was incorporated to allow for peptide conjugation. The liposomes showed mean diameters between 100 and 120 nm with PDI values below 0.20, as determined by dynamic light scattering. Stability studies performed at pH 7.4 and 37 °C demonstrated less than 16% cargo release over 48 hours. Encapsulation efficiencies of approximately 10% for BSH and 7% for Prohance were obtained. Cellular uptake studies were performed using the EGFR-expressing A549 NSCLC cell line. After 24 hours incubation, Ge11-functionalized liposomes exhibited significantly higher cellular uptake compared to non-targeted liposomes (Fig 1A), while no basal toxicity was observed for any formulation. Moreover, cells incubated with Ge11 conjugated liposomes showed a detectable signal intensity enhancement on T1-weighed images acquired at 7 T when compared to untreated cells (Fig 1B,C). Future studies will include neutron irradiation experiments to assess the potential of this platform for BNCT applications.

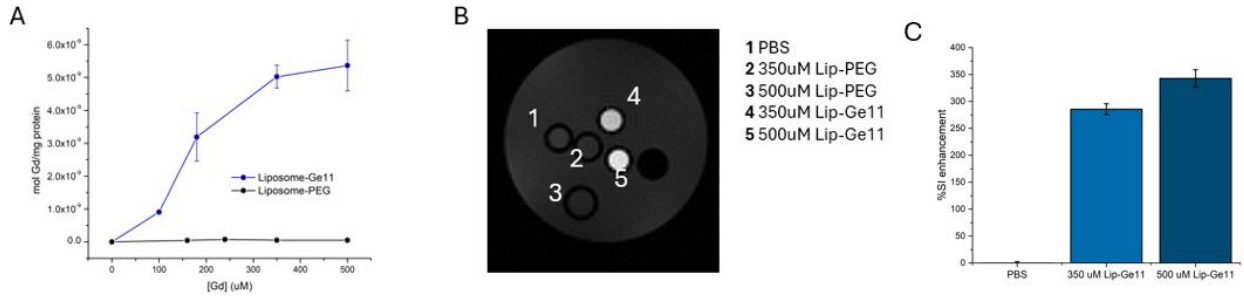

Fig. 1. **A** Uptake study performed on A549 cells with different concentrations of unmodified (Liposomes-PEG) or Ge11 conjugated liposomes **B** T1w image of A549 cells incubated with Ge11 conjugated liposomes (Lip-Ge11) or unmodified liposomes (Lip-PEG) **C** %T1w signal enhancement

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A glimpse in the metal ion selectivity rules: Zn(II), Cd(II) and Co(II) interplay with different protein coordination spheres in determining variable thermal stability and folding scenarios

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Keywords: solution NMR, biomolecules

Models designed to study protein/metal-ions interaction are helpful to provide insights into the rules of metal ion selectivity. In this context, the prokaryotic zinc-finger family Ros/MucR offers an example of several naturally occurring homologs binding a structural zinc ion with coordination spheres characterized by different amino-acids arrays. In particular, Ros87, the zinc binding domain of the protein Ros from *A. tumefaciens,* binds Zn(II) with a classical Cys_2His_2 coordination sphere, but most of its homologs show a substitution of the second cysteine by an aspartate [1].

In this study, the binding properties to several metal ions of the proteins Ros and Ros87-C27D, Ros87 mutant with a $CysAspHis_2$ coordination sphere, are investigated by means of UV-Vis, CD and NMR spectroscopies.

Structural effects, dissociation constants and the resulting mechanisms of folding are compared to underline how the interplay between the different metal ions and the coordinating amino-acid sets is determinant in modulating both the structural plasticity and the thermodynamic stability of the metal-binding site and dictate the protein-metal ion selectivity and its biological function.

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MONITORING DISORDERED PROTEIN-LIGAND INTERACTIONS THROUGH NMR

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Keywords:
solution NMR, small molecules, biomolecules.

Intrinsically disordered proteins (IDPs), unlike globular proteins, lack a stable three-dimensional structure and therefore do not possess well-defined binding pockets. Their pronounced conformational heterogeneity and dynamic nature make the rational design of ligands targeting IDPs particularly challenging. Given the increasing recognition of the biological and pathological relevance of IDPs, elucidating the molecular determinants underlying IDP–ligand interactions has become a crucial step toward the development of effective therapeutic strategies. In this context, solution-state NMR spectroscopy represents one of the most powerful techniques for investigating IDP conformational ensembles and their interactions at residue-level resolution under near-physiological conditions, providing unique insights into transient and dynamic molecular recognition processes. α-Synuclein (α-syn), a paradigmatic IDP predominantly localized at presynaptic terminals, is involved in several neurodegenerative disorders collectively known as synucleinopathies, including Parkinson's disease, where it accumulates into toxic oligomeric and fibrillar aggregates [1].
In this study, we explored a supramolecular approach based on a panel of rationally designed anionic calix[4]arenes to interfere with α-syn aggregation and toxicity. These aromatic macrocycles possess a conserved scaffold with selectively functionalizable upper and lower rims. Recent studies have shown that specific calixarene derivatives interfere with the aggregation of amyloidogenic proteins [2]. Among the investigated compounds, CLXP1, a tetraphosphonato calix[4]arene characterized by a rigid and preorganized cavity, emerged as the most effective inhibitor of α-syn aggregation in a validated yeast model of PD based on α-syn overexpression. Treatment with CLXP1 significantly improved cell viability, reduced the formation of toxic intracellular α-syn inclusions, and restored several dysregulated cellular pathways. To elucidate the molecular basis of these effects, we performed solution-state NMR investigations of α-syn:CLXP1 interaction. Two-dimensional ^{1}H-^{15}N HSQC spectra revealed selective chemical shift perturbations primarily localized within the N-terminal region of α-syn, a domain critically involved in membrane binding, oligomerization, and early aggregation events. Furthermore, ^{15}N longitudinal (R_1) and transverse (R_2) relaxation experiments highlighted local dynamic perturbations induced by CLXP1 binding, supporting the formation of a direct yet transient interaction involving lysine-rich segments of the protein [3].
These findings highlight the unique capability of NMR spectroscopy to characterize weak and dynamic interactions involving intrinsically disordered proteins and support the use of supramolecular compounds as promising modulators of pathological protein aggregation.

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INTEGRATING NMR STRUCTURE DETERMINATION AND LIGAND OPTIMIZATION OF THE CIAP2-BIR1 PROTEIN

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Keywords: solution NMR, small molecules, biomolecules.

Resistance to apoptosis is a fundamental hallmark of cancer and is often associated with the overexpression of Inhibitor of Apoptosis Proteins (IAPs) [1–3]. Through their conserved Baculoviral IAP Repeat (BIR) domains, these proteins suppress apoptotic signaling and promote tumor cell survival [4–5]. Within this family, cIAP2 plays a central role in regulating both apoptosis and inflammatory signaling, with its BIR1 domain, emerging as a promising therapeutic target, particularly in contexts where cIAP1 loss induces compensatory cIAP2 upregulation [6–7]. In this context, a structure-based virtual screening identified FC2, a benzodiazepinone compound, as a selective modulator of the BIR1 domain. Subsequently, a library of 22 structurally related derivatives was developed to optimize binding affinity and cytotoxic activity [8]. To support the rational optimization of cIAP2-BIR1-targeting compounds, a comprehensive solution NMR characterization of the uniformly ^{15}N, ^{13}C-labeled domain was performed. Multidimensional heteronuclear experiments enabled near-complete backbone and extensive side-chain resonance assignments. Chemical shift analysis performed using TALOS^{+} [9] confirmed a secondary structure consistent with the available crystal structure (PDB ID: 7NK0), while a dense network of Nuclear Overhauser Effect (NOE) distance restraints, obtained from 3D ^{5}N- and ^{13}C- edited NOESY-HSQC spectra, provided detailed information on the protein's tertiary structure in solution [10]. Furthermore, NMR relaxation measurements (T_1, T_2, and heteronuclear NOE) were used to characterize backbone dynamics and conformational flexibility under near-physiological conditions [11]. Overall, this high-resolution characterization provides a robust structural and dynamic foundation for the rational design of selective cIAP2 inhibitors, offering a promising strategy to restore apoptotic signaling in resistant tumor cells.

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NMR mapping of Cyclophilin D pockets using Ultra-Low Molecular Weight fragments

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Keywords: solution NMR, small molecules, biomolecules.

Cyclophilin D (CypD) is a mitochondrial peptidyl-prolyl isomerase implicated in the regulation of the mitochondrial permeability transition pore (PTP) and apoptosis. Dysregulation of CypD activity has been associated with mitochondrial dysfunction in several pathological conditions and has also been linked to the interaction with the OSCP (Oligomycin Sensitivity Conferral Protein) subunit of ATP synthase. [1] To better understand the molecular basis of this interaction, CypD mutants are being investigated to identify the structural determinants involved in OSCP recognition and complex formation.

A deeper understanding of these interactions may support the development of selective CypD inhibitors, which remains a challenging task due to the high conservation of the cyclophilin catalytic pocket across isoforms. [2] In this context, a mechanism-driven NMR-based Fragment Based Drug Discovery (FBDD) strategy was pursued, extending conventional fragment screening through the use of ultra-low molecular weight (ULMW) fragments. [3] Experimental conditions were validated using cyclosporin A, whose ^{1}H-^{15}N SOFAST-HMQC chemical shift perturbation (CSP) pattern on CypD was consistent with literature data. A library of Mini-Frags was screened at high protein-to-fragment molar ratios (1:1000 - 1:5000) to detect extremely weak interactions (K_D in the high mM range).

Binding events were quantified through CSP analysis of ^{1}H-^{15}N SOFAST-HMQC spectra, applying statistically defined thresholds based on standard deviation to distinguish specific perturbations from experimental noise. All interactions occurred in the fast-exchange regime, enabling residue specific mapping of binding sites and pocket level characterization. Some Mini-Frags showed localized and reproducible CSPs, highlighting potential interaction hot spots that may contribute to enhanced selectivity toward CypD over other cyclophilins.

Although ULMW screening allowed for detailed mapping of binding pockets, determining the orientation of the bound fragments under these experimental conditions remains challenging. This limitation is driving the development of alternative strategies for fragment epitope mapping, beyond conventional NMR approaches.

Nonetheless, the interaction motifs identified during ULMW screening provided a framework for the development of selective CypD ligands with potential relevance to mitochondrial dysfunction.

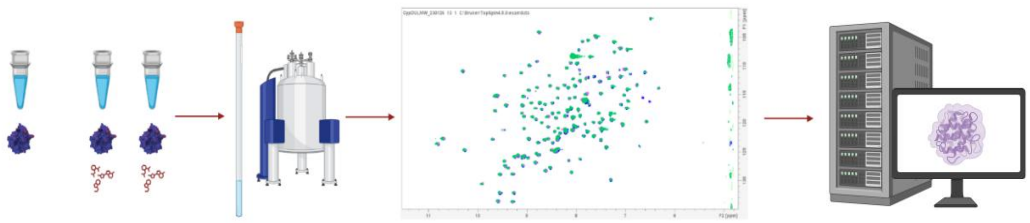

Figure 1: Workflow of the NMR-based ULMW screening approach

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NMR-based Solvent Paramagnetic Enhancement experiments on IDPs

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Keywords: solution NMR, biomolecules, contrast agents

NMR-based solvent paramagnetic relaxation enhancement (sPRE) has been widely used for globular proteins, but its application to intrinsically disordered proteins (IDPs) is still developing. Mapping sPRE along a protein sequence by measuring relaxation rates at increasing paramagnetic probe concentrations provides information on solvent exposure and conformational variability [1,2]. Here, α-synuclein is used as a model IDP to assess the performance of two high spin iron(III)-based co-solutes, Fe(NOTA) and Fe(DO3A) [3]. Then, we moved to a more structurally heterogeneous protein to evaluate sPRE efficacy as a descriptor of conformational heterogeneity. CBP-ID4, the fourth disordered linker of the CREB binding protein, was chosen for this scope because its previous NMR characterization showed the presence of two partially populated helices [4]. $^{1}H^{N}$ sPRE experiments on this protein were conducted at 298 K and 700 MHz in the presence of increasing concentration of Fe(DO3A), chosen as the optimal probe. To support sPRE data, ^{1}H-^{15}N CLEANEX and ^{15}N T_2 experiments were conducted in the same conditions. The data we obtained supports the different helix populations and highlights two additional regions with distinct features. Residues 50–70 show lower $^{1}H^{N}$ sPRE and CLEANEX alongside elevated ^{15}N R_2 values, while residues 130–145 display the opposite pattern. Notably, the polyP segment preceding the second helix shares the features of the 50–70 region, suggesting local conformational rigidity that may influence the second helix population. These results suggest that sPRE, combined with complementary NMR observables, holds strong potential for characterizing conformational ensembles of IDPs and monitoring their biologically relevant interactions.

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FAST FIELD-CYCLING RELAXOMETRY AS A TOOL TO INVESTIGATE PATHOLOGICAL STATES THROUGH WATER DYNAMICS IN TISSUES

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Conventional MRI, at high field strengths, excels in providing detailed morphological images; however, it has limitations in assessing key functional features, including those related to metabolism. In this study, we demonstrate that novel, easy to apply strategies performed at low and ultra-low magnetic fields allow to obtain relevant information that are invisible at standard, clinically used MRI. Our work developed along two main directions: one without and the second with the use of contrast agents.
The first is based on the observation that at low fields (in contrast to what is observed at clinically used magnetic fields), the recovery of 1H magnetisation depends on the rate of water exchange across the cell membrane. The extent of water cycling is related to the activity of membrane transporters, and the relaxometric readout then becomes a biomarker for the metabolic state of the tumour cells, providing important information on tumour aggressiveness and grading,1 which is fundamental for deciding on therapeutic strategies and for monitoring treatment in real time2,3, as recently demonstrated on a glioblastoma model. Furthermore, the method is also proposed for tumour margin assessment and lymph node status in breast conserving surgery.
The second propose two different innovative contrast enhancement strategies exploiting different contrast agents: i) a clinical approved iron oxide particle (Ferumoxytol). The method is based on R1 measurements at low field (0.01–1 MHz) to assess the presence of Tumour associated Macrophages (TAM) in melanoma tumours. R1 at low magnetic fields appears highly dependent on the intra or extra cellular localization of the nanoparticles thus allowing an unambiguous TAM quantification; ii) alternative contrast agents displaying remarkable relaxation effects in the absence of paramagnetic metal ions. They contain poly-histidine chains (poly- His), whose imidazole groups generate 14N-quadrupolar-peaks that cause a relaxation enhancement of water protons at a frequency (1.38 MHz) that is readily detectable from the frequencies associated with endogenous proteins, peaks whose characteristics can provide additional information on the investigated tumour tissues as the pH. Both approaches could significantly improve clinical diagnosis, treatment selection, and therapy monitoring in oncology. Although FFC relaxometry currently lacks spatial resolution, the knowledge gained from these studies open the way for the development of next-generation diagnostic tools and may accelerate the broader adoption of FFC-MRI scanners worldwide.

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Beyond Single-Field NMR: Insights into Dairy Structure and Function from Fast Field-Cycling Relaxometry

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Keywords: low field NMR, instrumentation

Dairy products are complex multi-phase systems in which water, proteins, fats and minerals continuously interact to determine texture, stability, and product quality. Understanding these interactions is essential for product development, authenticity assessment and quality control.

Fast Field-Cycling Nuclear Magnetic Resonance (FFC-NMR) relaxometry offers a unique way to investigate these systems by measuring spin-lattice relaxation over a wide range of magnetic fields and generating Nuclear Magnetic Resonance Dispersion (NMRD) profiles. Unlike conventional high-field NMR, which typically probes relaxation at a single magnetic field strength, FFC-NMR follows relaxation behaviour across several decades of Larmor frequency.

In this work, we demonstrate the use of FFC-NMR for the characterization of a range of dairy products, including different cheese varieties, fermented dairy products, and traditional and plant-based dairy matrices. The resulting NMRD profiles reveal differences in protein hydration, water mobility, and structural organization. These non-destructive measurements require minimal sample preparation and provide information that complements both conventional low-field and high-field NMR methods.

Recent advances in FFC instrumentation, reliable automated field cycling, precise temperature control, and integrated data analysis, have significantly broadened the practical use of relaxation dispersion measurements. With the help of these dairy products and recent methodological developments, this contribution shows how FFC-NMR provides insights beyond conventional single-field NMR experiments. While the focus is on dairy systems, the technique is valuable in food authentication, shelf-life studies, edible oils, biomaterials, and biomedical research, establishing FFC-NMR as a versatile complementary tool for the characterization of complex materials and food systems [1].

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Unlocking New Capabilities in Benchtop NMR: High-Resolution Analysis at 100 MHz

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Keywords:):
solution NMR, low field NMR, materials, small molecules, biomolecules, metabolomics, food, polymers, instrumentation.

The introduction of the Spinsolve 100 expands the capabilities of benchtop NMR, delivering enhanced spectral resolution and analytical performance in a compact instrument.

A key advantage of modern benchtop NMR is the ability to perform "no-prep" NMR directly on samples from the laboratory workflow. Because the instrument does not require deuterium for locking, samples can be analyzed in their native reaction solvents without solvent exchange. Combined with highly effective, push-button solvent suppression, this enables rapid acquisition of informative spectra from crude reaction mixtures with minimal user intervention.

