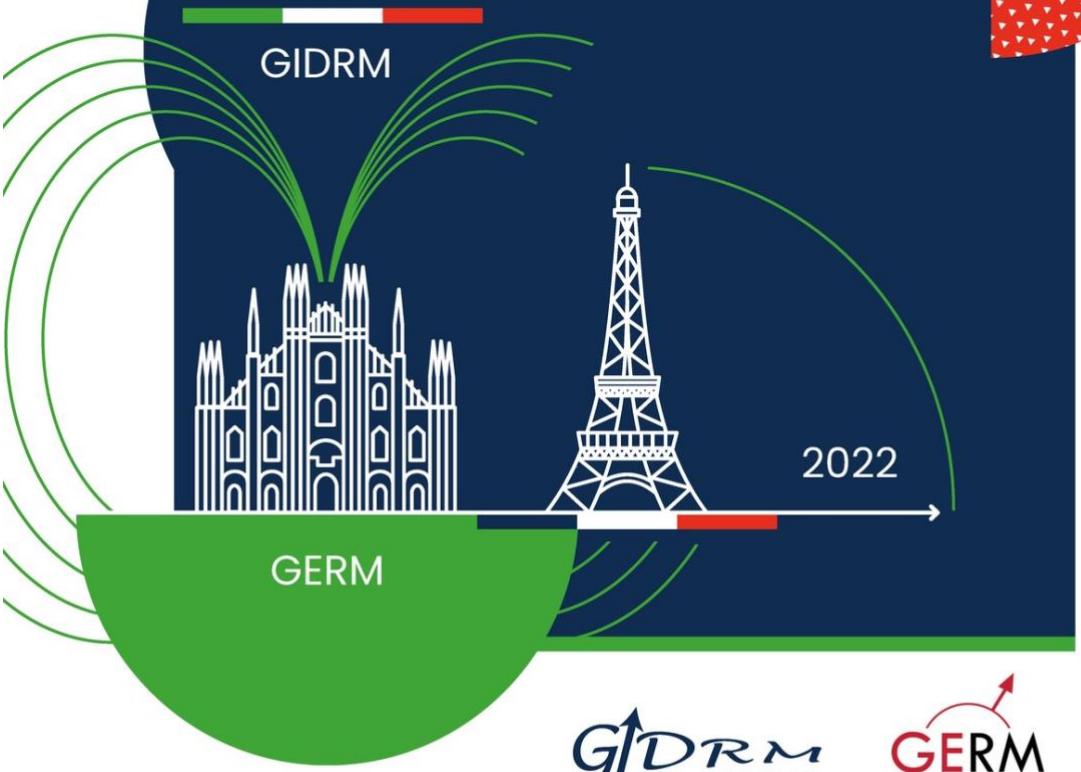
Italian-French International Conference on Magnetic Resonance

27-30 September 2022
University of Milano - Bicocca
Milan, Italy



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Italian-French International Conference on Magnetic Resonance

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Satellite Event : NMR in Industrial Applications

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General Information

Venue:

UNIVERSITY OF MILANO BICOCCA
DEPARTMENT OF BIOTECHNOLOGY AND BIOSCIENCES, BUILDING U3
P.ZZA DELLA SCIENZA 2
20126 - MILANO

Information about Posters

Poster session 1
Tuesday 27th, 16:30-17:30, ODD abstract numbers

Poster session 2
Wednesday 28th, 10:45-11:40, EVEN abstract numbers

Poster session 3
Wednesday 28th, 16:55-17:50, ODD abstract numbers

Poster session 4
Thursday 29th, 10:45-11:40, EVEN abstract numbers

Posters preferred size: A1 *(594 x 841 mm)*, maximum size: 700 mm width x 1000 mm height.

Under the Auspices of

INTERNATIONAL UNION OF
PURE AND APPLIED CHEMISTRY

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SCIENZA DEI MATERIALI

The GIDRM and GERM associations gratefully acknowledge their partners for financial support to the conference.

Réseau RMN structurale dans le Bassin Parisien
Fr2950 CNRS Fédération RMN Solide Hauts Champs
animées par Franck Fayon & Christian Bonhomme email : rmngbp@cnrs-orleans.fr

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JOINT ITALIAN-FRENCH MEETING ON MAGNETIC RESONANCE

MILAN, 27-30 SEPTEMBER 2022

SCIENTIFIC PROGRAM

Tuesday September 27st

9:00-13:00	**Registration**
9:30-11:00	**Jeol Satellite Meeting**
11:30-13:00	**Bruker Satellite Meeting**
13:00-14:00	**Lunch**

14:00-14:30	**Opening**

	Plenary session. **Chair: P. Sozzani**
14:30-15:15	**D. Bryce (University of Ottawa)** Sigma Hole Interactions Studied by Multinuclear Solid State Magnetic Resonance and Nuclear Quadrupole Resonance
15:15-16:00	**A. Webb (University of Leiden)** Low Field MRI: Physics, Engineering and Politics
16:00-16:20	**Sponsorship Lecture (Stelar): Ph. R. Bodart (University of Burgundy)** Polygalacturonic Gels Probed by Fast Field Cycling NMR
16:20-16:30	**IUPAC Presentation: S. Borsacchi**

16:30-17:30	**Coffee break + Poster session 1 (ODD abstract numbers)**

	Parallel session A **Chair: D. Bryce**	**Parallel session B** **Chair: A. Bifone**
17:30-17:50	**F. Pourpoint** - Probing Oxygen Exchange in Metal-Organic Frameworks and their Water Stability Using ^{17}O NMR	**D. Lalli** - High- and Low Resolution NMR Characterization of GdAAZTA Derivatives Functionalized with Amino Acids
17:50-18:10	**R. Chèvre** - NMR Spectroscopy, a Gateway for Kinetics and Structure Determination of Calcium Carbonate Hemihydrate	**E. Gianolio** - Design of MRI Contrast Agents: Matching enhanced relaxivity and stability in new Gd-HPDO3A analogues
18:10-18:30	**E. Carignani** - NMR for the Study of Dynamics in Lead Halide Perovskites: Applications to Mixed-Cations and 2D Phases	**F. Guerroudj** - NMR methods and devices for the characterization of flows and transfers in milli-channels
18:30-18:50	**F. Bravetti** - Solid-State NMR-Driven Crystal Structure Prediction of Organic Compounds with Ambiguous Proton Position	**R. Nasser Din** - Lanthanide-Containing Polyoxometalates as Enhanced MRI Contrast Agents at High Magnetic Fields
18:50-19:10	**A. Scarperi** - Structure and Dynamics of Crystalline Carbimazole: Combination Of Solid State Nmr, Quasielastic Neutron Scattering, Molecular Dynamics And DFT	

Wednesday September 28nd

	Plenary session Chair: A. Barnes
9:00-9:45	**M. Leskes (Weizmann Institute of Science)** PARAMAGNETIC METAL IONS DNP FOR BULK AND SURFACE SENSITIVITY IN INORGANIC SOLIDS
9:45-10:15	**P. Turano (University of Florence)** NMR OF BIOMOLECULES: FROM VERY LARGE TO VERY SMALL (AND VICE VERSA)
10:15-10:45	**A. Bifone (University of Torino)** TOWARDS HYPERPOLARIZATION WITH NITROGEN-VACANCY CENTERS IN DIAMOND
10:45-11:40	**Coffee break + Poster session 2 (EVEN abstract numbers)**

	Parallel session A Chair: P. Giraudeau	Parallel session B Chair: M. Leskes
11:40-12:00	**A. Sobolev** - NMR METABOLOMICS OF BRASSICA VEGETABLES: PRACTICAL IMPLEMENTATIONS IN AGRO-FOOD SUSTAINABLE SYSTEMS	**M. Lelli** - STRATEGIES FOR HIGH-TEMPERATURE AND FAST-MAS DYNAMIC NUCLEAR POLARIZATION
12:00-12:20	**E. Dufourc** - DYNAMIC SORTING OF MOBILE AND RIGID MOLECULES IN BIOMEMBRANES BY MAS ^{13}C-NMR	**S. Mamone** - PULSED PHIP-SAH METHODS TO PRODUCE HYPERPOLARIZED SUBSTRATES IN CLEAN WATER SOLUTIONS FOR BIOMEDICAL APPLICATIONS
12:20-12:40	**G. Petrella** - METABOLISM EVOLUTION OF PROSTATE CANCER CELLS DURING THE DEVELOPMENT OF CHEMORESISTANCE	**T. Georges** - INVESTIGATIONS OF Ca^{2+} AQUEOUS COMPLEXES THROUGH ^{43}Ca MAS-DNP NMR OF VITRIFIED SOLUTIONS
12:40-13:20	**GIDRM Under 35 award: V. Ghini and A. Vignoli** - NMR-BASED METABOLOMICS: A JOURNEY IN ITS APPLICATIONS IN CANCER RESEARCH, FROM BIOFLUIDS TO CELLS	**A. Frison** - AN INNOVATIVE SOLVENT-FREE POLYMER SAMPLE PREPARATION METHOD FOR DNP SSNMR **C. Praud** - DEVELOPMENT OF ULTRAFAST 2D NMR FOR DNP-HYPERPOLARISED METABOLIC MIXTURES
13:20-14:50	**Lunch**	

	Plenary session Chair: J. Martins
14:50-15:35	**A. Barnes (ETH Zurich, Switzerland)** ENABLING IN-CELL NMR WITH PULSED DNP, ELECTRON DECOUPLING, MAS SPHERES AND COMPACT 30 TESLA MAGNETS
15:35-16:05	**P. Giraudeau (University of Nantes, France)** DISSOLUTION DYNAMIC NUCLEAR POLARIZATION OPENS NEW PERSPECTIVES FOR METABOLOMICS
16:05-16:35	**H. Ratiney (CNRS Lyon, France)** IN VIVO MR SPECTROSCOPY QUANTIFICATION: LOCKS AND PROSPECTS
16:35-16:55	**Sponsorship Lecture (Jeol): M. Perez** DEVELOPING A NMR TOOL FOR ALL FROM SCRATCH
16:55-17:50	**Coffee break + Poster session 3 (ODD abstract numbers)**
from 17:50	**Social Event**

Thursday September 29rd

	Plenary session Chair: P. Turano
9:00-9:45	**M. Pons (University of Barcelona)** INTEGRATING ORDER AND DISORDER AN SRC CELL SIGNALLING: THE NMR APPROACH
9:45-10:15	**R. Fattorusso (University of Campania)** THE ROLE OF PROTEIN FOLDING MECHANISMS IN AMYLOID FIBRIL FORMATION
10:15-10:45	**F. Ochsenbein (CEA-Saclay, France)** STRUCTURE-FUNCTION STUDIES AND INHIBITOR DESIGN OF HISTONE CHAPERONES

10:45-11:40	**Coffee break + Poster session 4 (EVEN abstract numbers)**

	Parallel session A Chair: F. Ochsenbein	Parallel session B Chair: M. Pons
11:40-12:00	**G. Pintacuda** - FAST BIOMOLECULAR NMR WITH FAST MAS (WITHOUT AND WITH DNP)	**L. Fusaro** - INVESTIGATION OF THE EXTRAORDINARY SELF-ASSEMBLY OF A SIMPLE ORGANIC SALT BY MULTINUCLEAR NMR IN LIQUID-STATE
12:00-12:20	**A. Gallo** - STRUCTURAL BASIS FOR THE INTERACTION BETWEEN A PEPTIDYL CARRIER PROTEIN AND CONDENSATION DOMAIN IN THE ENACYLOXIN HYBRID PKS-NRPS	**I. Villa** - STUDY OF MAGNETIC PROPERTIES AND SPIN DYNAMICS IN MOLECULAR MAGNETS WITH INTEGER SPIN VALUES
12:20-12:40	**V. Bernard** - MICRORNA SPONGING BY HUR	**R.A. Salvino** - NMR-BASED METHODS FOR PHARMACEUTICAL INDUSTRY: AN APPLICATION OF THE ANALYTICAL PROCEDURES LIFECYCLE CONCEPT
12:40-13:00	**F. Munari** - NMR OF PROTEIN-PROTEIN INTERACTIONS OF TAU, A KEY PLAYER OF ALZHEIMER'S DISEASE	**S. Denis-Quanquin** - CAPTURING THE DYNAMIC ASSOCIATION BETWEEN A TRIS-DIPICOLINATE LANTHANIDE COMPLEX AND A DECAPEPTIDE: A COMBINED PARAMAGNETIC NMR AND MOLECULAR DYNAMICS EXPLORATION

13:00-14:20	**Lunch**

	Plenary session Chair: M. Geppi and C. Airoldi
14:20-14:50	**Winner of the GIDRM/GIRM Gold Medal 2022: P. Sozzani (University of Milan)** THE DYNAMICAL WORLD OF SOLIDS
	Chair: H. Ratiney
14:50-15:35	**D. Topgaard (University of Lund)** MODEL FREE APPROACH TO THE INTERPRETATION OF RESTRICTED AND ANISOTROPIC SELF DIFFUSION IN MAGNETIC RESONANCE OF BIOLOGICAL TISSUES
15:35-16:20	**A. Gronenborn (University of Pittsburgh, USA)** THE AWESOME POWER OF FLUORINE NMR - FROM DRUGS TO CELLS
16:20-16:40	**Sponsorship Lecture (Bruker): J. Coutant** SOFTWARE NEWS - TOPSPIN, SMARTDRIVENMR AND BRUKER CHEMIST SUITE
16:40-16:50	**Sponsorship Lecture (Magritek): D. Bouillaud** LATEST UPDATES ON BENCHTOP NMR: HOW IT CAN HELP YOUR DAILY WORK ?

16:50-17:15	**Coffee break**
17:15-19:15	**Assemblies of GIDRM and GERM**
20:30-24:00	**Social Dinner**

Friday September 30th

	Plenary session Chair: D. Topgaard
9:00-9:45	**J. Martins (University of Gent, Belgium)** AN NMR VIEW ON ANTIMICROBIAL CYCLIC LIPOPEPTIDES FROM PSEUDOMONAS
9:45-10:05	**Sponsorship Lecture (Extrabyte) – S. Sykora** DEVELOPMENTS IN THE EVALUATION OF DOSY DATA
10:05-10:45	**Best poster awards**

10:45-11:15	**Coffee break**

	Parallel session A Chair: R. Fattorusso	Parallel session B Chair: A. Gronenborn
11:15-11:35	**M.E. Di Pietro** - PLAYING AROUND WITH HYDROPHOBIC (DEEP) EUTECTIC SOLVENTS: WHAT WE CAN LEARN FROM NMR	**X. Falourd** - REVISITING 1H->13C POLARIZATION TRANSFER KINETIC TO INVESTIGATE INTERACTIONS IN POLYSACCHARIDE ASSEMBLIES
11:35-11:55	**A. Ducroix** - DYNAMICS OF WATER INSIDE BOEHMITE SUSPENSIONS PROBED BY FAST-FIELD CYCLING NMR	**T. Cartlidge** - PREDICTING SPIN RELAXATION IN POROUS MEDIA OF ARBITRARY COMPLEXITY
11:55-12:15	**T. Poumeyrol** - ^{1}H-^{1}H RESIDUAL DIPOLAR COUPLING MEASUREMENT FROM MULTIPLE-QUANTUM BUILD-UP EXPERIMENTS AT LOW MAGNETIC FIELD IN RUBBER INDUSTRY	**C. Lhoste** - ONLINE MONITORING OF AN ORGANOCATALYTIC REACTION BY BROADBAND ULTRAFAST 2D NMR IN FLOW CONDITIONS
12:15-12:35	**K. Bagheri** - STATE OF CHARGE OF THE LI-ION BATTERY ELECTRODES FROM THE DISTORTION OF THE 1H NMR SPECTRUM OF THE LIQUID ELECTROLYTE	**A. Briot-Dietsch** - UNTARGETED NMR ANALYSES OF PFAS IN POLLUTED ENVIRONMENTAL IN POLLUTED MEDIA

12:35-12:50	**Closing**

12:50-14:00	**Lunch**

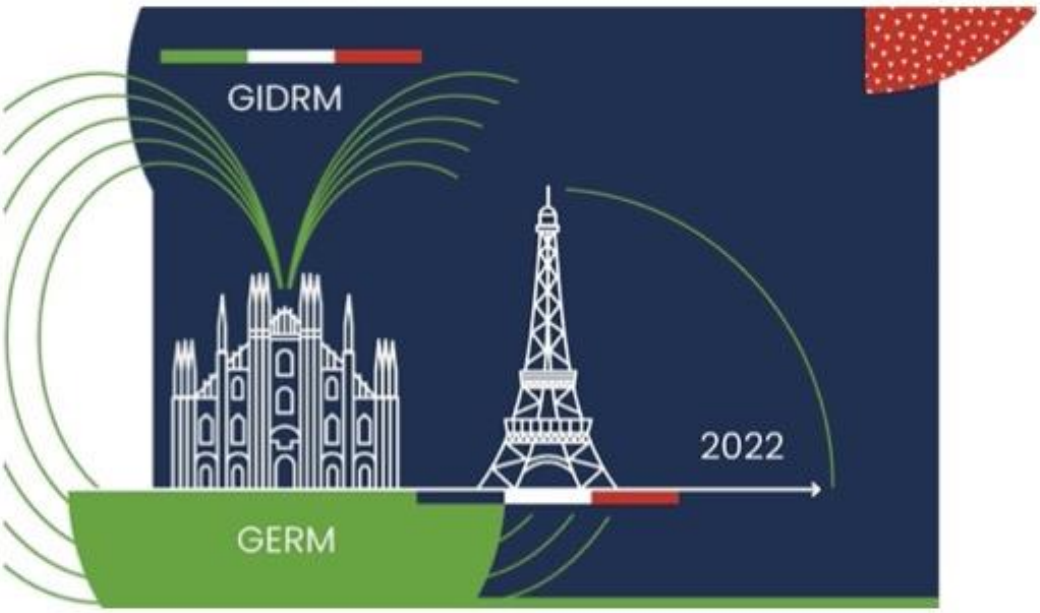
	Satellite Event : NMR in industrial applications Chair: C. Marchioro
14:00-14:25	**F. Reniero** - NMR ANALYSIS IN THE FRAME OF CUSTOMS CONTROLS
14:25-14:50	**V. Gallo** - NMR-BASED COMMUNITY-BUILT ANALYTICAL SYSTEMS IN FOOD CONTROL AND QUANTITATIVE ANALYSIS
14:50-15:15	**F. Berti** - NMR CHARACTERIZATION OF POLYSACCHARIDE-BASED VACCINES
15:15-15:40	**E. Moro** - CONFORMATIONAL ANALYSIS IN DRUG DESIGN: A SYNERGIC APPROACH OF NMR & COMPUTATIONAL CHEMISTRY
15:40-16:05	**L. Duciel** - RESCUE 3: VERSATILE DECISION-MAKING TOOL FOR NMR SPECTRAL ASSIGNMENTS OF PROTEINS
16:05-16:30	**D. Besghini** - LF-TD-NMR FOR THE DEVELOPMENT OF PRINTING BLANKETS

JEOL NMR Technical Symposium

27th September from 9,30 to 11,00

Italian-French International Conference on Magnetic Resonance

University of Milano Bicocca—Milan



JEOL ECZL spectrometer: see NMR in a new light
Dr. Adolfo Botana
JEOL UK Ltd

NMR-based metabolomics: a tool for present and future
Dr. Fabio Sciubba
Sapienza University of Rome, NMR-Based Metabolomics Laboratory

NMR-Based metabolomics: some examples from the Metabolome-MetaboHUB-Bordeaux platform
Dr. Catherine Deborde
INRAE Bordeaux - (Fruit Biology and Pathology), Metabolism Team

What's new on JEOL: hardware and software news
Dr. Manuel Perez
JEOL UK Ltd.

27TH SEPTEMBER

11:30	Introduction and welcome	Angelo Ripamonti
11:35	Bruker news	Eric Leonardis
11:50	News on service and aftermarket	Anna Minoja
12:05	Probe news	Olivier Assemat
12:20	Benchtop NMR	Francesca Benevelli
12:35	Software news	Jérôme Coutant
12:50	Q & A	Angelo Ripamonti

TUESDAY SEPTEMBER 27TH

9:00-13:00	**Registration**
9:30-11:00	**Jeol Satellite Meeting**
11:30-13:00	**Bruker Satellite Meeting**
13:00-14:00	**Lunch**

14:00-14:30	**Opening**

	Plenary session. **Chair: P. Sozzani**
14:30-15:15	**D. Bryce (University of Ottawa)** SIGMA HOLE INTERACTIONS STUDIED BY MULTINUCLEAR SOLID STATE MAGNETIC RESONANCE AND NUCLEAR QUADRUPOLE RESONANCE
15:15-16:00	**A. Webb (University of Leiden)** LOW FIELD MRI: PHYSICS, ENGINEERING AND POLITICS
16:00-16:20	**Sponsorship Lecture (Stelar): Ph. R. Bodart (University of Burgundy)** POLYGALACTURONIC GELS PROBED BY FAST FIELD CYCLING NMR
16:20-16:30	**IUPAC Presentation: S. Borsacchi**

16:30-17:30	**Coffee break + Poster session 1 (ODD abstract numbers)**

	Parallel session A **Chair: D. Bryce**	**Parallel session B** **Chair: A. Bifone**
17:30-17:50	**F. Pourpoint -** PROBING OXYGEN EXCHANGE IN METAL-ORGANIC FRAMEWORKS AND THEIR WATER STABILITY USING ^{17}O NMR	**D. Lalli -** HIGH- AND LOW RESOLUTION NMR CHARACTERIZATION OF GDAAZTA DERIVATIVES FUNCTIONALIZED WITH AMINO ACIDS
17:50-18:10	**R. Chèvre -** NMR SPECTROSCOPY, A GATEWAY FOR KINETICS AND STRUCTURE DETERMINATION OF CALCIUM CARBONATE HEMIHYDRATE	**E. Gianolio -** DESIGN OF MRI CONTRAST AGENTS: MATCHING ENHANCED RELAXIVITY AND STABILITY IN NEW GD-HPDO3A ANALOGUES
18:10-18:30	**E. Carignani -** NMR FOR THE STUDY OF DYNAMICS IN LEAD HALIDE PEROVSKITES: APPLICATIONS TO MIXED-CATIONS AND 2D PHASES	**F. Guerroudj -** NMR METHODS AND DEVICES FOR THE CHARACTERIZATION OF FLOWS AND TRANSFERS IN MILLI-CHANNELS
18:30-18:50	**F. Bravetti -** SOLID-STATE NMR-DRIVEN CRYSTAL STRUCTURE PREDICTION OF ORGANIC COMPOUNDS WITH AMBIGUOUS PROTON POSITION	**R. Nasser Din -** LANTHANIDE-CONTAINING POLYOXOMETALATES AS ENHANCED MRI CONTRAST AGENTS AT HIGH MAGNETIC FIELDS
18:50-19:10	**A. Scarperi -** STRUCTURE AND DYNAMICS OF CRYSTALLINE CARBIMAZOLE: COMBINATION OF SOLID STATE NMR, QUASIELASTIC NEUTRON SCATTERING, MOLECULAR DYNAMICS AND DFT	

SIGMA-HOLE INTERACTIONS STUDIED BY MULTINUCLEAR SOLID-STATE MAGNETIC RESONANCE AND NUCLEAR QUADRUPOLE RESONANCE

D. L. Bryce

Department of Chemistry and Biomolecular Sciences, University of Ottawa, Canada
E-mail: dbryce@uottawa.ca

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

Non-covalent interactions are critical to molecular and supramolecular structure and function in the gas, liquid, and solid phases. In addition to the ubiquitous hydrogen bond, the halogen bond is an example of an element-based electrophilic non-covalent interaction which is atom-centred. Halogen bonds are formed via the interaction of an area of depleted electron density and elevated electrostatic potential, i.e., the σ-hole, on a halogen atom with an electron donor in the same or another molecular entity. Similar definitions may be adopted for other element-based interactions including e.g., chalcogen bonds, pnictogen bonds, tetrel bonds, etc. [1] In this talk I will survey the information available from experimentally-measured solid-state NMR observables for the characterization of these various classes of non-covalent interactions in a range of cocrystalline materials. [2,3,4,5] These observables include chemical shift tensors, quadrupolar coupling tensors, and spin-spin coupling constants. In some cases, the presence of a non-covalent interaction is clearly evidenced by spectroscopic data while in other cases strong correlations between spectroscopic observables and specific structural features may be obscured by competing structural effects. In addition to high-field solid-state NMR spectroscopy of powders, results from single-crystal NMR and from nuclear quadrupole resonance experiments will be discussed. Density functional computations of NMR parameters using cluster models or periodic boundary conditions are useful in assessing the impact of such competing effects.

References

[1]. A. C. Legon, N. R. Walter, Phys. Chem. Chem. Phys. 20 (2018) 19332. https://doi.org/10.1039/C8CP03432J
[2] Y. Xu, P. M. J. Szell, V. Kumar, D. L. Bryce, Coord. Chem. Rev. 411 (2020) 213237. https://doi.org/10.1016/j.ccr.2020.213237
[3] S. A. Southern, T. Nag, V. Kumar, M. Triglav, K. Levin, D. L. Bryce, J. Phys. Chem. C 126 (2022) 851. https://doi.org/10.1021/acs.jpcc.1c10121
[4] V. Kumar, Y. Xu, D. L. Bryce, Chem. Eur. J. 26 (2020) 3275. https://doi.org/10.1002/chem.201904795
[5] P. M. J. Szell, L. Grebert, and D. L. Bryce, Angew. Chem. Intl. Ed. 58 (2019) 13479. https://doi.org/10.1002/anie.201905788

LOW FIELD MRI: PHYSICS, ENGINEERING AND POLITICS

A.G.Webb, T.O'Reilly, B.de Vos, J.Parsa, P.Boernert, K.Koolstra, and W.Teeuwisse.

C.J.Gorter MRI Centre, Department of Radiology, Leiden University Medical Center, Leiden. The Netherlands.
E-mail: a.webb@lumc.nl

Keywords: low field NMR, MRI, instrumentation.

Commercial magnetic resonance imaging (MRI) systems cost millions of pounds to purchase, require large electromagnetically shielded spaces to house, are extremely expensive to maintain and require highly trained technicians to operate. These factors together means that their distribution is confined to centrally-located medical centres in large towns and cities. Globally over 70% of the world's population has absolutely no access to MRI, and clinical conditions which could benefit from even very simple scans cannot be treated [1]. The magnetic fields typically used are very high, which means that there are severe contraindications so that, for example, MRI cannot currently be used in the emergency room. From the considerations above it is clear that if low-field MRI could be made more portable, accessible and sustainable then it would open up new opportunities in both developed and developing countries [2-4]. In order to achieve portability, we design systems that use thousands of very small low-cost permanent magnets, arranged in designs which have no fringe field and therefore very easy siting requirements. The low magnetic fields allow scanning of patients with implants, and the scanner could potentially be transported on an ambulance for differentiation of hemorrhagic or ischemic stroke, for example. This talk will cover aspects of magnet, gradient and RF coil design for low fields (~50 mT), as well as corrections for gradient- and B_0-distortions, and present the latest in vivo results as well as an outlook on future developments.

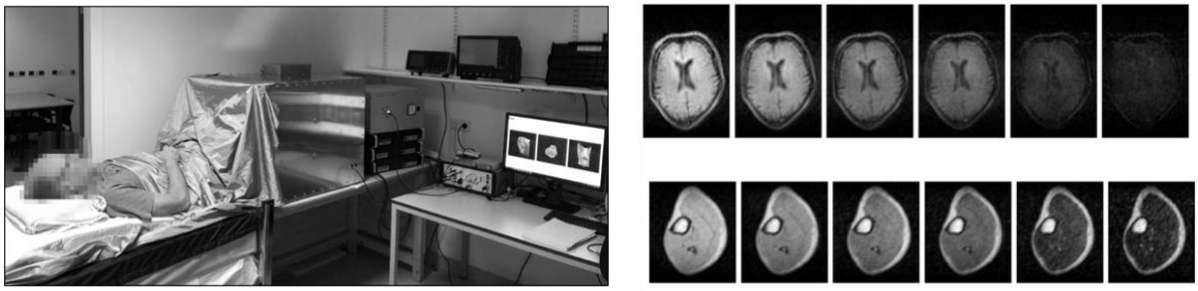


Fig. 1. Photograph of the low field portable 50 mT permanent magnet based system, with brain and knee images acquired in ~10 mins.

