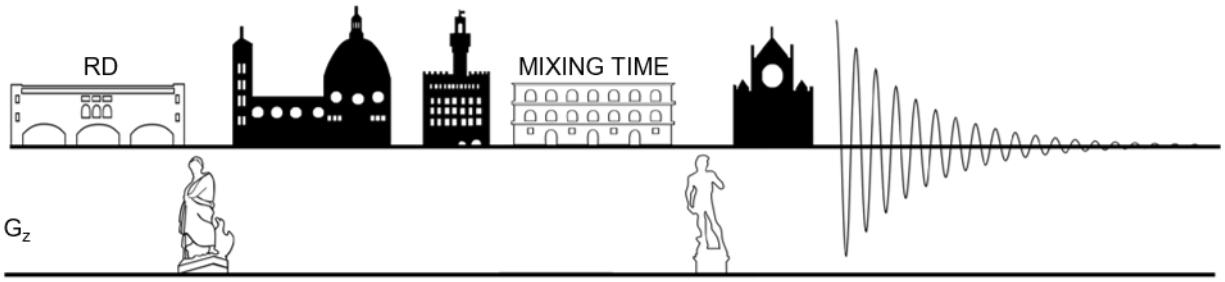
51st National Congress on Magnetic Resonance



September 4-6, 2024 | Florence

Building D4, University of Florence, Via delle Pandette 35, Florence

GIDRM
Gruppo Italiano Discussione Risonanze Magnetiche

Università degli Studi Firenze
Da un secolo, oltre.

51st National Congress on Magnetic Resonance

September 4-6, 2024 | Florence

Scientific Committee

Michele Remo Chierotti - *University of Turin*

Silvia Borsacchi - *CNR-ICCOM, Pisa*

Simonetta Geninatti Crich - *University of Turin*

Giacomo Parigi - *University of Florence*

Laura Ragona - *SCITEC-CNR, Milan*

Antonio Randazzo - *University of Naples Federico II*

Luigi Russo - *University of Campania Luigi Vanvitelli*

Organizing Committee

Veronica Ghini - *University of Florence*

Giacomo Parigi - *University of Florence*

Mario Piccioli - *University of Florence*

Leonardo Querci - *University of Florence*

Paola Turano - *University of Florence*

GENERAL INFORMATION

VENUE

Building D4 - Polo di Novoli
University of Florence
Via delle Pandette 35, 50127, Firenze

INVITED SPEAKERS

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

Maria Rosaria “Sasi” Conte - *King’s College London*

Dominik Kubicki - *University of Birmingham*

Mathilde Hauge Lerche - *Technical University of Denmark*

Claudio Luchinat - *University of Florence*

Alceo Macchioni - *University of Perugia*

Roberto Fattorusso, Winner of the GIDRM/GIRM Gold Medal 2024 – *University of Campania Luigi Vanvitelli*

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

Cristina Airoldi - *University of Milan Bicocca*

Francesca Cantini - *University of Florence*

Angelo Gallo - *University of Turin*

Cinzia Ingallina - *University of Rome La Sapienza*

Marilisa Leone - *CNR-IBB, Naples*

Alfonso Pedone - *University of Modena e Reggio Emilia*

Gabriele Stevanato - *University of Padua*

Claudia Testa - *University of Bologna*

POSTER SESSIONS

Poster session 1

Wednesday, September 4th, 16:05-17:00, ODD abstract numbers

Poster session 2

Thursday, September 5th, 10:30-11:20, EVEN abstract numbers

Poster session 3

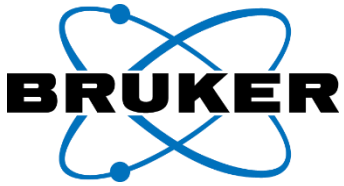
Thursday, September 5th, 12:50-14:10, ODD abstract numbers

Poster session 4

Thursday, September 5th, 16:10-17:30, EVEN abstract numbers

UNDER THE AUSPICES OF

GIDRM GRATEFULLY ACKNOWLEDGES ITS PARTNERS FOR FINANCIAL SUPPORT TO THE CONFERENCE



GRUPPO ITALIANO
DISCUSSIONE RISONANZE MAGNETICHE

51ST NATIONAL CONGRESS ON MAGNETIC RESONANCE

FLORENCE 04-06 SEPTEMBER 2024

SCIENTIFIC PROGRAM

Wednesday September 04th

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**
	Plenary session **Chair: A. Mucci and M.R. Chierotti**
14:30-15:20	**Plenary Lecture 1** **GIDRM/GIRM gold medal award** **Roberto Fattorusso (University of Campania Luigi Vanvitelli)** NMR SPECTROSCOPY APPLIED TO PROTEIN CONFORMATION EQUILIBRIA: MY SCIENTIFIC AND HUMAN TRIP
15:20-16:05	**Plenary Lecture 2** **Chair: A. Randazzo** **Maria Rosaria "Sasi" Conte (King's College London)** TELLING THE TALE OF LA-RELATED PROTEINS THROUGH NMR AND BIOPHYSICAL CHARACTERISATIONS
16:05-17:00	**Coffee break + Poster session (ODD abstract numbers)**

	Parallel session A **Chair: L. Russo**	**Parallel session B** **Chair: G. Parigi**
17:00-17:30	**Leone M.** SEARCHING FOR NOVEL ANTICANCER AGENTS BY NMR-BASED APPROACHES: SAM DOMAINS AS CHALLENGING TARGETS	**Stevanato G.** PARAHYDROGEN BASED PYRUVATE HYPERPOLARIZATION
17:30-17:45	**Castiglione F.** RECENT ADVANCES IN HYALURONIC ACID-BASED HYDROGELS: ^{1}H HR-MAS INVESTIGATION OF DIFFUSION MOTION	**Golini C.** PORTABLE LOW FIELD NMR FOR IN SITU DIAGNOSIS AND MONITORING OF CULTURAL HERITAGE
17:45-18:00	**Cerofolini L.** COMBINING SOLID-STATE NMR WITH STRUCTURAL AND BIOPHYSICAL TECHNIQUES TO DESIGN CHALLENGING PROTEIN-DRUG CONJUGATES	**Niccoli L.** NOVEL POLARIZING AGENTS FOR EFFICIENT HIGH FIELD AND FAST MAS DYNAMIC NUCLEAR POLARIZATION
18:00-18:15	**Di Carluccio C.** SIGLEC-7 NMR ASSIGNMENT AND BINDING SPECIFICITIES TO DISIALYLATED GANGLIOSIDES	**Licciardi G.** HIGH RESOLUTION RELAXOMETRY FOR MULTISCALE DYNAMICS IN COMPLEX SYSTEMS
18:15-18:30	**Bracaglia L.** STRUCTURALLY HETEROGENEOUS PROTEINS STUDIED BY RELAXATION-EDITED NMR EXPERIMENTS	**Pierigè M.** EFFECT OF RESINS ON SBR DYNAMICS BY SOLID STATE NMR AND NMR RELAXOMETRY
	Plenary session	

	Chair: S. Geninatti Crich
18:30-18:45	**Sponsorship Lecture (Stelar): A. Nagmutdinova** FFC NMR APPLICATION IN FOOD SCIENCE AND BEYOND
18:45-19:30	**Plenary Lecture 3** **Mathilde Hauge Lerche (Technical University of Denmark)** ENHANCING NMR SENSITIVITY FOR COMPLEX MIXTURE ANALYSIS THROUGH HYPERPOLARIZATION

Thursday September 05th

	Plenary session **Chair: G. Parigi**
8:45-9:30	**Plenary Lecture 4** **Claudio Luchinat (University of Florence)** IMPROVING THE INFORMATION CONTENT OF NMR SPECTRA OF METABOLOMIC SAMPLES
9:30-9:45	**Sponsorship Lecture (Bruker): A. Moreno** NEW EXPERIMENTS IN TOPSPIN: PURESHIFT, GEMSTONE, DOSY, AND MORE!
9:45-10:15	**Chair: M.R. Chierotti** **Under 35 GIDRM** **M. Spano (Sapienza University of Rome)** NMR METABOLOMICS TO BETTER DEFINE ALTERNATIVE AND SUSTAINABLE FOOD SOURCES
10:15-10:30	**Sponsorship Lecture (Jeol): A. Botana** THE MILLIGRAM CHALLENGE

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

	Parallel session A **Chair: A. Randazzo**	**Parallel session B** **Chair: D. Kubicki**
11:20-11:50	**Ingallina C.** METABOLOMIC MARVELS: DECODING THE FOOD WORLD THROUGH NMR SPECTROSCOPY	**Gallo A.** SOLID STATE NMR AS ELEGANT TOOL IN CRYSTAL STRUCTURE DETERMINATION OF SMALL ORGANIC MOLECULES
11:50-12:05	**Sozzi M.** CHALLENGES IN MEASURING ETHANOL CONTENT IN ALCOHOLIC BEVERAGES USING ^{1}H NMR: METHOD EVALUATION AND A WINE CASE STUDY	**Piva S.** DYNAMICS OF FAST-ROTATING ROTORS IN A METAL-ORGANIC FRAMEWORK AS EXPLORED BY SOLID-STATE NMR
12:05-12:20	**Cicero D.** NOVEL INSIGHTS INTO METABOLIC CROSSTALK IN MULTIMORBID PATIENTS USING NMR SPECTROSCOPY	**Bravetti F.** SYNTHESIS AND SOLID-STATE NMR-DRIVEN CRYSTAL STRUCTURE DETERMINATION OF THE FIRST CO-DRUG OF VALPROIC ACID AND L-CARNITINE
12:20-12:35	**Brigante F.** NOVEL CHARACTERIZATION OF PLANT-BASED BEVERAGES BY 1D AND 2D NMR	**Della Latta E.** USING ^{13}C HIGH-RESOLUTION SOLID-STATE NMR TECHNIQUES TO INVESTIGATE A Cu(II)-BASED PARAMAGNETIC MOF
12:35-12:50	**Zampieri S.** ^{1}H-NMR FOR MASLD DIAGNOSIS: MULTIVARIATE ANALYSIS OF SERUM METABOLITES TO DIFFERENTIATE DISEASE STAGES	**Martini F.** UNVEILING LOCAL DYNAMICS IN TRIPTYCENE-BASED POROUS POLYMERS: A SOLID-STATE NMR STUDY

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

	Plenary session **Chair: S. Borsacchi**
14:10-14:55	**Plenary Lecture 5** **Dominik Kubicki (University of Birmingham)**

	LOCAL STRUCTURE AND DIFFUSION OF SODIUM IN A HYBRID GLASS FROM HIGH-TEMPERATURE ^{23}Na MAS NMR AT 20 T
14:55-15:10	**Sponsorship Lecture (Bracco): A. Macula** ASSESSING THE RELIABILITY OF VIRTUAL CONTRAST: A GENERALIZATION STUDY ACROSS DIFFERENT TUMOR TYPES AND PATIENT DEMOGRAPHICS
15:10-15:25	**Sponsorship Lecture (Magritek/FKV): D. Bouillaud** NEW BENCHMARK FOR BENCHTOP NMR MAGNET HOMOGENEITY: HOW SPINSOLVE™ ULTRA NMRS BOOST SOLVENT SUPPRESSION PERFORMANCE FOR 1- AND 2D NMR
	Chair: L. Ragona **Segre-Capitani Fellowships**
15:25-15:40	**C. Papi (University of Turin)** A MRI-CEST METHOD FOR MAPPING WATER CYCLING ACROSS CELLULAR MEMBRANES
15:40-15:55	**A. Gambini (University of Modena and Reggio Emilia)** NMR-BASED METABOLOMICS IN MNGIE RARE DISEASE
15:55-16:10	**A. Barbanente (University of Bari)** AN NMR-BASED APPROACH TO ELUCIDATE THE LINK BETWEEN DIETARY ZINC STORAGE AND TRYPTOPHAN METABOLISM

16:10-17:30	**Coffee break + Poster session (EVEN abstract numbers)**
16:40-17:30	**GIRM assembly**
17:30-19.30	**GIDRM assembly + announcement of poster competition winner**

20:30	**social dinner**

Friday September 06st

	Plenary session **Chair: P. Turano**
8:45-9:30	**Plenary Lecture 6** **Alceo Macchioni (University of Perugia)** APPLICATION OF NMR TO THE STUDY OF CATALYTIC REACTIONS

	Parallel session A **Chair: S. Geninatti Crich**	**Parallel session B** **Chair: A. Macchioni**
9:30-10:00	**Testa C.** MAPPING BRAIN CONNECTIVITY THROUGH WATER MOTION	**Airoldi C.** NMR-BASED IDENTIFICATION AND DEVELOPMENT OF BIOACTIVE COMPOUNDS
10:00-10:15	**Brero F.** OPTIMIZATION OF IRON OXIDE MAGNETIC NANOPARTICLES FOR ENHANCED MRI CONTRAST AND HYPERTHERMIA TREATMENT	**Di Pietro M.E.** CHALLENGES IN USING NMR FOR SUSTAINABLE STRUCTURED SOLVENTS: CAUTION IN INTERPRETING INTERMOLECULAR NOE
10:15-10:30	**Quattrociocchi C.** IN VITRO AND IN VIVO MR IMAGING OF THE CAIX EXPRESSION IN BREAST CANCER	**Todisco S.** A MULTINUCLEAR NMR STUDY OF SELECTIVE DEGRADATION OF ORGANOSILICON COMPOUNDS BY OXALATE ION IN ACQUEOUS SOLUTION
10:30-10:45	**Porru M.** ZINC AND MANGANESE DOPED IRON OXIDE MAGNETIC NANOPARTICLES: EFFECT ON THE ^{1}H-NMR RELAXATION PROPERTIES	**Sabena C.** QUANTITATIVE SOLID-STATE NMR ANALYSIS BY CHEMOMETRIC APPROACH
10:45-11:00	**Macchia M.L.** ASSESSING THE IMPACT OF DONOR GROUPS ON THE COORDINATION AND MAGNETIC PROPERTIES OF MONOHYDRATED Fe(III) COMPLEXES FOR MRI APPLICATIONS	**Righetti G.** DEEP EUTECTIC SOLVENTS FOR HMF VALORIZATION

11:00-11:30	**Coffee break**	
	Parallel session A **Chair: M. Piccioli**	**Parallel session B** **Chair: S. Borsacchi**
11:30-12:00	**Cantini F.** SOLUTION NMR TO ADDRESS STRUCTURE AND INTERACTIONS OF METALLOPROTEINS: THE CASE OF THE IRON-SULPHUR CISD3 AND CALCIUM BINDING PROTEIN CIB2	**Pedone A.** ADVANCEMENT IN THE SIMULATIONS OF SOLID-STATE NMR SPECTRA OF OXIDE GLASSES: INTEGRATING AB INITIO CALCULATIONS AND MACHINE LEARNING
12:00-12:15	**Acconcia C.** NMR-GUIDED DESIGN OF SELECTIVE INTEGRIN LIGANDS	**Ravera E.** AN INTEGRATIVE VIEW ON BIOINSPIRED SILICA-LYSOZYME COMPOSITES
12:15-12:30	**Grasso D.** INVESTIGATION OF THE DYNAMICS/FUNCTION RELATIONSHIP OF PHOSPHORYLATED ONCOPROTEIN YB-1 BY NUCLEAR MAGNETIC RESONANCE	**Pizzanelli S.** STRUCTURE AND DYNAMICS OF HIGHLY CONCENTRATED LiCl METHANOLIC SOLUTIONS CONFINED IN A MESOPOROUS SILICA: AN NMR STUDY
12:30-12:45	**Grifagni D.** AN ABERRANT INTERACTION BETWEEN THE PATHOGENIC P144L MUTANT OF FDX2 AND FDXR PROVIDES THE MOLECULAR GROUNDS OF AN ULTRA-RARE HUMAN GENETIC DISORDER	**Fiorucci L.** REDEFINING THE TOOLBOX TOWARDS PRECISE PARAMAGNETIC NMR PREDICTIONS AT ULTRAHIGH FIELDS
	Plenary session **Chair: L. Ragona and M.R. Chierotti**	
12:45-13:25	**Poster competition winner lectures**	
13:25-13:30	**Closing**	
13:30-14:30	**Lunch**	

Wednesday 4th

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**
	Plenary session **Chair: A. Mucci and M.R. Chierotti**
14:30-15:20	**Plenary Lecture 1** **GIDRM/GIRM gold medal award** **Roberto Fattorusso (University of Campania Luigi Vanvitelli)** NMR SPECTROSCOPY APPLIED TO PROTEIN CONFORMATION EQUILIBRIA: MY SCIENTIFIC AND HUMAN TRIP
15:20-16:05	**Plenary Lecture 2** **Chair: A. Randazzo** **Maria Rosaria "Sasi" Conte (King's College London)** TELLING THE TALE OF LA-RELATED PROTEINS THROUGH NMR AND BIOPHYSICAL CHARACTERISATIONS
16:05-17:00	**Coffee break + Poster session (ODD abstract numbers)**

	Parallel session A **Chair: L. Russo**	**Parallel session B** **Chair: G. Parigi**
17:00-17:30	**Leone M.** SEARCHING FOR NOVEL ANTICANCER AGENTS BY NMR-BASED APPROACHES: SAM DOMAINS AS CHALLENGING TARGETS	**Stevanato G.** PARAHYDROGEN BASED PYRUVATE HYPERPOLARIZATION
17:30-17:45	**Castiglione F.** RECENT ADVANCES IN HYALURONIC ACID-BASED HYDROGELS: ^{1}H HR-MAS INVESTIGATION OF DIFFUSION MOTION	**Golini C.** PORTABLE LOW FIELD NMR FOR IN SITU DIAGNOSIS AND MONITORING OF CULTURAL HERITAGE
17:45-18:00	**Cerofolini L.** COMBINING SOLID-STATE NMR WITH STRUCTURAL AND BIOPHYSICAL TECHNIQUES TO DESIGN CHALLENGING PROTEIN-DRUG CONJUGATES	**Niccoli L.** NOVEL POLARIZING AGENTS FOR EFFICIENT HIGH FIELD AND FAST MAS DYNAMIC NUCLEAR POLARIZATION
18:00-18:15	**Di Carluccio C.** SIGLEC-7 NMR ASSIGNMENT AND BINDING SPECIFICITIES TO DISIALYLATED GANGLIOSIDES	**Licciardi G.** HIGH RESOLUTION RELAXOMETRY FOR MULTISCALE DYNAMICS IN COMPLEX SYSTEMS
18:15-18:30	**Bracaglia L.** STRUCTURALLY HETEROGENEOUS PROTEINS STUDIED BY RELAXATION-EDITED NMR EXPERIMENTS	**Pierigè M.** EFFECT OF RESINS ON SBR DYNAMICS BY SOLID STATE NMR AND NMR RELAXOMETRY

	Plenary session **Chair: S. Geninatti Crich**
18:30-18:45	**Sponsorship Lecture (Stelar): A. Nagmutdinova** FFC NMR APPLICATION IN FOOD SCIENCE AND BEYOND
18:45-19:30	**Plenary Lecture 3** **Mathilde Hauge Lerche (Technical University of Denmark)** ENHANCING NMR SENSITIVITY FOR COMPLEX MIXTURE ANALYSIS THROUGH HYPERPOLARIZATION

NMR SPECTROSCOPY APPLIED TO PROTEIN CONFORMATION EQUILIBRIA: MY SCIENTIFIC AND HUMAN TRIP

Roberto Fattorusso,[a]

[a]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Via Vivaldi 43, 81100, Caserta, Italy
E-mail: roberto.fattorusso@unicampania.it

Keywords:: solution NMR, biomolecules

NMR spectroscopy is an invaluable tool to investigate the relationship between structure, dynamics and activity of proteins in solution and cellular environment. Often, the three-dimensional protein structure fails to provide a complete understanding of its functional mechanism. In most biological functions, protein motions and conformational equilibria are the essential link to connect high-resolution structural features with cellular processes including protein folding, enzymatic catalysis, protein aggregation, solute transport and synaptic transmission. A personal view of the key NMR methodologies, challenges encountered, and solutions devised in understanding the interplay between protein structural dynamics and functions, as well as the role of dynamics on my daily NMR-related activities, will be presented.

TELLING THE TALE OF LA-RELATED PROTEINS THROUGH NMR AND BIOPHYSICAL CHARACTERISATIONS

G. Abis, R. Puglisi[a], Y. Gu[a], N. Agrawal[a], J. Sayegh[a], I. Cruz-Gallardo[a], T. Bui[a,b], J. Jarvis[a,b], G. Kelly[c], M. R. Conte[a]

[a]Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences, King's College London, UK
[b]Centre for Biomolecular Spectroscopy, King's College London, London SE1 1UL, UK
[c]MRC Biomedical NMR Centre, The Francis Crick Institute, London NW1 1AT, UK
E-mail: sasi.conte@kcl.ac.uk

Keywords:
Solution NMR, biomolecules.

LaRP4A and LaRP4B are members of the La-related protein (LaRPs) superfamily [1]. Both LaRP4 paralogs regulate translation, associates with polysomes and stabilise mRNAs subsets. They have also been implicated in cancer progression and development [1,2]. It has been suggested that LaRP4A and LaRP4B perform distinct functions, executed through recognition of their RNA and protein partners, but how RNA substrate discrimination is achieved remains an unsolved conundrum [1,3]. Previous work from our lab showed that in LaRP4A, the LaRP-conserved RNA binding platform, namely the La-module, unexpectedly played only a minor role in the interaction with oligoA RNAs. Instead, the binding was dominated by an N-terminal region (NTR) of the protein that exists in a semi-disordered state and lacks any recognisable RNA-binding motif [3]. LaRP4B does not interact with polyA, but associated with AU-rich regions present in 3'UTRs of many mRNAs. Here, RNA recognition is also mediated by the N-terminal domain (NTD) containing both the NTR and the La-module; however, contrary to LaRP4A, in LaRP4B the NTR and the La-module both contribute to binding affinity. This is quite unexpected as LaRP4A and LaRP4B NTD have high primary structure identity. Studies on the mechanisms of molecular recognition using Nuclear Magnetic Resonance (NMR) integrated with other methodologies will be presented here, revealing interesting similarities and differences between these two LaRP4 paralogs involved in regulation of translation and implicated in cancer biology. These molecular investigations will also inform on the growing class of RNA binding that use disordered non canonical regions to mediate tight and specific RNA interactions.

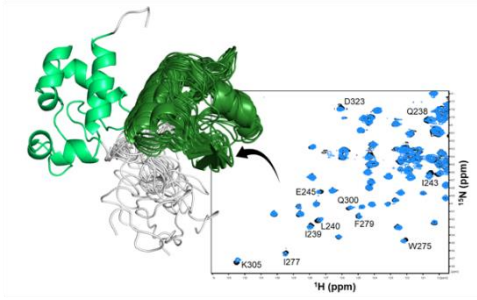


Fig. 1. NMR spectra and structure of LaRP4B.

References

[1] R. Maraia et al, *Wiley Interdiscip Rev RNA*. **8**(6). doi: 10.1002/wrna.1430 (2017)
[2] J C Coleman et al, *iScience.* **24**, 109288. (2024). doi: 10.1016/j.isci.2024.109288.
[3] I Cruz-Gallardo et al, *Nucleic Acids Res.* **47**, 4272-4291 (2019). doi: 10.1093/nar/gkz144.

SEARCHING FOR NOVEL ANTICANCER AGENTS BY NMR-BASED APPROACHES: SAM DOMAINS AS CHALLENGING TARGETS

M. Leone[a]

[a]Institute of Biostructures and Bioimaging (IBB-CNR), Via P. Castellino 111, 80131, Naples, Italy
E-mail: marilisa.leone@cnr.it

Keywords: solution NMR, biomolecules.

Sam domains represent small protein binding modules with a five-helix bundle fold. They are known for their versatility by playing roles in a variety of cellular processes. Although binding of Sam domains to lipids, RNA and other proteins has been reported, they show principally a propensity to form oligomers or polymers through Sam-Sam homotypic and heterotypic associations [1]. For quite a few Sam-Sam complexes a link to disease onset and progression has been established, thus, finding original molecular tools able to inhibit such interactions attracts interest in the drug discovery field. Sam-Sam complexes often adopt a canonical Mid Loop/End Helix structural model: the central regions of one Sam domain bind to the C-terminal helix and adjacent loops of another Sam domain. Sam-Sam binding interfaces are large and flat and consequently, their targeting by small molecules is very challenging while peptides represent ideal candidates as modulators of such interactions [2].
Our laboratory is focused on the discovery of peptide inhibitors of Sam-Sam interactions involving EphA2 [2]. EphA2 is a receptor tyrosine kinase that holds great interest in the anticancer drug discovery field, as it is overexpressed in diverse cancer types. EphA2 possesses a C-terminal Sam domain representing the site where protein regulators of receptor stability are recruited.
For our studies we are employing mixed experimental and computational approaches where NMR techniques are first employed for identification / validation of novel peptide ligands of Sam domains, and then to guide, through structural data, the optimization route. I will report on diverse examples related to linear and cyclic peptides targeting the EphA2-Sam interactome [2, 3].

References

[1] M. Vincenzi, F. A. Mercurio, and M. Leone *Curr. Med. Chem.* **27**, 450-476 (2020)
[2] F. A. Mercurio, M. Vincenzi, and M. Leone *Int. J. Mol. Sci.* **23,** 10397 (2022)
[3] M. Vincenzi, F. A. Mercurio, C. Di Natale, R. Palumbo, L. Pirone, S. La Manna, D. Marasco, E.M. Pedone, and M. Leone *Bioorg. Chem.* **122**, 105680 (2022)

Acknowledgement: The research leading to these results has received funding from AIRC under IG 2021 – ID. 26121 – P.I. Leone Marilisa.

RECENT ADVANCES IN HYALURONIC ACID-BASED HYDROGELS: ^{1}H HR-MAS INVESTIGATION OF DIFFUSION MOTION

F.Castiglione[a], V. Vanoli[a], M.Casalegno[a], F. Pizzetti[a], F. Rossi[a], A. Mele[a]

[a]Dipartimento di Chimica, Materiali e Ingegneria Chimica "G. Natta", Politecnico di Milano, via Mancinelli 7, I-20131 Milano (MI), Italy
E-mail: franca.castiglione@polimi.it

Keywords: High resolution magic angle spinning (HR-MAS) NMR, diffusion.

Hydrogels based on hyaluronic acid (HA) and agarose-carbomer (AC), due to their peculiar 3D architecture and biocompatibility, are promising candidates for pharmaceutical strategies based on the controlled delivery of single or multiple drugs to target different diseases with better patient compliance. The successful development of these applications requires a detailed understanding of the drugs transport motion in the gel matrix and release mechanism [1]. In this study, such an investigation is carried out on HA-AC hydrogels, prepared with variable mesh size, and then loaded with ethosuximide and sodium salicylate as single and dual drug formulations. Transport properties and intermolecular drug-drug/drug-polymer interactions are characterized by means of High Resolution Magic Angle Spinning NMR Spectroscopy (Fig. 1). Analysis of the experimental data, for the single drug formulation, provides evidence of superdiffusive motion for sodium salycilate while ethosuximide molecules exhibit unrestricted diffusion within the gel matrix [2]. Conversely, a similar diffusion dynamic within the hydrogel network is observed for the dual drug loading.
The effect of drug-drug interactions on release profiles is also investigated by means of *in vitro* release experiments.

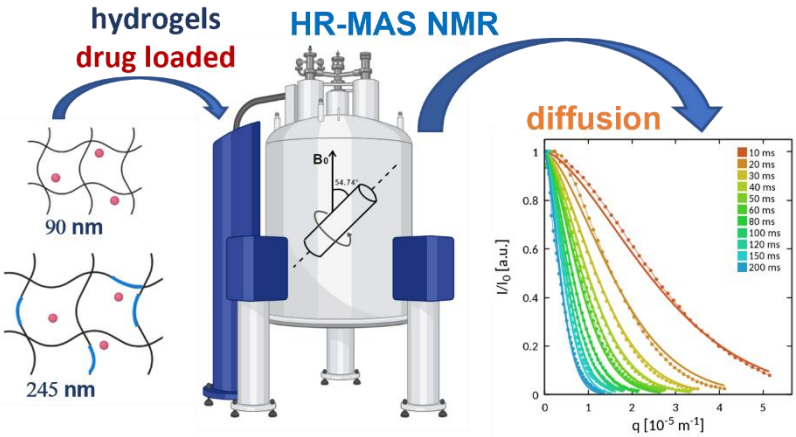


Fig. 1. Schematic representation of HR-MAS NMR spectroscopy application to study drug diffusion in hydrogels.

References

[1] F. Castiglione, M. Casalegno, M. Ferro, F. Rossi, G. Raos, A. Mele *Journal of Controlled Release* **305**, 110–119 (2019)
[2] V. Vanoli, S. Delleani, M.Casalegno, F. Pizzetti, P. Makvandi, H. Haugen, A. Mele, F. Rossi, F. Castiglione *Carbohydrate Polymers* **301,** 120309 (2023)

COMBINING SOLID-STATE NMR WITH STRUCTURAL AND BIOPHYSICAL TECHNIQUES TO DESIGN CHALLENGING PROTEIN-DRUG CONJUGATES

L. Cerofolini,[a,b,c] K. Vasa,[c] E. Bianconi,[d] M. Salobehaj [a,b,c], G. Cappelli,[c] A. Bonciani,[c] G. Licciardi,[a,b,c] A. Pérez-Ràfols, [a,e] L. Padilla-Cortés,[a,b,c] S. Antonacci,[a,c] D. Rizzo,[a,b,c] E. Ravera,[a,b,c] C. Viglianisi,[c] V. Calderone,[a,b,c] G. Parigi,[a,b,c] C. Luchinat,[a,b,c,e] A. Macchiarulo, [d] S. Menichetti,[c] M. Fragai. [a,b,c]

[a]Magnetic Resonance Centre (CERM), University of Florence, Via L. Sacconi 6, 50019, Sesto Fiorentino, Italy.
[b]Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP), Via L. Sacconi 6, 50019, Sesto Fiorentino, Italy.
[c]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3-13, 50019, Sesto Fiorentino, Italy.
[d]Department of Pharmaceutical Sciences, University of Perugia, Via Fabretti n.48, 06123, Perugia, Italy.
[e]Giotto Biotech s.r.l, Sesto Fiorentino, Via della Madonna del Piano 6, 50019, Florence, Italy.
E-mail: linda.cerofolini@unifi.it

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

Several protein-drug conjugates are currently being used in cancer therapy. These conjugates rely on cytotoxic organic compounds that are covalently attached to the carrier proteins or that interact with them via non-covalent interactions. Human transthyretin (TTR), a physiological protein, has already been identified as a possible carrier protein for the delivery of cytotoxic drugs. Here we show the structure-guided development of a new stable cytotoxic molecule based on a known strong binder of TTR and a well-established anticancer drug [1]. This example is used to demonstrate the importance of the integration of multiple biophysical and structural techniques, encompassing microscale thermophoresis, X-ray crystallography and NMR. In particular, we show that solid-state NMR has the ability to reveal effects caused by ligand binding which are more easily relatable to structural and dynamical alterations that impact the stability of macromolecular complexes.

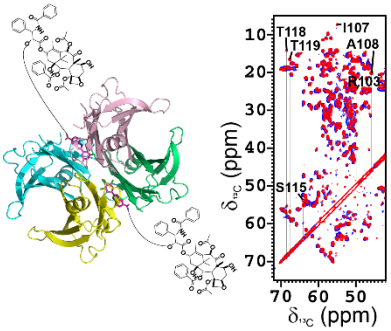


Fig. 1. Solid-state NMR analysis of protein-drug conjugate

References

[1] L. Cerofolini, K. Vasa, E. Bianconi, et al. *Angew Chem* **62**, e202303202 (2023)

SIGLEC-7 NMR ASSIGNMENT AND BINDING SPECIFICITIES TO DISIALYLATED GANGLIOSIDES

C. Di Carluccio[a,b], L. Cerofolini[c], L. Padilla-Cortés[c], G. R. Gheorghita[c], M. Tiemblo Martin[a], H-K. Tseng[d], A. Molinaro[a,b,e], R. Marchetti[a], M. Fragai[b], C. C. Lin[d] and A. Silipo[a,b,e]

[a] Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4I, 80126, Naples, Italy
[b] CEINGE-Biotecnologie Avanzate Franco Salvatore, Via Gaetano Salvatore, 486, 80145 Napoli, Italia
[c] Magnetic Resonance Centre (CERM), CIRMMP, and Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino 50019, Italy
[d] National Tsing Hua University, 101, Section 2, Kuang-Fu Road, Hsinchu 300044, Taiwan
[e] Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Osaka, Japan
E-mail cristina.dicarluccio@unina.it

Keywords: solution NMR, small molecules, biomolecules.

Gangliosides are glycosphingolipids composed of an extra-cellular carbohydrate moiety that is linked to ceramide, a hydrophobic lipid portion embedded in the cellular membrane. They are widely distributed in human cells and tissues and play crucial roles in cellular processes, such as neurotransmission, interaction with regulatory proteins of the nervous system, cell-cell recognition and modulation of signal transduction pathways. Notably, gangliosides containing sialic acids have been found to pronounce effects in cancers, influencing cell behaviors such as proliferation, migration, invasion, adhesion, and angiogenesis, but also mediating immunosuppression of tumors [1]. Certain Siglecs (sialic acid-binding immunoglobulin-like receptors), I-type lectins found on most white blood cells of the immune system, are engaged by endogenous gangliosides to trigger important physiological and pathophysiological signaling events [2]. For example, sialoglycans expressed on cancer cells surface can engage the inhibitory Siglec-7 on natural killer (NK) cells, leading to the inhibition of immune responses [3]. We here present a comprehensive analysis of the structure, conformation, and interactions of α2,8-linked gangliosides, including GD3 and its derivatives DSGb3α3 and DSGb3α6, as well as DSGB5 that contains both α2-3- and α2-6-linked sialic acids and densely populated on renal cell carcinoma (RCC) [4]. Understanding the dynamics of these interactions holds great promise for providing insights into disease mechanisms and potentially opening the door to the development of diagnostic and therapeutic strategies [5]. To this aim we combined structural biology methodologies, protein- and ligand-based NMR techniques, biophysical studies and computational approaches to provide information on binding affinities and 3D models of the complexes [6].

References

[1] R. Schnaar, *Glycoconjugate Journal* **40**,159–167 (2023)
[2] C. Di Carluccio, R.E. Forgione, A. Molinaro, P.R. Crocker, R.Marchetti, A. Silipo. *Carbohydr. Chem.*, **44**, 31–55 (2021).
[3] A. Ito, K. Handa, D.A. Withers, M. Satoh, S. Hakomori. *FEBS Lett* **504**, 82–86 (2001).
[4] P.J. Li, T. Angata, C.C. Lin et al., *Angew. Chem. Int. Ed*. **58**, 11273 –11278 (2019).
[5] J. Liu, *et al. Acta Pharmaceutica Sinica B* **8**, 713–720 (2018).
[6] a) C. Di Carluccio, M.C. Forgione, S. Martini, F. Berti, A. Molinaro, R. Marchetti, A. Silipo. *Carbohydrate Research* **503**, 108313 (2021). b) C. Di Carluccio, R. Marchetti, A. Silipo, et al., *ACS Cent.Sci.***10**, 447−459 (2024).

STRUCTURALLY HETEROGENEOUS PROTEINS STUDIED BY RELAXATION-EDITED NMR EXPERIMENTS

L. Bracaglia,[a,b] S. Oliveti,[a,b] I. C. Felli, [a,b] R. Pierattelli [a,b]

[a]Magnetic Resonance Center (CERM), University of Florence, Via Luigi Sacconi 6, 50019, Sesto Fiorentino, Italy
[b]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019, Sesto Fiorentino, Italy
E-mail: lorenzo.bracaglia@unifi.it

Keywords: Solution NMR, biomolecules, theory and methods.

CREB-binding protein (CBP) is a transcriptional coactivator involved in the transcription of several human genes as well as in many signalling pathways [1]. More than 60% of the protein's residues are predicted to be disordered, while the others are organized in seven globular domains. So far, characterization of both folded domains and disordered regions of CBP has been carried out by isolating them one by one. Although this approach greatly simplifies the *in-vitro* application of structural biology techniques such as NMR spectroscopy and X-ray crystallography, it allows for the study of a single domain at a time. In this work, we propose a different approach that aims to study two contiguous CBP domains with NMR spectroscopy, the CBP-TAZ4 construct. This construct is formed by the zinc-binding domain TAZ2 [2] and the flanking ID4 disordered region [3]. The two domains have very different relaxation properties, that reflect their structural features. Here we show that two- and three-dimensional NMR experiments can be tuned to exploit these differences to enhance spectral quality and obtain clean information about the two domains.
Moreover, our data show that the presence of ID4 alters the structural features of TAZ2 and vice versa. NMR spectroscopy, and ^{13}C-direct detected NMR in particular, has been proven to provide clean information about highly flexible disordered regions also when part of complex multidomain proteins [4]. With this work we further expand the NMR toolbox to address these complex systems.

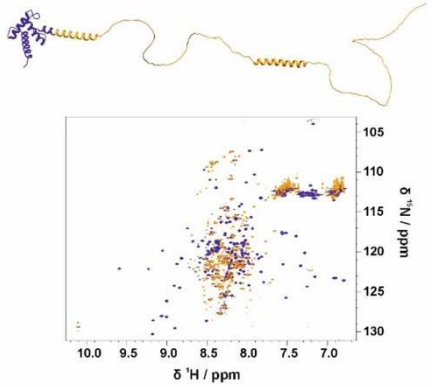


Fig. 1. Structural model of TAZ4 is reported on top of the figure. Relaxation-edited ^{1}H-^{15}N HSQC spectrum is reported on the bottom part of the figure.

References

[1] H. J. Dyson and P. E. Wright, *J. Biol. Chem.* **291**, 6714–6722 (2016).
[2] R. N. De Guzman, H. Y. Liu, M. Martinez-Yamout, H. J. Dyson and P. E. Wright, *J. Mol. Biol.*, **303**(2), 243-253 (2000)

[3]. A. Piai, O. E. Calçada, T. Tarenzi, A. Del Grande, M. Varadi, P. Tompa, I. C. Felli and R. Pierattelli, *Biophys. J.*, **110**(2), 372–381 (2016).
[4] I. C. Felli and R. Pierattelli, *Chem. Rev.* **122**, 9468–9496 (2022).

PARAHYDROGEN-BASED PYRUVATE HYPERPOLARIZATION

G. Stevanato,[a] Y. Ding,[b] S. Glöggler[b]

[a]Università degli Studi di Padova, Via Marzolo 1, Italia
[b]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany
E-mail: Gabriele.stevanato@unipd.it

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

Techniques for increasing the NMR signal have a lot of potential for addressing the basic sensitivity limit of nuclear magnetic resonance. The most well-known one, dynamic nuclear polarisation, has been around for more than 20 years and has progressed from being a tool for academics to becoming equipment that is already in some hospitals. The primary benefit of DNP is its capacity to magnify the relevant metabolites' NMR signal, which can yield insightful biology and clinical data. This is especially true for pyruvate, already in clinical trials, whose metabolism can distinguish healthy from diseased tissues. However, the expensive cost and intricate technical requirements of the necessary instrumentation somehow have so far prevented DNP technology from being widely used.
Here, we demonstrate the successful application of a far more accessible technology, named PHIP-SAH and that is based on parahydrogen, to pyruvate signal amplification for chemical and in-cell biological reaction monitoring. The NMR signal is amplified at room temperature, in solution, and in seconds, enabling real-time NMR monitoring in vitro and in cells. Pulsed strategies to selectively amplify the nuclear spins of interest in the molecule, together with a protocol ensuring the biocompatibility of the hyperpolarized pyruvate, are presented [1].
Finally, I will provide a brief overview of SABRE, a PHIP-SAH parent approach, as a potential way to overcome some of the present roadblocks to make hyperpolarized NMR a conventional tool for every laboratory.

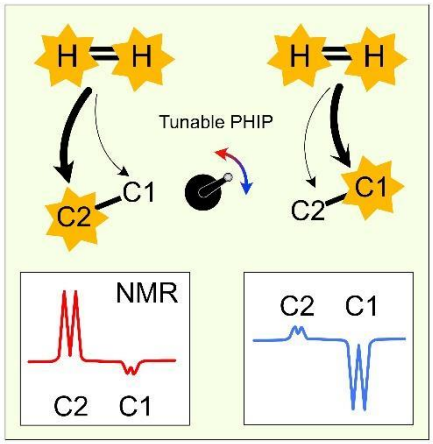


Fig. 1. Methodology for [1,2-^{13}C]pyruvate hyperpolarization

References

[1] Gabriele Stevanato, Yonghong Ding, Salvatore Mamone, Anil P. Jagtap, Sergey Korchak, and Stefan Glöggler *J. Am. Chem. Soc.* 2023, **145**, 10, 5864–5871

PORTABLE LOW FIELD NMR FOR IN SITU DIAGNOSIS AND MONITORING OF CULTURAL HERITAGE

C. Golini,[a] J. Frick,[b] L. Brizi[a] , B. Blümich[c], J. Anders[d]

[a] Department of Physics and Astronomy "Augusto Righi", University of Bologna, Italy
[b] Materials Testing Institute, University of Stuttgart, Germany
[c] Institute of Technical and Macromolecular Chemistry, University of Aachen, Germany
[d] Institute of Smart Sensors, University of Stuttgart, Germany
E-mail: carlo.golini2@unibo.it

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

Non-destructive techniques are key to assessing the effectiveness of treatments and the state of conservation of cultural heritage. Nuclear magnetic resonance (NMR) relaxometric profiling is an innovative, non-invasive, and non-destructive candidate to address these issues directly in situ using single-sided portable devices [1] [2]. Two applications are presented:
(a) Conservation methods for natural stone on buildings and monuments, such as consolidation and hydrophobisation treatments to improve their long-term durability [3]. NMR can localize the consolidation agent and the water uptake of the stone.
(b) Investigations of the structure of paintings providing essential information on their physical condition and pictorial technique to plan appropriate conservation measures [4]. NMR could avoid the sampling of painting fragments for stratigraphic analysis.
We present diagnostic and monitoring results from two sites in Germany: The *Hoppenlau Friedhof* in Stuttgart for stone monument conservation (results not shown) and the *Gründonnerstagsretabel* in the sacristy of Freising Cathedral for painting restoration (results in Fig. 1).

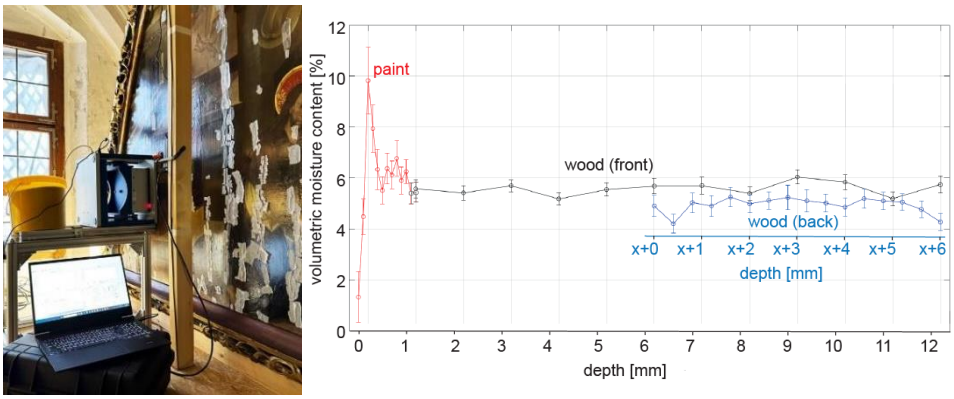


Fig. 1. (Left) Portable low field NMR device (Mouse PM25, *Magritek*) used in the restoration site of the *Gründonnerstagsretabel* in Freising, Germany. (Right) NMR profile results: volumetric moisture content profile across the painting. This is valuable information for conservation treatments.

References

[1] G. Eidmann, R. Savelsberg, P. Blumler, and B. Blümich *J. Magn. Reson.* **122,** 104-109 (1996)
[2] E. Danieli, and B. Blümich *J. Magn. Reson*. **229**, 142-154 (2013)
[3] L. Brizi, M. Camaiti, V. Bortolotti, P. Fantazzini, B. Blümich, and S. Haber-Pohlmeier *Micropor. Mesopor. Mat.* **269**, 186-190 (2018)
[4] L. Brizi, V. Bortolotti, G. Marmotti, and M. Camaiti *Magn. Reson. Chem.* **58**, 889-901 (2020)
This work was supported by the Deutsche Forschungsgemeinschaft, grants FR 2912/2-1 and AN 984/24-1.

NOVEL POLARIZING AGENTS FOR EFFICIENT HIGH FIELD AND FAST MAS DYNAMIC NUCLEAR POLARIZATION

L. Niccoli[a,b,c,d], G. Casano[e], G. Menzildjian[d], M. Yulikov[f], T. Robinson[d], Z.Wang[d], C. Reiter[g] , D. Siri[e], A. Venkatesh[h], L. Emsley[h], D. Gajan[d], F. Perras[i], O. Ouar[e], A. Lesage[d], M. Lelli[a,b,c]

[a]Center of Magnetic Resonance (CERM), University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy; [b]Consorzio Interuniversitario Risonanze Magnetiche Metallo Proteine (CIRMMP), Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy; [c]Department of Chemistry 'Ugo Schiff', University of Florence, Via della Lastruccia 13, 50019 Sesto Fiorentino, Italy; [d]Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCBL), 69100 Villeurbanne, France; [e]Aix Marseille Univ, CNRS, ICR, Marseille, France; [f]Department of Chemistry and Applied Biosciences, ETHZ, CH-8093 Zürich, Switzerland; [g]Bruker BioSpin, 76287 Rheinstetten, Germany; [h]Institut des Sciences et Ingénierie Chimiques (EPFL), CH-1015 Lausanne, Switzerland; [i]AMES Laboratory, U.S. DOE, Ames, Iowa 50011, USA

E-mail: lorenzo.niccoli@unifi.it

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

Dynamic Nuclear Polarization (DNP) applied to MAS solid-state NMR has proved to be a valuable technique to enhance the sensitivity of more than two orders of magnitude. The nature of the Polarizing Agent (PAs) plays an important role in determining the mechanism and the efficiency of the hyperpolarization process. It is known that the performances of PAs are strongly reduced passing from 9.4 T to 18.8 T [1], where the design of optimal PAs is still an open problem. Hybrid PAs such as HyTEK2 [2] or SNAPol [3] give excellent DNP performances but the synthesis of these diradicals involves complex protocols. Thus, refining the structure of dinitroxides to make them suitable for high-field NMR remains a valuable goal.

We present improved versions of TinyPol dinitroxides [4], two of which, dubbed O-TinyPol$(OH)_4$ and M-TinyPol$(OH)_4$, are able to significantly outperform current dinitroxide standards at 18.8 T and 40.0 kHz. They feature stereo-controlled structure and dihydroxypropyl chains on the spirocyclohexyl groups around the nitroxide rings. A combined approach of ^{2}H Electron Spin Echo Envelope Modulation (ESEEM) experiments in different DNP water/glycerol matrix and molecular dynamics simulations show a higher density of glycerol protons in the second solvation sphere around the nitroxides.

We thus propose that the dihydroxypropyl chains in M-TinyPol$(OH)_4$ and O-TinyPol$(OH)_4$ significantly improve the DNP efficiency by acting as a polarization escape way, facilitating the propagation of the hyperpolarization towards the bulk solution.

In order to rationalize our finding, our experimental investigation is also supported by simulations based on recent hybrid classical/quantum mechanical calculation schemes [5]. This simulation approach allows to consider thousands of spins simulation which is essential for a description of the spin diffusion process. These results will be also useful for an accurate *in-silico* design of novel PAs.

References

[1] Menzildjian, G.; et al. *Chem. Sci.*, 2023, ***14*** (23), 6120
[2] Wisser, D.; at al. *J. Am. Chem. Soc.* 2018, ***140*** (41), 13340–13349
[3] Cai, X.; et al. *Chem. – Eur. J.* 2021, ***27*** (50), 12758–12762
[4] Lund, A.; et al. *Chem. Sci.* 2020, ***11*** (10), 2810–2818
[5] Perras, F.; et al. *J. Chem. Phys*, 2022, **156** (12), 12411

HIGH-RESOLUTION RELAXOMETRY FOR MULTISCALE DYNAMICS IN COMPLEX SYSTEMS

G. Licciardi,[a,b,c] L. Fiorucci,[a,b,c] E. Ravera,[a,b,c] G. Parigi,[a,b,c] C. Luchinat,[a,b,c]

[a]Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, Sesto Fiorentino, 50019 Italy
[b]Magnetic Resonance Center (CERM), University of Florence, via Sacconi 6, Sesto Fiorentino, 50019 Italy
[c]Consorzio Interuniversitario Risonanze Magnetiche Metallo Proteine (CIRMMP), Sesto Fiorentino, Italy
E-mail: giulia.licciardi@unifi.it

Keywords: solution NMR, low field NMR, MRI, small molecules, biomolecules, metabolomics, food, contrast agents, theory and methods, instrumentation.

This work explores novel applications of High-Resolution Relaxometry (HRR), a cutting-edge NMR technique that offers unparalleled insight into dynamic processes within complex systems [1, 2]. HRR utilizes a high-field spectrometer's stray field for variable-field relaxation measurements, surpassing Fast Field Cycling (FFC) limitations and enabling high-resolution relaxation measurements over a wide timescale.
HRR was applied for the study of: protein-ligand interactions for understanding biological processes; interactions between metabolites and macromolecules in complex mixtures like urine; dynamics in complex viscous systems, combining FFC and HRR; dynamics in intrinsically disordered proteins. FFC and HRR measurements on potential contrast agents provided consistent data, significantly expanding the investigated spectral density function.
These preliminary studies demonstrated the versatility and effectiveness of HRR to unlock deeper understanding of multiscale dynamics. Additionally, the synergistic use of HRR with FFC offered significant insights, paving the way for further exploration of complex systems using these powerful NMR relaxometry methods.

References

[1] C. Charlier, S. N. Khan, T. Marquardsen, P. Pelupessy, V. Reiss, D. Sakellariou, G. Bodenhausen, F. Engelke, and F. Ferrage, *Journal of the American Chemical Society*, vol. 135, no. **49**, pp. 18665-18672 (2013)
[2] Z. Wang, S. Pisano, V. Ghini, P. Kaderavek, M. Zachrdla, P. Pelupessy, M. Kazmierczak, T. Marquardsen, J.-M. Tyburn, G. Bouvignies, G. Parigi, C. Luchinat & F. Ferrage, *Journal of the American Chemical Society*, *143*(**25**), 9393-9404 (2021)

EFFECT OF RESINS ON SBR DYNAMICS BY SOLID STATE NMR AND NMR RELAXOMETRY

M. Pierigé[a], F. Nardelli[b], L. Calucci[b,c], M. Cettolin[d], L. Giannini[d], A. Causa[d], M. Geppi[a,b,c], E.R. deAzevedo[e], F. Martini[a,c]

[a]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, 56124 Pisa, Italy
[b]Istituto di Chimica dei Composti OrganoMetallici, Consiglio Nazionale delle Ricerche, 56124 Pisa, Italy
[c]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), 56126 Pisa, Italy
[d]Pirelli Tyre SpA, 20126 Milano, Italy
[e]USP – Institute of Physics of São Carlos, 13566-590 São Carlos, São Paulo - Brazil
E-mail: michele.pierige@phd.unipi.it

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

In the tire industry, tackifying resins are essential ingredients because they modulate green tack and green strength of the uncured rubbers, facilitating their manipulation and preventing creep and tear of the final products. From a molecular perspective, the presence of the resin alters the dynamics of the polymer chain, resulting in the modification of the rheological and viscoelastic behaviour of the rubber compounds [1]. Since the mechanical response of the final compounds varies depending on the type of resin added, it is important to dissect the molecular origins of such differences. In this research, we compared the effect of a natural- and a petroleum-origin resins on the dynamics of Styrene-Butadiene Rubber (SBR) in compounds of interest for the tyre industry. To this end, we employed a variety of Solid-State Nuclear Magnetic Resonance experiments (SS-NMR) [2,3] at variable temperature. These include ^{1}H Field Cycling NMR, DIPolar chemical SHIFT correlation [4] and Centerband Only Detection of Exchange [5] experiments. These techniques enabled the study of polymer dynamics on a wide range of motion time scales, form the fast segmental motions related to glass transition to the slower and collective motions of the polymer chains. In addition, measurements of ^{1}H T_1 and $T_{1\rho}$ relaxation times provided information on the polymer-resin miscibility on the nanometer scale, which is essential for achieving compounds with the desired mechanical properties. Dipolar-Filtered Magic Sandwich Echo [6] experiments were also carried out to obtain information on the presence of domains with different degree of mobility, as well as on the onset of their motions with temperature. The SS-NMR results were compared with those from dynamic-mechanical, rheometric, calorimetric and chemical characterization. This approach provided valuable information to elucidate the complex relationship between molecular and macroscopic properties in rubber compounds, aiding the design of formulations with improved performances.