This presentation will showcase how Spinsolve benchtop NMR spectrometers enable direct analysis of complex samples and supports applications ranging from reaction monitoring to materials and industrial research. Through selected examples, we will demonstrate how rapid access to high-quality structural and compositional information can simplify workflows, increase analytical throughput, and bring NMR closer to the point of need.

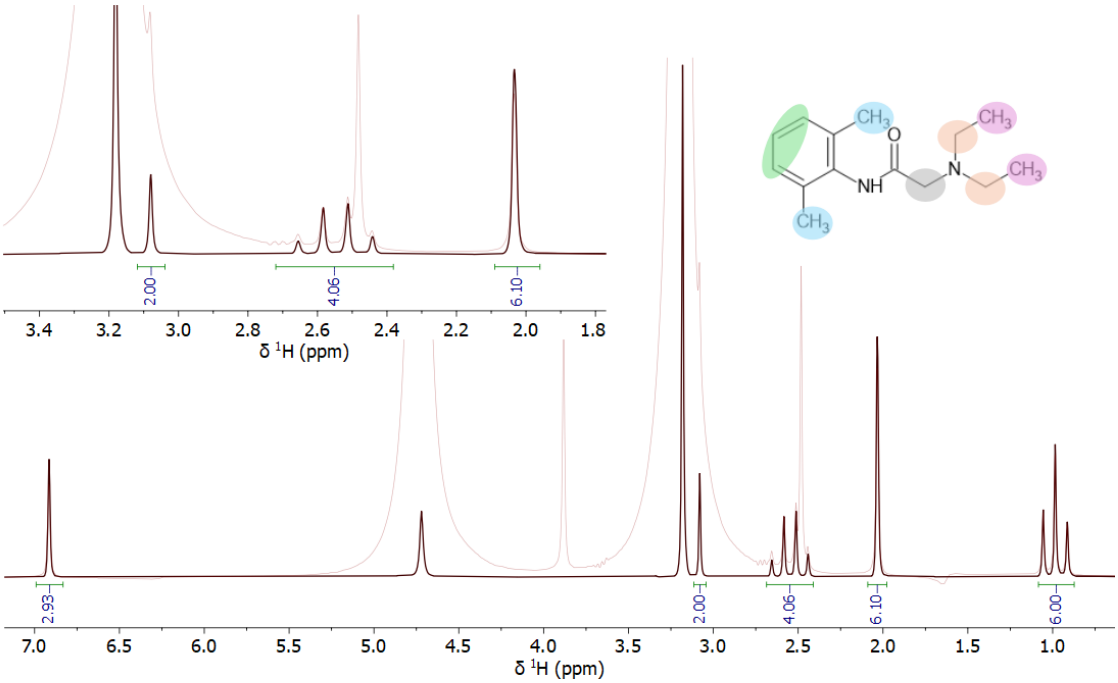

Fig. 1. Spectra of Lidocaine (50 mMolar) in protonated methanol. The spectrum was acquired in one minute using a WET solvent suppression sequence targeting the two methanol signals. This highly selective suppression enables the lidocaine peak at 3.1 ppm—which fully overlaps with methanol in a standard 1D spectrum (light red)—to be baseline resolved and accurately integrated.

ONCOGENIC NON-CODING RNA TARGETED BY SMALL MOLECULES: A NMR-BASED APPROACH

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Keywords:
solution NMR, small molecules, biomolecules

NMR is a powerful tool for drug discovery against RNA targets, as it can characterize ligand-RNA interactions in solution at the atomic level without requiring crystallization of highly flexible RNA targets.
Human miR-21 is a non-coding RNA playing a key role in tumour progression and resistance to anticancer therapies [1]. The final step in miR-21 maturation occurs in the cellular cytosol, where the RNase III enzyme DICER processes the precursor stem-loop RNA namely pre-miR-21 [2],[3]. Inhibition of pre-miR-21 maturation represents a promising anticancer strategy. However, to date, no drug capable of interfering with miR-21 biogenesis through binding to pre-miR-21 has been approved for treating human tumours, making this area a major focus of medicinal chemistry research.
Here, the integrated use of native mass spectrometry and NMR spectroscopy, complemented by biochemical and cellular assays, enabled the screening of a library of bicyclic aromatic compounds against pre-miR-21 and led to the identification of a hit molecule with promising binding profile and biological activity. To further validate our hit small molecule, advancing knowledge on its binding mechanism, we then applied both ligand-observed and RNA-observed NMR techniques. ^{1}H line-broadening and chemical shift perturbations analysis together with WaterLOGSY experiments enabled ligand epitope mapping, while RNA-observed NMR approaches identified the binding site on pre-miR-21. One-dimensional NMR experiments monitoring the imino protons of guanine and uracil bases in the stem region, combined with TOCSY and ^{1}H-^{13}C HSQC experiments targeting the rest of aromatic protons of pre-miR-21, revealed that the ligand binds to the upper stem region, located between the apical loop and the canonical stem of RNA construct. Intermolecular NOEs detected in NOESY spectra further defined the binding interface, revealing specific contacts between aromatic ligand protons and nucleobases within the binding site. Notably, this region largely overlaps with the DICER recognition site, providing a structural basis for the experimentally observed inhibition of DICER-mediated processing and miR-21 maturation. Remarkably, several NOE cross-peaks between nucleobase proton pairs were observed exclusively in the ligand-bound state and were absent in the apo form. These findings suggest that ligand binding stabilizes a distinct conformational state of pre-miR-21 characterized by increased structural compactness within the binding region. This previously unrecognized RNA conformation may provide a valuable framework for the structure-based design of novel inhibitors targeting DICER-mediated maturation of miR-21. Ongoing Molecular Dynamics (MD) simulations, guided by NMR-derived restraints, are currently being performed to generate an atomic-scale model of the complex between pre-miR-21 and the selected ligand.

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SOLID STATE NMR TOOLS IN THE EVALUATION OF WEAK INTERACTIONS

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

Weak intermolecular interactions are the fundamental driving forces governing the structure, dynamics, stability, and functional properties of molecular solids. Hydrogen bonds, π–π stacking, and the increasingly important family of σ-hole interactions (halogen, chalcogen, pnictogen, tetrel, osme, and matere bonds) dictate supramolecular organization in systems ranging from pharmaceutical multicomponent crystals to functional materials and host–guest assemblies. In this context, solid-state NMR spectroscopy has emerged as one of the most powerful approaches for probing weak interactions at the atomic scale, thanks to its unique sensitivity to local electronic environments and its applicability to both crystalline and disordered solids.[1] This lecture will present recent advances in the use of multinuclear and multiparametric SSNMR for the characterization of weak interactions in molecular crystals, with particular emphasis on developments achieved through the integration of high-field instrumentation, fast and ultrafast MAS, advanced multidimensional pulse sequences, and computational approaches. The discussion will focus on how SSNMR observables can be exploited to detect, classify, and quantitatively characterize weak intermolecular contacts, determine proton locations, and elucidate hydrogen-bond geometries. Selected examples will illustrate the role of SSNMR in modern crystal engineering and pharmaceutical sciences, including investigations of hydrogen-bonded molecular salts and cocrystals, salt–cocrystal continua, and tautomeric and zwitterionic equilibria.[2-4] Overall, the lecture will show how SSNMR is evolving from a primarily diagnostic technique into a predictive and design-oriented tool for supramolecular chemistry, pharmaceutical materials, and crystal engineering.

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RATIONAL OPTIMIZATION OF [1-^{13}C]PYRUVATE SABRE HYPERPOLARIZATION THROUGH CATALYST DESIGN, KINETIC MODELLING, AND TEMPERATURE CONTROL

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Keywords:
hyperpolarization, biomolecules, metabolomics, contrast agents.

Hyperpolarized [1-^{13}C]pyruvate is a key molecular probe for metabolic nuclear magnetic resonance (NMR). Parahydrogen based hyperpolarization methods have gathered considerable interest for enhancing NMR signals.[1-4] Signal Amplification by Reversible Exchange (SABRE) is a particularly attractive polarization method.[3] Efficient ^{1}[1-^{13}C]pyruvate signal amplification by SABRE remains challenging, being governed by catalyst structure, substrate binding, exchange kinetics, relaxation, temperature, and parahydrogen enrichment.[5]
Here, we report an integrated synthetic, experimental, and theoretical investigation of seven Ir–NHC catalysts for SABRE-SHEATH hyperpolarization of [1-^{13}C]pyruvate.[6] The benchmark IMes catalyst afforded approximately 3% ^{13}C polarization using 50% parahydrogen, corresponding to an extrapolated polarization of about 10% at full parahydrogen enrichment. The structurally distinct IPr and SIPr catalysts reached performances within approximately 20% of IMes and enabled single-scan detection of natural-abundance ^{13}C pyruvate on a 1.4 T benchtop NMR system.
Variable-temperature experiments showed that catalyst performance is primarily controlled by exchange dynamics. To rationalize the observed trends, we developed an efficient mechanistic spin-dynamics model that incorporates species concentrations, parahydrogen fraction, exchange kinetics, relaxation, and experimental control parameters.[6] By exploiting molecular symmetry to reduce Liouville-space dimensionality, the model provides a tractable and predictive framework for SABRE optimization. Guided by this analysis, we implemented a post-polarization temperature-jump protocol that selectively enhanced the free pyruvate signal by approximately 30% while reducing signals from catalyst-bound species. Overall, this work establishes a rational framework for catalyst and protocol optimization in biomedical hyperpolarized magnetic resonance.

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Structural investigation of Braun protein transpeptidation inhibitors in Gram-negative bacteria

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Keywords: solution NMR, small molecules, biomolecules

The project investigates the structure and function of L,D-transpeptidases which catalyze the attachment of Braun's lipoprotein (Lpp) to the peptidoglycan in *E. coli* [1]. As Lpp forms the only covalent link between the peptidoglycan and the outer membrane, disrupting this interaction compromise outer membrane stability [2] (**Fig.1**). Understanding the molecular basis of LDT-mediated Lpp attachment may support the development of novel inhibitors that weaken the outer membrane integrity and improve the effectiveness of existing antibiotics.
This work focuses first on the characterization of L,D-transpeptidase inhibition by existing antibiotics and novel compounds targeting the enzyme's active site. LdtB inhibition was assessed by mass spectrometry and solution NMR spectroscopy with carbapenems, cephems, and novel inhibitors such as N-thio-beta-lactams. Together these data indicate that these compounds bind covalently to the catalytic site. The investigation of the interaction mode of LdtB with the Braun protein using NMR and structural predictions suggest the existence of additional binding interfaces, besides the catalytic site, that could be targeted by next-generation antimicrobials.

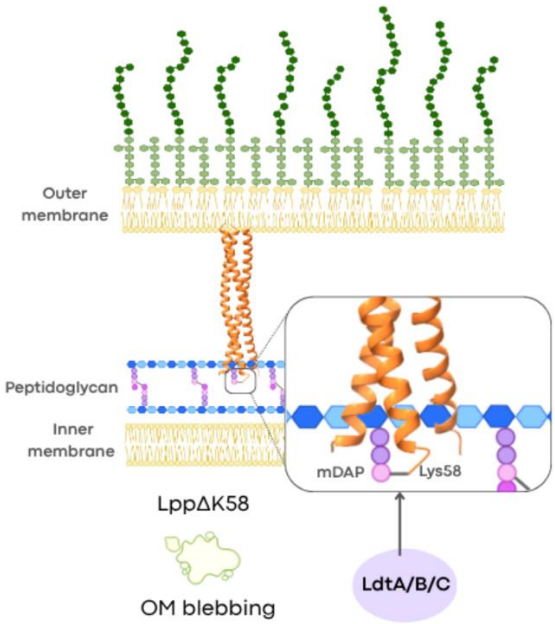

Fig. 1. Schematic representation of Gram-negative cell wall with peptidoglycan-bound Lpp.

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RNA MIMIC IN HuR-LIGAND STUDIES: INSIGHTS FROM STD-NMR AND PROTEIN-BASED NMR TECHNIQUES.

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Keywords: solution NMR, small molecules, biomolecules

HuR is a member of the Hu/ELAV family of RNA-binding proteins involved in the regulation of mRNA stability and translation, and its dysregulation has been associated with several cancers, making it an attractive therapeutic target [1,2]
Previous studies identified small drug-like molecules capable of disrupting HuR–RNA complexes through a combination of approaches, including in silico studies, STD-NMR, virtual screening, and fragment-based drug discovery strategies.[3]
The aim of the present study was to investigate whether the most promising compounds of the series of the BOPC family [3], A-BOPC3 effectively targets the RNA-binding interface of HuR and to evaluate the suitability of Peptide Nucleic Acids (PNAs) as RNA mimics for characterizing these interactions. (Figure 1) To this end, a PNA decamer was synthesized to mimic the natural RNA target of HuR based on the crystal structure of the complex (PDB 4ED5).
Protein-based NMR experiments using ^{15}N-labeled HuR and ^{15}N-HSQC titrations with RNA and PNA decamers enabled residue-specific monitoring of chemical shift perturbations.
The comparative analysis of the HSQC perturbation patterns induced by RNA and PNA demonstrated that the PNA decamer engages a similar binding interface on HuR, thus validating its use as a structural and functional mimic of the natural RNA target.
Subsequently, the interaction of A-BOPC3 was investigated using the same NMR approach. The resulting chemical shift perturbations revealed that the small molecule targets some residues involved in RNA recognition.
In parallel, Saturation Transfer Difference (STD) NMR experiments were performed on racemic and enantiomerically pure compounds to characterize ligand-binding epitopes and to carry out competition studies in the presence of the PNA decamer. [4] STD and competition experiments demonstrated that the two enantiomers, *(R,R)* and *(S,S)*, bind HuR but with distinct binding epitopes. Moreover, their displacement upon addition of the PNA decamer differed significantly: *(S,S)* was more effectively displaced, indicating a lower binding affinity, whereas *(R,R)* showed reduced displacement, consistent with a stronger and more stable interaction with the target site.

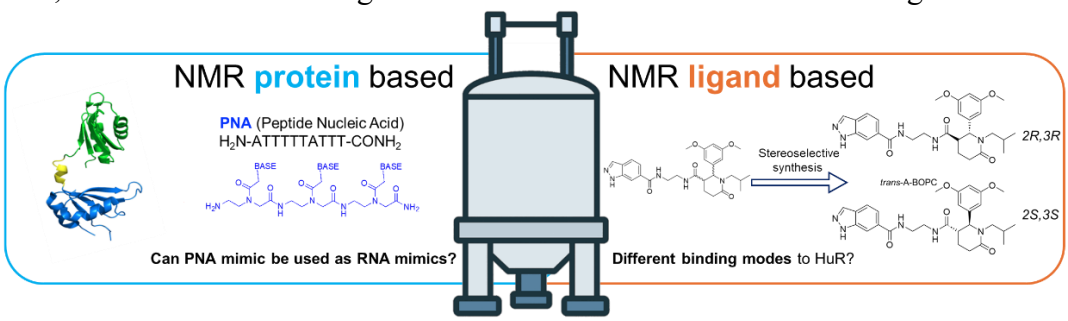

Fig. 1. Scheme of the strategy used to study PNA and ABOPC3 binding to HuR.

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EXPLORING ^{1}H-NMR DATA EXTRACTION POTENTIAL IN METABOLOMICS: UNVEILING AN OPIOID SIGNATURE

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Keywords: solution NMR, small molecules, metabolomics.

New Psychoactive Substances (NPS) are a diverse group of drugs of abuse designed to mimic traditional illicit drugs [1,2]. Opioids are the most widespread and lethal class: over 60 million people use them non-medically each year, driving a severe global overdose crisis. Their continuous emergence challenges conventional toxicological methods, which rely on prior structural knowledge and are easily evaded by novel chemical modifications. To address this, we introduce a metabolomics workflow that maximizes the information extracted from ^{1}H-NMR spectra, supported by mass spectrometry data. Using this approach, we investigate the systemic response to opioids, aiming to uncover a common metabolic signature that transcends specific chemical structures.
We evaluated the urinary metabolic profiles of CD-1 mice given equi-effective doses of four structurally diverse opioids: morphine, brorphine, etonitazene, and fentanyl. The ^{1}H-NMR spectra were processed by both intelligent bucketing and classical targeted profiling, the latter enhanced by mass spectrometry data via the SYNHMET method [3]. To test the hypothesis of a common signature that transcends chemical structure, we assessed whether the urinary metabolome carries enough information to predict opioid intake on the basis of this shared, class-wide metabolic response. We used a leave-one-out validation strategy: a model trained on the metabolic effects of three opioids predicted administration of the fourth, left-out opioid. Repeating this for each opioid tests whether the perturbation caused by one can be recognized from those of the others, indicating a class-wide signature rather than compound-specific effects.
Despite limitations (animal model, dose-dependence, circadian rhythms, unconfirmed class-specificity), this study establishes a robust NMR-based metabolomics methodology that maximizes spectral data extraction and accuracy, paving the way for a complementary tool to reduce reliance on structure-specific analyses for emerging substances.

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MODULATING HEPARANASE ACTIVITY WITH HEPARAN SULFATE MIMETICS: INSIGHTS FROM NMR AND MOLECULAR DYNAMICS INTO STRUCTURE–FUNCTION RELATIONSHIPS

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Keywords: solution NMR, biomolecules, polymers.