References

[1] U.C.Anazodo, J.J.Ng, B.Ehiogu et al, *MedRxiv*, doi: https://doi.org/10.1101/2022.05.02.22274588, (2022).
[2] T.O.Reilly, W.M.Teeuwisse, D.de Gans, K.Koolstra, A.G.Webb, *Magn.Reson.Med*. **85**, 495-505, (2021).
[3] Y.Liu, A.T.L.Leong, Y.Zhao et al. *Nature Communications*, **12**, 7238 (2021).
[4] S.C.L.Deone, P.Medeiros, A.T.Deoni et al. *Scientific Reports*, **12**, 5690, (2022).

POLYGALACTURONIC GELS PROBED BY FAST FIELD CYCLING NMR

P. R. Bodart,[‡*] P. Fouilloux, [‡] A. Lerbret, [‡] A. Rachocki[†], A. Assifaoui[‡]

[‡] Institut Agro Dijon, UMR PAM A02.102, Univ. Bourgogne Franche-Comté, France
[†] IFM PAN, Institute of Molecular Physics, Polish Academy of Sciences, Poland
E-mail: Philippe.bodart@u-bourgogne.fr

Keywords: low field NMR, food, polymers, Fast field Cycling, NMR relaxometry

Gels are essential materials of paramount importance in medicine, pharmacy and food science, to name but a few. In this work, we performed an extensive NMR analysis of the water dynamics and mesh size of heterogeneous polygalacturonic acid (polyGalA) hydrogels [1] used for encapsulation and release of active components. In gels, fast field cycling NMR [2] provides information hardly accessible by other technics. The results obtained with different dynamic models used to treat the FFC profile data are compared and put into perspective with outcomes from independent techniques (Molecular Dynamics, SANS, rheology).

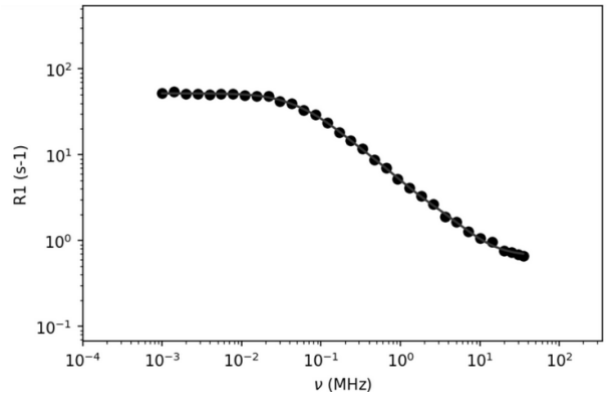


Fig. 1. 1H NMRD profile of Ca-polyGalA hydrogel

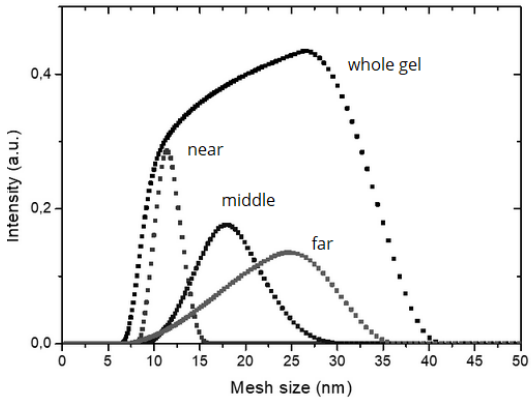


Fig. 2. Mesh size distribution in the complete and sliced heterogeneous Ca-polyGalA. hydrogel

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IUPAC Presentation

S. Borsacchi[‡]

[‡] IUPAC CHEMRAWN Committee and Italian National Commission for IUPAC, CNR
E-mail: silvia.borsacchi@cnr.it

The International Union of Pure and Applied Chemistry was established in 1919 with the aim of promoting exchanges between chemists from all over the world, also through the creation of a common and standardized language. Today, IUPAC is the world authority for the nomenclature of chemical compounds and the definition of chemical and chemical-physical quantities. Beside this, the Union is an important international, non-governmental and impartial scientific organization, committed to provide objective scientific expertise and develop the essential tools for the application and communication of chemical knowledge for the benefit of both humankind and the world, with great attention to sustainable development, young people and inclusiveness (http://www.iupac.org/ and www.iupac.cnr.it). In this presentation the main activities of IUPAC will be highlighted, with a special focus on global initiatives for building a sustainable future.

PROBING OXYGEN EXCHANGE IN METAL-ORGANIC FRAMEWORKS AND THEIR WATER STABILITY USING ^{17}O NMR

F. Venel,[‡] C. Volkringer,[‡] J. Špačková,[†] D. Laurencin[†], O. Lafon[‡], F. Pourpoint[‡]

[‡] Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181 – UCCS – Unité de Catalyse et Chimie du Solide, F-59000 Lille, France
[†]Institut Charles Gerhardt Montpellier, UMR-5253 CNRS-UM-ENSCM, 1919 route de Mende, 34095 Montpellier, France
E-mail: frederique.pourpoint@centralelille.fr

Keywords: solid state NMR, materials

Metal-Organic Frameworks (MOFs) are tunable microporous materials, which are promising for numerous applications, including the capture of gas or catalysis. These compounds are built from the three-dimensional association of metal clusters and organic ligands. Thanks to this hybrid framework, their porosity and functionalities can be tuned to optimize their properties for the desired application. Better understanding the stability of these materials in the presence of water is critical for their use in industrial processes, including the purification of flue gases or wastewater. In particular, the structure of defects caused by water is still an unsettled question.
As a local characterization endowed with atomic resolution, solid-state NMR is a promising tool to probe the structure of defects. We notably investigated using ^{17}O NMR the changes in the structure of Zr-based UiO-66 MOF (where UiO stands for *Universitetet i Oslo*), which is one of the most stable MOFs in the presence of water [1]. Different syntheses were tested for the ^{17}O isotopic enrichment, using as a source of ^{17}O isotope, either $H_2{}^{17}O$ or an ^{17}O-enriched terephthalate ligand prepared by mechanochemistry [2]. We demonstrate the interest of mechanochemistry, which allows an efficient enrichment with a significant reduction in cost.
These ^{17}O NMR experiments show distinct distributions of ^{17}O isotope between the μ_3-OH, μ_3-O^{2-} and COO^- sites of UiO-66 MOF, depending on the source of ^{17}O isotope as well as different reactivities of these sites with water. These experiments also prove the "lability" of the Zr-O bonds, even when the long-range structure is preserved. Characterizing this lability is crucial to understand the degradation of MOFs in the presence of water.

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NMR SPECTROSCOPY, A GATEWAY FOR KINETICS AND STRUCTURE DETERMINATION OF CALCIUM CARBONATE HEMIHYDRATE

R. Chèvre,[‡] S.F. Cousin,[‡] M. Juramy,[‡] F. Ziarelli,[†] S. Viel,[‡,§] C.E. Hughes,[¶] K.D.M. Harris,[¶] G. Mollica,[‡] P. Thureau.[‡]

[‡]Aix Marseille Univ, CNRS, ICR, Marseille, France
[†]Aix Marseille Univ, CNRS, Centrale Marseille, FSCM, Marseille, France
[§]Institut Universitaire de France, 75231 Paris, France
[¶]School of Chemistry, Cardiff University, Cardiff, Wales CF10 3AT, UK
E-mail: romain.chevre@univ-amu.fr

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

Calcium carbonate ($CaCO_3$) is one of the most abundant materials on Earth and therefore plays an important role in geology and biology. [1] The study of $CaCO_3$'s biomineralization processes is one of the keys to improve our understanding of phenomena like ocean acidification or rocks formation. $CaCO_3$ is also involved in the global carbon cycle.
$CaCO_3$'s crystallization can follow multiple pathways leading to different polymorphs. However, the non-classical crystallization mechanisms leading to the different forms are still ill-understood.[2,3] Recently, a new hydrated polymorph of $CaCO_3$ was discovered, the so-called hemihydrate polymorph (CCHH), which is believed to be a key metastable intermediate in the crystallization pathway of anhydrous $CaCO_3$. [4] However, the proposed structure of this new polymorph, essentially determined by PXRD, remains uncertain. In this work, we propose a strategy based on a combination of 1D and 2D solid-state NMR [5,6] experiments with DFT-D calculations [7] to investigate and propose a more accurate molecular structure for CCHH. Both DFT-calculations and Solid-State NMR experiments carried out in this study allowed to improve our knowledge of this new solid form and its crystallization pathway.

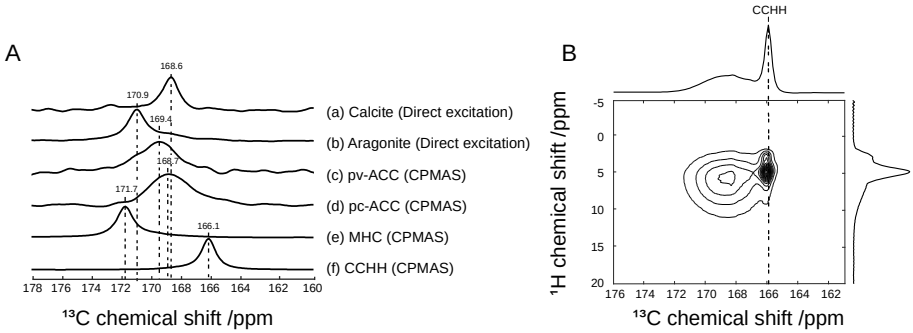

Figure 1. (A) ^{13}C solid-state NMR spectra of calcium carbonate phases: (a) aragonite; (b) calcite; (c) and (d) amorphous calcium carbonate ACC; (e) monohydrocalcite MHC; (f) CCHH. (B) ^{1}H-^{13}C FSLG HETCOR spectrum recorded with DNP conditions on a 9.4 T magnet at 100 K for a sample containing both amorphous phase and HHCC and impregnated with DNP juice.

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SOLID STATE NMR FOR THE STUDY OF DYNAMICS IN LEAD HALIDE PEROVSKITES: APPLICATIONS TO MIXED-CATIONS AND 2D PHASES

Elisa Carignani, [‡,†] Noemi Landi,[§] Silvia Borsacchi, [‡,†] Lucia Calucci, [‡,†] Marco Geppi[§,†]

[‡] Institute for the Chemistry of OrganoMetallic Compounds, National Research Council, CNR-ICCOM, via G. Moruzzi 1, 56124 Pisa, Italy
[†] Center for Instrument Sharing, University of Pisa (CISUP), 56126 Pisa, Italy
[§] Department of Chemistry and Industrial Chemistry, University of Pisa, via G. Moruzzi 13, 56124 Pisa, Italy
E-mail: elisa.carignani@cnr.it

Keywords: solid state NMR, materials

Lead Halide Perovskites (LHPs), of general formula $APbX_3$ (with A typically is methylammonium MA, formamidinium FA or Cs^+, and X = Cl^-, Br^-, I^-), have emerged as very promising classes of materials for applications in optoelectronic devices, such as solar cells, LEDs and lasers. The impressive interest aroused by LHPs is due to their remarkable optoelectronic properties with easy preparation, abundant constituent elements and wide compositional tunability [1]. Among the approaches devised to improve performances and stability of LHPs, the mixing of different cations and/or different halides gave very interesting results: mixed ion perovskites showed higher Power Conversion Efficiency (PCE) in solar cells, and higher moisture stability with respect to pure perovskites. Another strategy to improve the moisture stability consists in the use of 2D Ruddlesden-Popper (RP) phases. These structures are prepared by adding a large organic mono ammonium cation, L^+, the 3D structure of corner-sharing octahedra ($APbX_3$) is disrupted and a structure with a bilayer of spacer cations between metal halide sheets is formed ($L_2A_{n-1}Pb_nX_{3n+1}$). 2D perovskites are more stable with respect to their 3D analogues but show lower PCE.
Solid-State Nuclear Magnetic Resonance (SSNMR) stands out as characterization technique for LHPs for its ability to study ion dynamics, compositional variations and ion incorporation, chemical interactions, and degradation mechanisms [2].

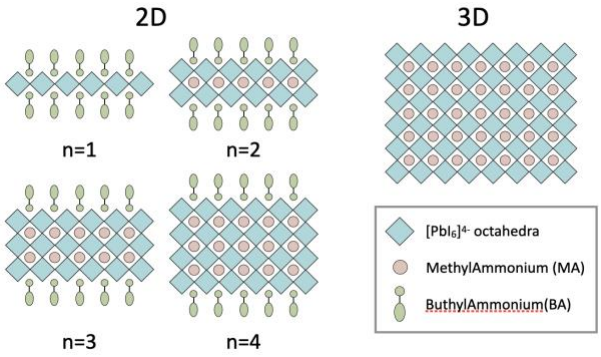

Figure 1. Schematic structure of 2D RP perovskites $BA_2MA_{n-1}Pb_nI_{3n+1}$ for n=1-4, and of the corresponding 3D perovskites $MAPbI_3$.

In this work, the potentialities of SSNMR are presented and discussed through the application to a multiple-cation lead mixed-halide perovskite $Cs_{0.05}FA_{0.81}MA_{0.14}PbI_{2.55}Br_{0.45}$, and 2D Ruddlesden-Popper (RP) phases containing ButylAmmonium as spacer ($BA_2MA_{n-1}Pb_nI_{3n+1}$ with n=1-4, Figure 1). In these two cases ^{207}Pb, ^{1}H, and ^{13}C SSNMR, both under Magic Angle Spinning and static conditions were applied. Some structural and dynamic features of these systems have been compared with those of 3D pure $MAPbI_3$ and discussed in relation to very recent literature [3].

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SOLID-STATE NMR-DRIVEN CRYSTAL STRUCTURE PREDICTION OF ORGANIC COMPOUNDS WITH AMBIGUOUS PROTON POSITION

F. Bravetti,[1] R. Russo,[2] S. Bordignon,[1] E. Alig,[3] D. Eisenbeil,[3] L. Fink,[3] M. U. Schmidt,[3] C. Nervi,[1] M. R. Chierotti[1]

[1]Università degli Studi di Torino, Department of Chemistry, via P. Giuria 7, 10125, Torino, Italy
[2]Università degli Studi di Camerino, Department of Chemistry, Via S. Agostino 1, 62032, Camerino, Italy
[3]Goethe University, Institute of Inorganic and Analytical Chemistry, Max-von-Laue-Str. 7, 60438 Frankfurt am Main, Germany
E-mail: federica.bravetti@unito.it

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

Crystal Structure Prediction (CSP) methods have recently turned out to be a powerful tool for the *ab-initio* structure solution of organic molecular crystals, with only the molecular structure given as input information.[1] Nonetheless, the content of the unit cell is not always definable *a priori*, in particular when the proton positions are ambiguous, *i.e.* when different tautomeric or zwitterionic forms, or salt and cocrystals in new multi-component systems, are possible. In these cases, several CSP calculations should be performed, to explore all different possibilities, considerably increasing the computational costs. Thus, CSP finds its perfect partner in solid-state NMR (SSNMR), which provides worthwhile information exploitable as constraints for the CSP. 1D (^{1}H MAS, ^{13}C and ^{15}N CPMAS) and 2D (^{1}H DQ MAS and ^{13}C-^{1}H HETCOR) SSNMR can be used to assess the content of the unit cell, (proton positions and number of independent molecules in the unit cell, Z'),[2] dramatically reducing the search space and increasing the reliability of the predicted structures. Information obtained by 1D and 2D experiments can also provide the local molecular arrangement in the unit cell. Furthermore, it is well known that SSNMR can also be used in the final step of the CSP for the selection of the correct crystal structure, comparing ^{13}C and ^{1}H experimental and computed chemical shifts.[3]
We successfully applied this combined method on two kinds of systems: mebendazole,[4] which crystallizes in three phases characterized by different tautomeric forms, and three structural isomers of pyridine dicarboxylic acids (quinolinic, dinicotinic and dipicolinic acid), that may crystallize as zwitterionic or non-zwitterionic forms.

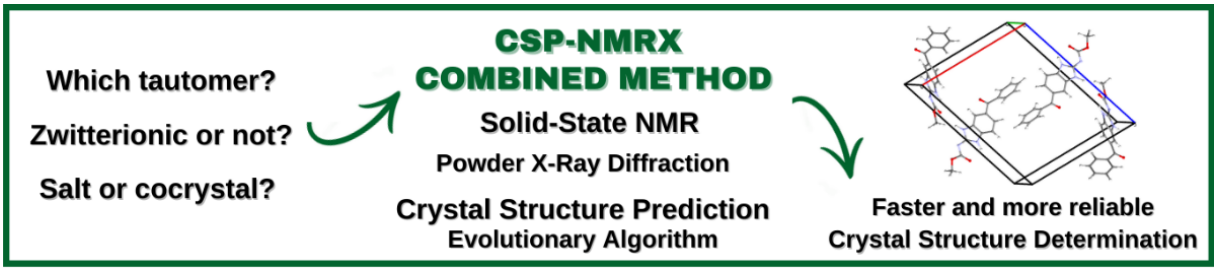

Fig. 1. Schematic description of the combined CSP-SSNMR method.

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STRUCTURE AND DYNAMICS OF CRYSTALLINE CARBIMAZOLE: COMBINATION OF SOLID STATE NMR, QUASIELASTIC NEUTRON SCATTERING, MOLECULAR DYNAMICS AND DFT

A. Scarperi,[‡] E. Carignani,[†,§] G. Barcaro,[‖] A. Pajzderska,[¶] F. Martini,[‡,§] M. Geppi[‡,§]

[‡] Department of Chemistry and Industrial Chemistry, University of Pisa, via G. Moruzzi 13, 56124 Pisa, Italy;
[†] Institute for the Chemistry of OrganoMetallic Compounds, Italian National Council for Research, CNR/ICCOM, via G. Moruzzi 1, 56124 Pisa, Italy;
[§] Center for Instrument Sharing, University of Pisa (CISUP), 56126 Pisa, Italy;
[‖] Institute for Chemical and Physical Processes, Italian National Council for Research, CNR/IPCF, via G. Moruzzi 1, 56124 Pisa, Italy;
[¶] Department of Radiospectroscopy, Faculty of Physics, Adam Mickiewicz University, Uniwersytetu Poznanskiego 2, 61-614 Poznan, Poland.

E-mail: andrea.scarperi@phd.unipi.it

Keywords: solid state NMR, small molecules

In the last two decades, the combination of Solid State NMR (SSNMR), diffractometric techniques and computational methods has been recognized as a powerful tool in the investigation of the structure of crystalline solids, and "NMR Crystallography" is seen as a rapidly maturing subject area in the crystallographic community [1,2]. On the other hand, SSNMR is a very powerful tool for the characterization of dynamic properties in solid phases on a broad time scale (form seconds to picoseconds) [3], and in combination with Quasielastic Neutron Scattering (QENS) the range can be extended to shorter times. Moreover, in the case of dynamics, computational methods add an unparalleled tool to achieve a more complex understanding.
In this work, the dynamic and structural properties of the crystalline form of carbimazole (see Fig. 1), a prodrug used in the treatment of hyperthyroidism, have been investigated in detail. The combination of DFT calculation with ^{1}H and ^{13}C 1D and 2D NMR experiments has allowed the elucidation of the drug crystal structure [4], resolving ambiguities in diffraction-derived structures previously reported. The measurement of spin-lattice relaxation times of ^{1}H and ^{13}C nuclei at variable temperatures, QENS and Molecular Dynamic simulations enabled the detailed characterization of the dynamic processes that the carbimazole molecule undergoes in the crystal lattice.

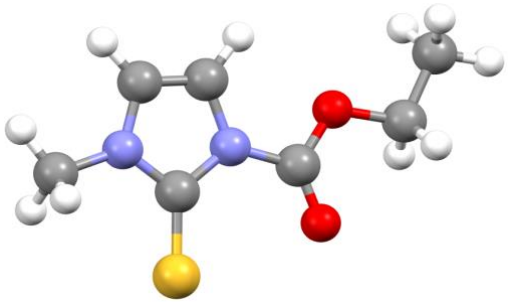

Fig. 1. Molecular structure of Carbimazole

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HIGH- AND LOW-RESOLUTION NMR CHARACTERIZATION OF GdAAZTA DERIVATIVES FUNCTIONALIZED WITH AMINO ACIDS

D. Lalli,[‡] M. Ricci, [‡] F. Carniato,[‡] L. Tei,[†] M. Botta[‡]

[‡] Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "A. Avogadro, Viale T. Michel 11, 15121 Alessandria, Italy
E-mail: daniela.lalli@uniupo.it

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

GdAAZTA is a mesocyclic complex of great interest for the design of new innovative Magnetic Resonance Imaging (MRI) probes, due to its remarkable magnetic properties, thermodynamic stability, kinetic inertness, and high chemical versatility. We developed new derivatives functionalized with four amino acids having different molecular weight and charge. Three are the main reasons for including amino acid moieties in the ligand structure: i) to study the chemical properties in aqueous solution of model compounds that mimic more complex structures based on polypeptide fragments; ii) to obtain probes with high relaxivity values at clinical fields (> 1 T) associated with a restriction of the rotational dynamics; iii) to promote non-covalent interactions with proteins in biological environments. The relaxometric properties of these chelates were analyzed by low- and high-resolution NMR spectroscopy, in order to evaluate the molecular parameters determining their performance as MRI probes. The relaxivity values of the novel chelates are higher than those of GdAAZTA over the entire range of applied magnetic fields, due to increased reorientational correlation time. Furthermore, tests in reconstituted human serum indicate the presence of weak interactions with proteins. Finally, EuAAZTA amino acid derivatives were characterized by ^{1}H NMR and time-resolved photoluminescence, in order to obtain structural information and determine the hydration state of the metal ion.

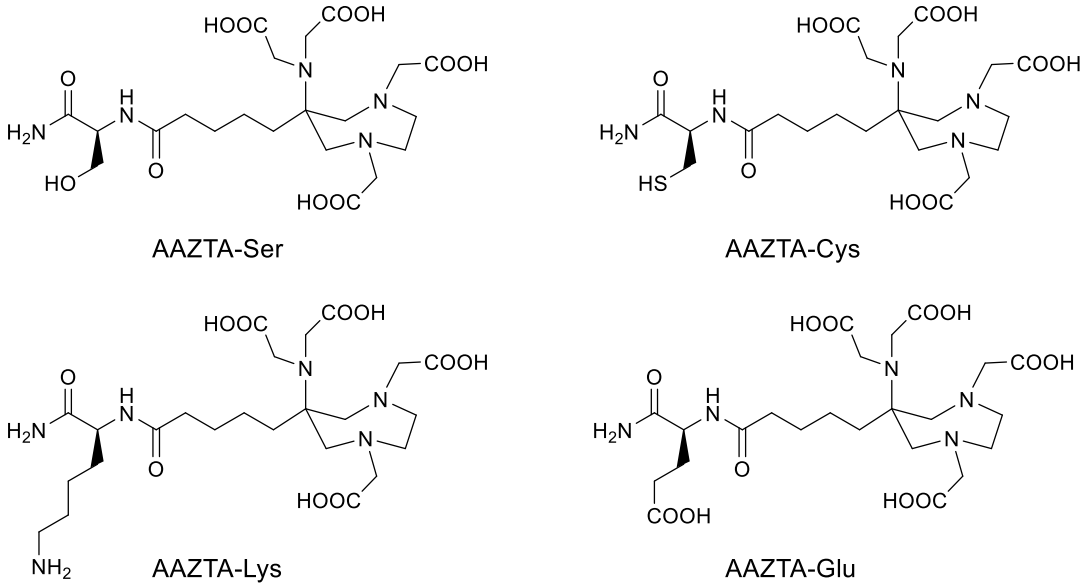

Fig. 1. Chemical structure of the AAZTA amino acidic derivatives.

TOWARDS AN IMPROVED DESIGN OF MRI CONTRAST AGENTS: MATCHING ENHANCED RELAXIVITY AND STABILITY IN NEW GD-HPDO3A ANALOGUES

E. Gianolio,[‡] C. Carrera[‡†], L. Tear,[‡] F. Hasallari[‡] and S. Aime[‡]

[‡]Dep. Molecular Biotechnologies and Health Science, University of Torino, Via Nizza 52, Torino, Italy
[†]Institute of Biostructures and Bioimaging (IBB), Italian National Research Council (CNR), Torino, Italy

Keywords: solution NMR, MRI, contrast agents, relaxometry

Gadolinium(III) complexes have been employed for more than 30 years as contrast agents in magnetic resonance imaging (MRI). In recent years there has been concern regarding the discovery of Gd deposition in the brain and other tissues of patients, after contrast-enhanced MRI scans. One of the contrast agents most commonly applied in clinical practice is Gd-HPDO3A (Prohance, Bracco), whose relaxometric properties have been extensively investigated and appears to be one of the agents with lower level of retention in the body, due to its high kinetic stability as a macrocyclic agent. In order to further improve the diagnostic accuracy or to provide comparable enhancement at a reduced administered dose, maintaining the high stability required for a safe use, our research was focused on the development new Gd(III)-complexes based on modified HPDO3A structure.
A series of HPDO3A derivatives, with small modifications to the hydroxyl arm, were investigated. A full ^{1}H and ^{17}O-NMR relaxometric analysis was conducted and demonstrated that increasing the distance of the OH group from the lanthanide centre significantly enhanced the water exchange rate, but with a subsequent reduction in kinetic stability. Alternatively, the introduction of an additional methyl group, which increased the steric bulk around the OH moiety, resulted in the formation of almost exclusively the TSAP isomer (95%) as identified by ^{1}H-NMR of the europium complex. The gadolinium analogue of this complex also exhibited a very fast water exchange rate, but with no detectable loss of kinetic stability, demonstrating a notable improvement over Gd-HPDO3A. Based on these findings two new Gd-HPDO3A HSA-binding contrast agents functionalized with a deoxycholic acid moiety were synthetized. The characterization included analysis of isomer composition, water-exchange rates, relaxivity profiles, and in vivo distribution. The significantly enhanced vascular retention time, led to enhanced contrast efficiency in a murine tumor model showing that these complexes present the opportunity to utilize the benefits of the Gd-HPDO3A structure in blood-pool imaging.

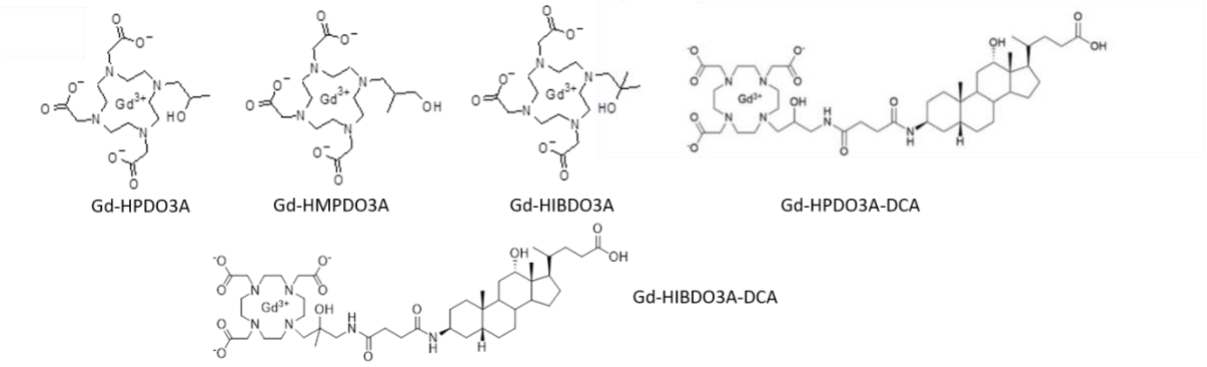

Fig. 1. Chemical structures of the investigated Gd-complexes.