References

[1] B. Rodgers, Rubber Compounding: Chemistry and Applications, Second Edition, CRC Press, Boca Raton (2015)
[2] M. Pierigé, F. Nerli, F. Nardelli, L. Calucci, M. Cettolin, L. Giannini, M. Geppi and F. Martini *Appl. Sci.*, **13**(3), 1939 (2023)
[3] M. Pierigé, F. Nardelli, L. Calucci, M. Cettolin, L. Giannini, A. Causa, F. Martini and M. Geppi *Polymers*, **16**(6), 834 (2024)
[4] M. G. Munowitz, R. G. Griffin, G. Bodenhausen, and T. H. Huang *J. Am. Chem. Soc.*, **103**(10), 2529–2533 (1981)
[5] E. R. deAzevedo, W. G. Hu, T. J. Bonagamba, and K. Schmidt-Rohr *J. Am. Chem. Soc.*, **121**(36), 8411–8412 (1999)
[6] J. G. Filgueiras, U. B. da Silva, G. Paro, M. N. d'Eurydice, M. F. Cobo and E. R. deAzevedo *Journal of Magnetic Resonance* **285**, 47–54 (2017)

FFC NMR APPLICATION IN FOOD SCIENCE AND BEYOND

A. Nagmutdinova[a], C. Testa[b], G. Landi[c], G. Ferrante[d], F. Zama[c], L. Brizi[b], R. Anedda[e], V. Bortolotti[a]

[a] DICAM University of Bologna, Italy
[b] DIFA University of Bologna, Italy
[c] Dep. Of Mathematics University of Bologna, Italy
[d] Stelar srl, Mede, Italy
[e] Porto Conte Ricerche srl (SS), Italy
E-mail: anastas.nagmutdinov2@unibo.it

Keywords: low field NMR, materials, food, theory and methods, instrumentation.

Fast Field Cycling (FFC) NMR is a non-destructive and relatively rapid technique that enables the continuous investigation of the same sample over time. It is a powerful method for characterizing various materials' structure, molecular motion, transport, and diffusion properties. FFC NMR stands out as the only low-field NMR technique allowing to perform efficient measurement of the longitudinal relaxation time $T1$ (or relaxation rate $R1=1/T1$) as a function of magnetic field strength across a broad range of frequencies [1]. The data collected through FFC NMR is represented in Nuclear Magnetic Resonance Dispersion (NMRD) profiles ($T1$ vs frequency).
Up to this day, the application of FFC NMR remains limited. This is likely due to the technical and theoretical complexities involved in the methodologies, which resulted in its slow development.
Some insights obtained at lower magnetic fields via FFC NMR are unattainable with Spectroscopy or fixed field measurements. This makes FFC NMR an invaluable tool for studying molecular dynamics and transport in complex systems, providing unique insights into sample properties.
This talk covers the theoretical background, hardware setup, and data processing methods of FFC NMR. We also present several NMRD profiles of industrial and artisanal cheeses (see Fig 1).

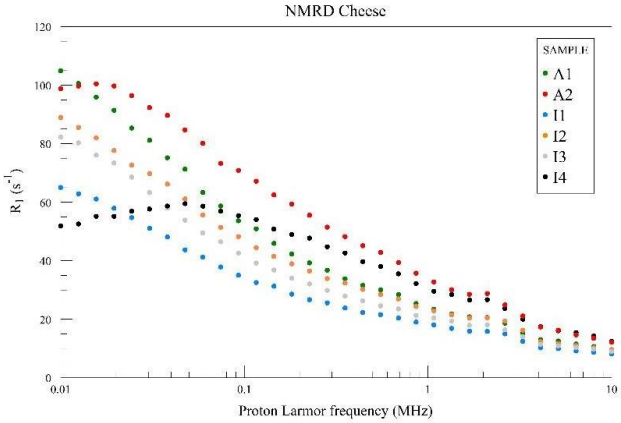


Fig. 1 NMRD profiles of different cheeses (A stands for artisanal and I for industrial cheese).

We demonstrate that the FFC-NMR technique could describe the microstructure of the cheese paste and the molecular dynamics of the main components (water, proteins, and fats) and solve the problem of recognizing and diagnosing heat treatments on milk through the analysis of aged cheese.

This work was performed as a part of the MRimprove activity.

References

[1] NMR RELAXOMETRY. From molecular dynamics to practical applications. Stelar Srl (2022).

ENHANCING NMR SENSITIVITY FOR COMPLEX MIXTURE ANALYSIS THROUGH HYPERPOLARIZATION

M. H. Lerche.

Department of Health Technology, Technical University of Denmark, DTU, Copenaghen

Advancements in hardware and intelligent acquisition design have significantly improved NMR sensitivity, yet quantitative NMR remains underutilized for analyzing compounds in complex mixtures with limited sample quantities. To address this, dissolution dynamic nuclear polarization (dDNP) enhances NMR sensitivity and resolution.
We developed a stable ^{13}C isotope tracer-based hyperpolarized NMR method to quantitatively measure metabolic flux with high sensitivity and contrast, facilitating the mapping of metabolic pathways and networks. This advancement enables new applications in fields characterized by sample complexity and low analyte concentrations.
Using this method, we investigated the metabolic signature of aggressive prostate cancer cells under hypotonic stress, comparing it to early-stage prostate cancer cells. Cellular enlargement due to hypotonicity dilutes metabolites and enzymes, potentially leading to enzymatic inhibition or activation and revealing metabolic survival strategies. Through this and other selected studies we outline advantages and limitations of the ^{13}C hyperpolarization method as well as giving insights into future developments.

Thursday 5th

	Plenary session **Chair: G. Parigi**
8:45-9:30	**Plenary Lecture 4** **Claudio Luchinat (University of Florence)** IMPROVING THE INFORMATION CONTENT OF NMR SPECTRA OF METABOLOMIC SAMPLES
9:30-9:45	**Sponsorship Lecture (Bruker): A. Moreno** NEW EXPERIMENTS IN TOPSPIN: PURESHIFT, GEMSTONE, DOSY, AND MORE!
9:45-10:15	**Chair: M.R. Chierotti** **Under 35 GIDRM** **M. Spano (Sapienza University of Rome)** NMR METABOLOMICS TO BETTER DEFINE ALTERNATIVE AND SUSTAINABLE FOOD SOURCES
10:15-10:30	**Sponsorship Lecture (Jeol): A. Botana** THE MILLIGRAM CHALLENGE

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

	Parallel session A **Chair: A. Randazzo**	**Parallel session B** **Chair: D. Kubicki**
11:20-11:50	**Ingallina C.** METABOLOMIC MARVELS: DECODING THE FOOD WORLD THROUGH NMR SPECTROSCOPY	**Gallo A.** SOLID STATE NMR AS ELEGANT TOOL IN CRYSTAL STRUCTURE DETERMINATION OF SMALL ORGANIC MOLECULES
11:50-12:05	**Sozzi M.** CHALLENGES IN MEASURING ETHANOL CONTENT IN ALCOHOLIC BEVERAGES USING ^{1}H NMR: METHOD EVALUATION AND A WINE CASE STUDY	**Piva S.** DYNAMICS OF FAST-ROTATING ROTORS IN A METAL-ORGANIC FRAMEWORK AS EXPLORED BY SOLID-STATE NMR
12:05-12:20	**Cicero D.** NOVEL INSIGHTS INTO METABOLIC CROSSTALK IN MULTIMORBID PATIENTS USING NMR SPECTROSCOPY	**Bravetti F.** SYNTHESIS AND SOLID-STATE NMR-DRIVEN CRYSTAL STRUCTURE DETERMINATION OF THE FIRST CO-DRUG OF VALPROIC ACID AND L-CARNITINE
12:20-12:35	**Brigante F.** NOVEL CHARACTERIZATION OF PLANT-BASED BEVERAGES BY 1D AND 2D NMR	**Della Latta E.** USING ^{13}C HIGH-RESOLUTION SOLID-STATE NMR TECHNIQUES TO INVESTIGATE A Cu(II)-BASED PARAMAGNETIC MOF
12:35-12:50	**Zampieri S.** ^{1}H-NMR FOR MASLD DIAGNOSIS: MULTIVARIATE ANALYSIS OF SERUM METABOLITES TO DIFFERENTIATE DISEASE STAGES	**Martini F.** UNVEILING LOCAL DYNAMICS IN TRIPTYCENE-BASED POROUS POLYMERS: A SOLID-STATE NMR STUDY

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

	Plenary session **Chair: S. Borsacchi**
14:10-14:55	**Plenary Lecture 5** **Dominik Kubicki (University of Birmingham)** LOCAL STRUCTURE AND DIFFUSION OF SODIUM IN A HYBRID GLASS FROM HIGH-TEMPERATURE ^{23}Na MAS NMR AT 20 T
14:55-15:10	**Sponsorship Lecture (Bracco): A. Macula** ASSESSING THE RELIABILITY OF VIRTUAL CONTRAST: A GENERALIZATION STUDY ACROSS DIFFERENT TUMOR TYPES AND PATIENT DEMOGRAPHICS
15:10-15:25	**Sponsorship Lecture (Magritek/FKV): D. Bouillaud** NEW BENCHMARK FOR BENCHTOP NMR MAGNET HOMOGENEITY: HOW SPINSOLVE™ ULTRA NMRS BOOST SOLVENT SUPPRESSION PERFORMANCE FOR 1- AND 2D NMR

	Chair: L. Ragona **Segre-Capitani Fellowships**
15:25-15:40	**C. Papi (University of Turin)** A MRI-CEST METHOD FOR MAPPING WATER CYCLING ACROSS CELLULAR MEMBRANES
15:40-15:55	**A. Gambini (University of Modena and Reggio Emilia)** NMR-BASED METABOLOMICS IN MNGIE RARE DISEASE
15:55-16:10	**A. Barbanente (University of Bari)** AN NMR-BASED APPROACH TO ELUCIDATE THE LINK BETWEEN DIETARY ZINC STORAGE AND TRYPTOPHAN METABOLISM

16:10-17:30	**Coffee break + Poster session (EVEN abstract numbers)**
16:40-17:30	**GIRM assembly**
17:30-19.30	**GIDRM assembly + announcement of poster competition winner**

20:30	**social dinner**

IMPROVING THE INFORMATION CONTENT OF NMR SPECTRA OF METABOLOMIC SAMPLES

C. Luchinat

CERM/CIRMMP and Department of Chemistry, University of Florence, Italy
E-mail: luchinat@cerm.unifi.it

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

NMR is emerging as an extremely powerful technique to provide metabolomic data through the analysis of biofluids that can be collected non-invasively.
To analyse NMR-metabolomic data, fingerprinting and profiling can be used [1]. In fingerprinting, the entire spectrum is analysed through a bucketing procedure. Fingerprinting needs absolutely optimal standard operating procedures. In profiling, the concentrations of all identifiable and quantifiable metabolites must be determined from the corresponding signal intensities. The accurate identification of the spectral resonances is the bottleneck of this approach. Blood derivatives (serum or plasma) are biofluids that are well controlled by homeostasis, and metabolite identification is relatively straightforward. On this basis we have successfully exploited NMR in different pathological contexts, providing significant information on a wide range of diseases, such as cancer [2], cardiovascular diseases [3], and COVID-19 [4].
Urine poses different challenges. On the methodology side, stabilization of urine samples, especially when collected at home, can be achieved by gelification of urines using silica particles, while addition of a small amount of paramagnetic gadolinium chelate permits faster acquisition thanks to the shortening of relaxation delays [5]. The main drawback for the full exploitation of the rich urine information content is the extreme variability of the urine matrix composition and, as a result, of the metabolite chemical shifts which makes full automated assignment close to impossible.
Our main goal is to overcome the difficulties in automated metabolite assignment in urine. To this end we have introduced a method to provide automated and accurate prediction of chemical shifts of about fifty metabolites based on the collection of a large “artificial urine” dataset to use for an extensive training of machine-learning programs [6]. Now we are further investigating the source of chemical shifts variations as due to metabolite-metabolite interactions. The far-reaching aims of this project are on the one hand to achieve a completely automated tool to predict the chemical shifts of all visible metabolites in any urine sample, and on the other hand to build a “urine metabolite interactome” on which accurate chemical shifts predictions could be based.

References

[1] V.Ghini, G.Meoni, A.Vignoli, F.Di Cesare, L.Tenori, P.Turano, C.Luchinat. Fingerprinting and profiling in metabolomics of biosamples. Progress in Nuclear Magnetic Resonance Spectroscopy 138–139, 2023, 105-135
[2] E.Risi , C.Lisanti , A.Vignoli , C.Biagioni , A.Paderi , S.Cappadona , F.Del Monte *et al*., Risk assessment of disease recurrence in early breast cancer: A serum metabolomic study focused on elderly patients Translational Oncology 27, 2023, 101585
[3] A.Vignoli, A.Fornaro, L.Tenori, G.Castelli, E.Cecconi, I.Olivotto, N.Marchionni, B.Alterini and C.Luchinat Metabolomics Fingerprint Predicts Risk of Death in Dilated Cardiomyopathy and Heart Failure, Frontiers in Cardiovascular Medicine, 9, 2022, 851905
[4] V.Ghini, W.Vieri, T.Celli, V.Pecchioli, N.Boccia, T.Alonso-Vásquez, *et al*. COVID-19: A complex disease with a unique metabolic signature. PLoS Pathog 19, 2023, 1011787.
[5] F. Mulder , L. Tenori , C. Licari , C. Luchinat Practical considerations for rapid and quantitative NMR-based metabolomics. Journal of Magnetic Resonance, 352, 2023, 107462
[6] Takis, P.G., Schäfer, H., Spraul, M. *et al.* Deconvoluting interrelationships between concentrations and chemical shifts in urine provides a powerful analysis tool. Nat Commun 8, 2017, 1662

NEW EXPERIMENTS IN TOPSPIN: PURESHIFT, GEMSTONE, DOSY, AND MORE!

A. Moreno,[a]

[a] Bruker Switzerland AG, Switzerland
E-mail: aitor.moreno@bruker.com

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

Over the past few years, significant innovations in the field of small molecule NMR have led to exciting new experiments. In this talk, we will discuss recently implemented experiments for small molecule NMR applications in the latest TopSpin version. We will also give a preview of upcoming experiments that will be available in one of the next TopSpin versions.

PureShift Experiments: These experiments suppress homonuclear coupling effects, resulting in proton spectra that provide only chemical shift information. Novel approaches have enhanced sensitivity, spectral purity, and tolerance of strong coupling compared to previous methods.

GEMSTONE (Gradient-Enhanced Multiplet-Selective Targeted-Observation NMR Experiment): GEMSTONE selectively targets one signal from overlapping multiplets by combining chemical shift filtering and spatial multiplexing.

DOSY (Diffusion-Ordered Spectroscopy): DOSY separates NMR signals based on diffusion coefficients. It provides insights into molecule size and shape, making it very useful for analyzing mixtures and studying interactions.

NMR METABOLOMICS TO BETTER DEFINE ALTERNATIVE AND SUSTAINABLE FOOD SOURCES

M. Spano [a,b]

[a] Laboratory of Food Chemistry, Department of Chemistry and Technology of Drugs, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[b] NMR-Based Metabolomics Laboratory (NMLab), Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
E-mail: mattia.spano@uniroma1.it

Keywords: solution NMR, metabolomics, food.

Food analysis has strongly benefited from the use of NMR spectroscopy, allowing the resolution of several issues namely metabolite profile elucidation, geographical origin determination, fraud tracking, quality analysis, etc. [1].
Among these applications, a field of research that has recently fascinated me regards the study of complex biological matrices as potential alternative food sources.
Mainly in the last years, an ever-increasing need of alternative and innovative food sources is occurring, with several matrices being introduced as Novel Foods. However, despite a matrix is accepted or proposed as Novel Food, the knowledge improving of its chemical composition remains a challenge to be pursued, to better understand its nutritional and biological values.
In this context, NMR metabolomics can be a potential tool to achieve this goal. Here, some applications are reported.
Starting from *Cannabis sativa* L. inflorescences [2,3,4], NMR has allowed to underline an interesting metabolite profile with potential nutritional applications, also basing on the used agronomical practices. Moreover, in the context of alternative protein sources, NMR metabolomics applied on edible insects [5] and medical mushrooms [6,7] has shown that these Novel Foods have larger potentials with respect to the only "protein use".
The study of other alternative matrices namely medicinal plants, agrifood wastes, and legume seeds treated with innovative processes has further confirmed that NMR spectroscopy is now an indispensable ally for a better study of new food sources.

References

[1] A. P. Sobolev et al. *Molecules* **27**, 7906 (2022)
[2] C. Ingallina et al. *Molecules* **25**, 1908 (2020)
[3] M. Spano et al. *Molecules* **26**, 2912 (2021)
[4] M. Spano et al. *Foods* **22**, 3659 (2022)
[5] M. Spano et al. *Foods* **12**, 2331 (2023)
[6] L. Goppa et al. *Foods* **12**, 2507 (2023)
[7] M. Spano et al. *LWT* **199**, 116123 (2024)

THE MILLIGRAM CHALLENGE

A. Botana,[a] R. Crouch,[b]

[a] JEOL UK, Welwyn Garden City, United Kingdom
[b] JEOL USA, Peabody, MA, United States
E-mail: adolfo.botana@jeoluk.com

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 circa 1 mg or less of sample for commonly run 1D and 2D experiments, facilitating as well the acquisition of more insensitive experiments.

References

[1] https://www.cif.iastate.edu/nmr/nmr-tutorials/sample-preparation
[2] https://www.hw.ac.uk/uk/schools/doc/egis/Sample_Preparation.pdf
[3] https://www.ucl.ac.uk/nmr/sample-preparation
[4] https://www.reading.ac.uk/caf/-/media/project/functions/research/chemical-analysis-facility/documents/nmrsamplepreparationpdf
[5] https://schuetz.cup.uni-muenchen.de/nmr-facility/nmr-sample-preparation/
[6] https://chem.ch.huji.ac.il/nmr/preparation/preparation.html
[7] A. T. Zens, *J. Magn. Res.* **316**, 106741 (2020)

METABOLOMIC MARVELS: DECODING THE FOOD WORLD THROUGH NMR SPECTROSCOPY

C. Ingallina,[a] D. Ambroselli, [a] F. Masciulli,[a] M. Spano,[a] G. Di Matteo,[a] A. P. Sobolev,[b] L. Mannina[a]

[a]Department of Chemistry and Technology of Drugs, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[b] "Annalaura Segre" Magnetic Resonance Laboratory, Institute for Biological Systems, CNR, Via Salaria, Km 29,300, 00015 Monterotondo, Italy
E-mail: cinzia.ingallina@uniroma1.it

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

The field of metabolomics applied to food matrices is continuously expanding, with the goal of deepening our understanding of food composition and its impact on health. One of the most notable advancements in this field is the use of NMR spectroscopy, which has proven to be an essential tool in research due to its robustness, non-invasiveness, and high-resolution capabilities for analysing the complex chemical compositions present in food.
This presentation will focus on the application of NMR spectroscopy in food characterization, particularly in identifying and quantifying metabolites, assessing food quality, evaluating the effect of pedoclimatic conditions, and characterizing food processing and byproducts. Case studies will be presented in three main areas to exemplify the valuable insights that can be derived from NMR data:
i) Food Resources: This involves studying the composition of plant-based foods, considering factors such as climate, land, and cultural practices ("terroir") that contribute to the traceability and characterization of authentic products [1,2].
ii) Food Processing: This entails analyzing the significant pre- and post-production manipulations on the original product, such as the impact of packaging and storage, the fermentation, safety and authenticity control, and management of waste and byproducts [3-5].
iii) Human Health: This encompasses studies on monitoring food consumption, investigating changes in the metabolome resulting from specific diets, and exploring the role of diet in treating and preventing diseases [6].
This comprehensive overview will highlight how NMR-based metabolomics can drive innovations in food science, improve nutritional studies, and benefit the global food industry through collaborative efforts and advancements.

References

[1] M. Spano, G. Di Matteo, C. Ingallina, A. P. Sobolev, A. M. Giusti, G. Vinci, S. Cammarone, C. Tortora, L. Lamelza, S. A. Prencipe, L. Gobbi, B. Botta, F. Marini, E. Campiglia, L. Mannina *Foods* **11**, 3658(2022).
[2] D. Ambroselli, F. Masciulli, E. Romano, R. Guerrini, C. Ingallina, M. Spano, and L. Mannina *Foods* **13**, 1642 (2024)
[3] A. P. Sobolev, A. Di Lorenzo, S. Circi, C. Santarcangelo, C. Ingallina, M. Daglia, and L. Mannina *Molecules* **26**, 5125 (2021).
[4] G. Di Matteo, P. Di Matteo, M. Sambucci, J. Tirillò, A. M. Giusti, G. Vinci, L. Gobbi, S. A. Prencipe, A. Salvo, C. Ingallina, M. Spano, A. P. Sobolev, N. Proietti, V. Di Tullio, P. Russo, L. Mannina, and M. Valente *Foods* **10**, 2440 (2021).
[5] A. Chiavaroli, F. Masciulli, C. Ingallina, L. Mannina, […] and C. Ferrante. *Food Res. Int.* **175**, 113654 (2024)
[6] A. P. Sobolev, A. Ciampa, C. Ingallina, L. Mannina, D. Capitani, […] and A. Pinto *Metabolites* **9**, 138 (2019).

CHALLENGES IN MEASURING ETHANOL CONTENT IN ALCOHOLIC BEVERAGES USING ^{1}H NMR: METHOD EVALUATION AND A WINE CASE STUDY

M. Sozzi,[a] V. Aru,[b] N. Cavallini,[a] B. Khakimov,[b] F. Savorani,[a] S. B. Engelsen,[b]

[a]Department of Applied Science and Technology, Polytechnic of Turin, Corso Duca degli Abruzzi 24, 10129, Turin, Italy
[b]Chemometrics & Analytical Technology, Department of Food Science, University of Copenhagen, Rolighdsvej 26, 1958 Frederiksberg C, Denmark
E-mail: mattia.sozzi@polito.it

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

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique that is now widely used in the field of food and beverages. Many recent applications are related to quality control, authentication, traceability, fraud detection but also process monitoring such as fermentation and aging effects.
Even if NMR spectroscopy is commonly used for quantification purposes, also with different research targets, in the recent literature there are few studies focused on the possibility of using this technique to quantify ethanol in alcoholic beverages [1]. Indeed, some recent works highlighted problems associated with this application, often related to low prediction accuracy [2], especially occurring with higher magnetic field intensities [3].
In this context we decided to explore the causes of these unexpected results by planning a detailed experimental design using a series of ethanol solutions with increasing concentrations and considering also different ^{1}H NMR acquisition parameters and different data processing techniques, the latter related to the ethanol quantification.
In this study, to avoid the known quantification problems [2,3] related to the use of the proton-proton coupling triplet signal at 1.18 ppm (i.e., one of the two "main" ethanol signals), the carbon-related satellite peak at 1.08 ppm was used instead, building upon the work of Lopez-Rituerto et al. [4]. The main and the satellite signals were modelled separately and compared, and a new correction method to make the satellites quantitative was developed. We therefore used this signal to quantify the ethanol content of solutions containing up to 40 % of alcohol.
In addition, from the data analysis and processing point of view, two different quantification approaches were tested (namely, row sum, and multivariate curve resolution). Also, to evaluate the effects of two deuterated solvents (D2O and DMSO) and of the suppression of the water signal, ANOVA Simultaneous Component Analysis (ASCA) was applied.
As a result, we were able to propose a new rapid and effective method for ethanol quantification in liquid samples based on Partial Least Squares (PLS) regression. To test and further evaluate the model performances, 20 different external wine samples were analyzed following an optimized operating procedure. Good prediction results were obtained, generally affected by a very reduced underestimation error (<1 %).

References

[1] Caleja-Ballesteros H.J.R., Ballesteros J.I., et al., Microchemical Journal, **164**, 105999 (2021).
[2] Matviychuk Y., Haycock S., Rutan T., et al., Analytica Chimica Acta, **1182**, 338944 (2021).
[3] Isaac-Lam M. F., International Journal of Spectroscopy, 2526946 (2016).
[4] Lopez-Rituerto E., et al., Journal of Agricultural and Food Chemistry, **57**, 2112-2118 (2009).

NOVEL INSIGHTS INTO METABOLIC CROSSTALK IN MULTIMORBID PATIENTS USING NMR SPECTROSCOPY

D.O. Cicero,[a] E. Pitti,[a] D. Vanni,[a] N. Viceconte,[b] A. Lembo,[a] G. Tanzilli,[b] V. Raparelli,[c] G. Petrella[a]

[a] Department of Chemical Science and Technology, University of Rome "Tor Vergata," 00133 Rome, Italy.
[b] Department of Cardiovascular, Respiratory, Nephrological, Anesthesiologic and Geriatric Sciences, Sapienza University of Rome, Policlinic Umberto I, 00161 Rome, Italy.
[c] Department of Experimental Medicine, Sapienza University of Rome, 00161 Rome, Italy
E-mail: cicero@scienze.uniroma2.it

Keywords: metabolomics, theory and methods.

Metabolomics is increasingly used to identify markers indicating the presence of specific diseases. Still, it's typically focused on individual conditions, which may not directly apply to people with multiple health issues. Our study takes a different approach by examining how metabolism interacts across various disease states. We conducted a survey of 306 patients at medium to high risk of developing coronary artery disease (CAD).
We used Nuclear Magnetic Resonance (NMR) to measure the plasma levels of 83 metabolites. We analyzed their connections with risk factors such as diabetes, hypertension, and dyslipidemia using linear regression and multivariate analysis. By examining the metabolic maps created from our analysis, we efficiently compared profiles and discovered opposing metabolic features among individual conditions and their combination.
We also found compensating metabolic effects between diabetes, hypertension, and dyslipidemia, mainly involving ketone body metabolism and fatty acid β-oxidation. Our study introduces a novel approach to investigating how metabolism responds to the simultaneous presence of multiple health conditions. This has allowed us to detect potential compensatory effects between diabetes, hypertension, and dyslipidemia, highlighting the complexity of metabolic interactions in patients with comorbidities. Understanding metabolic crosstalk like this could help develop targeted treatments and improve therapeutic outcomes.

References

[1] [1 Fortin M., Dionne J., Pinho G., *Ann Fam Med.* **4**, 104-108 (2006).
[2] Ke C., Zhu X., Zhang Y., *Metabolomics*. **14**, 1-11 (2018).
[3] Laffel L., *Diabetes Metab Res Rev*. **15**, 412-426 (1999).

NOVEL CHARACTERIZATION OF PLANT-BASED BEVERAGES BY 1D AND 2D NMR.

F. Brigante,[a] P. Solovyev,[b] L. Bontempo[c]

[a] Traceability Unit, Research and Innovation Center – Fondazione Edmund Mach (F.E.M), San Michele all' Adige, TN, Italy.
[b] Fondazione OnFoods, via Università n. 12 43121, Parma, Italy
E-mail: federico.brigante@fmach.it

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

Plant-based beverages (PBBs) are defined as emulsions obtained from different plant materials like legumes, cereals, pseudo-cereals, seeds, or nuts after the main steps of soaking, filtration, and thermal treatment. Naturally, they represent lactose-free options ideal for consumers with allergies or intolerance, and they possess environmental advantages compared to bovine milk, since the CO_2 fingerprint for their production is lower, their fiber content, and they favor animal welfare. They are also a staple food for those consumers that opt for new dietary habits such as veganism, vegetarianism, and flexitarianism [1,2]. In the last decade, efforts have been directed mostly towards their nutritional composition (in terms of macro and micronutrients), production processes, anti-nutritional factors (tannins, saponins, and enzyme inhibitors), their sensory acceptability, their protein availability, and the diversity of aroma-related compounds [1,3]. However, there is a large literature gap in the use of 1D, 2D NMR, and qNMR in the characterization of PBBs to gain deeper knowledge about their composition. The objective of this work was to characterize the polar extract of soy, oat, and almond PBBs by ^{1}H NMR, HSQC, and qNMR, using cow milk as a reference. ^{1}H NMR revealed that that the reference matrix was the one with the highest number of found compounds (68). The main chemical families were nitrogen compounds (excluding amino acids), carbohydrates, fatty acids, and organic acids. Then, soy PBB was the one closest to the reference in number of found and identified compounds, sharing fatty acids and carbohydrate signals but with distinct compounds like nucleosides, taurine, histidine, and histamine. Oat and almond PBBs showed around 30% less compounds (48 and 42, respectively) compared to the reference. Oat PBBs showed a richer carbohydrate profile with presence of mono, di, and oligosaccharides, especially maltose and raffinose. Almond PBB was the one with the lowest number of compounds among the 4 matrices, with pantothenate and *myo*-inositol as distinctive compounds. A total of 33 compounds were quantified, an analysis of variance with Tukey post-hoc test showed higher concentration of most organic acids and choline and its derivatives in cow milk. Soy PBB was enriched in compounds like stigmasterol and glucose-1-P, while oat PBB in beta-maltose, trigonelline, and valine. Finally, almond PBB showed significantly higher concentrations of tartrate, galacturonate, and malate.

References

[1] Hidalgo-Fuentes, B.; de Jesús-José, E.; Cabrera-Hidalgo et al., (2024). Plant-Based Fermented Beverages: Nutritional Composition, Sensory Properties, and Health Benefits. *Foods*, *13*, 844. https://doi.org/10.3390/foods13060844
[2] Popova, A.; Mihaylova, D. & Lante, A. (2023). Insights and Perspectives on Plant-Based Beverages. *Plants*, *12*, 3345. https://doi.org/10.3390/plants12193345
[3] Xie, A., Dong, Y., Liu, Z., Li, Z., Shao, J., Li, M. & Yue, X. (2023). A Review of Plant-Based Drinks Addressing Nutrients, Flavor, and Processing Technologies. *Foods*, *29*, 12(21):3952. https://doi.org/10.3390/foods12213952.

^{1}H-NMR for MASLD Diagnosis: Multivariate Analysis of Serum Metabolites to Differentiate Disease Stages

S. Zampieri[a*], G. Petrella[a], E. Nagni[a], S. Basili[b], F. Maiorca[b], L. Lombardi[b], A. Sabetta[b], L. Stefanini[b], D. O. Cicero[a]

[a] Department of Chemical Science and Technology, University of Rome "Tor Vergata," 00133, Rome, Italy.
[b] Department of Translational and Precision Medicine, Sapienza University of Rome, 00185, Rome, Italy.
E-mail: serena.zampieri@students.uniroma2.eu

Keywords: solution NMR, metabolomics, MASLD, multivariate analysis.

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a spectrum of liver diseases ranging from simple hepatic steatosis (MASL) to non-alcoholic steatohepatitis (MASH), all characterized by an excessive accumulation of fat in the liver.[1] Today, it is one of the most common liver diseases in the world, able to progress towards cirrhosis and/or hepatocellular carcinoma (HCC).[2] At present, the most reliable method to diagnose MASLD is through liver biopsy. This technique is the only one that can differentiate between various stages of disease progression, providing an accurate assessment of hepatic steatosis, hepatocellular damage, inflammation, and early fibrosis stages. However, metabolomics could offer a new way of discovering potential non-invasive biomarkers for the MASLD prognosis. To this end, we conducted a metabolomic analysis via NMR of the serum metabolic composition of MASLD patients enrolled at the Hospital Umberto I of Rome. The 88 serum samples were obtained from four different groups of patients with progressively worsening prognosis of liver disease, namely controls, MAFL, MASH, and Metabolic dysfunction-associated cirrhosis. It was possible to identify and quantify 62 metabolites through a manual deconvolution process of the spectra. A multivariate analysis was conducted to investigate the relationship between the systemic metabolic profile and the progression of the disease. Three Orthogonal Partial Least Square Discriminant Analyses (OPLS-DAs) were used to compare the serum metabolic profile of controls with those of MASL, MASH, and MASH/cirrhotic patients. This approach was used to qualitatively identify the specific metabolic fingerprints of each stage of the disease. Additionally, we analyzed whether the common part of the altered metabolism, compared to controls, showed a progressive increase or decrease over the stages of the disease. To investigate this correlation, an OPLS was used to study the relationship between metabolite levels and MAFLD progression. Many of the metabolites that were found to be altered in the serum of MAFLD patients are consistent with previous studies on this liver illness. However, no current research focuses on the relationship between metabolism and the disease course. These results can help in the future to improve our understanding of the biochemical mechanisms underlying the progression of MAFLD and to identify possible new therapeutic avenues.

References

[1] De, A.; Duseja, A.; Natural History of Simple Steatosis or Non-alcoholic Fatty Liver. *J Clin Exp Hepatol*. 2020, **10(3)**, 255-262.
[2] Li, B.; Zhang, C.; Zhan, Y-T.; Non-alcoholic Fatty Liver Disease Cirrhosis: A Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis, Management, and Prognosi. *Can J Gastroenterol Hepatol*. 2018, **2784537**.

SOLID STATE NMR AS ELEGANT TOOL IN CRYSTAL STRUCTURE DETERMINATION OF SMALL ORGANIC MOLECULES

F. Bravetti[a], S. Bordignon[a], A. Gallo[a], F. Rossi[b], C. Nervi[a], M. U. Schmidt[c], R. Gobetto[a], M. R. Chierotti[a]

[a]Dipartimento di Chimica, Università di Torino, via P. Giuria 7, 10125, Torino, Italy
[b]Dipartimento di Farmacia, Università di Torino, via P. Giuria 9, 10125, Torino, Italy
[c]Institut für Anorganische und Analytische Chemie, Johann Wolfgang Goethe-Universität, Max-von-Laue-Str. 7. D-60438, Frankfurt am Main, Germany
E-mail: angelo.gallo@unito.it

Keywords: solid state NMR, small molecules.

In the last two decades Solid State NMR (SSNMR) methods have turned out to be a powerful tool for the structure solution of small organic molecular in their crystal forms. Compared to the X-rays crystallography SSNMR can provide precise position of protons, can also define the weak interactions in the crystal structure, and can help in the definition of the tautomer present in the crystal structure. Here we present three clear examples of the elegant contribution of SSNMR which provides worthwhile information exploitable as constraints in structure determination.
Experiments in SSNMR such as ^{1}H MAS, ^{13}C and ^{15}N CPMAS, ^{1}H DQ MAS, ^{13}C-^{1}H HETCOR and several others can be used to assess the content of the unit cell, proton positions and number of independent molecules in the unit cell (Z')[1] and provide the local molecular arrangement in the unit cell.
We successfully applied this method in combination with crystal structure prediction on three different systems: mebendazole,[2] an active pharmaceutical ingredient which crystallizes in three phases characterized by different tautomeric forms, three structural isomers of pyridine dicarboxylic acids (quinolinic, dinicotinic and dipicolinic acid), that may crystallize as zwitterionic or non-zwitterionic forms[3], and leucopterin, a member of the class of pteridines that gives the white pigment in the wings of *Pieris brassicae* butterflies.[4]

References

[1] P. Hodgkinson, *Prog. Nucl. Magn. Reson. Spectrosc.*, **118–119**, 10–53 (2020)
[2] F. Bravetti, S. Bordignon, E. Alig, D. Eisenbeil, L. Fink, C. Nervi, R. Gobetto, M. U. Schmidt and M. R. Chierotti, *Chem. – Eur. J.*, **28**, e202103589 (2022)
[3] F. Bravetti, R.E. Russo, S. Bordignon, A. Gallo, F. Rossi, C. Nervi, R. Gobetto, M.R. Chierotti, *Molecules*, **28**, 1876 (2023)
[4] Bravetti, L. Tapmeyer, K. Skorodumov, E. Alig, S. Habermehl, R. Huhn, S. Bordignon, A. Gallo, C. Nervi, M. R. Chierotti and M. U. Schmid, *IUCrJ*, **10** (4), 448-463 (2023)

DYNAMICS OF FAST-ROTATING ROTORS IN A METAL-ORGANIC FRAMEWORK AS EXPLORED BY SOLID-STATE NMR

S. Piva,[a] J. Perego,[a] C. X. Bezuidenhout,[a] D. Kubicki,[a] P. Sozzani,[a] S. Bracco,[a] A. Comotti[a]

[a]University of Milano-Bicocca, Via R. Cozzi 55, 20125 Milano, Italy
E-mail: sergio.piva@unimib.it

Keywords: solid state NMR, materials, polymers.

The design and creation of solid materials containing fast-rotating components, such as molecular rotors, is highly attractive for developing advanced responsive machines capable of converting external chemical or physical stimuli into actions.[1] We successfully synthesised a new flexible pillared Metal-Organic Framework (MOF) named FTR-P2, whose structure comprises layers of byciclopentane dicarboxylate molecular rotors (BCP) and Zn metal nodes pillared by azobypyridyl ligands to generate a 3D network.[2] The framework structure consists of two centred interpenetrated nets that undergo reciprocal sliding along the pillar direction in the presence of guests in the pores. VT ^{13}C $\{^1H\}$ CP MAS spectra were collected down to 95 K (Fig. 1 Left) to study the structural changes. The BCP methylene groups always show a singlet indicating fast motional exchange, while more complex behaviour is observed for the pillars that have perfectly symmetric pyridyl rings. However, the carbons of the pyridyl rings only coalesce at temperatures above 150 K, attributed to the fast pedal-like motion of the azo group with $k_{exch} > 3600$ Hz. After adsorption of iodine molecules, the new off-centred structure (FTR-P2-I_2) presents two non-equivalent pyridyl rings: one (Ring A) is exposed to the channel and gains freedom, while the other (Ring B) sits in the plane of the 2D layers and forms hydrogen bonds with the oxygen atoms of the metal nodes that hinder its mobility. The coalesce peaks of the syn- and anti-conformations of C2 shift toward the central peak upon increasing temperature, according to the Boltzmann distribution of the two conformations. Finally, the dynamics of the BCP rotors were studied by VT ^{13}C and ^1H T_1 NMR relaxation times measurements, demonstrating the BCP hyper-mobility even at extremely low temperatures (Fig. 1 Right). Surprisingly, the structural changes induced by iodine molecules prompt an increase in cooperativity that enables even faster mobility with lower energy barriers, shifting the maximum relaxation rate (33.8 MHz) from 85 K to as low as 44 K.

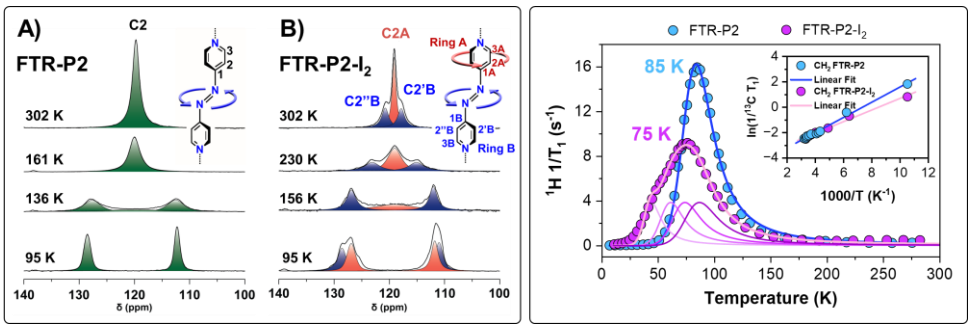


Fig. 1. Left: VT ^{13}C $\{^1H\}$ CP MAS spectra collected at variable temperatures of A) Azpy C2 signal of FTR-P2 and B) Azpy C2 signals of FTR-P2-I_2. Right: ^1H and ^{13}C T^1 NMR relaxation times of BCP moiety of FTR-P2 and FTR-P2-I2 collected at variable temperatures at 33.8 MHz.

References

[1] J. Perego et al. *J. Am. Chem. Soc.* **2021**, 143, 13082-13090
[2] J. Perego et al. *Angew. Chem. Int. ed.* **2024**, e202317094

SYNTHESIS AND SOLID-STATE NMR-DRIVEN CRYSTAL STRUCTURE DETERMINATION OF THE FIRST CO-DRUG OF VALPROIC ACID AND L-CARNITINE

F. Bravetti,[a,b] S. Bordignon,[b] A. Gallo,[b] R. Gobetto,[b] M. R. Chierotti[b]

[a]Institute of Inorganic and Analytical Chemistry, Goethe University, Max-von-Laue-Str. 9, 60438, Frankfurt am Main, Germany
[b]Department of Chemistry, University of Turin, via P. Giuria 7, 10125, Turin, Italy.
E-mail: Bravetti@chemie.uni-frankfurt.de

Keywords: solid-state NMR, small molecules.

Crystal engineering has been for many years a valuable strategy to adopt in the pharmaceutical field. Indeed, it has proved successful in countering the adverse properties associated to the administration of solid crystalline drugs, without altering their inherent pharmaceutical activity. Here, we focused on 2-propylpentanoic acid, commonly known as valproic acid (VPA), a synthetic anticonvulsant drug. Since VPA is liquid in ambient conditions, VPA-based drugs are administered in the form of sodium salts or amide derivatives. VPA is usually associated with a severe carnitine deficiency emerging in patients treated with this API, which leads to a dangerously inadequate lipid metabolism. Thus, amino acid L-carnitine (CNT) is frequently administered in combination with VPA [1]. In this work, we selected VPA and CNT to produce a drug-drug pharmaceutical cocrystal (or co-drug) in the co-administered dosage (1:1). The co-drug was obtained by mechanochemical methods, namely grinding equimolar amounts of VPA and CNT in an agate mortar [2]. VPA*CNT was initially analysed by means of multinuclear 1D and 2D SSNMR experiments (*i.e.*, ^{1}H MAS, ^{13}C and ^{15}N CPMAS, 2D off-resonance ^{1}H-^{13}C FSLG HETCOR), to confirm its actual stoichiometry and obtain information on the number of independent molecules in the unit cell, the protonation state of the two components, and intermolecular atom-atom proximities. The crystal structure of VPA*CNT was successfully determined from powder diffraction data, despite the challenge posed by the several conformational degrees of freedom of both VPA and CNT molecules. Indeed, the information provided by SSNMR on the hydrogen-bond network of the co-drug (see Fig. 1) proved fundamental during the structure determination process [3].

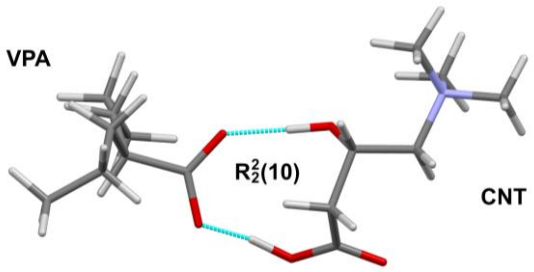


Fig. 1. Representation of the asymmetric unit of VPA*CNT and the characteristic $R_2^2(10)$ hydrogen-bond motif (turquoise dashed lines).

References

[1] P. E. R. Lheureux, P. Hantson, *Clin. Toxicol.*, **47** (2), 101–111 (2009)
[2] S. Bordignon, F. Bravetti, A. Gallo, R. Gobetto, M. R. Chierotti. Co-cristallo farmaco-farmaco di L-carnitina e acido valproico. Patent n. IT 102023000027489 (2023)
[3] K. D. M. Harris, *Crystals*, **12**, 1277 (2022)

USING ^{13}C HIGH-RESOLUTION SOLID-STATE NMR TECHNIQUES TO INVESTIGATE A Cu(II)-BASED PARAMAGNETIC MOF

E. Della Latta,[a] D.M. Dawson,[b] R.E. Morris, [b] S.E. Ashbrook[b]

[a]Department of Chemistry and Industrial Chemistry, University of Pisa, Via Giuseppe Moruzzi 13, Pisa, Italy
[b]School of Chemistry, EaStCHEM and Centre of Magnetic Resonance, University of St Andrews, St Andrews, UK
E-mail: elisa.dellalatta@phd.unipi.it

Keywords: solid state NMR, materials.

HKUST-1 is a Metal-Organic Framework (MOF) containing Cu(II) paddlewheel dimer secondary building units (SBUs) connected by 1,3,5-benzenetricarboxylate linkers (Fig. 1). In the as-synthesised form, solvent molecules occupy the axial positions, and these can be removed upon evacuation (*i.e.*, treatment under vacuum at high temperature) leaving coordinatively unsaturated sites available for the adsorption of guest molecules [1]. Diffraction techniques are usually employed to study MOFs, however solid-state NMR spectroscopy (SSNMR) can overcome the limitations of XRD, such as investigating guest molecules. ^{13}C is the most suitable nucleus for investigating the organic linker and host-guest interactions, however, the paramagnetic nature of the metal centres translates into a broad shift distribution and rapid relaxation. A typical HKUST-1 ^{13}C spectrum (Fig. 1d) shows three resonances related to the organic linker [2] two of which (1 and 2) vary significantly depending on the nature and the presence of guest molecules.

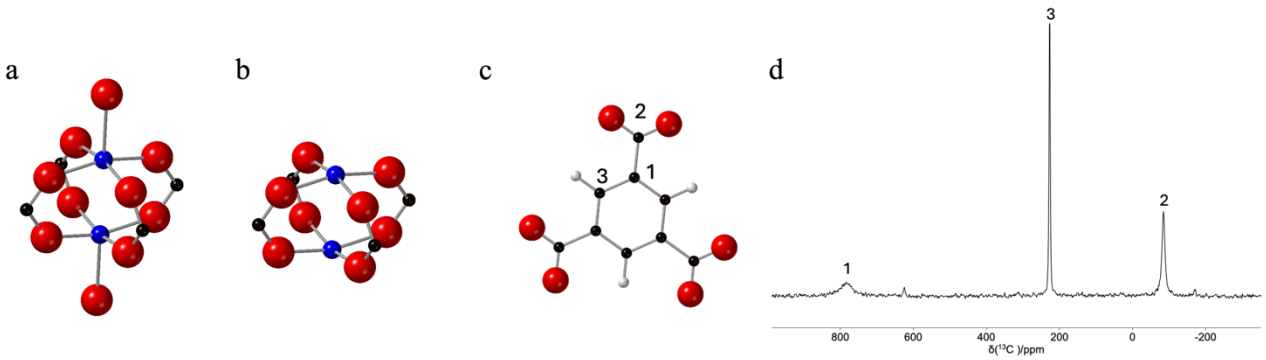


Fig. 1. Cu(II) paddlewheel dimers SBUs of HKUST-1 in the (a) hydrated form with water molecules occupying the axial position and in the (b) evacuated form. (c) The structure of the btc linker. (d) ^{13}C spectrum of evacuated HKUST-1.

In this work, we demonstrate how ^{13}C high-resolution SSNMR (*i.e.*, under Magic Angle Spinning) can be a useful probe to investigate the interaction of HKUST-1 with guest molecules. The dependence of the ^{13}C resonances with temperature was also explored by acquiring variable-temperature experiments [3].

References

[1] S. Chui, R. S. Lo, J. Charmant, A. Orpen and I.D. Williams *Science,* **283**, 1148-1150 (1999)
[2] D.M. Dawson, L. Jamieson, M. Mohideen, A.C McKinlay, I.A. Smellie, R. Cadou, N.S. Keddie, R. E. Morris, S.E. Ashbrook *Phys. Chem. Chem. Phys.* **15** 919-929 (2013)
[3] Z. Ke, D.M. Dawson, S.E. Ashbrook, M. Buehl, *Chem. Sci.* **13** 2674-2685 (2022)

UNVEILING LOCAL DYNAMICS IN TRIPTYCENE-BASED POROUS POLYMERS: A SOLID-STATE NMR STUDY

F. Martini,[a,b] E. Della Latta,[a] S. Borsacchi,[c,b] M. Warndorf,[d] K. R. Storme,[d] T. M. Swager,[d] M.Geppi[a,b]

[a]Dipartimento di Chimica e Chimica Industriale, Via Giuseppe Moruzzi 13, 56124 Pisa, Italy
[b]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), Lungarno Pacinotti 43, 56126 Pisa, Italy
[c]Istituto di Chimica dei Composti Organo Metallici, Consiglio Nazionale delle Ricerche (CNR-ICCOM), Via Giuseppe Moruzzi 1, 56124 Pisa, Italy
[d]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
E-mail: francesca.martini@unipi.it

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

Membrane-based gas separation technology is gaining interest as an energy-efficient alternative to traditional methods. Fluoropolymers with intrinsic microporosity stand out due to their high gas separation and sorption performances [1]. These polymers have bulky structural units, like triptycene groups, that hinder efficient packing of the polymer chains, resulting in a disordered network of pores [2]. Fluorinated groups form specific interactions with penetrants, enhancing gas sorption and separation capabilities. Besides structural features, dynamics play a crucial role in gas transport and sorption within porous polymers [3]. Gas molecules interact intricately with the local reorientations of polymer segments. Studying these properties is essential to understand gas transport and sorption mechanisms. Additionally, dynamics can reveal degradation and plasticization phenomena [4], which significantly impact membrane performance and durability.
This study presents an investigation of the local dynamics of a triptycene-containing fluorinated aryl ether (FLARE) polymer [1], both before and after the absorption of CO_2, by means of solid-state NMR spectroscopy (SSNMR). Different SSNMR techniques were employed, including measurements of ^{1}H, ^{19}F, and ^{13}C spin-lattice relaxation times (T_1), ^{19}F-^{13}C and ^{1}H-^{13}C Dipolar Chemical Shift Correlation (DIPSHIFT) experiments [5], and ^{2}H static spectra. All the gathered data were analyzed collectively, employing suitable dynamic models to achieve a quantitative characterization of dynamics of both the triptycene and the fluorinated biphenyl linkers constituting the polymer monomeric unit. Motion parameters such as activation energies and correlation times were determined. Furthermore, the dynamics of CO_2 in a $^{13}CO_2$-loaded sample were investigated by analyzing variable temperature ^{13}C spectra and ^{13}C T_1 data. This analysis provided valuable insights into the behavior of CO_2 within the polymer matrix, enhancing our understanding of gas adsorption and transport processes.
This research has been carried out with the financial support of the project PRIN PNRR 2022 "**In-MoTion**-Influence of dynaMics on the adsorption and Transport properties in polymeric materials for membrane technologies" funded by the European Union - Next Generation EU.