Heparanase (HPSE) is an endo-β-D-glucuronidase that cleaves heparan sulfate (HS) chains, contributing to the remodeling of the extracellular matrix [1]. Dysregulation of HPSE activity is implicated in several pathological conditions, including cancer and inflammation, where its overexpression enhances the release of growth factors and promotes angiogenesis [2]. Consequently, HPSE represents a therapeutic target for limiting tumor progression and metastasis.
Heparin inhibits HPSE due to its structural similarity to HS; however, its anticoagulant activity and susceptibility to enzymatic degradation restrict its clinical applicability in oncology. In contrast, chemically modified heparins, particularly glycol-split derivatives with reduced anticoagulant properties, have emerged over the past decade as promising HPSE inhibitors [3,4].
The molecular basis of HPSE recognition by glycol-split heparins was investigated using two synthetic hexasaccharides that differ only in the C5 configuration of the oxidized glycol-split uronic acid residue at the center of the oligosaccharide chain. Enzymatic assays revealed a marked difference in inhibitory potency, with one epimer exhibiting significantly stronger activity. To elucidate these differences, ligand-based NMR spectroscopy was combined with docking and molecular dynamics simulations. Both hexasaccharides bind within the catalytic cleft of HPSE, in proximity to glutamate residues E225 and E343, which are essential for the hydrolytic mechanism. Binding is primarily driven by electrostatic interactions with arginine- and lysine-rich regions of the active site. The glycol-split residue emerges as a key determinant of binding, while the surrounding saccharide units differentially modulate the stability and dynamics of the two complexes.
Overall, these findings define structural features governing HPSE recognition by glycol-split heparins and provide mechanistic insights that may support the rational design of more potent and selective HPSE inhibitors.

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SINGLE-SIDED NMR: STRENGTHS AND WEAKNESSES

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Keywords: low field NMR, single-sided NMR, non-invasive measurements, physicochemical properties.

Portable nuclear magnetic resonance (single-sided NMR) has emerged as a powerful tool for the non-invasive investigation of complex materials [1], particularly in cultural heritage [2,3]. Its main advantages include the ability to perform in situ measurements without sampling, preserving the integrity of valuable objects, and obtaining depth-resolved information on subsurface layers. Moreover, portable NMR enables the characterization of key physicochemical properties, such as moisture content, porosity, and fluid dynamics [4,5], making it highly suitable for monitoring processes and evaluating treatments [6].
However, this technique is affected by several intrinsic limitations related to its low-field configuration. The use of permanent magnets produces highly inhomogeneous magnetic fields, significantly limiting spectral resolution and preventing detailed chemical characterization, thus confining most applications to relaxometry-based approaches. In addition, the inherently low sensitivity of NMR, further reduced at low magnetic fields, together with the limited penetration depth, typically on the millimeter scale, constrains both signal detectability and the accessible sample volume. Data interpretation may also be challenging, as it often relies on indirect parameters and model-based analyses, especially in heterogeneous materials.
Approximately three decades after the introduction of portable NMR and its widespread application across diverse fields, a critical reassessment of its strengths and limitations is warranted, and this topic will be addressed in this talk.

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ACCESSING DYNAMICS OF SBR COMPOUNDS BY VARIABLE TEMPERATURE ^{1}H FC NMR AND DFMSE EXPERIMENTS

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

Polymers exhibit hierarchical dynamics over a wide range of time scales, from local segmental motions to slow cooperative processes [1]. ^{1}H Field Cycling (FC) NMR relaxometry is a powerful tool to investigate polymer dynamics around the glass transition by probing spin–lattice relaxation over several decades of Larmor frequency [2-4]. Here, ^{1}H FC NMR relaxometry is applied to study the effect of two tackifying resins, one petroleum-based and one of natural origin, on the segmental dynamics of styrene-butadiene rubber (SBR) in compounds of technological relevance for the tire industry. Uncured and vulcanized samples with different resin contents are investigated over a broad temperature range, enabling the construction of master curves spanning nine frequency decades (Figure 1a), from which segmental correlation times are determined and consistently described by Vogel–Fulcher–Tammann (VFT) behavior. FC NMR results are further correlated with low-field ^{1}H time-domain NMR measurements based on an improved Dipolar-Filtered Magic Sandwich Echo (DFMSE) approach [5] (Figure 1b), providing insight into the onset of the glass transition in the elastomer associated with cooperative segmental α-motions. The combined use of FC relaxometry and DFMSE experiments offers a comprehensive view of the dynamic heterogeneity of SBR/resin systems highlighting the different effects of resins of different origin and supporting the design of more sustainable elastomeric formulations.

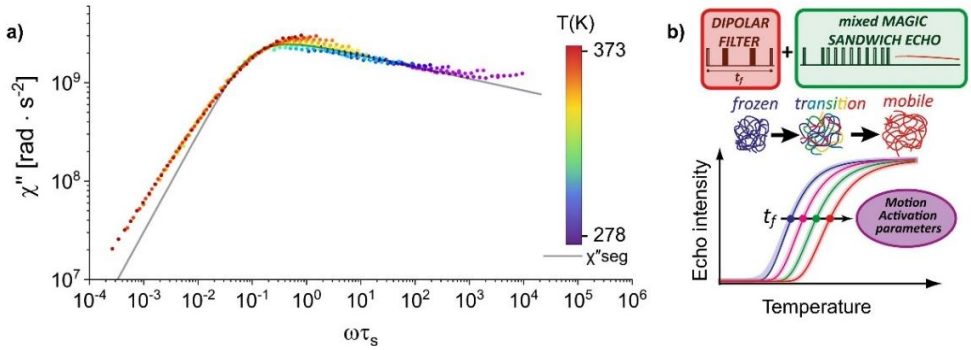

Fig. 1. a) SBR ^{1}H FC NMR master curves obtained at extended temperature range. b) Dipolar-filtered MSE pulse sequence.

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In-Situ Single-Sided NMR Profiling to Assess Cleaning Treatments on XI-Century Wall Paintings

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Keywords: low field NMR

The inner ceiling of the Collegiate Church of St. Pietro and Orso in Aosta houses a series of Ottonian wall paintings dating back to the XI century. In the years 1967 and 1968, the paintings were subjected to a restoration effort involving the application of a mixture of hydrophobic coatings which, by reducing the paintings' permeability, hindered their conservation. Our work, conducted in the context of a further restoration project, aimed to evaluate the permeability of the wall paintings before and after the removal of the hydrophobic coatings. The process involved the use of two different innovative electrospun tissues, based respectively on pullulan and polyamide 6, as carriers for the methyl ethyl ketone (MEK) cleaning agent. NMR measurements were performed using the NMR-MOUSE PM10 (Magritek, NZ; polarizing field B_0 = 0.327 T) single-sided device mounted on a micrometric-precision lift. The instrument allows for the implementation of the so-called profiling procedure [1], where pulse sequences are applied at different depths throughout the studied surface. The analysis focused on eight spots (outlined by dashed white line rectangles in Fig. 1 Left) of the wall paintings, chosen to account for possible effects on the cleaning process due to the different composition of each pigment and uneven coating distribution. Measurements were conducted in-situ, both before and after cleaning, by moistening the surfaces with water using a spray dispenser and then acquiring three CPMG profiles. The first profile, acquired immediately after moistening, with lift step size of 500 µm, was used to check the entire depth range to establish the boundaries for the two successive profiles, acquired respectively 20 and 40 minutes after moistening, with a step size of 200 µm. The acquired signal was then filtered following the acquisition of noise profiles and by weighted mobile averaging. By comparing the profiles, alongside their cumulative counterparts (Fig.1 Right), acquired before and after treatment of the wall paintings, it was possible to determine the efficacy of both carrier tissues. Specifically, the analysis showed how treatment influenced both the amount of absorbed water and its dynamics. Differences in performance between the two cleaning tests were also detected, thus aiding restorers in selecting the most appropriate MEK delivery tissue.

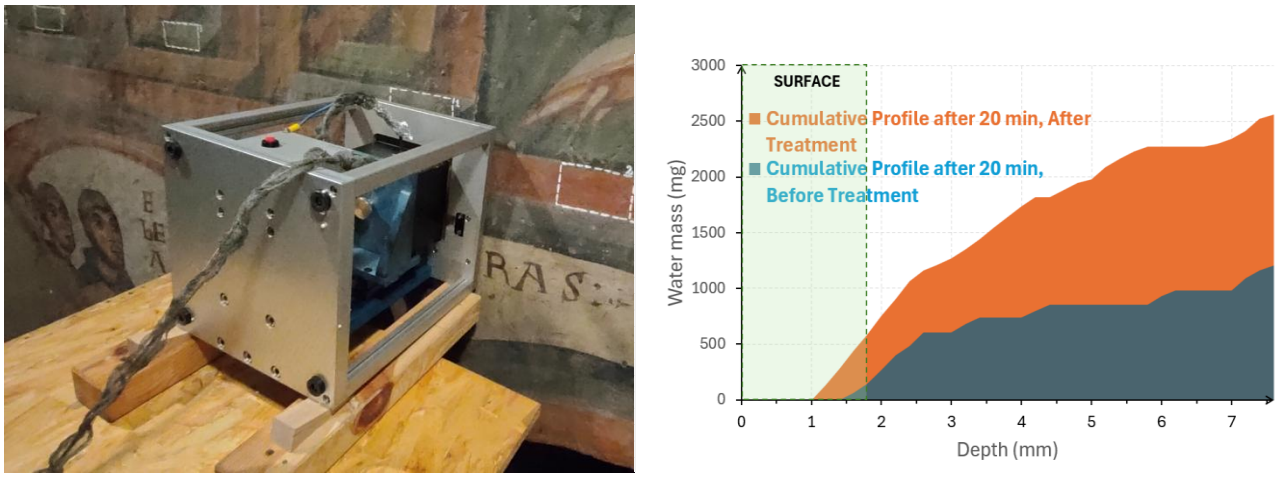

Fig. 1. Left: Experimental setup, the NMR-MOUSE is placed on a purposefully built structure to position its sensitive volume inside the wall paintings' surface. Right: Cumulative profiles (signal converted to water mass) acquired before and after treatment of a representative wall painting spot.

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Reactive Adduct Intermediates and Solvent-Cage Effects in Azo-Initiated Oxidation of Cinnamic Acid Derivatives Revealed by Solution NMR Spectroscopy

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Keywords: solution NMR, small molecules, theory and methods

Radical-mediated oxidation is central to processes spanning oxidative stress in biological systems, cellular redox imbalance, and antioxidant evaluation. Among the most widely used model systems, azo initiators such as 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) are generally assumed to generate a radical flux that directly follows initiator decomposition[1], while antioxidant activity is interpreted as a simple radical-scavenging event. Yet the molecular steps linking radical generation to substrate oxidation remain poorly resolved, particularly for bioactive natural products.[2]

Here, we combine quantitative solution-state NMR spectroscopy with LC–MS to establish a mechanistic framework for AAPH-induced oxidation of rosmarinic acid, a plant-derived natural product with reported antioxidant and neuroprotective activities.[3] Time-resolved measurements reveal that classical single-step scavenging models fail to reproduce the observed concentration-dependent kinetics. Instead, oxidation proceeds through the accumulation of multiple covalent radical adducts that remain kinetically active and participate in subsequent reaction pathways.

Using complementary NMR, high-resolution mass spectrometry, and LC–MS/MS, we directly characterize these transient species and identify imidazolidine-type heterocyclic adducts arising from radical addition to the α,β-unsaturated side chain.

By globally analyzing initiator decomposition, substrate consumption, and intermediate evolution, we directly determine the solvent-cage escape efficiency of AAPH-derived radicals ($f \approx 0.1$), demonstrating that the effective radical flux is strongly decoupled from the intrinsic rate of initiator thermolysis. These results establish AAPH-mediated oxidation of cinnamic acid derivatives as a flux-controlled, multistep radical process governed by solvent-cage dynamics and intermediate reactivity. More broadly, this work provides a quantitative framework for understanding the oxidative fate of natural products in biologically relevant environments, with implications for antioxidant assays, oxidative stress models, and screening platforms used at the chemistry–biology interface.

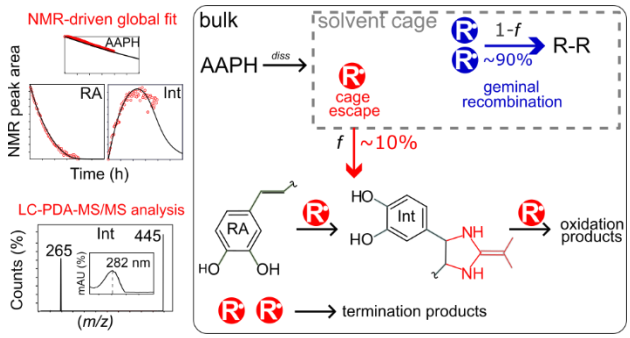

Fig. 1. Solvent-cage effects regulate the effective radical flux of AAPH, while reactive +84 Da adducts act as transient intermediates during oxidation of cinnamic acid derivatives.

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METABOLOMIC INSIGHTS INTO THE PROTECTIVE MECHANISMS OF THE NGF MUTANT CHF6467 IN AN ALZHEIMER'S DISEASE CELL MODEL

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Nerve Growth Factor (NGF) is a key neurotrophic factor that supports neuronal survival, plasticity, and growth by binding to two receptors: the low-affinity p75 neurotrophin receptor (p75NTR) and the high-affinity tropomyosin receptor kinase A (TrkA). Extensive evidence highlights a significant connection between NGF and the cholinergic system in the hippocampus. Additionally, changes in the brain's NGF metabolic pathway have been observed in Alzheimer's disease (AD). Building on these premises, research has thoroughly explored NGF's neuroprotective and regenerative effects on cholinergic neurons, resulting in positive findings in animal models of AD and clinical studies. [1, 2]
Despite these advantages, NGF therapy encounters two significant obstacles: its limited ability to cross the blood–brain barrier (BBB) and the potential for dose-dependent pain as a side effect. [3]
Regarding the second issue, a mutated form of NGF, CHF6467, has been developed. This variant carries two mutations compared to the wild-type NGF: P61S and R100E. Specifically, the R100E mutation selectively prevents NGF from binding to the p75NTR receptor, while preserving its affinity for TrkA. This modification enhances NGF's tolerability by decreasing pain-related side effects. [4]
Considering NGF's known neuroprotective role and the promising therapeutic potential of its mutant, CHF6467, understanding its mechanism of action (MoA) remains a major challenge in medicinal chemistry. Metabolomics, particularly in its untargeted form, is a highly effective approach for uncovering unforeseen biological responses. [5] Consequently, we consider this technique very suitable for a comprehensive assessment of CHF6467's overall effects.
Building on our previous research and recognizing the importance of amyloid β peptide as a critical pathological marker of AD and its role as an insult in *in vitro* AD models, we used an NMR metabolomic approach to examine how CHF6467 affects SH-SY5Y cells exposed to Aβ(1-42) peptide. [6-8]
Our study focused on examining the metabolomic profile of SH-SY5Y cells treated with homemade CHF6467, with and without the Aβ(1-42) peptide, using NMR spectroscopy. The analysis of intracellular and extracellular metabolites indicates that CHF6467 can influence the metabolic changes caused by Aβ(1-42). It promotes the production of vital intermediates for phospholipid synthesis, decreases glutamate-driven excitotoxicity, and helps restore balanced energy metabolism.

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NMR-BASED CHARACTERIZATION FOR THE DESIGN, STABILITY STUDIES, AND DELIVERY CONTROL OF BIOPHARMACEUTICALS

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Keywords: solid state NMR, biopharmaceuticals. drug design

The characterization of biopharmaceuticals, such as monoclonal antibodies, protein-drug conjugates, and theranostic platforms, represents a critical bottleneck in modern drug development. Our studies on different protein therapeutics and pharmaceutical targets demonstrate that solution and solid-state nuclear magnetic resonance (NMR) spectroscopy, used either standalone or integrated with other biophysical techniques, can effectively tackle these challenges [1-3]. By providing high-resolution data on higher-order structures, mapping complex binding interfaces, and evaluating therapeutic stability and drug delivery, NMR spectroscopy can pave the way to guarantee the efficacy, safety, and stability of next-generation biologics.

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A ^{19}F NMR STRUCTURAL CHARACTERIZATION OF SELUMETINIB AND TRAMETINIB β-CYCLODEXTRIN INCLUSION COMPLEXES

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Keywords: solution NMR, small molecules

Molecular and polymeric Cyclodextrins (CyDs) are widely used supramolecular hosts able to improve stability and solubility of small molecules through the formation of non-covalent inclusion complexes. Among them, β-cyclodextrin (β-CyD) have garnered significant attention in pharmaceutical applications due to their ability to form complexes with a variety of drugs and to their low toxicity[1].
Recently we analyzed the formation of inclusion complexes of the small molecules trametinib and selumetinib with two positively charged CyD polymers. These two agents are widely employed in the treatment of malignancies such as melanoma; however, their low solubility restricts their use in glioblastoma. We demonstrated that interaction with positively charged β-CyD significantly inhibits cell proliferation and ERK phosphorylation in glioblastoma cell lines enhancing the anticancer effects of these two MEK inhibitors[2].
Here, we report the study of the interaction of β-CD with selumetinib and trametinib exploiting ^{19}F NMR titration experiments. Changes in both proton and fluorine chemical shifts were followed, as indication of the changes in the magnetic environment associated with inclusion inside the cyclodextrin cavity highlighting the potential of ^{19}F NMR spectroscopy as an effective tool for studying cyclodextrin-based supramolecular systems. Furthermore, activity assays on eukaryotic cells will also be reported, which highlight a better efficacy of the complexed systems, favoring their delivery into the cell while maintaining the integrity of the cell membranes.