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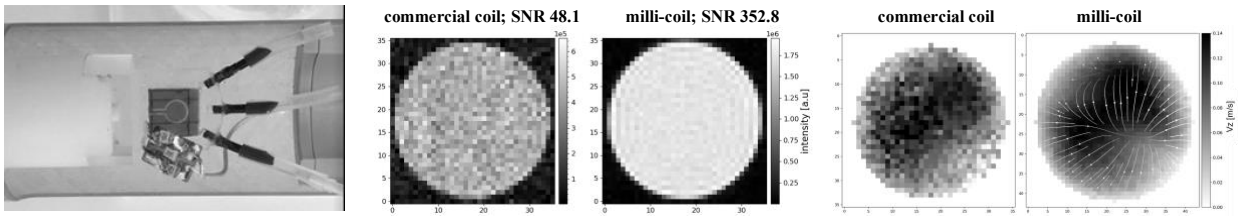
NMR METHODS AND DEVICES FOR THE CHARACTERIZATION OF FLOWS AND TRANSFERS IN MILLI-CHANNELS

F.Guerroudj,‡ L.Guendouz,‡ J.M. Commenge,† R. Hreiz,† N. Louvet,‡ J.Bianchin,‡ J.C Perrin‡
‡ Université de Lorraine, CNRS, LEMTA, F-54000 Nancy, France
† Université de Lorraine, CNRS, Lab React & Genie Proc, F-54000 Nancy, France
E-mail: feryal.guerroudj@univ-lorraine.fr

Keywords: instrumentation, MRI, materials, methods, solution NMR.

Milli-fluidics is the technology of flows in channels with characteristic dimensions of a few hundred microns. At this scale, capillary and viscosity effects are more important than volume forces and diffusion is often dominant over advection. The flows through milli-fluidic systems are implemented in several domains such as synthesis chemistry, biology or process engineering. The study of phenomena in milli-fluidic devices faces two major problems. One is that microfabrication techniques require costly investments and a good technological knowledge. Second, the geometric complexity of the systems induces difficulties due to optical access.
NMR/MRI methods are adapted to the study of such complex systems [1], provided that a specific instrumentation is developed to improve the sensibility of the measurement [2]. In this context, our study aims to implement, with a low-cost methodology, specific devices to study flows and transfer phenomena in milli-fluidic systems and to optimize NMR/MRI methods for their characterization.
Two milli-fluidic applications have been developed. The first one consists in a study of the growth of a biofilm in a capillary of submillimeter dimensions and the characterization of the hydrodynamics of the flow in presence of this biofilm. The second one is the study of the flow regime and hydrodynamic instabilities occurring in micromixers. For each application, a specific device was set up, including the milli-fluidic system and the radio frequency (RF) coil adapted to the geometry and dimensions of the system. The milli-coils were fabricated by etching on flexible copper/Kapton® substrates. With the considered geometrical parameters, RF simulations have shown that milli-coils produce an intense and homogeneous field and MRI measurements demonstrate an improvement of the signal-to-noise ratio compared to the commercial MicWB40 probe, which allows the detailed analysis of the above mentioned milli-fluidics phenomena (Fig. 1).



(a) (b) (c)

Fig. 1. Flow characterization in a micro-mixer. Comparison between commercial and milli coil (a). milli-fluidic device and milli-coil; (b). NMR signal (in-plane resolution: 28×28 µm, slice thickness: 200 µm); (c). velocity map (main flow direction, slice thickness: 200 µm, flow rate: 1.4ml/min): commercial Bruker MicWB40 (in-plane resolution: 30×30µm) and milli-coil (in-plane resolution: 23×23µm; representation of measured vortex in transverse direction – white arrows)

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LANTHANIDE-CONTAINING POLYOXOMETALATES AS ENHANCED MRI CONTRAST AGENTS AT HIGH MAGNETIC FIELDS

Rami Nasser Din[1], Steffen Krämer[1], Gisela Guthausen[2], Masooma Ibrahim[3], Aiswarya Chalikunnath Venu[3], Annie Powell[3,4]

[1]Univ. Grenoble Alpes, LNCMI UPR CNRS 3228, EMFL, 25 Av. des Martyrs, 38000 Grenoble, France
[2]Karlsruhe Institute of Technology (KIT), MVM-VM and EBI-WCT, Karlsruhe, Germany
[3]Karlsruhe Institute of Technology (KIT), Institute of Nanotechnology (INT), Karlsruhe, Germany
[4]Karlsruhe Institute of Technology (KIT), Institute of Inorganic Chemistry, Karlsruhe, Germany
E-mail: rami-nasser.din@lncmi.cnrs.fr

Keywords: contrast agents, instrumentation.

In nuclear magnetic resonance (NMR), paramagnetic relaxation enhancement (PRE) up to highest magnetic fields is an important research area related to the current trend for ultra-high-field magnetic resonance imaging (MRI). PRE changes the MRI image contrast by speeding up the nuclear spin relaxation of typically ^{1}H spins in water via their hyperfine interactions with unpaired electron spins of added contrast agents (CA). Since PRE of classical Cas containing Gd^{3+} ions generally decreases with magnetic field, alternative compounds are needed for high magnetic fields.

Recently, new Cas based on paramagnetic polyoxometalates (PM-POMs) have been synthesized. We studied their PRE over a wide frequency range from 20 MHz up to 1.4 GHz (33T) using resistive high field magnets and show that these compounds have a potential for application as Cas at high magnetic fields [1]. In addition, we developed NMR instrumentation and methods in order to overcome the lack of field homogeneity and field stability of resistive magnets including a wideband NMR setup up to 1.4 GHz for µl sample volume, single scan NMR, and tailored data analysis routines.

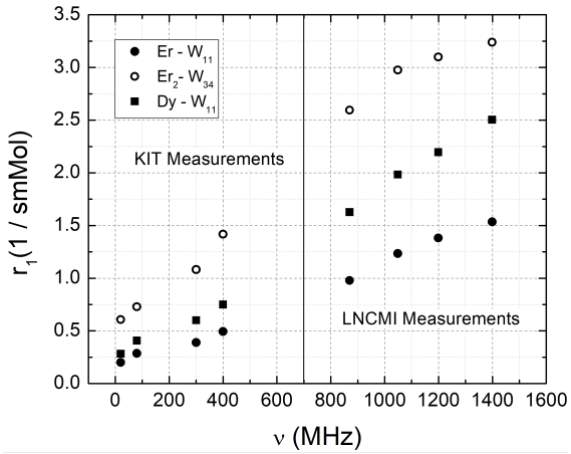

Fig. 1. Longitudinal relaxivity of PM-POMs as a function of frequency.

This work is supported by the French National Research Agency in the framework of the "Investissements d'avenir" program (ANR-15-IDEX-02).

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WEDNESDAY SEPTEMBER 28TH

	Plenary session Chair: A. Barnes
9:00-9:45	**M. Leskes (Weizmann Institute of Science)** PARAMAGNETIC METAL IONS DNP FOR BULK AND SURFACE SENSITIVITY IN INORGANIC SOLIDS
9:45-10:15	**P. Turano (University of Florence)** NMR OF BIOMOLECULES: FROM VERY LARGE TO VERY SMALL (AND VICE VERSA)
10:15-10:45	**A. Bifone (University of Torino)** TOWARDS HYPERPOLARIZATION WITH NITROGEN-VACANCY CENTERS IN DIAMOND

10:45-11:40	**Coffee break + Poster session 2 (EVEN abstract numbers)**

	Parallel session A Chair: P. Giraudeau	Parallel session B Chair: M. Leskes
11:40-12:00	**A. Sobolev** - NMR METABOLOMICS OF BRASSICA VEGETABLES: PRACTICAL IMPLEMENTATIONS IN AGRO-FOOD SUSTAINABLE SYSTEMS	**M. Lelli** - STRATEGIES FOR HIGH-TEMPERATURE AND FAST-MAS DYNAMIC NUCLEAR POLARIZATION
12:00-12:20	**E. Dufourc** - DYNAMIC SORTING OF MOBILE AND RIGID MOLECULES IN BIOMEMBRANES BY MAS ^{13}C-NMR	**S. Mamone** - PULSED PHIP-SAH METHODS TO PRODUCE HYPERPOLARIZED SUBSTRATES IN CLEAN WATER SOLUTIONS FOR BIOMEDICAL APPLICATIONS
12:20-12:40	**G. Petrella** - METABOLISM EVOLUTION OF PROSTATE CANCER CELLS DURING THE DEVELOPMENT OF CHEMORESISTANCE	**T. Georges** - INVESTIGATIONS OF Ca^{2+} AQUEOUS COMPLEXES THROUGH ^{43}CA MAS-DNP NMR OF VITRIFIED SOLUTIONS
12:40-13:20	**GIDRM Under 35 award: V. Ghini and A. Vignoli** - NMR-BASED METABOLOMICS: A JOURNEY IN ITS APPLICATIONS IN CANCER RESEARCH, FROM BIOFLUIDS TO CELLS	**A. Frison** - AN INNOVATIVE SOLVENT-FREE POLYMER SAMPLE PREPARATION METHOD FOR DNP SSNMR **C. Praud** - DEVELOPMENT OF ULTRAFAST 2D NMR FOR DNP-HYPERPOLARISED METABOLIC MIXTURES

13:20-14:50	**Lunch**

	Plenary session Chair: J. Martins
14:50-15:35	**A. Barnes (ETH Zurich, Switzerland)** ENABLING IN-CELL NMR WITH PULSED DNP, ELECTRON DECOUPLING, MAS SPHERES AND COMPACT 30 TESLA MAGNETS
15:35-16:05	**P. Giraudeau (University of Nantes, France)** DISSOLUTION DYNAMIC NUCLEAR POLARIZATION OPENS NEW PERSPECTIVES FOR METABOLOMICS
16:05-16:35	**H. Ratiney (CNRS Lyon, France)** IN VIVO MR SPECTROSCOPY QUANTIFICATION: LOCKS AND PROSPECTS
16:35-16:55	**Sponsorship Lecture (Jeol): M. Perez** DEVELOPING A NMR TOOL FOR ALL FROM SCRATCH

16:55-17:50	**Coffee break + Poster session 3 (ODD abstract numbers)**

from 17:50	**Social Event**

PARAMAGNETIC METAL IONS DNP FOR BULK AND SURFACE SENSITIVITY IN INORGANIC SOLIDS

Daniel Jardón-Álvarez [‡], Shira Haber[‡], Ayan Maity, Isaac Buchine, Michal Leskes[‡]

[‡]Department of Molecular Chemistry and Materials Science, Weizmann Institute of Science, Israel
E-mail: michal.leskes@weizmann.ac.il

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

Paramagnetic metal ions provide an efficient route for nuclear hyperpolarization in the bulk of inorganic solids.[1] In this talk I will describe recent developments of this approach, the conditions and mechanisms for gaining high sensitivity as well as some of the applications of metal ions DNP to gain structural insight into energy storage and conversion materials.
A key advantage of the approach is the ability to efficiently polarize low gamma and/or low abundance nuclei directly from the metal ion dopant with uniform polarization across the crystal.[2] This enables detection of 2D homonuclear correlations of extremely low sensitivity nuclei such as ^{89}Y, which provide medium range structural insight into oxygen vacancies distribution, a critical parameter in the design of solid electrolytes with high oxygen ion conductivity.[3] Furthermore, direct polarization from metal ions in the bulk extends to the material interface. I will show how the combination of endogenous interfacial polarization, from the bulk of the material, with exogenous polarization, from biradicals, emerges as a powerful structural tool for thin coatings and buried solid interphases.[4] Finally, I will present some of our ongoing efforts to characterize the paramagnetic dopants through their effect on nuclear relaxation times and how this effect is related to measured DNP enhancements.[5]

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NMR OF BIOMOLECULES: FROM VERY LARGE TO VERY SMALL (AND VICE VERSA)

P. Turano

CERM and Department of Chemistry, Polo Scientifico 50019 Sesto Fiorentino (FI), Italy
E-mail: turano@cerm.unifi.it

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

NMR spectroscopy has evolved over the past decades to enable studies on large biomolecular assemblies. Specific approaches have been developed to detect signals of large nanocage proteins such as ferritin (Fig. 1) and to monitor their interactions with organic and inorganic compounds or their surface functionalization [1-3]. Encapsulation of metal species in the ferritin cage is an efficient tool for the targeted delivery of metallodrugs towards certain cancer cells, while external surface decoration allows us to expand the panel of target receptors [4]. The efficiency of the delivery and the mechanism of action of encapsulated metal-based drugs is then evaluated by NMR-based metabolomics revealing dysregulation of several key small molecules at the level of the cellular endo- and exo-metabolome.
Furthermore, the use of ^{1}H NMR is here described as a tool to monitor the consequences of SARS-CoV-2 infection by monitoring metabolites and lipoproteins. The approach has allowed the identification of a clear fingerprint of the disease based on a pool of biomolecules, that can be used as monitoring biomarkers for the healing process and when assessing treatment response [5-7].

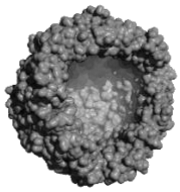

Fig. 1. Schematic representation of the ferritin cage.

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TOWARDS HYPERPOLARIZATION WITH NITROGEN-VACANCY CENTERS IN DIAMOND

A. Bifone

‡ *Department of Molecular Biotechnology and Health Sciences, University of Turin*, *Via Nizza, 52, Torino*, *I-10126 Italy*

E-mail: angelo.bifone@unito.it

Keywords: hyperpolarization, materials, theory and methods, instrumentation, exotica.

Nitrogen-vacancy centers (NVs) in diamond have attracted considerable attention as a means to hyperpolarize nuclear spins. Indeed, optical irradiation of negatively charged NV defects (NV^-) at room temperature results in a large spin-polarization that can be subsequently transferred to nuclear spins within the diamond lattice or to molecules adsorbed at its surface. This approach promises to overcome some of the limitations of current hyperpolarization methods, like the use of cryogenic temperatures and potentially toxic agents, and to be generally applicable to virtually all molecular substrates. However, despite much progress in the design and production of high-surface area NV-rich diamond materials, substantial hyperpolarization of nuclei outside the diamond has proven elusive. Here, I will describe Optically Detected Magnetic Resonance experiments in shallow NV centers, and report some unexpected effects of laser irradiation that may have limited successful polarization transfer to nuclei. I will also discuss materials and experimental conditions that promote the formation of dense, highly polarized ensembles of NV the proximity of the surface, and will present preliminary results that pave the way to the development of a diamond-based hyperpolarization device.

NMR METABOLOMICS OF BRASSICA VEGETABLES: PRACTICAL IMPLEMENTATIONS IN AGRO-FOOD SUSTAINABLE SYSTEMS

D. Giannino,[‡] G. Testone,[‡] E. Acciaro, [‡] A.P. Sobolev[‡]

[‡]Istituto per i Sistemi Biologici, CNR, Via Salaria Km 29.300, I-00015 Monterotondo, Italy

E-mail: anatoly.sobolev@cnr.it

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

^{1}H NMR based metabolite profiling in cruciferous crops has been developed [1,2] and up-dated practical implementations are strongly needed in the expanding and complex scenario of *Brassica spp.* vegetables. The present study focuses on the application of ^{1}H NMR metabolomics to *B. oleracea* (cauliflower) and *B. rapa* subsp. *sylvestris* ("cime di rapa"/broccoli-raab/rapini) as a tool for resolving specific practical issues. In one case, the study aimed at by-product valorization (leaves, stems and florets) derived from cauliflower production in a perspective of recovery and usage of bioactive compounds within a circular economy context [3]. A second study addressed the metabolic variation in leaves and florets of two rapini genotypes by comparing field harvested products (controls) against 4 day-long stored fresh and packaged products in order to valorize local vegetables and performance during shelf-life, and address genotype traceability [4]. Water-soluble metabolites from rapini tissues were extracted using a methanol-water mixture (1:1 v/v), whereas both water- and lipo-soluble fractions were extracted from cauliflower (chloroform/methanol/water biphasic solvent system) by the Bligh-Dyer protocol [5]. Overall, water-soluble extracts of all tissue types from both vegetables prevalently contained over thirty metabolites of common classes (sugars, organic acids, free amino acids) though in different amounts based on tissue-specificity. Moreover, the assignment of several species-specific secondary metabolites was widened and refined by including glucosinolates and other sulfur-containing compounds. Finally, metabolic profiles - supported by chemometric analysis (ANOVA and PCA) - showed that major differences were associated with organ diversity in rapini (leaves vs florets) and with the storage status (fresh vs packaged), while genotype divergences were less evident.

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DYNAMIC SORTING OF MOBILE AND RIGID MOLECULES IN BIOMEMBRANES BY MAS 13C-NMR

Estelle Morvan[a], Nada Taib-Maamar[b], Axelle Grélard[a,b], Antoine Loquet[a,b] and Erick J. Dufourc[a,b]

[a]Institut Européen de Chimie et Biologie, UMS3033, CNRS, Université de Bordeaux, INSERM
[b]Institute of Chemistry and Biology of Membranes and Nanoobjects, UMR5248, CNRS, University of Bordeaux, Bordeaux Polytechnic Institute. Allée Geoffroy Saint Hilaire 33600 Pessac France.

E-mail: erick.dufourc@cnrs.fr

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

Understanding the membrane dynamics of complex systems is essential to follow their function. As molecules in membranes can be in a rigid or mobile state depending on external (temperature, pressure) or internal (pH, domains, etc.) conditions, we have developed NMR methods to filter highly mobile molecular parts from others that are in more restricted environments. Cross Polarization (CP), Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) with reference to DP (Direct Polarization) ssNMR techniques used in combination with natural abundance MAS ^{13}C-NMR on rigid and fluid model membranes afforded demonstrating that INEPT will detect only very mobile lipid head groups in gel (*solid-ordered*) phases, the remaining rigid parts are only detected with CP. Interestingly, the ^{13}C-NMR chemical shift of lipid hydrocarbon chains can be used to track *order-disorder* phase transitions, calculate the fraction of defects and the part of the transition enthalpy due to bond rotamers. Cholesterol-containing membranes (*liquid-ordered* phases) can be dynamically contrasted as the rigid-body sterol is mainly detected by CP techniques and the phospholipid by INEPT (Fig. 1). A direct correlation is established between the normalized line intensity as obtained by CP and the C-H (C-D) order parameters that are measured from ssNMR or molecular dynamics: when the order is greater than 0.3 (maximum value S=0.5 for chain CH_2) only rigid parts can be filtered and detected using CP-MAS. This opens up a new, very simple and robust route for the determination of membrane dynamics from high resolution NMR.

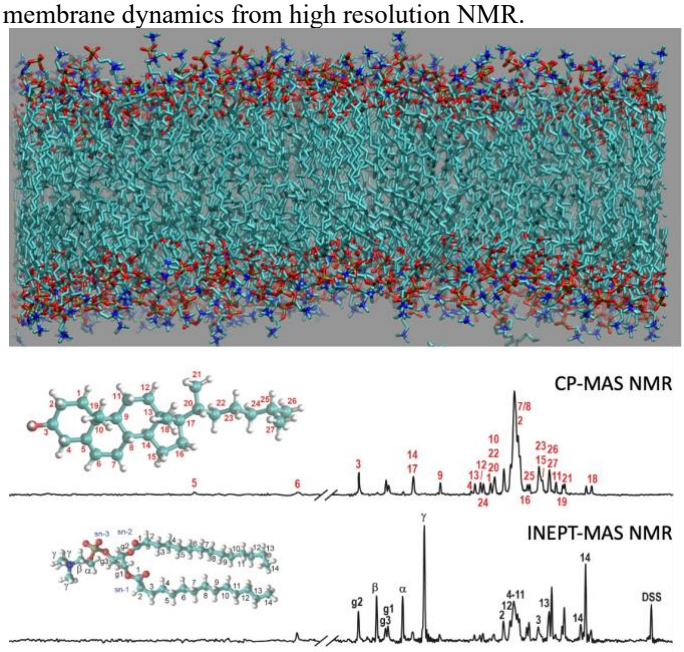

Fig. 1. Application of magic angle sample spinning and polarization transfer NMR techniques to bio-membranes (top) allows filtering of the ^{13}C-NMR spectrum (middle) of the rigid cholesterol body from the more mobile phospholipid resonances (bottom), within the same membrane.

METABOLISM EVOLUTION OF PROSTATE CANCER CELLS DURING THE DEVELOPMENT OF CHEMORESISTANCE

G. Petrella,[‡] G. Ciufolini,[‡] S. Germini,[‡] F. Corsi,[†] L. Ghibelli,[†] D.O. Cicero[‡]

[‡] Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", 00133 Rome, Italy
[†] Dipartimento di Biologia, Università di Roma "Tor Vergata", 00133 Rome, Italy
E-mail: petrella@scienze.uniroma2.it

Keywords: NMR spectroscopy, cellular metabolism, prostate cancer, chemoresistance

Chemo- and radiotherapies are mainly based on the induction of apoptosis in cancer cells. However, it is now emerging that apoptosis is not the end but just a turning point of the tissue dynamics alterations triggered by pro-apoptotic therapies [1]. Apoptotic cells secrete signals aimed at eliciting regeneration: this suggested the term "Phoenix Rising" (PR) [2], after the mythological bird that regenerates from its ashes. This process allows repopulating of injured tissues by coordinating cell death with proliferation, thus restoring a functional organ size. Cancer resistance to apoptosis and rapid repopulation after a regression phase are not biologically fully understood but necessary to understand therapy-associated tumor progression: to bypass this *in vivo* limitation, *in vitro* high-tech models are being developed to discriminate the two scenarios in real-time. One of the fields of application of this approach is prostate cancer (PCa), which accounts for 13.5% of worldwide cancer diagnoses among men.
Recently, a 2D *in vitro* model that mimics a monolayered epithelium such as the prostate has been proposed [3]. We used this model to study the evolution of the metabolic profile to the PR event of PC-3 cells following a chemotherapeutic insult with Etoposide.
To carry out this study, changes in the composition of the culture media of these cells were studied by nuclear magnetic resonance spectroscopy. This made it possible to follow nutrient utilization and excretion of products that reflect the metabolism of the different phases. The results of this work are the starting point for the development of adjuvant therapies to accompany chemotherapeutics that can shut down a metabolism related to the triggering of the PR mechanism in PCa.

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NMR-BASED METABOLOMICS: A JOURNEY IN ITS APPLICATIONS IN CANCER RESEARCH, FROM BIOFLUIDS TO CELLS

V. Ghini[‡,†], A. Vignoli[‡,†]

[‡]Center of Magnetic Resonance (CERM), University of Florence, via Luigi Sacconi 6, 50019, Sesto Fiorentino, Italy
[†]Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, 50019, Sesto Fiorentino, Italy E-mail: ghini@cerm.unifi.it; vignoli@cerm.unifi.it

Keywords: solution NMR, small molecules, metabolomics.

Metabolomics represents an important line of CERM research[1]. The NMR technique has been applied in various sectors, ranging from biomedicine[2,3,4] and medicinal chemistry[5], to foodomics[6] and microbial metabolism for biotechnological applications[7].

Here, we present two snapshots that best characterize our individual contributions to CERM metabolomics activities.

A.V. has carried out a multi-year activity on the characterization of the metabolic alterations at the systemic level of the profiles of oncological patients, particularly in breast cancer setting[8]. Through multi-center studies, the serum metabolomic fingerprint of breast cancer patients has demonstrated to be prognostic for breast cancer recurrence in the early setting[9]. Furthermore, the analysis of the serum metabolomic profiles has shown to be useful for the prediction of the outcome of neo-adjuvant chemotherapy in breast cancer patients with large tumors[10].

V.G. presents the use of NMR-based metabolomics in the evaluation of the mechanism of action of drugs in tumor cell lines. Untargeted NMR is a powerful tool to characterize the metabolome of cultured cancer cells, both at the levels of their endo- and exo-metabolome, to monitor the cellular responses to treatments. The results allow us to obtain hints on the mechanism of action and/or resistance of the different anticancer agents tested[5,11,12].

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STRATEGIES FOR HIGH-TEMPERATURE AND FAST-MAS DYNAMIC NUCLEAR POLARIZATION

Lorenzo Niccoli, [5,6] Georges Menzildjian,[1] Alicia Lund,[1] Dorothea Wisser,[1] Ganesan Karthikeyan,[2] Gilles Casano,[2] Hakim Karoui,[2] Maxim Yulikov,[3] David Gajan,[1] Gunnar Jeschke,[3] Olivier Ouari,[2] Lyndon Emsley,[4] Anne Lesage,[1] and Moreno Lelli,[5,6]

[1] Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCB Lyon 1), 69100 Villeurbanne, France
[2]AixMarseille Univ, CNRS, ICR, 13013 Marseille, France
[3]Department of Chemistry and Applied Biosciences, ETH Zürich, CH-8093 Zürich, Switzerland
[4]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[5]University of Florence, Department of Chemistry, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy.
[6]Magnetic Resonance Center (CERM/CIRMMP), Via Luigi Sacconi 6, 50019 Sesto Fiorentino (FI), Italy.

E-mail: moreno.lelli@unifi.it (Times 10)

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

MAS DNP is increasingly establishing itself as a powerful technique to boost sensitivity of NMR. At 9.4 T (400 MHz) and 100 K, DNP enhancements of 200–300 are now possible with several polarizing agents (PA) (AMUPol[1], TEKPol[2], and more recently SPIROPOL, PyPolPEG$_2$OH,[3] bcTol,[4] AsymPolPOK,[5] O-HydrOPol,[6] …). Despite the excellent performance at 9.4 T, these dinitroxide biradicals are much less efficient at 18.8 T or higher field, and the need for more efficient PA stimulated the research of biradical with a narrow EPR line,[7] such as for trityl in TEMTriPol[8] and more recently NATriPol.[9]
Here we show how hybrid biradicals, based on the Cross-Effect mechanism and designed by coupling BDPA with a nitroxide unit, provide very high DNP enhancements at 100 K: up to 185 at 18.8 T and 40 kHz MAS,[10] and up to 200 at 21.2 T and 65 kHz MAS.[11] We also show that this exceptional enhancement obtained with HyTEK2 is also persistent with temperature increase, when it is dissolved in a suitable rigid glassy phase like ortho-terphenyl (OTP)[12]. Enhancements up to 60 are observed at the glass transition temperature (243 K; –30 °C) and at 18.8T.[13]
In particular, we will discuss how the DNP performance of HyTEK2 depends on a combination of several factors, from the magnetic properties of the polarizing agent to the role played by spin-diffusion, including simulations to interpret the role of the electron relaxation times in the variable temperature performances. This radical formulation has the potential to make accessible the characterization of materials at high field over a large range of temperatures.

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PULSED PHIP-SAH METHODS TO PRODUCE HYPERPOLARIZED SUBSTRATES IN CLEAN WATER SOLUTIONS FOR BIOMEDICAL APPLICATIONS

Salvatore Mamone[†‡], Anil P. Jagtap[†‡], Sergey Korchak[†‡], Yonghong Ding[†‡], Sonja Sternkopf[†‡], Stefan Glöggler[†‡]

[†] NMR Signal Enhancement Group, Max Planck Institute for Multidisciplinary Sciences Am Fasssberg 11, 37077 Göttingen (DE)
[‡]Center for Biostructural Imaging of Neurodegeneration of UMG Von-Siebold-Straße 3A, 37075 Göttingen (DE)

E-mail: salvatore.mamone@mpinat.mpg.de

Keywords: hyperpolarization, contrast agents.

Nuclear Magnetic resonance (NMR) is a powerful analytical technique with applications to chemistry, physics, material science and biomedicine. Hyperpolarization methods aim to enhance the typical NMR signals by 4 orders of magnitude or more leading to improved sensitivity as well as temporal and spatial resolutions.
Hyperpolarized labelled metabolites are expected to lead to novel diagnostic methodologies in MRI. Para-hydrogen induced polarization (PHIP) is a convenient and effective hyperpolarization method.[1,2] The scope of PHIP has been extended by the introduction of side arm hydrogenation (SAH), in which a precursor with an unsaturated bond is used for hydrogenation and substrates are obtained by bond cleavage after polarization transfer.[3]
Here, we discuss novel PHIP-SAH methods to obtain high polarization levels in labelled substrates.[4,5] The methods rely on purposely designed sidearms in combination with effective pulse sequences to transfer spin order from para-hydrogen into net magnetization on the target heteronuclear spin. We introduce a procedure to transfer the hyperpolarized substrates in clean water solutions at physiological conditions in less than 20 s. We demonstrate the capability of the methods to monitor anaerobic glycolysis (a hallmark of cancer) in real time in cells. Metabolic imaging of pyruvate ↔ lactate conversion at 1 mm pixel resolution is demonstrated in a preclinical system. Finally, we discuss improvements that may ultimately lead to a widespread use of the method to obtain hyperpolarized substrates in magnetic resonance for biomedical applications.