References

[1] N.B. McKeown *Polymer* **202**, 122736 (2020)
[2] R.T. Long, T. Swager *J. Am. Chem. Soc.* **125**, 14113-14119 (2003)
[3] R. Zorn, W. Lohstroh, M. Zamponi, W.J. Harrison, P.M. Budd, M. Böhning, A. Schönhals *Macromolecules* **53**, 6731−6739 (2020)
[4] S.J.D. Smith, R. Hou, K. Konstas, A. Akram, C.H. Lau, M.R. Hill *Acc. Chem. Res.* **53**, 1381-1388 (2020)
[5] E.R. DeAzevedo, K. Saalwachter, O. Pascui, A.A. De Souza, T.J. Bonagamba, D. Reichert *J. Chem. Phys.* **128**, 104505 (2008)

LOCAL STRUCTURE AND DIFFUSION OF SODIUM IN A HYBRID GLASS FROM HIGH-TEMPERATURE ^{23}Na MAS NMR AT 20 T

P. Kolodzeiski[a], B. Gallant[b], D. J. Kubicki[b], S. Henke[a]

[a]Inorganic Chemistry, Department of Chemistry and Chemical Biology, TU Dortmund, Germany
[b]School of Chemistry, University of Birmingham, Birmingham, UK
E-mail: d.j.kubicki@bham.ac.uk

Keywords: solid state NMR, materials

The glassy state in coordination polymers, such as MOFs, is relatively rare, with only a handful of hybrid glasses known to date [1]. Crystal-liquid-glass transformations are difficult to design, and the resulting glasses are challenging to characterize because of their amorphous nature. However, they possess a range of exotic properties typically absent in conventional inorganic glasses, such as permanent porosity, gas permeability, ion conductivity, and anomalous thermal conductivity. Solid-state NMR has recently emerged as the ideal technique to study their local structure [2]. One of the main practical challenges is the missing understanding of their structure-property relationships that would enable tuning the glassy behavior and glass transition temperature to enable easier processability.
Here, I will talk about a new Na-containing additive that we discovered and that enables unprecedented tunability of the glass transition temperature in a hybrid Zn-based MOF glass. I will discuss high-temperature (300-700 K) ^{23}Na MAS NMR experiments which enabled us to determine the speciation of the dopant, quantify its dynamics and impact on the glassy behavior across a range of compositions. I will highlight how hybrid glasses combine structural features of conventional glasses with unique short-range order characteristics, and how solid-state NMR is an emerging way of studying this new, structurally diverse, and fascinating class of solids.

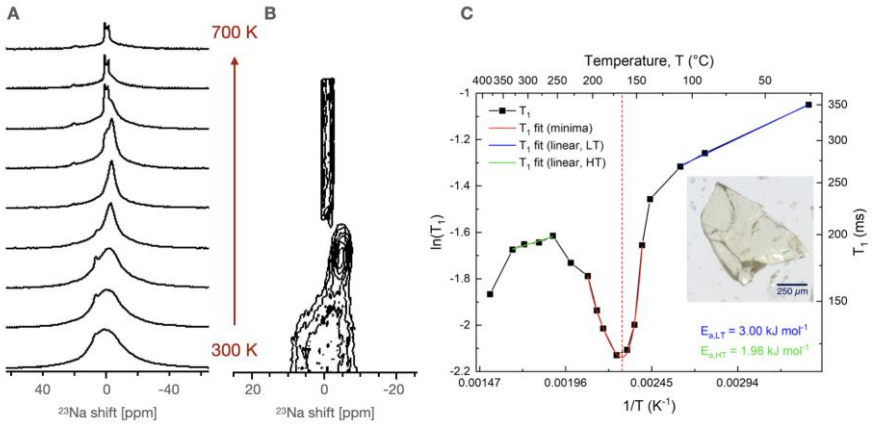


Fig. 1. Solid-state characterization of the doped hybrid glass using a 7 mm laser-heated MAS probe at 20 T (A) VT ^{23}Na MAS NMR, (B) pseudo-2D tracking of the line shape changes as the temperature is increased at a rate of 1 K/s, (C) ^{23}Na T_1 relaxation in the Na dopant evidencing a structural phase transition at 166 °C. The inset shows a shard of the hybrid glass.

References

[1] Ma, N.; Horike, S. *Chem. Rev.* **2022**, *122* (3), 4163–4203.
[2] Madsen, R. S. K.; et al. *Science* **2020**, *367* (6485), 1473–1476

ASSESSING THE RELIABILITY OF VIRTUAL CONTRAST: A GENERALIZATION STUDY ACROSS DIFFERENT TUMOR TYPES AND PATIENT DEMOGRAPHICS

A. N. Caragliano[1,2], A. Macula[1,3], S. C. Serra[1], A. F. Mingo[1], G. Morana[4], A. Rossi[5,6], M. Alì[7], D. Fazzini[7], F. Tedoldi[8], G. Valbusa[1], A. Bifone[9]

[1]Centro Ricerche Bracco, Bracco Imaging SpA, Colleretto Giacosa, Italy
[2]Research Unit of Computer Systems and Bioinformatics, Department of Engineering, Università Campus Bio-Medico di Roma. Rome, Italy, Europe.
[3]Department of Physics, Università degli Studi di Torino, Turin, Italy. [4]Neuroradiology Unit, A.O.U. Città della Salute e della Scienza di Torino, Turin, Italy [5]Neuroradiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy.
[6]Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy.
[7]Centro Diagnostico Italiano, Milan, Italy
[8]GR&D, Bracco Imaging SpA, Milan, Italy
[9]Department of Molecular Biotechnology and Health Sciences, Università degli Studi di Torino, Turin, Italy.
E-mail: anna.macula@bracco.com

Keywords: MRI, contrast agents, theory and methods, exotica.

Accurate and timely diagnosis of brain tumors is critical for patient management and treatment planning. Magnetic Resonance Imaging (MRI) is the method of election for brain tumor detection and characterization, often aided by the administration of gadolinium-based contrast agents (GBCAs) to enhance tumor visualization. Despite its effectiveness, the use of contrast agents has drawbacks, including the risk of allergic reactions, kidney-related complications, and economic burdens on patients and healthcare systems. Deep learning (DL) has emerged as possible solution to these limitations, providing models which predict contrast enhancement in brain tumor imaging directly from non-contrast MRI scans (Virtual Contrast,VC). However, a critical question remains regarding the generalizability of these models across different tumor types and diverse patient populations. Our research is focused on extending the promising performance obtained on a specific tumor type at a certain stage using a specific dataset (adults gliomas), to other tumor types and diverse patient populations. The results showed several limitations. Specifically, both in cases of adult patients with meningiomas and pediatric patients with gliomas, virtual contrast methods were not able to accurately predict contrast from non-contrast data, resulting in either complete or partial omission of lesions or highly inaccurate delineation of the enhancing region.

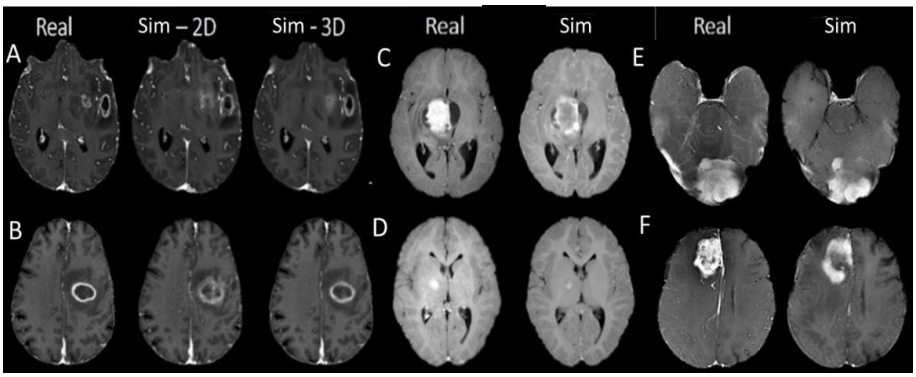


Fig. 1. Simulated versus real axial postcontrast T1w brain images. of adult patients affected by glioma (panels A,B), paediatric patients affected by glioma (panels C,D), and adult patients affected by meningioma (panels E,F). Each panel represents a separate patient, with real images on the left side of the panel and corresponding simulated images obtained with DL models on the right side. For A,B cases, two models (2D and 3D architectures) are shown.

NEW BENCHMARK FOR BENCHTOP NMR MAGNET HOMOGENEITY: HOW SPINSOLVE™ ULTRA NMRS BOOST SOLVENT SUPPRESSION PERFORMANCE FOR 1- AND 2D NMR

D. Bouillaud,* J.Kolz, F. Casanova

Magritek GmbH, Philipsstraße 8, Building MA, 52068 Aachen, Germany

E-mail: dylan@magritek.com

Typically, NMR samples are dissolved in deuterated solvents to avoid the overlapping of the large solvent peaks with the signal of the analytes. However, if samples need to be analyzed while they are being synthesized or if the components of a liquid formulation need to be quantified the use of deuterated solvents is not an option. In these situations, the alternative known from high-field NMR is to implement solvent suppression methods that are used to drastically attenuate the solvent signals. On benchtop NMR systems, however, it is challenging to obtain efficient suppression as these methods require a highly homogeneous magnetic field, like the ones generated by superconducting magnets.

Within this presentation, we´ll demonstrate the performance of the WET solvent suppression method implemented on Spinsolve™ ULTRA NMRs to attenuate the signals of some of the most common organic solvents. The ultra-high homogeneity of the Spinsolve™ ULTRA models makes it possible to significantly attenuate the solvent peaks by two to three orders of magnitude. In this way, the overlapping of the analyte and solvent signals is reduced to the point where the analytes can be detected baseline-separated after applying the WET sequence. Due to the external hardware lock of the Spinsolve™ NMR systems, protonated solvents can be directly employed without requiring tedious sample workups to replace the regular solvents used in the reactor for their deuterated counterparts. Finally, a Spinsolve™ protocol including a CPMG filter will be shown which allows the access of analytes being completely overlapped by broad matrix signals of ^{1}H NMR samples. Within this protocol, significant T_2 time differences are utilized to filter off unwanted peaks.

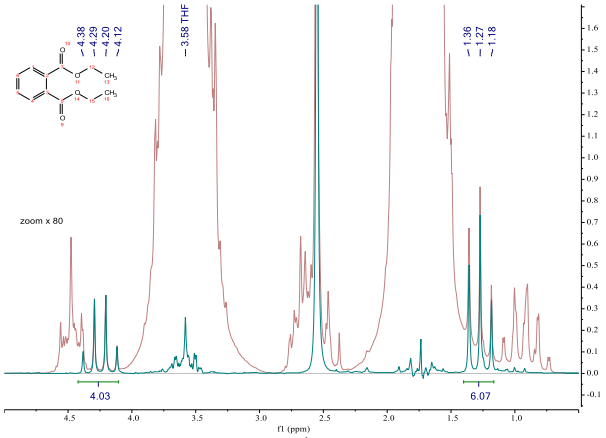


Figure 1: Zoomed comparison of a regular 1D ^{1}H NMR of diethyl phthalate in THF without ^{13}C decoupling and solvent suppression (red) and an applied 1D ^{1}H WET NMR protocol with ^{13}C decoupling and solvent suppression (cyan).

A MRI-CEST METHOD FOR MAPPING WATER CYCLING ACROSS CELLULAR MEMBRANES

E. Di Gregorio [a], C. Papi [a], L. Conti [a], A. Di Lorenzo [a], E. Cavallari [a], M. Salvatore [b], C. Cavaliere[b], G. Ferrauto [a], S. Aime [b]

[a] Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza 52, Torino (Italy)
[b] IRCCS SDN SynLab, Via E. Gianturco 113, Napoli (Italy)
E-mail: chiara.papi@unito.it

Keywords: MRI, contrast agents

Introduction: Water cycling across membrane transporters is a hallmark of cellular metabolism and could be crucial for diagnosing tumors and other diseases. This study reports an imaging method that provides insights into water exchange across compartments exploiting the response of the endogenous, intracellular CEST signal to the presence of an extracellular paramagnetic agent (Fig.1A).
Methods: The approach was tested through mathematical simulations, and then applied *in vitro* and *in vivo*. The paramagnetic effect of Gd-HPDO3A was assessed *in vitro* on the CEST response of molecules like creatine or Iopamidol, either when the paramagnetic agent and the CEST molecule are in the same compartment or in different compartments. Then, the effect of the paramagnetic perturbation brought by Gd-HPDO3A on the endogenous CEST signal was acquired on RBCs and on breast cancer (BCs) cells with a different degree of malignancy (4T1, TS/A and 168FARN). BALB/c mice were inoculated with BC cell lines to generate subcutaneous tumor models. CEST@2ppm [1,2] and DCE-MRI were acquired for 1h post i.v. injection of ProHance. The methodology was also applied to monitor Doxorubicin treatment.
Results: Mathematical simulations showed an exponential decay of the CEST signal with increasing Gd-HPDO3A concentration, appearing linear below 100 µM (tumor concentration). Then, the experiments on creatine-loaded liposomes with varying Gd-HPDO3A concentrations showed that the reduction of CEST response can be ascribed to differences in membrane water permeability. Applying the same method on cell lines, the following order of permeability was found 4T1>TS/A> 168FARN> RBC. *In vivo*, water permeability maps for BC murine models were generated, showing higher permeability in aggressive 4T1 cells and lower in less aggressive 168FARN cells. Maps were reported in Fig.1B-C-D and kinetic curves of the averaged $ST_{@2ppm}$ vs. time after in Fig.1E-F-G. The method was also applied to test the effect of Doxorubicin treatment in 4T1 mice. Doxorubicin treatment significantly decreased water permeability in 4T1 mice without a significant change in tumor size.

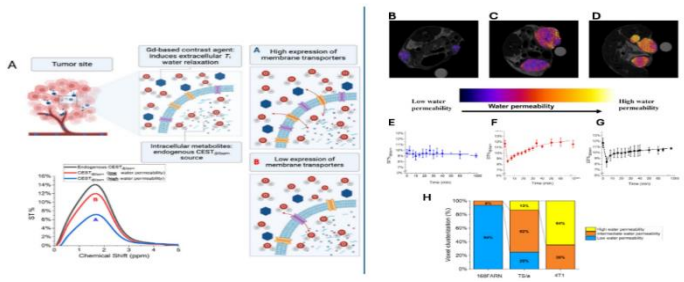


Fig. 1. (B-C-D) Water permeability MRI maps for 168FARN, TS/A and 4T1 murine models, respectively. (E-F-G) Kinetic curves of the averaged $ST_{@2ppm}$ vs. time after the i.v. injection of Gd-HPDO3A bolus (H)Histogram reporting grouped voxels per tumor model.

References

[1] Cai K. et al. NMR Biomed. 2015 Jan;28(1):1-8.
[2] Di Gregorio E. el al. Angew. Chem. Int. Ed. Engl. 2024 Feb 5;63(6):e202313485.

NMR-BASED METABOLOMICS IN MNGIE RARE DISEASE

A. Gambini,[a] E. Boschetti [b], S. Ratti [b], A. Mucci,[a] V. Righi[c]

[a] University of Modena and Reggio Emilia, Department of Chemical Sciences and Geology (DSCG), Via Giuseppe Campi 103 41125 Modena (MO), Italy
[b] Cellular Signaling Laboratory, Department of Biomedical and Neuro Motor Sciences (DIBINEM), Institute of Human Anatomy, University of Bologna, Via Irnerio, 48, 40126 Bologna, Italy
[c] University of Bologna, Department of Science for Quality of Life (QUVI), Corso d'Augusto 237, 47921 Rimini, Italy
E-mail: anna.gambini@unimore.it

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

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an ultra-rare autosomal recessive metabolic disorder caused by loss of function of thymidine phosphorylase. [1,2] The clinical manifestation of MNGIE is progressive and involves multiple systems. Due to the rarity and multisystem complexity of MNGIE, much remains unknown about its metabolic profiles and pathological mechanism. To investigate the metabolic disturbance, induce by MNGIE disease, we analyzed 9 liver tissue with HR-MAS NMR, of which 5 were collected from MNGIE patient and 4 were collected from healthy control. In addition, lipophilic (N=10) and hydrophilic (N=11) liver extract were analyzed by liquid NMR.
Principal component analysis was performed on the spectral data. Exploratory analysis of ^{1}H CPMG liver spectra highlighted that MNGIE sample are characterized by higher level of glucose and triglycerides content. A two tailed t-test was performed on the deconvoluted area of the metabolites and aspartate, β-OH butyrate, ethanol, scyllo-inositol and pyroglutamic acid were found to significantly higher ($p < 0.05$) in the control samples compared to MNGIE ones. Subsequently, metabolic data will be correlated with proteomic and transcriptomic data to investigate pathways at different levels. Finally, the distribution of molecular marker identified by the omics approach will be assessed by histological analysis.

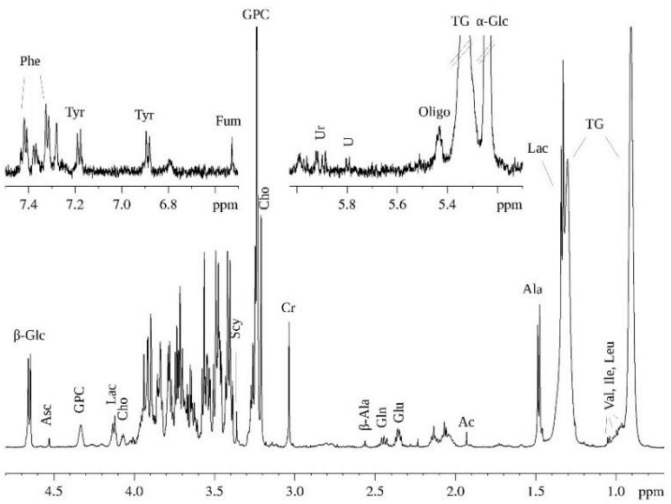


Fig. 1.^{1}H CPMG spectra of a MNGIE liver tissue.

References

[1] M. Hirano, V. Carelli, R . De Giorgio et al., *J. Inherit. Metab. Dis.*, **44**, 376–387 (2021)
[2] R. D'Angelo, R. Rinaldi, V. Carelli et al., *Neurol. Sci.*, **37**,1149–1151 (2016)

AN NMR-BASED APPROACH TO ELUCIDATE THE LINK BETWEEN DIETARY ZINC STORAGE AND TRYPTOPHAN METABOLISM

A. Barbanente,[a] D. Vitone,[a] E. Pignataro[b], S. D'Eusebio[b], C. Tejeda-Guzmàn[c], R. M. Marsano[b], F. Missirlis[c], F. Arnesano[a]

[a] Department of Chemistry, University of Bari "Aldo Moro", via E. Orabona, 4,70125 Bari, Italy [b] Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro" via E. Orabona 4, 70125 Bari, Italy
[c] Department of Physiology, Biophysics and Neuroscience, Cinvestav, 07360 Mexico City, Mexico;
E-mail: alessandra.barbanente@uniba.it
Keywords: solution NMR, small molecules, biomolecules.

Zinc (Zn) is essential for many physiological functions, and its intracellular levels are maintained through dynamic transport and vesicular storage processes. However, due to its labile nature, the Zn coordination environment in storage organelles and the chemical structure of intracellular Zn complexes remain elusive. Recently, we discovered that 3-hydroxykynurenine (3HK) and its derivative, xanthurenic acid (XA), are responsible for Zn binding and storage in the Malpighian tubules of *Drosophila*, which serve as the major Zn reservoir in insects [1]. Specifically, the precursor molecule kynurenine, released from insect fat bodies, induces the formation of Zn storage granules, where 3HK and XA act as endogenous Zn chelators. In this study, we further investigated the interaction of Zn with other kynurenine-related Trp metabolites, such as kynurenic acid (KYNA), xanthurenic acid 8-O-beta-D-glucoside (XAGly)[2], and kynurenine (Kyn), using solution NMR and other spectroscopic and biophysical methods These molecules are of interest due to their physiological relevance and structural similarities with 3HK and XA, aiming to unravel their Zn speciation in biological systems. Moreover, the strong link between Trp metabolism and intracellular Zn accumulation prompted us to consider 6-fluoro-L-tryptophan (6-F-Trp) as a suitable probe for monitoring Trp metabolites in Zn granules via ^{19}F NMR[3,4] (Fig.1). This approach enables the observation in *Drosophila* homogenates of ^{19}F signals of 6-F-Trp catabolites, which are key intermediates of the kyn pathway, with their intensity shown to be influenced by Zn supplementation to the diet.

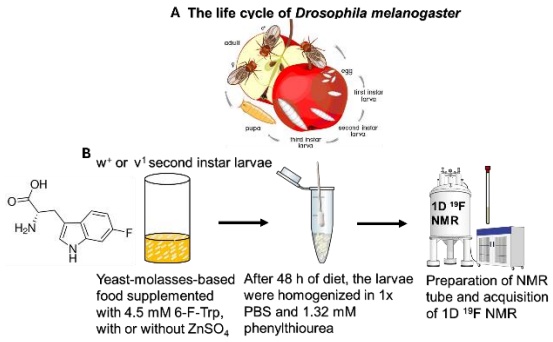


Fig. 1. **(A)** Representation of the life cycle of the fruit fly *Drosophila melanogaster*. **(B)** Schematic workflow of sample preparation and spectroscopical analysis of w+ or v1 *Drosophila* larvae homogenates after feeding with 6-F-Trp, with or without $ZnSO_4$.

References

[1] E. Garay, N. Schuth, A. Barbanente, C. Tejeda-Guzmán, D. Vitone, B. Osorio, A. H. Clark, M. Nachtegaal, M. Haumann, H. Dau, A. Vela, F. Arnesano, L. Quintanar, F. Missirlis, *Proc. Natl. Acad. Sci.* **2022**, *119*, e2117807119.
[2] J. Ferré, M. D. Real, J. L. Ménsua, K. B. Jacobson, *J. Biol. Chem.* **1985**, *260*, 7509–7514.
[3] H. Chen, S. Viel, F. Ziarelli, L. Peng, *Chem. Soc. Rev.* **2013**, *42*, 7971.
[4] R. J. Tombari, C. M. Saunders, C.-Y. Wu, L. E. Dunlap, D. J. Tantillo, D. E. Olson, *ACS Chem. Biol.* **2019**, *14*, 1866–1873.

Friday 6th

	Plenary session **Chair: P. Turano**
8:45-9:30	**Plenary Lecture 6** **Alceo Macchioni (University of Perugia)** APPLICATION OF NMR TO THE STUDY OF CATALYTIC REACTIONS

	Parallel session A **Chair: S. Geninatti Crich**	**Parallel session B** **Chair: A. Macchioni**
9:30-10:00	**Testa C.** MAPPING BRAIN CONNECTIVITY THROUGH WATER MOTION	**Airoldi C.** NMR-BASED IDENTIFICATION AND DEVELOPMENT OF BIOACTIVE COMPOUNDS
10:00-10:15	**Brero F.** OPTIMIZATION OF IRON OXIDE MAGNETIC NANOPARTICLES FOR ENHANCED MRI CONTRAST AND HYPERTHERMIA TREATMENT	**Di Pietro M.E.** CHALLENGES IN USING NMR FOR SUSTAINABLE STRUCTURED SOLVENTS: CAUTION IN INTERPRETING INTERMOLECULAR NOE
10:15-10:30	**Quattrociocchi C.** IN VITRO AND IN VIVO MR IMAGING OF THE CAIX EXPRESSION IN BREAST CANCER	**Todisco S.** A MULTINUCLEAR NMR STUDY OF SELECTIVE DEGRADATION OF ORGANOSILICON COMPOUNDS BY OXALATE ION IN ACQUEOUS SOLUTION
10:30-10:45	**Porru M.** ZINC AND MANGANESE DOPED IRON OXIDE MAGNETIC NANOPARTICLES: EFFECT ON THE ^{1}H-NMR RELAXATION PROPERTIES	**Sabena C.** QUANTITATIVE SOLID-STATE NMR ANALYSIS BY CHEMOMETRIC APPROACH
10:45-11:00	**Macchia M.L.** ASSESSING THE IMPACT OF DONOR GROUPS ON THE COORDINATION AND MAGNETIC PROPERTIES OF MONOHYDRATED Fe(III) COMPLEXES FOR MRI APPLICATIONS	**Righetti G.** DEEP EUTECTIC SOLVENTS FOR HMF VALORIZATION
11:00-11:30	**Coffee break**	
	Parallel session A **Chair: M. Piccioli**	**Parallel session B** **Chair: S. Borsacchi**
11:30-12:00	**Cantini F.** SOLUTION NMR TO ADDRESS STRUCTURE AND INTERACTIONS OF METALLOPROTEINS: THE CASE OF THE IRON-SULPHUR CISD3 AND CALCIUM BINDING PROTEIN CIB2	**Pedone A.** ADVANCEMENT IN THE SIMULATIONS OF SOLID-STATE NMR SPECTRA OF OXIDE GLASSES: INTEGRATING AB INITIO CALCULATIONS AND MACHINE LEARNING
12:00-12:15	**Acconcia C.** NMR-GUIDED DESIGN OF SELECTIVE INTEGRIN LIGANDS	**Ravera E.** AN INTEGRATIVE VIEW ON BIOINSPIRED SILICA-LYSOZYME COMPOSITES
12:15-12:30	**Grasso D.** INVESTIGATION OF THE DYNAMICS/FUNCTION RELATIONSHIP OF PHOSPHORYLATED ONCOPROTEIN YB-1 BY NUCLEAR MAGNETIC RESONANCE	**Pizzanelli S.** STRUCTURE AND DYNAMICS OF HIGHLY CONCENTRATED LiCl METHANOLIC SOLUTIONS CONFINED IN A MESOPOROUS SILICA: AN NMR STUDY
12:30-12:45	**Grifagni D.** AN ABERRANT INTERACTION BETWEEN THE PATHOGENIC P144L MUTANT OF FDX2 AND FDXR PROVIDES THE MOLECULAR GROUNDS OF AN ULTRA-RARE HUMAN GENETIC DISORDER	**Fiorucci L.** REDEFINING THE TOOLBOX TOWARDS PRECISE PARAMAGNETIC NMR PREDICTIONS AT ULTRAHIGH FIELDS
	Plenary session **Chair: L. Ragona and M.R. Chierotti**	
12:45-13:25	**Poster competition winner lectures**	
13:25-13:30	**Closing**	
13:30-14:30	**Lunch**	

APPLICATION OF NMR TO THE STUDY OF CATALYTIC REACTIONS

A. Macchioni[a]

[a]Department of Chemistry, Biology and Biotechnology and CIRCC, Via Elce di Sotto, 8, 06123–Perugia, Italy
E-mail: alceo.macchioni@unipg.it

Keywords: solution NMR, catalysis, energy.

This contribution will show how multinuclear and multidimensional NMR techniques can play a crucial role in understanding catalytic reactions mediated by transition metal complexes (Figure 1). It will be shown how the intra- and inter-molecular characterisation of the latter, also carried out through NOE and diffusional NMR techniques, combined with an in-depth study of their speciation in solution, under conditions comparable to those used in catalysis, leads to an in-depth knowledge of the key factors underlying their activity and deactivation processes. This knowledge, derived from the application of advanced NMR techniques, accessible to all with new instrumentation, can be exploited to design and manufacture homogeneous catalysts with superior performance. Selected examples from recent studies by our group, on homogeneous catalysts with applications in the stereospecific polymerisation of olefins [1] and in the energy field [2], will be shown.

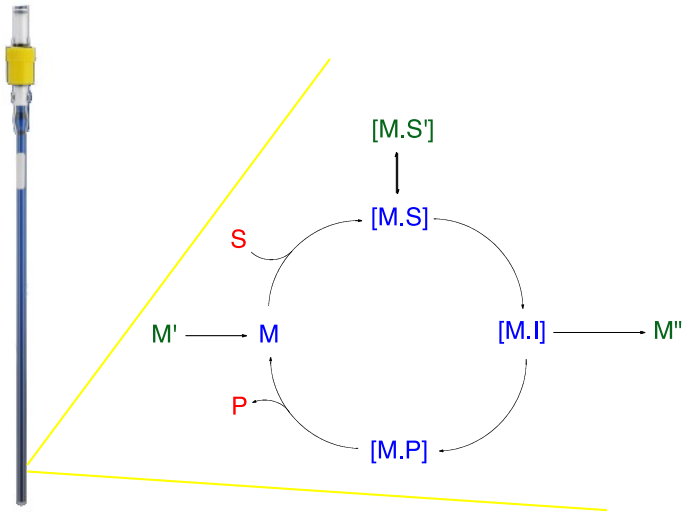


Fig. 1. A generic catalytic cycle mediated by a transition metal complex (M).

References

[1] F. Zaccaria, L. Sian, C. Zuccaccia, and A. Macchioni, *Adv. Organomet. Chem.* **73**, 1–78 (2020); A. Dall'Anese *et al*, *Inorg. Chem.* **62**, 16021−16037 (2023).
[2] G. Menendez Rodriguez, F. Zaccaria, L. Tensi, C. Zuccaccia, P. Belanzoni, and A. Macchioni, *Chem. Eur. J.* **27**, 2050–2064 (2021); C. Trotta, V. Langellotti, I. Manco, G. Menendez Rodriguez, L. Rocchigiani, C. Zuccaccia, F. Ruffo, and A. Macchioni, *ChemSusChem* doi.org/10.1002/cssc.202400612; C. Trotta, G. Menendez Rodriguez, C. Zuccaccia, and A. Macchioni, *ACS Catalysis* accepted for publication (2024).

MAPPING BRAIN CONNECTIVITY THROUGH WATER MOTION

C. Testa[a]

[a]Department of Physics and Astronomy, University of Bologna, viale Berti Pichat 6/2, Bologna, Italy.
E-mail: claudia.testa@unibo.it

Keywords: MRI.

Magnetic Resonance imaging offers the opportunity of non-invasively following the motion of water in the brain tissue. This allows to go beyond the knowledge of the structure of brain towards its microstructure. Diffusion of water is informative of the structural pathways of the brain, nerve fibers, using tractography and connectivity approaches. The study of structural connectivity has contributed to the development of a new "omics" discipline, connectomics, that is a complete map of the brain's structural connections [1]. A roundup of methods to identify anatomical connections in the human brain by characterizing the water motion within the brain tissue, which is sensitive to the presence and the orientation of white matter fibers in the brain will be presented. Examples applied to neurodegenerative diseases and tumor lesions characterization will be shown [2-5].

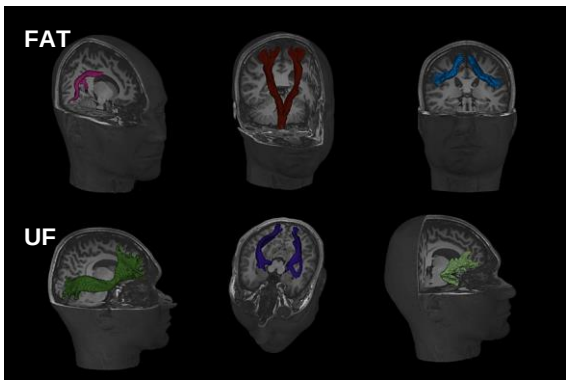


Fig. 1. Color-coded representation of some of the most important white matter tracts. AF: arcuate fasciculus; CST: cortico spinal tract; FAT: Frontal aslant tract; IFOF: Inferior fronto Occipital fasciculus; OR: optic radiation; UF: uncinate fasciculus.

References

[1] O. Sporns. *Dialogues Clin. Neurosci*. **15**, 247-62 (2013)
[2] L. Talozzi et al. *MRI*, **54**, 183-193 (2018)
[3] C. Testa et al. *MAGMA*, **30**, 265-180 (2017)
[4] M. Mitolo et al. *Front. Neurol.* **13**, 867048 (2022)
[5] M. Zoli et al. *Front. Neurol.* **12**, 633209 (2021)

OPTIMIZATION OF IRON OXIDE MAGNETIC NANOPARTICLES FOR ENHANCED MRI CONTRAST AND HYPERTHERMIA TREATMENT

F. Brero[a,b], M. Porru[a,b], A. Gallo-Cordova[c], M. Mariani[b,d], R. Herrera Aquino[c], F. Orsini[d,e] , P. Arosio[d,e], A. Lascialfari[a,b], M. del Puerto Morales[c]

[a] Department of Physics, University of Pavia, Pavia 27100, Italy
[b] National Institute of Nuclear Physics-INFN, Pavia 27100, Italy
[c] Instituto de Ciencia de Materiales de Madrid, ICMM/CSIC, Madrid 28049, Spain
[d] National Institute of Nuclear Physics-INFN, Milano 20133, Italy
[e] Department of Physics, University of Milano, Milano 20133, Italy
E-mail: francesca.brero@unipv.it

Keywords: solution NMR, contrast agents.

Iron oxide-based magnetic nanoparticles (MNPs) serve as dual-function agents for MRI contrast and tumor treatment via Magnetic Fluid Hyperthermia (MFH). Optimizing their shape, size, core type, magnetic ion, and coating is crucial for maximizing their efficacy.
This study investigates two types of core-shell MNPs—nanospheres and nanoflowers (composed of nanospheres)—with varying ferrite-core diameters. These MNPs are coated with biocompatible layers such as dimercaptosuccinic acid (DMSA), polyacrylic acid (PAA), and carboxymethyl-dextran (CM-dextran). Characterizations were performed using AFM, DLS, TEM, XRD, IR, and TG analyses. Relaxation properties were explored through ^{1}H-NMR measurements of longitudinal (T_1) and transversal (T_2) nuclear relaxation times as a function of frequency in the range 10 kHz-65 MHz, revealing contrast efficiency through r_1 and r_2 relaxivities. Core diameters ranged from 11 to 35 nm with coating thicknesses of 1-2 nm. It was singled out that MNPs' shape, size, and coating significantly influenced nuclear relaxation mechanisms and ^{1}H-NMRD frequency profiles. At a clinical field strength of 1.5 T, the particles exhibited r_2 values surpassing those of the Endorem® compound. The study also identified shape and size-dependent heating release mechanisms and Specific Absorption Rate (SAR) values, aligning with existing models. Understanding these factors aids in optimizing MNP parameters for improved MRI contrast and MFH heat release.

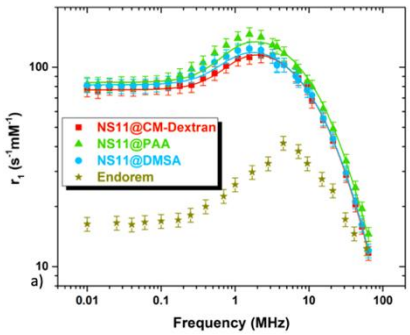


Fig. 1. Longitudinal relaxation profiles of 11-nm nanospheres adapted to the heuristic model of Roch-Müller-Gillis. The Endorem's longitudinal profile is also shown.

References

[1] M. Avolio, et al. *New Trends in Nanoparticle Magnetism*, 327-351 (2021)
[2] F. Brero, et al. *IL NUOVO CIMENTO C*, 46.4: 1-4 (2023)

IN VITRO AND *IN VIVO* MR IMAGING OF THE CAIX EXPRESSION IN BREAST CANCER

C. Quattrociocchi,[a] V. Menchise,[b] D. Delli Catselli [a]

[a]Dep. Molecular Biotechnologies and Health Science, University of Torino, Via Nizza 52, Torino, Italy.
[b]Institute of Biostructures and Bioimaging (IBB), Italian National Research Council (CNR), Torino, Italy
E-mail: claudia.quattrociocchi@unito.it

Keywords: MRI.

Introduction: Important advantages for cancer progression are conferred by the pH deregulation phenomena taking place in the intra-extra-cellular microenvironment. CAIX enzyme is a major player in the pH deregulation and is overexpressed in many solid tumors. The presence of a targetable extracellular domain and its limited expression in healthy tissues have contributed in making CAIX an increasingly important biomarker for cancer diagnosis and therapies[1]. Herein it is reported a new MRI approach for the imaging of the CAIX expression in breast cancer. **Methods:** A targeting peptide directed towards the PG domain of CAIX [2] (PepC) was designed and synthesized together with the scramble sequence (SCR). Two MRI nanoprobes (LIP_PepC/SCR), carrying PepC or SCR peptide on their surface respectively, and loaded with Gd based MRI contrast agent, were prepared. Mouse Mammary TS/A Cells, overexpressing CAIX, were incubated for 4 h with LIP_PepC, LIP_SCR and with a not functionalized liposome (LIP). Images of cells pellet have been acquired at 7T micro-imager immediately after the incubation or 24 hours later. ICP measurements of the mineralized samples have been performed to dose the amount of entrapped Gd [3]. Breast cancer murine models have been prepared by inoculating subcutaneously 300.000 TS/A cells and have been treated with the developed probes for MRI analysis.
Results/Discussion: MR images of a phantom containing cellular pellets incubated with either LIP_PepC, LIP_SCR or LIP are shown in Fig.1(a). The signal enhancement (SE) of the images is significantly higher cells incubated with LIP_PepC Fig.1(b). A comparison between TS/A and cells that shows a lower expression of the CAIX, MDA MB-231, was performed. The SE was significantly higher for TS/A than for MDA thus confirming the specificity of Lip_PepC. In vivo MRI experiments on murine models of breast cancer showed a good specificity of LIP_PepC for CAIX expression in vivo, displaying a maximum SE 8h after iv injection of probe at a dose of 0.1 mmoles Gd per kg (fig1.c).
Conclusion: This study represents the first MRI example of PG domain targeting. The results have demonstrated that the probe LIP_PepC shows high specificity for the CAIX isoform in labelling experiments. Experiments on murine model of breast cancer showed promising results, confirming the efficacy of the MRI strategy in vivo.

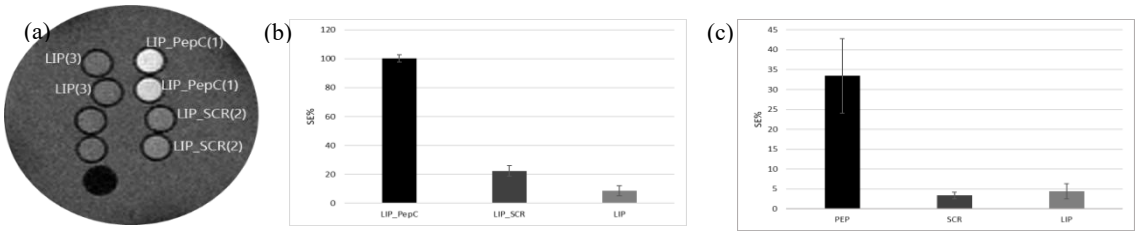


Fig. 1. (a) MRI T1W of TS/A cells incubated with LIP_PepC (1), LIP_SCR (2), LIP (3) and acquired after 24 h of incubation; (b) Histograms showing the SEs for Figure 1a. (c) Histogram showing the SE referred to in vivo images acquired 8h after injection.

References

[1]E. Langella, M. Buonanno, G. De Simone, and S. M. Monti, 'Intrinsically disordered features of carbonic anhydrase IX proteoglycan-like domain', *Cell. Mol. Life Sci.*, vol. 78, no. 5, pp. 2059–2067, Mar. 2021, doi: 10.1007/s00018-020-03697-3.[2]'John et al. - 2020 - Computational modeling of novel inhibitory peptide.pdf'.
[3]C. Quattrociocchi, A. Mangia, S. Aime, V. Menchise, and D. Delli Castelli, 'Molecular Resonance Imaging of the CAIX Expression in Mouse Mammary Adenocarcinoma Cells', *Pharmaceuticals*, vol. 16, no. 9, p. 1301,Sep.2023, doi: 10.3390/ph16091301.

ZINC AND MANGANESE DOPED IRON OXIDE MAGNETIC NANOPARTICLES: EFFECT ON THE ^{1}H-NMR RELAXATION PROPERTIES

M. Porru[a,b], F. Brero[a,b], M. Mariani[a,c], P. Arosio[d,c], F. Orsini[d,c], M. del Puerto Morales[e], A. Lascialfari[a,b]

[a] Department of Physics, University of Pavia, Pavia 27100, Italy
[b] National Institute of Nuclear Physics-INFN, Pavia 27100, Italy
[c] National Institute of Nuclear Physics-INFN, Milano 20133, Italy
[d] Department of Physics, University of Milano, Milano 20133, Italy
[e] Instituto de Ciencia de Materiales de Madrid, ICMM/CSIC, Madrid 28049, Spain
E-mail: margherita.porru01@universitadipavia.it

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

Magnetic nanoparticles (MNPs) have a major role in the biomedical field, particularly in diagnostics [1,2]. Their magnetic nature induces sizable inhomogeneities in the local magnetic field, perturbing the surrounding hydrogen nuclei present in the biological tissues according to their biodistribution. These fluctuations at the nuclear sites leads to the shortening of *spin-lattice* (T_1) and *spin-spin* (T_2) nuclear relaxation times, enhancing the contrast in the MRI images, that is quantified by the longitudinal and transverse relaxivity (r_1 and r_2, respectively). The physical mechanisms underlying the contrast enhancement in MRI are strongly influenced by factors such as core size, shape, coating and doping. This study delves into the impact of core composition (specifically magnetic ions) and particle size modifications on MNPs' relaxivities. Two sets of magnetite-based MNPs ($Mn_xZn_yFe_{3-x-y}O_4$), with core diameters of around 4 nm and 15 nm are studied; the smaller set consists of 1 undoped sample, 3 samples doped with Zn^{2+} (y= 0.1, 0.7, 0.9) and 2 samples where Zn^{2+} is held constant while Mn^{2+} is gradually introduced (y= 0.5, x= 0.2, 0.4); the larger set includes 1 undoped sample, 3 samples with increasing amount of Zn^{2+} (y=0.1, 0.3, 0.5) and 3 with constant Zn^{2+} (y=0.3) and increasing amount of Mn^{2+} (x=0.1, 0.2, 0.3). Both sets were coated with CM-Dextran and polyacrylic acid-PAA to ensure stability and functionality. Morpho-structural and magnetic analyses, including ICP-OES, VSM, DLS, TEM, XRD, IR, and TG, were employed for characterization. The frequency-dependent nuclear relaxivities were studied through ^{1}H-NMRD relaxation times in a frequency range of 0.01 - 64 MHz. It is evidenced how the different physical mechanisms leading the nuclear relaxation affect the spin dynamics, which is strongly influenced by the morpho-structural characteristics and the Zn^{2+}/Mn^{2+} doping level of the nanocompounds. We compare these findings with current literature models for $r_{1,2}$ vs frequency. These results should help to fine-tune the chemical-physical characteristics of ferrite-based nanoparticles for MRI contrast agent applications.

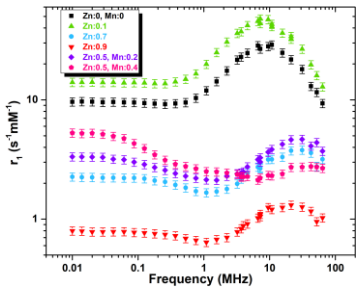


Fig. 1. Longitudinal NMRD profile (r_1) of 4 nm MNPs, with different doping.

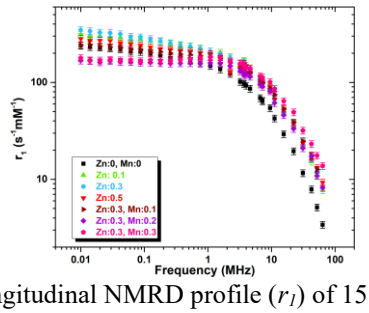


Fig. 1. Longitudinal NMRD profile (r_1) of 15 nm MNPs, with different doping.

References

[1] M. Basini et al. *J. Chem. Phys,* **146**, 034703 (2017)
[2] F. Brero et al. *Nanomaterials,* **13**, 804 (2023)

ASSESSING THE IMPACT OF DONOR GROUPS ON THE COORDINATION AND MAGNETIC PROPERTIES OF MONOHYDRATED Fe(III) COMPLEXES FOR MRI APPLICATIONS

M. L. Macchia,[a] A. Nucera,[a] M. Ricci,[a] F. Carniato,[a] M. Botta[a]

[a] *Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "A. Avogadro", Viale T. Michel 11, 15121 Alessandria, Italy.*

E-mail: maria.macchia@uniupo.it

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

Recent interest in the search for more sustainable alternatives to Gd(III) [1] has led to an increased focus on biologically relevant metals such as Mn(II) and Fe(III). This study investigated the coordination chemistry of monohydrated high-spin Fe(III) complexes in aqueous solution [2]. Four amide-functionalized CDTA derivatives ([Fe(CD2A-monoDEA)], [Fe(CD2A-monoBUT)], $[Fe(CD2A\text{-}bisDEA)]^+$, and $[Fe(CD2A\text{-}bisBUT)]^+$) were synthesized, and their corresponding Fe(III) complexes were prepared. The modifications to the CDTA structure were designed to obtain neutral and positively charged chelates to explore the effects of different donor atoms and the overall charge on the parameters defining their efficacy (relaxivity). Global analysis of integrated ^{1}H NMRD and ^{17}O NMR relaxation data as a function of temperature and magnetic field allowed the assessement of the relaxation parameters. The mean residence lifetime of the inner-sphere water molecule (t_M) increases from $[Fe(CDTA)]^-$ to $[Fe(CD2A\text{-}bisDEA)]^+$ and $[Fe(CD2A\text{-}bisBUT)]^+$, consistent with what has been observed for monohydrated Gd(III) and Mn(II) complexes [3,4].
In addition, the thermodynamic stability and kinetic inertness of the Fe(III) chelates were investigated by pH potentiometry, capillary zone electrophoresis (CZE), and UV-Vis spectroscopy. These data provide a comprehensive description of metal-ligand speciation as a function of pH and dissociation kinetics. The substitution of carboxyl groups results in a decrease in the stability and kinetic inertness of the complexes, although the stability constants remain robust in the range of 10^{20}-10^{22}. The redox activity of [Fe(CDTA-monoDEA)] has been investigated by monitoring the reduction of ascorbate, emphasizing the challenges associated with the reducing biological environment.
This work allowed us to verify the unsuitability of the amide donor group to stabilize Fe(III) and their inefficacy in modulating electronic relaxation parameters that limit the relaxivity values at high magnetic fields. On the other hand, we demonstrated that shifting from negatively to positively charged complexes allows us to transition from an intermediate to a slow water exchange regime without compromising relaxation efficiency (see Fig. 1).

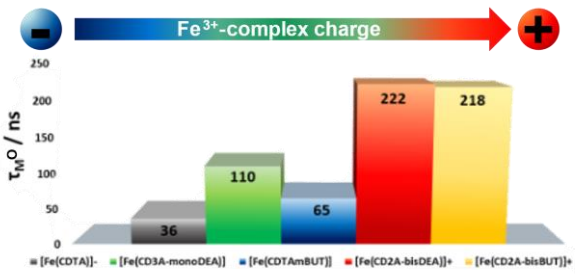


Fig. 1. Water exchange lifetime (τ_M) of Fe(III) CDTA-derivatives complexes.

References

[1] K. Schmidt, M. Bau, G. Merschel, N. Tepe, *Sci. Total Environ*. **687**, 1401–1408 (2019)

[2] Z. Baranyai, F. Carniato, A. Nucera, D. Horváth, L. Tei, C. Platas-Iglesias, M. Botta, *Chem. Sci.* **12**, 11138–11145 (2021)
[3] P. Hermann, J. Kotek, V. Kubíček, I. Lukeš, *Dalton Trans*., **23**, 3027-3047 (2008)
[4] M. Botta, F. Carniato, et al., *Future Med. Chem.* **11**, 1461-1483 (2019)

NMR-BASED IDENTIFICATION AND DEVELOPMENT OF BIOACTIVE COMPOUNDS

C. Airoldi,[a] A. Palmioli,[a] L. Moretti,[a] L. Molteni[a]

[a]BioOrgNMRLab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
E-mail: cristina.airoldi@unimib.it

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

NMR-based molecular recognition studies allow the characterization of a wide variety of ligand-receptor pairs with biological and biomedical relevance [1].
We will report and discuss some selected examples covering binding events involving amyloidogenic proteins [2], oncoproteins [3], cell wall or membrane receptors [4], as well as the development of methodologies for the analysis of heterogeneous systems, such as samples containing cells [1,3b,4], or complex mixtures, such as natural extracts, also requiring the application of metabolomic approaches [1,2].

Acknowledgements
PRIN 2022 - Self-assembly of bacteria-targeting materials across the mesoscale (SAMBA) (PNRR per la Missione 4, componente 2 Investimento 1.1- Avviso 104/2022).
Next Generation EU – "Age-It – Ageing well in an agening society" project (PE000015), National Recovery and Resilience Plan (NRRP) – PE8 – Mission 4, C2, Intervention 1.3".

References

[1] C. Ciaramelli, A. Palmioli, C. Airoldi, *NMR Spectroscopy for Probing Functional Dynamics at Biological Interfaces*, The Royal Society of Chemistry, **6**, 142 (2022).
[2] A. Palmioli, C. Airoldi, *ChemPlusChem*, e202400243 (2024).
[3] a) A. Palmioli, C. Ciaramelli, R. Tisi, M. Spinelli, G. De Sanctis, E. Sacco, C. Airoldi, *Chem. Asian J.*, **12**, 18, 2457–2466 (2017); b) A. Palmioli, C. Ceresa, F. Tripodi, B. La Ferla, G. Nicolini, C. Airoldi, *Bioorg. Chem.*, **99**, 103861 (2020).
[4] a) A. Palmioli, P. Sperandeo, S. Bertuzzi, A. Polissi, C. Airoldi, *Bioorg. Chem.*, **112**, 104876 (2021); b) A. Palmioli, L. Moretti, C. A. Vezzoni, L. Legnani, P. Sperandeo, L. Baldini, F. Sansone, C. Airoldi, A. Casnati, *Bioorg. Chem.*, **138**, 106613 (2023).

CHALLENGES IN USING NMR FOR SUSTAINABLE STRUCTURED SOLVENTS: CAUTION IN INTERPRETING INTERMOLECULAR NOE

M. E. Di Pietro,[a] A. Mannu,[a] F. Castiglione,[a] A. Mele[a]

[a]Dept. Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133, Milano, Italy
E-mail: mariaenrica.dipietro@polimi.it

Keywords: solution NMR, materials, theory and methods.

The nuclear Overhauser effect (NOE) plays a unique role in the large repertoire of NMR techniques, as it depends on spatial dipolar interactions of nuclei rather than chemical connectivity via chemical bonds. NOE is then a well-recognized tool for characterizing structure and interactions in liquids, and its application has been extended to detecting intermolecular contacts in neoteric structured liquids such as ionic liquids and deep eutectic solvents [1].
However, its use in intermolecular studies is fundamentally limited by an unspecific long-ranged interaction behavior [2], often leading to confusion and overinterpretation in rapidly growing research areas like intermolecular interactions in sustainable solvents.
An approach to overcome this limitation is to select general cases where a short-distance interpretation of NOE is applicable without contradicting the general theory of intermolecular cross-relaxation [3]. This contribution shows that the long-ranged contributions of the spectral density function can be minimized by maximizing the frequency difference between the selected interacting isotopes. For isotopes with similar gyromagnetic ratios, e.g. the homonuclear ^{1}H-^{1}H extreme case, as well as the heteronuclear ^{1}H-^{19}F case, the NOEs are long-ranged. In contrast, selecting isotopes with significantly different gyromagnetic ratios promotes a short-ranged behavior (Fig. 1). This is illustrated here using HOESY build-up curves for the ^{1}H-^{7}Li pair in an ionic liquid-based electrolyte [4] and the ^{1}H-^{31}P pair in hydrophobic type V deep eutectic solvents.