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Structural and Dynamic Features of Diastereomeric (Deep) Eutectic Systems (DIADES)

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Keywords: solution NMR, small molecules

Chirality is a fundamental property of biological systems and of many chemically relevant compounds, which often occur in a single enantiomeric form. In recent years, Deep Eutectic Solvents (DES) have also been prepared from homochiral building blocks, many of which are readily accessible from the natural chiral pool. [1][2]
This work investigates a new and still largely unexplored class of low-melting liquids, called diastereomeric deep eutectic systems (DIADES), formed by pairs of enantiopure chiral components. The aim is to demonstrate that the four possible stereochemical combinations generate distinct liquid environments with predictable thermodynamic, structural, and dynamic properties.
A preliminary mapping is carried out adopting a multidisciplinary approach combining thermodynamics, conventional NMR [Fig. 1, 2] , infrared (IR) and Raman spectroscopy, X-ray scattering, molecular dynamics simulations, and pharmaceutical formulation science. Further information will be obtained from the dissolution of chiral solutes and the investigation of the interactions pattern depending on the absolute configuration of the components.
Overall, our results provide a rational framework for understanding solvation in these novel systems as a consequence of their stereochemistry, establishing a basis for the targeted design of enantioselective media for advanced chemical and pharmaceutical applications.

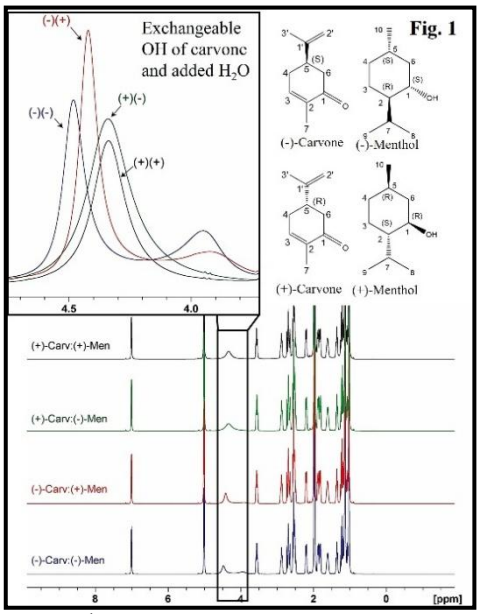

Fig 1. ^{1}H spectra of carvone:menthol.

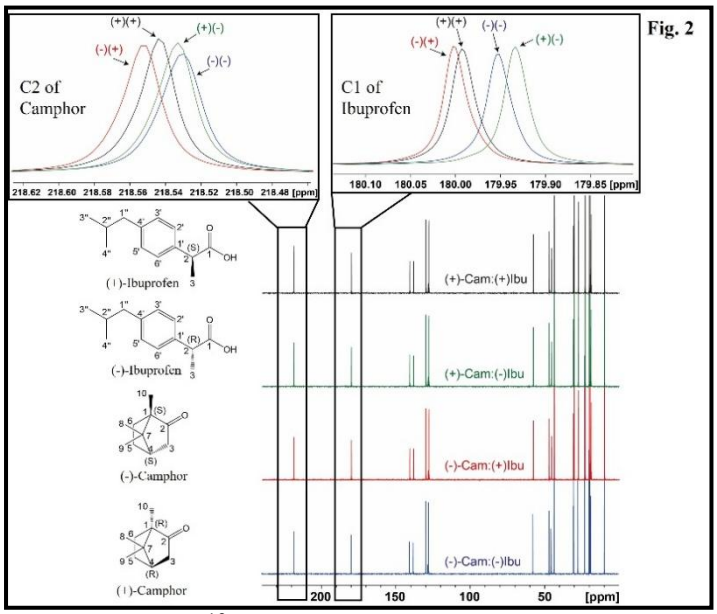

Fig. 2. ^{13}C spectra of camphor:ibuprofen.

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NMR Strategies for Multidomain Intrinsically Disordered Proteins: Application to the SARS-CoV-2 Nucleocapsid Reveals Structure, Dynamics, and Binding Mechanisms

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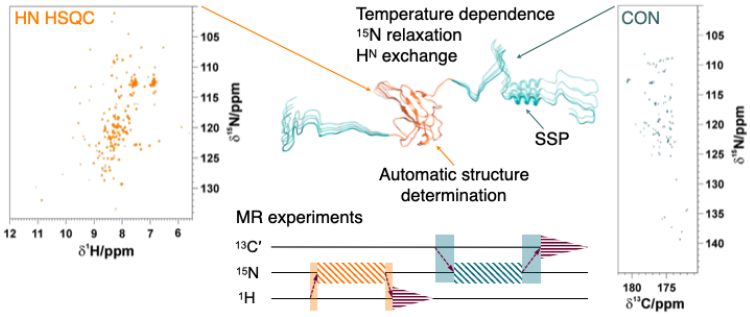
Keywords:

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

Intrinsically disordered proteins (IDPs) and multidomain systems pose significant challenges for structural biology due to their conformational heterogeneity, extensive dynamics, and spectral complexity. Solution NMR spectroscopy provides unique opportunities to investigate these systems at atomic resolution, but often requires dedicated methodological adaptations to overcome limitations associated with spectral overlap, exchange broadening, and the coexistence of ordered and disordered regions [1].

In this work, advanced NMR strategies were developed and implemented to address these challenges, with particular emphasis on ^{13}C direct-detected experiments and multiple-receiver acquisition schemes. These approaches were optimized to improve the characterization of disordered regions both in isolation and within larger multidomain architectures, enabling efficient resonance assignment and detailed structural and dynamical investigations.

The methodologies were applied to the SARS-CoV-2 nucleocapsid (N) protein, a paradigmatic multidomain system comprising folded domains interconnected by extensive intrinsically disordered regions. A progressive analysis ranging from the isolated N-terminal domain to larger constructs and the full-length protein provided residue-specific insights into how structural disorder modulates protein behavior across increasing levels of complexity [2].



This detailed structural and dynamical characterization established the basis for addressing biologically relevant questions related to molecular recognition. Building on this framework, NMR was employed to investigate interactions with heparin-derived ligands of increasing oligosaccharide length, illustrating how residue-specific information can be used to dissect recognition processes in structurally heterogeneous systems. These studies revealed a significant contribution of intrinsically disordered regions to ligand binding and highlighted the importance of investigating increasingly complex and native-like protein constructs to fully capture the molecular determinants of multivalent interactions [3].

Overall, this work demonstrates how tailored NMR methodologies can expand the accessible range of structurally heterogeneous biomolecular systems and provides a framework for investigating the interplay between structure, dynamics, and molecular recognition in complex proteins of biomedical relevance.

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PROBING STRUCTURE, DYNAMICS AND LIGHT-INDUCED EFFECTS IN HALIDE PEROVSKITES VIA SOLID STATE NMR AND NQR

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Keywords: solid state NMR, materials, instrumentation.

Metal halide perovskites continue to attract significant interest due to their remarkable optoelectronic properties and promising applications in a variety of fields, such as photovoltaics. However, their high compositional flexibility, intrinsic disorder, and relative instability pose major challenges for local characterization. The combination of solid-state NMR and Nuclear Quadrupole Resonance (NQR) is particularly effective in addressing these challenges. In this contribution, I will present insights into the structure and dynamic properties of halide perovskites obtained by combining multinuclear (^{1}H, ^{13}C, ^{207}Pb, ^{133}Cs) solid-state NMR and ($^{79/81}$Br, ^{127}I) NQR, also applied under in situ illumination conditions, to simulate solar irradiation in a photovoltaic cell [1, 2, 3].
Together, these results highlight the versatility of nuclear resonance spectroscopies in exploring structure, dynamics, and light-induced phenomena in halide perovskites.

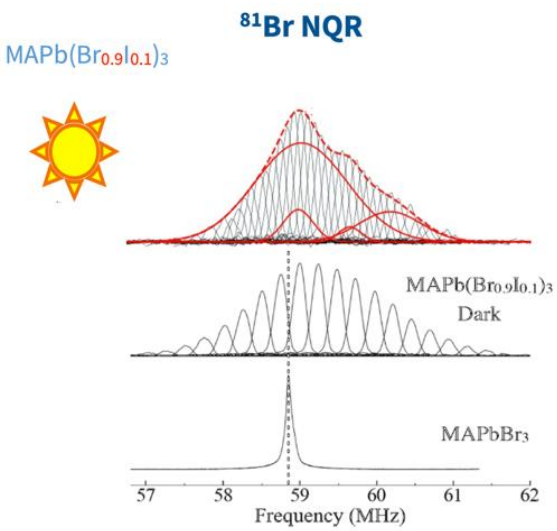

Fig. 1. ^{81}Br NQR spectra of $MAPbBr_3$ (bottom) and of the mixed halide perovskite $MAPb(Br_{0.9}I_{0.1})_3$ in the dark (middle) and under in situ illumination (top).

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SOLID-STATE NMR INSIGHTS INTO HYDRATION, FRAMEWORK FLEXIBILITY, AND GUEST DYNAMICS IN CALF-20

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

Metal-organic frameworks (MOFs) are a versatile class of porous materials with high surface areas and tunable pore architectures, making them attractive for CO_2 capture and separation [1]. However, their practical deployment is often limited by poor hydrothermal stability and H_2O competitive adsorption. CALF-20 (Calgary Framework-20, [Zn_2(1,2,4-triazolate)$_2$(oxalate)]) represents a notable exception, combining high CO_2 uptake under humid conditions with remarkable stability and demonstrated industrial scalability [2-4].
Despite extensive investigation, its hydration behavior remains debated, with discrepancies arising from differences in sample history, experimental conditions, and structural assignments of hydrated phases. Current understanding converges toward a reversible transition between an open-pore, weakly hydrated state and a contracted, water-stabilized phase at intermediate humidity, involving significant structural reorganization [3, 5, 6, 7].
In this work, we employ multinuclear solid-state NMR spectroscopy to gain atomic-level insight into the structural evolution of CALF-20 upon hydration and CO_2 adsorption. By monitoring changes in local environments under controlled hydration levels, we clarify the nature of the phase transition and its reversibility, as well as the role of framework-water interactions in stabilizing distinct hydration regimes.
A key aspect of this study is the investigation of guest dynamics in the CALF-20 framework. While previous studies have proposed possible CO_2 adsorption sites [2,3,5], experimental information on molecular dynamics remains scarce. Here, we analyze the temperature dependence of static ^{13}C lineshapes of adsorbed CO_2 in differently hydrated CALF-20 to probe its reorientational dynamics within the pores. In addition, water dynamics are explored by ^{2}H lineshape analysis of D_2O-loaded CALF-20 at different hydration levels and temperatures. Together, these measurements reveal a strong dependence of guest mobility on both temperature and hydration state, providing insight into the interplay between confined molecules and framework flexibility.

This study 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 "DAM4CO_2".

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^{14}N SOLID-STATE NMR QUADRUPOLAR PRODUCTS AS QUANTITATIVE PROBES OF HYDROGEN-BOND ENVIRONMENTS IN PYRIDINIC SOLIDS

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

Hydrogen bonds in molecular solids often span a continuum of interaction regimes, where subtle variations in proton position and hydrogen-bond geometry may lead to distinct solid forms and affect physicochemical properties [1]. Probing these fine structural differences can be experimentally challenging [2]. In this study, we explored the use of ^{14}N solid-state NMR as a sensitive probe of hydrogen-bond geometry, exploiting its quadrupolar nature and intrinsic sensitivity to local electronic environment. Since direct detection of ^{14}N is often impractical, we focused on indirect, ^{1}H-detected, ^{14}N experiments under fast magic-angle spinning [3]. In particular, we identified the ^{14}N quadrupolar product (P_Q) as an easily accessible parameter, extracted by combining ^{1}H–^{14}N T-HMQC spectra with ^{15}N CPMAS [4]. A series of crystalline systems containing pyridinic nitrogen atoms involved in different hydrogen bonds was investigated, spanning protonated, shared-proton, neutral, weakly hydrogen-bonded, and free nitrogen environments. Systematic variations of P_Q values were observed across the series, revealing a clear dependence on hydrogen-bond type. Moreover, independent ^{1}H–^{14}N distance measurements were performed using PM-S-RESPDOR [5] and selective overtone-based (OT-RESPDOR) [6] experiments. A strong linear correlation was found between P_Q values and the inverse third power of the experimentally determined N–H distances, providing direct experimental validation of the physical relationship between the quadrupolar interaction and hydrogen-bond geometry. These results establish ^{14}N quadrupolar products as robust and quantitative descriptors of hydrogen bond environments in solids, providing a valuable framework for NMR crystallography across the salt–cocrystal continuum.

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STRUCTURAL INSIGHTS INTO A NEW DUAL-DRUG CO-CRYSTAL CHARACTRISED BY NMR CRYSTALLOGRAPHY AND ELECTRON DIFFRACTION

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

Multi-component pharmaceutical solids such as co-crystals represent an effective strategy to tune the physicochemical properties of active pharmaceutical ingredients (APIs) while preserving their molecular integrity[1]. In this work, the formation of a new co-crystal between levetiracetam and R-flurbiprofen, whose structure is not known[2], was investigated through an NMR crystallography approach combining MAS solid-state NMR (ssNMR), Dynamic Nuclear Polarization (DNP)-enhanced ssNMR, powder X-ray diffraction (PXRD), and electron diffraction (ED). The co-crystal was obtained by solvent evaporation and liquid-assisted grinding. ^{13}C ssNMR and ^{1}H spin-lattice relaxation times (T_1) measurements enabled the identification of a new crystalline phase and provided insight into local molecular environments, intermolecular interactions, and the number of crystallographically inequivalent molecules in the unit cell. DNP-enhanced ssNMR allowed ^{13}C-^{13}C, ^{1}H-^{15}N and ^{13}C-^{15}N correlation experiments useful for the investigation of structural features associated with co-crystal formation. PXRD confirmed phase purity, while ED enabled preliminary structural determination. These results highlight the potential of ssNMR within an NMR crystallography framework for the structural characterization of pharmaceutical co-crystals.

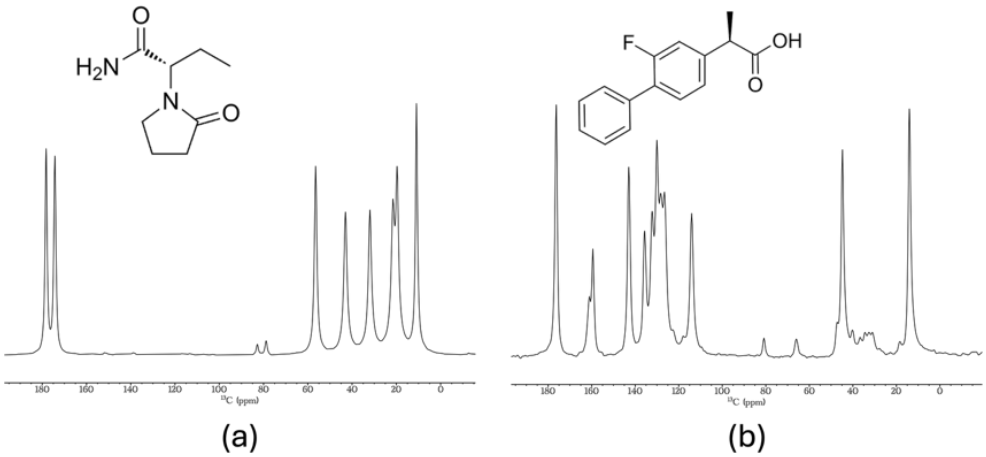

Fig. 1. ^{13}C MAS ssNMR spectra of (a) levetiracetam and (b) (R)-flurbiprofen

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POSTERS

EXPLORING THE MT1 BINDING MECHANISM BY MELATONIN USING A COMBINED NMR-BASED APPROACH

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Keywords: small molecules, biomolecules, solution NMR

Melatonin (MLT) regulates circadian rhythms through activation of MT1 and MT2 receptors and exerts several neuroprotective and antioxidant effects [1,2]. Despite recent structural studies of MT1 in complex with synthetic agonists, the molecular basis of melatonin recognition under near-physiological conditions remains poorly understood. Here, to investigate MT1-melatonin interactions, we combined high-resolution NMR experiments performed using isolated cellular membranes, overexpressing the human MT1 receptor, with homology modeling, molecular docking, and molecular dynamics simulations. Our results indicate that melatonin binds the orthosteric pocket mainly through hydrophobic interactions enhancing receptor dynamics and promoting conformational rearrangements in the intracellular region that are potentially associated with early activation events [3,4]. These findings provide new insights into the structural and dynamic determinants of melatonin recognition by MT1 and contribute to a better understanding of melatonergic signaling.

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NANOSCALE CLUSTERED SOLID SOLUTIONS: UNCOMMON MATERIALS IDENTIFIED BY SSNMR

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Keywords: solid state NMR, materials, small molecules

SSNMR is a useful tool in materials science, being able to provide precious insight into local structure and dynamics. In this case study, a series of KBH_4–$RbNH_2$ mixed-anion samples were investigated, with different stoichiometric BH_4^-/NH_2^- ratios. While ^1H T_1 relaxation times and 2D variable-temperature ^1H DQ/SQ spectra initially pointed to our samples being physical mixtures of the two parent compounds, 2D ^1H SQ/SQ spectra were fundamental to assess the existence of spin diffusion at the nanometer scale among all protons of the probed materials. These results, in agreement with Raman and X-ray diffraction evidence, indicate that the analyzed compositions do not form homogeneously mixed phases at the atomic scale; instead, they are found to be nanoscale clustered solid solutions, in which BH_4^- and NH_2^- remain crystallographically distinct while being intimately intergrown over nanometer length scales.