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INVESTIGATIONS OF CA2+ AQUEOUS COMPLEXES THROUGH 43CA MAS-DNP NMR OF VITRIFIED SOLUTIONS

T. Georges,[‡] R. Chèvre,[†] P. Thureau,[†] G. Mollica,[†] T. Azaïs[‡]

[‡]Sorbonne Université, CNRS, Laboratoire de Chimie de la Matière Condensée de Paris (LCMCP), F-75005 Paris, France
[†]Aix Marseille Univ, CNRS, Institut de Chimie Radicalaire (ICR), 13397 Marseille, France
E-mail: tristan.georges@upmc.fr

Keywords:
solid state NMR, solution NMR, hyperpolarization, materials, small molecules, biomolecules, theory and methods, exotica.

Calcium is an abundant element in both geological and biological worlds [1]. In vivo, calcium ion is involved in cell metabolism, muscle contraction or biomineralization [2]. To do so, various proteins interact with Ca^{2+} ions by means of complexation. Due to the huge amount and variety of these proteins, in-vivo calcium complexation appears as a key point to investigate in various fields of research such as biochemistry or biomineralization. Thus, 43Ca NMR appears as a promising technique, because of its ability to probe dynamics, structure and interactions at the atomic scale. However, the use of 43Ca NMR is still limited because of some inherent weak sensitivity induced by its low gamma, its quadrupolar nature or its low natural abundance (0.135%). Moreover, these sensitivity issues are further enhanced in case of biological-like concentrations (≈ mM) [3].

In this study, we propose a novel strategy based on 43Ca MAS-DNP NMR to perform solid-state NMR on vitrified solutions containing aqueous calcium complexes (see Fig. 1). We take advantage of the cryogenic temperature (100 K) that drastically reduces the dynamics of Ca-complexes, to performed solid-state NMR experiments such as 1H-43Ca CP MAS. In particular, we compared the efficiency of different 1H-43Ca CP MAS conditions including “high” and “low” RF power, generally used for 1/2 or quadrupolar spins, respectively. To interact with calcium ions, we used EDTA as a model of complexing agent and aspartic acid in reason of its implication in biomineralization processes. We show that the complexation site can be detailed through 2D 1H-43Ca HETCOR experiments. This approach can be extended to *ex-situ* monitoring of calcium phosphate precipitation. More generally, this study opens new perspectives for the investigation of physico-chemical processes involving Ca^{2+}-complexes.

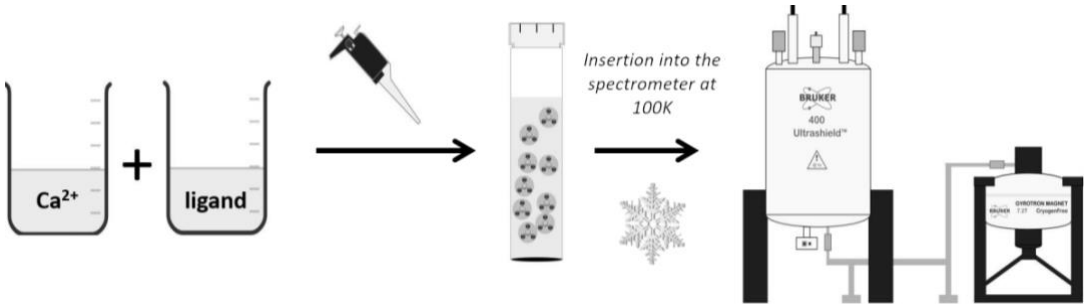

Fig. 1. MAS-DNP of vitrified solutions methodology

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AN INNOVATIVE SOLVENT-FREE POLYMER SAMPLE PREPARATION METHOD FOR DNP SSNMR

A. Frison,[‡,†] Y. Masmoudi,[§] M. Schneider,[§] F. Ferrer,[†] P. Thureau,[†] E. Badens,[§] F. Ziarelli,[‡] S. Viel[†,‡‡]

[‡] Aix Marseille Univ, CNRS, Centrale Marseille, FSCM, Marseille, France
[†] Aix Marseille Univ, CNRS, ICR, Marseille, France
[§] Aix Marseille Univ, CNRS, Centrale Marseille, M2P2, Marseille, France
[‡‡] Institut Universitaire de France, Paris, France
E-mail: amelie.frison@univ-amu.fr

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

Solid-state NMR (SSNMR) is highly suitable for analyzing polymers but has low sensitivity. This limitation can be overcome using dynamic nuclear polarization (DNP), a technique that enhances NMR sensitivity by transferring the electronic spin polarization of polarizing agents to surrounding nuclei [1]. However, mainly soluble polymers can be analyzed by DNP SSNMR because efficient methods for preparing the samples for DNP usually require initial polymer solubilization [2]. In this work, we will present an innovative solvent-free sample preparation method based on the use of supercritical CO_2 technology. More precisely, supercritical CO_2 is used as a plasticizing agent to lower the polymer glass transition/melting points, allowing thus an efficient mixing of polarizing agents within the resulting soften/molten polymer matrix and therefore a homogeneous and in-depth loading, similarly to a conventional polymer extrusion process but at relatively moderate temperatures (40–50°C for the investigated polymers). This method was applied to incorporate either TEKPol [3] or AMUPol [4] as polarizing agents within different polymers. The resulting samples were characterized by CW EPR and DNP SSNMR. The results were compared to conventional polymer sample preparation methods (glass forming, film casting, etc.) (Fig.1) highlighting the potential of the proposed process as a new and efficient method to prepare polymer samples for DNP.

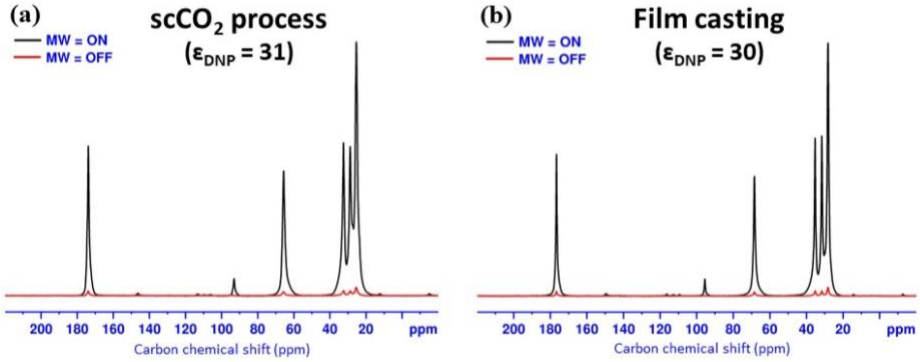

Fig. 1. ^{13}C CPMAS DNP SSNMR spectra of a polycaprolactone sample obtained by (a) supercritical preparation method, (b) film casting preparation method

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DEVELOPMENT OF ULTRAFAST 2D NMR FOR DNP-HYPERPOLARISED METABOLIC MIXTURES

C. Praud,‡ J. Mandral,‡ A. Dey,‡ B. Charrier, ‡ J.-N. Dumez‡ and P. Giraudeau‡

‡Nantes Université, CNRS, CEISAM UMR 6230, 44000 Nantes, France
E-mail : clement.praud@univ-nantes.fr

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

NMR is well suited for the analysis of complex mixtures as it provides both structural and quantitative information in a repeatable and non-destructive way. Despite this, it suffers from a low sensitivity and, in the case of 1D ^{1}H NMR spectroscopy, from the overlap of multiple signals. 2D NMR, and in particular heteronuclear ^{1}H-^{13}C experiments, can spread the chemical information over a second axis, which leads to improved peak discrimination and makes it easier to assign signals from mixture constituents. However, 2D NMR is struggling in the case of low-concentration analytes because of its low general sensitivity. This limitation can be circumvented by dissolution Dynamic Nuclear Polarization (d-DNP), which has been applied to solve various analytical chemistry problems [1]. DNP can increase NMR sensitivity by 4 or 5 orders of magnitude, but d-DNP is an irreversible process which is not compatible with the multiple transients needed to record conventional 2D NMR spectrum. Ultrafast (UF) NMR is based on a spatial encoding strategy and it can deliver a 2D spectrum in a single-scan in a few milliseconds, making it the perfect candidate for d-DNP applications [2]. The coupling of UF 2D NMR and d-DNP has already been demonstrated but at an early stage of development and several improvements are needed so that UF 2D experiments can be used for analytical chemistry applications on complex mixtures [1,3,4]. Here, we will report the development and optimisation of an UF heteronuclear pulse sequence called long-range HETCOR, designed for its coupling with d-DNP. This sequence capitalises on ^{13}C hyperpolarized states and long-range scalar couplings to observe long range ^{1}H-^{13}C couplings, typically involving quaternary ^{13}C nuclei. Multiple optimisations have been done concerning the different delays, such as the use of a filter and a 180° pulse to optimise the selection of coherence pathways and maximise the resulting signal. Experimental conditions have also been adapted to minimize convection effects after sample injection, that can highly impact the quality of spatial encoding. We will present results of d-DNP boosted ultrafast 2D experiments on metabolite mixtures to assess the potential of this new coupling. Results were obtained with a d-DNP prototype recently installed in our lab and dedicated to the exploration of analytical chemistry applications of hyperpolarization.

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Enabling In-Cell NMR with Pulsed DNP, Electron Decoupling, MAS Spheres and Compact 30 Tesla Magnets

A. Barnes

Dep. of Chemistry and Applied Biosc., Lab. für Physikalische Chemie, HCI D 225, Vladimir-Prelog-Weg 1-5/10, 8093 Zürich, Switzerland

E-mail : alexander.barnes@phys.chem.ethz.ch

Proteins and drugs that function in cells are best studied in cells. It is far better to investigate biological structure in a cellular context, with all of the diverse proteins, lipids, salts, and small molecules present that ultimately govern cellular function and regulation, also present. As an example, I will present DNP enhanced MAS NMR showing distinct changes in molecular conformation of phorbol esters within intact human cells, as compared to in vitro membrane preparations. However, even with high-field DNP, NMR sensitivity is still challenged, and hereto insufficient, to characterize molecular structures at endogenous, or related pharmacologically utilized concentrations on the nanomolar level. Better technology and methodology is required to improve NMR sensitivity by an additional few orders of magnitude. I will show how we will accomplish such sensitivity gains through 1) Time domain dynamic nuclear polarization > 28 Tesla; 2) Electron spin decoupling and chirped microwave pulses; 3) Cryogenic magic angle spinning spheres below 6 Kelvin; 4) Compact high temperature superconducting (HTS) magnets >28 Tesla and 5) Frequency agile gyrotrons. Particular emphasis will be put on HTS magnets—HTS is severely underutilized for both gyrotron and also high-field NMR magnets. I will present my vision, with supporting evidence, that HTS magnets will bring us to 100 Tesla for NMR within this decade. Lastly, I will also briefly introduce our idea, and progress, using optical tweezers to suspend ~30 micron sizes rotors in vacuum to access MAS frequencies > 1 MHz.

DISSOLUTION DYNAMIC NUCLEAR POLARIZATION OPENS NEW PERSPECTIVES FOR METABOLOMICS

P. Giraudeau[‡]
[‡] Nantes Université, CEISAM, CNRS UMR 6230, 2 rue de la Houssinière, F-44000 Nantes, France
E-mail: patrick.giraudeau@univ-nantes.fr

Keywords: solution NMR, hyperpolarization, small molecules, metabolomics

NMR spectroscopy is a major analytical tool in metabolomics, owing to its ability to provide highly reliable structural and quantitative information. Most NMR-based metabolomics studies are based on ^{1}H 1D spectra which are sensitive and high-throughput, but severely impacted by peak overlap. Heteronuclear ^{13}C 1D and 2D experiments would be ideally suited to tackle the complexity of complex metabolite mixtures, but they are barely used in practice due to their low sensitivity. To tackle such limitations, we recently started to explore the potential of dissolution dynamic nuclear polarization (D-DNP) [1] for ^{13}C NMR metabolomics. Such exploration raises a number of methodological and analytical challenges, since D-DNP is still a recent method relying on complex experimental settings, whose potential to study complex biological mixtures at natural abundance remains unexplored. After preliminary studies that showed the potential of D-DNP for such samples [2,3], we recently developed a complete untargeted NMR-based metabolomics workflow based on D-DNP. Using a D-DNP prototype dedicated to analytical chemistry applications, we showed the ability of this approach to statistically distinguish two group of plant sample extracts, and to highlight relevant biomarkers [4]. We have also explored the potential of D-DNP to analyze biofluids at natural abundance, making it possible to detect and identify several tens of relevant metabolites from ^{13}C hyperpolarized spectra of freeze-dried urine samples [5].

In parallel with these promising metabolomics applications, we are constantly improving the performance of the D-DNP analytical workflow to increase its applicability for the analysis of complex metabolic mixtures. Thanks to a fine optimization of the many parameters involved in the D-DNP experiment, we have been able to significantly increase the sensitivity, the precision and the robustness of our experimental setting [6]. In addition, we are exploring the potential of ultrafast 2D NMR methods to provide single-scan homo- and hetero-nuclear correlations following D-DNP, in order to improve the separation of overlapped metabolite signals [7]. We will describe these recent developments and applications, highlighting the potential of D-DNP for metabolomics, as well as the questions raised by the development of this new analytical approach.

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IN VIVO MR SPECTROSCOPY QUANTIFICATION: LOCKS AND PROSPECTS

Hélène Ratiney[‡]
[‡] Univ Lyon, INSA-Lyon, Université Claude Bernard Lyon 1, UJM-Saint Etienne, CNRS, Inserm, CREATIS UMR 5220, U1294, F-69621, LYON, France
E-mail: helene.ratiney@creatis.insa-lyon.fr

Drawing its sources from high resolution magnetic resonance but having developed in 40 year history of use its own specificities and issues, *in vivo* MR spectroscopy remains the unique tool for non-invasive measurement of biochemical compounds (metabolites, lipids). It provides, in addition to anatomical magnetic resonance imaging, unique and valuable information for the diagnosis and therapeutic follow-up of many brain diseases (epilepsy, multiple sclerosis, cancer) and it shows more and more applications in other organs than the brain (liver, prostate, breast, muscle etc…). Applied on a preclinical system, it helps to study animal models. However, this technique is not yet routinely used for clinical diagnosis or for preclinical studies. This can be explained both by the difficulty of acquiring quality data and by the various pitfalls that can be encountered during the analysis. This presentation will focus on the quantification of brain metabolites but also on lipid quantification. Through these examples, some of the thoughts and solutions that have been explored will be presented, as well as the emerging avenues that could be foreseen**.** The most widely developed methods are based on parametric adjustment using classical mathematical optimization methods. The *in vivo* NMR spectroscopy community has proposed many methods for brain metabolite quantification, and is now confronted with the variability of the results from the different packages proposed [1]. Quantification of these signals is difficult because of the large number of metabolites with overlapping spectral components, the presence of macromolecules and the low signal-to-noise ratio of the signals. However in this case, the choice of the mathematical model, what it implies in its handling, the taking into account of the prior knowledge are all sources of variability. Facing this kind of complexity, one can favor the robustness of the adjustment by strongly constraining and linking the parameters according to a given prior knowledge, and using a different sampling. This model-based approach provides results with low variances at the expense of a possible bias and can be profitable for lipid quantification[1]. In the same vein, this kind of analysis can be useful for dynamic metabolic MR imaging of hyperpolarized 13C [3]. Then, to find solutions and move forward, we can try to multiply the ways of looking at the signal, and include in the analysis the measurement of the relaxation times of the metabolites, to realize multiparametric MRS, an approach of the "MR fingerprinting" type adapted to the *in vivo* MR spectroscopy[4]. Finally integrating AI into processing are more and more explored for the quantification or the correction of MR *in vivo* data and should help robustify and speed up solutions[5][6].

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DEVELOPING A NMR TOOL FOR ALL FROM SCRATCH

Manuel Pérez, ‡ Vadim Zorin‡

‡JEOL UK Ltd, 4 Bankside, Long Hanborough, OX298LJ, UK

E-mail: Manuel.Perez@JEOLUK.com

Keywords: NMR, software development, NMR simulation, qNMR, NMR prediction, NMR signal deconvolution, frequency-domain deconvolution, NUS, peak peaking approaches, Savitzky-Golay

The development of a tool to process, analyse and report different types of NMR data is challenging. We will detail the algorithm implemented for signal deconvolution, multiplet analysis, DOSY-transform, our novel NMR prediction approach, as well as future plans for our software suite.

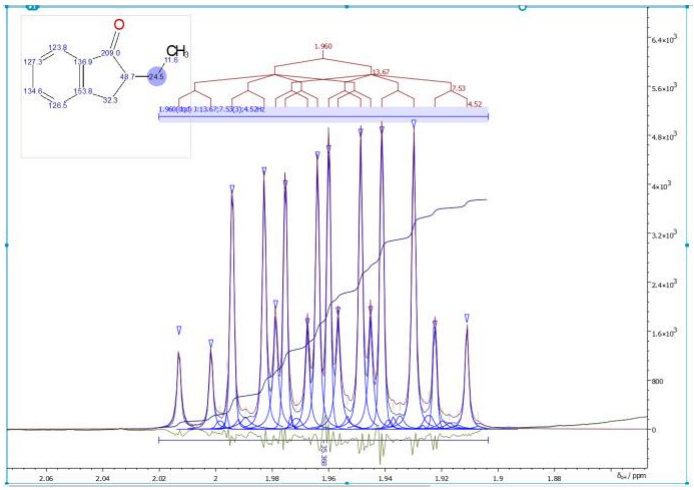

Fig. 1. Peak deconvolution, showing the automatically calculated multiplet analysis. The results of the deconvolution are portrayed by showing the Peak Models, the Sum and the Residual from the fitting.

THURSDAY SEPTEMBER 29TH

	Plenary session **Chair: P. Turano**
9:00-9:45	**M. Pons (University of Barcelona)** INTEGRATING ORDER AND DISORDER AN SRC CELL SIGNALLING: THE NMR APPROACH
9:45-10:15	**R. Fattorusso (University of Campania)** THE ROLE OF PROTEIN FOLDING MECHANISMS IN AMYLOID FIBRIL FORMATION
10:15-10:45	**F. Ochsenbein (CEA-Saclay, France)** STRUCTURE-FUNCTION STUDIES AND INHIBITOR DESIGN OF HISTONE CHAPERONES

10:45-11:40	**Coffee break + Poster session 4 (EVEN abstract numbers)**

	Parallel session A **Chair: F. Ochsenbein**	**Parallel session B** **Chair: M. Pons**
11:40-12:00	**G. Pintacuda** - FAST BIOMOLECULAR NMR WITH FAST MAS (WITHOUT AND WITH DNP)	**L. Fusaro** - INVESTIGATION OF THE EXTRAORDINARY SELF-ASSEMBLY OF A SIMPLE ORGANIC SALT BY MULTINUCLEAR NMR IN LIQUID-STATE
12:00-12:20	**A. Gallo** - STRUCTURAL BASIS FOR THE INTERACTION BETWEEN A PEPTIDYL CARRIER PROTEIN AND CONDENSATION DOMAIN IN THE ENACYLOXIN HYBRID PKS-NRPS	**I. Villa** - STUDY OF MAGNETIC PROPERTIES AND SPIN DYNAMICS IN MOLECULAR MAGNETS WITH INTEGER SPIN VALUES
12:20-12:40	**V. Bernard** - MICRORNA SPONGING BY HUR	**R.A. Salvino** - NMR-BASED METHODS FOR PHARMACEUTICAL INDUSTRY: AN APPLICATION OF THE ANALYTICAL PROCEDURES LIFECYCLE CONCEPT
12:40-13:00	**F. Munari** - NMR OF PROTEIN-PROTEIN INTERACTIONS OF TAU, A KEY PLAYER OF ALZHEIMER'S DISEASE	**S. Denis-Quanquin** - CAPTURING THE DYNAMIC ASSOCIATION BETWEEN A TRIS-DIPICOLINATE LANTHANIDE COMPLEX AND A DECAPEPTIDE: A COMBINED PARAMAGNETIC NMR AND MOLECULAR DYNAMICS EXPLORATION

13:00-14:20	**Lunch**

	Plenary session **Chair: M. Geppi and C. Airoldi**
14:20-14:50	**Winner of the GIDRM/GIRM Gold Medal 2022: P. Sozzani (University of Milan)** THE DYNAMICAL WORLD OF SOLIDS
	Chair: H. Ratiney
14:50-15:35	**D. Topgaard (University of Lund)** MODEL FREE APPROACH TO THE INTERPRETATION OF RESTRICTED AND ANISOTROPIC SELF DIFFUSION IN MAGNETIC RESONANCE OF BIOLOGICAL TISSUES
15:35-16:20	**A. Gronenborn (University of Pittsburgh, USA)** THE AWESOME POWER OF FLUORINE NMR - FROM DRUGS TO CELLS
16:20-16:40	**Sponsorship Lecture (Bruker): J. Coutant** SOFTWARE NEWS - TOPSPIN, SMARTDRIVENMR AND BRUKER CHEMIST SUITE
16:40-16:50	**Sponsorship Lecture (Magritek): D. Bouillaud** LATEST UPDATES ON BENCHTOP NMR: HOW IT CAN HELP YOUR DAILY WORK ?

16:50-17:15	**Coffee break**
17:15-19:15	**Assemblies of GIDRM and GERM**
20:30-24:00	**Social Dinner**

INTEGRATING ORDER AND DISORDER IN SRC CELL SIGNALLING: THE NMR APPROACH

A. Fernández,[‡] A. Lang[‡], M. Gairí, [†] M.T. González, [†] F. Cárdenas,[†] M.Pons[‡]

[‡]Biomolecular NMR Lab. Department of Inorganic and Organic Chemistry, Universitat de Barcelona (UB). Baldiri Reixac, 10-12 08028-Barcelona, Spain
[†]Centres Científics I Tecnològics de la Universitat de Barcelona (CCiTUB), Baldiri Reixac, 10-12 08028-Barcelona, Spain
E-mail: mpons@ub.edu

Keywords: solution NMR, biomolecules, fuzzy complexes, phosphorylation.

The non-receptor tyrosine kinase c-Src is the leading member of the Src family of kinases (SFK) and is involved in signaling processes regulating cell growth, migration, invasion and survival. High levels of c-Src are associated with poor prognosis in colorectal, prostate and breast-cancers. The SFK members share a common domain structure formed by three globular domains, including the kinase domain and the regulatory SH3 and SH2 domains, and a N-terminal disordered region including the lipidated SH4 domain and the Unique domain. The term "Unique" refers to the lack of homology of this region among SFK members that contrasts with the sequence conservation in the other domains. Canonical regulation of c-Src activity involves a switch between a closed/inactive state in which the SH3 and SH2 domains are packed against the kinase domain, and an open form in which the SH3 and SH2 domains are released and the kinase domain becomes active. The SH4 domain sole known role is as membrane anchor. The function of the Unique domain remains obscure.
Using NMR, our group has identified specific regions in the Unique domain predicted to be functionally important. Mutations in one of these regions, called the Unique Lipid Binding Region (ULBR), results in 50% decrease in the invasive capacity of colorectal cancer cells and a similar decrease in tumor growth rate [1].
NMR has also revealed that the entire disordered region forms a intramolecular fuzzy complex nucleated around the SH3 domain and the important role of the SH4 domain, beyond membrane anchoring, as part of the fuzzy complex even in non-myristoylated constructs [2]. In addition, NMR shows that the SH3 domain contains a myristoyl binding site that modulates membrane anchoring by competing with the insertion of the myristoyl group in the lipid bilayer [3].
The fuzzy complex is strongly affected by phosphorylation of serine and threonine residues in the Unique domain, as seen by the widespread changes observed in HSQC spectra. 31P-NMR has been used to measure the phosphorylation kinetics at the various sites and has revealed that the actual phosphorylation sites by ERK2 are modulated by allosteric interaction of the disordered regions of c-Src with the kinase, confirmed by NMR.

Acknowledgements.
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THE ROLE OF PROTEIN FOLDING MECHANISMS IN AMYLOID FIBRIL FORMATION

R. Fattorusso

Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100, Caserta, Italy
E-mail: roberto.fattorusso@unicampania.it

Keywords: solution NMR, biomolecules.

Protein misfolding disorders are caused, in most cases, by the aggregation of unstructured peptides, intrinsically disordered proteins and fragments of otherwise structured proteins. However, globular proteins, having persistent and well defined secondary and tertiary structures, have also been described to undergo aggregation causing amyloid diseases. It is clear that the possibility to form amyloid structures is not an exclusive mark of proteins directly associated with neurodegenerative diseases but is an inherent property in all polypeptide sequences Protein mutations, environmental changes, chemical modifications and metal ions have been reported to influence amyloid formation. The analysis of how these factors alter protein folding pathways, inducing the formation of stable misfolded intermediate states, may provide decisive clues about the propensity to form soluble aggregates and fibrils. [1]. Here, we describe a couple of NMR-based studies where the protein folding and misfolding mechanisms have been clearly correlated to amyloid fibril formation [1, 2, 3].

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STRUCTURE-FUNCTION STUDIES AND INHIBITOR DESIGN OF HISTONE CHAPERONES

Francoise Ochsenbein[1]

[1] CEA Saclay, Direction de la Recherche Fondamentale (DRF), Institut de Biologie Integrative de la Cellule (I2BC), SB2SM, Laboratoire de Biologie Structurale et Radiobiologie (LBSR), 91191 Gif sur Yvette cedex

E-mail: francoise.ochsenbein@cea.fr

Histone chaperones are proteins that escort store and deliver histones into the nucleus, assemble histones with the DNA, and regulate histones dynamics within chromatin[1]. These chaperones are key players for maintenance of genome and epigenome integrity. We applied integrative structural biology to unravel the action mechanism of histone chaperones involved in the deposition of histones coupled to DNA synthesis, in particular the chaperones ASF1 (anti-silence function 1) [2-4], the N-terminal tail of MCM2, the subunit 2 of the replicative helicase [5] and the Chromatin Assembly Factor 1, CAF-1 [6]. Results highlight the importance of flexible regions to handle histones and favor their interactions with DNA. Among histones chaperones, ASF1 was recently identified as a promising anticancer target [7-8]. We took advantage of the high resolution structure of the human ASF1A-histone H3-H4 complex to design inhibitory peptides and peptidomimetics through a rational design strategy combining epitope tethering, optimization of interface contacts [9]. These peptides show promising anti-tumor activity in cells and in mouse tumor grafted models. We next derived peptide-mimetics with high affinity for ASF1 that recapitulate the binding mode of the peptides and show major improvement of their stability against proteolysis [10-11]. .

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FAST BIOMOLECULAR NMR WITH FAST MAS (WITHOUT AND WITH DNP)

G. Pintacuda‡

‡ Lyon High-Field NMR Center (CNRS/ENS Lyon/UCBL), Villeurbanne, France
E-mail: guido.pintacuda@ens-lyon.fr

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

For large molecules, ultrafast (100 kHz) spinning narrows spectral resonances better than Brownian motion does for solution NMR, removing a fundamental barrier to the NMR study of large systems with ^{1}H detection. Nonetheless, performing the assignment of all resonances remains a rate-limiting step in protein structural studies, and even the latest optimized protocols fail to perform this step when the protein size exceeds ~20 kDa. We introduce two approaches that address this issue, simultaneous parallel detection[1] and projection spectroscopy of hyperdimensional datasets,[2] allowing to accelerate acquisition and data analysis and at the same time to lift the molecular size barrier of the targets amenable to NMR analysis.
We additionally discuss the applicability of ^{1}H detection methods under cryogenic conditions to perform structural studies with DNP.