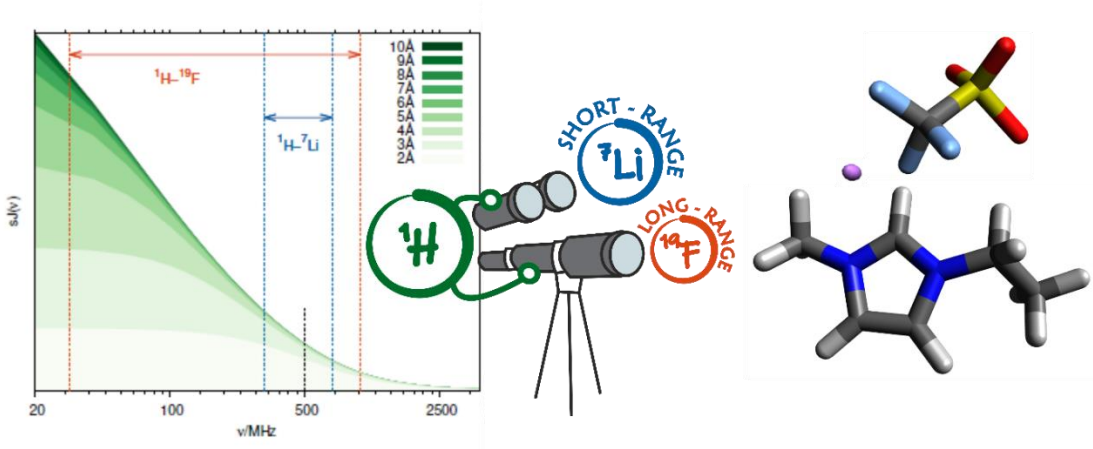


Fig. 1. Proper selection of interacting isotopes resulting into mainly short- or long-ranged behavior.

References

[1] A. Mele, G. Romanò, M. Giannone, E. Ragg, G. Fronza, G. Raos, V. Marcon *Angew. Chem. Int. Ed. Engl.* **45**, 1123-1126 (2006).
[2] S. Gabl, O. Steinhauser, H. Weingärtner *Angew. Chem., Int. Ed.* **52**, 9242−9246 (2013).
[3] F. Castiglione, G. B. Appetecchi, S. Passerini, W. Panzeri, S. Indelicato, A. Mele *J. Mol. Liq.* **210**, 215−222 (2015).
[4] P. Honegger, M. E. Di Pietro, F. Castiglione, C. Vaccarini, A. Quant, O. Steinhauser, C. Schröder, A. Mele *J. Phys. Chem. Lett.* **12**, 8658−8663 (2021).

A MULTINUCLEAR NMR STUDY OF SELECTIVE DEGRADATION OF ORGANOSILICON COMPOUNDS BY OXALATE ION IN ACQUEOUS SOLUTION

S. Todisco,[‡] B. Musio,[‡] R. Ragone,[‡] M. Trisolini,[‡] M. Triggiani,[‡] M. Latronico,[‡,†] P. Mastrorilli,[‡,†] V. Gallo[‡,†]

[‡]DICATECh, Polytechnic of Bari, Via E. Orabona 4, Bari, Italy
[†]Innovative Solutions, Spin Off of Polytechnic of Bari, Zona H, 150/B, 70015 Noci BA, Italy
E-mail: stefano.todisco@poliba.it

Keywords: QNMR, ^{29}Si NMR, Standard NMR reference, Oxalate, Degradation.

Organosilicon compounds represent a class of organic compounds with a mainly hydrophobic character endowed with notable stability in air exploited in countless applications in the field of materials chemistry. An important application of organosilanes is in the field of NMR spectroscopy where their signals are used for calibration of ^{1}H, ^{13}C and ^{29}Si spectra and for evaluating the quality and homogeneity of magnetic field.
To solubilize the organosilanes in aqueous solutions they are usually functionalized with polar groups producing amphiphilic molecules that, as a drawback, are exposed to intermolecular interactions with proteins, which affects the sharpness of the organosilanes NMR signal. In fact, the most common polar organosilanes employed as standard in quantitative NMR (3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt, TSP and (3-Trimethylsilyl)propanesulfonic-2,2,3,3,4,4-d_6 acid sodium salt, DSS) can be employed in agrifood or biological matrices only after deproteinization of the sample. [1]
Moreover, it has been ascertained that when TSP or DSS are used in water solutions containing $HC_2O_4^-/C_2O_4^{2-}$ buffer (which warrants great stability of the sample over time [2-4]) an unexpected reactivity between the TSP, or DSS, and oxalate ions was observed, leading to slow but unremitting degradation of the amphiphilic organosilanes (Figure).

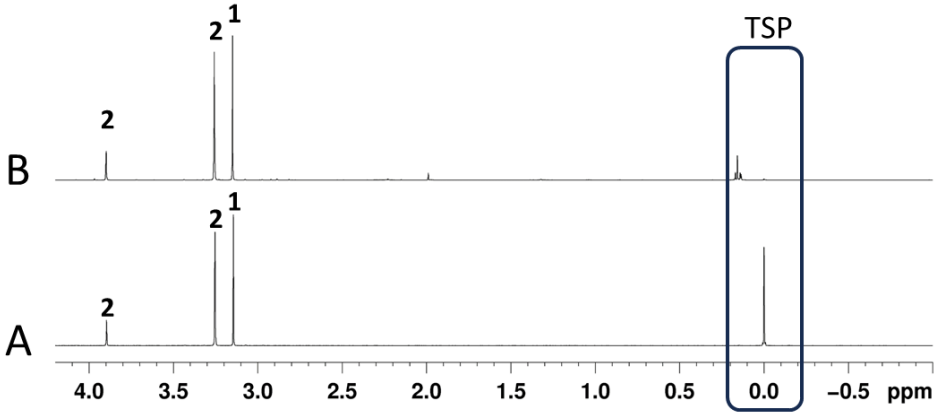


Fig.1. Portion of the ^{1}H NMR spectra of a H_2O/D_2O dimethylsulfone (**1**) + betaine (**2**) solution containing TSP and $HC_2O_4^-/C_2O_4^{2-}$ buffer: A) freshly prepared; B) after two weeks at rt.

References

[1] J. Wallmeier, C. Samol, L. Ellmann, H. U. Zacharias, F. C. Vogl, M. Garcia, K. Dettmer, P. J. Oefner, W. Gronwald, GCKD Study Investigators, *J. Proteome Res.* 2017, **16**, 1784−1796
[2] R. Ragone, S. Todisco, M. Triggiani, S. Pontrelli, M. Latronico, P. Mastrorilli, N. Intini, C. Ferroni, Biagia Musio, V. Gallo, *Food Chemistry* **332**, (2020), 127339
[3] B. Musio, S. Todisco, M. Antonicelli, C. Garino, M. Arlorio, P. Mastrorilli, M. Latronico, V. Gallo, *Appl. Sci.* 2022, **12**, 2583
[4] A. Elhussein, B. Musio, S. Todisco, P. Mastrorilli, V. Gallo, M. Saponari, F. Nigro, Stefania Gualano, F. Santoro, *Molecules* 2023, **28**, 7512

QUANTITATIVE SOLID-STATE NMR ANALYSIS BY CHEMOMETRIC APPROACH

C. Sabena,[a] C. Rosso,[a] E. Alladio, [a] L. Castellino, [a] A. Gallo,[a] R. Gobetto,[a] M.R. Chierotti [a]

[a] Department of Chemistry, University of Turin, Pietro Giuria 7, 10125, Turin, Italy
E-mail: chiara.sabena@unito.it

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

Most of the drugs currently on market, approximately 80-90 %, are administered as solid formulations [1]. For this reason, the investigation of the solid-state properties of Active Pharmaceutical Ingredients (APIs) has gain significant importance for the pharmaceutical industries. Indeed, APIs can exist in various crystalline – polymorphs, solvates/hydrates, salts and cocrystals – or amorphous forms and these different forms can exhibit substantial variations in chemical and physical properties of the API, such as solubility, stability, and bioavailability [2]. To avoid patent infringements/litigations, it is necessary for the pharmaceutical companies to identify, characterize and quantify the different forms of the API to be sure that only the desired form is in the drug product. Therefore, for regulatory and property reasons, it is crucial to have a good method to detect the possible presence of contaminating forms, both amorphous and crystalline, even in small amount.
This work is focused on developing a fast and reliable SSNMR-based quantification method for mixtures of crystalline and amorphous forms. The quantification method is based on the combination of SSNMR with chemometric approaches, in particular by using MCR-ALS (Multivariate Curve Resolution-Alternating Least Squares) for data processing. MCR-ALS is a chemometric technique used for the analysis of multivariate datasets, particularly in spectroscopic applications, and it is specifically designed to resolve complex mixtures into their pure component spectra and concentration profiles, even in the presence of overlapping signals [3].
The method was tested on two APIs for which various crystalline and amorphous forms are reported in the literature. Specifically, indomethacin (INDO), a non-steroidal anti-inflammatory drug, and mebendazole (MBZ), an anthelmintic drug, were selected. For both APIs, the different solid forms were obtained and characterized using FT-IR, Raman, and SSNMR analyses. Subsequently, mixtures of known composition were prepared to evaluate the reliability of the quantification method both on amorphous-crystalline (INDO) and crystalline-crystalline (MBZ) mixtures. In particular, binary mixtures between the amorphous form and the γ polymorph were prepared for INDO, while for MBZ, mixtures were made between polymorphs A and C. All mixtures were analyzed using ^{13}C CPMAS SSNMR spectroscopy (that can easily distinguish different crystal forms, due to differences in chemical shift, and amorphous forms, due to the presence of signals with very large linewidths), and the acquired spectra were pre-processed by using *i*coshift (a tool useful to aligned spectra [4]) and then treated by using MCR-ALS.
The proposed method provided excellent quantitative data, effectively distinguishing and quantifying both crystalline and amorphous fractions. This approach presents a performant, fast, and effective alternative to traditional quantification methods, overcoming quantification issues due to the CPMAS technique and offering promising results for pharmaceutical analysis and quality control.

References

[1] B.M. Couillaud, P. Espeau, N. Mignet, Y. Corvis. *ChemMedChem*. **14** (1), 8-23 (2019).
[2] A. Karagianni, M. Malamatari, K. Kachrimanis. *Pharmaceutics*. **10** (1), 18 (2018).
[3] M. Garrido, F.X. Rius, M.S. Larrechi. *Anal Bioanal Chem*. **390** (8), 2059-2066 (2008).
[4] F. Savorani, G. Tomani, S. Engelsen. *Journal of Magnetic Resonance*. **202** (2), 190-202 (2010).

DEEP EUTECTIC SOLVENTS FOR HMF VALORIZATION

G. I. C. Righetti,[a] M. E. Di Pietro,[a] A. Mele[a]

[a] Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Via Mancinelli 7, Italy
E-mail: graziaisacarla.righetti@polimi.it

Keywords: solution NMR, small molecules.

The development of sustainable and efficient processes for biomass exploitation represents a primary target both in industrial and academic research. 5-Hydroxy methylfurfural (HMF) has gained increasing attention as it is a valuable platform chemical that can act as precursor for the synthesis of high value-added bio-based molecules. [1-3] New and sustainable chemical routes can be developed by using alternative reaction media such as mixtures of Hydrogen Bond Donors and Acceptors (HBDs, HBAs). In this context, HMF interaction with selected type V Deep Eutectic Solvents (DESs) has been assessed by multinuclear NMR, FTIR and Raman spectroscopy. Based on previous HMF-DES systems scouting, HMF reactivity in the most promising DESs has been studied (Figure 1).

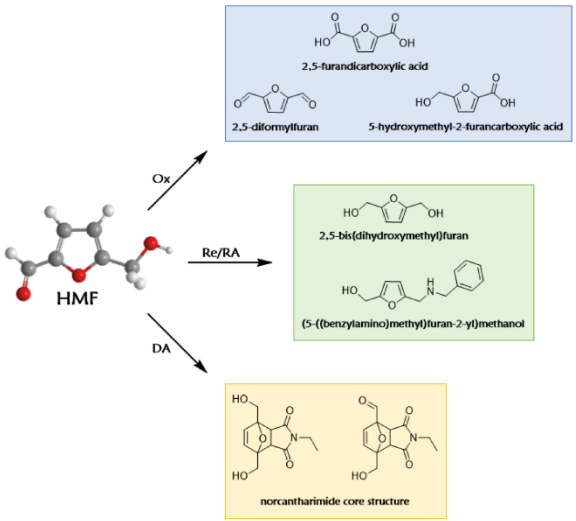


Fig. 1. Proposed HMF transformation in DES.

Finally, green metrics such as atom economy, solvent recycling, environmental factor and EcoScale have been calculated for the most relevant scenarios.

Acknowledgement: The SEED4GREEN project has been funded by European Union – Next Generation EU in the framework of the PRIN 2022 (project SEED4GREEN - Code 20223W4RT9).

References

[1] Nolan M. D., Mezzetta A., Guazzelli L., Scanlan E. M., *Green Chemistry*, **24**, 1456-1462 (2022)
[2] Arnaboldi S., Mezzetta A., Grecchi S., Longhi M., Emanuele E., Rizzo S., Arduini F., Micheli L., Guazzelli L., Mussini P. Electrochimica Acta, **380**, 138-189 (2021)
[3] Iris K.M. Yu, Daniel C.W. Tsang, Biosourced Technology, **238**, 716-732 (2017

SOLUTION NMR TO ADDRESS STRUCTURE AND INTERACTIONS OF METALLOPROTEINS: THE CASE OF THE IRON-SULPHUR CISD3 AND CALCIUM BINDING PROTEIN CIB2

F. Cantini[a]

[a]Magnetic Resonance Center CERM and Department of Chemistry "Ugo Schiff", University of Florence, Florence, Italy
E-mail: francesca.cantini@unifi.it

Keywords: solution NMR.

Solution NMR, with its broad range of applications, from structural to dynamic characterization of macromolecules, from the study of single molecules to macromolecular complexes, can contribute to the description of the mechanisms of functioning of a biological system at the atomic level. I will present how solution NMR contributes to describe the function of two proteins involved in calcium-mediated signal transduction and iron-sulphur protein biogenesis respectively.
Human Calcium- and Integrin-Binding protein 2 (CIB2) is a small protein involved in the Ca(II)-mediated signal transduction in hearing and probably in sight. It belongs to a family of calcium and integrin-binding proteins containing four EF-hand domains that change conformation upon Mg(II) and/or Ca(II) binding [1]. Its broad expression levels in a variety of tissues suggests its involvement in a multiplicity of physiological and disease-associated processes. At variance with the other proteins of this family, an atomic level characterization of CIB2 in the presence of both Ca(II) and Mg(II) ions is missing. NMR interaction studies and dynamic properties of CIB2 with both metal ions and with the $\alpha7\beta$ integrin peptide will be shown. These results offer novel insights into the dynamic regulation of target recognition and unravel the role of metal binding events in CIB2 [2].
CISD3 is a [2Fe-2S] binding protein belonging to the NEET proteins family which is involved in cancer, diabetes and neurodegenerative disorders. The NEET proteins have been proposed to act as redox switches with their active states being associated to the oxidation of their cluster. Combining cellular, biochemical and biophysical approaches, we show that CISD3 exhibits an intrinsic sensitivity of its cluster to oxygen, sensitivity to pH variation and to various stress conditions (iron deficiency, exposure to hydrogen peroxide, or nitric oxide) different from the other members of the family [3]. The characterization of human CISD3 provides a better understanding of the mechanisms underlying its function and permits the comparison with the other human NEET proteins, mitoNEET, and CISD2.

References

[1] R. Vallone, G. Dal Cortivo, M. D' Onofrio and D. Dell'Orco *Front Mol Neurosci.* **11**, eCollection (2018)
[2] G. Olivieri, G.Dal Cortivo, S. Zanzoni, V. Marino, R. Del Conte, F. Cantini, D. Dell'Orco *submitted*
[3] D. Grifagni, J. M. Silva, L. Querci, M. Lepoivre,C. Vallieres, L. O. Louro, L. Banci, M. Piccioli, M-P. Golinelli-Cohen, F. Cantini *J. Biol.Chem.* **300**, 105745 – 105771(2024)

NMR-GUIDED DESIGN OF SELECTIVE INTEGRIN LIGANDS

C. Acconcia,[a] A. Paladino,[b] B.Farina[b,c], A. Del Gatto[b,d], S. Di Gaetano[b,d], D. Capasso[d,e], M.T Gentile[a], M. Saviano[d,f], R. Fattorusso[a], L. Zaccaro[b,d], L. Russo[a]

[a]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli" Caserta, Italy;
[b]Institute of Biostructures and Bioimaging, CNR, Via Castellino 101, Naples, Italy;
[c]Advanced Accelerator Applications, A Novartis Company, Via Ribes, Colleretto Giacosa, Italy;
[d]Interdepartmental Center of Bioactive Peptide, University of Naples Federico II, Via Mezzocannone 16, Naples, Italy;
[e] "Ettore Pancini" Physics Department, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo - Via Cinthia, 21 - 80126, Naples, Italy;
[f]Institute of Crystallography, CNR, URT Caserta, Via Vivaldi 43, Caserta, Italy.
E-mail: clementina.acconcia@unicampania.it

Keywords: solution NMR, RGDechi, molecular dynamics, tumor progression.

Integrins, comprising non-covalently associated α and β subunits, constitute a family of heterodimeric membrane receptors [1]. Their pivotal involvement in tumor progression and the formation of metastases has ignited interest in devising novel pharmaceutical agents, capable of regulating integrin activity to introduce new therapeutic approaches [2]. Integrins able to bind the RGD motif, such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta$, play a significant role in various tumor processes [3]. Over the last decade, we designed and characterized a bi-functional peptide (called RGDechi-hCit), that is composed of a cyclic RGD pentapeptide for integrins binding, covalently linked by a spacer at a modified C-term echistatin fragment to confer a high selectivity for the β_3 receptor [4]. *In vitro* and *in vivo* data demonstrated that RGDechi-hCit is able to selectively bind $\alpha_v\beta_3$ integrin, and structural investigations, based on a combination of NMR and computational techniques, using $\alpha_v\beta_3$ embedded in cancer cell membranes, highlighted the molecular details of the binding [5]. To validate the recognition model and the key role of homocitrulline residue (h-Cit) in the recognition of the β_3 chain, we designed a derivative peptide, called RGDechi15D, in which the h-Cit was substituted with an aspartic acid [5]. Interestingly, this substitution shifted the selectivity from $\alpha_v\beta_3$ to $\alpha_v\beta_5$, in accordance with the different amino acid composition of the two integrin subunits. Here, we report an alternative on-cell NMR approach, developed to investigate the structural details driving the formation of the RGDechi15D/$\alpha_v\beta_5$ complex, under native conditions [6]. Additionally, to shed light on the integrin recognition mechanism by RGDechi-hCit, structural-dynamic NMR and MD data collected for two derived RGDechi-hCit peptides (RGDechi1-14 and ψRGDechi), obtained by chemical modification of the C-terminal part of the peptide, will also be presented [7].

References

[1] Takada, Y.; Ye, X.; Simon, S. Genome. Biol. 8, 215, 2007.
[2] Desgrosellier, J.S.; Cheresh, D.A. Nat. Rev. Cancer, 10, 9–22, 2010.
[3] Giancotti, F.G.; Ruoslahti, E. Science, 285, 1028–1032, 1999.
[4] Del Gatto A, Zaccaro L, Grieco P, Novellino E, Zannetti A, Del Vecchio S, et al. J Med Chem 2006;49:3416–20.
[5] Farina B, de Paola I, Russo L, Capasso D, Liguoro A, Gatto AD, et al. Chemistry. 2016;22 (2):681–93.
[6] Farina B., Corvino A., Del Gatto A., Comegna D., Di Gaetano S., Capasso D., Paladino A., Acconcia C., et al. Computational and Structural Biotechnology J. 19 (2021) 3303-3318.
[7] Acconcia C., Paladino A., della Valle M., Farina B., Di Gaetano S., Capasso D., Gentile M.T., et al. International Journal of Molecular Science, 23,11.3, 2022.

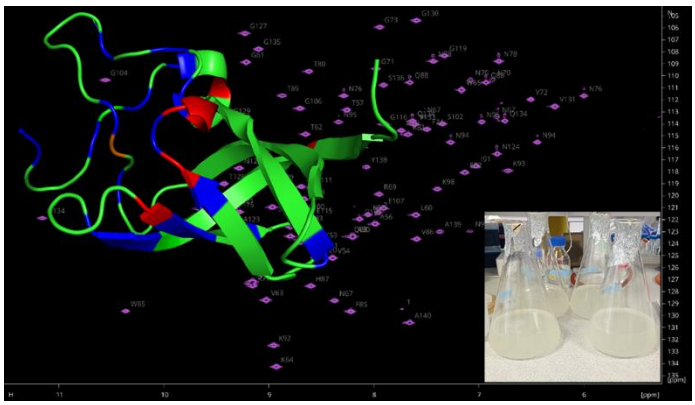
INVESTIGATION OF THE DYNAMICS/FUNCTION RELATIONSHIP OF PHOSPHORYLATED ONCOPROTEIN YB-1 BY NUCLEAR MAGNETIC RESONANCE

D. Grasso, [a, b] A. Bernini, [a] F. Prischi [b]

[a] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy.
[b] Randall Centre, King's College London, United Kingdom.
E-mail: daniela.grasso@student.unisi.it, andrea.bernini@unisi.it, filippo.prischi@kcl.ac.uk

Keywords: solution NMR, biomolecules.

The human Y-box-binding protein 1 (YB-1) is a multifunctional DNA/RNA binding oncoprotein, allowing it to interact with specific regulatory regions in the genome and modulate gene expression. In tumours from cancer patients, YB-1 is highly phosphorylated, and it has been shown that phosphorylation of Ser 102 affects both DNA and RNA binding by inducing a conformational change in the cold shock domain (CSD). However, other neighbouring amino acids undergo phosphorylation with unknown structural/functional outcomes. DNA binding from YB-1 could be affected by phosphorylations in a sequence-dependent fashion, regulating the expression of different genes. This study aims to characterise YB-1 phosphorylation-induced structural and dynamics changes and DNA binding capability by combining NMR and in vitro studies using RSK4 Kinase (Ribosomal S6 Kinase 4).



Results: YB-1 CSD was expressed as 15N enriched, and backbone amide assignment was carried out by 2D and 3D NMR double resonance spectroscopy. The kinetics of CSD phosphorylation was monitored using 15N SOFAST HMQC spectroscopy upon incubation of CSD with RSK4 under proper conditions. T1 and T2 15N-relaxation measurements also evaluated backbone dynamics for both phosphorylated and non-phosphorylated CSD. Phosphorylation sites of YB-1 CSD and full-length protein were confirmed by mass spectroscopy. The chemical shift perturbation and backbone dynamics analysis of NMR spectra identified the protein regions mostly influenced by phosphorylation, showing dynamical changes possibly involved in the DNA binding modulation. Last, MD simulations of CSD and phosphorylated-CSD provided a rational for the identified experimental changes.

Conclusions: Significant changes were observed in the YB1-CSD 15N NMR spectra upon phosphorylation, suggesting a change in the conformation and dynamics of residues Lys58, Trp65, Phe66, Asn67, Tyr72, Gly73, Phe74, Asn76, Val86, His87, Gln88, Thr89, Ala90, Ile91, Lys92, Lys93, Asn95, Arg101, Ser102, Gly104, Gly119, Val131, Val133, Gln134, Ala139, Ala140 the region involved in the DNA binding. Moreover, the attenuation of other AAs in the neighbourhood of Ser102 hints at the possible phosphorylation of this residue, also suggested by MS, which could trigger the changes in the DNA-binding region, thus modulating the binding to nucleic acids and, in turn, gene expression. Our results suggest that YB-1 phosphorylations do not cause conformational changes in the conserved CSD, but alter protein dynamics, which may be key in regulating DNA/RNA binding. Future aspects involve biomarker research since YB1 is secreted in blood, and investigation through liquid biopsy can be envisaged.

AN ABERRANT INTERACTION BETWEEN THE PATHOGENIC P144L MUTANT OF FDX2 AND FDXR PROVIDES THE MOLECULAR GROUNDS OF AN ULTRA-RARE HUMAN GENETIC DISORDER

D. Grifagni[a], D. Doni[b], B. Susini[a], P. Costantini[b]*, S. Ciofi-Baffoni[a]*

[a] Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019, Sesto Fiorentino, Florence, Italy.
[b] Department of Biology, University of Padova, 35121, Padova, Italy.

E-mail: grifagni@cerm.unifi.it

Keywords: (solution NMR, low field NMR, biomolecules, exotica.)

FDX2 plays a crucial role as electron source required for the assembly of both [2Fe-2S] and [4Fe-4S] clusters in mitochondria. A rare, orphan autosomal recessive disorder (episodic mitochondrial myopathy with or without optic atrophy and reversible leukoencephalopathy, MEOAL) is caused by mutations of FDX2 gene [1,2]. Recently, a homozygous missense mutation in FDX2 (c.431C > T, p.P144L) was found [3], but how the mutation of this highly conserved proline residue damages the iron-sulfur cluster biosynthesis is still elusive. In this work, we present a structural and dynamic characterization of the pathogenic P144L FDX2 mutant, performed by solution NMR, and we investigate its interaction with its electron donor, the ferredoxin reductase, by monitoring electron transfer efficiency and structurally characterizing complex formation between FDX2 and FDXR in comparison with the wild-type system. These data allow us to provide a molecular picture of how the mutation negatively affects the electron transfer pathway required for assembling mitochondrial iron-sulfur clusters (Figure 1).

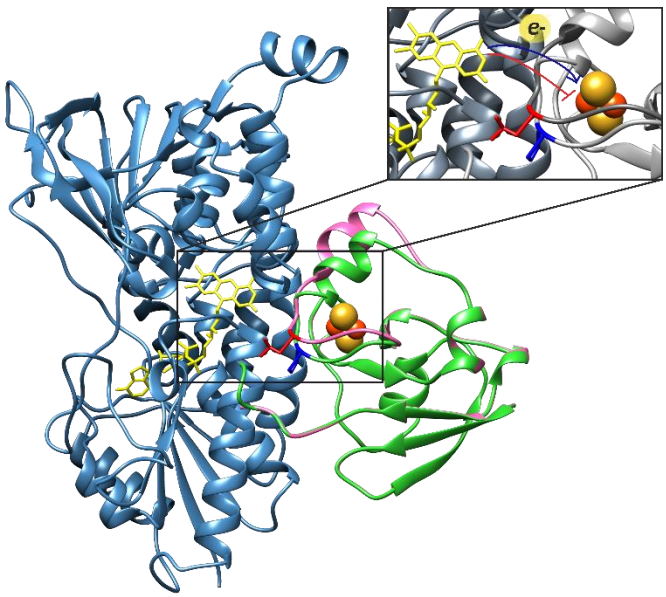


Fig. 1. Representation of the aberrant interaction between FDXR and FDX2 caused by P144L pathogenic mutation (in blue FDXR, in green WT FDX2 and in pink P144L FDX2).

References

[1] Spiegel R. *et al.*, *Eur J Hum Genet* **22**, 902-906 (2014)
[2] Lebigot E. *et al.*, Mol Genet Metab **112**, 85-94 (2017)
[3] Schulz V. *et al.*, Nat. Chem Biol. 19 (2), 206-217 (2023)

ADVANCEMENT IN THE SIMULATIONS OF SOLID-STATE NMR SPECTRA OF OXIDE GLASSES: INTEGRATING AB INITIO CALCULATIONS AND MACHINE LEARNING

A. Pedone,[a] M. Bertani,[a] T. Charpentier[c]

[a]*University of Modena and Reggio Emilia, Department of Chemical and Geological Sciences, via Campi 103, Modena, Italy*
[b]*Université Paris-Saclay, CEA, CNRS, NIMBE, 91191 Gif-sur-Yvette, France*
E-mail: alfonso.pedone@unimore.it

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

Solid-state NMR spectroscopy is a powerful tool for investigating the structure of oxide glasses, providing essential insights into coordination numbers, network connectivity, oxygen speciation, and cation mixing. However, the amorphous nature of glasses complicates the interpretation and deconvolution of NMR spectra, especially for complex compositions. This is due to the broadening of spectra caused by the diverse topological and chemical environments present in the glass matrix.
To address these challenges, in the past we have integrated Molecular Dynamics (MD) simulations with Density Functional Theory (DFT) calculations using the Gauge-Including Projector Augmented Wave (GIPAW) method.[1-3] This MD-GIPAW approach generates and optimizes glass structures, subsequently calculating NMR parameters. However, the high computational cost limits its application to small system sizes (up to ~1000 atoms) and does not account for dynamical effects at room temperature.
In recent years, Machine Learning (ML) has emerged as a promising alternative to overcome the limitations of DFT calculations.[4] ML techniques can predict various properties with near-DFT accuracy while significantly reducing computational time. In this communication, we will revise our previous studies and we will show how ML techniques can be successfully used to predict NMR chemical shifts in silicate glasses enabling the simulations and analysis of larger and more complex glass systems, paving the way for deeper structural insights and more efficient spectral interpretations.

References

[1] T. Charpentier, M. C. Menziani, A. Pedone, *RSC Adv.* **2013**, *3*, 10550–10578.
[2] M. Bertani, N. Bisbrouck, J.-M. Delaye, F. Angeli, A. Pedone, T. Charpentier, *Journal of the American Ceramic Society* **2023,** 106**,** 5501-5521.
[3] C. J. Pickard, F. Mauri, *Phys. Rev. B* **2001**, *63*, 245101.
[4] Z. Chaker, M. Salanne, J.-M. Delaye, T. Charpentier, *Physical Chemistry Chemical Physics* **2019**, *21*, 21709–21725.

AN INTEGRATIVE VIEW ON BIOINSPIRED SILICA-LYSOZYME COMPOSITES

E. Ravera,[a,b,c,d]

[a] Centro di Risonanze Magnetiche, Università degli Studi di Firenze, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy;
[b] Department of Chemistry Ugo Schiff, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy;
[c] Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy;
[d] Florence Data Science, Università degli Studi di Firenze
E-mail: ravera@cerm.unifi.it

Keywords: solid state NMR, materials, biomolecules.

Hen egg-white lysozyme promotes the formation of condensed silica microparticles from silicic acid solutions under milder conditions than the standard sol-gel synthesis [1]. A similar chemistry appears to be applicable to titania and other IV-oxidation state oxides. However, little is known about the mechanism of the reaction, and even less so about the fate of the protein after the reaction is completed.
Over the past years, we applied a wide range of structural biology methodologies - including MAS-NMR and spin-labelling EPR - for understanding the interaction between the protein and the precursor [2], and for studying the protein-silica interface [3-5]. Our results indicate that the nature of the protein-precursor interaction is mainly electrostatic in nature, and this has been recently confirmed by molecular simulations [6]. After the reaction, the main contribution to the confinement of the protein within the composite is steric, even though electrostatics provide some orientational preference.
From the NMR standpoint these composite represent a significant challenge because of the dilution that the matrix imposes on the protein component and *vice versa*. To overcome this limitation, we have applied DNP [3] but also developed alternative processing methods for increasing the SNR *a posteriori* [7,8].

This work has been supported by:
Project no. IR0000009 - call MUR 3264/2021 PNRR M4/C2/L3.1.1 and Project no. P2022JSC5Z – call MUR 1409/2022 PNRR M4/C2/L1.1 CUP D53D23016850001 (European Union NextGenerationEU)

References

[1] Heather R Luckarift et al., Small, 2006, 2 (5), 640–643
[2] Lucia Gigli et al., Biomolecules, 2021, 11 (1), 43
[3] Enrico Ravera et al., ChemPhysChem, 2015, 16 (13), 2803-2809
[4] Francesco Bruno et al., Langmuir, 2022, 38 (26), 8030-8037
[5] Francesco Bruno et al., Journal of Composites Science, 2023, 7, (5), 188
[6] Marina Macchiagodena et al., Chemistry – A European Journal, 2024, in press
[7] Francesco Bruno et al., Analytical Chemistry, 2020, 92 (6), 4451-4458
[8] Francesco Bruno et al., Magnetic Resonance in Chemistry, 2023, 61, 373-379

STRUCTURE AND DYNAMICS OF HIGHLY CONCENTRATED LiCl METHANOLIC SOLUTIONS CONFINED IN A MESOPOROUS SILICA: AN NMR STUDY

S. Pizzanelli,[a] F. Nardelli[a]

[a] National Research Council, Institute of chemistry of organometallic compounds, Via Giuseppe Moruzzi 1, 56124 Pisa, Italy
E-mail: silvia.pizzanelli@pi.iccom.cnr.it

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

Electrolyte solutions confined in nanopores are technologically relevant as they play a crucial role in applications in the fields of energy conversion, nanofluidics, catalysis, and desalinization. The structure and dynamics of aqueous solutions of inorganic salts confined in silica materials have been extensively investigated [1], whereas non-aqueous electrolyte solutions have received less attention. We present experimental evidence on the structure and dynamics of highly concentrated methanolic LiCl solutions confined in a mesoporous silica gel by means of NMR spectroscopy. This system finds application in adsorption cooling technology, where the incorporation of the salt into the porous matrix enhances the affinity of methanol for the adsorbent and results in improved adsorption cycle performances [2].
Methanol and LiCl methanolic solutions with increasing concentrations were confined in the porous matrix. A collection of NMR techniques was employed to access different properties, including ^{1}H chemical shifts, self-diffusion of methanol by ^{1}H pulse field gradient techniques (Figure 1), and ^{7}Li and ^{1}H longitudinal relaxation times (T_1), the latter obtained through Fast Field Cycling NMR relaxometry.

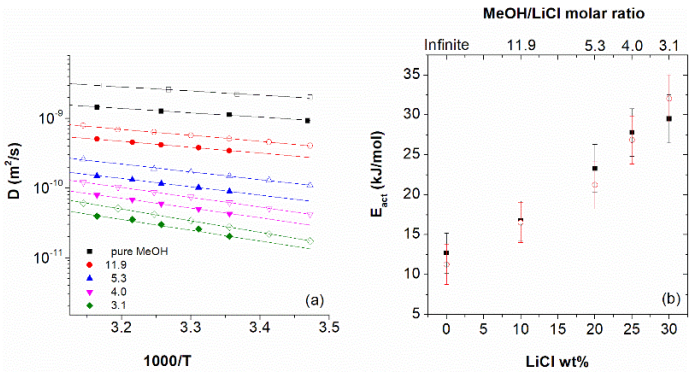


Fig. 1. (a) Self-diffusion coefficient of methanol as a function of temperature in the confined (filled symbols) and bulk (empty symbols) solutions at the indicated MeOH/LiCl molar ratios. The straight lines represent Arrhenius fits. (b) Activation energy values derived from the Arrhenius fits of the data reported in panel (a) as a function of LiCl concentration for the confined (filled black squares) and bulk (empty red circles) solutions.

References

[1] H. R. Corti, G. A. Appignanesi, M. C. Barbosa, J. R. Bordin, C. Calero, G. Camisasca, M. D. Elola, G. Franzese, P. Gallo, A. Hassanali, K. Huang, D. Laria, C. A. Menéndez, J. M. Montes de Oca, M. P. Longinotti, J. Rodriguez, M. Rovere, D. Scherlis, I. Szleifer *Eur. Phys. J. E* **44,** 136 (2021).
[2] L. G. Gordeeva, Y. I. Aristov *Energy* **36,** 1273-1279 (2011).

Acknowledgments. This research was partially funded by the Italian Ministry of University and Research (project PRIN 2022 FLARECO - Eco-friendly, washing-durable FLAme-REtardant finishing for cotton fabrics by COvalent grafting of α-amino acid-derived polyamidoamines, code 202237JYZN).

REDEFINING THE TOOLBOX TOWARDS PRECISE PARAMAGNETIC NMR PREDICTIONS AT ULTRAHIGH FIELDS

L. Fiorucci[† ‡§], L. Lang[#], E. Ravera[† ‡§], G. Parigi[† ‡§], C. Luchinat[† ‡§]

‡ Magnetic Resonance Center (CERM), University of Florence, via L. Sacconi 6, 50019 Sesto Fiorentino, Italy;
† Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, 50019 Sesto Fiorentino, Italy
§Consorzio Interuniversitario Risonanze Magnetiche Metallo Proteine (CIRMMP), Sesto Fiorentino, 50019, Italy
Institut für Chemie, Theoretische Chemie/Quantenchemie, Technische Universität Berlin, Sekr. C7, Straße des 17. Juni 135, 10623 Berlin, Germany
E-mail: fiorucci@cerm.unifi.it

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

The presence of paramagnetic centers impacts both shifts and relaxation rates in NMR experiments through the hyperfine interaction. The hyperfine interaction is an extremely valuable source of long-range restraints and electronic structure information, but increasing evidence has highlighted that a correct modelling is not trivial. For example, the possibility of acquiring paramagnetic NMR data at very high magnetic field values revealed the need for suitable equations for describing the hyperfine interaction that remain valid even at such high fields: experiments conducted on paramagnetic transition metal complexes and lanthanoid complexes at 28.2 T (1.2 GHz), pointed out a non-negligible field dependence of hyperfine shifts (that can be as large as 12 ppm at 1.2 GHz for a highly anisotropic complex like DyDOTA). To cope with this, we developed and tested new equations based on the definition of an hypersusceptibility tensor, representing the second non-zero term in the induced field expansion over the applied external magnetic field (analogously to what Ramsey did for diamagnetic complexes[1]). Part of this field-induced correction comes from the same physical phenomena, i.e. energy levels saturation, that has been usually described with the Brillouin equation, that causes a decrease (in absolute value) of the chemical shifts. In addition to this, high magnetic fields produce, in presence of an anisotropic contribution to the magnetic susceptibility, a partial orientation in solution, that causes an increase (in absolute value) of the hyperfine shifts. This latter effects comes directly from the orientational averaging of this high order derivative. The developed equations are valid within the point-dipole approximation and can be expressed both in the spin hamiltonian formalism and in the Kurland-McGarvey fashion [2,3]. This derivation is completely general, therefore it is applicable to any d^n or f^n configuration. This treatment, coupled with Quantum Chemistry prediction of the parameters and the application of crystal field/ligand field programs, allowed us to reproduce the experimentally observed magnetic field effects with a high level of accuracy for a number of small inorganic complexes. This gave us an in-depth view of the effects contributing to the field-related hyperfine shift variation, their angular dependence and relative contribution to the total effect. This could lead to the inclusion of this field effect in the hyperfine shift simulations for paramagnetic protein structure refinement, as well as for the characterization of the electronic structure of paramagnetic tags, MRI contrast agents and single-ion magnets.

References

[1] Ramsey, N. F. *Phys. Rev. A*, *1*(5), 1320 (1970)
[2] Kurland, R. J., & McGarvey, B. R. *Journal of Magnetic Resonance*, *2*(3), 286-301 (1970).
[3] Lang, L., Ravera, E., Parigi, G., Luchinat, C., & Neese, F. *J. Phys. Chem. Lett.*, 11, 20, 8735–8744 (2020)

Aknowledgments

L.F. acknowledges the support of the H2020 project HIRES-MULTIDYN (Grant 899683)

POSTERS

THE INFLUENCE OF BIODEGRADABLE PACKAGING MATERIAL ON THE QUALITY AND SHELF LIFE OF FRESH APPLES

D. Ambroselli,[a,b] V. Vergine,[a] C. Ingallina,[a,b] G. Adiletta,[c] P. Russo,[c] M. E. Crestoni,[a] L. Mannina,[a,b]

[a] Department of Chemistry and Technology of Drugs, Sapienza University of Rome, P. le Aldo Moro 5, 00185 Rome, Italy
[b] NMR Lab, Sapienza University of Rome, P. le Aldo Moro 5, 00185 Rome, Italy
[c] Department of Chemical Engineering Materials Environment, Sapienza University of Rome, Via Eudossiana 18, 00184 Rome
E-mail: donatella.ambroselli@uniroma1.it

Keywords: metabolomics, food.

Fruits and vegetables are highly perishable and prone to post-harvest losses, which is one of the major obstacles to making these nutritious commodities available to consumers.
Packaging is one of the most important post-harvest operations, with a significant role in controlling postharvest damages and physical, chemical, and biological contaminants. However, conventional food packaging greatly contributes to an exponentially growth of packaging waste and pollution globally. Eco-sustainable bio-packaging in food wrapping represents an alternate option to reduce negative environmental impact, keeping the product's long shelf-life and consumers' health.
Minimally processed apples are highly susceptible to tissue softening, enzymatic browning, and microbiological development, thus hindering their commercial marketing.
Within the Agritech project [1], this study aims to evaluate the effect of two biodegradable packaging materials on the quality and safety of fresh-cut apples during cold storage (21 days, 5 °C) in comparison with packaging in conventional polymer pouches (low-density polyethylene (LDPE) film). The two commercial biodegradable films have the following compositions: i) corn starch, cassava and eucalyptus; ii) polylactic acid from corn starch. Additionally, a commercial LDPE film was used as the reference packaging material.
Analyses to monitor chemical profile and new and (re)-emerging hazards in stored funnels at three time points (0, 14 and 21 days) were carried out by means of untargeted (FT-ICR MS, NMR) and targeted methodologies (HPLC-DAD, HPLC-MS/MS). In addition, chemical, mechanical, microbiological quality and safety of use assessments have been carried out on fresh and packaged samples. In particular, the primary metabolites, including amino acids, carbohydrates, organic acids, lipids and fatty acids, and the secondary metabolites, such as polyphenols and terpenes, were identified and quantified.

References

[1] The research was conducted as part of the Agritech project and received funding from the European Union European Next-Generation EU (NATIONAL REPRESENCE AND RESILIENCE PLAN (PNRR) - MISSION 4 COMPONENT 2, INVESTMENT 1.4 - D.D. 1032 17/06/2022, CN000022).

[P-2]

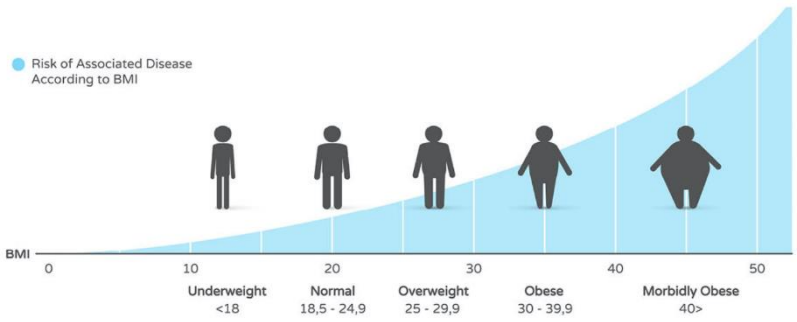
METABOLOMIC PROFILING OF MORBID OBESITY INDIVIDUALS BEFORE AND AFTER BARIATRIC SURGERY

V. Balloni[1], S. Brocchi[1], K. Rahmouni[1], E. O. Gorleku[1], M. Luconi[2], G. Cantini[2], D. Grasso[1], A. Bernini[1]

[1] Structural Biology Lab, Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy.
[2] Dipartimento di Scienze Biomediche, Sperimentali e Cliniche 'Mario Serio', University of Florence, Italy.
E-mails: valentina.balloni@unisi.it

Keywords: solution NMR, small polar molecules, biomolecules, metabolomics, untargeted analysis, morbid obesity, serum.

Class III obesity, or morbid obesity (MO), is characterised by a significant increase in body mass index (BMI), mainly due to fat accumulation. A person with Class III obesity has a BMI of 35 or higher. Recent statistics indicate that over 2 billion individuals worldwide are affected by obesity or overweight, which is about 30% of the global population. However, while BMI is a useful index for diagnosis, it does not evaluate the metabolic alterations and the outcomes usually associated with this condition. Epidemiological data suggest that obesity is linked to a 30–70% increased risk of colorectal cancer (CRC), type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), and other non-communicable chronic diseases. Due to the complexity and controversy regarding whether obesity should be considered a disease, we propose metabolic profiling of serum as a useful tool to stratify MO individuals and predict long-term responses to different bariatric surgery procedures, allowing for more personalised interventions.



Results

Although investigating a condition hallmarked by excessive lipid accumulation through the profiling of serum polar metabolites seems counterintuitive, it must be considered that fatty acid metabolism involves basic molecules such as acetate, butyrate, citrate. Such metabolites can be easily identified and quantified in an untargeted fashion by NMR of biofluids. By comparing the profile of MO and healthy individuals, we revealed the dysregulation of several metabolites connected with lipid metabolism, e.g. ketone bodies, citrate, SCFA, but also biomarkers connected with purine salvage pathway and protein methylation. Comparison of metabolic profiles of the same individuals after bariatric surgery allowed to stratify patients as respondent and non-respondent. The latter group is much more largely populated, suggesting surgery ameliorating BMI and connected risks but not necessarily the molecular basis of the condition.

Conclusions

Metabolic profiling of circulating small molecules in individuals pre/post bariatric surgery allowed for determining the molecular signature of MO and patient stratification. The next step is the molecular characterisation of body tissues of the same subjects to differentiate overall and regional metabolism dysregulation.

[P-3]

THE ASSESSMENT OF TUMOUR GRADE IN BREAST CANCER BY MEANS OF FIELD CYCLING RELAXOMETRY

S. Baroni,[a] V. Bitonto,[a] S. Rakhshan,[a] A. Zareach,[a] A. Pittaro,[b] , I. Castellano[b] and S. Geninatti Crich[a]

[a]Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza, 52, 10126, Torino, Italy
[b]Department of Medical Sciences, University of Turin, 10126 Torino, Italy
E-mail: simona.baroni@unito.it

Keywords: low field NMR, theory and methods.

A tumour is classified according to its stage and grade, which are the parameters to stratify patients according to their risk of recurrence and to determine the most suitable therapeutic treatment. The grade indicates the level of differentiation and aggressiveness and is currently assigned on the basis of the histologic examination conducted by the anatomopathologist. In addition to being operator-dependent, the assignment does not refer to universally accepted and uniform guidelines [1].
The field cycling (FC) relaxometry at low magnetic field strengths (below 0.2 T) has the potential to discriminate between cancerous and healthy cells. This technique has demonstrated the ability to characterise tumour margins [2]. Indeed, the relaxation rates R_1 data of biological tissues provide insight into the physio-pathological transformations occurring within the cells and at the level of membrane water permeability.
In this study, we applied relaxometric investigation to assess the tumour grade of small freshly excised tissue samples from patients undergoing breast cancer surgery, in comparison to the gold standard histological evaluation. The acquisition of R_1 was performed on a SpinMaster FFC-NMR relaxometer (Stelar S.r.l., Mede, Italy), equipped with a microcoil of 10 mm diameter. The water exchange rate constant across the cell membrane (k_{io}) and the extracellular volume fraction were determined by analysing the data using the 2SX model [3]. The preliminary data indicated that the relaxometric method provided a sensitivity of 93% and a specificity of 86% in classifying samples.

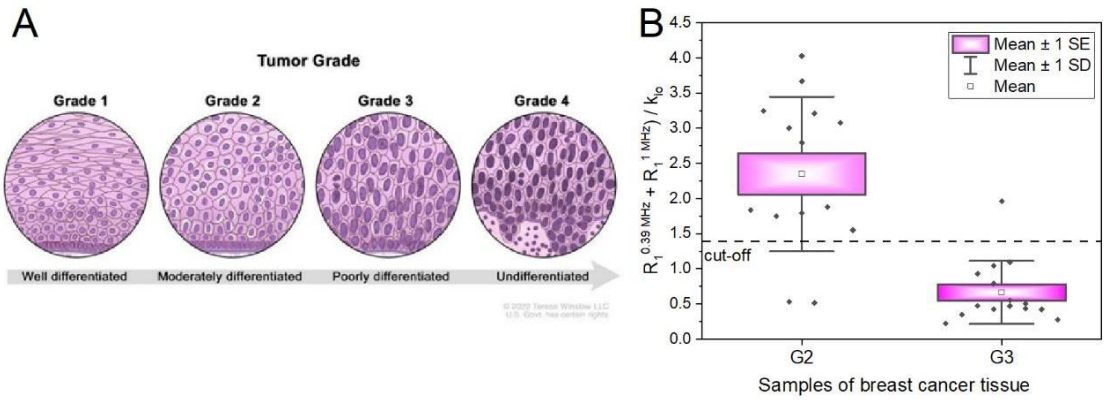


Fig. 1. A) Schematic representation of tumour grade. B) Relaxometric classification of G2 or G3 grade breast cancer tissue samples.

References

[1] C.W. Elston and I.O. Ellis *Histopathology* **19**, 403-410 (1991)
[2] V. Bitonto, M.R. Ruggiero, A. Pittaro, I. Castellano, R. Bussone, L.M. Broche, D.J. Lurie, S. Aime, S. Baroni and S. Geninatti Crich S.G Cancers **13**, (2021)
[3] X. Li, S. Mangia, J. Lee, R. Bai and C.S. Springer *Magn. Reson. Med.* **82**, 411-424 (2019)

[P-4]

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

T. Bolognesi[a,b], M. Schiavina[a,b], I. C: Felli[a,b], R. Pierattelli[a,b]

[a] Magnetic Resonance Center, University of Florence, Via L. Sacconi 6, Sesto F.no (IT)
[b] Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, Sesto F.no (IT)
E-mail: tessa.bolognesi@unifi.it

Keywords: solution NMR, biomolecules.

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

References

[1] L. Pontoriero, M. Schiavina, S. M. Korn, A. Schlundt, R. Pierattelli, I. C. Felli, *Biomolecules* **12**, 929 (2022)
[2] M. Schiavina, L. Pontoriero, G. Tagliaferro, R. Pierattelli, I. C. Felli, *Biomolecules* **12**, 1302 (2022)
[3] M. Schiavina, M. G. Murrali, L. Pontoriero, V. Sainati, R. Kümmerle, W. Bermel, R. Pierattelli, I. C. Felli, *Biophys J* **117**, 46–55 (2019)

Acknowledgments:

The support of the CERM/CIRMMP center of Instruct-ERIC is gratefully acknowledged. The Covid19-NMR consortium is greatly acknowledged for stimulating discussions. This work was supported in part by the project "Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC" (ITACA.SB, Project no. IR0000009) and by the project ID-COVID (FISR2020IP_02112).

[P-5]

VT-^{1}H SSNMR APPLIED TO SOLID ACIDS: CORRELATION BETWEEN DYNAMICS AND SUPERPROTONIC TRANSITIONS

S. Bordignon,[a] M. C. Chierotti,[a] S. Ocak,[b] S. d'Agostino,[b] D. Braga[b]

[a] Department of Chemistry and NIS Centre, University of Torino, 10125 Torino, Italy
[b] Department of Chemistry, University of Bologna, 40126 Bologna, Italy
E-mail: simone.bordignon@unito.it

Keywords: solid state NMR, materials, small molecules.

Solid electrolytes are materials which exhibit fast ion conduction features. They have gained considerable attention in the last decades because of their applicability in electric devices such as batteries, fuel cells and sensors. molecular sensors, supercapacitors, batteries, and fuel cells [1,2]. In this context, solid acids such as alkali-metal salts of H_2SO_4, H_2SeO_4 and H_3AsO_4 were found to manifest superprotonic phase transitions, i.e., reversible solid-solid transitions involving dynamically disordered hydrogen-bond networks [3]. This in turn confers to such materials an increased protonic conductivity. Additionally, when using crown ethers as coordinating agents towards the alkali cations of these solid acids, superprotonic phase transitions appear to be favored, possibly in association with dynamical processes involving both the crown ether and the acid proton [4].
Here, ^{1}H variable-temperature (VT)-SSNMR was employed to assess variations in the dynamics of 18-crown-6·$KHSO_4$ and 18-crown-6·$RbHSO_4$, and to evaluate the E_a associated to the different observed motion regimes involving the crown ether. Specifically, this was possible by measuring the ^{1}H T_1 values relative to the ^{1}H solid-state signal of 18-crown-6 while increasing the temperature [5]. The results of the SSNMR study, combined with VT-X-ray diffraction, differential scanning calorimetry and electrochemical impedance spectroscopy, indeed indicated a correlation between the presence of several motion regimes with the occurrence of superprotonic transitions, which led to heightened ion transport features.