SOLID STATE NMR UNRAVELS CO_2 INTERACTIONS AND DYNAMICS IN TWO Zr-FUMARATE METAL-ORGANIC FRAMEWORKS AND THEIR MIXED-MATRIX MEMBRANES

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Keywords:
solid state NMR, materials.

Zr-based Metal–Organic Frameworks (MOFs), particularly those built from simple C_4 dicarboxylic ligands such as fumaric acid and its analogues, have attracted significant attention for gas separation applications due to their high hydrothermal stability, environmentally friendly synthesis routes, and excellent CO_2 adsorption properties.[1] Among the most widely studied members of this family are MOF-801,[2] with general formula $[Zr_6(\mu_3\text{-}O)_4(\mu_3\text{-}OH)_4(\text{fumarate})_6]$, and MIP-203,[3] with general formula $[Zr_6(\mu_3\text{-}O)_4(\mu_3\text{-}OH)_4(\text{fumarate})_4(\text{formate})_2(OH)_2(H_2O)_2]$, which exhibit different framework topologies and pore structures.

In this work, we present a comparative study of MOF-801 and MIP-203 aimed at understanding how structural differences influence the adsorption behavior, intermolecular interactions, and dynamics of CO_2 confined within the porous frameworks.[4] ^1H and ^{13}C solid-state NMR (SSNMR) 1D and 2D experiments, complemented by PXRD and gas sorption measurements, revealed different interactions and dynamic properties of CO_2 within the two distinct frameworks. In particular, interactions between CO_2 molecules and the MOF linkers were probed by ^1H–^{13}C CP MAS and HETCOR experiments, whereas CO_2 dynamics was characterized by variable temperature ^{13}C NMR static spectra.

To correlate molecular-scale behavior with macroscopic CO_2 capture and separation performance, both MOFs were incorporated as fillers into mixed-matrix membranes (MMMs) prepared with the intrinsic microporosity polymer PIM-1. The SSNMR results indicate that the distinctive CO_2 dynamics observed in the pristine MOFs are preserved within the MMMs.

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 "DAM4CO_2".

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NMR STRUCTURAL INVESTIGATION OF THE TPP1 OB DOMAIN: FROM LIGAND SCREENING TO BOUND-STATE CHARACTERIZATION

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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 [1]. Many cancer cells exploit this mechanism by employing the telomerase enzyme, assisted by the Shelterin protein TPP1, to elongate telomeric DNA strands [2,3], thereby evading cellular senescence and conferring immortality [4,5]. Inhibiting the telomerase–TPP1 complex represents an attractive yet underexplored strategy to induce senescence in cancer cells. Through a targeted virtual screening of the OB domain of TPP1, we identified two promising small-molecule ligands, whose interaction with TPP1 was confirmed by STD [6] and WaterLOGSY [7]. To obtain residue-specific information on the TPP1 OB domain in its free and bound forms, we acquired a complete set of heteronuclear NMR spectra, on uniformly $^{15}N/^{13}C$-labeled samples, for backbone assignment further complemented by relaxation experiments (T_1, T_2, and heteronuclear NOE) [8] and chemical shift-based secondary structure analysis (TALOS-N) [9]. To characterize the three-dimensional structure of the TPP1 OB domain in its ligand-bound state, intramolecular distance restraints derived from ^{13}C-NOESY-HSQC and ^{15}N-NOESY-HSQC [10] spectra were used, complemented by (^{13}C, ^{15}N)-double-filtered NOESY and ^{13}C-edited/^{13}C-filtered NOESY experiments [11] to probe the intermolecular environment and confirm the presence of protein–ligand contacts. Structure calculations performed using intra-protein restraints exclusively yielded a high-quality ensemble of the TPP1 OB domain in its bound conformation. This experimentally determined bound-state structure is currently being employed as input for molecular docking and molecular dynamics (MD) simulations, aimed at defining the binding pose of the ligand and rationalizing the key interactions driving complex formation at the atomic level. The pharmacological activity of the ligands was assessed through anti-proliferative, pro-senescence, and telomeric DNA shortening experiments. By integrating computational and experimental NMR methodologies, we aim to fully characterize the inhibitory potential of our compounds, underscoring TPP1 as a promising therapeutic target for future anticancer drug design campaigns.

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^{1}H NMR PROFILING OF HIGH-OLEIC SUNFLOWER OILS: CALCULATION STRATEGIES AND CHEMOMETRIC FINGERPRINTING

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

High-oleic sunflower oils are matrices of increasing interest in food technology due to their high Oleic Acid (OA) content and improved oxidative stability compared with conventional oils. The development of recently selected hybrid varieties with increasingly OA levels highlights the need for rapid methods for compositional screening and quality control, also in industrial contexts.
^{1}H NMR spectroscopy is a well-established tool for the rapid determination of fatty acid composition in edible and vegetable oils [1-3]. In this work, NMR spectroscopy was evaluated as an integrated platform for the analysis of high- oleic sunflower oils obtained from hybrid cultivars of *Helianthus annuus* L., grown in different areas of Calabria and at different altitudes. The approach combines targeted estimation of OA percentage, based on the integration of diagnostic acyl-chain signals, with chemometric analysis of ^{1}H NMR spectral data (PCA and, where applicable, PLS-DA), aimed at exploring compositional differences associated with cultivar, geographical area, extraction process and matrix quality. NMR results were compared with GC-MS/GC-FID data to assess the reliability of OA estimation and the robustness of the applied calculation strategies.
A central aspect of the work is the critical evaluation of the integration and calculation strategies used for OA and fatty acids (FAs) content estimation [1-3]. Signals associated with glycerol, acyl chains and free fatty acids (FFAs) were considered to verify whether the lipid composition and the degree of hydrolysis may affect the accuracy of compositional estimates [4]. Spectral markers related to the glycerol backbone and functional groups of acyl chains suggest that even small changes in the lipid matrix may affect the reliability of the applied calculation models. The results obtained show good agreement between ^{1}H NMR and GC, while also highlighting possible effects of the extraction process and the initial quality of the lipid matrix. Overall, this work supports the use of ^{1}H NMR as a rapid tool for the compositional screening of new varieties of Calabrian high-oleic sunflower oils, including quantification, lipid fingerprinting and critical evaluation of the real matrix.

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CAN URINARY METABOLOMICS DETECT MUSCLE-INVASIVE BLADDER CANCER? AN NMR AND LC-HRMS COMBINED METABOLOMIC APPROACH

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Bladder cancer (BC) diagnosis and staging, particularly the differentiation between non-muscle-invasive (NMIBC) and muscle-invasive bladder cancer (MIBC), currently rely on invasive procedures such as Transurethral Resection of Bladder Tumor (TURBT). TURBT presents inherent risks, including the potential to promote tumor cell dissemination into the bloodstream [1,2]. Existing imaging modalities lack the resolution to reliably discriminate NMIBC from MIBC prior to surgery, leaving a substantial unmet clinical need for non-invasive staging tools. To address this, a multi-platform urinary metabolomic approach combining 1H-NMR spectroscopy and LC-HRMS was developed to profile 180 urine samples (76 NMIBC, 48 MIBC, 56 controls).

To maximize quantitative accuracy, an integration strategy based on the SYNHMET method [3] was applied. Mass spectrometric data were utilized as an orthogonal analytical dimension to correct and validate NMR quantifications. This synergy mitigated intrinsic limitations such as NMR signal overlap and MS matrix effects. Subsequent deconvolution of the processed 1H-NMR spectra enabled the precise identification and absolute quantification of 43 urinary metabolites.

Multiple linear regression, adjusted for age, sex, and hospital of origin, identified seven metabolites (Lactate, Leucine, Pseudouridine, Pyruvate, Tryptophan, 3-Indoxylsulfate, Creatinine) that significantly differentiated MIBC from NMIBC. These alterations reflect interconnected pathophysiological mechanisms, including enhanced glycolytic flux, increased RNA turnover, and the reorientation of tryptophan catabolism from the gut microbiota–indole axis toward the immunosuppressive kynurenine pathway. A LASSO-penalized logistic regression model was trained on a refined 4-metabolite signature (3-indoxylsulfate, leucine, pseudouridine, tryptophan), yielding an Area Under the Curve (AUC) of 0.791, with 81% sensitivity and 66% specificity. The logistic score was translated into a clinical risk stratification table with six interpretable tiers. Despite limitations such as cohort size and the lack of independent external validation, this integrated NMR-MS framework represents a proof of concept demonstrating that a non-invasive urinary metabolomic panel can meaningfully contribute to pre-surgical NMIBC/MIBC discrimination.

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EXPLORING THE INTERACTION BETWEEN CYCLOPHILIN A AND THE AIF-DERIVED PEPTIDE CC1 BY NMR SPECTROSCOPY

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The interaction between apoptosis-inducing factor (AIF) and cyclophilin A (CypA) plays a key role in caspase-independent programmed cell death, making the disruption of the AIF-CypA complex an attractive therapeutic strategy [1, 2]. Recently, we designed and synthetized a disulfide-bridged peptide, named AIF(381-389), showing a similar in vitro affinity for CypA, an improved antiapoptotic activity in cells and an enhanced proteolytic stability compared to the parent peptide AIF(370-394). Moreover, NMR data confirmed that AIF(381-389) adopts in solution a native-like β-hairpin conformation and it is able to bind CypA into a shallow groove flanking the protein catalytic site [3]. Starting from AIF(381-389), we identified a novel AIF-derived peptide called CC1 (primary sequence C^{381}IKLK**LL**RKVC^{389}) by performing combinatorial screening of internal residues within the turn region (D385 and G386). Here, we investigated the structural peculiarities of CC1 and its ability to bind CypA by using and integrated approach in which NMR structural data were integrated with computational techniques. Hα and Cα secondary chemical shift (SCS) analysis showed that CC1 behaves predominantly as an intrinsically disordered peptide, displaying only a local and limited α-helical propensity. Comparison of the NMR spectra acquired for the peptide in the absence and presence of CypA indicated that the binding occurs in a slow-exchange regime on the NMR timescale with distinct resonances corresponding to free and bound states. Importantly, CC1 remains largely disordered upon binding while preserving its helical tendency at the C-terminus. Overall, this work offers a detailed characterization of the CypA-CC1 interaction and provides a structural framework for the rational design of AIF-derived inhibitors targeting the pathogenic AIF-CypA complex.

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PROBING THE HOST–GUEST INTERACTIONS OF CURCUMIN ENCAPSULATED WITHIN ZIF-8 VIA SOLID-STATE NMR

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The need for controlled drug release to reduce toxicity and enhance therapeutic efficacy has driven the development of Metal-Organic Frameworks (MOFs) as drug carriers [1]. ZIF-8 is particularly promising due to its biocompatibility and pH-responsive nature [2]. A major pharmacological challenge is the delivery of Curcumin (*cur*), a natural bioactive polyphenol renowned for its antioxidant, anti-inflammatory and anticancer properties, with low aqueous solubility and poor stability [3,4]. Encapsulating *cur* within ZIF-8 offers a strategic solution to improve the stability of the molecule and its bioavailability [5]. Understanding how drugs interact at a molecular level with porous nanocarriers is fundamental to guide the rational design of advanced drug delivery systems. This work exploits solid-state NMR (ssNMR) as a unique atomic-level probe of structure and dynamics in *cur*@ZIF-8 systems. This technique provides definitive evidence of whether the drug is truly encapsulated, adsorbed on the surface, or present as an amorphous/crystalline bulk phase. By analyzing samples with different curcumin initial concentrations (0.5, 1, and 2 mg/mL – referring to the amount of curcumin dissolved in MeOH for the synthesis [6]), we provide detailed insights into host-drug interactions and structural modifications induced by loading, enabling the identification of stability thresholds and distinct confinement regimes within the framework. A multinuclear (^{1}H, ^{13}C, ^{15}N) high-resolution ssNMR strategy was used to investigate the structure of both the framework and the drug and, in particular, the interactions between curcumin and ZIF-8. Furthermore, spin-lattice (T_1) and spin-spin (T_2) relaxation measurements were exploited to reveal linker and guest dynamics under confinement across multiple timescales. To gain a deeper understanding of the host-guest interactions, 2D ssNMR experiments were also performed, complemented by DFT calculations to provide both structural validation and characterization of the non-covalent forces at play.

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STRUCTURE AND DYNAMICS OF NOVEL HUMAN 20S PROTEASOME ACTIVATORS PROBED BY NMR

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Keywords: solution NMR, small molecules, biomolecules

Protein degradation is a fundamental aspect of cellular protein homeostasis. Understanding the mechanisms of protein degradation is essential to elucidating the molecular causes of numerous pathologies associated with misfolding and aggregation of proteins [1, 2]. In this scenario, the proteasome represents a key molecular machine having critical functions in cell cycle regulation, protein quality control, apoptosis, inflammation, transcription and many more biological processes; it is therefore an interesting therapeutic target. Proteasomal activity is strongly regulated through conformational changes in the core particle (CP) triggered by its interaction with specific regulatory particles (RPs) [3]. Recent studies have demonstrated that small compounds and peptides can allosterically activate the 20S gate of human proteasome. These findings suggest new possibilities for developing alternative therapeutic strategies that modulate the activity of protein systems that have been identified as challenging targets [4, 5].
Based on these premises, our aim is to improve the structure-activity relationship (SAR) studies of proteasome regulators through the inclusion of greater chemical diversity. A structural and functional characterization of novel synthesized peptide-activators has been performed by using high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy in combination with computational techniques such as molecular docking and molecular dynamics simulations.

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^{1}H-NMR metabolomics approach to study the chemical composition changes of lettuce grown with different nutrient solutions

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Lettuce (*Lactuca sativa* L.), member of the Asteraceae family, is a leafy vegetable widely grown and consumed worldwide, fresh cut or minimally processed, constituting a rich dietary source of natural antioxidants and bioactive compounds. Compared to many other vegetable crops, lettuce has a notably short growth cycle, approximately 30–60 days from sowing to harvest. Several factors such as different genotypes, climatic conditions, and agronomic practices can influence the plant development and, consequently, the chemical profile.
In the present project, the effect of several nutrient solutions on the chemical composition of lettuce was investigated by high-field NMR as a powerful techniques with a metabolomic approach to understand the effects of different supplementation on the qualitative and quantitative change of metabolites extracted either from the lettuce leaves. Lettuce plants were cultivated with eight different nutritive solutions: different urea levels with or without organic compounds. The levels of four sugars (fructose, glucose, sucrose and myo-inositol, five organic acids (citrate, formate, fumarate, acetate and malate), twelve amino acids (alanine, arginine, asparagine, aspartate, GABA, glutamine, isoleucine, leucine, pyro-glutamate, threonine, tyrosine and valine) and choline were determined in all the samples. Finally, statical analysis was performed to determine the metabolites most responsible for discriminating among the sample groups.

^{1}H-NMR metabolomics for the characterization of fennel quality during storage

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Fresh-cut vegetables undergo metabolic changes during storage that may affect their quality and shelf-life. In this work, ^{1}H-NMR metabolomics was applied to characterize the polar metabolites of fennel (*Foeniculum vulgare* Mill.) and to evaluate the effects of different packaging systems during refrigerated storage. A sustainable biodegradable packaging system was evaluated for its ability to preserve the shelf-life and quality of fresh-cut fennel in comparison with conventional low-density polyethylene (LDPE) packaging. Fennel slices were packaged in biodegradable films or LDPE reference films, either alone or in combination with active packaging components, including moisture absorbers, oxygen absorbers, and ethylene scavengers, and stored at 5 °C for 14 days.
NMR analysis allowed the identification and quantification of the main polar metabolites in fennel, including amino acids, organic acids, sugars, choline, and phosphorylcholine. At time zero, sugars represented the predominant metabolite class, with glucose, fructose, and sucrose as the main compounds. Malic acid was the most abundant organic acid, while asparagine and glutamine were the main amino acids. After 14 days of storage, clear metabolic changes were observed according to the packaging system. Amino acid levels generally increased, suggesting relevant post-harvest metabolic remodeling. Organic acid profiles were also affected, with increased levels of succinate, acetate, lactate, fumarate, and formate particularly evident in samples stored in biodegradable packaging. Conversely, malate, quinate, and citrate remained relatively stable. Regarding sugars, conventional packaging showed a more stable profile, whereas biodegradable packaging was associated with a decrease in glucose and, more markedly, sucrose.
PCA analysis based on quantitative NMR data highlighted sample clustering according to the packaging material, confirming that the storage system influenced the metabolic profile of fennel. Overall, this study demonstrates the potential of ^{1}H-NMR metabolomics as a powerful tool to monitor storage-induced biochemical changes and to assess the impact of sustainable packaging strategies on fresh-cut vegetables.