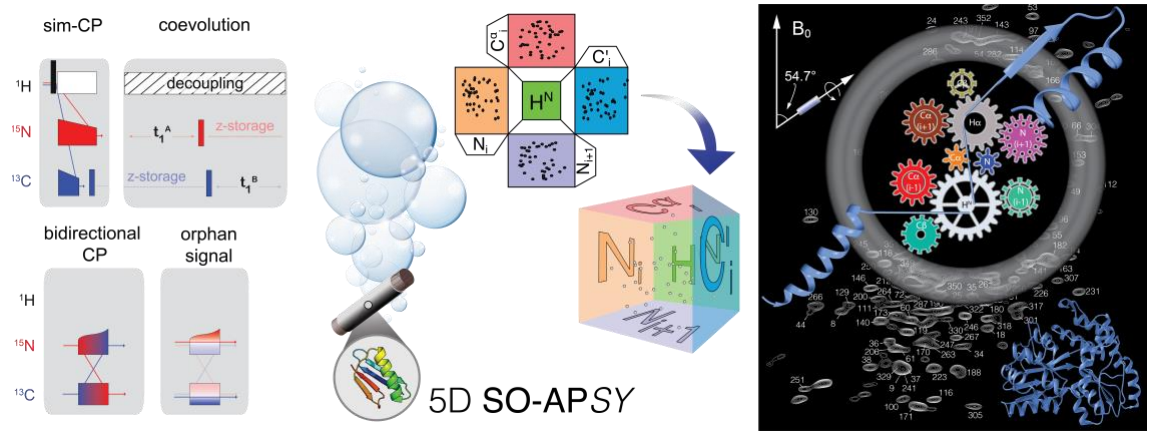

Fig. 1. Left: scheme of radiofrequency building blocks for multiple pathway coherence transfers, key for the simultaneous acquisition of a single self-consistent data set composed of multiple ^{1}H-detected 3D spectra. Middle: correlations of five resonance frequencies of nuclear spins within one NMR experiment are now feasible by using projection spectroscopy at 100 kHz MAS. Right: 100 kHz magic-angle spinning NMR allows automatic fingerprinting of large proteins, as demonstrated here on the 42.5 kDa maltose binding protein, the largest protein assigned to date in the solid state.

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STRUCTURAL BASIS FOR THE INTERACTION BETWEEN A PEPTIDYL CARRIER PROTEIN AND CONDENSATION DOMAIN IN THE ENACYLOXIN HYBRID PKS-NRPS

A. Gallo,[‡*] S. Kosol,[‡] D. Griffiths,[‡] T. Valentic,[†] J. L. Masschelein,[‡] M. Jenner,[‡] E. de los Santos,[¥] L. Manzi,[§] P. K. Sydor,[‡] D. Rea,[¥] V. Fülöp,[¥] N. J. Oldham,[§] S-C. Tsai,[†] G. L. Challis,[‡] and J. R. Lewandowski[‡]

[‡]Department of Chemistry, University of Warwick, Gibbet Hill Road, CV4 7AL, Coventry, UK
[†]Departments of Molecular Biology and Biochemistry, Chemistry, and Pharmaceutical Sciences, University of California Irvine, 517 Bison Ave CA 92697, Irvine, USA
[¥]Department of Life Sciences, University of Warwick, Gibbet Hill Road, CV4 7AL, Coventry, UK
[§]School of Chemistry, University of Nottingham, Nottingham NG7 2RD, UK
*Current address: Department of Chemistry University of Turin, via Pietro Giuria 7, 10126 Torino Italy

E-mail: angelo.gallo@unito.it

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

Modular polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are giant bacterial multi-enzymes (1-3 MDa), which biosynthesize structurally complex bioactive natural products. Modular nature of PKSs makes them suitable for bioengineering provided important factors responsible for control of the biosynthesis, such as protein-protein interactions, are considered. Here we report on protein-protein interactions implicated in the chain termination in the biosynthesis of enacyloxin; an antibiotic that is active against multidrug-resistant *A. baumannii*: the number one organism on the list of the WHO priority pathogens. [1]
Combining solution NMR, solid-state NMR, mass spectrometry based carbene footprinting, X-ray crystallography and molecular dynamics (MD) we are able to elucidate the interactions between a peptidyl carrier protein (Bamb_5917 PCP, ~11 kDa) and a condensation domain (Bamb_5915 C, ~56 kDa).
Solution NMR and carbene footprinting studies show that interaction between the two proteins involve the C-terminal intrinsically disordered docking domain of Bamb_5917 PCP and β-hairpin docking domain of Bamb_5915 C and as well as globular segments of the proteins. ^{15}N CEST on Bamb_5917 PCP provides clues about changes in conformation of the C-terminal intrinsically disordered region upon binding. [2] All these data suggest large conformational changes of both Bamb_5917 PCP and Bamb_5915 C upon binding, which are consistent with correlated motions observed in MD simulations. [2] Fast and ultra-fast MAS solid-state NMR of sedimented the protein complex is used to obtain an atomic resolution view of Bamb_5917 PCP within the ~70 kDa complex. Docking calculations using HADDOCK provide insights into the interactions between the two proteins and biochemical assays demonstrate that the condensation reaction critically depends on the presence of the docking domains. [2]
Our results suggest an intriguing general allosteric regulation mechanism responsible for directionality of a condensation reaction and provide a basis for a synthetic biology approach to create hybrid PKS/NRPSs systems to produce new antibiotics.

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MICRORNA SPONGING BY HUR

V. Bernard,[‡] J. Rengifo Gonzalez,[‡] K. El Hage,[‡] B. Goswami,[†] A. Bouhss,[‡] O. Maciejak,[‡] P. Ray,[†] D. Pastré,[‡] E. Steiner,[‡]

[‡]Laboratory SABNP (INSERM U 1204), Evry University, Paris-Saclay University, Evry, France
[†]Dept. of Biological Sciences, IISER, Kolkata, India
E-mail: virginie.bernard@univ-evry.fr

Keywords: solution NMR, biomolecules

Over the last decade, interest has grown towards two classes of regulatory molecules, represented by RNA-binding proteins (RBPs) and microRNAs (miRNAs). While these regulators have shown to be involved in inflammatory response and tumorigenesis, recent studies have highlighted a functional interplay among themselves,[1,2] where miRNAs and RBPs either cooperate or counteract in the regulation of shared messenger RNAs (mRNAs).

Among reported RBPs, Human Antigen R (HuR/ELAVL1) is one of such protein. Ubiquitously expressed, HuR positively regulates mRNA stability and translation by binding AU-rich elements in mRNA 3' UTR, competing with miRNAs and preventing their targeting. Moreover, HuR has been recently reported as the first RBP capable of miRNA sponging, thus repressing furthermore their function on target mRNA. To understand how HuR specificity towards miRNAs is driven, our work is focused on the interplay between the oncogenic miR-21 and HuR, which has shown to be preventing miR-21 mediated translation repression of PDCD4 mRNA, a gene controlling apoptosis during inflammation.[3]

Whereas most structural studies have been dedicated to HuR in complex with short cognate RNA,[4–6] we have investigated by NMR the mechanistic details underlying the binding to the mature miR-21 by deciphering the respective involvement of HuR RNA Recognition Motifs (RRMs), *i.e.* the residues essential for miR-21 recognition. More interestingly, NMR dynamics experiments have also highlighted the formation of a dimer along the 22 nucleotides of miR-21, which could be critical for HuR to function in cells.

We expect our results to unveil a new generalized mode of post-transcriptional gene regulation as HuR might disengage many miRNAs from their mRNA targets, making it an attractive miRNA exporter for therapeutic innovation.

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NMR OF PROTEIN-PROTEIN INTERACTIONS OF TAU, A KEY PLAYER OF ALZHEIMER'S DISEASE

F. Munari[‡], L. Mollica[†], M. D'Onofrio[‡], S. Capaldi[‡], M. Assfalg[‡].

[‡]Department of Biotechnology, University of Verona, Strada Le Grazie 15, Verona, Italy.
[†]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.
E-mail: francesca.munari@univr.it

Keywords: solution NMR, biomolecules.

Tau is a microtubule associated protein that promotes the assembly and stability of microtubules, with important roles in regulating axonal stability and transport, neurite outgrowth and synaptic function. In Alzheimer's disease Tau converts into toxic aggregated species and self-assembles into filaments that accumulate within neurons [1]. Enhancing the cellular pathways that promote the removal of abnormal proteins from neurons represents a valid strategy to limit the accumulation of aggregated tau and its neurotoxicity. A key mechanism governing protein turnover is the covalent attachment of ubiquitin multimers to target proteins that work as signal for their proteasomal-degradation [2]. The ubiquitin-dependent degradation of Tau is controlled by CHIP (carboxyl terminus of Hsp70 interacting protein), an E3 ligase that target misfolded proteins for degradation [3].
Despite the pivotal role of CHIP in the clearance of Tau, structural aspects describing the mechanism of their molecular recognition is still unknown. In this study, by using high resolution nuclear magnetic resonance combined to biochemical and computational methods, we elucidate the structural basis governing the multi-domain dynamic interaction between the ligase and its intrinsically disordered substrate Tau [4].

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INVESTIGATION OF THE EXTRAORDINARY SELF-ASSEMBLY OF A SIMPLE ORGANIC SALT BY MULTINUCLEAR NMR IN LIQUID-STATE

L. Fusaro

Namur Institute of Structured Matter (NISM) University of Namur, Namur, Belgium
E-mail: luca.fusaro@unamur.be

Keywords: (solution NMR, small molecules)

Recently, we have isolated four new crystalline phases of fampridine hydrochloride (4-APH+Cl−), a simple organic compound used for the symptomatic treatment of multiple sclerosis [1]. The four crystalline phases comprise two analogous sub-hydrate forms 4-APH+Cl−·1/12H_2O (phase **1**) and 4-APH+Cl−·1/90H_2O (phase **2**), a monohydrate, 4-APH+Cl−H_2O (phase **3**) and an anhydrous form, 4-APH+Cl− (phase **4**).

Of particular interest was the observation of phases **1** and **2** (see Fig 1), featuring polyhedral molecular self-assemblies similar to those observed in clathrate hydrates, and a large number of molecules in the asymmetric unit (Z'=30 and 15, respectively). This serendipitous discovery represented the first observation in small organic molecules of Frank-Kasper (FK) phases [2], a particular family of topologically close-packed structures identified more than 50 years ago in metal alloys and, over the recent years, also observed in a variety of systems.

To broaden the understanding of how such a simple molecule may crystallise as an FK phase, we monitored the crystallization of complex phase **1** and simple phase **3** by liquid-state NMR, to identify any differences in the composition, intermolecular interactions and aggregation between the precursors of phase **1** and **3**.[1] In particular, the samples were investigated by 1H, 13C, 14N, and 35Cl NMR as a function of the concentration of 4-APH+Cl− until the moment when precipitation of the crystalline phases occurred. Variations of chemical shifts, T1 relaxation times of 13C signals, and full-width at half-maximum of the signals of quadrupolar 14N and 35Cl nuclei were measured. The results indicate the formation of different self-assembled clusters prior to the nucleation of complex phase **1** and simple phase **3.**

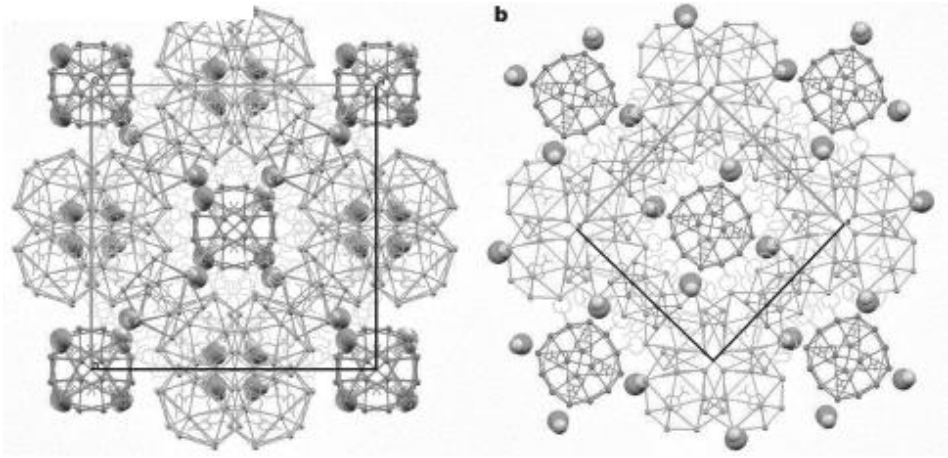

Fig. 1. Crystal packing and self-assembly of phases **1** and **2**.

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STUDY OF MAGNETIC PROPERTIES AND SPIN DYNAMICS IN MOLECULAR MAGNETS WITH INTEGER SPIN VALUES

I. Villa[1], F.A. Rusnati[2], S. Sanna[3], F. Adelnia[4,5], A. Radaelli[2], M. Mariani[5], A. Chiesa[6], C. Sangregorio[7], R. Winpenny[8], G. Timco[8], S. Carretta[6], F. Borsa[5] and A. Lascialfari[5]

[1]Department of Clinical, Surgical and Paediatric Sciences, and INFN, Università degli studi di Pavia, Pavia, Italy
[2]Department of Physics, Università degli studi di Milano, Milano, Italy
[3]Department of Physics and Astronomy, Università degli studi di Bologna, Bologna, Italy
[4]Vanderbilt University Medical Center, Nashville, Tennessee (US)
[5]Department of Physics, Università degli studi di Pavia, INFN and INSTM, Pavia, Italy
[6]Department of Mathematical, Physical and Computer Sciences, Università degli studi di Parma, Parma, Italy
[7]ICCOM-CNR and INSTM, Sesto F.no (FI), Italy
[8]School of Chemistry, Manchester University, Manchester (UK)
E-mail: ilaria.villa@unipv.it

Keywords:
materials, small molecules

In the present work, we investigate the spin dynamics of one-dimensional spin integer single-molecule magnets (SMMs) $((CH_3)_2NH_2)V_7MF_8(O_2C^tBu)_{16}2C_7H_8$, namely V_7M (M=Ni, Mn) [1,2,3] through magnetization, susceptibility and Muon Spin Rotation (MuSR) measurements. These heterometallic nanomagnets contain seven vanadium ions (spin $s=1$) and one Ni^{2+} ($s=1$) or Mn^{2+} ($s=5/2$) ion, arranged in the form of regular rings. In the case of closed periodic spin chains, a discrete energy levels structure is expected due to quantum properties [4,5]. The theoretical studies of rings with a finite number of integer spins indicate a gapped ground state but also a significant deviation from the Landé rule (i.e. $E(S) = (P/2)\cdot S\cdot(S+1)$ and $P = 4J/N$, with S=total spin and J=exchange constant), valid for semi-integer spins. It is worth mentioning that the infinite spin-integer chain exhibits a topological Haldane gap between the ground state and the first excited state [6]. The ground state of V_7Ni and V_7Mn is expected to be antiferromagnetic, as the analogue SMM V_7Zn [1,2,7], and the average exchange coupling constant among the nearest neighbour magnetic ions is estimated to be of the order of a few Kelvin degrees. Susceptibility and magnetization measurements at low temperatures display anisotropy effects when an external magnetic field is applied. The muon longitudinal relaxation rate λ at magnetic fields $\mu_0H\geq500$ G as a function of temperature in the range $1.5<T<100$ K follows a heuristic Bloembergen-Purcell-Pound (BPP) model [8]. On the other hand, at lower field $\mu_0H=300$ G, the intensity of the curve $\lambda(T)$ does not rescale as expected from the BPP model, indicating the possible presence of an internal magnetic field of the order of hundreds Gauss. No effect related to a topological gap was evinced.

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NMR-BASED METHODS FOR PHARMACEUTICAL INDUSTRY: AN APPLICATION OF THE ANALYTICAL PROCEDURES LIFECYCLE CONCEPT

R.A. Salvino,[‡] G. Boccardi,[‡] L. Mauri,[‡] D. Paganini,[‡] M. Mandalari,[‡] C. Cosentino,[‡] M. Guerrini[‡]

[‡]Institute for Chemical and Biochemical Research "G. Ronzoni", via G. Colombo 81, 20133 Milan, Italy
E-mail: salvino@ronzoni.it

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

Specifications and analytical procedures are one part of the control strategy of the quality of pharmaceutical substances and products. According to the old concept, procedure parameters were frozen at the end of analytical development, evaluated during validation, and then tightly applied in routine analysis. Since more than a decade this concept was overcome by the idea of a lifecycle of the analytical procedure, now adopted by the ICH Q14 guideline published for comments, giving more flexibility at each change of the product process, of the analytical parameters or of the Analytical Target Profile [1]. The new landscape is much more favorable to the continuous improvement required by advanced techniques like NMR where it inconceivable to use frozen methods for more than few years.
In this contribution the case of an NMR-based analytical method developed by the "G.Ronzoni" research institute for the quantitative study of heparins is presented [2-4]. The development and validation steps of the published method will be briefly provided and used as basis for the discussion of the lifecycle of the 2D NMR analytical method [3]. Key features of the method were a control strategy based on a System Suitability Test (SST) ensuring suitable shimming and sensitivity and flexible setting of the number of scans to meet SST criteria and to allow method transfer to different spectrometers. Changes suggested by experience in routine analysis were implemented and the possibility of improving NMR- acquisition and processing conditions was explored. The HSQC method was rediscussed with general considerations and with experimental data: pulse sequence, acquisition parameters and the use Non-Uniform Sampling were the factor investigated also by introducing DoE strategy.
The aim of the method moved gradually from a research method with no acceptance criteria to a procedure to confirm production consistency and to a candidate method for batch release: this required the formulation of a suitable Analytical Target Profile based on past performance and on possible specification setting. A protocol for method revalidation will be discussed according to the elements of the new ICH guideline Q2(R2) [5].

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CAPTURING THE DYNAMIC ASSOCIATION BETWEEN A TRIS-DIPICOLINATE LANTHANIDE COMPLEX AND A DECAPEPTIDE: A COMBINED PARAMAGNETIC NMR AND MOLECULAR DYNAMICS EXPLORATION

S. Denis-Quanquin[‡], O. Maury[‡], F. Riobé[‡], E. Dumont[‡] & N. Giraud[†]

[‡] UMR 5182, Laboratoire de Chimie, ENS Lyon (ENS/CNRS), 46 allée d'Italie, 69364 Lyon7, France
[†] Université Paris Cité, CNRS, Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologiques, F-75006 Paris, France
E-mail: sandrine.denis-quanquin@ens-lyon.fr

Keywords: solution NMR, small molecules, biomolecules

Lanthanides complexes show very interesting properties for protein structure determination. They may be used as luminescent bioprobes or as anomalous scatterers for diffraction studies and for as non covalent paramagnetic tags for NMR experiments [1]. Tris-dipicolinate lanthanide complexes $[Ln(DPA)_3]^{3-}$ is one of the first system of its kind whose ability to develop supramolecular interactions with proteins has been reported.

We will present the study of the interaction between a lanthanide probe $[Ln(DPA)_3]^{3-}$ and a intrinsically disordered decapeptide (see Fig. 1) using both NMR experiments (PCS, PRE, diffusion measurements, T1 measurements) and molecular dynamics simulations [2]. Although a rather trivial, electrostatically driven interaction was expected, the combination of experimental and calculated data reveals a highly dynamic association process. It provides extensive insights into the interaction sites and their occupancy. This study highlights the importance of a large conformational sampling to reconcile characteristic time in NMR with molecular dynamics simulations, where sampling in the microsecond range is needed. This study thus opens the door for a detailed mechanistic elucidation of the early steps of lanthanide complex-peptide interaction or self-assembly processes.

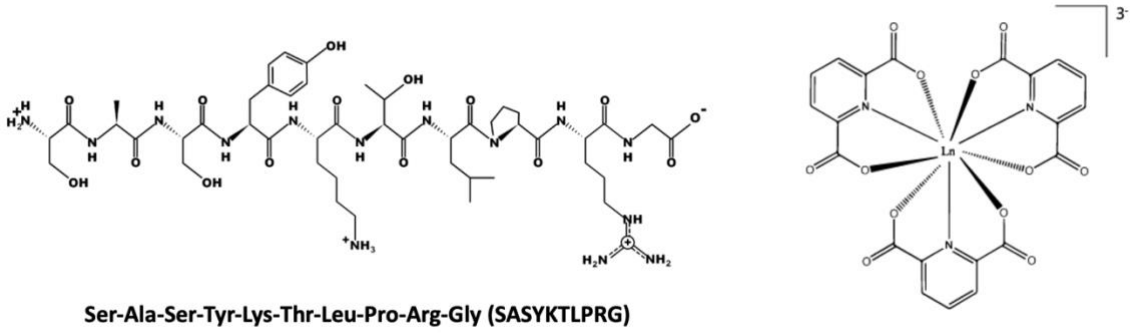

Fig. 1. Decapeptide sequence (on the left) and lanthanide complex $[Ln(DPA)_3]^{3-}$ (on the right)

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THE DYNAMICAL WORLD OF SOLIDS

Piero Sozzani

Department of Materials Science, University of Milano Bicocca, Italy
E-mail: piero.sozzani@unimib.it

Solids are usually conceived as passive pieces of matter which are scarcely prone to adapt to the environment. Solid state NMR can provide an alterative view by recognizing molecular reorientation within the correct timescale. Motion can be either spontaneous or induced by responsiveness to irradiation or other stimuli, yielding rapidly reorientable rotors, switches and even motors. Additionally, porous solids provide the playground to manipulate polymer chains and gases. This fascinating area encompasses several multinuclear and multidimensional NMR approaches, which span from multinuclear and multidimensional experiments to motion-modulated anisotropic profiles and relaxation phenomena. Additionally, porous matrices can imprint anisotropy to diffusing-in gas spectra.

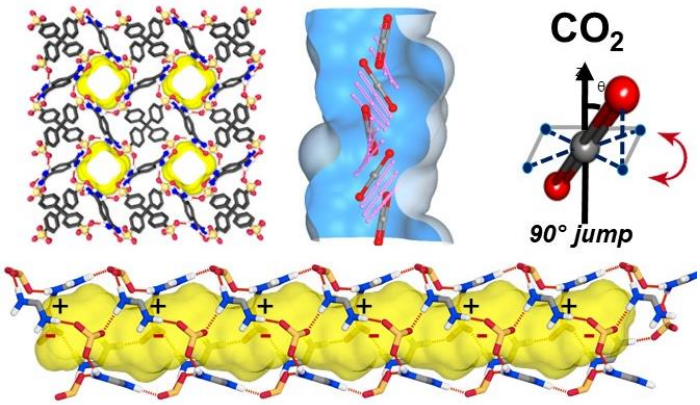

Fig. 1. Dynamics of CO_2 in nanochannels.

Results collected during the last few years are indicative of a rich variety of intriguing phenomena, such as CO_2 diffusion and reorientation in porous crystals (Fig. 1)[1].
Unique examples of recognition of interaction sites of gases within solid matrices were shown by multinuclear 2D NMR, highlighting through-space magnetization transfer.

Hyperpolarized xenon, exploring multiple cavities in MOF mixed crystals, showed in a unique way the homogeneity of photoactive ligand architecture [2]. 3D structure of ultrafast rotor-containing Fe-MOFs were described unconventionally from the paramagnetic sphere-of-influence of the metal nodes [3].The detection of rotary motion at very low temperature is a challenge, nevertheless we recorded fast rotation in the MHz regime even at 1.6 K - 4 K by multinuclear T_1 relaxation times [4]. Rapid relaxation rates at such low temperatures remark the unprecedented behaviour of solid materials. A complete development of Kubo-Tomita from 2 K to 5 K reveals a negligeable activation energy 0.024 kcal/mol for rotor revultion, allowing for several unidirectional turns unaffected by the minimal thermal energy at a few kelvin[5].

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MODEL-FREE APPROACH TO THE INTERPRETATION OF RESTRICTED AND ANISOTROPIC SELF-DIFFUSION IN MAGNETIC RESONANCE OF BIOLOGICAL TISSUES

D. Topgaard,[‡]

[‡]Lund University, SE-22100 Lund, Sweden
E-mail: daniel.topgaard@fkem1.lu.se

Keywords: MRI, materials, theory and methods.

Magnetic resonance imaging (MRI) is the method of choice for noninvasive studies of micrometer-scale structures in biological tissues via their effects on the time/frequency-dependent ("restricted) and anisotropic self-diffusion of water. Traditional MRI relies on pulsed magnetic field gradients to encode the signal with information about translational motion in the direction of the gradient, which convolves fundamentally different aspects—such as bulk diffusivity, restriction, anisotropy, and flow—into a single effective observable lacking specificity to distinguish between biologically plausible microstructural scenarios [1]. To overcome this limitation, we introduce a formal analogy between measuring rotational correlation functions and interaction tensor anisotropies in nuclear magnetic resonance (NMR) spectroscopy and investigating translational motion in MRI [2], which we utilize to convert data acquisition and analysis strategies from NMR of rotational dynamics in macromolecules [3] to MRI of diffusion in biological tissues, yielding model-independent quantitative metrics reporting on relevant microstructural properties with unprecedented specificity. This lecture includes the basic physical principles of using spectrally modulated gradient waveforms [4] to separate and correlate specific aspects of translational motion, as well as examples of applications to liquid crystals, yeast cells, tumors, and brains.

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THE AWESOME POWER OF FLUORINE NMR - FROM DRUGS TO CELLS

Angela M. Gronenborn

Department of Structural Biology, School of Medicine, Department of Bioengineering, Swanson School of Engineering, Department of Chemistry, Dietrich School of Arts and Sciences, University of Pittsburgh, Biomedical Science Tower 3, Room 1051, 3501 Fifth Avenue, Pittsburgh, PA 15260, USA
E-mail: amg100@pitt.edu

Keywords: solid state NMR, solution NMR

Nuclear magnetic resonance (NMR) spectroscopy is a versatile tool for probing structure, dynamics, folding, and interactions at atomic resolution. While naturally occurring magnetically active isotopes, such as 1H, 13C, or 15N, are most commonly used in biomolecular NMR, with 15N and 13C isotopic labeling routinely employed at the present time, 19F is a very attractive and sensitive alternative nucleus, which offers rich information on biomolecules in solution and in the solid state. This presentation will summarize the unique benefits of solution, solid-state and in-cell 19F NMR spectroscopy for the study of biomolecular systems. Particular focus will be placed on the most recent studies and on unique and important potential applications of fluorine NMR methodology.

RECENT ADVANCES IN SELECTIVE EXCITATION

J. Coutant[‡]

[‡]Bruker France, 34 rue de l'industrie 67160 Wissembourg France
E-mail: jerome.coutant@bruker.com

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

Selective excitation is often used in structure elucidation by solution state NMR. It enables to excite a specific signal while leaving other resonances untouched, making it possible to get information on a particular spin in an organic molecule. However, in case of severe overlap between signals, single excitation cannot be performed without perturbing neighbouring resonances.
Recently, several methods have been proposed which provide efficient tools for selective excitation:
Kiraly *et al.*[1] have introduced a new NMR experiment called Gradient-Enhanced Multiplet-Selective Targeted-Observation NMR Experiment (GEMSTONE) to excite a specific proton signal from an overlapped multiplet.
Jenne *et al.*[2] have developed the technique named Designed Refocused Excitation And optional Mixing Targets In vivo and Mixture Elucidation (DREAMTIME), which allows to selectively excite and detect the compound(s) of interest inside a complex mixture.
Implementation of both of these methods in TopSpin will be described, and practical examples will be shown.

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Latest updates on Benchtop NMR: how it can help your daily work?

Dylan Bouillaud[†], Jürgen Kolz[†], Federico Casanova[†]

[†]Magritek GmbH, Philipsstrasse 8, 52068 Aachen, Germany
E-mail: dylan@magritek.com

Keywords:
solution NMR, low field NMR, instrumentation, exotica.

With the launching of the Spinsolve 90 ULTRA Multi X, Magritek has taken another step towards achieving even higher resolution, versatility, and sensitivity on the bench. These powerful magnets are now available with automatic multinuclear probes which make it possible to measure multiple nuclei on the same instrument.
In this lecture, the latest Spinsolve 90 benchtop NMR applications will be shown with extensive examples of NMR data (e.g. 1D and 2D NMR structure verification of Brucine) highlighting the ever-increasing potential of these systems. The Spinsolve 90 has the highest sensitivity that enables advanced experiments, such as HSQC-ME, to be acquired in just 2 minutes. It´s unparalleled magnet design enables highly efficient solvent suppression performance making it possible to measure samples in protonated solvents as they come from the reactor. The versatility of the Spinsolve Multi X probes, which can automatically switch between several X-nuclei, like ^{13}C, ^{31}P, ^{7}Li, ^{29}Si, (among others) will be shown. Thanks to the compactness and portability of these systems, a wide range of applications e.g. on-line monitoring of chemical reactions or qNMR studies are possible as the NMR spectrometers can directly be installed next to the chemical reactors or in the production sites [1,2].