References

[1] D. A. Boysen, T. Uda, C. R. I Chisholm and S. M. Haile *Science* **303**, 68-70 (2004)
[2] L. Fan, Z. Tu and S. H. Chan *Energy Reports* **7**, 8421-8446 (2021)
[3] A. V. Belushkin, C. J. Carlile and L. A. Shuvalov *Ferroelectrics* **167**, 21-31 (1995)
[4] D. Braga, S. d'Agostino, F. Grepioni, M. Gandolfi and K. Rubini *Dalton Trans.* **40**, 4765-4777 (2011)
[5] S. Ocal, S. d'Agostino, G. Venturini, F. Soavi, S. Bordignon, M. R. Chierotti and D. Braga *J. Phys. Chem. C* **128**, 4789-4795 (2024)

[P-6]

THE MIXTURE DEMIXER AND OTHER STORIES

F. Bruno[‡§#], L. Fiorucci[‡§#], E. Ravera[‡§#]

‡ Magnetic Resonance Center (CERM), University of Florence, via L. Sacconi 6, 50019 Sesto Fiorentino, Italy;
§ Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, 50019 Sesto Fiorentino, Italy;
# Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP), via L. Sacconi 6, 50019 Sesto Fiorentino, Italy.
Email: bruno@cerm.unifi.it

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

NMR spectroscopy is an incredibly powerful analytical technique, because every chemical species has -in principle- a unique spectrum, and the response factor of each nucleus solely depend on the number of equivalent nuclei. These characteristics make NMR suitable for studying the composition of mixtures, which is a common problem in a wide range of applications: reaction monitoring, food control, quality checks, biological fluids…
For this purpose we present pyIHM, an open-source python package that aims to fit the spectrum of a mixture using the information gathered from the pure components as basis set, following the Indirect Hard Modelling approach. The deconvolution of the spectra is a reliable alternative to the traditional peak-integration approach for quantification, which is often not feasible because of the intrinsic poor sensitivity of the technique and of the signal overlapping in complex mixtures.
In pyIHM, the NMR signals are simulated in the time domain using the Voigt model. The parameters of the Voigt (frequency, linewidth, relative intensity and Lorentzian to Gaussian ratio) can be obtained either from the experimental spectrum of the pure components, or simulated according to already existing spectral assignments.
The chemical shifts of the model signals are then aligned to the ones of the mixture spectrum by minimizing a target function computed on the integrals of the spectra, in order to overcome the secondary minima arising from partial superimposition of complex features.
Finally, the all parameters of the model spectra are fitted in the least-squares sense against the mixture spectrum, and the composition of the mixture is calculated.
We tested pyIHM on mixtures of known composition. The program proved to be more robust with respect to the traditional peak integration approach, providing comparable results.

σ-HOLE INTERACTIONS INVOLVING OSMIUM TETROXIDE OsO_4: A COMPLETE CHARACTERIZATION VIA SOLID-STATE NMR AND SINGLE CRYSTAL XRD

M. Calabrese,[a] A. Daolio,[a] A. Pizzi,[a] G. Terraneo,[a] G. Resnati,[a] S. Bordignon,[b] and A. Frontera[c]

[a]Department of Chemistry, Materials, and Chemical Engineering "Giulio Natta", Polytechnic of Milan, via Mancinelli 7, 20131 Milano (Italy)
[b]Department of Chemistry, University of Turin, Via Pietro Giuria 7, 10125 Torino (Italy)
[c]Department of Chemistry, Universitat de les Illes Balears Crta. de Valldemossa, 07122, Palma de Mallorca, (Spain)
E-mail: miriam.calabrese@unito.it

Keywords: solid-state NMR (SS-NMR), materials, theory and methods

σ-hole interactions, a subclass of noncovalent interactions, are involved in different interesting compounds such as gold-based drugs [1] or functional molecular materials [2]. The occurrence of the σ-hole interactions is attributable to the anisotropic distribution of the electron density on the outer regions of bonded atoms, firstly this phenomenon has been found in *p*-block elements and subsequently in the *d*-block elements. Following this approach, here, new cocrystals between osmium tetroxides OsO_4 (Os, Group 8) and pyridine or pyridine N-oxide derivatives are presented [3]. An in-depth characterization of the obtained adducts, through the combination of complementary techniques, ^{15}N CPMAS NMR and Single Crystal XRD, has led to the rationalization of the short interactions, Os···N/O, formed between OsO_4 and nitrogen or nucleophiles, as σ-hole interactions, the so-called Osme Bonds. Furthermore, computational tools have been employed and have allowed the confirmation of the attractive nature of these short interactions.

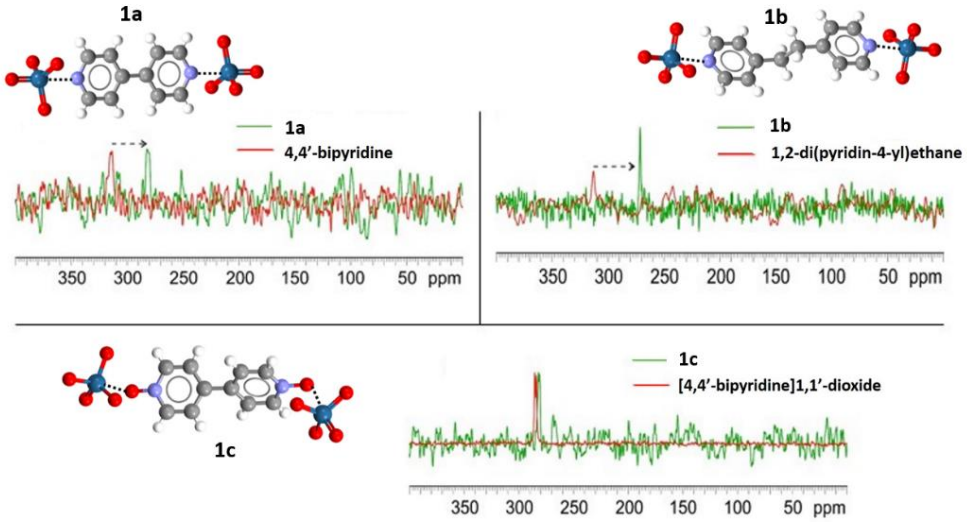


Fig. 1. Overlap of the ^{15}N CPMAS NMR spectra of adducts **1a**, **1b** and **1c** (green lines) and respective pure starting pyridine derivatives (red lines). Ball and stick crystal structures of adducts **1a**, **1b**, and **1c** are given close to relative spectra. Osme Bonds are represented as black dotted lines. Color code: *whitish*, hydrogen; *gray*, carbon; *indigo*, nitrogen; *red*, oxygen; *navy*, osmium.

References

[1] A. Pizzi, M. Calabrese, A. Daolio, M. Ursini, A. Frontera, G. Resnati, *CrystEngComm*, **24**, 3846-3851 (2022).
[2] M. Calabrese, A. Pizzi, R. Beccaria, A. Frontera, G. Resnati, *ChemPhysChem*, **24**, e202300298 (2023).
[3] A. Daolio, A. Pizzi, M. Calabrese, G. Terraneo, S. Bordignon, A. Frontera, G. Resnati, *Angew. Chem.*, **133**, 20723–20727 (2021).

[P-8]

PHYSI- AND CHEMISORBED CO_2 IN AMINE-PIM-1 MEMBRANE AS STUDIED BY SOLID STATE NMR

F. Nardelli[a], C. Rizzuto[b], M. Carta[c], B. Comesana-Gandara[d], N. McKeown[e], A. Fuoco[b], E. Tocci[b], J. C. Jansen[b], L. Calucci[a,f,c]

[a] Istituto di Chimica dei Composti OrganoMetallici, Consiglio Nazionale delle Ricerche, Pisa, Italy
[b] Istituto per la Tecnologia delle Membrane, Consiglio Nazionale delle Ricerche, Rende (Cs), Italy
[c] Department of Chemistry, Faculty of Science and Engineering, Swansea University, Swansea, UK
[d] IU CINQUIMA, University of Valladolid, Valladolid, Spain
[e] EastChem, School of Chemistry, University of Edinburgh, Edinburgh, UK
[f] CISUP- Centro per l'Integrazione della Strumentazione dell'Università di Pisa, Pisa, Italy
E-mail: lucia.calucci@pi.iccom.cnr.it

Keywords: solid state NMR, materials, polymers.

Membranes based on polymers of intrinsic microporosity (PIMs) are widely used for applications in the field of gas and vapour separation [1]. The high free volume available for gas permeation arising from inefficient packing of rigid molecular moieties allows membranes to be obtained with high performance in terms of permeability and good selectivity for the main gas pairs (CO_2/CH_4 and CO_2/N_2). In amine-PIM-1 an enhancement of the affinity for CO_2 has been achieved by the conversion of the nitrile group of PIM-1 in the primary amine group [2,3].
Given that CO_2 adsorption efficiency and selectivity are strongly related to the adsorption mechanism in the membrane, it is crucial to investigate the identity of chemisorbed and physisorbed species. To this aim, solid state NMR spectroscopy has revealed as one of the most powerful techniques to identify, quantify and characterize CO_2 species in solid sorbents derivatized with amine groups [4-7]. In the present work, ^{13}C 1D and 2D magic angle spinning (MAS) SSNMR experiments were applied to amine-PIM-1 and $^{13}CO_2$-loaded amine-PIM-1 membranes for investigating the structure and dynamics of the species formed upon adsorption of CO_2. It was found that the reaction between CO_2 and the primary amine groups of amine-PIM-1 leads to the formation of two carbamic acid species with different degree of hydrogen bonding, as also confirmed by ^{15}N MAS spectra. Moreover, physisorbed CO_2 with different mobility degree was detected, in agreement with computational data.

Acknowledgments
This work 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". We acknowledge the Ministry of University and Research (MUR) for financial support under the program PRIN 2020 (project 2020P9KBKZ doMino).

References

[1] P. Budd et al. *J. Membr. Sci*. **251**, 263-269 (2005).
[2] C. R. Mason et al. *Macromolecules* **47**, 1021-1029 (2014).
[3] B. Satilmis et al. *J. Membr. Sci*. **555**, 483-496 (2018).
[4] D. Pereira et al. *Curr. Opin. Colloid Interface Sci*. **64**, 1-19 (2023).
[5] S. M. Pugh, A. C. Forse, *J. Magn. Reson*. **346**, 107343 (2023).
[6] J. K. Moore et al. *J. Phys. Chem. C* **122**, 4403-4408 (2018).
[7] J. K. Moore et al. *J. Phys. Chem. C* **117**, 22995-22999 (2014).

EFFECT OF METAL BINDING ON THE STRUCTURE OF THE Ros87_C27D ZINC FINGER PROTEIN

G. Caputo,[a] M. Dragone,[a] G. Shitaye,[a] G. D'Abrosca,[b] L. Russo,[a] I. Baglivo,[a] P. V. Pedone,[a] R. Fattorusso,[a] G. Malgieri,[a] C. Isernia[a]

[a]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta, Italy
[b]Department of clinical and experimental medicine, University of Foggia, Foggia, Italy
E-mail: gaetano.caputo@unicampania.it

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

The possibility of choices of protein ligands and coordination geometries leads to diverse metal binding sites in proteins, allowing a range of important biological roles. The prokaryotic Cys_2His_2 zinc finger domain (found in the Ros protein from *A. tumefaciens*) [1] tetrahedrally coordinates a structural zin ion through two cysteine and two histidine residues [2]. It is the first structurally characterized member of a large family of bacterial proteins [3] that presents several amino acid changes in the positions occupied in Ros by the zinc coordinating residues [4]. In particular, the second position is very often occupied by an aspartic acid. Since the coordination of structural zinc by an aspartate in a zinc finger is very unusual, to also elucidate whether and how Ros homologues bind the structural metal ion when the coordinating cysteine is replaced by an aspartate, we here report the characterization of a functional point mutant of Ros87, Ros87_C27D.
UV, CD and NMR techniques are here exploited to provide an accurate description of the effects of different metal binding on the structure of Ros87_C27D.

References

[1] A. Y. Chou, J. Archdeacon, C.I. Kado *Proc. Natl Acad Sci* **95**, 5293-5298 (1998)
[2] S. Esposito, I. Baglivo; G. Malgieri; L. Russo; L. Zaccaro; L.D. D'Andrea; M. Mammucari; B. Di Blasio; C. Isernia; R. Fattorusso; P.V. Pedone, *Biochem.* **45**, 10394-10795 (2006)
[3] G. Malgieri, L. Russo, S. Esposito, I. Baglivo, L. Zaccaro, E.M Pedone, B. Di Blasio, C. Isernia, P.V. Pedone, R. Fattorusso, *Proc. Natl. Acad. Sci.* **104**, 17341-17347 (2007)
[4] I. Baglivo, L. Russo, S. Esposito, G. Malgieri, M. Renda, A. Salluzzo, B. Di Blasio, C. Isernia, R. Fattorusso, P.V. Pedone *Proc Natl. Acad. Sci.* **106**, 6933-6941 (2009)

[P-10]

QUANTITATIVE CHARACTERIZATION OF DYNAMIC DISORDER IN 2D RUDDLESDEN–POPPER LEAD IODIDE PEROVSKITES BY SOLID-STATE NMR SPECTROSCOPY AND RELAXOMETRY

N. Landi,[a] S. Borsacchi,[b,c] L. Calucci,[b,c] M. Geppi[a,c] and E. Carignani[b,c]

[a] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via G. Moruzzi 13, 56124 Pisa, Italy
[b] Istituto di Chimica dei Composti OrganoMetallici, Consiglio Nazionale delle Ricerche, CNR/ICCOM, via G. Moruzzi 1, 56124 Pisa, Italy
[c] Centro per l'Integrazione della Strumentazione dell'Università di Pisa, (CISUP), 56126 Pisa, Italy
E-mail: elisa.carignani@cnr.it

Keywords: solid state NMR, materials.

In the fast-developing research of improved and sustainable materials for optoelectronics, 2D Lead Halide Perovskites (LHP) have attracted considerable attention because they offer the possibility of tunable band gap and enhanced environmental stability with respect to the corresponding 3D perovskites. 2D Ruddlesden–Popper (RP) perovskites can be prepared by adding a large organic mono ammonium cation, L^+, in order to form a structure with a bilayer of spacer cations between metal halide sheets is formed ($L_2A_{n-1}B_nX_{3n+1}$). For example, butylammonium (BA^+) is a suitable organic cation to force the archetypical perovskite $MAPbI_3$ into 2D RP perovskites $BA_2MA_{n-1}Pb_nI_{3n+1}$ (Figure 1), which are the object of the present study. The layer thickness of metal halide sheets is specified by n and can be adjusted by tuning precursor stoichiometry.
Solid-State NMR stands out as characterization technique for LHP for its ability to study ion dynamics, compositional variations and ion incorporation, chemical interactions, and degradation mechanisms [1,3]. In this work, the 2D RP perovskites $BA_2MA_{n-1}Pb_nI_{3n+1}$ with n=1-4 have been characterized by ^{207}Pb, ^{1}H, and ^{13}C Solid-State NMR, both under Magic Angle Spinning and static conditions. In particular, the reorientational dynamics of butylammonium cations has been selectively and quantitatively characterized by measurement and analysis of ^{13}C nuclear relaxation times.

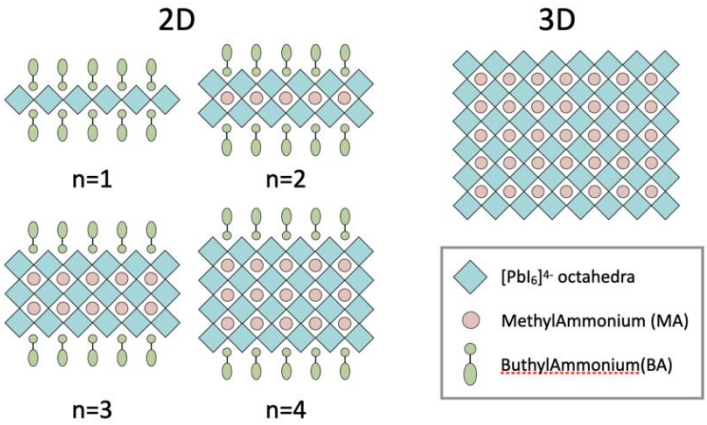


Fig. 1. Schematic structure of 2D RP perovskites $BA_2MA_{n-1}Pb_nI_{3n+1}$ for n=1-4, and of the corresponding 3D perovskites $MAPbI_3$.

References

[1] W. M. J. Franssen, A. P. M. Kentgens, *Solid State Nucl. Magn. Reson*., 100 (2019) 36-44
[2] L. Piveteau, V. Morad, M. V. Kovalenko, *J. Am. Chem. Soc. 142* (2020) 19413-19437
[3] D. J. Kubicki, S. D. Stranks, C. P. Grey, L. Emsley *Nat Rev Chem* 5 (2021) 624–645

[P-11]

HYDROPHOBIC EUTECTICS AND EUTECTOGELS IN WATER REMEDIATION: AN NMR PERSPECTIVE

C. Carotti, A. Rossetti, L. Riva, A. Mannu, G. I. C. Righetti, F. Briatico Vangosa, C. Punta, A. Mele, M. E. Di Pietro

Dept. Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Italy
Email: poseidon.prin22pnrr@gmail.com

Keywords: solution NMR, small molecules, materials.

Water is indispensable for human survival, yet only 40% of surface water bodies currently maintain a "good ecological status" [1]. Current methods for removing industrial contaminants of emerging concern (ICECs) rely on toxic volatile organic solvents and unsustainable sorbents with limited capacity and selectivity. This highlights the need for new, environmentally friendly, and cost-efficient extraction systems to remediate water pollution. The POSEIDON project (hydroPhObic eutectic SolvEnts In water remeDiatiON) meets this need by designing a novel class of sustainable extractants based on Hydrophobic Eutectic Solvents (HES) [2], combined into Hydrophobic EutectoGels (HEG) [3] and HES-loaded Cellulose nanoSponges (HECS) [4].
This contribution emphasizes the dual role of NMR spectroscopy in the project. Quantitative NMR is applied to evaluate the HES/HEG performance in terms of extraction ability and HES leaching (Fig. 1), and, along with other macroscopic properties, to screen and rank the initial set of extractants. The complex interplay of intermolecular interactions between HES component, gel matrix, and ICEC, is then investigated using different NMR methods.

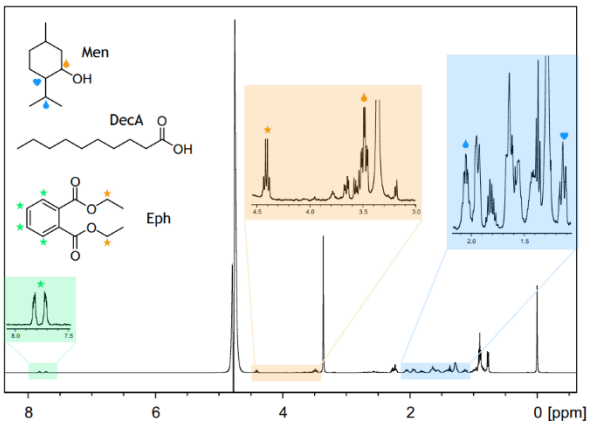


Fig. 1. Representative 1D ^{1}H spectrum showing the extraction of diethylphthalate (EPh) by menthol:decanoic acid HES (Men:DecA 1:1).

This work has been funded by European Union – Next Generation EU in the framework of the PRIN 2022 PNRR project POSEIDON - P2022J9C3.

References

[1] EU Commission, Water, https://environment.ec.europa.eu/topics/water_en
[2] D. J. G. P. Van Osch, C. H. J. T. Dietz, S. E. E. Warrag, M. C. Kroon, *ACS Sustain. Chem. Eng.*, **8**, 10591-10612 (2020)
[3] G. de Araujo Lima e Souza, M. E. Di Pietro, A. Mele, *RSC Sustainability*, **2**, 288-319 (2024).
[4] L. Melone, B. Rossi, N. Pastori, W. Panzeri, A. Mele, C. Punta, *Chempluschem*, **80**, 1408-1415 (2015).

[P-12]

THE POTENTIAL OF 3D CELL CULTURES TO MIMIC *IN VIVO* ENVIRONMENTS: AN NMR AND LC-MS METABOLOMIC STUDY

S. Cesaroni[a], G. Ciufolini[a], S. Zampieri[a], D. O. Cicero[a], C. Raggi[b], G. Petrella[a]

[a]Department of Chemical Science and Technology, University of Rome "Tor Vergata," Rome, Italy
[b]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
E-mail: simonacesaroni09@gmail.com

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

Accurate *in vitro* models closely replicating *in vivo* tumor environments are essential for advancing cancer research and therapeutic development. Conventional two-dimensional cell cultures (2D) often fail to replicate tumor tissue's intricate structural and functional diversity. Conversely, three-dimensional culture systems (3D) provide a more physiologically relevant milieu by mimicking key aspects of the tumor microenvironment, including cell-cell communication, oxygen and nutrient gradients, and waste accumulation [1]. Despite the extensive research conducted to compare the two culture methodologies, unresolved queries still need to be addressed to demonstrate the improved accuracy of spheroids in replicating *in vivo* conditions [2].
Our study highlights the metabolic differences between 2D and 3D culture systems, focusing on the interplay between metabolite exchange rates and internal pools. Using Nuclear Magnetic Resonance Spectroscopy (NMR) and Liquid Chromatography coupled to Mass Spectrometry (LC-MS) data, we examine the exo- and endo-metabolomic profiles of three different CCA cell lines, CCLP1, HUCCT1, and SG231, cultured as monolayers and spheroids. NMR analysis of the metabolite concentrations in the culture medium resulted in the measurement of 38 exchange rates, obtained by calculating the ratio between the variations in metabolite concentration in the blank and spent medium and the area under the cell growth curve [3]. We explore the correlation between internal pool concentrations obtained by LC-MS and exchange rates of those metabolites found to be important for 2D/3D discrimination. We notice that while metabolite exchange primarily reflects the outer layer of spheroids, internal metabolite levels reveal the metabolic heterogeneity within the 3D structure. Combining both data sets, we observe significant changes in central carbon metabolism, with pyruvate and glutathione playing key roles, affecting glycolytic activity and cellular redox balance. The difference between the metabolism of cells in spheroids and those cultured in monolayers is similar to that observed between tumor and healthy cells *in vivo*, underscoring the superior ability of 3D cultures to replicate the tumor microenvironment and their suitability for studying tumor cell metabolism.

References

[1] I. Papapostolou, *et al. Methods and Protocols 2023, Vol. 6, Page 94* 6, 94 (2023)
[2] O. Habanjar, *et al. International Journal of Molecular Sciences* 22, (2021)
[3] G. Petrella, *et al. Int J Mol Sci* 21, 1–13 (2020)

CHALLENGES AND OPPORTUNITIES IN HYDROGEN STORAGE: INSIGHTS FROM SOLID-STATE NMR

N.A. Consoli,[a,b,c] G. Provinciali,[d] A.L. Rollet,[e] A. Rossin,[d] M.Lelli [a,b,c]

[a] Center of Magnetic Resonance (CERM), University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy. [b] Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, Sesto Fiorentino, 50019 Italy [c] Consorzio Interuniversitario Risonanze Magnetiche MetalloProteine (CIRMMP), via Luigi Sacconi 6, Sesto Fiorentino, 50019 Italy. [d] Istituto di Chimica dei Composti Organometallici (ICCOM CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy. [d] Physicochimie des Electrolytes et Nanosystèmes interfaciaux (PHENIX) Laboratory, Sorbonne University, Case Courrier 51, 4 place Jussieu, Paris, France
E-mail: naomianna.consoli@unifi.it

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

The awareness of environmental issues and energy shortage has driven the development of clean and efficient energy sources. Hydrogen, as powerful energy carrier offers a sustainable, and flexible choice however, its low volumetric energy density at room temperature and safety concerns poses challenges for widespread adoption and urgently demands for a safe and sustainable hydrogen storage methods [1].
One promising approach involves incorporating hydrogen into porous materials or stable chemical compounds [2]. We focused on a composite system where lightweight hydrides (Ammonia Borane, $BH_3{\cdot}NH_3$, AB) [3], and hydrazine bisborane ($BH_3{\cdot}NH_2\text{-}NH_2{\cdot}BH_3$) [3]- are confined within the nanopores of two different Metal Organic Frameworks (MOFs) [4]. These hydrides can release hydrogen just for pyrolysis, and the interaction between the AB and MOF scaffold promotes the hydrogen incorporation and release at mild temperatures.
NMR spectroscopy is fundamental to characterize these systems. Thanks to the combination of mono and bidimensional NMR experiments on the all the active NMR nuclei (^{1}H, ^{11}B, ^{13}C,^{15}N) we were able to distinguish all the species inside our system and monitor the formation of new species once the hydride is confined. Additionally, we have studied the transformation occurring in the hydride species upon hydrogen releasee and loading. These data are compared with Relaxation Dispersion NMR profiles that give us information about the dynamic of molecular hydrogen inside the pores.
The NMR data provides important information that complements crystallographic analysis and allow us to design new systems with better performances both in term of stability and efficiency in hydrogen capacity.

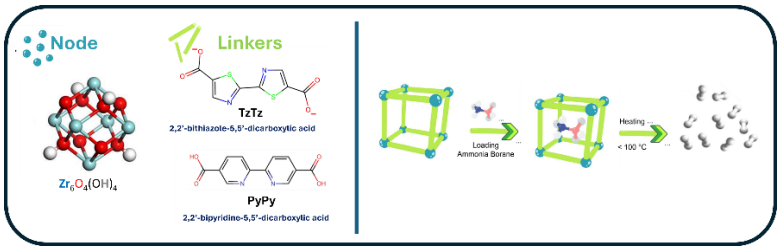


Fig. 1. On the left side of the panel the Zirconium node and the two organic ligands that compose the two different MOFs. On the right side, a schematic representation of the nanoconfinement strategy for [AB@MOF] composites is provided.

References

[1] A. Chapman, H. Farabi-Asl and Y. Nakahara Int. J. Hydrogen Energy, **45**, 3883-3898 (2020)
[2] Z. Chen, K.O. Kirlikovali, K.B. Idrees, M.C. Wasson and O.K. Farha Chem, **8**, 693–716 (2022) [3] A. Staubitz, A. P. M. Robertson and I. Manners Chem. Rev, **7**, 4079-4124 (2010)
[4] S.P. Shet, S. Shanmuga Priya, K. Sudhakar and M. Tahir Int. J. Hydrogen Energy, **46**, 11782–11803 (2021)
[5] A. Rossin, G.Tuci, L. Luconi and G. Giambastiani ACS Catal, **7**, 5035−5045 (2017)

[P-14]

AUTOPHAGY AS A POSSIBLE THERAPEUTIC TARGET ASSISTING CHEMOTHERAPY IN LUNG CANCER CELLS

F.Cortese,[a] G. Petrella,[b] P. Kalantari,[c] G. Ciufolini,[b] S. Zampieri,[b] B. Macchi,[b] D.O. Cicero[b]

[a]Department of Electronic Engineering, University of Rome "Tor Vergata," Via del Politecnico 1, 00133, Rome, Italy
[b]Department of Chemical Science and Technology, University of Rome "Tor Vergata," Via della Ricerca Scientifica 1, 00133, Rome, Italy
[c]Department of Biology, University of Rome "Tor Vergata," Via della Ricerca Scientifica 1, 00133, Rome, Italy
E-mail: federico.cortese@alumni.uniroma2.eu, pegah.kalantari@students.uniroma2.eu

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

Lung cancer, being the most common tumor worldwide in 2022 [1] and notoriously difficult to treat, necessitates innovative therapeutic approaches. In the search for new ways to treat lung cancer, recent data suggest the potential benefits of combining autophagy inhibitors with chemotherapy treatment [2]. In this context, this study aims to perform a metabolomic analysis of lung cancer cells, leveraging NMR spectroscopy, to provide new insights into tumor cell behavior regarding chemotherapy-induced autophagy.
The study was conducted using the in vitro lung cancer cell line A549. Cell cultures were treated with varying concentrations of cisplatin to determine the optimal dosage for metabolic adaptation studies. NMR spectroscopy was employed to analyze the exchange rates of 30 metabolites between the cells and the culture media, performing an exometabolomic experiment. This information was used to evaluate the changes in treated versus untreated cells.
Four cell culture experiments yielded 36 cell culture media samples analyzed by NMR. Significant differences were observed in metabolic fluxes between treated and untreated samples. Key metabolites predictive of cisplatin treatment included acetate, alanine, fructose, glucose, glutamate, and lactate. The metabolic profiles were analyzed using Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), demonstrating clear separation between control and treated groups. An ELISA kit was used to evaluate the expression of autophagy in untreated and treated cells for the detection of the LC3-II marker of autophagy. A control experiment for autophagy was performed using cell cultures incubated in starvation media. The results showed that the expression of LC3-II in cisplatin-treated cells was even higher than in cells incubated in starvation media, while untreated cells showed little to no LC3-II expression.
This research provides a comprehensive metabolomic profile of lung cancer cell response to cisplatin treatment. The identified metabolites offer insights into the metabolic pathways affected by chemotherapy, suggesting potential biomarkers for treatment response. While the experiments so far have confirmed the presence of chemotherapy-induced autophagy, the experiment to correlate cisplatin treatment biomarkers with autophagy has yet to be performed. Nonetheless, these findings lay the groundwork for further biological investigations into the metabolic effects of cisplatin in lung cancer.

References

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. *CA Cancer J Clin.* 74(3):229-263 (2024)
[2] Hao C, Liu G, Tian G. *Ther Adv Respir Dis.* (2019)

CONTROLLING THE INCORPORATION OF FLUORINATED AMINO ACIDS IN HUMAN CELLS AND ITS STRUCTURAL IMPACT

Azzurra Costantino,[a] Lan B. T. Pham,[a] Letizia Barbieri,[a,b] Vito Calderone,[a,c] Gili Ben-Nissan,[d] Michal Sharon,[d] Lucia Banci,[a,b,c] Enrico Luchinat [a,b,c]

[a] CERM – Magnetic Resonance Center, Università degli Studi di Firenze, via Luigi Sacconi, 6, 50019, Sesto Fiorentino, Italy
[b] Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine – CIRMMP, via Luigi Sacconi, 6, 50019, Sesto Fiorentino, Italy
[c] Dipartimento di Chimica, Università degli Studi di Firenze, Via della Lastruccia, 3, 50019, Sesto Fiorentino, Italy
[d] Department of Biomolecular Sciences, Weizmann Institute of Science, 234 Herzl St., Rehovot, Israel
E-mail: azzurra.costantino@unifi.it

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

Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for investigating protein structures, interactions, and dynamics both in vitro and in cells [1,2]. However, traditional NMR-active nuclei like ^{1}H, ^{13}C, and ^{15}N can be challenging for complex proteins due to signal overlap and reduced sensitivity. To overcome these issues, the incorporation of ^{19}F into proteins has emerged as a promising solution due to its high sensitivity and broad shift range.[3,4].
In this study we investigate the effects of fluorinated amino acids (FAAs) incorporation in proteins expressed directly in human HEK293T cells, focusing on the probability of incorporation (p_F) and its structural impact. We identified two optimal expression conditions: one to maximize multiple FAA incorporations per protein molecule, increasing signal intensity in ^{19}F NMR spectra, and the other to limit incorporation to a single FAA per molecule, minimizing any structural perturbations. The overall incorporation of FAAs was studied using NMR spectroscopy, and the frequency distribution of each fluorinated protein form was investigated by mass spectrometry (MS). Furthermore, potential structural alterations induced by FAAs were explored in a model protein, carbonic anhydrase type 2 (CA2), via x-ray crystallography.
Results of NMR and MS reveal that FAA incorporation is purely stochastic and amino acid position-independent, although its efficiency is significantly dependent on the expression conditions. However, x-ray crystallography of CA2 shows selective exclusion of fluorine at certain positions, indicating site-induced conformational changes. Finally, we propose a predictive model for FAA incorporation efficiency based on FAA concentration in the expression medium.
In conclusion, this work offers a comprehensive analysis of FAA incorporation in proteins expressed in human cells, providing insights for controlled protein fluorination in human cells and enhancing ^{19}F NMR spectroscopy applications in protein research.

References

(1) Hu, Y.; Cheng, K.; He, L.; Zhang, X.; Jiang, B.; Jiang, L.; Li, C.; Wang, G.; Yang, Y.; Liu, M. NMR-Based Methods for Protein Analysis. *Anal. Chem.* **2021**, *93* (4), 1866–1879. https://doi.org/10.1021/acs.analchem.0c03830.
(2) Theillet, F.-X. In-Cell Structural Biology by NMR: The Benefits of the Atomic Scale. *Chem. Rev.* **2022**, *122* (10), 9497–9570. https://doi.org/10.1021/acs.chemrev.1c00937.
(3) Luchinat, E.; Cremonini, M.; Banci, L. Radio Signals from Live Cells: The Coming of Age of In-Cell Solution NMR. *Chem. Rev.* **2022**, *122* (10), 9267–9306. https://doi.org/10.1021/acs.chemrev.1c00790.
(4) Dr. Wenkai Zhu,Dr. Alex J. Guseman,Dr. Fatema Bhinderwala,Dr. Manman Lu,Prof. Dr. Xun-Cheng Su,Prof. Dr. Angela M. Gronenborn. Visualizing Proteins in Mammalian Cells by 19F NMR Spectroscopy. March 12, 2022. https://doi.org/10.1002/anie.202201097.

SOLID STATE NMR SPECTROSCOPY AND RELAXOMETRY INVESTIGATION OF Hg(II)-BISPIDINE 1D COORDINATION POLYMERS FOR VOC ADSORPTION

F. Della Croce,[a] G. Bizai,[b] A. Savarese,[b] E. della Latta,[b] M. Cametti,[c] F. Martini,[b,a,d] M. Geppi,[b,a,d] L. Calucci[a,d]

[a]ICCOM-CNR, via G. Moruzzi 1, 56124 Pisa, Italy
[b]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via G. Moruzzi 13, 56124 Pisa, Italy
[c]Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano, via Luigi Mancinelli, 7, 20133, Milano, Italy
[d]CISUP- Centro per l'Integrazione della Strumentazione dell'Università di Pisa, lungarno Pacinotti 43, 56126 Pisa, Italy
E-mail: fdc.francesco@gmail.com

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

Coordination Polymers (CPs) are metal-organic materials built upon metal ions or clusters bound through organic linkers to give long range ordered frameworks. Thanks to their crystal flexibility, 1D CPs are of great interest for many applications, including the adsorption of volatile organic compounds [1]. To better understand their final macroscopic properties, dynamic and structural characterization at the molecular level is necessary. To this aim, Solid-State Nuclear Magnetic Resonance (SSNMR) is one of the most powerful techniques, thanks to its capability of investigating wide spatial and time scales.

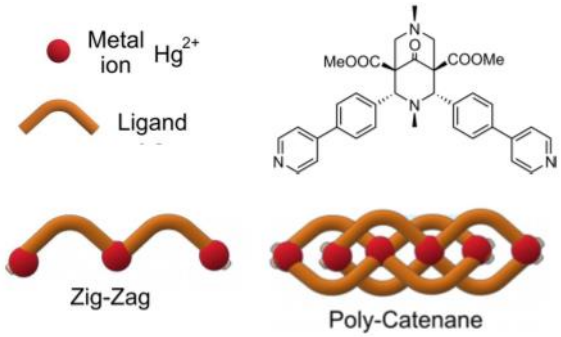


Fig. 1. Organic linker and topologies of investigated CPs.

In this work, we present an SSNMR characterization of two 1D CPs, both containing bispidine as organic linker, Hg(II) as metal center, and chlorobenzene as adsorbate trapped in the porous structure [2]. Both samples are characterized by 1D linear arrays with either zig-zag or polycatenane topology (see Fig. 1). Structural information on the linkers was obtained by high resolution ^{1}H Direct-Excitation and ^{1}H-^{13}C Cross-Polarization NMR experiments. The dynamics of linkers and chlorobenzene was investigated in detail by analyzing ^{13}C T_1 relaxation times obtained from high resolution experiments, as well as ^{1}H T_2 and T_1 relaxation times from time-domain experiments. The motions of chlorobenzene in the polymer cavities were further characterized by line shape analysis of ^{2}H NMR static spectra recorded on samples containing chlorobenzene-d_5.

Acknowledgements
We gratefully acknowledge the Ministry for University and Research (MUR) for financial support under the program PRIN 2022 (project 2022JB2F7H CLEANAIR).

References

[1] M. Lippi, M. Cametti, Coord. Chem. Rev. 430 (2021) 213661.
[2] M. Lippi, A. Maurelli, P. Rossi, P. Paoli, M. Cametti, Chem. Eur. J. 28 (2022) e202200420.

[P-17]

NMR SCREENING OF A MOLECULAR FRAGMENT LIBRARY FOR THERAPIES TARGETING THE PED/PEA15-PLD1 INTERACTION IN TYPE II DIABETES

M. della Valle,[a,d] I. Mercurio,[b,d] G. D'Abrosca,[c] G. Malgieri,[d] C. Isernia,[d] L. Russo,[d] S. Di Gaetano,[e] E. M. Pedone,[e] L. Pirone,[e] A. Del Gatto,[e] L. Zaccaro,[e] D. Alberga,[b] G. F. Mangiatordi,[b] M. Saviano,[a] R. Fattorusso[d]

[a]Institute of Crystallography, CNR, Via Vivaldi 43, 81100, Caserta, Italy
[b]Institute of Crystallography, CNR, Via Amendola 122/o, 70126 Bari, Italy
[c]Department of Clinical and Experimental Medicine, University of Foggia, Viale Pinto 1, 71122 Foggia, Italy
[d]Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania – Luigi Vanvitelli, via Vivaldi 43, 81100 Caserta, Italy
[e]Institute of Biostructures and Bioimaging, CNR, Via P. Castellino 111, 80131 Naples, Italy
E-mail: maria.dellavalle@unicampania.it

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

PED/PEA15 (Phosphoprotein Enriched in Diabetes) is a small multifunctional protein scaffold expressed in human cells, which regulates various cellular functions (e. g. glucose metabolism, proliferation, and apoptosis) through its interaction with other proteins. [1, 2, 3] It is known that PED interacts with PLD1 and PLD2, the isoforms of phospholipase D. High levels of PED, in cultured muscle, adipose cells, and peripheral tissues of transgenic mice, lead to increased expression of the classical protein kinase C (PKC) isoform PKC-α, negatively affecting insulin-stimulated GLUT4 translocation and glucose transport. This suggests that PED overexpression may contribute to insulin resistance in type 2 diabetes (T2D). Therefore, the PED inhibition, and the modulation of its interaction with PLD1, could potentially restore proper glucose transport, highlighting the PED/PLD1 complex as a promising target for the development of novel antidiabetic drugs. [4]
In the present study, a characterization of molecular interaction between PED and PLD1 in a cellular lysate environment, through Nuclear Magnetic Resonance methodologies, was performed, following an NMR screening of several small organic molecules as protein ligands. The outlined structural determinants, influencing PED-PLD1 binding, allowed the discovery of BPH03, as the best lead compound [5] able to disrupt the interface between PED and PLD1, displacing this latter from its interaction with the protein. Moreover, a computational analysis has been employed, supporting NMR data and evidencing the presence of a hidden pharmacologically targetable cavity within PED structure. [6]

References

[1] F. Fiory, P. Formisano, G. Perruolo, F. Beguinot *Am. J. Physiol. Endocrinol. Metab.* **297**, E592−E601 (2009)
[2] K. Callaway, O. Abramczyk, L. Martin, K. N. Dalby *Biochem.* **46**, 9187-9198 (2007)
[3] A. Trencia, A. Perfetti, A. Cassese, G. Vigliotta, C. Miele, F. Oriente, S. Santopietro, F. Giacco, G. Condorelli, P. Formisano, F. Beguinot *Mol. Cell. Biol.* **23**, 4511-4521 (2003)
[4] B. Farina, N. Doti, L. Pirone, G. Malgieri, E. M. Pedone, M. Ruvo, R. Fattorusso *Biochim. Biophys. Acta* **1834**, 1572-1580 (2013)
[5] B. Farina, L. Pirone, G. D'Abrosca, M. della Valle, L. Russo, C. Isernia, M. Sassano, A. Del Gatto, S. Di Gaetano, L. Zaccaro, G. Malgieri, E. M. Pedone, R. Fattorusso *ACS Chem. Biol.* **16**, 2798-2807 (2021)
[6] I. Mercurio, G. D'Abrosca, M. della Valle, G. Malgieri, R. Fattorusso, C. Isernia, L. Russo, S. Di Gaetano, E. M. Pedone, L. Pirone, A. Del Gatto, L. Zaccaro, D. Alberga, M. Saviano, G. F. Mangiatordi *Comput. Struct. Biotechnol. J.* **23** 2001-2010 (2024)

[P-18]

NMR METABOLOMICS TO FOLLOW THE METABOLITES PROGRESSION ALONG THE GASTROINTESTINAL TRACT: THE CASE STUDY OF THE FEMALE INFLORESCENCES OF ZUCCHINI

G. Di Matteo,[a] G. Romeo,[a] V. Di Clemente,[a] M. Spano,[a] S. Lombardi,[b] L. Izzo,[b] L. Mannina[a]

[a]Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[b] Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
E-mail: giacomo.dimatteo@uniroma1.it

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

The aim of the project is to deeply investigate different foodstuff matrices, characterizing not only the raw products but also the chemical profile during the gastrointestinal tract progression. The female inflorescences of zucchini were chosen as interesting case study. Zucchini (Cucurbita pepo L.) belong to the Cucurbitaceae family and present male and female flowers on the same plant. Female flowers are rich nutritional components like carbohydrates, proteins, lipids and polyphenols.
In the present study, the raw and the digested samples were analysed by 1H-NMR used as a metabolomic tool for the chemical characterization of the foodstuff whole composition and the identification of bioactive compounds. The Bligh-Dyer method was applied to extract both the hydroalcoholic and the organic fractions [1]. The INFOGEST protocol was applied in order to study the release of food components under simulated gastrointestinal conditions [2]. Both the two extracts were analysed through a Bruker AVANCE 600 spectrometer operating at the proton frequency of 600.13 MHz realizing both 1D and 2D experiments, namely 1H-1H TOCSY, 1H-13C HSQC, 1H-13C HMBC, JRES and DOSY. The levels of four sugars (fructose, glucose, sucrose and myo-inositol), seven organic acids (formate, fumarate, malate, citrate, lactate, succinate and acetate), seventeen amino acids (alanine, arginine, asparagine, aspartate, GABA, glutamine, glutamate, histidine, isoleucine, leucine, lysine, phenylalanine, pyro-glutamate, serine, threonine, tryptophane and valine), and other compounds such as choline, uridine, trigonelline and 4-Hydroxyphenyllactate, a typical polyphenol compound of zucchini were quantified and compared before and after the gastrointestinal digestion.

References

[1] Bligh, E.G. and Dyer, W. J. Can. J. Biochem. Physiol. 1959, 37 (8).
[2] Minekus, M., et al. Food Funct. 2014, 1113–1124 (5).

Acknowledgments

This work was supported by the PRIN 2022 project FLOWER, funded by the Italian Ministry of Education, University and Research (grant no. 2022FWK54E)

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title “ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods”.

PHIP-SAH [1-^{13}C]PYRUVATE FOR METABOLIC INVESTIGATION OF PC3 CANCER CELLS

G. Di Matteo,[a] C. Carrera,[b] F. Reineri[a]

[a] Department of Molecular Biotechnology and Health Sciences, Center of Molecular Imaging, University of Turin, Turin, Italy
[b] Institute of Biostructures and Bioimaging, National Research Council, Turin, Italy
E-mail: ginevra.dimatteo@unito.it

Keywords: solution NMR, hyperpolarization, metabolomics, contrast agents.

NMR and MRI are powerful tools for the non-invasive detection of metabolic changes related to many diseases (such as cancer) both in cell cultures and living systems. The main limitation for the application of these technique is the intrinsic low sensitivity, which makes the metabolites almost undetectable. For this reason, in the last decades a method termed hyperpolarization has been used to obtain metabolites with more intense signals that can be injected on cells or in vivo to follow the metabolism. In particular, the PHIP-SAH method[1] allows to obtain hyperpolarized (HP) [1-^{13}C]pyruvate in a fast, simple and cheap way. The aim of this work is to investigate the metabolic changes in PC3 prostate cancer cells, kept in normoxic (20% O_2) and hypoxic (1% O_2) conditions, using parahydrogen polarized [1-^{13}C]pyruvate. The expression of MCT1[2], MCT4 and GLUT1 (pyruvate, lactate and glucose transporters) is upregulated in PC3 cells kept in hypoxia for 24h and ^{1}H-NMR metabolomics analysis also reveals a higher glucose consumption and lactate production in this condition. Some preliminary hyperpolarized metabolic experiments have been carried out on 4 M PC3 cells cultured in normoxia, suspended in their culture medium (~100 µL) and perfused with the HP [1-^{13}C]pyruvate (3±0.5 mM in 400 µL). A series of low flip angle (15°) ^{13}C spectra was acquired after the aqueous solution injection directly on cells suspension and the buildup of the lactate signal was observed. Further hyperpolarized experiments using PHIP-SAH [1-^{13}C]pyruvate on PC3 cells cultured in hypoxia will be carried out to investigate the metabolic changes driven by the oxygen deficiency.

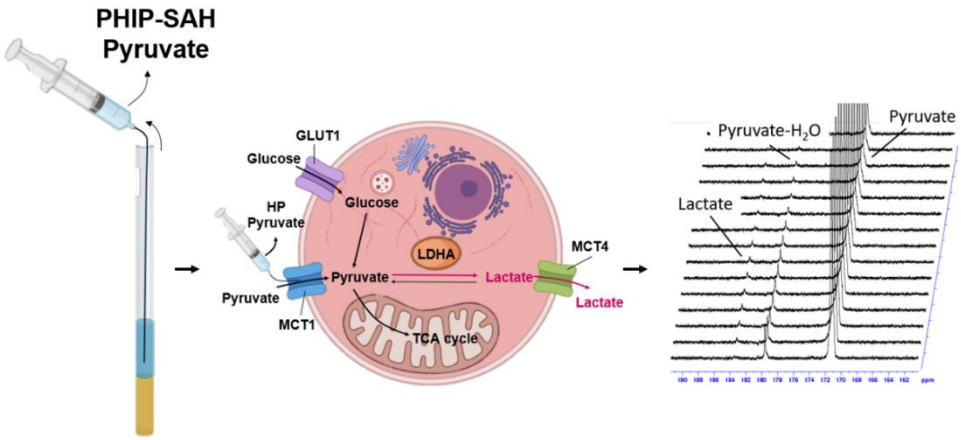


Fig. 1. Procedure for hyperpolarized experiments on PC3 prostate cancer cells. PHIP-SAH [1-^{13}C]pyruvate is quickly prepared and injected on PC3 cell suspension just before starting ^{13}C spectra acquisition.

References

[1] Reineri F, Boi T, Aime S. ParaHydrogen induced polarization of ^{13}C carboxylate resonance in acetate and pyruvate. *Nat Commun*. (2015)
[2] Fryer, Pertega-Gomes, 'A glycolytic phenotype is associated with prostate cancer progression and aggressiveness: a role for monocarboxylate transporters as metabolic targets for therapy', *J Pathol* 236: 517–530, 2015

[P-20]

THE NMR CHARACTERIZATION OF PHOX2B STRUCTURE AND INTERACTION WITH THE DNA: INSIGHTS INTO THE PATHOGENICITY OF THE VARIANT + 7ALA

D. Diana,[a] L. Russo,[b] L. Pirone[a], E. M. Pedone,[a] G. Malgieri,[b] R. Fattorusso[b]

[a]Istituto di Biostrutture e Bioimmagini, C.N.R., via Pietro Castellino 111, 80145, Napoli, Italy
[b]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania "Luigi Vanvitelli", via Vivaldi 43, 81100, Caserta, Italy.
E-mail: donatella.diana@cnr.it

Keywords: solution NMR, biomolecules.

PHOX2B is a transcription factor that plays a key role in the development of the autonomic nervous system and neural structures involved in the control of respiration [1-3]. About 90% of congenital central hypoventilation syndrome (CCHS) patients, characterized by defective autonomic control of breathing, show polyalanine triplet expansions, ranging from +5 to +13 alanine residues, of the 20 alanine stretch region of transcription factor PHOX2B [4].
Recent studies demonstrated that the alanine tract expansion influences PHOX2B folding and activity. Therefore, structural information on PHOX2B is an important target for obtaining clues to elucidate the insurgence of the alanine expansion-related syndrome and also for defining a viable therapy. Here we report the NMR structural characterization of the homeodomain (HD) of PHOX2B and HD + C-terminus PHOX2B protein, free and in the presence of the target DNA. The obtained structural data are then exploited to obtain a structural model of the PHOX2B–DNA interaction. In addition, the variant +7Ala, responsible for one of the most frequent forms of the syndrome, was analysed, showing different conformational proprieties in solution and a strong propensity to aggregation. Our data suggest that the elongated poly-alanine tract would be related to disease onset through a loss-of-function mechanism [5]. Overall, this study paves the way for the future rational design of therapeutics, suggesting as a possible route the use of specific anti-aggregating molecules capable of preventing variant aggregation and possibly restoring the DNA-binding activity of PHOX2B.

References

[1] A. Pattyn, X. Morin, H. Cremer, C. Goridis and J. F. Brunet *Nature* **399**, 366-370 (1999)
[2] A. Pattyn, C. Goridis and J. F. Brunet *Mol. Cell. Neurosci.* **15**, 235-243 (2000)
[3] R. L. Stornetta, T. S. Moreira, A. C. Takakura, B. J. Kang, D. A. Chang, G. H. West, J. F. Brunet, D. K. Mulkey, D. A. Bayliss and P. G. Guyenet *J. Neurosci.* **26**, 10305-10314 (2006).
[4] I. Matera, T. Bachetti, F. Puppo, M. Di Duca, F. Morandi, G. M. Casiraghi, M. R. Cilio, R. Hennekam, R. Hofstra,J. G. Schöber, R. Ravazzolo, G. Ottonello and I. Ceccherini *J. Med. Genet.*, **41**, 373-380 (2004).
[5] D. Diana, L. Pirone, L. Russo, G. D'Abrosca, M. Madheswaran, R. Benfante, S. Di Lascio, L. Caldinelli, D Fornasari, C. Acconcia, A. Corvino, N. Ventserova, L. Pollegioni, C. Isernia, S. Di Gaetano, G. Malgieri, E. Pedone, R. Fattorusso *Chem. Sci.* DOI: 10.1039/D3SC06427A (2024).

[P-21]

CONFORMATIONAL ANALYSIS AND MOLECULAR RECOGNITION OF THE VIRAL ENVELOPE DERIVED PEPTIDES WITH THE SECOND PDZ DOMAIN OF ZO1 BY NMR SPECTROSCOPY.

V. Pennacchietti,[a] A. Arcovito [b], N. Giacon [b], R. Fattorusso [c], A. Toto[a], D. Diana [d]

[a] Dipartimento di Scienze Biochimiche "A. Rossi Fanelli", Sapienza Università di Roma, 00185, Rome, Italy.
[b] Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Largo F. Vito 1, 00168 Roma, Italy.
[c] Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università degli Studi della Campania "Luigi Vanvitelli", via Vivaldi 43, 81100, Caserta, Italy.
[d] Istituto di Biostrutture e Bioimmagini, C.N.R., via Pietro Castellino 111, 80145, Napoli, Italy.
E-mail: donatella.diana@cnr.it

Keywords: solution NMR, biomolecules.