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STRUCTURAL INSIGHTS INTO THE MECHANISM OF PHOX2B VARIANTS AGGREGATION LEADING TO AMYLOID FORMATION

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Congenital central hypoventilation syndrome (CCHS) is a rare neonatal disorder characterized by impaired autonomic control of respiration, resulting in abnormal ventilatory responses to hypoxia and hypercapnia [1]. The disease is primarily caused by mutations in the PHOX2B gene, including polyAla expansions (95%) and, less frequently, frameshift mutations (5%) [2]. In this scenario, we investigated the structural, dynamical and functional properties of Phox2B protein and its pathological variants [3]. In detail, we performed the structural characterization of the homeodomain (HD) of PHOX2B and HD + C-terminus PHOX2B protein, free and in the presence of the target DNA [4]. We also demonstrated that the HD is structurally coupled, via transient interactions, to the intrinsically disordered C-terminal domain, in which the polyAla tract adopts a stable α-helical conformation [4]. This highlighted inter-domain coupling mechanism may play a protective role in preventing misfolding and aggregation, analogous to molecular mechanisms described in other aggregation-prone proteins [5]. This hypothesis is supported by ThT aggregation experiments showing that pathological variants, such as the +7Ala mutant, exhibit a strong propensity to aggregate into insoluble amyloid fibrils, suggesting a potential contribution to disease development. Building on these findings, we characterized, using a multidisciplinary approach combining biochemical, spectroscopic and computational techniques, the molecular determinants driving in the initial stages of the aggregation process of Phox2B variants. In addition, advanced real-time NMR experiments provided atomic resolution structural insights into the assembly mechanism identifying key HD residues involved in the early steps of the nucleation process. These structural insights will guide the rational design of peptide-based inhibitors targeting aggregation-prone HD regions, with the goal of preventing aggregation processes by which Phox2B variants form, through the formation of soluble oligomeric species, amyloid fibrils that in turn may have a crucial role in CCHS disease progression.

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INTERACTION OF NANOPLASTICS WITH THE N-TER DOMAIN OF HUMAN PRION PROTEIN DRIVES CYTOTOXIC OLIGOMER FORMATION

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solution NMR, biomolecules.

Widespread distribution of nano-plastics (NPs) in the environment is increasingly recognised as a global health concern [1]. NPs can enter the human body and can accumulate over time, and induce toxic effects and damage to cells, tissues and organs, including the brain. Recently, NPs have attracted growing interest and were found to either accelerate or inhibit protein fibrillation, depending on NP properties, conditions, and protein aggregation propensities [2]. Our research is focused on understanding if and how NPs may contribute to the development of neurodegenerative diseases, particularly prion diseases.
Here, we investigated the interaction between anionic polystyrene NPs with the N-Terminal Domain (NTD, residues 23-89) of Human Prion Protein (HuPrP). Unlike the C-Terminal Domain (CTD, residues 90-231), which is primarily responsible for the protein misfolding and, subsequently, prion aggregation [3]; the NTD, which is released in the extracellular space from the full-length protein after a proteolytic cleavage, does not show any aggregation propensity under physiologic conditions [4]. Using Dynamic Light Scattering (DLS) measurements, Nuclear Magnetic Resonance (NMR) methodologies and fluorescence spectroscopy studies, we have demonstrated that the NTD is absorbed on NPs surface and that this interaction generates NTD oligomers (NTD(o)), whose formation is driven by an interplay between hydrophobic and hydrophilic interactions. Moreover, we have performed biological assays to evaluate the cytotoxicity in vitro of NTD(o) on neuronal cells. We have also developed a synthetic strategy to site-specifically label the NTD with a fluorescent probe to explore the cellular localization of NTD(o) in the presence of NPs by fluorescence microscopy. Our findings highlight the significant impact of NPs on NTD amylogenic proprieties and suggest a potential role in the onset and progression of prion diseases.

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FROM SEMIQUANTITATIVE ANALYSIS TO QUANTITATIVE REPRODUCIBILITY BY A HARMONIZED NMR STRATEGY FOR BETAINE DETERMINATION IN DURUM WHEAT AND PASTA

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

Can a semiquantitative NMR workflow deliver truly quantitative results? This study shows that, under carefully harmonized conditions and with gravimetrically traceable reference standards, the answer can be yes. In a field where semiquantitative 1H NMR is routinely used for non-targeted metabolite profiling, demonstrating quantitative reproducibility from the same spectra would represent a major analytical advantage for food applications.
To address this question, an interlaboratory comparison was carried out on the determination of betaine in aqueous extracts of durum wheat (cvv. Marco Aurelio and Iride) and the corresponding pasta products. A common set of samples was analyzed using a harmonized acquisition protocol across 50 spectrometers operating at magnetic field strengths from 80 to 700 MHz. Two data-processing strategies were compared: distributed operator-dependent processing, involving multiple operators and software environments, and centralized processing performed by a single operator using five different software platforms. Quantification was carried out by both internal and external standard methods, using TSP-d4, $DMSO_2$, and betaine as reference compounds.
The results clearly demonstrate that the metrological reliability of the approach strongly depends on the reference standard employed. TSP-d4 systematically overestimated betaine concentration and introduced additional dispersion, whereas DMSO2 and betaine yielded accurate and highly precise results. Moreover, it was demonstrated that a non-targeted NMR workflow, commonly regarded as semiquantitative, can provide quantitatively robust determination of target analytes, when anchored to traceable standards with documented uncertainty. More broadly, the work highlights the possibility of using a single harmonized NMR experiment to obtain both reliable metabolite profiling and metrologically sound quantification in complex food matrices.

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Understanding the metabolic effect of PFBS on human SH-SY5Y cell line with NMR-Metabolomics

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

Although perfluorobutanesulfonic acid (PFBS) was introduced as a safer alternative to the Stockholm Convention-regulated pollutant perfluorooctanesulfonic acid (PFOS), its neurotoxic potential remains insufficiently explored. This knowledge gap is mainly attributable to the shorter human half-life of PFBS relative to PFOS, as well as the limited availability of direct tissue concentration data. [1,2]. To evaluate its effects on the central nervous system, we employed NMR-based metabolomics to investigate the impact of subtoxic PFBS exposure on human neuroblastoma cells (SH-SY5Y) after 48, 72, and 96 hours of treatment.
Principal Component Analysis (PCA) clearly distinguished high- and low-dose exposure groups at all investigated time points. Moreover, separation between control and low-dose samples emerged after 96 hours of exposure, revealing increased levels of GPC, choline, hypotaurine, uridine, and uracil, alongside decreased levels of PC, creatine, glycine, and taurine in treated cells compared with controls (Figure 1). Pathway analysis identified taurine and hypotaurine metabolism, together with glutathione metabolism, as the most significantly affected metabolic pathways.
Since differentiation between controls and low-dose samples was observed only at the longest exposure time, these preliminary results support the notion that PFBS exhibits lower short-term toxicity than its long-chain predecessors. Nevertheless, its potential long-term neurotoxicity warrants careful attention, given the high chemical stability and environmental persistence of PFBS.

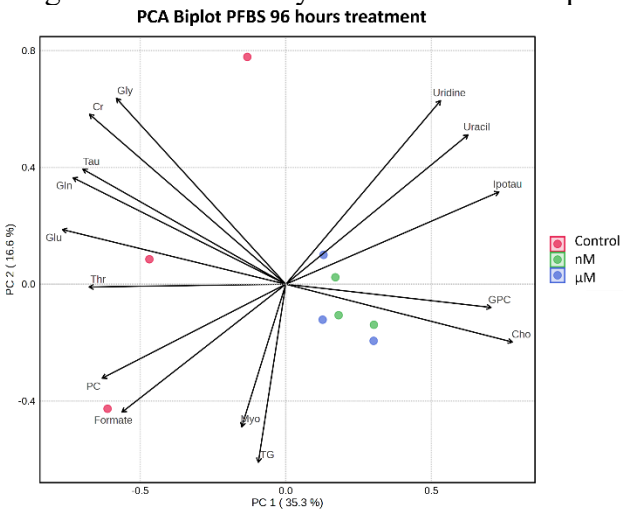

Fig. 1. Principal Component Analysis Biplot of low-dose samples treated for 96 hours with PFBS

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WOMEN'S HEALTH AND EARLY DIAGNOSIS: MULTI-OMICS LIQUID BIOPSY AGAINST GYNAECOLOGICAL CANCERS

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Keywords: Nuclear magnetic resonance (NMR); Metabolomics; Lipoproteomics, Lipidomics, Gynaecological cancer, Minimal residual disease (MRD), Liquid biopsy.

Gynaecological cancers often have high recurrence rates and poor outcomes, primarily because of minimal residual disease (MRD). MRD refers to small groups of tumour cells that survive surgery and chemotherapy but are undetectable by standard clinical and imaging techniques. These residual cells are major contributors to relapse and resistance to therapy, emphasizing the importance of developing sensitive, minimally invasive methods for early detection and ongoing disease monitoring.

Multi-omics liquid biopsy techniques using nuclear magnetic resonance (NMR) metabolomics, lipoproteomics, and lipidomics have emerged as a promising approach to explore systemic and local molecular changes linked to tumour persistence and recurrence [1]. The study involved 50 patients with gynaecological cancers, from whom serum and ascites or peritoneal washing samples were collected at pre-treatment and follow-up stages. Quantitative NMR analysis identified metabolites related to energy metabolism, inflammation, oxidative stress, and lipid remodelling, along with detailed profiles of circulating lipoproteins and lipid species.

Lipoproteomic and lipidomic changes could offer further understanding of MRD biology, since gynaecological cancer progression is closely linked to disrupted lipid metabolism, increased lipid uptake, membrane changes, and inflammation that influence circulating lipoprotein particles. Changes in lipoprotein subclasses and lipid makeup may thus indicate tumour-driven metabolic shifts and residual disease activity, even when standard biomarkers are negative [2].

Integrating metabolomic, lipoproteomic, and lipidomic signatures with clinical data can aid in discovering new biomarkers for early diagnosis, MRD detection, and patient stratification [3]. This integration may advance precision medicine approaches for tailored monitoring and treatment of gynaecological cancer patients.

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Urinary Metabolomic Profiling by ^{1}H-NMR Spectroscopy in Acute Pyelonephritis

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Keywords: solution NMR, metabolomics.

Acute pyelonephritis (APN) is a bacterial infection that causes inflammation of the kidneys and is among the most prevalent kidney diseases, with higher incidence in women aged 15 to 65. This project was carried out in collaboration with the University Hospital of Alessandria SS. Antonio e Biagio e Cesare Arrigo that collected the urine samples of 13 female patients hospitalized with a diagnosis of APN at the time of admission to the nephrology department (T0) and 3 months after diagnosis (T3), in conditions of documented recovery from APN and confirmed clinical stability.
We performed the NMR analysis of 21 urine samples (13 for T0 and 8 for T3), following the analytical protocol reported in the literature. [1] The 1D ^{1}H-NOESY spectra collected on the T0 and T3 samples were analysed with two different approaches, one based on untargeted and the other on targeted analysis. For the untargeted metabolomics, Principal Component Analysis (PCA) was performed with the software *R* [2] to explore patterns within the NMR data. The main differences between the two groups are due to signals in the region between 3.0 – 4.5 ppm, and in the aromatic region. For the targeted analysis, we used *Chenomx* software to identify and quantify urinary metabolic markers, to evaluate the differences between T0 and T3 sample and associate them with the APN. The long-term aim of the project is to identify urinary metabolite candidates potentially associated with APN recurrence.

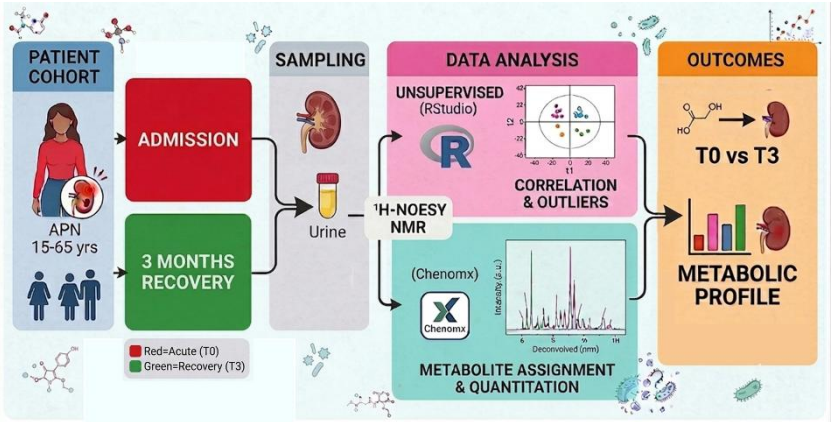

Fig. 1. Project overview

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EVALUATION OF CYCLIC PEPTIDES AS INHIBITORS OF SAM-SAM INTERACTIONS INVOLVING THE EPHA2 RECEPTOR THROUGH NMR STRUCTURAL AND BINDING STUDIES.

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Keywords: solution NMR, docking, cyclic peptide inhibitors, Sam domains, cancer.

EphA2 is a tyrosine kinase receptor involved in cancer diseases through the regulation of pro- and anti-oncogenic routes [1]. EphA2 domain organization includes in the C-terminal intracellular side a Sam (Sterile alpha motif) domain (EphA2-Sam) that is able to form heterotypic complexes with Sam domains from several proteins. The interaction between EphA2-Sam and the Sam domain from the lipid phosphatase Ship2 (Ship2-Sam) is of particular interest as it negatively regulates the process of receptor endocytosis with pro-oncogenic effects [1]. Thus, inhibition of the EphA2-Sam/Ship2-Sam association through molecules (such as peptides) represents an interesting drug discovery route to fight cancer pathologies. Previously we identified the KRI3 peptide, which is a weak inhibitor of the EphA2-Sam/Ship2-Sam interaction [2,3]. This peptide is disordered in aqueous milieu but assumes a helical fold, possibly reflecting the peptide bioactive conformation, when in presence of the structuring co-agent TFE (2,2,2, trifluoroethanol). A model of the Ship2-Sam/KRI3 complex was generated by docking techniques [3]. A structure-based approach relying on analysis of the contacts occurring at the protein/peptide binding interface suggested different KRI3 cyclization strategies to stabilize peptide secondary structure and improve ability to interact with Ship2-Sam while increasing drug-like properties. Herein, we will report on NMR studies of four cyclic versions of the KRI3 peptide. NMR conformational analyses were performed in PBS buffer and in presence of TFE to reveal structural tendencies of the designed peptides. NMR chemical shift perturbation studies were also carried out to investigate the binding between Ship2-Sam and each peptide.

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NMR-BASED STRUCTURAL INVESTIGATION OF PANTININ-DERIVED PEPTIDES AGAINST VETERINARY HERPESVIRUSES

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Keywords: solution NMR, biomolecules.

Pantinin peptides, isolated from the venom of the scorpion Pandinus imperator, are cysteine-free antimicrobial peptides with broad-spectrum activity [1]. Their membrane-interacting properties, together with the relatively favorable cytotoxicity profile reported for selected pantinin peptides, make these molecules of interest for possible therapeutic applications [2]. In this study, the pantinin-derived antimicrobial peptides pantinin-1 and pantinin-2 were investigated by natural-abundance nuclear magnetic resonance (NMR) spectroscopy in the absence and presence of 30% trifluoroethanol (TFE/H2O). The aim was to characterize their conformational behavior in solution and in the presence of TFE. NMR analysis showed that both peptides mainly adopt random-coil, disordered conformations in aqueous solution. Pantinin-2, however, displayed a slightly higher tendency to form secondary-structure elements. In the presence of TFE/H2O, both peptides underwent a clear conformational transition toward α-helical structures. This conformational behavior is consistent with the membrane-interacting nature of antimicrobial peptides and may be relevant to their antiviral mechanism of action.

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IDENTIFICATION OF AGEING-RELATED BIOMARKERS THROUGH NMR METABOLIC PROFILING OF HUMAN PLASMA

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Keywords: solution NMR, small molecules, metabolomics

With the progressive increase in life expectancy, age-related pathologies are becoming a major public health challenge [1]. This scenario highlights the need for reliable diagnostic and prognostic tools capable of supporting the early identification and monitoring of ageing-associated functional and cognitive decline.

In this field, NMR spectroscopy allows both the metabolic profiling and fingerprinting of biological samples [2,3,4], thus providing a comprehensive overview of their molecular composition. Here we describe the application of this technique to the profiling of human plasma samples within the multicentric randomized controlled trial IN-TeMPO (ItaliaN Study With Tailored Multidomain Interventions to Prevent Functional and Cognitive Decline in Community-dwelling Older Adults, ID NCT06248723), which aims to evaluate the efficacy of guided multidomain interventions in preventing age-related cognitive and functional decline [5].

To identify potential diagnostic and prognostic biomarkers related to ageing and age-associated decline, plasma samples collected at t0 and t1 in the IN-TeMPO trial, corresponding respectively to baseline and after the 12-month intervention, are subjected to multivariate statistical analyses combining NMR-derived metabolic profiles with clinical, functional, and demographic patient metadata.

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METABOLIC REPROGRAMMING ASSOCIATED WITH FERROPTOSIS PROTECTION BY AN INDOLE-BASED ANTIOXIDANT IN Aβ (25–35)-TREATED SH-SY5Y CELLS

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Keywords: solution NMR, biomolecules, metabolomics.