Fig. 1. Spinsolve 90, the fastest compact NMR spectrometer

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FRIDAY SEPTEMBER 30TH

	Plenary session Chair: D. Topgaard
9:00-9:45	**J. Martins (University of Gent, Belgium)** AN NMR VIEW ON ANTIMICROBIAL CYCLIC LIPOPEPTIDES FROM PSEUDOMONAS
9:45-10:05	**Sponsorship Lecture (Extrabyte) – S. Sykora** DEVELOPMENTS IN THE EVALUATION OF DOSY DATA
10:05-10:45	**Best poster awards**

10:45-11:15	**Coffee break**

	Parallel session A Chair: R. Fattorusso	Parallel session B Chair: A. Gronenborn
11:15-11:35	**M.E. Di Pietro** - PLAYING AROUND WITH HYDROPHOBIC (DEEP) EUTECTIC SOLVENTS: WHAT WE CAN LEARN FROM NMR	**X. Falourd** - REVISITING 1H->13C POLARIZATION TRANSFER KINETIC TO INVESTIGATE INTERACTIONS IN POLYSACCHARIDE ASSEMBLIES
11:35-11:55	**A. Ducroix** - DYNAMICS OF WATER INSIDE BOEHMITE SUSPENSIONS PROBED BY FAST-FIELD CYCLING NMR	**T. Cartlidge** - PREDICTING SPIN RELAXATION IN POROUS MEDIA OF ARBITRARY COMPLEXITY
11:55-12:15	**T. Poumeyrol** - ^{1}H-^{1}H RESIDUAL DIPOLAR COUPLING MEASUREMENT FROM MULTIPLE-QUANTUM BUILD-UP EXPERIMENTS AT LOW MAGNETIC FIELD IN RUBBER INDUSTRY	**C. Lhoste** - ONLINE MONITORING OF AN ORGANOCATALYTIC REACTION BY BROADBAND ULTRAFAST 2D NMR IN FLOW CONDITIONS
12:15-12:35	**K. Bagheri** - STATE OF CHARGE OF THE LI-ION BATTERY ELECTRODES FROM THE DISTORTION OF THE 1H NMR SPECTRUM OF THE LIQUID ELECTROLYTE	**A. Briot-Dietsch** - UNTARGETED NMR ANALYSES OF PFAS IN POLLUTED ENVIRONMENTAL IN POLLUTED MEDIA

12:35-12:50	**Closing**

12:50-14:00	**Lunch**

	Satellite Event : NMR in industrial applications Chair: C. Marchioro
14:00-14:25	**F. Reniero** - NMR ANALYSIS IN THE FRAME OF CUSTOMS CONTROLS
14:25-14:50	**V. Gallo** - NMR-BASED COMMUNITY-BUILT ANALYTICAL SYSTEMS IN FOOD CONTROL AND QUANTITATIVE ANALYSIS
14:50-15:15	**F. Berti** - NMR CHARACTERIZATION OF POLYSACCHARIDE-BASED VACCINES
15:15-15:40	**E. Moro** - CONFORMATIONAL ANALYSIS IN DRUG DESIGN: A SYNERGIC APPROACH OF NMR & COMPUTATIONAL CHEMISTRY
15:40-16:05	**L. Duciel** - RESCUE 3: VERSATILE DECISION-MAKING TOOL FOR NMR SPECTRAL ASSIGNMENTS OF PROTEINS
16:05-16:30	**D. Besghini** - LF-TD-NMR FOR THE DEVELOPMENT OF PRINTING BLANKETS

AN NMR VIEW ON ANTIMICROBIAL CYCLIC LIPOPEPTIDES FROM PSEUDOMONAS

J.C. Martins[‡]

[‡] Dept. Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281 S4, 9000 Gent
E-mail: jose.martins@UGent.be

Keywords: solution NMR, small molecules, biomolecules.

Pseudomonas are ubiquitous bacteria and outstanding producers of bioactive secondary metabolites in support their eclectic lifestyle (e.g., iron scavenging, swarming motility, biofilm formation, pathogenicity, cooperation or antagonism)[1]. Of these metabolites, cyclic lipopeptides ¬ – CLIPs in short – have enjoyed increasing attention because of their antimicrobial activity profile and anti-proliferative properties, which holds some potential for biomedical applications[2]. Their biosynthesis through non-ribosomal peptide synthetases creates a large structural diversity, introducing opportunities to learn how Nature uses the same molecular blueprint to generate a swiss-army like diversity of effects.[2]

It is generally accepted that CLiPs exert their effects through perturbation and/or permeation of the cellular membrane [2]. Thus, the conformation needs to be investigated under membrane mimicking circumstances rather than merely aqueous solution. The determination of the conformation of various CLiPs in such conditions as well as the investigation of location and orientation of the CLiP across the water/lipid interface using NMR and modelling, and insight derived therefrom will be discussed [3]. In particular, the added value generated by the possibility to biosynthetically introduce ^{13}C and ^{15}N isotope labelling using the Pseudomonas own NRPS will be highlighted. Their insertion allows the application of NMR methodologies allowing to extract conformations sensitive scalar couplings while directly identifying and monitor hydrogen bonds under a variety of conditions. Using these, conformational changes upon insertion in DPC or SDS micelles can be investigated.

The accidental discovery of the self-assembly of certain CLiPs into supramolecular structures in low polarity solvents will also be touched upon. While it remains unclear if their formation is of biological relevance, it proved a rather interesting playground for its investigation by diffusion based methods and heteronuclear relaxation studies at natural abundance [4,5].

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DEVELOPMENTS IN THE EVALUATION OF DOSY DATA

S. Sykora[1], M. Abele[2], G. Selva[1], E. M. Vasini[1]

[1] Extra Byte, p.zza Mazzini 80, 20022 Castano Primo (MI) Italy
[2] Evonik Operations GmbH, Smart Materials, Untere Kanalstrasse 3, 79618 Rheinfelden, Germany
e-mail: sykora@extrabyte.eu

Keywords:
NMR, spectroscopy, DOSY, diffusion, mixture analysis, multinuclear, Bayesian, methods, software, algorithms

NMR DOSY (diffusion- ordered spectroscopy) is an NMR technique employing magnetic field gradient pulses strategically coordinated with an RF pulse sequence generating a spin echo of some type. The principle [1] and the salient computational and experimental aspects [2,3] of DOSY are well known since a long time. The outcome of a DOSY experiment is 2D array with the usual chemical shifts along the 'spectral' X-axes and the diffusion constant value along the 'diffusion' Y-axis. When the sample contains a mixture of compounds (molecules) with different diffusion coefficients, each compound's spectrum appears aligned along a different horizontal trace, thus permitting a virtual separation of the mixture components.

A widespread application of DOSY is somewhat hindered by the following obstacles:

1. One needs a special probehead equipped with the gradient-generating coils.
2. With some older evaluation algorithms, data evaluation of COSY spectra was lengthy
3. The quantitation of the DOSY components is often rather approximate.
4. Evaluation encounters problems in spectral areas where the spectra of two or more components of the mixture overlap (multi-exponential decays).

Starting from an older version of Bayesian DOSY approach [4], we have developed a considerably more refined software algorithm the that greatly alleviates obstacles (2 and 3), making the DOSY transform lightning fast, void of undesired artefacts, and quite well normalized. We have also combined it with features such as automatic spectra apodization, phasing, baseline correction, and generation of all kinds of horizontal and vertical cuts and projections. Particular attention was paid to the vertical width of the DOSY cross peaks (we can separate up to 8 distinct peaks per one decade along the diffusion constant projection).

Another substantial boost in the power of DOSY studies is more methodological. It consists in measuring two DOSY spectra of the same sample, using two different nuclides X and Y (for example X = 1H and Y = 29Si), and then comparing the two DOSY spectra against each other. Obviously, the diffusion constant of a given molecule must be the same regardless of the nucleus used to measure it. Our special MnDOSY (multi-nuclear DOSY) software does all the required preprocessing of the individual X and Y DOSY spectra, as well as the final correlation between 2D cross-peaks in the (X,Y) pair. This considerably reduces the incidence of spectral peaks overlaps (obstacle 4), and also facilitates use of the DOSY spectra for joint assignments.

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PLAYING AROUND WITH HYDROPHOBIC (DEEP) EUTECTIC SOLVENTS: WHAT WE CAN LEARN FROM NMR

Maria Enrica Di Pietro,[‡] Giselle de Araujo Lima e Souza,[‡] Valeria Vanoli,[‡] Matteo Busato,[†] Giorgia Mannucci,[†] Paola D'Angelo,[†] Franca Castiglione,[‡] Andrea Mele[‡]

[‡]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, 20133 Milan, Italy
E-mail: mariaenrica.dipietro@polimi.it
[†] Department of Chemistry, University of Rome "La Sapienza", 00185 Rome, Italy

Keywords: solution NMR, materials, HRMAS.

Deep eutectic solvents (DES) are sustainable materials that gained the attention of the scientific community over the last two decades. Among them, the recently introduced hydrophobic non-ionic type V (deep) eutectic solvents (HES) [1] are emerging as the rising stars. Compared to conventional DES, HES typically display lower viscosities, are chloride-free, and can be used in applications where even low water contents represent an issue [2]. As a further step, we recently developed a simple one-step procedure to immobilize HES in a gel structure by adding low-molecular-weight gelators, obtaining hydrophobic eutectogels (HEG) with a distinguished solid-like behavior.
If considerable progress has been achieved in the understanding of the intermolecular interactions within traditional hydrophilic DESs (mainly based on H-bonds), very little is known for HES (where dispersive forces may play a significant role) [3] and no study is available for HEG so far.
Here I show how the application of liquid-state and high-resolution magic angle spinning (HRMAS) NMR methods can help in shedding light into the intermolecular network in both liquid and semi-solid samples. By a rational choice of the components, it is possible to alter the balance between H-bond and dispersive forces, thus offering guidelines for a proper design of HES. The understanding of such interactions is also crucial when selecting the HES to form a supramolecular gel, as their interplay leads to a peculiar diffusive behavior in the studied HEG.

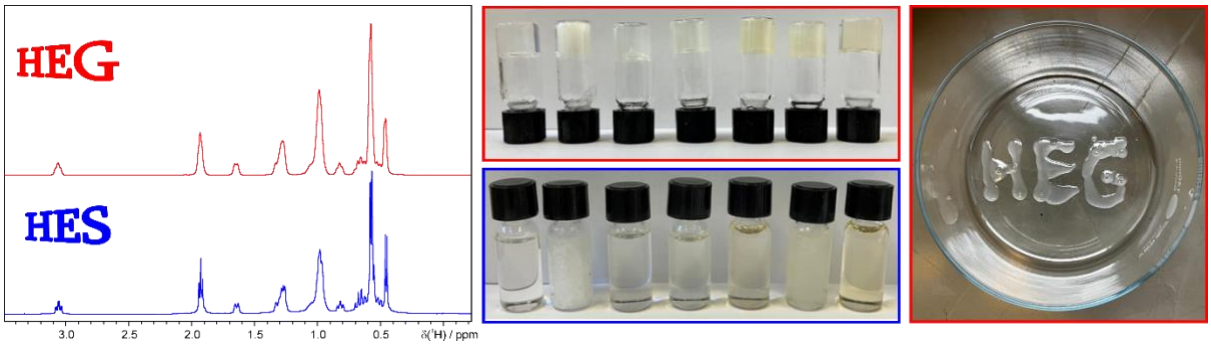

Fig. 1. (a) Liquid-state and HRMAS ^{1}H spectra of a representative HES and HEG, (b) pictures of the full set of developed HES (bottom) and HEG (top) ad (c) photograph of a representative HEG.

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DYNAMICS OF WATER INSIDE BOEHMITE SUSPENSIONS PROBED BY FAST-FIELD CYCLING NMR

Alice Ducroix[‡†], Thibaud Chevalier[‡], Jan Verstraete[ǂ], Pierre Levitz[†], Anne-Laure Rollet[†]

[‡] IFP Energies Nouvelles, 1 et 4 avenue de Bois-Préau, 92852 Rueil-Malmaison, France
[†] Laboratoire PHENIX, Sorbonne Université, CNRS, 4 place Jussieu 75005 Paris, France
[ǂ] IFP Energies Nouvelles, rond-point de l'échangeur de Solaize, BP 3, 69360 Solaize, France
E-mail: alice.ducroix@sorbonne-universite.fr

Keywords: low field NMR, materials

γ-Alumina is a widely used heterogeneous catalytic support, particularly in the field of petrochemistry and biochemistry. To enhance its catalytic performance, it has been the subject of many studies on the optimization of its textural, mechanical and physico-chemical properties [1–3]. To perfect this optimization, the accessibility of the molecules to active sites and more generally the molecular transport inside the support need to be fully assessed. Thus, interactions between surface and reactant and phenomena occurring at the surface will be better understood. As γ-alumina is obtained through a topotactic transformation, most of its properties are inherited by the boehmite and its process [4]. The peptization, where the agglomerates of the raw boehmite powder break into aggregates and then reorganize themselves, is one of the key steps for the final structure and properties of the support [5].

In this work, we perform a multi-scale analysis of the water dynamics inside boehmite suspensions. Global diffusion is assessed on a second-to-millisecond time scale by PFG-NMR while dynamics at the interface and particularly bulk mediated surface diffusion is probed by Fast-Field Cycling NMR (NMRD) on a microsecond time scale. We especially study the influence of the aggregates organization on the solvent dynamics. To this aim, several boehmite suspensions have been prepared by varying the ionic force and the volume fraction of boehmite. At the microsecond time scale, the prepared boehmite suspensions display almost identical NMRD profiles. It means that the long-range geometry of the system does not seem to influence the dynamics at this timescale. Furthermore, we were able to prove that the observed water dynamics comes from the adsorption layer (first few water monolayers from the surface). NMRD is not probing the dynamics at the interface between water from the bulk and the adsorption layer as anticipated but rather the surface dynamics. At this scale, the water dynamics seem to be controlled only through strong interactions with the surface of aggregates. Hence, we found that surface diffusion of water in boehmite is slow. Recently, it has been shown that PFG-NMR diffusion can be separated into a surface and an in-pore diffusion [6], NMRD information is thus of interest to better understand the global dynamics probed by PFG-NMR.

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^{1}H-^{1}H RESIDUAL DIPOLAR COUPLING MEASUREMENT FROM MULTIPLE-QUANTUM BUILD-UP EXPERIMENTS AT LOW MAGNETIC FIELD IN RUBBER INDUSTRY

T. Poumeyrol,[†] H. Ménil,[†] S. Mayer,[†] M. Couty[†]

[†] Manufacture Française des Pneumatiques MICHELIN, Centre de Ladoux, 23 place des Carmes, 63000 Clermont-Ferrand, France
E-mail: thomas.poumeyrol@michelin.com

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

Tyres are an assembly of tens of individual pieces, each of them being a complex composite material designed to reach specific properties dedicated to its function. Among the structural parameters at the molecular level which can be modified to reach the desired mechanical properties, the length of elastically effective chains in elastomer appears as one of the most important. The density of crosslinks and trapped entanglements made during the vulcanization process must be monitored either to fine tune the material composition and improve the properties or for quality control purpose.
The measurement of homonuclear ^{1}H-^{1}H Residual Dipolar Coupling in elastomers using Multiple-Quantum polarization build-up as proposed by K. Saalwächter [1] has appeared as a breakthrough for the study of elastomer chain networks and has been widely applied in rubber science. However, an application of such a measurement in an industrial context needs some improvements: the experimental duration has to be low to study a large amount of samples, the precision of the measured parameter must be high and well known and the method must be easy to use to make it accessible to the widest range of users.
This communication will show that the experimental duration can be highly reduced by avoiding the use of phase cycling to select the desired coherence pathways and a measurement uncertainty can be computed for each sample. The final method leads to a better understanding of interactions between components in the composition of the composite materials and helps material designers to improve their conception rules.

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STATE OF CHARGE OF THE LI-ION BATTERY ELECTRODES FROM THE DISTORTION OF THE ^{1}H NMR SPECTRUM OF THE LIQUID ELECTROLYTE

Khashayar Bagheri,[‡ †] Michaël Deschamps,[‡ †] Elodie Salager [‡†]

[‡]CNRS, CEMHTI UPR3079, Université d'Orléans, Orléans, France
[†]Réseau sur le Stockage Electrochimique de l'Energie (RS2E), FR CNRS 3459, Amiens Cedex, France.
E-mail: Khashayar.bagheri@cnrs-orleans.fr

Keywords: materials, exotica, solution NMR

Electrochemical energy storage devices, such as Li-ion batteries, supercapacitors, and hybrid capacitors, are essential for the energy transition and have shown their promising application in electric cars and portable electronic products. The entire device characterization (in situ) is critical to understanding the charge and degradation processes and increasing their electrochemical performance.

In situ ^{7}Li NMR is one of the techniques of choice to attain this goal [1]. Following the lithiation state of the electrodes inside the device is difficult due to resolution and sensitivity issues, so that ^{7}Li in situ NMR was mainly used to follow graphite and silicon lithiation and lithium plating on graphite and anode-less electrodes. Although the liquid electrolyte in the battery provides a stronger and sharper NMR signal, it has not been fully exploited until now. Liquid electrolyte is usually composed of a lithium salt (e.g., $LiPF_6$) dissolved in carbonated solvents.

Herein we present our efforts to use the liquid NMR signal of the electrolyte solvent (here dimethyl carbonate, DMC) to "spy" on the electrodes while benefitting from the high sensitivity of 1H NMR. We demonstrate that the state of charge of the electrodes can be tracked indirectly (through magnetic susceptibility changes) by distortion in the DMC spectrum near the battery components. We analyze the effect of battery components such as current collectors, separators, and electrodes with various states of charge. We identify the descriptors of the DMC spectrum that are relevant for in situ studies.

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REVISITING ^{1}H –> ^{13}C POLARIZATION TRANSFER KINETIC TO INVESTIGATE INTERACTIONS IN POLYSACCHARIDE ASSEMBLIES

X. Falourd[‡], L. Rutin[‡], V. Aguié-Beghin[*], C. Rondeau-Mouro[†], M. Lahaye[‡]

[‡] INRAE, UR1268 BIA, F-44316, Nantes, France
[†] INRAE, UR1466 OPAALE, 17 Avenue de Cucillé, CS 64427, F-35044 Rennes, France
[*] Université de Reims Champagne Ardenne, INRAE, FARE, UMR A614, 51097 Reims, France.

E-mail: xavier.falourd@inrae.fr

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

The supramolecular structure of cellulose is of great interest for the pulp, paper and fiber industry and recently for renewable fuel production. The organization of the cellulose chains, as well as their integrity within the investigated biomass significantly influences its physical and chemical properties [1].
Information at the nanometric scale can be accessed by solid state NMR spectroscopy. In particular, the study of the polarization transfer ^{1}H-> ^{13}C kinetics allows measuring different relaxation times including the $T_{1\rho}^{H}$, which evaluates molecular ordering within a volume of 2 to 30 nm^3.
The VCT-CPMAS (Variable Contact Time-Cross Polarization Magic Angle Spinning) sequence used to assess molecular dynamics ($T_{1\rho}^{H}$, T_{HH} spin diffusion time) in solids is generally underused due to the associated time constraints (~23 hours of data acquisition for 20 kinetic points and ~6 hours for processing). Moreover, to extract the dynamical parameters from the experimental data, several mathematical models exist in literature, that can be very confusing for a neophyte user. These models are very rich since they give rise to several dynamical parameters. Therefore, they need a large number of experimental points for fitting analysis, which is time consuming. All these experimental constraints have motivated us to optimize the VCT-CPMAS measurements and processing, for applications in biopolymer characterizations. The present work shows results obtained for cellulose nanocrystals (CNC) by collecting more than 300 kinetic points in 32 hours for a better curve fitting [2].
By comparing cellulose of different origins and types at “native” hydration, we succeeded to demonstrate that T_{HH} was sensitive to crystal size in addition to the well-known relationship between $T_{1\rho}^{H}$ and crystallinity index. Adding variable amounts of water to CNC, an evolution of the polarization transfer kinetics was observed, which demonstrated that VCT-CPMAS analysis provides information on the establishment of the hydrogen bonds network and/or on their implication in the nanocrystals structure.
To further assess the value of this approach, binary assemblies of cellulose and glucomannan were analyzed under different conditions of hygrometry, to evaluate the limits of the method on more complex macromolecular assemblies
This approach was demonstrated to be a mean of studying interactions in macromolecular assemblies at a nanometer scale that complements other classical methods (QCM-D, contact angle…). By modeling the oscillating part, we defined three different spin diffusion times (T_{HH}) which provide information on the localization of water and the involvement of different proton pools in the hydrogen bond network

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PREDICTING SPIN RELAXATION IN POROUS MEDIA OF ARBITRARY COMPLEXITY

T. A. A. Cartlidge,[‡] T. B. R. Robertson,[‡] M. Utz,[‡] G. Pileio[‡]

[‡]School of Chemistry, University of Southampton, Southampton, UK
E-mail: taac1g17@soton.ac.uk

Keywords: solution NMR, theory and methods

NMR is a versatile technique used extensively to probe and quantify structural features of porous media. The technique is renowned for its applicability in a range of studies from the growth of regenerative tissues to the efficiency of electrodes. In some cases, the method used relies on signal attenuation of T_2 to probe the environment and quantify parameters such as restricted diffusion coefficients, porosity, and tortuosity [1]. This signal attenuation is related to the differences in the magnetic susceptibility between the solid matrix and the surrounding liquid causing localized distortions in the magnetic field. This spatially dependent demagnetization field is known to cause drastic reduction in the lifetime of T_2. In small pores, the magnetic susceptibility induced relaxation mechanism prevents any form of quantitative analysis as the lifetime of T_2 may be shorter than the required delays within the pulse sequence. Although this effect has been experimentally observed [2], there is currently no complete theory detailing the effect of the demagnetization field on relaxation rates for porous structures of arbitrary complexity. By taking a joint analytical, computational and experimental approach, this project aims to derive this theory and apply the findings to the integration of long-lived states (LLS) into diffusion NMR. This contribution outlines the steps taken towards producing a simulation framework capable of calculating the propagator of the spin Hamiltonian responsible for this susceptibility induced relaxation. Details of which include the use of μCT for digitizing the porous structure, calculation of the demagnetization field, and Monte-Carlo simulations to simulate Brownian motion. Both the simulation and experiments follow the evolution of nuclear spins diffusing through the demagnetization field during a single echo pulse sequence. The effect on T_2 is studied across varying field strengths, susceptibility differences, and pore sizes to evaluate suitable conditions under which LLS may be utilized.

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ONLINE MONITORING OF AN ORGANOCATALYTIC REACTION BY BROADBAND ULTRAFAST 2D NMR IN FLOW CONDITIONS

C. Lhoste,[‡] M. Bazzoni,[‡] J. Bonnet,[‡] K. E. Konan,[‡] A. Bernard,[‡] F.-X. Felpin,[‡] P. Giraudeau,[‡] J.-N. Dumez [‡]

[‡] Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France
E-mail: celia.lhoste@univ-nantes.fr

Keywords: solution NMR, small molecules.

Monitoring reactions is important to understand their behavior and to optimize reaction conditions. NMR spectroscopy is a powerful tool for reaction monitoring, that provides extensive structural information, requires minimal sample separation, and is non-destructive. 1D ^{1}H NMR is commonly used for monitoring, but is limited by ubiquitous peak overlap which makes compound identification and quantification more challenging. 2D NMR spectroscopy can address these two issues, but conventional experiments require longer acquisition times and this can prevent the study of reactions on timescales of less than one hour.

Ultrafast (UF) 2D NMR is a powerful approach that overcomes the speed problem of conventional 2D experiments. UF 2D NMR is based on the spatial parallelization of sub-experiments along the NMR sample tube [1] and is leading to very short experiment durations: 300 ms against at least 15 min with conventional NMR. Recently, the use of flow NMR on standard high-field NMR spectrometers has emerged as a promising tool to monitor reactions in realistic conditions. This is particularly useful for kinetic studies and reaction optimization. UF 2D NMR is a powerful tool for such real-time monitoring applications [2], but the acquisition of UF 2D NMR spectra on flowing samples is particularly challenging [3].

We have developed homonuclear UF 2D NMR experiments for online reaction monitoring by flow NMR at high field. We first achieved efficient solvent suppression that is compatible with both spatial encoding and flow. We then developed approaches to record 2D spectra with suitable spectral widths achieved using interleaving methods to address the limitations of UF 2D NMR. The interferences between sample flow and spatial encoding have been addressed exploiting multiple gradient axes. The resulting pulse sequences yield broadband UF 2D spectra in 64 seconds, for samples flowing at up to 2.5 mL/min. To assess the potential of the method, we monitored an organocatalyzed condensation reaction with the optimized ultrafast WET COSY experiments in flow conditions, thanks to a flow-tube device. The results obtained so far open many avenues for the development and application of online monitoring by UF 2D NMR in organic chemical synthesis.

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UNTARGETED NMR ANALYSES OF PFAS IN POLLUTED ENVIRONMENTAL MEDIA

A.Briot-Dietsch,[‡] L.Duciel,[‡] M-A. Delsuc[†]

[‡] Casc4de, Pôle API, 300 Bd Brant 67400 Illkirch, France
[†] IGBMC, 1 rue Laurent Fries 67404 Illkirch, France & Casc4de, Pôle API 300 Bd Brant 67400 Illkirch, France
E-mail: anne.briot-dietsch@casc4de.eu

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

Per- and polyfluoroalkyl substances (PFAS) are toxic emerging environmental pollutants. Due to characteristics of fluorine, they show interesting repellent or nonstick properties which are widely used by the industry in everyday objects. As of today, more than 12 000 highly fluorinated compounds have been inventoried by the USEPA - United States Environmental Protection Agency. They are now recognized as a major environmental and health problem of global dimension. Indeed, there is an increasing need for a target-free, quantitative, robust and easy-to handle methods to monitor fluorinated contaminants in polluted soils or water samples.
The predominant current approaches typically involve the use of high pressure liquid chromatography and mass spectrometry (HPLC-MS) but samples have to be prepared according to time-consuming specific protocols before injecting.
Based on ^{19}F NMR spectroscopy and Machine Learning (ML) techniques, our FLUOVIAL platform allows to identify fluorinated compounds from NMR spectra. It offers interesting features: a non-targeted compounds determination, almost no preparation of sample except a solvent based extraction for soil samples, a dedicated algorithm to take advantage of the information rich NMR spectra and reproducible results.

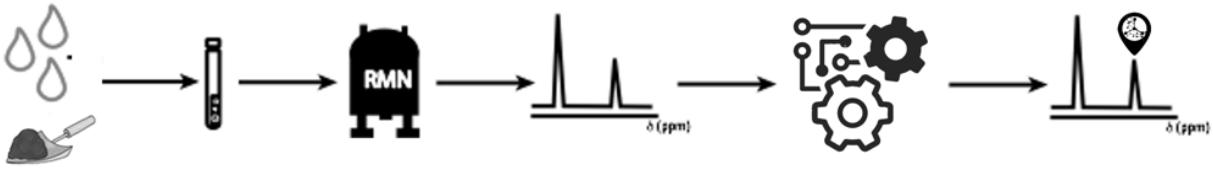

Fig. 1. FLUOVIAL approach using ^{19}F NMR and Machine Learning

To achieve acquisition, we use the OPERA pulse [1] which offers a broad excitation bandwidth and a small flip angle suitable for the observation in one experiment of the large chemical shift dispersion of ^{19}F NMR and which has been validated for quantification.
Thus, the proposed method fits in terms of detection, characterization and quantification of PFAS in polluted environmental media. Through our NMR experiments we can achieve quite easily calculation of average chain length using CF_3/CF_2 ratio, polydispersity and establish functionalization of the per- or polyfluoroalkyl chains. Further in-depth analysis of PFAS mixtures require spectral assignment which remains tricky even with the presence of some characteristic signals especially the terminal CF_3 or its adjacent CF_2 moiety and the last CF_2 near of the functional group of the molecule. The development of our analytic ML tool therefore greatly accelerates our analyses and enable a higher throughput.
Regarding performance characteristics of our method, detection (LOD) and quantification (LOQ) limits when determining PFAS concentration, first explorations exhibit promising LOD at 10 ppb and LOQ at 30 ppb of total fluorine in less than an hour. As NMR sensitivity keeps pushing forward, the method has a bright future.