The Envelope (E) protein is one of the main structural proteins encoded by the genome of SARS-CoV, SARS-CoV-2 and MERS-CoV Coronaviruses and participates in many aspects of the viral life cycle such as virus maturation, assembly, and virulence mechanisms [1]. The E protein is characterized by the presence of a PDZ-binding motif (PBM) at its C-terminus which allows it to interact with several PDZ-containing proteins in the host cell [2]. One of the main binding partners of the Coronavirus E protein is the PDZ2 domain of ZO1, a protein with a crucial role in the formation of epithelial and endothelial tight junctions (TJs) [3]. To date the molecular details of the interaction between PDZ2-ZO1 and the E protein have not been established.
Here, we report the structural characterization, by solution NMR spectroscopy, of three peptides mimicking the C-terminal portion of the Coronavirus E protein from SARS-CoV, SARS-CoV-2 and MERS-CoV and their interaction with the ZO1-PDZ2, providing novel insights for the elucidation of the molecular mechanisms involved in the insurgence of the pathology.

References

[1] M.S., Balda, K. Matter *EMBO J*. **19**:2024-33, (2000).
[2] A., Toto, S., Ma, F., Malagrinò, L., Visconti, L., Pagano, K., Stromgaard, S. Gianni. *Protein Sci*., **29**:2038-2042, (2020).
[3] V. Pennacchietti, A. Toto. *Sci Rep.* **16**, (2023).

[P-22]

NON COVALENT HOST GUEST INTERACTIONS IN PENTAPETIDES/β-CD COMPLEXES

M. Dragone [a], G. D'Abrosca [b], R. Grazioso [a], G. Shitaye [a,c], A. D'Aniello [a], S. Tomassi [d], R. Fattorusso[a], L. Russo [a], C. Isernia [a], S. Di Maro [a], M. Saviano[e], G. Malgieri [a], R. Iacovino [a]

[a]Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania Luigi Vanvitelli Caserta, Italy
[b]Department of Clinical and Experimental Medicine, University of Foggia, Italy
[c]Department of Biomedical Sciences, School of Medical Sciences, Bahir Dar University, Ethiopia
[d]Department of Pharmacy, University Federico II, Italy
[e]Institute of crystallography, CNR, Caserta , Italy
E-mail: martina.dragone@unicampania.it

Keywords: solution NMR, biomolecules.

Host guest interactions involve two molecules that can form complexes through unique structural relationships and noncovalent bindings [1]. β-cyclodextrin (β-CD) has truncated cone-like structure composed by 7 glucopyranose units. Their inner cavity has a slightly hydrophobic nature that allows forming spontaneously host-guest complexes with several non-polar compounds.
Since aromatic side chains of amino acids such as tryptophan, phenylalanine, and tyrosine are known to play a significant role in the host–guest interactions due to their ability to fit the hydrophobic cavity of β-CD [2], in a previous work [3], we have investigated the inclusion properties of β-CD with various tripeptides. The peptides consisting of two L-alanines (A) and one aromatic amino acid (either L-tryptophan, L-phenylalanine, or L-tyrosine) were designed permuting the position of the aromatic residue. Among the sequences, the tripeptide Ac-AYA-NH_2 exhibited the highest affinity for β-CD, suggesting that the positioning of the aromatic tyrosine residue significantly enhances the formation of the inclusion complex thus indicating that the spatial arrangement of the aromatic side chain within the peptide sequence is crucial for the optimization of the interaction with the β-CD cavity.
Here, we extend our study to five model pentapeptides, each consisting of four L-alanine residues and one aromatic residue (L-tyrosine) in different positions in the sequence. To investigate the peptide-cyclodextrin host-guest inclusion complexes formation, we employed a combination of experimental and computational techniques, including UV-Vis spectroscopy, NMR spectroscopy, and molecular docking or molecular dynamics analyses.
Understanding positional effects can guide the design of more effective peptide-CD complexes. This is particularly valuable in drug delivery systems, where cyclodextrins are used to improve the solubility and stability of peptide drugs.

References

[1] E. Seyrek, G. Decher, *Polymer Science: A Comprehensive Reference, Elsevier,* **7** 159–185, (2012)
[2] T. Irie, K. Uekama *Advanced Drug Delivery Reviews*, **36** (1), 101–123 (1999)
[3] J.V. Caso, L. Russo, M. Palmieri, G.Malgieri, S. Galdiero, A. Falanga, C. Isernia, and R. Iacovino. *Amino Acids* **47** (10), 2215–2227, (2015)

[P-23]

REVISITING FATTY ACIDS METABOLISM USING NMR: A NOVEL REACTION IDENTIFIED IN MITOCHONDRIAL β-OXIDATION

S. Fabbian,[a] E. Schievano[b] and G. Giachin[b].

[a]Department of Pharmaceutical and Pharmacological Sciences (University of Padua), Via Marzolo 5, Italy
[b]Department of Chemical Sciences (University of Padua), Via Marzolo 1, Italy
E-mail: simone.fabbian@unipd.it

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

Fatty acid β-oxidation (FAO) is a crucial pathway for cellular energy production [1], and deficiencies in FAO enzymes are responsible for a wide range of pathological conditions [2]. The lack of effective therapies underlies the urgency of deepening our understanding of FAO molecular mechanisms. To date, the atomic-level characterization of each FAO reaction has not been performed. NMR is a powerful technique for this purpose [1], capable of accurately reconstructing the structure of FAO products in a native environment essential for enzymatic catalysis. NMR analysis can also be extended to mitochondria for real-time detection under both physiological and pathological conditions, with significant implications for the diagnosis and treatment of FAO disorders.

Here, we present the molecular characterization of the first reaction in fatty acid β-oxidation though the integrative use of NMR and MS. In detail, we studied the α-β-dehydrogenation of palmitoyl-CoA catalyzed by two homologous acyl-CoA dehydrogenases: very-long chain acyl-CoA dehydrogenase (VLCAD) and acyl-CoA dehydrogenase family member 9 (ACAD9), whose deficiencies are associated with severe FAO disorders [2],[3].Through ^{1}H-^{13}C HSQC analysis followed by ^{1}H-^{1}H TOCSY assignment, we identified not only the expected E-2-hexadecenoyl-CoA but also a significant percentage of a second product resulting from different dehydrogenation of palmitoyl-CoA. This novel reaction could be involved in previously unknown mechanisms regulating FAO or may serve as a hallmark of acyl-CoA dehydrogenase defects, potentially exacerbated by pathogenic mutations linked to FAO disorders.

Our findings enhance the understanding of fatty acid β-oxidation and pave the way for atomic-level investigations of FAO disorders using NMR analysis. Extending our study to enzymes with pathogenic mutations, will provide new insights into FAO disorders, for the development of targeted therapies. Additionally, our methodology can be a valuable tool for discovering new enzymatic functions of FAO enzymes, with potential applications in lipid modification technologies.

References

[1] S. Demine, N. Reddy, P. Renard, M. Raes, and T. Arnould *Metabolites.* **4**, 831-878 (2014)
[2] A. Nsiah-Sefaa, and M. McKenzie *Bioscience Rep.* **36**, 1-19 (2016)
[3] M. Schiff, B. et al. *Human Mol. Gen.* **24**, 3238-3247 (2015)

ENDOGENOUS ^{31}P CEST: A TOOL FOR MONITORING GLYCOLYSIS WITHOUT CONTRAST AGENTS

C. Fiorucci[a] G. Vassallo[a] , F. Garello[a], S. Aime[a] and D. Delli Castelli[a]

[a] Department of Molecular Biotechnology and Health Science, via Nizza 52 Turin, Italy,
E-mail: cecilia.fiorucci@unito.it

Keywords: MRI.

Introduction: The dysregulation of glycolysis stands as a metabolic anomaly observed across various pathologies. Among these dysregulations, foremost is the Warburg effect occurring in many solid tumor. In clinical and pre-clinical settings, multiple imaging techniques have been successfully used to detect aberrant glycolysis such us ^{18}FDG-PET, HP ^{13}C MRI, DMI (Deuterium Metabolic imaging) and ^{1}H-CEST-MRI [1]. All these techniques share the limitation of relying on exogenous contrast agents to observe the *in vivo* fate of injected metabolites. Indeed, the MRI approach owns a detection limit that does not permit the visualization of metabolites from the glycolytic pathway at their endogenous concentrations, even when the detection deals with a magnetically active species such as ^{31}P whose nat. abundance is 100%. On this basis, we deemed it interesting to design a novel method for detecting relevant glycolysis substrates at endogenous concentration. Dealing with endogenous molecule the natural choice is to use ^{31}P signals belonging to Pi (inorganic phosphate)-PCr or –y-ATP as bulk while saturating the signals of the phosphate groups belonging to the phosphorylated molecules of the glycolytic cascade. The innovation here relies on the possibility to exploit the amplification effect of the CEST contrast modality to detect signals from phosphorylated metabolites, which are below the S/N threshold in MR spectroscopy, via the most abundant signal of the bulk that can be detected by MRS. **Methods:** In vitro experiments were performed on three mammary carcinoma cell lines from mouse tissues, well known in the literature for their varying aggressiveness: 168FARN, TS/A, and 4T1. Cells were cultured to reach about 40-60 x 10^6 cells, then detached, washed in HEPES buffer, and finally centrifuged into a capillary for the NMR measurement. Z-spectra (Irradiation power: 3 μT, Irradiation time: 2s, ns=64 per point), centered on the ^{31}P inorganic phosphate signal in a range comprise between ±5, were performed on a Bruker Avance 600 operating at 14T at a temperature of 37°C. Control experiments were conducted by incubating cells with glycolysis inhibitors. For in vivo experiments, 4 mice were inoculated subcutaneously with the mammary breast cancer cell lines TS/A or 4T1. After two weeks, localized ^{31}P-CEST MRI experiments were performed in the tumor mass (voxel size about 0.8 cc) with the ISIS sequence implemented with the saturation transfer module. Z-spectra were acquired centered on the PCr signal intensity in a range of ppm comprised between ±9, using the following parameters (TR=3s; ns=8; Irradiation time=2s; irradiation power 2 μT; total acquisition time 1h 29', 3 minutes per point) in a Bruker Pharma scan 7T equipped with a ^{1}H/^{31}P dual-tuned volume coil. **Results and discussion:** Z-spectra acquired on the 4T1, TS/A and 168-FARN displayed different amount of saturation transfer in correspondence to the chemical shift of the glycolytic substrates (G6P, F6P, F1,6BP, G3P, 2PG, 3PG and PEP). Control cells treated with glycolysis inhibitors (NaF) displayed significantly differences with untreated cells. In vivo (Fig.1), it was possible to observe saturation transfer coming from glycolytic substrates in the breast cancer models (either 4T1 or TS/A). As control Z spectra on the muscle of the hindlimb were acquired and no ST have been measured in correspondence to that metabolites. This new methodology makes it possible to distinguish different metabolisms based on tumor aggressiveness. The sensitivity and resolution of the method is the same as ^{31}P-MRS but the detection threshold is at least three order lower. The advantage of this method over the existing ones to monitor glycolysis is related to the possibility to perform the analysis without the administration of contrast agents.

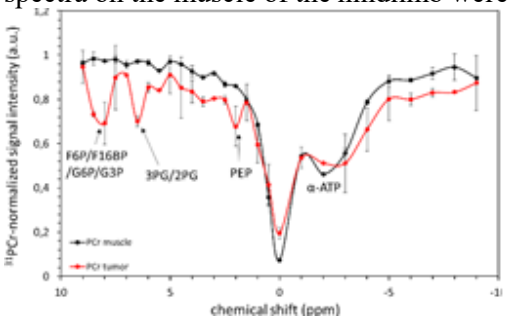


Fig.1: Localized ^{31}P Z-spectra on the tumor mass (red circle) and in the hindlimb muscle (clack circles) acquired on a Bruker Pharmascan 7T.

References: [1] Kim M, *et al.* Magn Reson Med 2009;61:1441-145.

EXPLORING THE ROLE OF PIN1 IN MOUSE SKELETAL MUSCLE THROUGH NMR-METABOLOMICS

A. Gambini,[a] A. Mucci,[a] M. Grosso,[b] S. Molinari,[b] V. Righi[c]

[a] Università degli Studi di Modena e Reggio Emilia, Dipartimento di Scienze Chimiche e Geologiche, Via Giuseppe Campi 103, 41125 Modena, Italy
[b] Università degli Studi di Modena e Reggio Emilia, Dipartimento di Scienze della Vita, Via Giuseppe Campi 287, 41125 Modena, Italy
[c] Università di Bologna, Dipartimento di Scienze per la Qualità della Vita, Corso d'Augusto 237, 47921 Rimini, Italy
E-mail: anna.gambini@unimore.it

Keywords: small molecules, metabolomics.

We investigated the metabolic changes caused by *Pin1* depletion in mammalian skeletal muscles using a mouse model. Our goal was to understand the roles of PIN1 enzyme in controlling the metabolic behaviour of muscle myofibers. [1] In particular, a metabolomic study on slow-twitch soleus (SO) and fast-twitch tibialis anterior (TA) muscles, from *Pin1* knock out (KO, N=10) and wild type (WT, N=10) mice, was carried out using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy, directly applied on tissue samples, without any pre-treatment. [2]
Multivariate statistical analysis highlighted metabolic differences between SO and TA muscles. The SO samples are richer in lipids, fumarate, succinate, glutamate and glutamine, instead TA samples show higher content of creatine, glucose, lactate and pyruvate. These results agree with the notion that slow-twitching SO have an oxidative metabolism, whereas fast-twitching TA are characterized by glycolytic metabolism.
We detected metabolic changes in *Pin1* depleted muscles that suggest a tendency of TA to become more oxidative, whereas those observed on SO are more nuanced.

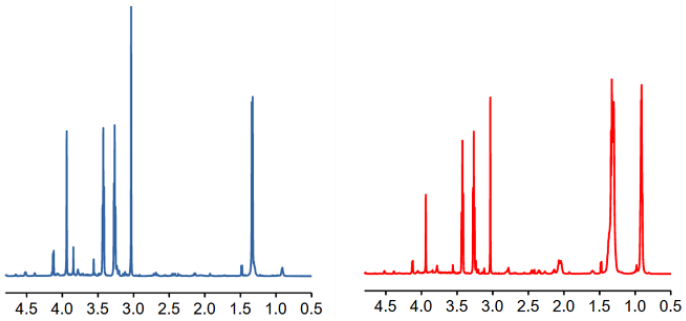


Fig. 1. ^{1}H HR-MAS NMR spectra of TA (blue) and SO (red) muscles.

References

[1] Y.C. Liou, X.Z. Zhou, K.P. Lu, *Trends Biochem. Sci.*, **36**, 501-514 (2017)
[2] L. Schenetti, A. Mucci, F. Parenti, R. Cagnoli, V. Righi, M.R. Tosi, V. Tugnoli, *Concepts Magn. Reson.* Part A, **28A**, 430-443 (2006)

DIFFERENT DYNAMIC BEHAVIOUR OF THE TWO POLYMORPHS OF LEFLUNOMIDE AS SEEN BY SOLID-STATE NMR SPECTROSCOPY AND RELAXOMETRY

A. Ghelardi,[a] E. Carignani,[b,c] M. Geppi[a,b,c]

[a]Department of Chemistry and Industrial Chemistry, University of Pisa, 56124 Pisa, Italy
[b]Institute for the Chemistry of Organometallic Compounds, National Council of Research (ICCOM-CNR), 56124 Pisa, Italy
[c]Center for Instrument Sharing of the University of Pisa (CISUP), 56124 Pisa, Italy
E-mail: arianna.ghelardi@phd.unipi.it

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

Active pharmaceutical ingredients (APIs) in solid formulations are influenced by their chemical and physical properties in the solid-state, impacting crucial characteristics like solubility and stability[1]. Polymorphism (the ability of a compound to exist in multiple solid forms) and dynamic processes also affects significantly these properties[2], making necessary a detailed characterization of the solid state of pharmaceutical compounds not only from a structural but also from a dynamic perspective. Leflunomide, a Disease Modifying Antirheumatic Drug (DMARD) primarily used for rheumatoid arthritis[3], exhibits two known polymorphs, Form α and Form β[4,5]. While their structures are disclosed[4,5], dynamic insights remain unexplored. Here, we utilized solid-state Nuclear Magnetic Resonance spectroscopy (ssNMR) to characterize and compare the structural and dynamic aspects of leflunomide polymorphs[6]. High-resolution spectra of various nuclei allowed for the differentiation of the two forms, confirmation of their structural properties, and provided further insights into their intermolecular environments. The measurement and analysis of spin-lattice relaxation times allowed the obtainment of quantitative dynamic parameters, such as the activation energies and correlation times. This comprehensive study enhanced our understanding of leflunomide polymorphs and underscores the utility of ssNMR in pharmaceutical research.

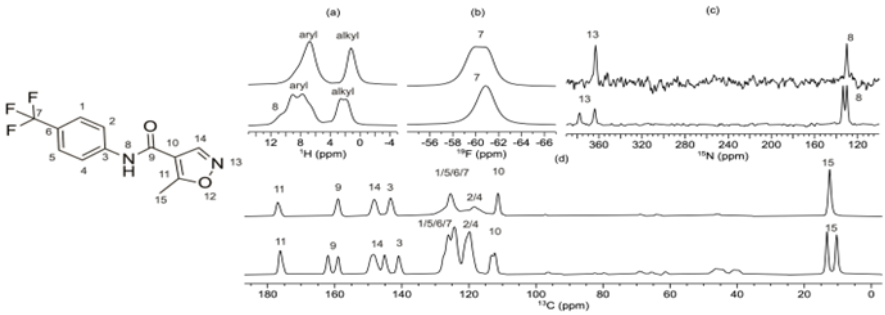


Fig. 1. ^{1}H (a), ^{19}F (b), ^{15}N (c) and ^{13}C (d) MAS spectra of the two different polymorphs of Leflunomide, ⟨ and ®, with signal assignment .

References

[1] H. G. Brittain *In. Pol. Pharm.-Solids_ 2nd ed.*, **192**, (1999).
[2] H. G. Brittain, D. J. Grant and P. B. Myrdal *In. Pol. Pharm.-Solids*, **192**, 436-480, (2009).
[3] R. R. Bartlett, H. Anagnostopulos, T. Ziefinski, T. Mattar and R. Schleyerbach, **14**, (1993).
[4] D. Vega, A. Petralli, D. Fernandez and J. A. Ellena *J. Pharm. Sci.*, 1075-1083, (2006).
[5] P. Sharma, D. Grangopadhyay, S. Umrao, S. Kumar, A. K. Ghosh, P. C. Mishra and R. K. Singh *J. Raman Spect.*, **47**, 4, 468-475, (2016).
[6] M. Geppi, G. Mollica, S. Borsacchi and C. A. Veracini *Appl. Spect. Rev.*, **43**, 2, 202-302, (2008).

[P-27]

NMR DETECTION OF THE CELLULAR EFFECTS OF GOLD-BASED ANTICANCER COMPOUNDS.

V. Ghini,[a] P. Turano[a,b]

[a] Department of Chemistry, University of Florence, via della Lastruccia 3 50019, Sesto Fiorentino
[b] Center of Magnetic Resonance, University of Florence, via Sacconi 6 50019, Sesto Fiorentino
E-mail: ghini@cerm.unifi.it

Keywords: solution NMR, small molecules, metabolomics.

^{1}H NMR provides a powerful tool to investigate the metabolic perturbations induced by gold-compounds [1] in cancer cells. The chemical identity and concentration of metabolites detected in cell lysates and their respective growing media by NMR can be viewed as a global fingerprint that unambiguously describes the response to drug treatment [2].
In this framework, we have carried out comparative NMR metabolomics studies to analyze the responses of A2780 human ovarian cancer cells to a panel of selected gold-compounds, including Auranofin, Aurothiomalate, Au(NHC)Cl and $[Au(NHC)_2]PF_6$. Due to the intrinsic nature of these metal centers, these molecules are supposed to give rise to multiple intracellular interactions with many functional proteins, rather than with a single enzyme or protein. Interestingly, with the proposed methodological approach, information on the predominantly affected biochemical pathways as well as on the protein targets of each metallodrug tested could be obtained and compared [3,4].

References

[1] S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini and L. Messori. *Medicinal Research Reviews*, **30**, 550–580 (2010).
[2] A. Vignoli, V. Ghini, G. Meoni, C. Licari, PG. Takis, L. Tenori, P. Turano, C. Luchinat. *Angew. Chem. Int. Ed. Engl.* **58**, 968-994 (2019).
[3] V. Ghini, T. Senzacqua, L. Massai, T. Gamberi, L. Messori, P. Turano, *Dalton Trans.* **50**,6349-6355 (2021).
[4] V. Ghini, M. Mannelli, L. Massai, A. Geri, S. Zineddu, T. Gamberi, L. Messori, P. Turano. *RSC Adv*. **13**, 21629-21632 (2023).

[P-28]

PORTABLE LOW FIELD NMR TO STUDY STRUCTURAL DIFFERENCES IN THE ARTICULAR CARTILAGE TISSUE

C. Golini,[a] C. Testa,[a] L. Brizi[a]

[a] Department of Physics and Astronomy "Augusto Righi", University of Bologna, Italy
E-mail: carlo.golini2@unibo.it

Keywords: low field NMR, biomolecules, theory and methods, instrumentation.

Osteoarthritis is a common degenerative musculoskeletal disease characterized by the degradation of articular cartilage and pathophysiological changes in the underlying subchondral bone [1]. Low-field NMR single-sided devices have demonstrated their potential to study both soft and mineralized tissues [2]. In the last decade, few preliminary studies [3] have been conducted on the articular cartilage using the NMR-MOUSE [4]. This study aims to quantify the role of cartilage dehydration and develop a procedure capable of quantitatively evaluating the structure of the three cartilage layers.
Forty osteochondral cylindrical specimens (Ø = 10mm, h = 10mm) were extracted from the knees of the bovine and analyzed by a low field NMR single-sided device (Mouse PM10, *Magritek*).
The depth and thickness of the three cartilage layers were identified and four NMR parameters T_1, T_2, D, and α (extracted from Double-Quantum-like sequence [5]) were obtained. Significant discrimination of the three cartilage layers was determined by T_2, D, α, and T_1. The NMR dataset was sensitive to the structural differences among the layers, like water confinement, proteoglycans organization, and collagen structure (see Fig. 1). The results pave the way for a procedure to evaluate cartilage changes due to cartilage diseases.

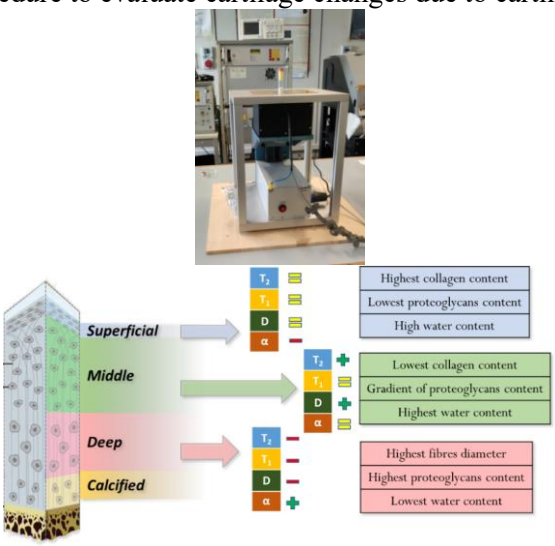


Fig. 1. (Left) Portable low field NMR device to obtain signal profiles at different depths of the cartilage sample. (Right) Sketch of the cartilage structure. The measured trends of T_2, T_1, D and α along the layers are reported (+ and – represent the highest and lowest values, while = is in the middle between them). The different organization and content of chondrocytes, collagen, and proteoglycans lead to different conditions of water restrictions in the intra- and extra-cellular space.

References

[1] M. Barbieri, P. Fantazzini, V. Bortolotti, F. Baruffaldi, A. Festa, D.N. Manners, C. Testa, and L. Brizi *Magn. Reson. Med*. **85**, 3353-3369 (2021)
[2] D. Muratovic, D.M. Findlay, F.M. Cicuttini, A.E. Wluka, Y.R. Lee, S. Edwards, and J.S. Kuliwaba *Osteoarthr. Cartil.* **27**, 1653-1662 (2019)
[3] E. Rossler, C. Mattea, A. Mollova, and S. Stapf *J. Magn. Reson.* **213,** 112-118 (2011)
[4] G. Eidmann, R. Savelsberg, P. Blumler, and B. Blümich *J. Magn. Reson.* **122,** 104-109 (1996)
[5] B. Blümich, J. Perlo, and F. Casanova *Prog. Nucl. Magn. Reson. Spectrosc*. **52**, 197-269 (2008)

GBCA-LOADED PEPTIDIC HYDROGELS AS HIGHLY SENSITIVE MRI TEMPERATURE REPORTERS

F. Hasallari[a,b], E. Gallo[c], A. Accardo[d], M. Salvatore[c], G. Digilio[b], S. Aime[a,c], E. Gianolio[a]

[a]Department of Molecular Biotechnology and Health Sciences, University of Torino - Torino, Italy
[b]DiSIT - Department of Science and Technological Innovation, University of Eastern Piedmont "A. Avogadro", Alessandria, Italy
[c] IRCCS SYNLAB SDN, Napoli, Italy
[d] Department of Pharmacy, University of Naples Federico II, Napoli, Italy
E-mail: ferdeze.hasallari@uniupo.it

Keywords: solution NMR, MRI, contrast agents.

Magnetic Resonance Imaging (MRI) stands as a distinctive modality for thermal treatment monitoring, offering various methods to measure temperature within the human body. Most NMR/MRI parameters are in fact affected by temperature. Among these, the predominant approach is based on the temperature sensitivity of the water proton resonance frequency (PRF) shift (approximately –0.010 ppm/°C). However, limitations of this method include its reduced sensitivity in adipose tissues and bones, along with its capacity to measure only relative temperature changes. T_1 of water protons can also be considered for MR-thermometry, as its value is directly proportional to temperature, especially if amplified in the presence of paramagnetic relaxation agents such as Gadolinium Based Contrast Agents (GBCA). The temperature dependence of the relaxivity of a given GBCA can be high but the use of the observed R_1 values for the measure of the temperature is hampered by the difficulties encountered in the determination of the actual concentration of the agent. For certain applications, more than the use of GBCAs flowing in the vascular system, it appears more suitable the design of a biocompatible device that can be located close to the site where the temperature has to be measured. Thus, we deemed of interest to investigate the effect of temperature on the relaxation properties of GBCA-loaded peptidic HydroGels. Several GBCA-containing HydroGels (HG) were prepared using the Fmoc-K2 peptide and the relaxation rates measured at 20 and 40 MHz over the temperature range from 25 to 45°C. After a thorough screening it was seen that the higher responsiveness to temperature was had by a Gd(III)-DTPA derivative containing three phenyl groups (Gd-1) (Fig.1) showing a remarkable increase of R_1 (and r_1) on going from solution to HG, maintaining constant the concentration, and increasing the temperature. The obtained Δr_1/°C for the HG containing 0.11 mM Gd-1 at 40 MHz was 0.545. The stability of the HG-thermometer in contact with biological matrices at 25 and 37°C and the reversibility after a cycle of heating/cooling were investigated. The results showed very good stability and response reversibility. The potential of the selected system to act as temperature reporting probe in T_{1w} MR images was assessed in vitro on a phantom containing the Gd-1(0.11mM)/HG and PBS/HG imaged on a Bruker Icon scanner operating at 1T (40MHz) at four different temperature values. The good linearity of R_1 vs. T indicates that the observed R_1 values can indeed unambiguously report on the actual sample temperatures.

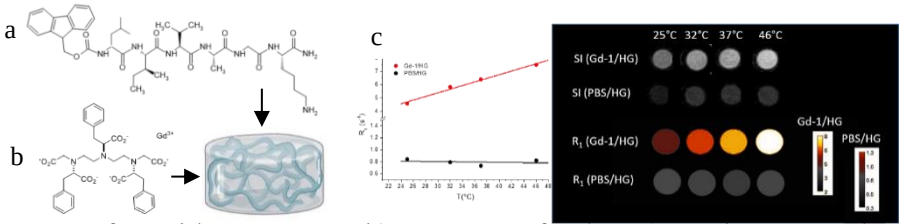


Fig.1 a) structure e of peptide Fmoc-K2; b) structure of Gd-1; c) T_{1w} images and T_1 maps at 1T.

[P-30]

DESIGN NEUROPROTECTIVE PEPTIDES AGAINST PRION DISEASES

M. Kamarehei,[a] V. Fusco,[b] L. Celauro,[c] L. D. D'Andrea,[d] C. Isernia,[a] R. Fattorusso,[a] G. Legname,[c] L. De Rosa,[b] L. Russo[a]

[a]Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche, Università degli Studi della Campania 'Luigi Vanvitelli', Caserta, Italy
[b]Istituto di Biostrutture e Bioimmagini, CNR, Naples, Italy
[c]Department of Neuroscience, Laboratory of Prion Biology, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy
[d]Istituto di Scienze e Tecnologie Chimiche 'G. Natta', CNR, Milan, Italy
E-mail: maryam.kamarehei@unicampania.it

Keywords: Solution NMR, prion, neurodegeneration, biomolecules.

Prions are the cause of deadly neurodegenerative diseases known as prion diseases or transmissible spongiform encephalopathies (TSEs). These disorders represent a significant public health threat due to the lack of effective treatments and the need for ongoing research into therapeutic interventions. The central molecular event in prion disease development is the conformational conversion of the cellular prion protein (PrP^{C}) into its misfolded form, scrapie prion (PrP^{Sc}). This conversion initiates a cascade of neurodegenerative processes, ultimately leading to the severe symptoms and fatal outcomes associated with prion diseases [1]. Given the severe implications of prion diseases and the current lack of treatments, focused research on therapeutic interventions remains a critical priority in addressing this public health concern. Recently, we identified the structural and dynamical determinants controlling the prion misfolding process by which PrP^{C} HuPrP(90-231) converts to an amyloid fibril through the formation of a β-sheet-enriched intermediate state (β-PrPI) involved in the initial stages of prion fibrillation. Moreover, our study demonstrated the importance of the coupling, through transient electrostatic interactions, between the N- and C-terminal domains of PrP^{C} in modulating long-range microsecond-millisecond conformational dynamics, which in turn regulate the folding process and prevent the formation of β-PrPI and prion aggregation [2]. In this study, taking advantage of our findings, we intend to target the prion pathogenic conversion by developing peptide-based strategies able to interfere with the initial stages of prion misfolding and aggregation process avoiding the formation of stable intermediate states and/or oligomeric species involved in the amyloid assembly mechanism. Here, we report the investigation of the conformational features and the evaluation of the binding interactions with HuPrP(90-231) of a twenty-one amino acid peptide, encompassing the region from Lys^{23} to Ser^{43} of the N-terminal domain of Human prion (HuPrP), called MANTRAP 1. NMR data indicate that MANTRAP 1, displaying a high degree of conformational flexibility without adopting any preferential secondary structure, is able to transiently interact with HuPrP(90-231).

References

[1] NJ. Cobe and WK. Surewicz *Biochem*. 48, 2574-2585 (2009)
[2] L. Russo, G. Salzano, A. Corvino, E. Bistaffa, F. Moda, L. Celauro, G. D'Abrosca, C. Isernia, D. Milardi, G. Giachin and G. Malgieri *Chem. Sci.* 13, 10406-10427 (2022)

[P-31]

RELAXOMETRIC CHARACTERIZATION OF PROTEINS TAGGED WITH Gd(III) COMPLEXES AS POTENTIAL MRI THERANOSTIC AGENTS

A. Kubrak,[a] J. Bindi,[a] M. Fragai,[a] P. Sebastiao,[b] G. Parigi[a]

[a]Magnetic Resonance Center (CERM), University of Florence, and CIRMMP, via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy
[b]Center of Physics and Engineering of Advanced Materials, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
E-mail: kubrak@cerm.unifi.it

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

Fast field-Cycling (FFC) NMR Relaxometry is a well-established technique that enables one to study the molecular dynamics of macromolecules as proteins by measuring relaxation rates R_1 as a function of the applied magnetic field. The characterization of paramagnetic compounds potentially useful as contrast agents in MRI is an important application of ^{1}H FFC-NMR Relaxometry, which can shed light on structural and dynamic properties important for the understanding of their mechanism of action. Contrast agents based on gadolinium(III) complexes are widely used in clinical diagnostics, but due to the high toxicity of the free metal ion, there are concerns about their safety [1]. Striving towards a decrease of the injected Gd(III) complex, several studies aim to enhance the relaxivity and thus the efficacy of the MRI agents. To this aim, low molecular weight Gd(III) complexes have been developed to be covalently bound to macromolecules with molecular reorientation times in the nanosecond time scale. Gd-tagged proteins, such as Gd-DOTA attached to asparaginase, show a high increase in paramagnetic relaxivity and were suggested as potential theranostic agents [2].
Here, we investigate human transthyretin (TTR), which is a carrier protein for the delivery of cytotoxic drugs, as a potential theranostic agent [3]. The paramagnetic Gd-C4-IA complex is used as a tag. Previous studies revealed its promising relaxivity enhancement after conjugation with protein cages [4].
The analysis of the acquired FFC NMR Relaxometry (or Nuclear Magnetic Relaxation Dispersion, NMRD) profiles is performed through the application of relaxation models able to describe interactions and motional processes occurring in the system. Several models have been developed in the years to account for different conditions and assumptions, in which the relaxation rates are described using well defined sets of parameters. A new fitting software with a user-friendly web interface is under development that will easily allow for the analysis of the relaxation profiles of paramagnetic molecules, proteins and nanoparticles.

References

[1] E. Gianolio et al. Radiology 2017, 285, 839–849
[2] G. Licciardi et al. Bioconjugate Chemistry 2022, 33, 2411–2419
[3] L. Cerofolini et al., Angewandte Chemie International Edition 2023, 62
[4] M. A. Kaster et al. ACS Applied Bio Materials 2023, 6, 591–602

TOWARDS REDOX-RESPONSIVE TRANSITION METAL CONTRAST AGENTS FOR MRI APPLICATIONS

D. Lalli,[a] C. Platas-Iglesias,[b] M. Botta[a]

[a]Universitá del Piemonte Orientale, Dipartimento di Scienze e Innovazione Tecnologica, Viale T. Michel 11, 15121 Alessandria, Italy
[b]Universidade da Coruña, Centro Interdisciplinar de Química e Bioloxía (CICA) and Departamento de Química, Facultade de Ciencias, 15071, A Coruña, Spain
E-mail: daniela.lalli@uniupo.it

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

Gadolinium-based contrast agents (GBCAs) are widely used as diagnostic probes in Magnetic Resonance Imaging. However, despite their remarkable safety profile, recent concerns about their safety and environmental impact have opened the field for safer, endogenous and earth-abundant metal ions. Mn(II) represents a valuable alternative to Gd(III), as it is an effective T_1 relaxation agent with efficiencies comparable to those of the traditional GBCAs. Also Co(II), and Ni(II) have shown promise as paramagnetic Chemical Exchange Saturation Transfer (CEST) MRI CAs.
Here we present the synthesis and characterization of a series of Mn(III), Co(III) and Ni(II) complexes with cross-bridge cyclam derivatives containing acetamide or acetic acid pendant arms, to access their potential application as redox-responsive transition metal CAs. In particular, we investigated the Mn(III)-complexes as T_1 relaxation agents, and the Co(III) and Ni(II) complexes as chemical exchange saturation transfer (CEST) agents [1].

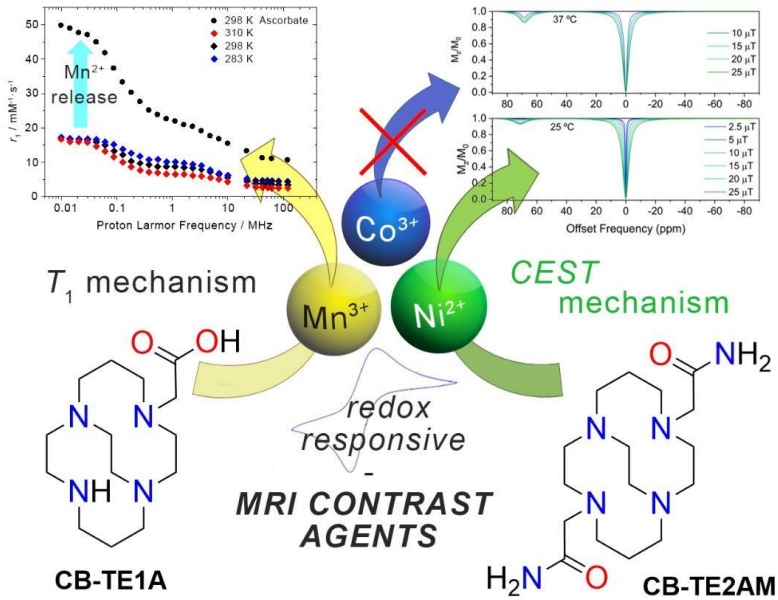


Fig. 1. Cross-bridge derivatives of Mn(III), Co(III) and Ni(II) show potentials as MRI contrast agents taking advantage of both the classical T_1 relaxation CEST mechanisms.

References

[1] R. Rocío Uzal-Varela et al. *Inorg. Chem.* **63**, 1575–1588 (2024)

[P-33]

COMBINING MAGNETIC RESONANCE EXPERIMENTS AND MOLECULAR SIMULATIONS FOR PROTEIN–MATERIALS INTERACTIONS

J. Lanuza,[a,b,c] J. Bindi,[a,b,c] F. Bruno,[a,b,c] G. Parigi,[a,b,c] M. Fragai,[a,b,c] E. Ravera[a,b,c,d]

[a] Centro di Risonanze Magnetiche, Università degli Studi di Firenze, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy;
[b] Department of Chemistry Ugo Schiff, Università degli Studi di Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy;
[c] Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy;
[d] Florence Data Science, Università degli Studi di Firenze
E-mail: josemanuel.lanuzadelgado@unifi.it

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

Protein-material interactions are common in biological and biotechnological contexts. When a protein interacts with a material, the resulting composite has properties that extend beyond—and may be significantly different—from those of the individual components.
A molecular understanding of interface processes is required for the efficient development of functional composites for consumer and industrial applications. However, current structural techniques and molecular simulations are incapable of answering these questions by themselves. Our group uses an integrative approach based on magnetic resonance-based experiments and molecular simulations to probe and rationalise biomolecule–material interactions in order to answer the question on how protein A interacts with material B.

This work has been supported by:
Project no. IR0000009 - call MUR 3264/2021 PNRR M4/C2/L3.1.1 and Project no. P2022JSC5Z – call MUR 1409/2022 PNRR M4/C2/L1.1 CUP D53D23016850001 (European Union NextGenerationEU)

SPIN DYNAMICS IN RARE–EARTH IONS NANOMAGNETS STUDIED BY NMR

M. Mariani,[a] A. Lascialfari,[a] M. Filibian,[a] I. Villa,[a] M. Porru,[a] L. Sorace,[b] M. Fittipaldi,[c] G. Latino,[c] A. Rettori,[c] F. Cinti,[c] A. Cini,[c] F. A. Rusnati,[d] P. Arosio,[d] F. Orsini,[d] F. Brero,[a] E. Giroletti,[a] D. Redigolo,[e] C. Cialdai,[e] P. Santini,[f] G. Poneti[g]

[a] Dept. of Physics, University of Pavia, and INFN, Pavia, Italy
[b] Dept. of Chemistry «Ugo Schiff», University of Firenze, and INFN, Sesto Fiorentino (FI), Italy
[c] Dept. of Physics and Astronomy, University of Firenze, and INFN, Sesto Fiorentino (FI), Italy
[d] Dept. of Physics «Aldo Pontremoli», University of Milano, and INFN, Milano, Italy
[e] Firenze INFN RU, Sesto Fiorentino (FI), Italy
[f] Dept. of Mathematical, Physical and Computer Sciences, University of Parma, Parma, Italy
[g] Instituto de Química, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
E-mail: alessandro.lascialfari@unipv.it

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

Since 80s, molecular magnetism [1-5] provided model systems to study new phenomena at the mesoscopic level, remarkably in zero-dimensional and one-dimensional systems. In this framework, single molecule magnets (SMMs) and single ion magnets (SIM) played a crucial role; they are molecular crystals, generally characterized by strong uniaxial anisotropy and progressive slowing down of the magnetization, where adjacent molecules are well-separated by shells of organic ligands. Thus, such materials can be considered as constituted of quasi-non-interacting magnetic units and possess characteristic quantum effects due to low dimensionality. Both these kinds of compounds have increasingly attracted the attention of the scientists for fundamental physics, chemistry and applications [3-7]. One of the aims of the researchers is to improve the performances of such molecular nanomagnets, for example by synthesizing moieties with an increased anisotropy barrier and a minimum level of dominating relaxation mechanisms at low temperature [8]. We investigated the magnetic properties and the spin dynamics of four lanthanide-based complexes (Ln[DTBSQ][$HBPz_3$]$_2$ and Ln[Trp][$HBPz_3$]$_2$, Ln-SQ and Ln-Trp in short, with Ln = Tb, Dy) [9,10], as a function of temperature, in the range 1.9K < T < 300K, and magnetic field ($\mu_0 H$ = 0.5, 1.5, 3.46 Tesla). As experimental tool we used ^{1}H NMR, measuring absorption spectra, spin-lattice (T_1) and spin-spin (T_2) nuclear relaxation times, and dc/ac susceptibility techniques. This study allowed us to single out: (i) the role of a different Ln magnetic centre (Tb^{3+} or Dy^{3+}) for the spin dynamics of the system; (ii) the influence of the exchange interaction on the physical properties, when the Ln magnetic ion is bound to the paramagnetic SQ (s = 1/2) ligand; here the magnetic anisotropy and crystal field are different with respect to the case of systems where the Ln centre is bound to the diamagnetic Trp (s = 0) radical; (iii) the complementarity of NMR and ac susceptibility as for frequency range of investigation, that allow to evince the molecular spin dynamics.

References

[1] D. Gatteschi et al *"Molecular Nanomagnets"* (Oxford University Press, 2006)
[2] A. Caneschi et al *Angew. Chem. Int. Ed.* **40**, 1760 (2001); M. Mariani et al *Inorg. Chim. Acta* **360**, 3903-3908 (2007); F. Cinti et al *Phys. Rev. Lett.* **100**, 057203 (2008)
[3] S.H. Baek et al *Phys. Rev. B* **70**, 134434 (2004); M.Belesi et al *Phys. Rev. Lett.* **102**,177201 (2009)
[4] N. Ishikawa et al *J. Am. Chem. Soc.* **125**, 8694 (2003)
[5] R. Sessoli et al *Nature* **65** , 141 (1993); D. Gatteschi et al *Science* **265**, 1054 (1994)
[6] S. Carretta et al *Phys. Rev. Lett.* **98**, 168401 (2007); L. Sorace et al *Chem. Soc. Rev.* **40**, 3092 (2011); F. Donati et al *Science* **352**, 318-321 (2016); F. D. Natterer et al *Nature* **543**, 226 (2017)
[7] F. Troiani et al *Phys. Rev. Lett.* **94**, 207208 (2005); A. Conrad et al *Nature* **548**, 439 (2017)
[8] S. T. Liddle et al *Chem. Soc. Rev.* **44**, 6655 (2015)
[9] M. Mariani et al *Phys. Rev. B* **97**, 144414 (2018); E. Lucaccini et al *ibidem* **100**, 174416 (2019)
[10] A. Caneschi et al *Dalton Trans.* **7**, 1048 -1055 (2004)

[P-35]

INVESTIGATING THE ROLE OF TEMPERATURE IN SOLID-STATE MAS DNP NMR

L. Niccoli,[a,b,c,d] A. Lesage,[d] L. Emsley,[e] and M. Lelli, [a,b,c,]

[a]Centre of Magnetic Resonance (CERM), Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy; [b]Department of Chemistry "Ugo Schiff", University of Florence, Via Della Lastruccia 3, 50019 Sesto Fiorentino, Italy; [c]Consorzio Interuniversitario Risonanze Magnetiche MetalloProteine (CIRMMP) Via Luigi Sacconi 6, 50019 Sesto Fiorentino. [d]Centre de la RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCBL), 69100 Villeurbanne, France; [e]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.
E-mail: moreno.lelli@unifi.it

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

Dynamic Nuclear Polarization (DNP) applied to MAS solid-state NMR has proved to be a valuable technique to enhance the sensitivity of more than two orders of magnitude. The nature of the polarizing agent plays an important role in determining the efficiency of the hyperpolarization process. Cross-effect polarizing agents (PA) are among the most efficient radicals and di-nitroxides AMUPol[1] and TEKPol[2] lead to enhancement factors of up to about 250 at 100 K and 9.4 T.
Nevertheless, the DNP enhancement is strongly dependent on temperature and, in commonly used solvents, it rapidly decreases to almost 1 above 200 K. The development of a DNP at higher temperature is an important goal, since it increases the application fields, especially in biological solid-state NMR, where the low temperature used in DNP strongly increases the resonance linewidth compromising the resolution.
Here we show that using polarizing agents with long electron relaxation times, like BDPA or HyTEK-2, and choosing the nature of the solvent matrix it is possible to increase the DNP above 200 K obtaining enhancements of the order of 10 even at room-temperature.
With the help of computational simulations, here we show how the electron T_1 and T_2 are involved in the cross-effect DNP mechanism, especially for hybrid biradicals like HyTEK2. Their role is not only limited to the electron saturation event, but they also regulate the preservation of the polarization difference between the two electrons until occurrence of the cross-effect event. Quantum spin simulations allow us to predict the role of electron-relaxation time and give indication to how further improve the DNP efficiency at higher temperature.

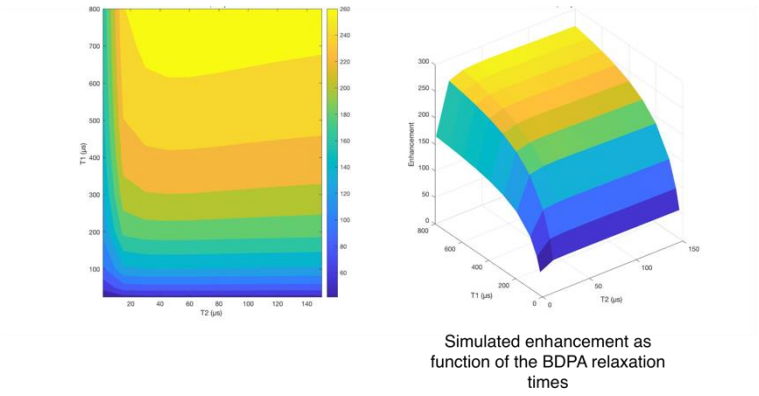


Fig. 1. Simulated DNP Enhancement of HyTEK-2 as a function of BDPA relaxation time.

References

[1] C. Sauvée, *et al. Angew. Chem. Int. Ed.* **2013**, 52, 10858–10861.
[2] A. Zagdoun, *et al. J. Am. Chem. Soc.* **2013**, 135, 12790–12797.
[3] Wisser, D. *et al J. Am. Chem. Soc*. **2018**, 140 (41), 13340–13349.
[4] Menzildjian, G. *et al. J. Phys. Chem. B* **2021**, 125 (48), 13329–13338.

INVESTIGATION OF THE ROLE OF MECHANICAL FORCES ON MECHANO-ENZYMATIC MECHANISM OF TRANSTHYRETIN BY NMR

Lourenço, I.O.[1]; Cantarutti, C.[1]; Mimmi, M. C.[2]; Verona, G.[3]; Giorgetti, S.[2]; Mangione, P.P.[2,3]; Bellotti, V.[3,4]; Corazza A.[1]

[1]Department of Medicine, University of Udine, 33100 Udine, Italy.
[2]Department of Molecular Medicine, Institute of Biochemistry, University of Pavia, 27100 Pavia, Italy.
[3]Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, University College London, London NW3 2PF, UK.
[4]Scientific Direction, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy.
E-mail: isabella.otteniodelourenco@uniud.it
Keywords: solution NMR, biomolecules, polymers

Transthyretin amyloidosis (ATTR) is a progressive and fatal disease characterized by amyloid deposition, derived from either variant or wt TTR, leading to severe organ damage. This disease is associated with aging or inherited variants in the TTR genetic sequence. Despite efforts, the biological mechanism behind the amyloidogenic conversion of native TTR remains elusive. High-resolution crystallography or cryo-EM haven't provided clear insights into this process. Studies propose that TTR dissociation and fibril formation involve complex mechanisms, including truncated TTR forms [1]. A physiological model suggests a combination of biomechanical forces and specific proteolytic enzymes that destabilize the TTR tetramer, by disassembly and monomer misfolding [2], generating the highly amyloidogenic 49-127 fragment. While certain variants, such as S52P, may undergo natural proteolytic cleavage, the wt and other variants require destabilization through mechanical forces and/or hydrophobic surfaces [3]. Notably, the non-amyloidogenic T119M variant remains uncleaned under similar conditions [3]. The proteases involved in TTR cleavage are unidentified, although the highly amyloidogenic fragmentation pattern suggests potential involvement of trypsin-like proteases [1]. To elucidate the role of shear forces on TTR mechano-enzymatic fibrillogenesis, NMR spectroscopy has been employed. The strategy towards the comprehensive characterization of TTR behavior under shear stress involves using a Rheo-NMR apparatus to replicate forces acting on TTR *in vitro* fibrillogenesis assays. The application of this technique makes it possible to characterize the effect of the shear forces on TTR structure and dynamics at single residues resolution and follow the aggregation and/or fibril formation in real time. Both static and shearing at 40 Hz conditions have been explored to discern the influence of mechanical forces on the aggregation process of TTR. The results suggest that neither cleavage nor shear forces are sufficient to shift the balance towards fiber formation. Although some residues from TTR have been identified as affected by the application of forces, shear forces alone are not capable of promoting aggregation. This research endeavors to deepen our molecular understanding of the mechanism behind biomechanical factors and proteolytic events in ATTR amyloidogenesis, offering potential prospects for therapeutic intervention in ATTR.

References

[1] P.P. Mangione, R. Porcari, J.D. Gillmore, P. Pucci, M. Monti, M. Porcari, S. Giorgetti, L. Marchese, S. Raimondi, L.C. Serpell, W. Chen, A. Relini, J. Marcoux, I.R. Clatworthy, G.W. Taylor, G.A. Tennent, C.V. Robinson, P.N. Hawkins, M. Stoppini, S.P. Wood, M.B. Pepys, V. Bellotti, Proteolytic cleavage of Ser52Pro variant transthyretin triggers its amyloid fibrillogenesis, PNAS. 111 (2014) 1539–1544.
[2] W. Colon, J.W. Kelly, Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro, Biochemistry. 31 (1992) 8654–8660.
[3] J. Marcoux, P.P. Mangione, R. Porcari, M.T. Degiacomi, G. Verona, G.W. Taylor, S. Giorgetti, S. Raimondi, S. Sanglier-Cianférani, J.L. Benesch, C. Cecconi, M.M. Naqvi, J.D. Gillmore, P.N. Hawkins, M. Stoppini, C.V. Robinson, M.B. Pepys, V. Bellotti, A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis, EMBO Mol Med. 7 (2015) 1337–1349.

NMR INVESTIGATION OF TOLUENE AND TEMPERATURE-INDUCED MICELLIZATION OF PLURONIC F127: INFLUENCE ON HOLLOW SILICA NANOSTRUCTURES

V. Marsala[a], L. Fusaro[a*], C. Aprile[a,*]
UCNANO, Department of Chemistry, NISM, University of Namur, Rue de Bruxelles 61, Belgium
E-mail: vittorio.marsala@unamur.be

Keywords: Solid-state NMR, solution NMR, materials, polymers.