Ferroptosis is a crucial process linking iron imbalance, oxidative stress, and neurodegeneration in diseases related to amyloid. [1,2] Building on our earlier research that identified an indole-based compound - named compound 20 - as a promising antioxidant and neuroprotective agent, this study investigates its role in preventing amyloid-induced ferroptosis in human neuroblastoma SH-SY5Y cells treated with Aβ(25-35). [3]
Using biochemical assays, organelle-specific analyses, and gene expression studies, we found that compound 20 significantly alleviated Aβ(25-35)-induced ferroptotic damage. It achieved this by restoring intracellular glutathione levels, decreasing the labile iron pool, reducing lipid peroxidation, improving mitochondrial membrane potential, diminishing ER stress and inflammatory markers. Although increasing evidence indicates a correlation, the precise role of ferroptosis in cellular response to Aβ(25–35) and the influence of amyloid-targeting antioxidants at the metabolic level remain insufficiently understood. [4] Based on this, we employed an NMR metabolomic approach to analyze how Aβ(25-35) affects the SH-SY5Y endometabolome and exometabolome. This investigation aimed to identify metabolic changes caused by the treatment and how they relate to known effects of ferroptosis. Subsequently, to gain a comprehensive understanding of compound 20's mechanism of action against ferroptosis toxicity induced by Aβ(25-35), we examined the metabolic profile of cells co-treated with compound 20 and Aβ(25-35). This method ensures a comprehensive system-level investigation, allowing verification of the previously obtained results and exploration of collateral effects caused by compound 20 that could not be detected in other targeted experiments. Accordingly, the untargeted ^{1}H-NMR metabolomic analysis confirmed the activation of antioxidant pathways and revealed significant changes in energy metabolism and GABA-related pathways, essential for redox balance and neuronal resilience.
This work demonstrates how NMR metabolomics can be integrated into a multi-layered approach to confirm the activation of specific cell death pathways and clarify the complex mechanisms of action of new molecular entities.

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^{1}H-NMR METABOLOMICS FOR FARMING SYSTEM AUTHENTICATION: CASE STUDIES ON ITALIAN RICE AND CAULIFLOWER

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Keywords:
^{1}H-NMR, conventional and organic agriculture, metabolomics markers.

Agricultural authentication is increasingly critical as global demand for organic products grows, driven by consumer health and environmental concerns. This study demonstrates the efficacy of ^{1}H-NMR-based metabolomics in discriminating between organic and conventional farming systems through two distinct case studies: Italian rice and cauliflower.

In the first study, NMR metabolomic fingerprinting combined with multivariate statistics (PCA, HCA, and OPLS-DA) successfully classified Italian brown and white rice samples according to cultivation practice [1]. Specific metabolites, including amino acids (aspartate, lysine, leucine, and tryptophan), sugars (maltose, sucrose, and glucose), and purines (adenosine, guanosine, and oxypurinol), were identified as key markers for rice authentication. Quantitative analysis revealed that organic samples were characterized by higher levels of amino acids and nitrogenous compounds, while conventional brown rice was uniquely distinguished by high purine content.

The second case study utilized ^{1}H-NMR targeted metabolomics to characterize cauliflower corymbs over two consecutive years [2]. The approach accurately classified farming systems (conventional, organic, and agroecological), identifying choline, isobutyrate, formate, and alanine as robust markers independent of the cultivation year. Similar to the rice study, organic cauliflower exhibited higher nutritional quality, with significantly higher concentrations of organic acids and nitrogen-containing compounds. In contrast, conventional farming practices in both matrices tended to favor higher carbohydrate concentrations, likely due to the immediate availability of synthetic nitrogen and reduced pest stress.

Together, these findings underscore the analytical robustness, reproducibility, and high-throughput potential of ^{1}H-NMR spectroscopy for food traceability. By establishing distinct metabolic signatures for organic produce across different species, NMR-based metabolomics provides a powerful and efficient tool for routine quality control and regulatory oversight in the food industry.

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MECHANISTIC INSIGHTS INTO PROTEIN MISFOLDING AND AGGREGATION PROCESSES OF AMYLOIDOGENIC GELSOLIN G2 DOMAIN BY REAL TIME SOLUTION NMR APPROACHES

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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].
We have recently developed a DOSY-ILT NMR approach in which NMR diffusion measurements are coupled with the Inverse Laplace Transform reconstruction method to detect the rapidly interconverting visible species and effectively characterize the early steps of the aggregation process. The method has been successfully applied to elucidate the time-dependent oligomer distribution of the Aβ1-40 amyloid peptide and assess the effect of Epigallocathechin gallate, a known remodeling agent of amyloid fibrils, on Aβ1-40 species distributions [2]. New mechanistic insights on the G2-D187N aggregation process and its pH dependence, are discussed. DOSY-ILT NMR approach is further presented as a strategy for rapidly elucidating the mechanism of action of aggregation inhibitors, thereby supporting the systematic optimization of compounds potency during the early stages of drug discovery.

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NEW FLAVONOID GLYCOSIDES FROM PARSLEY FRUITS

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

The chemical composition of the volatile, fixed oil and flavonoid fraction extracted from the fruits of parsley was investigated in two cultivar and a local ecotype of the species [1]. Nine compounds were detected in the flavonoid fraction, being glycosyl and glycosyl malonate derivatives of apigenin and chrysoeriol the most abundant [2]. Among these, the compounds apigenin 7-O-[apiofuranosyl(1→2)-(6''-O-malonyl-β-glucopyranoside)] and chrysoeriol 7-O-[apiofuranosyl(1→2)-(6''-O-malonyl-β-glucopyranoside)], were detected for the first time in the fruits of the species. The complete identification of these compounds (fig.1), performed by NMR techniques, is reported.

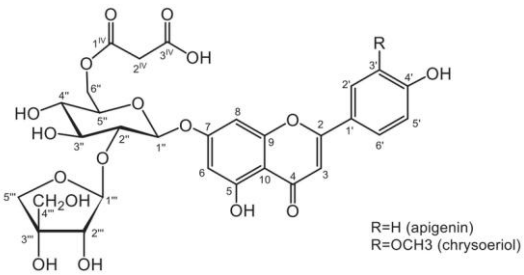

Fig. 1. Structure of apigenin and chrysoeriol

This study, aimed at the complete identification and quantitation of different classes of metabolites, represents to date the most exhaustive report on the evaluation of bioactive compounds in parsley fruits [3], a particular food resource endowed with promising nourishing features.

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AN INTEGRATED SOLID-STATE NMR AND PAIR DISTRIBUTION FUNCTION APPROACH FOR STRUCTURE ELUCIDATION OF NANOCRYSTALLINE ORGANIC COMPOUNDS

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

Investigating the structure of molecular solids is critical for establishing structure–property relationships and controlling material performance, but remains challenging when long-range order is reduced or lost [1]. Pair Distribution Function (PDF) analysis offers a valuable approach for probing the local structure of poorly crystalline, nanocrystalline, and amorphous inorganic materials by investigating interatomic distances [2]. More recently, the development of the Global-PDF-Fit method has extended PDF-based structure determination to organic compounds [3], although intrinsic limitations can complicate unique structure identification [4,5].
To overcome this, we recently developed a synergistic workflow integrating Global-PDF-Fit with solid-state NMR (SSNMR) for the structure determination of nanocrystalline organic compounds [6]. In this approach, SSNMR-derived restraints and constraints allow to guide the PDF-based structure search, reduce computational time, improve structure ranking, and increase reliability, ultimately enabling unambiguous structure determination. This method was initially demonstrated on nanocrystalline organic systems with crystallite sizes above 40 nm, where Bragg diffraction features are still resolved for traditional diffraction-based structure determination.
In the present study, the application of the Global-PDF-Fit/SSNMR workflow is extended to more challenging nanocrystalline systems of approximately 20 nm, using melatonin as a model compound. Here, significant Bragg peak broadening and overlap, together with diffuse scattering, make conventional diffraction methods for structure determination unfeasible. The results obtained so far demonstrate how SSNMR-derived local structural information can support PDF data, highlighting the potential of the integrated Global-PDF-Fit/SSNMR approach for reliable structure elucidation of nanocrystalline organic compounds with very reduced crystallite sizes.

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^{1}H NMR Serum Metabolomics Reveals Metabolic Alterations in Advanced Stage CKD Pathophysiology

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Abstract

Understanding metabolic alterations in chronic kidney disease (CKD) is essential, as serum creatinine-based diagnosis alone lacks sufficient precision and may affect key clinical decisions. In this study, a ^{1}H NMR-based serum metabolomics approach was applied to investigate metabolic perturbations associated with advanced-stage CKD and to further explore how diabetes influences the metabolic pathophysiology of CKD.

Serum samples from advanced-stage CKD patients (Stage 4(S4) and 5(S5); n = 52) and healthy controls (HC) (n = 25) were first analyzed to identify metabolic alterations linked to CKD progression. Statistical analyses revealed distinct metabolic patterns between groups. Compared with HC, CKD patients showed significant alterations in creatinine, urea, myo-inositol, choline, and N,N-dimethylglycine, along with decreased tyrosine, all demonstrating potential diagnostic value (AUC > 0.8). Correlation analysis indicated positive associations of myo-inositol, dimethylamine, N,N-dimethylglycine, and choline with creatinine levels, while tyrosine showed a negative correlation. Amino acid metabolism was further downregulated in S5 CKD, reflecting increased disease severity. Additional metabolites, including glutamate, glutamine, alanine, threonine, citrulline, citrate, and betaine, highlighted metabolic alterations associated with CKD progression, with pathway analysis identifying five key metabolic pathways involved.

Keywords: Chronic Kidney disease, NMR-based metabolomics, Metabolic biomarkers

Molecular Dynamics of Animal- and Plant-Based Cheese Matrices via Temperature-Dependent FFC-NMR Relaxometry

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Keywords: low field NMR, instrumentation, food

This study investigates the molecular dynamics of diverse cheese matrices using Fast Field Cycling (FFC) NMR relaxometry, a non-invasive technique that enables detailed characterization of water mobility and structural transitions. Traditional animal-based cheeses (Cheddar, Gouda, and White) were compared with their plant-based analogs formulated with coconut oil (23–29%) and modified starch, as well as creamy Labne varieties (regular vs. lactose-free). FFC-NMR measurements were conducted across a frequency range of 0.01 to 8 MHz at 20°C, 40°C, and 60°C to evaluate the effect of thermal energy on molecular mobility. Preliminary results show a clear temperature dependence: increasing temperature correlates with a significant increase in spin-lattice relaxation time (T1), reflecting enhanced molecular mobility within both casein-based networks of animal cheeses and the starch-fat matrices of plant-based analogs. Among the sustainable alternatives, Greek White plant-based cheese exhibited the highest T1 values, while Gouda and Cheddar analogs showed comparable relaxation behaviors. The influence of the lactase enzyme on the hydrogen-bonding network of creamy Labne was also analyzed. These findings contribute to a deeper understanding of how composition affects food structure, quality, and the development of sustainable and health-oriented dairy alternatives [1]

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STRUCTURAL ELUCIDATION THROUGH NMR ANALYSIS OF SPECIALIZED METABOLITES FROM *MYRTACEAE* PLANTS AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES.

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

Myrtaceae represents one of the most significant flowering plant families in the world, recognized as the nineth largest globally, with numerous genera of considerable ecological and economic importance and a cosmopolitan distribution spanning multiple geographical regions. [1] A wide range of bioactive specialized metabolites can be produced by these genera. Apart from phloroglucinols derivates, the most extensively studied specialized metabolites from *Myrtaceae,* plants of this genus are also a valuable source of flavonoids [2].
In this study we report on the bio-guided phytochemical investigation of leaves crude extracts of several *Myrtaceae* plants: *Psidium cattleianum, Psidium myrtoides*, *Psidium guinensee*, *Psidium friedrichsthalianum*, *Myrrhinium atropurpureum* and *Hexachlamis edulis.* Leaves crude extracts of all the species were tested for their antimicrobial activity against two strains of *Staphylococcus aureus*: ATCC 29213 and 43300 (a methicillin-resistant *Staphylococcus aureus* strain, MRSA). In light of the promising results, the most active extracts were subsequently fractionated using chromatographic techniques and fractions obtained from the purification of the active extracts were then studied through spectroscopic techniques: 2D-NMR experiments (HSQC, HMBC, HSQC-TOCSY) and spectrometric analyses (HR-MS).

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Water dynamics in biomolecular condensates and saturated salt solutions probed through multinuclear NMR

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Keywords: solution NMR, water dynamics, biomolecules

Biomolecular phase separation is a fundamental process observed among all kingdoms of life, leading to the formation of dynamic cellular assemblies known as biomolecular condensates. One such highly dynamic cellular assembly is the Cajal body, which exhibits rapid exchange of its components and plays a crucial role in supporting the compositional dynamics of the RNA splicing machinery. The protein coilin is prone to RNA-induced phase separation and serves as a scaffold for Cajal bodies. The present study is performed to understand the water dynamics (translational/rotational) in constrained environment such as biomolecular condensates, including RNA-induced coilin droplets that exhibit highly dynamic liquid-like behavior [1]. To investigate water dynamics in such crowded conditions that are typically enriched by highly charged nucleic acid molecules, water in saturated salt solutions were taken as a reference, where water molecules are predominantly within the hydration shell of ions and typically shared between ion pairs (cations and anions) [2]. In this study, we take advantage of multi-spin NMR spectroscopy that directly determine ^{1}H and ^{17}O nuclei as probes of water dynamics and ^{23}Na as cations and ^{19}F, ^{35}Cl, ^{79}Br and ^{127}I as anions and study water and ion dynamics in a series of saturated sodium halide solutions (NaF, NaCl, NaBr and NaI). We monitored structure and dynamics of water and ions (cations and anions) through NMR chemical shifts, diffusion coefficients and spin relaxation rates. The present study demonstrates that ^{1}H and ^{17}O chemical shifts of water are affected by anionic size in the following order: $F^- < Cl^- < Br^- < I^-$. In addition, the translational diffusion and rotational mobility of water molecules follow a similar trends $F^- < Cl^- < Br^- < I^-$. This study provides molecular level insight into dynamical behavior of water and ions in saturated salt solutions and pave the way for a more quantitative understanding of water and ion dynamics within biomolecular condensates, which is essential for understanding the physicochemical basis of LLPS driven cellular organization.

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A NOVEL ^{1}H NMR METHOD FOR SIMULTANEOUS DETERMINATION OF DIASTASE AND INVERTASE ACTIVITY IN HONEY

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Keywords: solution NMR, biomolecules, food

Enzyme activity is a key quality parameter in honey, as it reflects its freshness and thermal history. Diastase activity has long been a standard regulatory parameter in honey quality control (Codex Alimentarius). Invertase activity, following recent updates to European honey quality regulations, has been reintroduced as a relevant quality marker, making the simultaneous determination of both enzymatic activities particularly timely and analytically relevant.
Colorimetric approaches are inherently affected by several limitations: they involve multiple dilutions and volume additions that accumulate analytical error and are susceptible to matrix interference effects. In honey analysis specifically, these issues are further compounded by the wide variability in enzyme activity across botanical origins. These limitations affect both the classical Schade procedure and the commercial Phadebas method for diastase, as well as the standard invertase assays, ultimately compromising the accuracy and reproducibility of the measurements. Furthermore, current colorimetric assays rely on artificial substrates that do not reflect physiological ones, introducing additional bias.
Here we present a novel ^{1}H NMR-based method for the simultaneous determination of diastase and invertase activity in a single assay, based on the selective monitoring of natural substrate disappearance — sucrose for invertase and maltotriose for diastase. The method exploits the absence of mutual interference between the two enzymatic activities under the assay conditions, allowing their simultaneous and independent determination. A linear relationship between the amount of substrate consumed and its initial concentration was demonstrated over a wide range, ensuring that the activity measurement is independent of the initial substrate concentration, a critical feature given that sucrose is naturally present in honey at variable levels and maltotriose occurs in certain honey types such as honeydew.
The method was validated on over 50 honey samples spanning different botanical origins. Good correlation with standard colorimetric methods was established (R^2 = 0.97–0.98).
The proposed method represents a robust and reliable alternative to classical colorimetric procedures, offering simultaneous determination of both enzymatic activities in a single, rapid and accurate measurement, with significant advantages in terms of simplicity, reproducibility and analytical throughput.

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NMR IDENTIFICATION AND QUANTIFICATION OF GALACTINOL IN GREEN *COFFEA ARABICA*: IMPLICATIONS FOR TRACEABILITY AND AUTHENTICITY

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Keywords: solution NMR, small molecules, biomolecules.

Among coffee low-molecular weight carbohydrates, galactinol plays an important role in coffee physiology and quality, having received attention also for its potential use as a marker of geographical origin. Nevertheless, data on the occurrence of this sugar in green coffee beans remain limited. In this study, galactinol was isolated for the first time from green Coffea arabica beans and unambiguously identified by high-field NMR spectroscopy. A quantitative HPLC-RID screening of 100 green Arabica coffee samples from 16 geographical origins showed that galactinol was detected in 31% of the samples, with consistent occurrence in Ethiopian coffees but sporadic or absent detection in several other origins. Notably, the chromatographic analysis revealed a previously unreported co-elution of galactinol with ciceritol, a chickpea-derived cyclitol of relevance for coffee adulteration detection. These results highlight both the potential and the limitations of galactinol as a geographical marker and stress the importance of combining chromatographic and structure-specific spectroscopic approaches to ensure reliable interpretation in coffee traceability and authenticity studies.

MOLECULAR CHARACTERIZATION OF THE CALCIUM-DEPENDENT CALMODULIN–TAU INTERACTION AND ITS IMPACT ON TAU AGGREGATION

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Keywords: solution NMR, biomolecules

Calcium dyshomeostasis is an early hallmark of neurodegenerative disorders, including Alzheimer's disease, and plays a central role in the onset and progression of Tau pathology. Calmodulin (CaM), the primary intracellular Ca^{2+} sensor, becomes activated upon calcium elevation and represents a key molecular link between Ca^{2+} imbalance and Tau dysfunction [1]. Activated CaM regulates Ca^{2+}/CaM-dependent kinases, including CaMKII, thereby promoting Tau hyperphosphorylation and reducing its affinity for microtubules, while also modulating phosphatases such as calcineurin. In parallel, CaM and pathological Tau exert opposing effects on Ca^{2+}-handling proteins, establishing a feedback loop that further amplifies calcium dysregulation and neuronal stress. Beyond these indirect mechanisms, CaM has been reported to bind directly to the microtubule-binding domain of Tau, impairing its microtubule-stabilizing function and potentially affecting its conformation and aggregation behavior [2]. However, the molecular basis of the Tau–CaM interaction and its functional implications remain poorly understood.