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SATELLITE EVENT : NMR IN INDUSTRIAL APPLICATIONS

NMR analysis in the frame of Customs controls

F. Reniero,[‡] C.Guillou,[‡] M.Holland,[‡] G.Palmieri[†]

[‡] European Commission, Joint Research Centre, Directorate F-Health, Consumers and Reference Materials, Ispra, Italy
[†] Agenzia delle Accise, Dogane e Monopoli, DT I- Lombardia, Ufficio Antifrode, Sezione Laboratori, Milano, Italy

E-mail: fabiano.reniero@ec.europa.eu

Keywords: solution NMR, low field NMR, small molecules

Customs authorities are responsible for the control of products entering the European Community market within the frame of Regulation (EC) No 765/2008 [1]. This applies also to chemical products among which, pharmaceutical products, medicines, research chemicals and many other kinds of chemical substances.
Sophisticated analytical equipment such as high-resolution NMR spectrometers can be required for the identification of new chemical structures or verification of customs declaration of certain substances. The majority of customs laboratories is not equipped with such instrumentation.

In agreement with DG TAXUD, the JRC (Joint Research Centre) of the European Commission established the project CLEN2SAND to provide scientific support for identification of unknown compounds controlled by European Customs, in particular New Psychoactive Substances (NPS).
Furthermore, the new generation of low field NMR is of high interest for the Customs Laboratories European Network (CLEN). The Customs Control Equipment Instrument (CCEI) Regulation will help the acquisition of such instrumentation by Customs laboratories [2].

In this presentation, we will describe the activities performed by the JRC in the frame of the CLEN2SAND project regarding NMR applications for the identification of unknown substances, chemical analysis to facilitate the correct application of customs' tariffs and the support in the use of NMR spectrometers and interpretation of spectra as well as the sharing of data and the implementation of databases.

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NMR-based community-built analytical systems in food control and quantitative analysis

Vito Gallo,[1,2] Piero Mastrorilli,[1,2] Mario Latronico,[1,2] Biagia Musio,[1] Maurizio Triggiani,[1,2] Marica Antonicelli,[1] Rosa Ragone,[1] Stefano Todisco[1]

‡ Politecnico di Bari, DICATECh, via Orabona 4 – CAMPUS, I-70125, Bari, Italy
† 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, metabolomics, food

NMR spectroscopy is gaining ever-growing importance in analytical chemistry. In the last decade, quantitative NMR (qNMR) and non-targeted approaches allowed for great improvements in both quantification of molecules in complex mixtures and identification of product features in suitable sample pools. Metrological traceability of products can be successfully achieved by qNMR even when no certified reference materials are commercially available. Along with quantification and purity assessment of molecules, NMR is emerging also as a powerful tool in food chemistry, especially when searching for product features such as cultivar, geographical origin, typicality of the production process, etc.
The great application potential of NMR spectroscopy derives from the fact that, based on theory, the ratio between a signal generated by the molecule under investigation and the signal of a reference molecule depends exclusively on the corresponding mole ratio. In other words, when a given sample is analyzed by different spectrometers, the same output is obtained in terms of signal ratios independently of the hardware configuration. This offers the unique opportunity to develop community-built analytical systems capable of identifying sample features and quantifying a number of molecules.
In this presentation, based on our previous studies carried out with different NMR spectrometers (300 to 700 MHz),[1,2] the first examples of community-built analytical systems will be shown. In particular, the case study of a data-driven grape juice identification system will be presented along with the advantages and the limitations of using non-targeted NMR analyses performed at different magnetic fields. Moreover, quantification of metabolites in grape juices by using a community-built calibration tool will be also shown.[3] The feasibility of NMR spectroscopy to generate statistically equivalent NMR signal ratios from a number of different spectrometers will be demonstrated also for other complex mixtures such as aqueous extracts of wheat and flour.[4] Finally, potential use of benchtop NMR in both quantification of standard molecules and valorization of food biodiversity will be introduced.

Acknowledgements
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NMR CHARACTERIZATION OF POLYSACCHARIDE-BASED VACCINES

F. Berti

GSK Vaccines, Via Fiorentina, 1, 53100 Siena, Italy.

E-mail: francesco.x.berti@gsk.com

Keywords: biomolecules, polysaccharide-based vaccines

Polysaccharide-protein conjugate vaccines are among the safest and most successful vaccines developed during the last 40 years. Since the first semisynthetic chemical conjugate vaccine licensed in the 1980's to protect human against *Haemophilus influenzae* type b infection, conjugate vaccines against *Neisseria meningitidis* and *Streptococcus pneumoniae* have been developed and registered using the same approach (i.e. bacterial growth to produce capsular polysaccharide antigen and chemical coupling to carrier protein).
Physicochemical techniques are a powerful tool for the structural characterization of those vaccines. High-field Nuclear Magnetic Resonance (NMR) spectroscopy has been established as an extremely useful and robust method for tracking the industrial manufacturing process of these vaccines from polysaccharide bulk antigen through to the final formulation.
A description of the use of proton NMR for structural identity and conformity testing of carbohydrate-based vaccines is provided.

CONFORMATIONAL ANALYSIS IN DRUG DESIGN: a synergic approach of NMR & Computational Chemistry

E. Moro, [‡] C. De Santi, [‡] Iuni M. L. Trist, [†] Diego Fiorucci[†], Silvia Davalli

[‡]NMR Spectroscopy DD&D, Campus Levi-Montalcini, Via Alessandro Fleming, 4, 37135 Verona
[†]Molecular Architets, Campus Levi-Montalcini, Via Alessandro Fleming, 4, 37135 Verona

E-mail: elisa.moro@evotec.com

Keywords: solution NMR, small molecules

The bioactive conformation that a molecule must adopt to be recognized by a receptor and its relationship to biological activity response are of fundamental importance to drug discovery.
Understanding the free ligand conformational preferences and dynamics of small molecules in solution can guide and support in drug design hypotheses and can help to increase productivity in drug discovery. Experimental NMR data, combined with computational approaches, allow refining the design of potentially active compounds leading to fasten the discovery process with a lower number of synthesized molecules and higher probability of success.
In the last years, Evotec started investing in this approach by implementing a workflow able to address the combination of NMR data with *in silico* conformational analysis. The improvement to the discovery process will be highlighted by showing real case studies.

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RESCUE 3: Versatile decision-making tool for NMR spectral assignments of proteins.

L. Duciel,[‡] T. Malliavin,[†] M.-A. Delsuc[*]

[‡] Casc4de, Pôle API, 300 Bd Brant 67400 Illkirch, France
[†] Laboratoire de Physique et Chimie Théorique UMR CNRS 7019. Université de Lorraine Bd des Aiguillettes 54506 Vandœuvre-lès-Nancy, France
[*] IGBMC, 1 rue Laurent Fries 67404 Illkirch, France & Casc4de, Pôle API 300 Bd Brant 67400 Illkirch, France

E-mail: laura.duciel@casc4de.eu

Keywords: biomolecules

Spectral assignments of proteins in NMR is crucial as, even today, this generally requires lots of manual work and good expertise. The development of automatic techniques would therefore greatly accelerate analyses and thus enables a higher throughput in cases where this step is required, such as for ligand interaction screening, protein-protein recognition, NMR structure determination and/or when assignment information is not obtainable (e.g non labeled proteins, methyl-based approaches).
RESCUE is a statistical approach developed in 1999 for NMR spectral assignment of proteins through a simple artificial neural network (perceptron) [1]. It allowed the type of amino acid to be determined from the observed ^{1}H chemical shift. It used as a training set the data available in the BMRB (bmrb.io) at the time. It was extended in 2004 to the whole spin set, using a "Naive Bayes" probabilistic model for each amino acid type from a set of given chemical shifts [2]. Predictions accuracy of this second version reaches up to 75%.
The recent development in Deep Learning (DL) as well as the current size of the BMRB database available today allow to get better assignment predictions. We developed a Deep Neural Network (DNN) together with a cleaned database by removing duplicates, strongly homolog entries, non-protein sequences and scarcely assign entries and by realigning chemical shift references. We obtain a final database from the BMRB including of 485264 sets of chemical shifts and a representation of the 20 classical amino acids to feed the neural network.
We developed a 7 dense layers neural network using the open-source Keras Python library. An adamax optimizer and a categorical crossentropy loss were used, with training on 25 epochs. We then built scenarios to filter the chemical shift sets according to what is acquired experimentally. This allows us to adapt the DNN to each sets of available experiments.
The newly developed algorithm has been tested in several situations reproducing possible scenarios and allowing to evaluate the efficiency of the program in different cases. In all cases the algorithm shows very good results and is able to make relevant amino acid predictions with assessment of prediction accuracy.

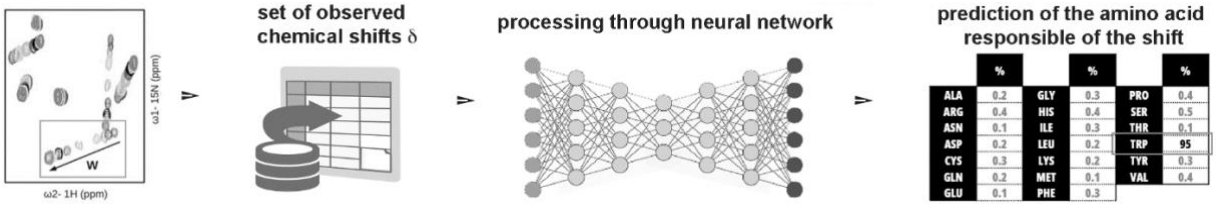

Fig. 1. RESCUE 3 approach

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LF-TD-NMR FOR THE DEVELOPMENT OF PRINTING BLANKETS

D. Besghini,‡ P. D'Onofrio‡

‡Trelleborg Printing Solutions, SP140, Lodi Vecchio (LO), Italy
E-mail: denise.besghini@trelleborg.com

Keywords: low field NMR, materials, polymers.

Printing blankets are composite systems of thin layers (few hundreds microns maximum) of rubbers and fabrics. To study these rubbers, which are mainly composed of Nitrile Butadiene Rubber (NBR), and their crosslinks density, advanced techniques are necessary, since traditional methods for rubbers, for example swelling, cannot be applied. Low-Field Time-Domain NMR (LF-TD-NMR) was demonstrated to be a powerful tool to monitor easily the effect of different parameters[1] (sulfur and accelerators quantity, mixing, additives, vulcanization temperature, etc…) on crosslinking, applying a plethora of NMR sequences, in particular Hahn-Echoes[2] and the Baum-Pines[3] sequence.
We will show, through some examples (Fig.1), how LF-TD-NMR can help understanding NBR compounds properties at microscopic level, thus guiding the industrial development of new compounds and, consequently, new products.

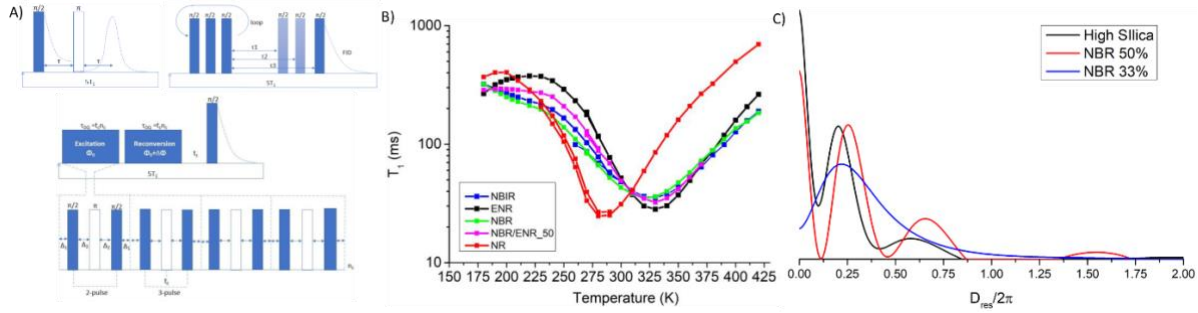

Fig. 1. A) NMR sequences used to analyze NBR rubbers: Hahn-Echo, Saturation Recovery and Baum-Pines; B) T_1 dispersions for different rubbery polymers; C) D_{res} distributions for two NBR 33% nitrile formulations containing different silica quantity and NBR 50% nitrile.

Our discussion will deal with different topics: the extraction of NMR parameters, such as residual dipolar couplings, T_2 or T_1, allowed the determination of the microscopic structure of NBR, containing 33% of nitrile groups, in comparison with other polar and apolar rubbery polymers; we could determine the crosslinks density in compounds made with NBR with very high content of nitrile groups, monitoring the effect of different vulcanization systems; and we evaluated the effect on crosslinking of different silane quantities. This wide range of possibilities highlights the relevant role that LF-TD-NMR can hold for rubber manufacturers.

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POSTERS

[P-1] HIGH-RESOLUTION STRUCTURAL INVESTIGATION OF MT1 MELATONIN RECEPTOR ACTIVATION MECHANISM USING NMR TECHNIQUES

C. Acconcia,[‡] F. Scebba,[†] D. Angeloni,[†] S. Comai,[#] L. Russo.[‡]

[‡]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "L. Vanvitelli" Caserta, Italy.
[†]Institute of Life Sciences, Scuola Superiore Sant'Anna; Pisa, Italy.
[#]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy; Department of Biomedical Sciences, University of Padua, Padua, Italy; Department of Psychiatry, McGill University, Montreal, QC.
E-mail: clementina.acconcia@unicampania.it

Keywords:
solution NMR, melatonin, membrane receptor, biomolecules.

Melatonin is a neurohormone produced in a circadian rhythm in the pineal gland which is involved in numerous physiological functions, including sleep and temperature regulation, through its G-protein coupled receptors MT1 and MT2 [1-4]. Different preclinical studies using knockout animals for MT1 and MT2 receptors are showing that these melatonin receptors may have complementary and physiological functions [4]. In our case, we investigated the structural model of interaction between Melatonin and MT1 receptor from Nuclear Magnetic Resonance, Molecular Modelling and Molecular Docking methodologies. Initially, we analyzed NMR saturation transfer difference (STD) and T1ρ-based experiments, to identify the binding portions of Melatonin both in the presence and absence of cell membrane containing the MT1 receptor. Then, we defined a three-dimensional model of the melatonin/MT1 complex by combining computational methodologies with NMR data. Finally, our data demonstrate that the interaction between MT1 receptor and Melatonin is determined by different regions of the ligand that induce conformational changes on the cytoplasmatic side of the receptor.

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[P-2] KINETIC STUDIES OF DEGRADATION OF NEW NEUROTOXIC ORGANOPHOSPHORUS AGENTS DURING DECONTAMINATION PROCESS

Jobard Elodie‡, Laurence Tambour‡, Charlotte Mappa‡, Julien Enche‡, Christine Albaret‡

‡*DGA CBRN Defence, Analytical Chemistry Department, Vert-le-Petit, France*
E-mail: christine.albaret@intradef.gouv.fr

Keywords: solution NMR, small molecules.

Following the recent inclusion by the OPCW of the new schedule chemicals in the CWC Annex including new neurotoxic organophosphorus agents, a plenty of studies have to be realized, in order to characterize these new agents and to manage the associated risk. To conduct these studies, safety conditions have to be firstly considered in term of protection and decontamination. These considerations are also useful for operational forces. Two types of new neurotoxic agents can be distinguished: on the one hand, organophosphorus agents (OP) divided in two families (amidine – code 1.A.13 & 1.A.14) and a derivative (substituted guanidine, code 1.A.15) and on the other hand the family of carbamates (code 1.A.16).
In this context, one of the primary objective to allow handling such chemicals was to study their degradation process in a decontamination solution. In a first time, four OP NNA have been studied (see Fig. 1):

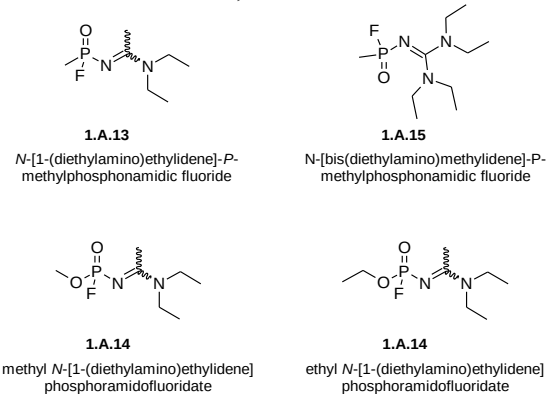

Fig. 1: Formulae and IUPAC name of new studied OP agents

The decontamination solution, commonly used for well-know organophosphorus chemicals is alcoholic sodium hydroxide (water, ethanol 96%, sodium hydroxide 35%, 7/3/1 v/v/v).
The kinetic study of degradation was followed by $^{19}F\{^{1}H\}$ NMR at one or two concentrations. The identification of degradation products was achieved by NMR (and by LC-HRMS in some cases). The kinetic constant (between 0.5 min^{-1} & 2.19 min^{-1}) and the half life time (between 18.9 sec & 71.7 sec) of the degradation reaction were determined according to a model of first order.
In conclusion, for the four studied compounds, the decontamination solution (H_2O/C_2H_5OH/NaOH, 7/3/1 v/v/v) used at DGA MNRBC is effective. The next step is to control the degradation process in decontamination solution for carbamates.

[P-3] CAULIFLOWER BY-PRODUCTS VALORIZATION BY APPLYING NMR BASED METABOLOMICS

D. Ambroselli[1], F. Masciulli[1], E. Romano[1], A. Sobolev[2], L. Mannina[1]

[1]Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[2] Institute for Biological Systems, Magnetic Resonance Laboratory "Segre-Capitani", CNR, Via Salaria Km 29.300, 00015 Monterotondo, Italy
E-mail: donatella.ambroselli@uniroma1.it

Keywords: solution NMR, metabolomics, food

Worldwide, thousands of tons of agricultural food waste and by-products are generated along the fruit and vegetable supply chain. The conventional treatment of agro-waste implies economic, social and environmental impacts, thus becoming a global issue to face by governments and institutions, including FAO, United Europe and ONU, that are encouraging and supporting innovative solutions towards a zero waste future [1].
Plant material is a valuable source of nutrients and secondary metabolites that may be linked to several health benefits. For this reason, agro-waste, the non-edible part of the plant, represents an important raw-material to be used for different purposed, according to its chemical-biological composition.
Among fruit and vegetable crops, cauliflower (*Brassica oleacea*) bears a large amount (approximately 45-60% of the total weight) of waste and by-products, thus being characterized by a high waste index.
The aim of the present study, developed within the project Ri-cicloHorto supported by Regione Lazio [2], is to valorize the chemical profile of cauliflower by-products by using untargeted NMR approach.
Freeze-dried stems and leaves of cauliflower by-products were subjected to the Bligh–Dyer extraction protocol [3] to obtain both hydroalcoholic and organic fractions. The ^{1}H NMR spectral assignments of both hydroalcoholic and organic fractions were carried out by means of 2D experiments (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) and literature data [3]. Different classes of compounds, namely sugars, organic acids, free amino acids, and other compounds were identified and quantified in hydralcoholic extracts, whereas fatty acids, sterols, and phospholipids were detected in the organic ones. Therefore, the chemical profile characterization of cauliflower by-products represents a starting point towards the valorization of these food wastes. The knowledge of the chemical composition is crucial to identify the proper application for the development of new nutraceutical products, functional foods, or biostimulants for the agricultural sector.

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[P-4] SOLID STATE NMR APPROACHES FOR THE COMPREHENSION OF BIOMINERALIZATION PROCESSES

T. Azaïs,[‡] T. Georges,[‡] L. Epasto,[†] D. Kurzbach, [†] V. Ramnarain,[+] O. Ersen, [+] C. Sanchez[‡+]

[‡]Sorbonne Université, CNRS, Laboratoire de Chimie de la Matière Condensée de Paris (LCMCP), F-75005 Paris, France
[†]Faculty of Chemistry, Institute of Biological Chemistry, University Vienna, 1090 Vienna, Austria
[+]Institut de Physique et Chimie des Matériaux Strasbourg, 23 Rue du Loess Strasbourg Cedex2, France

E-mail: thierry.azais@sorbonne-universite.fr

Keywords:
Solid state NMR, Hyperpolarization, Materials.

Biomineralization is a fascinating and complex biological process leading to the formation of mineral phases *in vivo*. Biominerals are found in very diverse species and mineralized tissues including corals, sea urchins spines, crustaceous cuticles or mollusks shells for invertebrates and bones or teeth in case of vertebrates. All these biomineralized tissues have in common their hydride nature where the mineral crystals (*i.e.* calcium carbonate or calcium phosphate) are 3D organized within an organic extracellular matrix. As a consequence, biomineralized structures exhibit mechanical properties that exceed those of their individual building blocks. Moreover, biomineralization occurs at room temperature, using naturally-occurring chemicals, and in fully aqueous conditions, which is much more sustainable than how engineering materials are processed. However, chemists are still unable to duplicate this remarkable chemistry because molecular-level mechanisms of biomineralization are still poorly understood.

In recent years, our team demonstrated the ability of solid-state NMR spectroscopy to describe at the molecular level biomineralized structures including biomineral surfaces [1] or organo-mineral interactions [2], and such for various mineralized tissues such as bone [3,4], spines from sea urchins [5] or mollusks shells [6]. More recently, we have also demonstrated that *ex situ* and *in situ* solid-state NMR is appropriate for the comprehension of ongoing biomineralization processes. The use of biomimetic models allows the monitoring of the crystallization of amorphous phases in hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ [7,8] or monohydrocalcite $CaCO_3.H_2O$ [9].

In this communication, we show that hyperpolarized methods, such as dynamic nuclear polarization (DNP), combined to NMR allows the comprehension of the very first steps of calcium carbonate or calcium phosphate nucleation including non-classical pathways for which prenucleation clusters are in equilibrium with individual solvated ions. Example of a (meta)stable calcium phosphate solution, namely simulated body fluid, will be detailed [10] and compared to a supersaturated solution for which calcium phosphate precipitation appears within seconds. For the latter, the use dissolution DNP methods are mandatory to observe prenucleation clusters formation and aggregation [11]. Finally, we show that DNP methods in the solid state (DNP MAS) are also useful to describe the atomic structure of $CaCO_3$ prenucleation clusters stabilized by amino-acids [12].

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[P-5] New solid-state NMR and DNP methods for 19F detection in pharmaceuticals

Martins Balodis, Judith Schlagnitweit[†], David Gajan[†], Gilles Casano[††], Ouari Olivier[††], Guido Pintacuda,[†] Anne Lesage[†]

[†] Université de Lyon, Centre de RMN à Tres Hauts Champs (UMR 5280 CNRS/UCBL/ENS Lyon), 69100, Villeurbanne (France)
[††]Institut de Chimie Radicalaire, Aix Marseille Université, F-13013 Marseille (France)
E-mail: martins.balodis@ens-lyon.fr

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

High resolution structural information is the key ingredient for rational drug design and development. Usually, this information is acquired for unmodified drugs in their free form, but rarely for the final form of the drug after it is melded into more complex drug formulation. Unfortunately, the structure may change upon this inclusion after the interaction with the excipients. Therefore, methods allowing the detection of different polymorphs and the determination of structural changes upon formulation of the drug with various excipients (e.g. in tablets) are highly valuable to ensure safety, efficacy and bioavailability of the final medication.

Solid-state NMR, particularly when combined with DNP is a powerful method to obtain high resolution structural information of APIs in complex mixtures [1] as well as structural information of delivery systems on the nano-meter length scale [2]. Different components and APIs in complex mixtures can be distinguished based on different isotopes (e.g. ^{13}C or ^{15}N labelling), different chemical environments (chemical shifts), or based on specific nuclei. An important nucleus of interest is ^{19}F as of today about 30% of APIs contain at least one fluorine atom. ^{19}F is particularly valuable because hardly any excipients contain fluorine making it a selective probe towards the structure of the API. Following the recent developments in ^{19}F NMR [3,4] here we are showing new strategies for improving the detection of ^{19}F in pharmaceutical formulations used in structural characterization.

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[P-6] MULTINUCLEAR METAL-BINDING ABILITY OF THE N-TERMINAL REGION OF HUMAN COPPER TRANSPORTER CTR1: DEPENDECE UPON pH AND METAL OXIDATION STATE

A. Barbanente,[‡] M.I. Nardella,[‡] M. Fortino[†], G. Natile[‡], A. Pietropaolo[†], F. Arnesano[‡]

[‡] Department of Chemistry, University of Bari, via E. Orabona, 4, 70125 Bari, Italy
[†] Dipartimento di Scienze Della Salute, University of Catanzaro, viale Europa, 88100 Catanzaro, Italy
E-mail: alessandrabarbanente@libero.it

Keywords: solution NMR, biomolecules.

The 14mer peptide corresponding to the N-terminal region of human copper transporter Ctr1 was used to investigate the intricate mechanism of metal binding to this plasma membrane permease responsible for copper import in eukaryotic cells. The peptide contains a high-affinity ATCUN Cu(II)/Ni(II)-selective motif, a methionine-only MxMxxM Cu(I)/Ag(I)-selective motif and a double histidine HH(M) motif, which can bind both Cu(II) and Cu(I)/Ag(I) ions. Using a combination of NMR spectroscopy and electrospray mass spectrometry, clear evidence was gained that the Ctr1 peptide, at neutral pH, can bind one or two metal ions in the same or different oxidation states. Addition of ascorbate to a neutral solution containing $Ctr1_{1-14}$ and Cu(II) in 1:1 ratio does not cause an appreciable reduction of Cu(II) to Cu(I), which is indicative of a tight binding of Cu(II) to the ATCUN motif. However, by lowering the pH to 3.5, the Cu(II) ion detaches from the peptide and becomes susceptible to reduction to Cu(I) by ascorbate. It is noteworthy that at low pH, unlike Cu(II), Cu(I) and Ag(I) ions stably bind to methionines of the peptide (see Fig. 1, *left panel*). The redox reaction could take place in the lumen of acidic organelles after Ctr1 internalization. Unlike $Ctr1_{1-14}$-Cu(II), bimetallic $Ctr1_{1-14}$-2Cu(II) is susceptible to partial reduction by ascorbate at neutral pH, which is indicative of a lower binding affinity of the second Cu(II) ion. The reduced copper remains bound to the peptide, most likely to the HH(M) motif (see Fig. 1, *right panel*). By lowering the pH to 3.5, Cu(I) shifts from HH(M) to methionine-only coordination, an indication that only the pH-insensitive methionine motif is competent for metal binding at low pH. The easy interconversion between different coordination modes of monovalent cations was supported by DFT calculations [1].

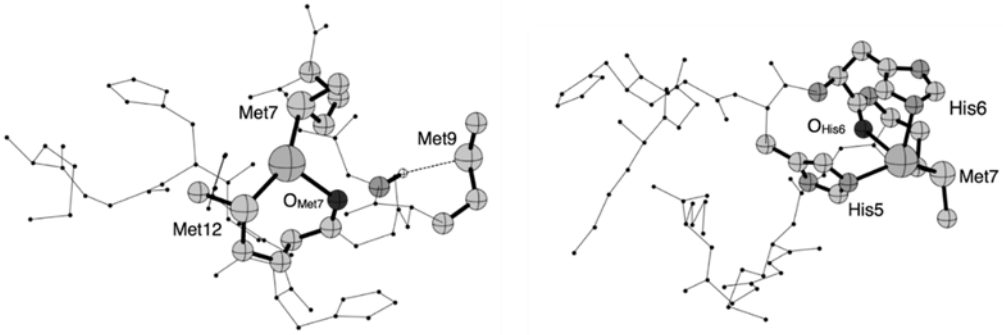

Fig. 1. Two optimized structural models predicted for Ag(I)-$Ctr1_{1-14}$.