In the last 20 years, many low-dimensional porous silica-based nanomaterials have been synthesized via the sol-gel process and the micelles-templated syntheses of mesoporous solids. Among them, hollow nanospheres and nanotubes (Fig. 1) have withdrawn a great interest because of their high specific surface area and large pores diameter, which are favorable features for catalytic applications.[1] Moreover, the isomorphic substitution of the Si with a metal cation allows obtaining an acid heterogeneous catalyst.[2]
Interestingly, the synthesis protocol for both silica nanotubes and hollow nanospheres is very similar. These materials are obtained using triblock copolymer (Pluronic F127) micellar systems as soft templates, combined with a swelling agent as toluene. The only significantly different parameters in the two syntheses are the stirring speed and the time between the addition of a swelling agent and the silica precursor (tetraethyl orthosilicate, TEOS).[1]

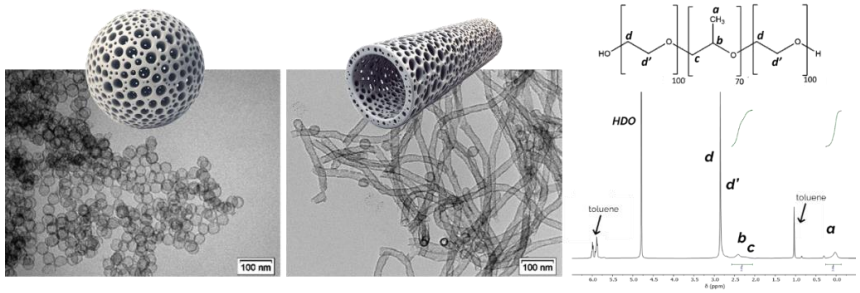


Fig. 1. TEM micrographs of silica hollow nanospheres (left) and nanotubes (center); ^{1}H-NMR of Pluronic F127 ($(PEO)_{100}(PPO)_{70}(PEO)_{100}$) in the presence of toluene (right).

Earlier research had proposed that hollow nanospheres are formed through the fragmentation of nanotubes. More recently, our research group definitively established that the crucial parameter determining whether tubular or spherical nanostructures form is the amount of surfactant-stabilized toluene present in the reaction mixture.[3]
Herein, we used solution NMR techniques like ^{1}H NMR, 2D COSY, and diffusion NMR to understand more in-depth how the process of Pluronic F127 micellization is influenced by the toluene amount and the temperature, and how these parameters affect the formation of hollow nanospheres. Specifically, 1D and pseudo-2D NMR experiments have been acquired at different temperatures and toluene concentrations to obtain structural and dynamic information.
Finally, tin-doped nanomaterials have been synthesized and characterized through static and MAS solid-state NMR experiments of ^{29}Si and ^{119}Sn, as well as other techniques like N_2-physisorption and Transmission Electron Microscopy (TEM).

References

[1] Huang, L.; Kruk, M., Chem. Mater. 2015, 27, 3, 679-689.
[2] Soumoy, L., Célis, C. Debecker, D.P., Armandi, M., Fiorilli S., Aprile, C., Journ. Cat. 2022, 411, 41-53
[3] Soumoy, L., Fusaro, L., Debecker, D.P., Aprile, C., Chem. Mater. 2023, 35, 5, 1877–1890

[P-38]

NMR CHEMICAL CHARACTERISATION OF LIGNIN EXTRACTED FROM FOOD WASTE

F. Masciulli,[a,b] M. Spano,[a,b] C. Ingallina,[a,b] Khuloud Al-Jamal,[c] and L. Mannina,[a,b]

[a] Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[b] NMR-Based Metabolomics Laboratory (NMR Lab), Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[c] Institute of Pharmaceutical Science, Nanomedicine Lab, King's College London, London SE1 9NH, UK. 1
E-mail: fabrizio.masciulli@uniroma1.it

Keywords: solution NMR, food, polymers

Thousands of tons of food waste are generated annually along the fruit and vegetable supply chain. The volume of trash from the entire food chain, until domestic consumption, became a global issue to face by governments and institutions, encouraging and supporting innovative solutions towards a zero-waste future. Lignin is one of the most abundant waste materials in the plant world and can be recovered cheaply from food byproducts and raw material waste [1].
The present study aimed to extract and characterise this biopolymer from the woody [2], non-edible part of dried fruit (the exocarp of almonds, walnuts, hazelnuts, and peanuts) and consider it a possible green tool useful in the pharmaceutical field.
After extraction from the dried fruit shell, the lignin structure was determined by NMR spectroscopy to characterise it and identify its monomers compared with the standard. Through one-dimensional and two-dimensional experiments (^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC), the NMR analysis of extracted lignin revealed its aromatic units, whose interaction generates polymer heterogeneity depending on its botanical origins. In each one, the sugar xylose was a contaminant. However, due to lignin's polymeric nature, the diversity of protons from various structures, and the irregular linkages between building units, the ^{1}H-NMR spectrum of lignin is somewhat overlapped and difficult to interpret accurately.
The recovery and reuse of chemically and physically characterised food byproducts valorised them and allowed the production of high-quality green polymers useful in nanomedicine. Nanoparticles made from lignin found in dried fruit exocarp could be utilised in the health and well-being industry, aligning with the European Community's goals to reduce daily waste through a circular economy approach.

References

[1] W. D. Hahn Schneider, A. J. P. Dillon, and M. Camassola *Biotechnol. Adv.* **47**, 107685 (2021)
[2] D. Fan, X. Xie, C. Li, X. Liu, J. Zhong, X. Ouyang, Q. Liu, and X. Qiu *J Agric Food Chem* **69** (37), 10838-10847 (2021)

[P-39]

ADVANCEMENTS IN LUNG CANCER DIAGNOSIS AND TREATMENT: INTEGRATING CONVENTIONAL CT AND HIGH-RESOLUTION MICRO-CT/MRI IMAGING FOR ENHANCED ANALYSIS

S. Megalizzi,[a] C. Bortolotto,[a,b] F. Brero,[c,d] R. Cabini,[d,e,f] M. Filibian,[d,g] R. Gioia,[g] A. Lascialfari,[c,d] L. Preda[a,b]

[a] Department of Radiology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy.
[b] Department of Clinical, Surgical, Diagnostic, and Pediatric Sciences, University of Pavia, Italy.
[c] Department of Physics, University of Pavia, Pavia, Italy.
[d] Istituto Nazionale di Fisica Nucleare, Sezione di Pavia, Pavia, Italy.
[e] Euler Institute,Università della Svizzera Italiana, Lugano, Switzerland.
[f] Department of Mathematics, University of Pavia, Pavia, Italy.
[g] Centro Grandi Strumenti, University of Pavia, Pavia, Italy.
E-mail: silvia.megalizzi@gmail.com

Keywords: : MRI, contrast agents, theory and methods, instrumentation.

The integration of Magnetic Resonance Imaging (MRI) with Micro-Computed Tomography (micro-CT) offers an innovative approach to non-invasive, high-resolution imaging for the study of biological samples [1]. This study specifically focuses on the Non-Small Cell Lung Carcinoma (NSCLC) in the form of two variants, Adenocarcinoma (ADC) and Squamous Cell Carcinoma (SCC) within NSCLC. The goal is to develop and validate a protocol for extracting and analyzing radiomic features from micro-CT, MRI and conventional CT images. Starting with the image acquisition of tissue blocks from NSCLC patients (obtained through biopsies of the ex-vivo samples of lung), micro-CT generates ultra-thin, high-resolution images, while preclinical MRI provides superior soft tissue contrast and functional imaging [2]. To enhance image contrast and detail, tissue samples are stained with contrast agents. Semi-automatic segmentation technique identifies the regions of interest, and radiomic features are extracted providing a large amounts of quantitative data from images, related to the tissue composition [3]. Features selection is conducted through machine learning algorithms to identify correlations between features and clinical outcomes, while AI-driven statistical methods [4] compare data across micro-CT, CT, and MRI imaging modalities to identify biomarkers of tumor aggressiveness, metastatic potential, and therapeutic response [5]. This integrative approach aims to enhance our understanding of tumor biology and could improve personalized NSCLC treatment [6] through advanced imaging techniques and analysis.

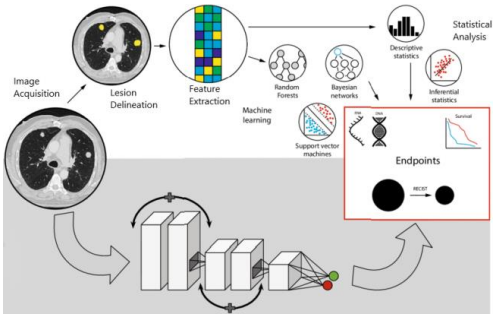


Fig. 1. Workflow of Radiomics Analysis on Lung Cancer Images.

References

[1] de Bournonville et al. *Contrast media & molecular imaging* (2019).
[2] Dizbay S. et al. *Diagnostics*, **13**, 3262 (2023).
[3] Cabini R.F. et al. *Insights into Imaging*, **13**, 38 (2022).
[4] Mayerhoefer M. et al. *Journal of Nuclear Medicine*,**61**,488-495 (2020).
[5] Binczyk F. et al., *Translational lung cancer research*, **10**,1186 (2021). [6] Bortolotto C. et al. *Cancers*,**15**, 3252(2023)

[P-40]

NMR-BASED METABOLOMIC APPROACH TO ESTIMATE CHEMICAL AND SENSORIAL PROFILES OF OLIVE OIL

G. Meoni[a], L. Tenori[a], C. Cherubini[b], L. Mazzanti[b], C. Luchinat[c]

[a] Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, 50019, Sesto F.no, Florence, Italy (tenori@cerm.unifi.it)
[b]ANALYTICAL S.R.L., Florence, Italy
[c] Consorzio Interuniversitario Risonanze Magnetiche Metallo Proteine (CIRMMP) and Giotto Biotech S.r.l., Sesto Fiorentino, Italy
E-mail: meoni@cerm.unifi.it

Keywords: solution NMR, metabolomics, food.

Olive oil (*Olea europea* L.) has been consumed by humans since antiquity and it remains a highly valued food today[1]. Due to the sensory and nutritional quality, there is a growing interest in extra virgin olive oils in the world market. Since 1991 international regulations have established analytical criteria to define olive oil genuineness and quality grade (European communities 1991, International olive oil council 1995, Characterization of Italian extra virgin olive oils using ^{1}H-NMR spectroscopy, 1998). Generally, the assessment of olive oil grade is carried out by standard analytical methods[2,3]. These methods are often time-consuming, elaborate, and expensive, and require a large number of samples and solvents. A fast and viable alternative is represented by ^{1}H-NMR spectroscopy combined with chemometrics[4-5] and this approach has been proposed in this study. Chemometric regression technique as Random Forest (RF) is used to highlight the correlations between the NMR spectra and the parameters of interest. We recorded the proton NMR spectra of olive oil with a 400 MHz spectrometer. RF regression models for prediction were built using the following analytical and sensorial data: UV spectrophotometric indices (K232, K268), acidity (free fatty acids), peroxide value, the composition of fatty acids (FA), tocopherols, the composition of biophenols, total biophenols, bitter, sweet, spicy, gustatory intense, olfactory fruity, olfactory intense, and overall pleasantness. Therefore, in this work, ^{1}H-NMR coupled with the RF regression technique is used to perform the simultaneous determination of 50 olive oil quality parameters. The models created were used to forecast chemical and sensorial parameters for unknown olive oil samples. Considering the large number of analyses required to determine all the mentioned parameters, as well as the use of toxic solvents and waste generation, our approach is suggested to reduce the time of the analyses, the number and the quantity of samples, and the waste production. Indeed, we demonstrated that, using ^{1}H-NMR, measurements can be performed quickly and simultaneously for multiple analytes present in a complex mixture.

References:

[1] Uylaşer, V.; Yildiz, G. *Crit. Rev. Food Sci. Nutr*., **54**, 1092–1101 (2014).
[2] Di Lecce, G.; Piochi, M.; Pacetti, D.; Frega, N. G.; Bartolucci, E.; Scortichini, S.; Fiorini, D. *Foods,* **9**, 904 (2020).
[3] Tena, N.; Wang, S. C.; Aparicio-Ruiz, R.; García-González, D. L.; Aparicio, R. J. Agric. Food Chem., 63, 4509–4526 (2015).
[4] Dourou, A.-M.; Brizzolara, S.; Meoni, G.; Tenori, L.; Famiani, F.; Luchinat, C.; Tonutti, P. Food Res. Int., 129, 108861 (2020).
[5] Mannina, L.; Sobolev, A. P. Mag. Reason. Chem., 49, S3–S11 (2011).

Acknowledgments: This work was made by a researcher with a research contract co-funded by the European Union - PON Research and Innovation 2014-2020 in accordance with Article 24, paragraph 3a), of Law No. 240 of December 30, 2010, as amended and Ministerial Decree No. 1062 of August 10, 2021.

[P-41]

PEPTIDES TARGETING THE HETEROTYPIC INTERACTION BETWEEN THE EPHA2 RECEPTOR AND THE SHIP2 LIPID PHOSPHATASE: NMR STRUCTURAL AND INTERACTION STUDIES

F.A. Mercurio,[a] M. Vincenzi,[a] M. Leone[a]

[a]Institute of Biostructures and Bioimaging (IBB-CNR), Via P. Castellino 111, 80131, Naples, Italy
E-mail: flaviaanna.mercurio@cnr.it

Keywords: solution NMR, peptide inhibitors, computational studies, cancer.

EphA2 is a receptor tyrosine kinase playing a complex role in cancer through the regulation of pro- and anti-oncogenic pathways [1]. EphA2 possesses within its modular domain organization a Sam (sterile alpha motif) domain (EphA2-Sam) forming heterotypic complexes with Sam domains from several proteins. In particular, the interaction between EphA2-Sam and the Sam domain from the lipid phosphatase Ship2 (Ship2-Sam) negatively regulates the receptor endocytosis process with pro-oncogenic outcomes [1]. Thus, molecule antagonists of the EphA2-Sam/Ship2-Sam association hold a certain interest in the anti-cancer drug discovery field. Starting from the KRI3 peptide previously identified in our laboratory, which is a weak inhibitor of the EphA2-Sam/Ship2-Sam interaction [2,3], and from a docking model of Ship2-Sam/KRI3 complex [3], we set up a computational approach to obtain optimized KRI3 analogs. In detail, in silico studies were carried out to predict KRI3 mutant peptides specifically targeting Ship2-Sam regions responsible for the interaction with EphA2-Sam with improved binding affinity. Herein, we will report on NMR studies of a few of the best predicted peptides. Conformational analyses were first performed in PBS buffer and in presence of the structuring co-solvent TFE to unveil structural features characteristic of the novel peptides. In addition, chemical shift perturbation studies allowed to validate the binding between Ship2-Sam and each peptide and to precisely localize the binding sites thus providing useful constraints to build 3D models of peptide/protein complexes with the support of molecular docking.

References

[1] F. A. Mercurio, M. Vincenzi, and M. Leone *Int. J. Mol. Sci.* **23,** 10397 (2022)
[2] F. A. Mercurio, C. Di Natale, L. Pirone, R. Iannitti, D. Marasco, E.M. Pedone, R. Palumbo, M. Leone *Sci Rep.* 7, 17474 (2017)
[3] M. Vincenzi, F. A. Mercurio, C. Di Natale, R. Palumbo, L. Pirone, S. La Manna, D. Marasco, E.M. Pedone, and M. Leone *Bioorg. Chem.* **122**, 105680 (2022)

Acknowledgement: The research leading to these results has received funding from AIRC under IG 2021 – ID. 26121 – P.I. Leone Marilisa. E.M. Pedone and L. Pirone are kindly acknowledged for the expression of recombinant Sam domains.

EXPLORING BORON NEUTRON CAPTURE THERAPY(BNCT) EFFECTS ON AMYLOID AGGREGATES

S. Micocci[1], S. Parisotto[2], V. Bitonto[1], D. Alberti[1], P. Renzi[2], A. Lanfranco[2], S. Altieri[3], N. Protti[3,4], A. Deagostino[2], S. Geninatti-Crich[1].

[1]Department of Molecular Biotechnology and Health Sciences, University of Turin, Via Nizza 52, 10125, Turin, Italy.
[2]Department of Chemistry, University of Turin, Via P. Giuria 7, 10125, Turin, Italy.
[3]Department of Physics, University of Pavia, Via A. Bassi 6, 27100, Pavia, Italy.
[4]National Institute of Nuclear Physics INFN, Pavia Unit, Via A.Bassi 6, 27100 Pavia, Italy
E-mail: sebastianomariasalomone.micocci@unito.it
Keywords: solution NMR, biomolecules.

Alzheimer's disease (AD) is a neurodegenerative pathology that, following the amyloid-beta (A®) hypothesis, is mainly caused by A® abnormal accumulation and aggregation in the brain. A® aggregation leads to the formation of oligomers, protofibrils, and fibrils. Among these, oligomers have the most neurotoxic effect, but larger aggregates can act as a sink, releasing them [1]. This project aims to exploit the high-selective BNCT to reduce the quantity of larger aggregates and aid the clearance of the smaller ones, stimulating the microglia. Disaggregation can be obtained thanks to the synergy between an external beam of thermal neutrons and 10B-containing probes that selectively bind to the A® aggregates. In literature curcumin affinity for A® fibrils and its therapeutic effects for AD are well known, so a new class of boronated monocarbonyl analogs of Curcumin (BMACs) that contain a 10B-enriched ortho-carborane has been developed[2]. The generated alpha particle in BNCT is responsible for its therapeutic effect and can cause localized oxidation in the A® protein (fig.1). A® aggregates have been obtained in-vitro and characterized by FESEM microscopy and by fluorescence, using thioflavin T (ThT) as a reporter. We determined the probes affinity for A® by competition assay with ThT and their inhibition constant values using the Cheng-Prusoff equation [3]. A® aggregates, after previous incubation with BMACs, were irradiated and analyzed by FESEM microscopy. A significant disaggregation effect was observed only in the samples treated with both neutrons and BMACs. The irradiated samples were then analyzed by 1H-NMR spectroscopy to identify the possible chemical changes in the protein structure. These preliminary studies have shown the disaggregation effect of BNCT on A® aggregates. Preliminary results, showing the possible structural damage of beta-amyloid (A®) aggregates induced by ionizing radiations generated by neutron capture, will be also described.

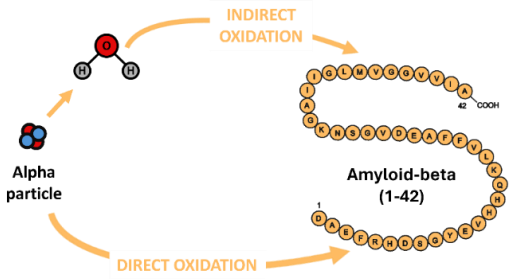


Fig. 1. Hypotized oxidation mechanism for amyloid-beta due to BNCT.

References

[1] A. Thapa, S.D. Jett, E.Y. Chi, ACS Chem Neurosci, 7(1):56-68 (2016).
[2] E. Azzi, D. Alberti, S. Parisotto, A. Oppedisano, N. Protti, S. Altieri, S. Geninatti-Crich, A. Deagostino. Bioorg Chem. 93:103324 (2019).
[3] Yung-Chi, C. & Prusoff, W. H. Biochemical Pharmacology 22, 3099–3108 (1973).

[P-43]

BIOINFORMATICS ANALYSIS OUTLINES THE DIFFUSION OF A NOVEL H-NS-LIKE PROTEIN FAMILY LARGELY PRESENT IN PROTEOBACTERIA

M. Hossein Mosalaeizadeh[a], G. D'Abrosca[a], L. Russo[a], C. Isernia[a], G. Malgieri[a], R. Fattorusso[a]

[a]Department of Environmental, Biological and Pharmaceutical Science and Technology, Second University of Naples, Via Vivaldi 43, 81100 Caserta, Italy
E-mail: mohammadhossein.mosalaeizadeh@unicampania.it

Keywords: solution NMR, biomolecules.

The MucR family transcriptional regulator Ros, initially identified in *Agrobacterium tumefaciens* -a member of the alpha proteobacteria family- is demonstrated to be a zinc finger protein [1]. Ros/MucR proteins are crucial in regulating the expression of genes involved in various cellular processes, including virulence, biofilm formation, and stress response [2]. These regulators typically function by binding to DNA sequences through their zinc finger motifs, which contain conserved cysteine and histidine residues- although aspartate is more frequent than cysteine in the second coordinating position- that coordinate zinc ions for structural stability [3,4].
This study aims to determine the homology of Ros/MucR protein across diverse bacterial families, including both Gram-negative and Gram-positive bacteria, as well as eukaryotes. Special attention is given to the conserved position of the four amino acids containing the putative metal binding site. Our comprehensive bioinformatics analysis revealed significant homology of Ros/MucR protein among various bacterial families. Remarkably, our results also highlighted an unexpected similarity between Ros/MucR protein, and six proteins found in eukaryotic organisms: *Cyprideis torosa, Ptychographa xylographoides, Ricinus communis*, *Friedmanniomyces endolithicus, Drosophila suzukii,* and *Aspergillus fumigatus*.
Our bioinformatics results have allowed the choice of a Ros/MucR homologue to be structurally characterized through NMR and Cryo-EM techniques.

References

[1] G. Malgieri, L. Russo, S. Esposito, I. Baglivo, L. Zaccaro, E. M. Pedone, B. Di Blasio, C. Isernia, P. V. Pedone, and R. Fattorusso, Proc. Natl. Acad. Sci. U.S.A. 104, 17341-17346 (2007)
[2] G. Malgieri, M. Palmieri, L. Russo, R. Fattorusso, P. V. Pedone, and C. Isernia, FEBS J. 282, 4480-4496 (2015)
[3] M. A. Islam, J. A. Nupur, M. Shafiq, Q. Ali, A. Sami, and M. A. Shahid, BMC Genomics 24, 603 (2023)
[4] S. Aizenshtein-Gazit and Y. Orenstein, Bioinformatics 38, ii62-ii67 (2022)

[P-44]

THE PHDVC5HCH TANDEM DOMAIN OF NSD2 IS A COMBINATORIAL READER OF UNMODIFIED H3K4 AND TRI-METHYLATED H3K27 THAT REGULATES TRANSCRIPTION OF CELL ADHESION GENES IN MULTIPLE MYELOMA

A. Berardi[a,b†], C. L. Kaestner[c†], G. Quilici[a,d], P. Cocomazzi[e], J. Li[c,f], F. Ballabio[a,g], C. Zucchelli[a], S. Knapp[h], M. Ghitti[a], J. D. Licht[c], G. Musco[a]

[a]Biomolecular NMR Laboratory, c/o Ospedale San Raffaele Via Olgettina 58 20132 Milano-Italy
[b] present address: The AIRC Institute of Molecular Oncology | Via Adamello 16, 20139 Milan, Italy
[c] Division of Hematology/Oncology, The University of Florida Health Cancer Center, 2033 Mowry Road, Gainesville, FL 32610
[d] present address:Parco Area delle Scienze, 23/A – 43124 Parma
[e] Department of Biosciences, University of Milan, via Celoria 26
[f] Department of Pharmacology, Physiology and Cancer Biology, Thomas Jefferson University, Philadelphia, PA
[g] present address:Università di Milano Dipartimento di Biosceinze
[h] Goethe Universitaet, Frankfurt
† These authors contributed equally to this work'
E-mail: musco.giovanna@hsr.it

Keywords: (NSD2, PHD finger, multiple myeloma)

Histone methyltransferase NSD2 overexpression in multiple myeloma (MM) patients plays a significant role in the development of this specific subtype of disease [1]. Through the expansion of gene activation associated H3K36me2 and suppression of repressive H3K27me3 marks, NSD2 activates an aberrant set of genes that contribute to myeloma growth adhesive and invasive activities. NSD2 transcriptional activity also depends on its non-catalytic domains, which facilitate its recruitment to chromatin through histone binding. In this study, using NMR, ITC and molecular dynamics simulations, we show that the tandem PHD domain of NSD2 ($PHDvC5HCH_{NSD2}$) is a combinatorial reader of unmodified histone H3K4 and three-methylated H3K27 (H3K27me3). This is the first PHD tandem cassette known to decode the methylation status of H3K27. Importantly, in a NSD2-dependent MM cellular model, we show that expression of NSD2 mutants, engineered to disrupt the interaction between H3K27me3 and PHDvC5HCH, display in comparison to wild-type NSD2: i. incomplete loss of H3K27 methylation throughout the genome, ii. decreased activation of adhesive properties and cell adhesion genes, and iii. a decrease of the corresponding H3K27ac signal at promoters. Collectively, these data indicate that PHDvC5HCH of NSD2 plays an important role in modulating gene expression and chromatin modification, thus opening new opportunities for pharmacological intervention

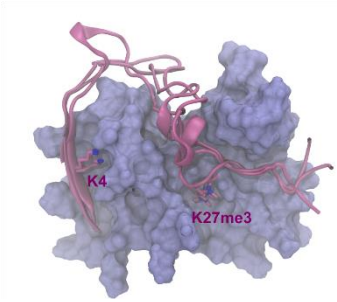


Figure 1: Model of $PHDvC5HCH_{NSD2}$:H3K4K27me3 complex. Superposition of three representative binding poses extracted from three independent MD calculations

References

[1] Morishita,M. and di Luccio,E. (2011) Cancers and the NSD family of histone lysine methyltransferases. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1816, 158–163.

[P-45]

^{1}H NMR PROFILING OF PNEUMONIA

V. Pecchioli,[a] V. Ghini,[b,c] P. Turano,[b,c]

[a]CIRMMP, Via Luigi Sacconi 6, Sesto Fiorentino, 50019 (FI), Italy
[b]CERM, University of Florence, Via Luigi Sacconi 6, Sesto Fiorentino, 50019 (FI), Italy
[c]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastrucci 3, Sesto Fiorentino, 50019 (FI), Italy
E-mail: pecchioli@cerm.unifi.it

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

In this work, that arises from the COMETA project (founded by Regione Toscana), ^{1}H NMR spectroscopy was used to analyze the differences in the metabolomic and liproproteomic profiles of EDTA-plasma samples from patients affected by COVID-19 pneumonia compared to patients affected by non-COVID ones. All the samples analyzed in the study were collected at the Santa Maria Nuova hospital in Florence. Samples from patients suffering from non-COVID pneumonia, were divided into two groups: interstitial and lobar pneumonia, according to the radiographic report.
In our previous works on COVID-19 [1][2][3], we had seen how, in a group of 510 samples, the COVID-19 metabolomic and lipoproteomic profiles of the infection were well characterized compared to a control group made up of 95 subjects recovered from the infection.
Significant alterations in the concentrations of several metabolites and lipoprotein components were observed, which show characteristic trends as a function of the disease severity [3].
In this work we have compared the COVID-19 metabolomic profiles with those of non-COVID interstitial and lobar pneumonia. Interestingly, the profile of non-COVID pneumonia appears to be very similar to COVID-19 one in both lipoproteins and metabolites alterations. Instead, the lobar ones were found to give rise to some characteristic alterations, that will be shown in detail.

Acknowledgements: This work was funded by Regione Toscana, COMETA project.

References

[1] V. Ghini, G. Meoni, L. Pelagatti et al. Profiling metabolites and lipoproteins in COMETA, an Italian cohort of COVID-19 patients. *PLoS Pathog*, **18**(4): e1010443 (2022)
[2] G. Meoni, V. Ghini, L. Maggi et al. Metabolomic/lipidomic profiling of COVID-19 and individual response to tocilizumab. *PLoS Pathog*, **17**(2): e1009243 (2021)
[3] V. Ghini, W. Vieri, T. Celli, V. Pecchioli, et al. COVID-19: A complex disease with a unique metabolic signature. *PLoS Pathog*, **19**(11):e1011787 (2023)

[P-46]

MOLECULAR BASES OF CLUSTER RELEASE AND DESTABILIZING EFFECTS OF NITRIC OXIDE IN IRON-SULFUR PROTEINS: THE CASE OF THE NEET PROTEIN CISD3

L. Querci,[a] D. Grifagni,[a,b] J. M. Silva[a], M. Lepoivre[b], C. Vallières[b], R. O Louro[c], L. Banci[a], M. Piccioli[a], M. P. Golinelli-Cohen[b], F. Cantini[a]

[a] Magnetic Resonance Center and Department of Chemistry, University of Florence, Via L. Sacconi 6 50019 Sesto Fiorentino, Italy
[b] Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198 Gif-sur-Yvette, France
[c] Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Universidade Nova de Lisboa, Av. da República (EAN), 2780-157 Oeiras, Portugal
E-mail: leonardo.querci@unifi.it

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

Of the human NEET proteins, CISD3 is least understood, with its functional role largely unknown. We have investigated the biochemical features of CISD3 at the atomic and in cellulo levels upon challenge with different stress conditions i.e., iron deficiency, exposure to hydrogen peroxide (H_2O_2) and nitric oxide (NO). In vitro, the redox states of CISD3 as well as its interaction with NO and H_2O_2 has been studied though a combination of EPR, NMR, electronic absorption spectroscopies and cyclic voltammetry. Oxidized CISD3 is particularly sensitive to the presence of hydrogen peroxide in vitro, whereas only the reduced form is able to bind nitric oxide. Tailored NMR approaches allowed an unprecedented, extensive assignment of ^{1}H, ^{13}C and ^{15}N resonances, also in proximity of the [Fe_2S_2] cluster[1]. Paramagnetic NMR provides clear evidence that upon NO binding, the cluster disassembles and converts into a dinitrosyliron complex (DNIC), which remains bound to the protein. Although EPR measurements identified DNIC as a minor species, our NMR data demonstrates that DNICs are significantly produced upon NO binding. Additionally, NMR spectroscopy provided insights into the preferential structural stability of the reduced state of CISD3 over the oxidized state. Chemical shift perturbation measurements suggest that, upon cluster oxidation, the protein undergoes a conformational change at the C-terminal CDGSH domain, which determines the instability of the oxidized state[2]. This redox-associated conformational change may be the source of cooperative electron transfer via the two [Fe_2S_2] clusters in CISD3, which displays a single sharp voltammetric signal at -31 mV vs SHE. Furthermore, in cellulo CISD3 is unaffected by oxidative stress induced by hydrogen peroxide but it becomes highly unstable in response to nitric oxide treatment. In our cellular experiments, CISD3 exhibited an almost ten-fold longer half-life than mitoNEET, implying a specific function. Cyclic protein film voltammetry revealed only two distinct redox states for CISD3, as confirmed by UV and EPR spectroscopies.

References

[1] Grifagni, D., Silva, J. M., Cantini, F., Piccioli, M., and Banci, L. (2023), J Inorg Biochem 239, 112089
[2] Grifagni, D., Silva, J. M., Querci, L., Lepoivre, M., Valliéres, C., Louro, R.O., Banci, L., Piccioli, M., Golinelli-Cohen, M.P. Cantini, F., (2024), J Mol. Biol. 300(3), 105745

[P-47]

DIFFUSION ORDERED SPECTROSCOPY TO TRACK THE EVOLUTION OF OLIGOMERIC SPECIES POPULATION DURING THE AMYLOID AGGREGATION PROCESS

K. Pagano,[a] L. De Rosa,[b] S. Tomaselli,[a] H. Molinari,[a] L. D. D'Andrea,[c] L. Ragona[a]

[a]Istituto di Scienze e Tecnologie Chimiche "G. Natta", Consiglio Nazionale Delle Ricerche, Laboratorio NMR, Via Corti 12, 20133, Milano - Italy
[b]Istituto di Biostrutture e Bioimmagini, Consiglio Nazionale Delle Ricerche, Via Pietro Castellino 111, 80131, Napoli – Italy
[c]Istituto di Scienze e Tecnologie Chimiche "G. Natta", Consiglio Nazionale Delle Ricerche, Via M. Bianco 9, 20131, Milano - Italy
E-mail: laura.ragona@scitec.cnr.it

Keywords: solution NMR, biomolecules

The accumulation of misfolded proteins leading to amyloid fibrils formation is a hallmark of various human diseases, with Alzheimer's disease (AD) being particularly prominent. Within the intricate cascade of events culminating in amyloid aggregation, attention is focused on oligomers emerging during the early steps of amyloid aggregation as they are believed to be responsible for the neurotoxic damage associated with AD. [1-2] Little is still known about the detailed mechanism of their formation, mainly due to the transient and unstable nature of the oligomeric states explored by Aβ peptide and the difficult in preparing homogeneous and seed-free Aβ peptide solutions [3].
Seed-free solutions were ensured by optimizing an experimental protocol for preparing 26-O-acyl isoAβ1-40, a modified Aβ1-40 peptide that retains the monomeric unordered state until incubated at neutral pH, where it undergoes rapid and quantitative conversion into the native amide peptide and initiates the aggregation process [4]. The aggregation kinetics of Aβ1-40 were monitored through 1D 1H NMR experiments and 2D diffusion-ordered spectroscopy (DOSY) spectra at various time points. We demonstrate the efficacy of coupling NMR DOSY with the Inverse Laplace Transform (ILT) reconstruction method to elucidate the distribution of oligomeric species in rapid exchange with monomers. This innovative approach efficiently maps oligomers distributions across a wide spectrum of initial peptide concentrations, offering unique insights into the evolution of oligomers relative populations over time. We demonstrate the efficacy of DOSY-ILT proposed approach by assessing the impact of Epigallocathechin gallate, a known remodeling agent of amyloid fibrils, on the oligomeric distributions of aggregated Aβ1-40 [4].
The DOSY-ILT approach provides a powerful strategy for rapidly gaining insight into potential inhibitors' impact on protein aggregation processes, at different stages of aggregation, thus emerging as a valuable tool in drug discovery.

Acknowledgements
The authors acknowledge financial support of Fondazione Antonio De Marco.

References

[1] D. J. Rinauro et al. *Molecular Neurodegeneration* https://doi.org/10.1186/s13024-023-00651-2 (2024)
[2] K. Pagano et al. *Front. Neurosci.–Neurodegeneration,* https://doi.org/10.3389/fnins.2020.619667 (2020)
[3] K. Pagano et al. *Mol. Neurobiol*., **56**(3), 1957-1971 (2019)
[4] K. Pagano et al. *Chemistry a Eur. Journal.* https://doi.org/10.1002/chem.202400594 (2024)

[P-48]

CONNECTIVITY AND MICROSTRUCTURAL PROPERTIES OF THE CINGULUM BUNDLE AND ITS SUBDIVISIONS IN ALZHEIMER'S PROGRESSION

M. Ricchi[a,b], G. Campani[c], A. Nagmutdinova[d], V. Bortolotti[d], D. Greco[e,f], L. Brizi[g], C. Testa[b,g] for the Alzheimer's Disease Neuroimaging Initiative

[a] University of Pisa, Largo Bruno Pontecorvo 3, Pisa 56127, Italy
[b] INFN, Bologna Division, Bologna, Italy
[c] European Institue of Oncology (IEO), Via Adamello 16, Milano 20139, Italy
[d] University of Bologna, via Umberto Terracini 28, Bologna 40131, Italy
[e] Politecnico di Milano, DIG Via Lambruschini 4/b, 20156, Italy
[f] Università Degli Studi di Genova, via Dodecaneso 35, Genova 16146, Italy
[g] University of Bologna, viale Berti Pichat 6/2, Bologna 40126, Italy
E-mail: mattia.ricchi@phd.unipi.it

Keywords: MRI, theory and methods.

Using MR diffusion tractography, brain structural connections are identified by analyzing water movement in brain tissue. This study focuses on the cingulum bundle (CB), a complex tract linked to functions such as emotion, reward, pain, and memory [1]. Given the CB's role in neurodegenerative diseases like Alzheimer's, this work aims to develop an automated protocol to identify CB subdivisions and analyze their microstructural properties, tracking dementia progression [2]. The reconstructed subdivisions exhibit different pathways (Fig. 1), terminations, and structural characteristics. We observed differences in diffusivity metrics among the CB subdivisions and between Alzheimer's disease patients and control subjects (Fig. 2). These findings are promising as they enable future tractography analyses to isolate fiber groups with common terminations, allowing for individual examination of these subdivisions. This approach can help associate specific functions with distinct CB fiber groups and correlate variations in their properties with pathologies.

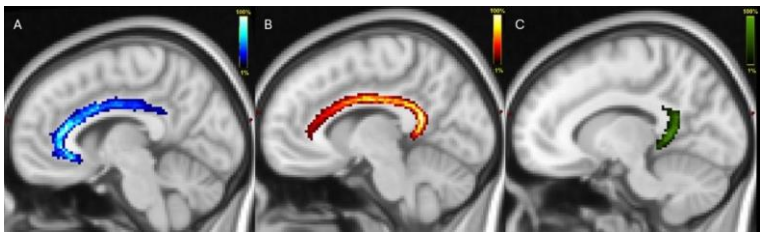


Fig. 1. A. Subgenual, B. Retrosplenial, C. Parahippocampal CB subdivisions

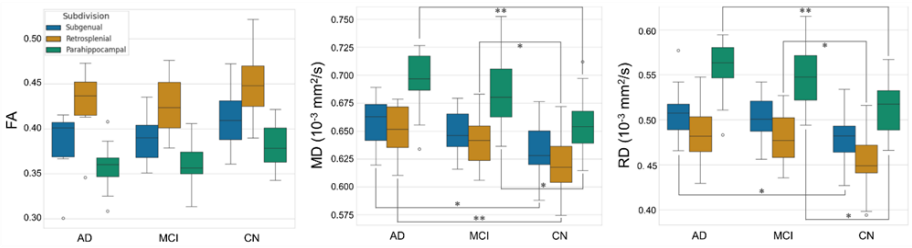


Fig. 2. Comparison between the different subdivisions and groups for the Fractional Anisotropy (FA), Mean Diffusivity (MD) and Radial diffusivity (RD) diffusion metrics.

References

[1] M. Beckmann, et al. *The Journal of neuroscience*, 29(4), 1175–1190 (2009)
[2] M. Bergamino, et al. *Scientific reports*, 11(1), 6990 (2021)

COMPREHENSIVE NMR STUDY OF FLUORIDE BINDING IN LANTHANIDE(III) COMPLEXES

L. Risolo, D. Lalli, M. Botta

Department of Sciences and Technological Innovation, University of Eastern Piedmont "Amedeo Avogadro", Viale Teresa Michel 11, 15121-Alessandria, Italy
E-mail: lorenzo.risolo@uniupo.it

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

The recognition of anions by inorganic complexes remains a significant challenge in coordination chemistry and it is attracting increasing interest due to its potential applications in technological and biomedical fields [1]. In this work, we investigated the interaction between fluoride ion and two Ln(III)-complexes [Ln(III) = Gd(III), Y(III)] characterized by different structures, coordination geometries, and hydration numbers (q = 1, 2) (see Fig. 1) [2]. We employed a combination of high- and low-resolution NMR techniques to provide a comprehensive characterization of the kinetics and thermodynamics of fluoride binding, offering detailed insights into the structural and dynamic properties of the ternary complexes with fluoride. Specifically, analyses of the water proton longitudinal relaxation rates as function of the magnetic field (^{1}H NMRD profiles), and ^{17}O transverse relaxation rates and shifts *vs* temperature were performed on the Gd(III)-complexes, which allowed evaluating the relaxation parameters of the ternary adducts (*i.e.* exchange dynamics, molecular tumbling etc.). The affinity constant (K_A) of the complexes with F^- was determined *via* relaxometric titration. Additionally, complementary studies on the diamagnetic Y(III) complexes by variable temperature ^{19}F NMR spectroscopy provided information on kinetics and thermodynamics associated with fluoride binding [3]. The results of this study represent an important step towards the development of new Ln(III)-receptors with enhanced anion affinity and selectivity.

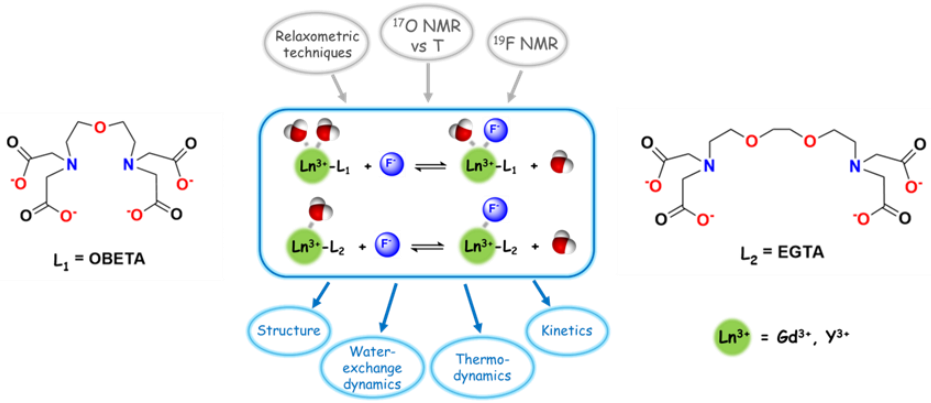


Fig. 1. Low- and high-field NMR techniques used to study the interactions between Ln(III)-complexes and fluoride.

References

[1] S. E. Bodman, and S. J. Butler *Chem. Sci.* **12**, 2716-2734 (2021)
[2] Z. Baranyai, M. Botta, M. Fekete, G. B. Giovenzana, R. Negri, L. Tei, and C. Platas-Iglesias *Chem. Eur. J.* **18**, 7680 – 7685 (2012)
[3] D. Lalli, F. Carniato, L. Tei, C. Platas-Iglesias, and M. Botta *Inorg. Chem.* **61**, 496-506 (2022)

[P-50]

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

C. Romiti[a], D. Alberti[a], S. Aime[b], S. Geninatti Crich[a]
[a]Department of Molecular Biotechnology and Health Sciences, University of Turin, Molecular Biotechnology Center "G.Tarone", Via Nizza 52, 10126, Turin, Italy
[b]IRCCS SDN SynLab, Napoli
E-mail: chiara.romiti@unito.it

Keywords: MRI, contrast agents.

Extracellular vesicles (EVs) are nanosized membranous subcellular structures naturally released by all cells for horizontal transport of intracellular cargos. In the last decades, their application to drug delivery purposes has garnered growing interest. Their natural origin has been associated with a better cell-specific tropism than synthetic nanoparticles and easiness of crossing physiological barriers, making them suitable to explore less conventional routes of administration (*e.g.* oral and intranasal) [1]. Despite mammalian cells-derived EVs, edible plant-derived EVs are highly available in nature, easy to isolate in a standardized fashion, and more stable at the acidic pH of the stomach environment [2]. However, to date, few efforts have been made to characterize these novel delivery platforms and their targeting efficiency through robust imaging techniques. MRI noninvasiveness and anatomical resolution may help to provide information about the localization and accumulation of the EVs and their therapeutic cargo *in vivo*, as well as the interaction with the target tissue to optimise dosage and delivery methods. Herein, orange-derived EVs have been isolated through ultracentrifugation and characterized for their size, surface ζ potential and concentration in the solution. 70-100 nm EVs were incubated with MRI contrast agents (CAs) via osmotic stress, and subsequently washed from unbound CA through 3 dialysis cycles (Fig. 1A). To find the most suitable CA for EVs labelling, Gadoteridol and the ammino-substituted FF759 (Fig. 1B) were compared in terms of Gd concentration inside EVs versus incubated concentration, assessed through ICP-MS, measures of Relaxivity at 21 MHz, and T_1 contrast enhancement of *in vitro* phantoms at 300 MHz (Fig, 1C, D). Positively charged CAs like FF759 may represent the optimum for EVs labeling, probably due to the interaction with their negatively charged surface. In this respect, more compounds will be investigated as EVs labeling and imaging protocols are truly needed to go more in deep into the knowledge of these novel nanocarriers of high interest.

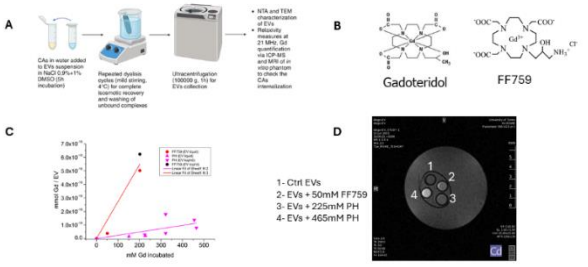


Figure 1. (**A**) EVs incubation protocols. (**B**) Structures of incubated CAs. (**C**) Incubation efficiency of Gadoteridol (purple) vs FF759 (red and black) inside EVs. (**D**) T_{1w} image of *in vitro* phantom of EVs incubated with Gadoteridol at increasing concentration (3 and 4) and FF759 (2).

References

[1] Gai C, Pomatto MAC, Deregibus MC, Dieci M, Piga A, Camussi G. Edible Plant-Derived Extracellular Vesicles for Oral mRNA Vaccine Delivery. Vaccines (Basel).2024 Feb15.
[2] Zhao Y, Tan H, Zhang J, Pan B, Wang N, Chen T, Shi Y, Wang Z. Plant-Derived Vesicles: A New Era for Anti-Cancer Drug Delivery and Cancer Treatment. Int J Nanomedicine.2023 Nov18;

PROBING HYDROGEN BONDS IN ANION···ANION HYDROGEN-SELENITE ADDUCTS BY SOLID-STATE NMR

C. Rosso,[a] F. Bravetti,[a] A. Gallo,[a] R. Beccaria,[b] A. Pizzi,[b] C. Lo Iacono,[b] G. Resnati,[b] M. R. Chierotti,[a] R. Gobetto[a]

[a] Department of Chemistry, University of Turin, Via P. Giuria 7, 10125 Turin, Italy
[b] NFMLab, Department of Chemistry, Materials, and Chemical Engineering "Giulio Natta", Politecnico di Milano, Via L. Mancinelli 7, I-20131 Milano, Italy
E-mail: ch.rosso@unito.it

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

It is well known that hydrogen bond (HB) interaction plays a fundamental role in solid-state supramolecular chemistry due to its directionality, specificity, strength, and selectivity [1]. While the establishment of HBs between neutral and ionic structural units (charged-assisted HB) has been extensively studied and exploited in various applications and industrial fields, in recent years there has been an increased interest in the use of inter-anionic hydrogen bonds, *i.e.* deliberately forming hydrogen bonds between anions [2]. HB-based and counterion-assisted anion-anion assemblies have proven to be effective and versatile enough to produce materials with different functionalities and applications [2,3]. In the analysis of intermolecular interactions and, especially, of HBs in solid-state systems, solid-state NMR (ssNMR) is the leading spectroscopic technique, as it allows the atoms directly involved in the bonding interaction to be specifically and accurately investigated.
In this work, two new and different anionic assemblies consisting of hydrogen-selenites, $HSeO_3^-$, and stabilised by the presence of two different organic counterions, 3-aminopyridazine and 4-aminopyridine, are presented. An in-depth and comprehensive characterization using ssNMR is here proposed. One-dimensional, ^{1}H MAS, ^{13}C and ^{77}Se CPMAS, and two-dimensional, ^{14}N-^{1}H J-HMQC and ^{1}H DQ MAS, experiments were carried out in combination with ab initio and GIPAW calculations performed on the crystallographic structures experimentally obtained by SCXRD.

References

[1] M.R. Chierotti, R. Gobetto, *Chem. Commun.* **14**, 1621–1634 (2008)
[2] W. Zhao, A. H. Flood, N. G. White, *Chem. Soc. Rev.* **49**, 7893–7906 (2020)
[3] R. Beccaria, A. Dhaka, M. Calabrese, A. Pizzi, A. Frontera, G. Resnati, *Chem. – Eur. J.* **30**, (2024)

[P-52]

STRUCTURE, DYNAMICS AND WATER STATUS IN A POLYKETONE-BASED ANION EXCHANGE MEMBRANE FOR ELECTROCHEMICAL APPLICATIONS: A SOLID STATE NMR STUDY

S. Rotundo,[a] A. Giovanelli,[a] F. Nardelli,[b] E.Carignani,[b] F. Martini,[a,b,c] A. Pucci,[a,c] M. Geppi[a,b,c]

[a]Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, 56124 Pisa, Italy
[b]Istitute of Chemistry of Organometallic Compounds, National Research Council, 56124, Pisa, Italy
[c]CISUP, Center for Instrument Sharing-University of Pisa, Lungarno Pacinotti 43, 56126, Pisa, Italy
E-mail: s.rotundo2@studenti.unipi.it

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

In recent years, research has focused on developing new strategies and technologies to produce greener and more sustainable materials and energy resources, driven by the climate and energy crisis. Among these, green hydrogen represents one of the most interesting materials, with applications in the domestic, industrial and automotive sectors. One promising technology for producing green hydrogen relies on the electrolysis of water using anion exchange membranes (AEMs) [1]. AEM are typically based on polymer backbones with charged groups, whose chemical structure can be adjusted to enhance and optimize the membrane's performance, efficiency, and characteristics. The final properties of the membrane are also closely linked to the structural and dynamic properties of the polymer network at both the molecular and supramolecular levels. Another crucial aspect is the state of water and its degree of interaction with the polymer network, which determines the formation of the so-called ionic channels. Investigating these properties is essential to understand the mechanism of ion transport within these membranes.
In the present work, we characterized the structural and dynamic properties of AEMs for water electrolyzers based on low molecular weight polyketone (PK) by means of solid state NMR spectroscopy (SSNMR). The membranes were functionalized through a Paal-Knorr reaction with a diamine, which was quaternized with alkyliodides. Dialkyliodides were employed as crosslinking agents. Various (di)alkyliodides, including (1,4-di)iodobutane, were studied to explore their effect on the properties of the AEMs. Different alkyliodide/dialkyliodide ratios were also probed [2],[3]. ^{1}H and ^{13}C high-resolution SSNMR spectra provided information on the structural properties of the polymer network and the degree of functionalization. Variable temperature ^{1}H T_1 relaxation times revealed insights into the dynamics of the polymer chains and functional groups, as well as sample homogeneity on the nanometer scale. Additionally, ^{2}H spectra of water in membranes hydrated with deuterium oxide were analyzed to study water dynamics and interaction with the polymer matrix, varying both the membrane composition and water content.

Acknowledgements: This research has been carried out with the financial support of the project PRIN PNRR 2022 "In-MoTion-Influence of dynaMics on the adsorption and Transport properties in polymeric materials for membrane technologies" funded by the European Union – Next Generation EU.

References

[1] I. Vincent, D. Bessarabov, *Renew. And Sustain. Energy Rev.* **81**, 1690-1704 (2018)
[2] O. Racchi, R. Baldassari, E. Araya-Hermosilla, V. Mattoli, P. Minei, A. Pozio, A. Pucci *Polymers* **15** (2023)
[3] G. Benzahia, M. I. Ferahi, E. Magnani, A. Giovanelli, A. Pucci, R. Meghbar *Pol. For Adv. Tech.* **35** (2024)

[P-53]

A FIRST LOOK AT URINE METABOLITE INTERACTOME THROUGH NMR METABOLOMICS

M. Salobehaj,[a,b] L. Tenori,[a,b] C. Luchinat[c,d]

[a]CERM, University of Florence, Via Luigi Sacconi 6, Sesto Fiorentino, 50019 (FI), Italy
[b]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastrucci 3, Sesto Fiorentino, 50019 (FI), Italy
[c]CIRMMP, Via Luigi Sacconi 6, Sesto Fiorentino, 50019 (FI), Italy
[d]Giotto Biotech S.r.l., Via Madonna del Piano 6, Sesto Fiorentino, 50019 (FI), Italy
E-mail: maria.salobehaj@gmail.com

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

Metabolomics involves the comprehensive analysis of low molecular weight metabolites in biological specimens [1]. Urine, with over 2000 endogenous metabolites, serves as a rich source of metabolic data, with 250-400 metabolites detectable by NMR spectroscopy within minutes [2]. Automating metabolite signal identification and quantitation in urine using NMR is hindered by variable chemical shifts, influenced by pH and ion concentrations [3]. To address this challenge, we aim to use a standard urine sample, comprising a mixture of individuals, as a universal reference. This approach will enable us to systematically observe shift variations and metabolite interactions. By establishing a universal urine metabolite interactome, we seek to enhance the reliability and efficiency of automated NMR-based urine analysis. This development holds the potential to facilitate high-throughput metabolomics studies, improving biomarker discovery, disease diagnostics, and personalized medicine applications.