In this study, we characterize the Ca^{2+}-dependent interaction between CaM and Tau at high molecular resolution. Using solution NMR spectroscopy together with complementary biophysical approaches, including isothermal titration calorimetry and fluorescence spectroscopy, we investigated the structural and thermodynamic features of the CaM–Tau complex in both calcium-bound and calcium-free conditions. Our results demonstrate that Ca^{2+} modulates both the affinity of the interaction and the binding interface. NMR titration experiments identified specific interaction hotspots, in agreement with predictions from calmodulin-binding databases (Calmodulation). Paramagnetic relaxation enhancement (PRE) NMR experiments further refined the mapping of the CaM–Tau interaction interface. Functionally, we show that CaM binding reduces Tau aggregation in vitro, as demonstrated by ThT fluorescence assays and transmission electron microscopy (TEM). We also observed the colocalization of CaM and Tau within liquid–liquid phase-separated (LLPS) droplets, suggesting that this interaction may influence the properties of early protein condensates.

Taken together, these findings provide a detailed molecular description of the CaM–Tau interaction and its calcium dependence, highlighting how CaM binding modulates Tau conformation and aggregation propensity. This work will help to establish a mechanistic framework for the Ca^{2+}/CaM/Tau axis in neurodegeneration and may contribute to the development of therapeutic strategies targeting this interaction.

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THE ROLE OF SOLID-STATE NMR IN PHARMACEUTICAL COCRYSTAL SCREENING

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Keywords: (solid state NMR, small molecules)

In recent years, many efforts have been devoted to the study of pharmaceutical crystal forms as a strategy for modulating physicochemical properties of active pharmaceutical ingredients (APIs).[1] They include mono-component (polymorphs) and multi-component forms (hydrates/solvates, salts and cocrystals).[2] In particular, pharmaceutical cocrystals are defined as single-phase crystalline solids formed by two or more molecular components in a stoichiometric ratio, where at least one of the components is an API. They are linked by non-covalent interactions, e.g. hydrogen bond, halogen bond, π stacking, etc…[3] yielding either a binary system formed by two components or a higher order cocrystal containing more than two components. Both forms lead to an improvement of physicochemical properties of APIs, but higher order cocrystals often outperform binary systems in terms of functionality.
This work presents a characterization protocol optimized for the screening of binary and ternary cocrystals. The protocol consists in the comparison of the spectra (vibrational, NMR and PXRD patterns) of the starting materials with those of the reaction products to evaluate possible changes. All samples are initially analyzed by ATR and Raman spectroscopy as these techniques provide rapid and simple analysis using small amounts of powder.[2] The positive outcomes from vibrational spectroscopies are further characterized to confirm cocrystal formation and structural features. The X-ray diffraction from single crystal (SCXRD) is usually used to get an in-depth structural analysis, however, it cannot always be performed because it requires high-quality single crystals; in these cases, powder X-ray diffraction (PXRD) and solid-state nuclear magnetic resonance (SSNMR) provides an excellent method to characterize multicomponent crystal adducts and to distinguish different crystal forms. PXRD allows to distinguish the obtained adducts from other crystalline forms or specific polymorphs of the starting materials used for the synthesis.[4] The contribution of SSNMR enabled to identify the nature of the adduct obtained by studying the induced shifts in the ^{13}C and ^{15}N signals of the involved groups and providing information on the ionic or neutral character of the hydrogen bonds in the cocrystals. Advantages and limitations of all techniques are highlighted.

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LOW-FIELD NMR AS A TOOL TO CONTROL PURITY OF ORGANOFLUORINE KETON NOVEC™ 1230

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

Perfluoro-2-methyl-3-pentanone, marketed under the name NOVEC™ 1230, is currently used as a fire extinguishing agent due to its ability to evaporate rapidly and absorb heat. The marketing of this perfluoro-ketone, along with that of many other organofluorine compounds, is subject to strict traceability controls by customs authorities because they have been classified as potent greenhouse gases. The regulation of these substances has led to the emergence of an illicit market where the degree of purity often does not correspond to quality standards. For this reason, it is necessary to increase the number of controls to be carried out by control agencies.
The introduction of new Low-Field benchtop NMR systems has made ^{19}F NMR spectroscopy a potential complementary technique to current protocols based on the use of the valid GC/MS tool.
The aim of the study was to develop a robust analytical method by combining theoretical calculations (DFT) and multinuclear High-Field NMR. First, the spectroscopic features and possible simultaneous presence of conformational isomers in solution were identified. Subsequently, the study then focused on evaluating the stability of NOVEC™ 1230 towards common deuterated solvents.
The results showed that conformational isomers do not form in any common solvents, and that the perfluoro-ketone exhibited marked reactivity towards residual water of deuterated solvents. The reaction follows a base-catalysed mechanism and no take place in not anhydrous chloroform; all degradation products were identified and characterized.[1]
In conclusion, the study showed that a stable and rapid Low-Field NMR method can be used alongside GC/MS to certify the purity of the NOVEC™ 1230.

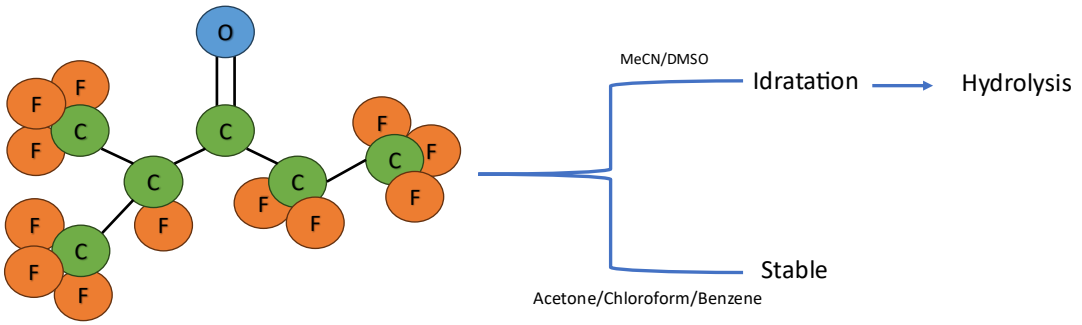

Fig 1. Stability of Perfluoro-2-methyl-3-pentanone in common deuterated organic solvents.

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NMR Characterization of α-Synuclein Interactions with GCase-Targeting Pharmacological Chaperones

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Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for characterizing intrinsically disordered proteins (IDPs) and their interactions at atomic resolution with binding partners, such as small molecules and metal ions. [1] This study focuses on human α-synuclein (α-syn), an IDP closely associated with neurodegenerative disorders, including Parkinson's disease (PD). Mutations in the *GBA1* gene, which encode the lysosomal enzyme glucocerebrosidase (GCase), are responsible for Gaucher disease and represent one of the most common genetic risk factors for PD. Reduced GCase activity promotes α-syn aggregation, thereby contributing to PD pathogenesis. [2]

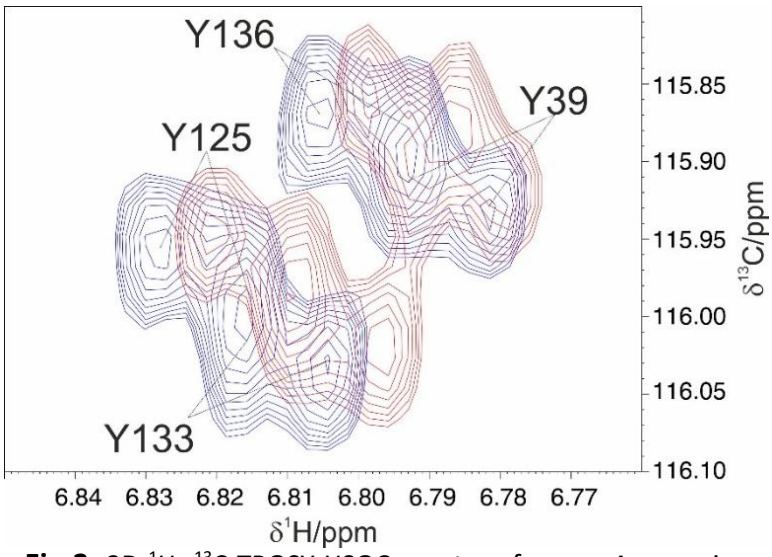

Fig 2. 2D ^{1}H- ^{13}C TROSY HSQC spectra of α-syn. A zoom in the region where the resonances of tyrosine side chains (^{1}H$^{\varepsilon}$- ^{13}C$^{\varepsilon}$) are detected is shown in its free form (blue) and upon the addition of ambroxol (red) corresponding to a molar ratio of 64:1 ambroxol: α-svn.

Following a "dual-target" approach, we investigated ambroxol, a mucolytic drug under clinical evaluation for GCase-related disorders that acts as a pharmacological chaperone (PC) for GCase and also interacts with the intrinsically disordered monomeric form of α-syn.[3] To study the interplay between ambroxol and α-syn, we employed high-resolution NMR spectroscopy, utilizing both ^{13}C- and ^{1}H-detected experiments, focusing on backbone and side-chain changes upon interaction (Fig 1). Side chains generally play a key role in mediating and modulating molecular recognition. Monitoring chemical-shift changes in amino-acid side-chain nuclear spins brings us closer to the main interaction sites, providing additional insight.

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STRUCTURAL AND PHYSICOCHEMICAL ANALYSES OF SINAPIC ACID-LOADED LIPOSOMES

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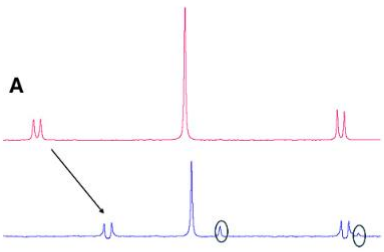
Keywords: solution NMR, small molecules, food

This study is framed within the context of the circular bioeconomy, applied to the field of biocosmetics. Starting from natural matrices and agri-food waste, bioactive compounds are isolated and subsequently encapsulated into lipid-based nanocarriers. This approach aims to protect fragile and volatile molecules from degradation, enhance encapsulation efficiency, and improve their stability within complex formulations [1], such as creams and serums, while employing biodegradable and low-toxicity materials. Among the first biomolecules investigated for loading into lipid nanoparticles, there is sinapic acid, a hydroxycinnamic acid well known for its antioxidant activity and widely present in plant matrices, including rocket salad (*Eruca Sativa*), from Brassicaceae Family. The formulation development, carried out using a microfluidic system, was followed by stability studies performed via Dynamic Light Scattering (DLS) [2], as well as encapsulation efficiency assessment using HPLC-DAD/MS. In addition, structural characterization was conducted through Nuclear Magnetic Resonance (NMR) [3], highlighting a conformational rearrangement associated with the transition from the free to the phospholipid membrane-bound state. Future investigations will include advanced structural analyses, such as Small-Angle X-ray Scattering (SAXS) [4], Fourier Transform Infrared Spectroscopy (FTIR), and Differential Scanning Calorimetry (DSC), to gain deeper insight into the nanoscale organization, molecular interactions, and thermal behavior of the lipid carriers, further elucidating the interaction between Sinapic Acid and the phospholipid bilayer.



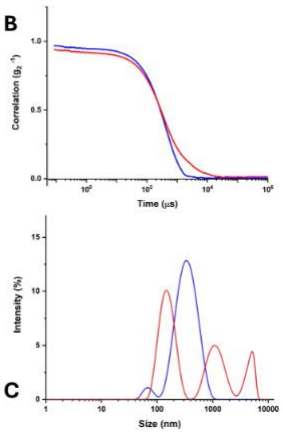


Fig. 1. ^{1}H NMR spectra of free and encapsulated Sinapic Acid, in magenta and blue, respectively.
Fig. 2 One month-stability of loaded (blue) vs. empty liposomes (red) by DLS correlogram (2B) and Intensity-based Size Distribution (2C).

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CYCLOPHILIN D: INTERACTION STUDIES WITH SMALL MOLECULES BY NMR AND NATIVE MS

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Keywords: solution NMR, small molecules, biomolecules.

Cyclophilin D (CypD) is a peptidyl-prolyl isomerase (PPIase) involved in the regulation of the opening of mitochondrial permeability transition pore (mPTP), a channel located in the inner mitochondrial membrane and presumably formed by the F_1F_0-ATP synthase. mPTP opening can be caused by Ca^{2+} overload, increased ROS levels or the interaction with CypD, which sensitizes the pore opening to Ca^{2+}. [1]
A well-known CypD inhibitor is Cyclosporine A (CsA); however, it is not selective and causes numerous side effects due to its immunosuppressive nature, in addition to exhibiting limited permeability across the blood-brain barrier. For this reason, the search for new CypD inhibitors is important, since its prolonged opening is associated with cell death and several neurodegenerative diseases.
We therefore focused on the identification of small molecules that interact with CypD, to test them by 2D NMR spectroscopy and native mass spectrometry.
Three small molecules were identified from the literature. The first, F9850666 from the ChemDiv database, was previously tested by surface plasmon resonance (SPR) and showed a nanomolar K_D value, forming hydrogen bonds with the active site of CypD. [2]
(-)-Epigallocatechin-3-gallate (EGCG) was identified as another compound with a micromolar K_D. To further investigate this reported interaction, we focused our study on gallic acid as a core scaffold for a potential class of inhibitors. [3]
The final compound investigated was Ebselen, a bioavailable molecule capable of crossing the blood-brain barrier and with cytoprotective properties. In vivo studies demonstrated its ability to inhibit mPTP opening, while the crystal structure of the CypD-Ebselen complex revealed the formation of a covalent S-Se bond with a cysteine residue in the active site of CypD. [4]
Not all the compounds proved to be promising candidates; however, the S-Se covalent bond may represent an interesting starting point to allow modulation rather than complete inhibition of CypD activity.

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Fast Field Cycling Relaxometry: From Molecular Dynamics to Industrial Applications

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Keywords: theory and methods, instrumentation, Fast Field Cycling NMR.

Fast Field Cycling NMR Relaxometry (FFC NMR) is a powerful NMR technique that enables the measurement of longitudinal relaxation times (T_1) over a broad range of magnetic fields, spanning several orders of magnitude without changing the spectrometer frequency. The resulting Nuclear Magnetic Relaxation Dispersion (NMRD) profiles provide valuable insights into molecular dynamics by probing motion over a wide range of correlation times.
In particular, access to low Larmor frequencies allows the investigation of slow molecular processes, including surface dynamics, collective motions, and intermolecular interactions, which are often difficult to characterize using conventional spectroscopic techniques. Because radiofrequency radiation can penetrate a wide variety of materials, FFC NMR is particularly suited to heterogeneous and complex systems, including liquids, solids, gels, and aggregated materials.
In this work, recent developments in FFC NMR are presented together with selected applications demonstrating its potential as a tool for structural and dynamic characterization. NMRD profiles are used to investigate molecular mobility, identify weak intermolecular interactions, and provide qualitative and quantitative information on the structure and dynamics of complex materials.
Beyond its value as a fundamental research technique, these capabilities highlight the potential of FFC NMR for practical applications in material characterization, quality assessment, and off-line process monitoring. The development of advanced FFC NMR instrumentation and methodologies represents an important step toward transferring NMR relaxometry from a predominantly research-oriented technique to a more accessible and versatile industrial analytical tool.

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^{1}H NMR as a Tool for Monitoring Extracellular Medium Evolution in Microbial Cultures

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

Xylella fastidiosa is a slow-growing and nutritionally demanding bacterium, making robust analytical workflows essential for investigating its extracellular metabolic activity under in vitro conditions. In this work, ^{1}H NMR spectroscopy was applied as a non-destructive tool to monitor the temporal evolution of bacterial culture supernatants grown in two media with different chemical complexity: XFM, a defined minimal medium, and PD3, a richer cultivation medium.[1]
Supernatants were collected at five time points, centrifuged to remove cells, aliquoted, and stored at -80 °C until analysis. Sample preparation and acquisition conditions were optimized to obtain reproducible 1D ^{1}H NMR spectra from complex extracellular matrices. Different pulse sequences were compared, and CPMG provided better baseline quality and signal resolution than NOESY, especially in samples containing proteinaceous components.
The optimized workflow revealed clear time-dependent spectral changes in both media and confirmed the suitability of ^{1}H NMR for monitoring extracellular medium evolution in slow-growing microbial systems. This approach provides a practical methodological basis for future exometabolomic and footprinting applications.[2]

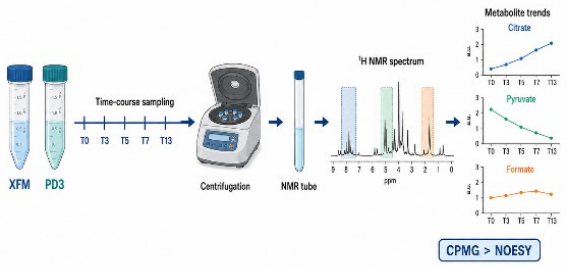

Fig. 1. Workflow for time-resolved ^{1}H NMR monitoring of bacterial culture supernatants in two media

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