References

[1] Wishart, D. S. et al. HMDB 3.0-The Human Metabolome Database in 2013. *Nucleic Acids Res.* **41**, D801–D807 (2013).
[2] Bouatra, S. et al. The human urine metabolome. *PLoS ONE* **8**, e73076 (2013).
[3] Gil, R. B., Lehmann, R., Schmitt-Kopplin, P. & Heinzmann, S. S. 1H NMR- based metabolite profiling workflow to reduce inter-sample chemical shift variations in urine samples for improved biomarker discovery. *Anal. Bioanal. Chem.* **408**, 4683–4691 (2016).

[P-54]

DEVELOPMENT AND CHARACTERIZATION OF NANOSYSTEMS FOR INNOVATIVE APPLICATIONS OF MAGNETIC RESONANCE IMAGING

A. Santangelo,[a] F.Garello[a], E.Cavallari[a] , S.Aime[a,b] ,E.Terreno[a]
[a]Department of Molecular Biotechnology and Health Science, Molecular and Preclinical Imaging Center, Piazza Nizza 44/bis, Turin, Italy
[b] IRCCS SDN SynLAB, Via Gianturco 113, Naples, Italy.
E-mail: santangelo.alice@unito.it

Keywords: solution NMR, MRI, materials, polymers, contrast agents.

The possibility of visualizing selected nanoparticles through Magnetic Resonance Imaging (MRI) without the use of contrast agents has been recently reported, using the Chemical Shift Imaging (CSI) technique [1]. Through CSI, it is indeed possible to directly visualize certain drug nanocarriers that exhibit a strong and defined NMR signal, generally associated with their components, such as phospholipids, surfactants, and polymers, achieving non-invasive monitoring of their delivery and pharmacokinetics. Furthermore, the CSI technique is inherently quantitative, allowing for the determination of the quantity of nanosystems delivered in the area of interest, in order to predict the therapeutic response in individual patients. In this context, the present work aims to synthetize different biocompatible nanosystems and investigate the possibility of visualizing them through chemical shift imaging (CSI).
More specifically, four different nanosystems were studied: PLGA-based nanoparticles (PLGA-NP), polymethylmethacrylate nanoparticles (PMMA-NP), pluronic micelles (F127-COOH) and a nanoemulsion containing both the surfactant Kolliphor® P188 and a perfluorocarbon (PFCE-NE). For each type of nanoparticle, the following haracteristics were determined: proton concentration, transverse and longitudinal relaxation times, size and stability in physiological solution, morphology using field emission scanning electron microscopy (FESEM), and the limit of detection using the ^{1}H CSI technique. The best performing nanosystem was then selected to perform preliminary ^{1}H CSI assays in vitro in murine macrophages (RAW 267.4 cells).
All the developed nanosystems demonstrated one or more well-defined and intense ^{1}H NMR peak between 1 and 4 ppm (Figure 1A), with a protonic concentration in the range of 100-6000 mM. The T_1 relaxation time were around 200-600 ms respectively for PLGA-NP, pluronic micelles, and PFCE-NE and around 2.0 s for PMMA-NP. Most of the nanosystems were stable in physiological solution, except for PMMA-NP. ^{1}H CSI acquisitions of phantoms containing serial dilutions of each nanosystem were performed (Figure 1B).

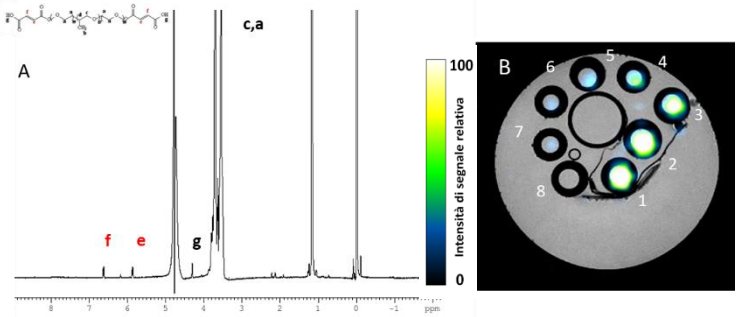


Fig. 1. A) Proton spectrum of micelles based on F127-COOH acquired at 600 MHz at 298 K B) CSI ^{1}H of the peak at 3.7 ppm superimposed on T_2-weighted (grayscale) MRI ^{1}H images of a dummy containing the micelles at decreasing concentrations from 1 (most concentrated sample) to 8 (control sample without the NPs)

The intrinsic quantitative nature of the technique allows for the calculation of the SNR and the limit of detection (approximately 15-50 mM of equivalent protons). According to the results obtained, the best performing system was the PFCE-NE, that was further incubated with cells, demonstrating for the first time the possibility of visualizing by ^{1}H CSI the nanosystem-associated signal in vitro, following cell internalization. Due to the presence of ^{19}F in the nanoemulsion a comparison between ^{19}F MRI and ^{1}H CSI was also carried out. In conclusion, the results identified key nanosystem traits for CSI visualization, guiding the creation of CSI-eligible molecules for new nanosystems.

References

[1] Garello F, Cavallari E, Terreno E. MRI detection of free-contrast agent nanoparticles. 19th Annual Meeting of the European Society for Molecular Imaging (EMIM 2024), 12-15/03/24, Porto, Portugal.

NQR SPECTROSCOPY FOR THE STUDY OF MAPb(Br_xI_{1-x})$_3$ PEROVSKITES AND THEIR PHOTOINDUCED HALIDE SEGREGATION

A. Scarperi,[a] N. Landi,[b] E. Carignani[b, c], S. Borsacchi[b, c], M. Geppi[a, c]

[a] Department of Chemistry and Industrial Chemistry, University of Pisa, via G. Moruzzi 13, 56124 Pisa, Italy;
[b] Institute of Chemistry of Organometallic Compounds, Italian National Research Council (ICCOM-CNR), via G. Moruzzi 1, 56124 Pisa, Italy;
[c] Center for Instrument Sharing of the University of Pisa (CISUP), 56124 Pisa, Italy;
E-mail: andrea.scarperi@phd.unipi.it

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

Over the past decade, metal halide perovskites have emerged as one of the most promising classes of materials in photovoltaics due to their remarkable optoelectronic properties. A key factor in their success is the ability to tune their properties by varying their composition, which simultaneously makes the structural characterization of these materials particularly challenging due to the high degree of structural and dynamic disorder [1].
Recently, Nuclear Quadrupole Resonance (NQR) spectroscopy has gained increasing interest its ability to provide in-depth local investigations of halide environments [2-4], which are notoriously difficult to study directly using Solid-State Nuclear Magnetic Resonance (SSNMR).
In this study, we investigated the halogen chemical environments of various compositions of mixed-halide perovskites MAPb(Br_xI_{1-x})$_3$, where MA is Methylammonium, using ^{127}I and $^{79/81}$Br NQR. Additionally, through *in situ* illumination experiments, we observed for the first time the reversible effect of photoinduced halide segregation in mixed-halide perovskites [5] by means of nuclear resonance spectroscopy (Fig. 1). This kind of study opens up the possibility of obtaining local structural information associated with this phenomenon, thus increasing the experimental data available to understand its origins.

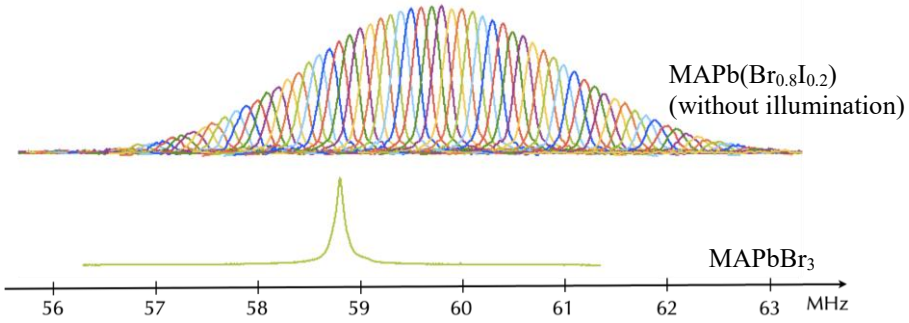


Fig. 1. ^{81}Br NQR spectra of $MAPbBr_3$ and MAPb($Br_{0.8}I_{0.2}$)$_3$

References

[1] S. Stranks, H. Snaith, *Nature Nanotech* **10**, 391–402 (2015)
[2] L. Piveteau, V. Morad, M. V. Kovalenko, *J. Am. Chem. Soc*. **142**, 19413−19437 (2020)
[3] M. Aebli, N. Porenta, N. Aregger, M. V. Kovalenko, *Chem. Mater*. **33**, 6965−6973 (2021)
[4] R. W. Hooper, K. Lin, J. G. C. Veinot, V. K. Michaelis, *J. Magn. Reson.* **352**, 107472 (2023)
[5] M. C. Brennan, A. Ruth, P. V. Kamat, M. Kuno, *Trends in Chemistry* **2**, 282-301 (2020)

This work is financially supported by the European Union "NextGenerationEU" through the PRIN 2022 program promoted by Italian Ministry of University and Research (MUR), project NUTSHELL (2022P3B4YJ)

EXPLOITING MULTIPLE RECEIVERS AT 1.2 GHZ TO INVESTIGATE INTRINSICALLY DISORDERED PROTEINS

M. Schiavina,[a] S. Knoedlstorfer,[b] M.A. Rodella[a], R. Kuemmerle[c], R. Konrat[b], I.C. Felli[a], R. Pierattelli[a]

[a] Department of Chemistry 'Ugo Schiff' and Magnetic Resonance Center (CERM), University of Florence, Florence, Italy
[b] Department of Computational and Structural Biology, Max Perutz Labs, University of Vienna, Vienna, Austria
[c] Bruker BioSpin AG, Fällanden, Switzerland
E-mail: schiavina@cerm.unifi.it

Keywords: solution NMR, theory and methods, instrumentation.

Increased magnetic field strengths and console improvements drive progress in biomolecular NMR applications. Ultra-High-Field NMR instruments enable the investigation of complex IDPs. However, the crowding of NMR spectra frequently hampers their study. High-field ^{13}C-detection offers excellent resolution to mitigate this problem [1]. However, due to ^{13}C's lower sensitivity and longer relaxation times compared to protons, obtaining well-resolved spectra typically requires time-consuming experiments. To address this, we are developing Multiple-Receivers NMR experiments (mr_NMR), where one experiment is acquired during the recycle delay of another (Fig. 1a). The recovery delay of ^{13}C nuclei is long enough to acquire a second NMR experiment during this period, saving time and providing complementary information from different correlations [2]. Previously developed, mr_NMR experiments like mr_CON//HN (Fig. 1b) and mr_CON//H$^{\alpha}$ CAN were used to obtain at 1.2 GHz the fingerprint and sequence-specific information of the structurally heterogeneous CBP-ID4 protein. The new mr_CACO//btHN pulse sequence offered insights into Myc:Max and BRCA protein interaction (Fig. 1c), collecting complementary structural and dynamic information of IDPs and their interactions.

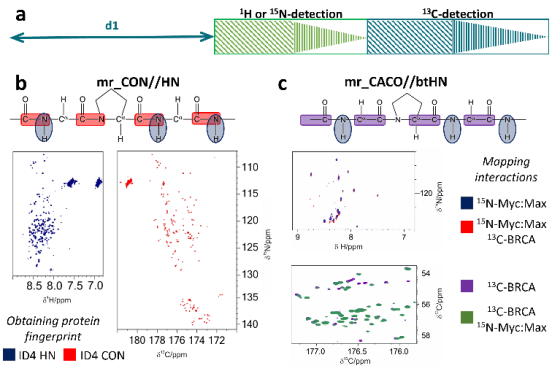


Fig. 1. **(a)** The mr_NMR strategy with one experiment (green) is acquired during the recovery delay of a second, ^{13}C-detected, one (blue). **(b)** The mr_CON//HN experiment demonstrating its application in obtaining the fingerprint of the ID4 protein. **(c)** The mr_CACO//btHN experiment mapping the interaction between Myc:Max and BRCA using specific labelling schemes.

References

[1] M. Schiavina, L. Bracaglia, M.A. Rodella et al. *Nat. Protoc.* **19(2)**, 406-440 (2024)
[2] M. Schiavina, MG Murrali, L. Pontoriero et al. *Biophys. J.* **117(1)**, 46-55 (2019)

[P-57]

NMR METABOLOMICS OF ORGANIC "PAN DI ZUCCHERO" CHICORY: CHANGES IN NUTRIENT CONTENT IN RESPONSE TO BIOINOCULANT TREATMENT UNDER WATER-SAVING CONDITIONS

G. Scioli,[a] L. Pin,[a] G. Testone [a], G. Arnesi[b], D. Giannino,[a] A.P. Sobolev[a]

[a] Institute for Biological Systems (ISB-CNR), Council of National Research of Italy (CNR); Via Salaria km 29.300, 00015 Monterotondo-Rome, Italy
[b] Enza Zaden Itaia , Tarquibia, Viterbo, Itay
E-mail: giuseppe.scioli@isb.cnr.it

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

"Pan di zucchero" (PDZ) or "Radicchio di Milano" is a vegetable of the leafy cicorium/radicchio group (*Cicorium intybus* L. var. *foliosum* Bischoff) of the cultural heritage of Italy, Switzerland, and France, similar to lettuce, it differs in its taste, which is sour and bitter, and is highly appreciated in the fresh-cut industry for its long shelf-life, maintaining quality and freshness. In the context of organic farming, where PDZ is grown in open fields (Biocaramadre company, Lazio), climate change and water shortages affect yield and quality, causing variations in key nutrients. Fungal bioinoculants (biostimulants) are promising tools for agronomic techniques to mitigate the negative effects of abiotic stresses such as heat waves or drought. This study used quantitative NMR spectroscopy to assess the metabolic variation of water-soluble compounds in PDZ heads grown under water-saving conditions, namely progressive water depletion of 15 and 30% over controls and treated with a bioinoculant (Micosat F). Metabolites were extracted from freeze-dried leaves of heads at harvest with a mixed solvent of water-acetonitrile 1:1 v/v and subsequently dissolved in deuterated water with phosphate buffer (400 mM, pH =7). Thirty-two metabolites, mainly amino acids, sugars, and organic acids were assigned and quantified. Principal component analysis showed that at maximum irrigation, differences between treated and control plants were minimal, whereas water depletion caused a clean separation of treated and control samples due to stress-related metabolic changes. According to ANOVA, biostimulant treatment, water depletion and their interaction caused the level variation of all metabolites (except FOR ethanolamine, choline, malic and tartaric acids). The most significant changes included several amino acids (leucine, tyrosine, phenylalanine, histidine) and carbohydrates (glucose, fructose and galactose). The variation in metabolite levels was not linearly proportional to the degree of water depletion (e.g. the decrease in metabolite content was not 15 and 30% compared to fully watered controls), bringing out complex regulatory mechanisms, probably mediated by the biostimulant.

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

[P-58]

NMR ANALYSIS OF FRUIT PROCESSING WASTES TAILORED FOR VALORIZATION AND REUSE

A.P. Sobolev,[a] G. Scioli,[a] S. Demaria,[b] C. Baldisserotto[b]

[a] Institute for Biological Systems (ISB-CNR), Council of National Research of Italy (CNR); Via Salaria km 29.300, 00015 Monterotondo-Rome, Italy
[b] Department of Environmental and Prevention Sciences, University of Ferrara, C.so Ercole I d'Este, 32, 44121 Ferrara, Italy
E-mail: anatoly.sobolev@cnr.it

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

Comprehensive chemical characterization of food processing wastes is a prerequisite for their valorization and the development of new perspective ways of their reuse as a part of the circular economy. NMR spectroscopy, as a versatile tool for the identification and quantification of bioactive compounds, was chosen to characterize by-products of the industrial transformation of fruits (pear, apple, apricot, and peach). Water-soluble extracts of by-products were obtained by centrifugation of the pulp or by extraction from lyophilized samples with water. Quantitative NMR metabolite profiling revealed the prevalence of common classes of metabolites (sugars, organic acids, free amino acids), in varying amounts, specific to each by-product. Peach by-product extracts were tested in parallel with pure sugars (glucose, sucrose, or their 1:1 mixture) as substrates for accelerating the growth *of Chlorella protheicoides* microalgae. Sugar consumption and the eventual release of their biotransformation products in a growth medium were monitored by NMR. NMR data indicated microalgae preferences for glucose with respect to sucrose and the release of small amounts of gluconic, lactic, and 3-hydroxybutyric acids as products of glucose transformation. The study of the influence of sugars in the growth medium on the metabolite profile of *Chlorella protheicoides* microalgae is in progress.

This work was supported through European Union Funding – Next Generation EU - M4C2, investment 1.1 ALBA-VITA, CUP B53D23017950006.

[P-59]

HEMP METABOLITE PROFILING BY NMR: VARIETY AND IRRIGATION EFFECTS

E. Serni,[a] G. D'Orazio,[a] V. Montesano,[b] M. Centritto,[c] A.P. Sobolev[a]

[a] Institute for Biological Systems (ISB-CNR), Council of National Research of Italy (CNR); Strada Provinciale 35d, 9 – 00010 Montelibretti (RM)
[b] Institute for Sustainable Plant Protection (IPSP-CNR), Council of National Research of Italy (CNR); Via Amendola 122/D 70126 Bari, Italy
[c] Institute for Sustainable Plant Protection (IPSP-CNR), Council of National Research of Italy (CNR); Via Madonna del Piano 10 – 50019 Sesto Fiorentino (FI), Italy
E-mail: enrico.serni@cnr.it

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

Rising temperatures caused by climate change affect the yield and quality of industrial crops, which are mostly grown in open fields and undergoing abiotic stress such as drought, thus causing variations in the content of important nutritive compounds.
Hemp (*Cannabis sativa* L.) is a well-known fast-growing and high-biomass crop, that has been used for centuries for multiple purposes, especially for fiber and seed oil production. Different genotypes with low levels of psychoactive cannabinoids were selected through breeding in the last decades, thus suitable for non-pharmaceutical uses like biofuel and textile fiber production. A putative correlation between metabolic profile and stress resistance could help to elucidate the mechanisms of resilience, allowing the selection of cultivars with clearer criteria and development of agronomical practices for their production.
This study used NMR spectroscopy to assess the variations in the metabolic profile of water-soluble compounds in industrial hemp inflorescences due to both genetic background and water depletion. Hemp plants of four well-established industrial cultivars (Fedora, Futura 75, Felina 32 and Earlina) were grown in an open field with well-watered controls and reduced irrigation plots.
The water-soluble metabolites were extracted from lyophilized inflorescences using simultaneous chloroform-methanol-water liquid extraction (Bligh-Dyer protocol [1]). Hydroalcoholic extracts were dried and re-dissolved in deuterated water with phosphate buffer (400 mM, pH =7).
Twenty-two metabolites, mostly amino acids, sugars and organic acids, were identified in the aqueous extracts. PCA pointed out the differences between the metabolite profiles of the four cultivars irrespective of irrigation levels, whereas the reduced irrigation effect was only noticeable for Futura inflorescences. ANOVA confirmed the results of PCA highlighting that the levels of 7 out of 22 metabolites (such as GABA, asparagine, citric acid, sucrose, myo-inositol and quebrachitol) were significantly influenced by the cultivar, while the levels of glutamic acid, aspartic acid and asparagine were increased in Futura 75 inflorescences due to reduced irrigation.

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

References

[1] E. G. Bligh, W. J. Dyer *Can. J. Biochem. Physiol*. **37**, 911-917 (1959)

[P-60]

USE OF NUCLEAR MAGNETIC RESONANCE IN CHEESE ANALYSIS

P. Solovyev,[a] V. Maestrello,[a,b] A. Stroppa,[c] P. Franceschi,[a] E.Franciosi,[a] J. Andersen,[a] L. Bontempo[a]

[a]Fondazione Edmund Mach, via Edmund Mach 1 38098, San Michele all'Adige TN, Italy
[b]C3A-Centro Agricoltura Alimenti Ambiente, University of Trento, via Edmund Mach 1 38098, San Michele all'Adige TN, Italy
[c]Consorzio Tutela Grana Padano, Via XXIV Giugno 8, 25010, San Martino Della Battaglia, Desenzano del Garda, BS, Italy
E-mail: pavel.solovyev@fmach.it

Keywords: solution NMR, biomolecules, metabolomics, food.

NMR spectroscopy has been long used for the analysis of cheese products, starting from the simple moisture determination in the 1960s and 70s [1,2] but the more in-depth studies of these matrices using high resolution magnets have started only at the dawn of the 21st century [3], and remains a pertinent field of interest up to the present day [4]. Recently, our research group has applied this approach in several case studies. The first one consisted in nutritional value study of cheeses enriched with blackcurrant and Cornelian Cherry. Here, the targeted NMR results indicate that it is possible to differ the blackcurrant-modified cheeses (that turned out to possess increased bioactive potential) from the others [5]. Another one was the dataset comprising two Italian PDO cheeses and several competitor varieties. Both aqueous and chloroform extracts demonstrated that after the statistical analysis (Random Forest approach) the PDO cheese can be discriminated from others with 92-93% predictivity [6,7]

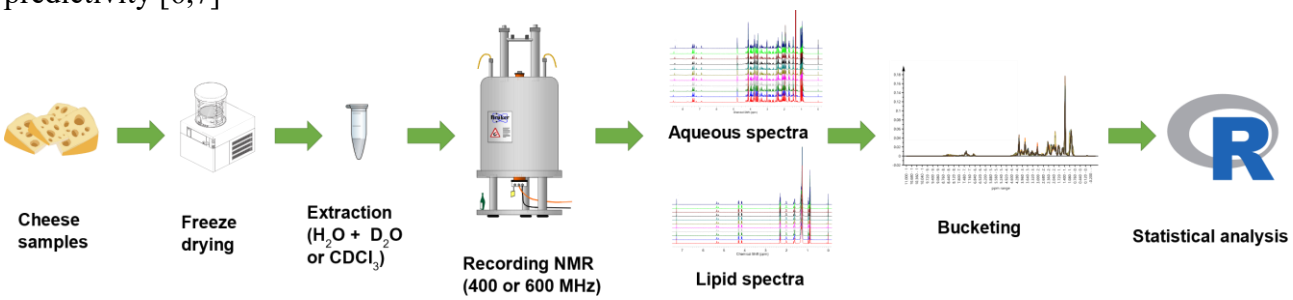


Fig. 1. Experimental workflow of cheese analysis.

In each case study the experiment design (Fig. 1) consisted of freeze drying the grated cheese followed by extraction of the residue either with water or chloroform to obtain aqueous and lipid fractions, recording proton NMR spectra for each fraction with subsequent post-processing, binning and statistical analysis using specified packages in R programming language environment as well as measurement of concentrations for individual components. Thus, NMR spectroscopic analysis of cheeses has been shown to be a promising technique in detection of mislabeling and nutrition studies.

References

[1] W. D. Pohle and R. L. Gregory *J. Am. Oil Chem. Soc*. **45**, 11, 775-777 (1968)
[2] R. E. Hester and D. E. C. Quine *J. Food. Technol*. **11**, 4, 331.339 (1976)
[3] S. de Angelis Curtis et al. *Food Chem*. **71**, 495-502 (2000)
[4] M. Tarapoulouzi, I. Pashalidis and C. R. Theocharis *Appl. Sci*.**14**, 6, 2584 (2024)
[5] J. Andersen et al. *Front. Nutr*. **9**, 1023490 (2023)
[6] V. Maestrello et al. *Foods* **13**, 3, 358 (2024)
[7] V. Maestrello et al. *Submitted manuscript* (2024)

[P-61]

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

A. S. Tino[a,b,c], M. Quagliata[a], M. Schiavina[b], A. M. Papini[a,c], R. Pierattelli[a,b], and I. C. Felli[a,b]

[a] Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, Sesto F.no (IT)
[b] Magnetic Resonance Center, University of Florence, Via L. Sacconi 6, Sesto F.no (IT)
[c] Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, University of Florence, Via Madonna del Piano 6, Sesto F.no (IT)
E-mail: angelasofia.tino@unifi.it

Keywords: solution NMR, biomolecules.

Numerous RNA-binding proteins (RBPs) exhibit modular structures containing folded domains and intrinsically disordered regions (IDRs). Investigating the role of these domains and their potential interaction is essential for understanding protein function and developing intervention strategies. The Nucleocapsid protein (N) of SARS-CoV-2 is a pivotal example of RBP. Its complex structure encompasses two folded domains and three IDRs. In particular, the globular N-terminal domain (NTD) is responsible for the viral RNA interaction and the two flanking IDRs play an important synergic role (1). The aim of my PhD project is to design and synthesize molecules able to interfere with the protein function, monitoring the interaction through solution NMR titrations. In particular, taking into account the structural characteristics of the protein, a first peptide was designed to simulate the main interactions driving the viral RNA binding. Then, a series of different peptides with slight modifications were synthesized to discern residues or motifs essential to interact in the protein target site. This collection of peptides has been tested by NMR titrations to identify the sequence displaying the highest affinity with the protein NTD and also with the NTR construct, comprising also the two flanking IDRs. Different NMR experiments were performed to enable the simultaneous observation of globular and disordered regions both with atomic resolution; in particular the interaction was followed through ^{1}H-^{15}N HSQC experiments but also by exploiting the ^{13}C detection, essential to investigate flexible regions. This study is now being improved with the design and synthesis of a peptide-PNA chimera, replacing certain amino acid residues of the parental peptide sequence with four PNA building blocks, selecting four G as nucleobases (2), aiming to better mimic the RNA nature. This promising molecule was tested with both protein constructs in the same experimental conditions. For now, this study yielded two main and clear results: the comparison among the titrations carried out first with the peptides and then with the peptide-PNA chimera has revealed a significantly greater affinity between the protein and the chimera with the respect to the peptide molecules. Additionally, in both cases, the presence of IDRs in the protein NTR construct has shown visibly more pronounced effects in the HSQC spectra compared to the NTD alone, suggesting an important contribution of these flexible regions in the interaction.

References

[1] Pontoriero, L.; Schiavina, M.; Korn, S.M.; Schlundt, A.; Pierattelli, R.; Felli, I.C. Biomolecules 12, 1 (2022).
[2] Sekine R, Tsuno S, Irokawa H, Sumitomo K, Han T, Sato Y, Nishizawa S, Takeda K, Kuge S. J Biochem 173, 6 (2023).

[P-62]

EXPLORING THE DRUGGABILITY OF INTRINSICALLY DISORDERED PROTEINS INVOLVED IN NEURODEGENERATIVE DISORDERS THROUGH NMR SPECTROSCOPY

F. Turchi[a,b], G. Tagliaferro[a,b], M. Schiavina[a,b], F. Clemente[a], F. Cardona[a], R. Pierattelli[a,b] and I. Felli[a,b]

[a] Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019 Sesto F.no (IT)
[b] Magnetic Resonance Center, Via L. Sacconi 6, 50019 Sesto F.no (IT)
E-mail: filippo.turchi@unifi.it

Keywords: Solution NMR, small molecules, biomolecules.

Intrinsically disordered proteins (IDPs) are highly flexible and dynamic molecules often linked to the onset of incurable diseases. Despite their great therapeutic potential, IDPs are often considered as undruggable because of the lack of defined binding pockets which are at the basis of current drug discovery approaches. Nuclear magnetic resonance (NMR) spectroscopy represents the method of choice in characterizing the dynamic and structural properties of IDPs at atomic level [1].
The objective of this research is to focus on α-synuclein (α-syn), a soluble IDP involved in the onset of synucleinopathies, a series of neurodegenerative disorders which also include Parkinson's disease (PD). Aggregates of α-syn (oligomers and fibrils) are found within dopaminergic neurons of people affected by these diseases. A very interesting strategy for treating synucleopathies, involves the use of small molecules, called chemical chaperones (CCs), that interact with the monomeric form of α-syn by modulating its aggregation. In this context, we use several advanced NMR methods, to assess how small molecules, such as nitrogen-containing glycomimetics (e.g. iminosugars), can interact with the monomeric state of α-syn (Fig. 1). The identification of CCs *vs* α-syn among iminosugars already recognized as pharmacological chaperones for α-glucocerebrosidase (GCase) [2], an enzyme involved in the pathogenesis of PD, will provide the great advantage to obtain dual targeting compounds able to enhance GCase activity and stabilize α-syn.

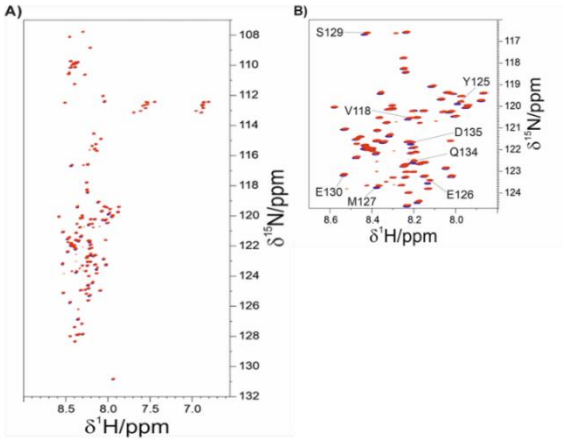


Fig. 1. (a) 2D HN NMR spectra acquired on α-synuclein without (blue) and with (red) iminosugars. (b) A zoom of the 2D HN HSQC spectra is shown to illustrate residues experiencing changes in chemical shifts.

References

[1] I. Felli & R. Pierattelli, *Chem. Rev.* **122**, 9468–9496 (2022)
[2] F. Clemente et al., *Bioorganic Chem*. **98**, 103740 (2020)

[P-63]

NMR METHODS FOR THE IDENTIFICATION AND CHARACTERIZATION OF SMALL ANTICANCER MOLECULES IN COMPLEX MIXTURE

G. Valentino[a]

[a] Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", via Vivaldi 43, 81100, Caserta, Italy
E-mail: giovanna.valentino@unicampania.it

Keywords: solution NMR, small molecules, metabolomics.

Despite medical advances, cancer is still a fast-spreading lethal disease. Finding novel anticancer drugs is crucial. Natural products and their derivatives hold significant potential in this field due to their structural diversity and pharmacological properties [1]. However, to develop new chemical products it is necessary to understand molecules' structure and behavior. In complex systems, it can be extremely difficult to obtain the required information efficiently.
In principle, NMR is expected to be able to meet this need, being one of the most powerful and versatile techniques for studying structural proprieties of molecules in solution [2]. Nevertheless, in the case of complex mixtures, conventional NMR methods struggle to extract clear. Therefore, to discover and characterize potential anti-cancer bioactive compounds from plants. Novel NMR-based methodologies are urgently needed to reduce the time and improve the spectral resolution needed
Here, we propose the application of alterative NMR methods to perform an accurate characterization of complex mixtures. In details, the identification of spectral bioactive regions was obtained by combining NMR-based metabolomics approaches with biological activity assay data [3]. Yet, following our strategy, a virtual separation of components based on their different diffusion properties was achieved by DOSY pulse sequences [4]. After that, putative bioactive compounds were identified and characterized through spectral simplification by combining conventional NMR approaches with pure shift and homonuclear spectral editing experiments [5].
Thanking advantage of the information obtained by in-mixture NMR experiments we carried out an *ad hoc* phytochemical strategy (L-L ext., CC, HPLC) and five bioactive sesquiterpene lactones were rapidly isolated and characterized.

References

[1] T. Rodrigues, D. Reker, P. Schneider and G. Schneider, *Nat Chem*, ***8***, 531-541 **(**2016)
[2] C. Deborde, A. Moing, L. Roch, D. Jacob, D. Rolin and P. Giraudeau, *Prog Nucl Magn Reson Spectrosc* ***102-103***, 61-97 (2017)
[3] G. Valentino, V. Graziani, B. D'Abrosca, S. Pacifico, A. Fiorentino and M. Scognamiglio, *Molecules* ***25*** (2020)
[4] M. Foroozandeh, R. W. Adams, N. J. Meharry, D. Jeannerat, M. Nilsson and G. A. Morris, *Angew Chem Int Ed Engl* ***53***, 6990-6992 (2014)
[5] P. Kiraly, M. Nilsson, G. A. Morris and R. W. Adams, *Chem Commun (Camb)* ***57***, 2368-2371 (2021)

NMR AS A TOOL TO INVESTIGATE THE LINK BETWEEN ION TRANSPORT AND LOCAL SEGMENTAL DYNAMICS IN POLYMERIC ELECTROLYTES.

V. Vanoli[a] , F. Castiglione[a]. R. Pasquino[b] F. Lorandi[c]

[a]Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Milan, Italy
[b] Università degli Studi di Napoli Federico II, Department of Chemical Engineering, Materials and Industrial Production, Naples, Italy
[c]University of Padova, Department of Chemical Sciences, Padova, Italy
E-mail: valeria.vanoli@polimi.it

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

Rechargeable Li batteries using solid electrolytes are safer alternatives to current Li-ion batteries that employ flammable liquid electrolytes [1]. Moreover, the promise to achieve higher energy densities makes solid-state battery desirable candidates to meet the urgency of decarbonizing transportation and improving air quality. In this work we study PEGMA-based graft polymers designed to present backbones with different flexibility, parameter that affects the mobility of side chains, which determines the mobility of Li-ions. To elucidate the role of structural uniformity on Li-ion mobility and the influence of backbone flexibility, we analyse the dynamics of polymer chains and Li ions over a broad range of timescales using NMR techniques (High Resolution and Solid State NMR) [2]. A comprehensive understanding of relaxation and ion-solvation phenomena in relation to structural features will be helpful to define structure-properties relationships to guide the design of highly-conductive PEs.

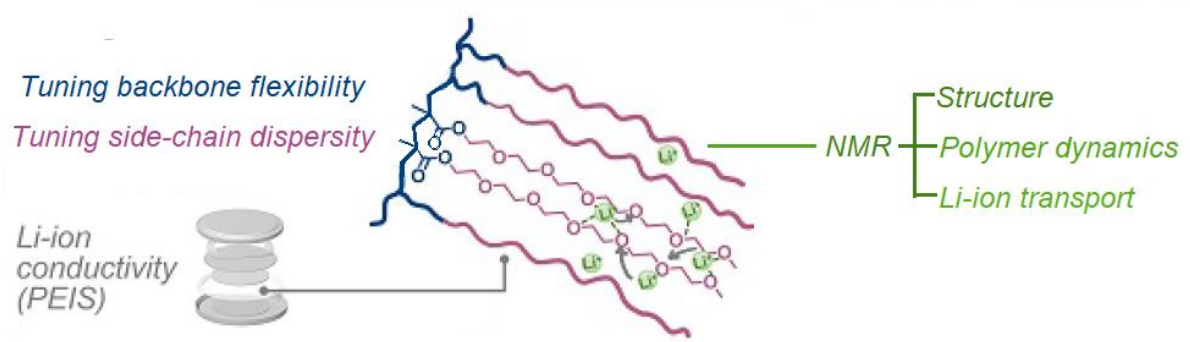


Fig.1 – Schematic representation of the NMR approach to study the influence of structural features on Li^+ dynamics.

References

[1] M. Ma, M. Zhang, B. Jiang, Y. Du, B. Hu, and C. Sun, "A review of all-solid-state electrolytes for lithium batteries: high-voltage cathode materials, solid-state electrolytes and electrode–electrolyte interfaces," *Mater Chem Front*, vol. 7, no. 7, pp. 1268–1297, 2023.
[2] C. Vinod Chandran and P. Heitjans, "Solid-State NMR Studies of Lithium Ion Dynamics Across Materials Classes," 2016, pp. 1–102.

[P-65]

PROBING THE PATHOLOGICAL CONFORMATIONAL TRANSITION OF HUMAN PRION PROTEIN BY CEST NMR SPECTROSCOPY

N. Ventserova[a], Luigi C.[b], G. Malgieri[a], G. D'Abrosca[a], C. Isernia[a], G. Legname[b], L. Russo[a] and R. Fattorusso[a]

[a] Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania Luigi Vanvitelli, Caserta, Italy
[b] Laboratory of Prion Biology, Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy.
E-mail: nataliia.ventserova@unicampamia.it

Keywords: solution NMR, biomolecules

Protein dynamics are fundamental for its function. In some cases, these motions involve the interconversion between highly populated "ground state" and less populated, higher energy "invisible" state in μs–ms (microsecond-millisecond) timescale [1]. In case of Human Prion Protein (HuPrP(90-231)), "invisible" states might be associated with cytotoxic oligomeric species, forming during the conformational conversion of the cellular prion protein (PrPC) into protease-resistant, β-sheet-rich scrapie form (PrPSc), which is the key infectious agent in prion diseases[2]. Recently, using Nuclear Magnetic Resonance (NMR) spectroscopy, we detected and characterized the on-pathway β-PrPI intermediate state that acts as a precursor of prion protein aggregation and amyloid fibril assembly [3]. In the current study, we eager to provide, an accurate high-resolution structural characterization of the oligomeric species driving the fibril aggregation process activated by the stable β-enriched intermediate state (β-PrPI) by Chemical Exchange Saturation Transfer (CEST) NMR experiments [4]. These latter NMR techniques allow to quantify slow chemical exchange providing a description of kinetic and thermodynamics parameters of the exchange process as well as the chemical shifts of the excited-state nuclei. Firstly, we demonstrated slow exchange conditions between monomer and oligomers (β-PrPI(O)) by analyzing 1H-15N HSQC spectra acquired at 15 °C on freshly prepared HuPrP(90-231) and β-PrPI (O) sample, obtained by incubating HuPrP(90-231) at 61 °C for 15 hours, thus, opening up the possibility to apply advanced spin relaxation methods, such CEST-NMR. As results, in the absence of β-PrPI (O), 15N CEST intensity profile acquired at low temperature (15°C) indicates only ground state with 15N CEST chemical shifts perfectly aligned with ^{15}N shifts assigned for the monomeric HuPrP(90-231) . On the contrary, in the presence of β-PrPI(O), 15N CEST intensity profile analysis revealed that the monomeric form of HuPrp(90-231) is in slow conformational exchange with "invisible" oligomeric states, having a noticeable ®-strand character. Interestingly, 15N chemical shift values of β-PrPI(O) are aligned with the Cryo-EM structure of prion fibrils [5], demonstrating that the N-term tail of HuPrP(90-231) undergoes structural rearrangements at earlier stages of the β-PrPI-induced oligomerization process.

References

[1] A.J. Baldwin, L E. Kay, *Nature chemical biology* **5.11,** 808-814 (2009)
[2] H. Wille, J. R. Requena. *Pathogens* **7.1**, 20 (2018)
[3] L. Russo et al. *Chem. Sci*. **13**, 10406-10427 (2022)
[4] T.R. Alderson, L.E. Kay, *Current opinion in struct. Biology* **60**, 39-49 (2020)

[P-66]

NMR-BASED METABOLOMICS IN ALZHEIMER'S DISEASE RESEARCH: FROM CELLS TO BIOFLUIDS

A. Vignoli,[a,b] C. Luchinat,[c,d] L. Tenori[a,b]

[a]Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3-13, 50019, Sesto Fiorentino, Italy
[b]Magnetic Resonance Center - CERM, University of Florence, via Luigi Sacconi 6, 50019, Sesto Fiorentino, Italy
[c]Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine - CIRMMP, via Luigi Sacconi 6, 50019, Sesto Fiorentino, Italy
[d]Giotto Biotech S.r.l., via Madonna del Piano 6, 50019, Sesto Fiorentino, Italy
E-mail: vignoli@cerm.unifi.it

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

Metabolomics can play a pivotal role in unraveling the intricate mechanisms underlying age-related diseases. By studying the dynamic profile of small molecules, metabolomics can uncover alterations in metabolic pathways, cellular processes and novel biomarkers offering powerful insights into the metabolic changes occurring with aging and age-related disorders. Furthermore, metabolomics can facilitate a comprehensive understanding of the interplay between genetics, environment, and lifestyle factors in shaping the aging process [1].
Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that slowly destroys memory, thinking skills, and ultimately the ability to accomplish even the simplest daily tasks. It is the most common neurodegenerative disorder in the elderly population, affecting about 5% to 7% of the population over 60 years of age [2]. In clinical practice, brain MRI coupled with abnormal levels of the cerebrospinal fluid core AD biomarkers, total tau (t-tau), phosphorylated tau (p-tau) proteins and amyloid beta 1-42 peptide(αβ42), enable the identification of patients affected by AD. Nevertheless, the molecular pathways involved in AD onset and progression are not fully understood. Metabolomics may provide an interesting approach to identify alterations in multiple biochemical processes associated with AD [3].
We have investigated via NMR-based metabolomics the underling biochemistry of AD at two different levels: 1) studying the effects of soluble oligomers of Abeta (ADDLs) and glutamate addition (below toxicity levels) on neuroblastoma (SH-SY5Y human derived cell line) cell cultures as compared to untreated cultures to investigate the effects of Alzheimer's molecular stressors in concentrations characteristic of the first stage of this disease. 2) analyzing blood serum samples of patients that covers the entire spectrum of cognitive impairment, from mild cognitive impairment (MCI) to pre-dementia (MCI-AD) and to overt AD dementia with the aim of proving novel diagnostic and prognostic tools. Using a LASSO regression approach, we found the best combination of metabolites and lipoproteins to discriminate AD and MCI patients. Moreover, we built a model able to discriminate MCI-AD patients at fast and slow progression rate to dementia.

References

[1] D. J. Panyard, B. Yu, M. P. Snyder, *Science Advances* **2022**, *8*, eadd6155.
[2] S. F. Foley, K. E. Tansey, X. Caseras, T. Lancaster, T. Bracht, G. Parker, J. Hall, J. Williams, D. E. J. Linden, *Biological Psychiatry* **2017**, *81*, 154–161.
[3] A. Vignoli, L. Tenori, *Front Mol Biosci* **2023**, *10*, 1308500

[P-67]

EXPLORING THE URINARY METABOLOMIC FINGERPRINT OF HUMAN CYTOMEGALOVIRUS: A ^{1}H-NMR STUDY ON CONGENITALLY INFECTED NEWBORNS

M. Zoccarato[b], A. Spadavecchia[a], G. Tedone[a], M. Biolatti[c], V. Dell'Oste[c], A. Leone[a], A. Cossard[b], M. Sozzi[d], E. Bertino[a], R. Gobetto[b], A. Coscia[a], A. Gallo[b]

[a]Neonatal Unit, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy.
[b]Department of Chemistry, University of Turin, Turin, Italy.
[c]Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy.
[d]Department of Applied Science and Technology, Polytechnic of Turin, Turin, Italy try
E-mail: marta.zoccarato@unito.it

Keywords: Solution NMR, 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 congenital HCMV infection (cHCMV) 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 cHCMV infection has still to be completely characterized. In particular, little is known about the metabolic response triggered by HCMV in congenitally infected newborns. As such, urinary metabolic profiling by ^{1}H-nuclear magnetic resonance (NMR) might represent a promising tool to be exploited in the context of cHCMV. This study aims to investigate the impact of HCMV infection on the urine metabolome in a population of newborns by ^{1}H-NMR spectroscopy combined with multivariate statistical analysis. Thirty-five newborns diagnosed with cHCMV and fifteen uninfected controls were recruited. The ^{1}H-NMR spectra of patients and controls allowed the identification of an overall amount of 55 metabolites. Principal Component Analysis (PCA) and clustering are able to correctly classify newborns into the infected and control group. Partial least squares-discriminant analysis (PLS-DA) revealed that newborns with cHCMV resulted to have increased betaine, citrate, succinate, acetate, urea, galactose, glycolate, and formiate levels in the urine. On the other hand, healthy controls showed increased 1-methylnicotinamide, myoinositol, ethanolamine, glycine, taurine, fumarate, creatinin, and creatinin-phosphate levels. Specifically, succinate emerged as the discriminating metabolite in cHCMV newborns, whereas glycine and taurine were characteristic of healthy controls. These results showed a clear difference in terms of metabolomic fingerprint between newborns with cHCMV infection and healthy controls. Thus, metabolomics can be considered a new promising diagnostic and prognostic tool in the clinical management of cHCMV patients.

[P-68]

CHARACTERIZATION OF DUX4 – MATR3 COMPLEX TO DESIGN PEPTIDOMIMETICS FOR FSHD THERAPY

C. Zucchelli[a], A. Berardi[a,b], G. Quilici[a,c], V. Runfola[d], M. Pannese[d], P. Ghezzi[d], D. Gabellini[d], G. Musco[a]

[a]Biomolecular NMR Unit, IRCCS Ospedale San Raffaele, Via Olgettina 60, 20132 Milano
[b]current address: IFOM ETS - The AIRC Institute of Molecular Oncology, Via Adamello 16, 20139 Milano
[c]current address: Università di Parma, Centro Interdipartimentale Misure, Parco Area delle Scienze 23/A, 43124, Parma
[d] Gene Expression Regulation Unit, IRCCS Ospedale San Raffaele, Via Olgettina 60, 20132 Milano
E-mail: zucchelli.chiara@hsr.it

Keywords: solution NMR, small molecules, biomolecules.

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common genetic neuromuscolar diseases, with no cure despite several clinical trials. It is caused by inappropriate reactivation of DUX4 gene due to copy number and/or epigenetic alterations. DUX4 is an embryonic-restricted transcription factor whose reactivation in skeletal muscle leads to apoptosis and impairs muscle differentiation. Our collaborator Dr. Gabellini discovered that Matrin 3 (MATR3) protein binds to the two DUX4 DNA-binding domains (DUX4dbd) and blocks DUX4 activity, rescuing cell viability and myogenic differentiation of FSHD muscle cells [1]. Moreover, a N-terminal 46-residue-long MATR3 fragment (MATR3_46) has the same inhibitory acitivity. Thus, MATR3, and in particular MATR3_46, represent a novel therapeutic opportunity in FSHD. Using NMR spectroscopy and BioLayer Interferometry (BLI) we characterized MATR3_46 - DUX4dbd interaction. Our ultimate goal is to design MATR3_46-based peptidomimetics (mPeps) to be tested for DUX4dbd binding in vitro and for DUX4 inhibition in FSHD cellular models. We expressed in E. coli and purified recombinant istopically labelled human MATR3_46 and DUX4dbd. Chemical shift perturbation analysis revealed the binding surfaces on both proteins. We generated a structural model of the complex, consistent with the NMR data, using AlphaFold2 Multimer (AF). NMR binding analysis and BLI affinity measurements of MATR3_46 alanine mutants allowed for the identification of key residues critical for DUX4dbd binding, confirming some interactions predicted by the AF complex model. Based on these findings we designed an initial set of mPeps, shortening MATR3_46 sequence to determine the minimal region necessary and sufficient for DUX4dbd binding. We identified a 20-residue-long mPep (MATR3_20) recapitulating MATR3_46 binding pocket on DUX4dbd and with an affinity that is one order of magnitude better than the one of MATR3_46. NMR-based secondary structure prediction suggests that this enhanced affinity may be due to an increased helical propensity of the peptide, which might facilitate DUX4dbd binding. The AF complex model indeed predicts that MATR3_46 adopts a helical conformation upon binding.
In conclusion, the structural investigation of MATR3_46 – DUX4dbd interaction has guided our mPeps design strategy, leading to the identification of a promising mPep. We are currently testing this and other mPeps for their ability to inhibit DUX4 in FSHD cellular models. Additionally, to further enhance affinity, we are generating MATR3_20 sequence variants, harbouring also chemical modifications, to enable new contacts with DUX4dbd and stabilize the mPep structure in the bound conformation. Our project represents the proof of concept for a drug-like approach to block DUX4 activity for FSHD treatment.

References

[1] V. Runfola et al. *Cell Rep.* 2023 Sep 26;42(9):113120.

[P-69]

ADVANCEMENTS IN qNMR AND COLLABORATIVE ANALYTICAL SYSTEMS: APPLICATIONS IN FOOD CHEMISTRY AND THE INAUGURAL MAGNETHON CHALLENGE

B. Musio,[a] A. Rizzuti,[a] R. Ragone,[a] M. Trisolini,[a] S. Todisco,[a] M. Triggiani,[a,b] M. Latronico,[a,b] P. Mastrorilli,[a,b] V. Gallo[a,b]

[a] Politecnico di Bari, DICATECh, via Orabona 4 – CAMPUS, I-70125, Bari, Italy
[b] Innovative Solutions S.r.l., Zona H 150/B, I-70015, Noci (BA), Italy
E-mail: vito.gallo@poliba.it

Keywords: Solution NMR, low field NMR, small molecules, metabolomics, food.

NMR spectroscopy has gained increasing importance in analytical chemistry, offering significant advancements in the quantitative analysis of complex mixtures and the identification of product characteristics. The development of quantitative NMR (qNMR) and non-targeted approaches has enabled precise quantification and purity assessment of molecules, even in the absence of certified reference materials. This capability extends to food chemistry, where NMR is utilized for determining attributes such as origin and authenticity.
The fundamental principle that the ratio of signals from analyte and reference molecules depends solely on their mole ratio, independent of the spectrometer hardware, underpins the reliability and reproducibility of NMR analyses. This characteristic facilitates the creation of community-built analytical systems, which can collaboratively identify sample features and quantify multiple analytes. This presentation will showcase pioneering examples of such systems,[1-3] with a focus on the identification of wheat and pasta samples, as well as the quantification of betaine. The benefits and constraints of collaborative, non-targeted NMR analyses across different magnetic fields will be critically examined. Furthermore, the establishment of effective systems for the collection and management of reference materials, crucial for ensuring the metrological traceability and consistency of NMR data across diverse applications, will be discussed.
Additionally, the inaugural experimental NMR Challenge, named Magnethon, will be presented. Magnethon is set to take place in November 2025 in Bari, Italy. This international event offers a unique platform for exploring cutting-edge methods in teaching, learning, and communication, bringing together researchers, students, and professionals from around the world. Magnethon caters to both novices and experienced practitioners, providing practical sessions, expert guidance, and opportunities to connect with the global NMR community. Participants will deepen their understanding of NMR spectroscopy, refine their technical skills, and contribute to ongoing advancements in the field. The competition encourages innovative approaches, such as cooperative, experiential, and flipped learning, while fostering essential soft skills in an international and multicultural context. Scientifically, Magnethon promotes collaborative analysis in the agri-food sector using NMR, with a focus on a notable Apulian product of broad interest. Teams will compete both in-person and remotely, making it an inclusive event that celebrates scientific exploration and cultural exchange. Stay updated at www.magnethon.com and be part of this exciting journey in Puglia!

References

[1] V. Gallo, P. Mastrorilli, M. Latronico, M. Triggiani, et al., *Analytical Chemistry* 2015, **87**, 6709–6717.
[2] R. Ragone, S. Todisco, M. Triggiani, S. Pontrelli, M. Latronico, P. Mastrorilli, N. Intini, B. Musio, V. Gallo, *Food Chemistry* 2020, **332**, 127339.
[3] V. Gallo, R. Ragone, B. Musio, S. Todisco, A. Rizzuti, P. Mastrorilli, M. Triggiani, M. Latronico, et al., *Food Analytical Methods* 2020, **13**, 530–541.