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[P-7] ULTRAFAST COSY FOR STRUCTURAL ANALYSIS OF SMALL MOLECULES

A. Bernard,‡ P. Giraudeau,‡ J.-N. Dumez‡

‡Nantes Université, CNRS, CEISAM, UMR6230, F-44000 Nantes, France
E-mail: aurelie.bernard@univ-nantes.fr

Keywords:
solution NMR, small molecules, theory and methods

Structural analysis by NMR spectroscopy relies on a wide range of 2D experiments. In their conventional form, 2D NMR experiments are time-consuming as they require the acquisition of a large number of increments in order to sample the indirect dimension with sufficient resolution. This can lead to a significant load of the spectrometers. Many methods have been developed by the NMR community to accelerate 2D data acquisition, such as non-uniform sampling (NUS), Hadamard encoding, fast-repetition methods, or aliasing. Among these methods, ultrafast 2D NMR (UF) allows the acquisition of the entire indirect dimension in a single scan, by combining spatial encoding of the indirect dimension with spectroscopic imaging (EPSI). [1] [2]
The COSY method is one of the most widely used 2D experiments for the structural elucidation of small molecules. In this preliminary study, we wanted to explore the potential of the UF COSY experiment for structural analysis and to optimise the parameters of the pulse sequence to obtain a complete 2D spectrum in less than 2 minutes. We work with cyclosporine A as a standard yet complex test sample. Several aspects were addressed: optimisation of the parameters to obtain a complete map, sensitivity, phase cycling, impact of J-modulation. It was thus possible to record a UF COSY spectrum covering the entire spectral range of cyclosporine A (9 ppm) in 1.5 minutes. However, some of the correlation spots could not be obtained at this stage. Several avenues are being explored to try to remedy this, including the use of alternative COSY-based UF pulse sequences, to better understand the effects that modulate signal intensities in UF 2D NMR
These initial tests will be extended to other homonuclear techniques (TOCSY, diffusion, etc.) and applied to structural analysis, as well as to the monitoring of non-equilibrium mixtures.

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[P-8] A SPECTROSCOPIC INVESTIGATION OF EARLY PHASE OCHRONOTIC PIGMENT DEVELOPMENT IN ALKAPTONURIA.

D. Grasso, I. Tazza, A. Santucci, A. Bernini.

Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy.
E-mail: andrea.bernini@unisi.it

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

Alkaptonuria (AKU), a rare genetic disorder, is characterised by the accumulation of homogentisic acid (HGA) in organs due to a deficiency in functional levels of the enzyme homogentisate 1,2-dioxygenase (HGD), required for the breakdown of HGA, because of mutations in the HGD gene [1]. Over time, HGA accumulation causes the formation of the ochronotic pigment, a dark deposit that leads to tissue degeneration and organ malfunction [2]. Such behaviour can also be observed in vitro for HGA solutions or HGA-containing biofluids (e.g. urine from AKU patients) upon alkalinisation. However, a comparison at the molecular level between the laboratory and the physiological conditions is lacking. Indeed, independently of the conditions, such a process is usually explained by forming 1,4-benzoquinone acetic acid (BQA) as the product of HGA chemical oxidation, mainly based on structural similarity between HGA and hydroquinone that is known to be oxidised to the corresponding para-benzoquinone. To test such correlation, a comprehensive, comparative investigation of HGA and BQA chemical behaviours was carried out by a combined approach of spectroscopic techniques (UV spectrometry, Nuclear Magnetic Resonance, Electron Paramagnetic Resonance, Dynamic Light Scattering) under acid/base titration both in solution and in biofluids (see Fig. 1). New insights on the process leading from HGA to ochronotic pigment have been obtained [3], spotting the central role of radical species as intermediates not reported so far. Such evidence opens the way for molecular investigation of HGA fate in cells and tissue to find new targets for Alkaptonuria therapy.

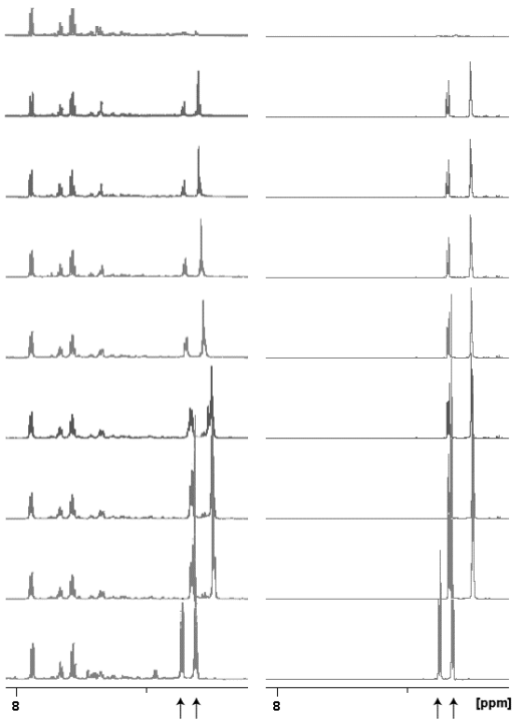

Fig. 1. Reaction under alkaline conditions was monitored by 1H spectroscopy for a urine sample (left panel) and HGA solution (right panel) from 0’ up to 14 days, from bottom to top. Loss of signal intensity for HGA peaks (indicated by arrows) down to the noise level with the same rate is apparent for both samples.

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[P-9] A GENERAL PARADIGM FOR CONSTRUCTING ADAPTIVE AND EFFICIENT MULTISPECTRAL IMAGING FILTERS: APPLICATIONS TO NUCLEAR MAGNETIC RELAXOMETRY IN BRAIN

J.M. BONNY[‡], M. BOUHRARA[†]

[‡] AgroResonance, INRAE, UR QuaPA, 63122 Saint-Genès Champanelle France
[†] Magnetic Resonance Physics of Aging and Dementia Unit, Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA

E-mail: jean-marie.bonny@inrae.fr

Keywords: MRI, theory and methods.

Based on the Bayesian theorem, we introduced a new paradigm for the design of high-performance image filters. This comprehensive statistical framework is applicable to most imaging modalities where multispectral images, that is, frames with different contrasts, can be acquired from the same subject, or sample, under investigation. Unlike the classical nonlocal filtering approaches [1,2], our formalism permits incorporation of adaptive fusion operators to calculate and merge the frame-dependent weights within the multispectral images. We show that the conventional, and widely used, multispectral nonlocal means filtering represents only a special case of our generalized framework. Through extensive numerical and in-vivo analyses, conducted on NMR images for myelin water fraction (MWF) determination, we demonstrate the flexibility and superior performance of our formalism for accurate and precise MWF mapping. Our results indicate that the use of adaptive fusion operators provides an advanced degree of freedom for the multispectral filtering leading to higher quality filtering with details preservation in derived MWF maps as compared to the conventional approaches. We also provide a mathematically based formulation for the calculation of the weight of the central voxel for which the signal intensity has to be restored. This issue has previously been overlooked, with only empirical solutions have been suggested. Our definition of the self-similarity here is easily extendable to various fusion operators and addresses this outstanding issue. This work opens the way to further stabilize quantitative MR imaging for advanced applications in many fields such as preclinical and clinical investigations. We note that, beside MR imaging applications, our filtering paradigm is readily applicable to other multispectral imaging modalities.

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[P-10] MULTIPARAMETRIC MRI IN INVESTIGATION OF AN EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS

P. Bontempi[1], A. Busato[1,*], P. Marzola[1], E. Nicolato[2], G. Piccolantonio[1], G. Constantin[3], Anita Conti[4], Gabriele Angelini[3], Alessandro Bani[3], Nicola Lopez[3]

[1]Department of Computer Sciences, University of Verona, Italy
[2]Department Technological Platform Center of University of Verona, Italy
[3]Department of Medicine, Section of General Pathology, University of Verona, Italy
[4]Department of Neurosciences, Biomedicine and Movement, University of Verona, Italy
[*]Current affiliation: Safety Assessment Dept, Aptuit S.r.l, an Evotec Company, Verona, Italy

Corresponding author e-mail: pietro.bontempi@univr.it

Keywords: **MRI**

In this work, multiparametric MRI was applied to the investigation of disease evolution in an experimental model of multiple sclerosis. Considering that the development of innovative therapeutic strategies that promote functional recovery is a major goal of multiple sclerosis research, functional imaging was applied together with high resolution morphological imaging and DTI. Chronic Experimental Autoimmune Encephalomyelitis (EAE) was induced in 21 C57BL/6 female mice by subcutaneous immunization and 21 normal mice were used as control. MR images were acquired at three time points after induction (about 15, 25, and 50 days post induction). Within a single experiment session, BOLD images for rsfMRI, DTI images and a high resolution T2w images were acquired. Preprocessing of structural T2w images and functional images was performed with FMRIB's Software Library (FSL-VBM and MELODIC). Independent Component Analysis (ICA) was performed with Group ICA of fMRI Toolbox (GIFT) to infer the functional connectivity (FC) and identify the resting-state networks (RSNs). The rsfMRI networks, reported in literature for the mouse brain [1], were well-identified in the two groups. No significant statistical difference in FC was detected between groups for the identified RSNs. VBM analysis of high resolution T2w images detected gray matter (GM) atrophy in EAE mice at all the time points in the left motor and left somatosensory areas. Region of increased GM content in the cerebellum area of EAE mice was also identified, probably corresponding to inflammatory reaction. Analysis of DTI data is still in progress. In conclusion, no significant alteration in FC between the EAE and control group was detected. Further investigations using Seed-Based Correlation Analysis with ROIs in thalamic and cerebellum regions, as reported in human studies [2, 3], are in progress. VBM approach is able to detect neuroimaging biomarker of the pathology, allowing to monitor the disease progression.

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[P-11] MULTINUCLEAR SOLID-STATE NMR APPLIED TO ENERGY STORAGE MATERIALS

S. Bordignon,[‡] C. Pistidda,[†] T. T. Le,[†] M. R. Chierotti[‡]

[‡]Università degli Studi di Torino, Department of Chemistry and NIS Centre, via P. Giuria 7, 10125, Torino, Italy
[†]Institute of Hydrogen Technology, Helmholtz-Zentrum hereon GmbH, Max-Planck-Straße 1, D-21502 Geesthacht, Germany
E-mail: simone.bordignon@unito.it

Keywords: solid state NMR, materials

Metal borohydrides, amides of alkaline and alkaline-earth metals, and metal hydride solid-state materials are currently considered a promising option to effectively and safely store hydrogen. In particular, metal amide-hydride mixtures have been extensively studied as potential hydrogen storage media for mobile and stationary applications, owing to their high hydrogen storage capacity and favorable thermodynamics, which in many cases allow releasing hydrogen at temperatures below 150 °C in a reversible way. Since these materials are usually synthesized through mechanochemical reactions, their characterization by means of single-crystal X-ray diffraction proves quite challenging. In the present work, we show the ability of solid-state NMR in providing structural information on a series of energy storage materials. Its multinuclear approach (*e.g.,* ^{1}H, ^{7}Li, ^{11}B, ^{15}N…) allows elucidating polymorphism, phase purity, reaction outcome, site symmetry and dynamics. Calculations are also a fundamental tool in assisting the chemical shift assignment and in assessing the structure.
Some of the presented cases will be the following: KNH_2–KH [1] and $RbNH_2$–RbH [2] solid solutions; $Ca(BH_4)_2$–Mg_2NiH_4 system [3]; MgB_2 under reaction with H_2 [4].

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[P-12] INNOVATIVE HYBRID MATERIALS FOR BONE CANCER TREATMENT: A SOLID STATE NMR INVESTIGATION

S. Borsacchi,[a] E. Carignani,[a] L. Altomare,[b] M.G. Raucci,[c] L. Calucci[a]

[a]Institute for the Chemistry of OrganoMetallic Compounds, Italian National Research Council (ICCOM-CNR), via G. Moruzzi 1, 56124 Pisa, Italy
[b]Department of Chemistry, Materials, and Chemical Engineering "G. Natta"- Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy
[c]Institute of Polymers, Composites and Biomaterial, Italian National Research Council (IPCB-CNR), Viale J.F. Kennedy, 54 Mostra d'Oltremare Pad. 20, 80125 Naples, Italy
E-mail: silvia.borsacchi@cnr.it

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

Bone cancer and bone metastases are usually associated with severe bone pain and osteolysis, the latter being also accompanied by increased bone fragility and susceptibility to fracture. Nowadays, chemotherapy and/or radiotherapy represent the main treatments able to block the metastases progression. However, these treatments inhibit cell division without distinction between healthy and cancer cells, thus inducing many side effects in patients. Hence the need for developing innovative treatments able to inhibit metastases progression and, at the same time, to promote the formation of new tissue. In this contribution we present a multinuclear Solid State NMR and relaxometric investigation [1, 2] aimed at shedding light on innovative injectable materials with the dual function of inhibiting cancer cells proliferation and inducing new bone tissue formation/mineralization. They have been designed to have a suitable hybrid organic-inorganic nature and to contain 2D graphene oxide (GO), active as photodynamic therapy (PDT) agent for cancer treatment [3]. The application of several ^{31}P, ^{1}H and ^{13}C Solid State NMR high-resolution experiments and the measurement of ^{1}H relaxation times at low magnetic field allowed us to gain insights into these complex hybrid materials, identifying and monitoring the formation of the inorganic osteo-inductive calcium phosphate phases, characterizing the organic (cellulosic) components and GO, and obtaining information on the materials' dynamic properties.

This research was funded by the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR), grant number PRIN 'ACTION' 2017SZ5WZB.

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[P-13] INVESTIGATING BIOINSPIRED SILICA-LYSOZYME COMPOSITE TROUGH A MULTI-TECHNIQUE APPROACH

Francesco Bruno[‡†], Enrico 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.
E-mail: bruno@cerm.unifi.it, ravera@cerm.unifi.it

Keywords: solid state NMR, materials, biomolecules.

We investigate the peculiar interaction occurring at the surface of silica in a lysozyme-silica hybrid composite using solid-state NMR under magic-angle spinning (MAS), Small-Angle X-ray Scattering (SAXS) and Scanning Electron Microscopy (SEM). As these hybrid interfaces are non-crystalline in nature, MAS NMR spectroscopy is the ideal tool to unravel short- (1.5-5.0 Å) and medium- (5.0-10.0 Å) range atomic-level distances that define their structure, and it provides us with further insight on how bioinspired silica interacts with lysozyme [1]. Observing the protein resonances through ^{13}C MAS NMR, we can infer that the folding of the protein is preserved inside the composite, given the presence of the distinctive features of the folded protein. In parallel, ^{29}Si-MAS NMR allows to evaluate the degree of condensation of the silica matrix through the ratio Q^4/Q^3: we observed that the hybrid lysozyme-silica compound is much more condensed than silica gel prepared without the protein, and becomes even more condensed upon removal of lysozyme. These observations are consistent with the structural data obtained by SAXS and SEM. In order to study the protein-silica interface, we have acquired ^{1}H-^{13}C and ^{1}H-^{29}Si 2D correlation CP-HETCOR spectra at increasing contact times, and discriminated among the protons that act as polarization sources for the heteronuclear sites. All 2D NMR spectra were processed with Multivariate Curve Resolution to increase the sensitivity [2]. Collectively, the experimental observations suggest a non-covalent interaction between lysozyme and silica matrix in this material. However, protein and inorganic scaffold are still in tight contact in this composite. From the fact that lysozyme can only be quantitatively removed from the silica by denaturation, regardless of the ionic strength of the medium, in combination with a multi-technique spectroscopic approach to assess the local atomic structure, a model of lysozyme being trapped inside a silica cage via steric effect is supported.

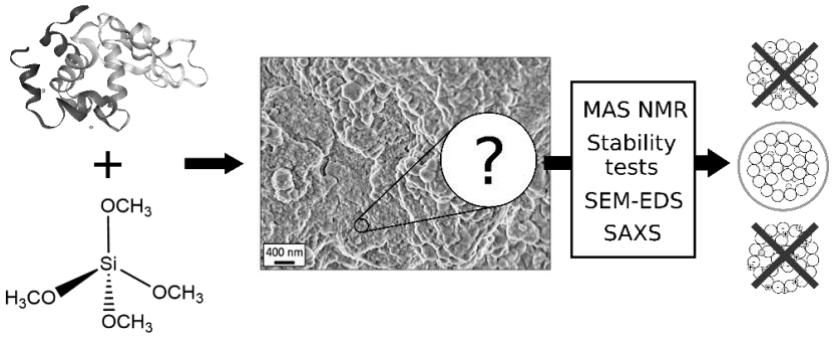

Fig. 1. Graphical abstract that sums up the rationale of this study.

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[P-14] FAST DEEP LEARNING RECONSTRUCTION TECHNIQUES FOR MAGNETIC RESONANCE FINGERPRINTING

R.F. Cabini,[a,b] L. Barzaghi,[a,c] D. Cicolari,[d,b] A. Pichiecchio,[c,e] S. Figini,[b,f] P. Arosio,[g] M. Filibian,[h,b] S. Carrazza,[g] A. Lascialfari[d,b]

[a]University of Pavia, Department of Mathematics, Via Adolfo Ferrata 5, 27100 Pavia, Italy.
[b]INFN, Istituto Nazionale di Fisica Nucleare – Pavia Unit, Via Bassi 6, 27100, Pavia, Italy.
[c]Neuroradiology Department, IRCCS Mondino Foundation, Via Mondino 2, 27100 Pavia, Italy.
[d]University of Pavia, Department of Physics, Via Bassi 6, 27100, Pavia, Italy.
[e]University of Pavia, Department of Brain and Behavioural Sciences, Via Mondino 2, 27100 Pavia, Italy.
[f]University of Pavia, Department of Social and Political Science, Corso Carlo Alberto 3, 27100 Pavia, Italy.
[g]University of Milan, Department of Physics and INFN – Milano Unit, Via Celoria 16, 20133 Milan, Italy.
[h]Centro Grandi Strumenti, University of Pavia, Via Bassi 21, 27100 Pavia, Italy.
E-mail: raffaellafiamm.cabini01@universitadipavia.it

Keywords: MRI, theory and methods, instrumentation.

Magnetic Resonance Fingerprinting (MRF) simultaneously measures multiple tissue properties through a more time-efficient acquisition routine than standard mapping techniques. Because the traditional dictionary-based MRF post-processing framework requires significant computational time and storage capacity, we propose a DL method and an hyperparameters optimization strategy to reconstruct T_1 and T_2 maps acquired with two MRF sequences. The dataset consists of 6 slices of two ex-vivo brain rat phantoms: 5 slices are from the first phantom and one slice from the second one. We performed the two Gao [1] and Zhao [2] MRF sequence routines and the reference T_1 and T_2 mapping sequences (Inversion-Recovery and Spin-Echo) on a 7-Tesla Bruker Scanner. Gao and Zhao MRF dictionaries were simulated by Extended-Phase-Graph formalism. We defined two different architectures of the DL model: a multilayer perceptron and a recurrent neural network. Both the models were trained and validated on 5 slices (80/20%) of the first phantom, using the Mean Squared Error as loss function. The slice of the second phantom excluded from the training process was used as an independent test set. The set of optimal hyperparameters of the model and the training algorithm were established through an optimization procedure.
Concerning the DL reconstruction method, the mean percentage error (MPE) for the Zhao dataset is equal to 6%±8% for T_1 (7%±9% for the Gao dataset) and 12%±11% for T_2 (12%±16% for the Gao dataset). For the dictionary-based method, the MPE for the Zhao dataset is equal to 16%±8% for T_1 (19%±8% for the Gao dataset) and 20%±25% for T_2 (35%±29% for the Gao dataset). We evaluated the reconstruction performances of the DL algorithm and of the dictionary-based one with different acquisition sequence lengths of both datasets. For the Gao dataset a good agreement between the DL reconstructed and true maps was obtained for a sequence length of at least 60 time-points for T_1 (100 for the Zhao dataset) and at least 100 time-points for T_2 (100 for the Zhao dataset), compared to the 300 time-points necessary for the dictionary-based reconstruction (400 for the Zhao dataset).
Through a lower number of images than the standard post-processing, DL-based method and automatic hyperparameters optimization strategy deliver parametric maps with similar accuracy as the dictionary-based methodology. These results envisage a significant time shortening of MRI investigation.

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[P-15] Characterization of biocompatible polymeric hydrogel for IVD structures replacement/integration @REGENERA Project.

Emanuela Callone,[‡] Elia Bissoli[†], Devid Maniglio, [†] Antonella Motta [†], and Sandra Dirè,[‡]

[‡]University of Trento, Department of Industrial Engineering, "Klaus Müller" Magnetic Resonance Lab, via Sommarive 9, 38123 Trento, Italy
[†] University of Trento, Department of Industrial Engineering, BIOtech Research Center, via delle Regole 101, 38123 Trento, Italy
E-mail: emanuela.callone@unitn.it

Keywords: solid state NMR, materials, polymers.

The use of biomaterials, which include both materials of natural origin and synthetic polymers, has seen a significant development for applications in the biomedical sector. In parallel with this success, requests for optimization and multiplication of their functionalities have increased. [1] In the mainframe of the Regenera project (under the Program Department of Excellence 2018-22, awarded to the Department of Industrial Engineering) [2], we focused our attention to the intervertebral disk (IVD) remediation, since damages due to unproper loading or degeneration result in back pain, a common pathology experienced by an increasing number of patients.

The IVD is a largely avascular and anerval fibro-cartilaginous structure that, together with two neighboring vertebrae, represents the human spine functional motion unit. (Fig 1) The disc is formed by three different parts: the middle *nucleus pulposus* (NP), an outer ring called *anulus fibrosus* (AF) and the cartilaginous endplates, which anchor the discs to adjacent vertebrae. Among them, AF is made of concentric layers of differently oriented collagen fibers to enable multidirectional resistance and contrast the lateral expansion of the NP when subjected to vertical load, varying its main composition from the outside in order to control and limit motion between vertebrae during bending and twisting of the spine thanks to its shear properties.

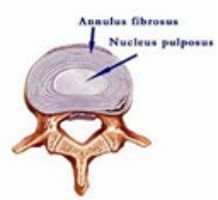

Fig. 1. Scheme of IVD

The damages usually drive to a total disc replacement. This can be performed using metallic or non-metallic materials, or a combination of the two. Non-metallic devices usually rely on finding polymers or elastomers with mechanical performances similar to the natural disc. Accordingly, hydrogels are highly attractive materials for developing synthetic analogs thanks to their ability to simulate the nature of soft tissues.

The presented work is devoted to the evaluation of highly hydrophilic, biocompatible and tunable three-component hydrogels, by comparing structural information, gained though solid state NMR analyses, with their rheological and swelling behaviors, with the aim to design a versatile, easy bio-functionalizable platform for addressing AF repair or regeneration strategies. [3]

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[P-16] THE AMYLOIDOGENIC PROTEIN TRANSTHYRETIN REVEALS A COMPLEX DYNAMIC LANDSCAPE ON MULTIPLE TIMESCALES

C. Cantarutti,[‡,$] W. Mandaliti,[‡] G. Verona,[†] P. Mangione,[*] V. Bellotti[#,$] and A. Corazza[‡,$]

[‡] Department of Medicine, University of Udine, P.le Kolbe 4, 33100 Udine, Italy.
[$] INBB, Viale delle Medaglie d'Oro 305, 00136 Roma, Italy.
[†] Wolfson Drug Discovery Unit, Center for Amyloidosis and Acute Phase Proteins, University College London, London, NW3 2PF, UK.
[*] Department of Molecular Medicine, Institute of Biochemistry, University of Pavia, Via Taramelli 3b, 27100 Pavia, Italy.
[#] Scientific Direction, Fondazione IRCCS Policlinico San Matteo, Viale Camillo Golgi 19, 27100 Pavia, Italy.
E-mail: cristina.cantarutti@uniud.it

Keywords: solution NMR, biomolecules.

Transthyretin (TTR) is an amyloidogenic protein responsible for systemic amyloidosis which in its wild-type form affects 25% of the population over 80s [1]. A specific proteolytic cleavage between K48 and T49 at the end of strand C (Fig. 1) has been identified as a key step for the dissociation of the TTR tetramer and amyloidogenesis [2,3]. However, the reason why TTR undergoes this aggregation process is not yet understood. To investigate the inherent plasticity of TTR in solution, we applied an NMR-based approach to measure its dynamic properties on a timescale from ps to days. The following methods were used: 1) real-time NMR to study the motion properties (min-h) of TTR via hydrogen-deuterium exchange (HDX); 2) Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion to analyze intermediate exchange (μs-ms); 3) ^{15}N nuclear spin relaxation (hNOE, R_1, R_2) interpreted in terms of reduced spectral density mapping and model-free approach to access ps-ns dynamics. Our results highlight specific protein regions that undergo characteristic motions. In particular, we found that the central strands of the inner sheet (G and H strands) and the residues located at the subunit interfaces are affected by intermediate regime dynamics. In addition, the C and D strands stand out from other secondary structure elements showing particularly fast and slow dynamics at their edges. These findings suggest that the peculiar dynamic behavior of TTR regions involved in proteolytic cleavage (strand C) and protein dissociation (inner strands and residues at the subunit interfaces) could be crucial for the process of fibrillogenesis.

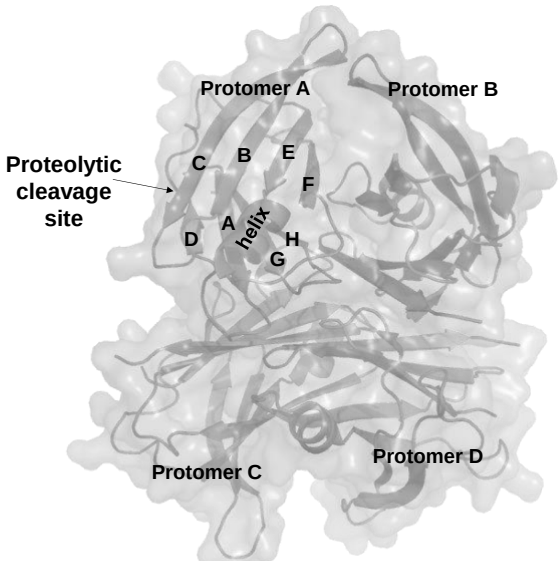

Fig. 1. TTR structure.

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[P-17] CHARACTERIZATION OF THALAMIC BOLD SIGNAL ALTERATIONS IN CHEYNE STOKES RESPIRATION

S. Cauzzo,[‡*] M. S. Morelli,[†] F. Frijia,[†] A. Giannoni,[†] D. Montanaro, C. Passino,[†] M. Emdin,[†] N. Vanello[*]

[‡]Istituto di Scienze della Vita, Scuola Superiore Sant'Anna, via Santa Cecilia 3, Pisa, Italy
[†]Fondazione Toscana G. Monasterio, Consiglio Nazionale delle Ricerche di Pisa, Pisa, Italy
[*]Centro di Ricerca "E. Piaggio", Università di Pisa, Pisa, Italy
E-mail: cauzzo.simone@gmail.com

Keywords: MRI, theory and methods.

fMRI is a promising solution to unravel chemoreception alterations in the Cheyne-Stokes Respiration (CSR) central abnormal breathing pattern, and to evaluate new targeting solutions for this predictor of Heart Failure (HF) mortality [1]. Physiological noise in subcortical regions in forced or pathological breathing patterns push the technique to its limits [2].
Here we use masked Independent Component Analysis (mICA [2]) on brainstem and subcortical nuclei to analyze, with fMRI at 3T, the BOLD response to hypercapnia in 10 healthy subjects (HS) during breath hold (BH) and 12 HF patients during rest. Patients are split in a HF group (N=6) and a periodic breathing (PB) group (N=6) in terms of diurnal Apnea/Hypopnea Index (dAHI≥25). We tailor the preprocessing pipeline to the study of CO_2 oscillations in subcortical regions [3]. Concurrently recorded end-tidal CO_2 ($P_{ET}CO_2$) is used in a cross-correlation analysis to define $P_{ET}CO_2$-related components and estimate lags for IC associated time courses to $P_{ET}CO_2$. IC stability across groups is assessed by spatial correlation.
Limiting our discussion to the thalamus, we recognize bilaterally one central and one posterior $P_{ET}CO_2$-related component, stable across analyses. We observe longer lags for the PB group with respect to both HS and HF groups, with stronger activations for BH in HS with respect to patients. There is an indication for overactivations in PB with respect to HF (Fig. 1).
We hypothesize CO2-dependent bottom-up connections for non-sensory respiratory feedback to cortical autonomic areas, and a role for elongated lags in CSR physiopathology. We sustain the feasibility of studying CSR alterations with standard clinical equipment.

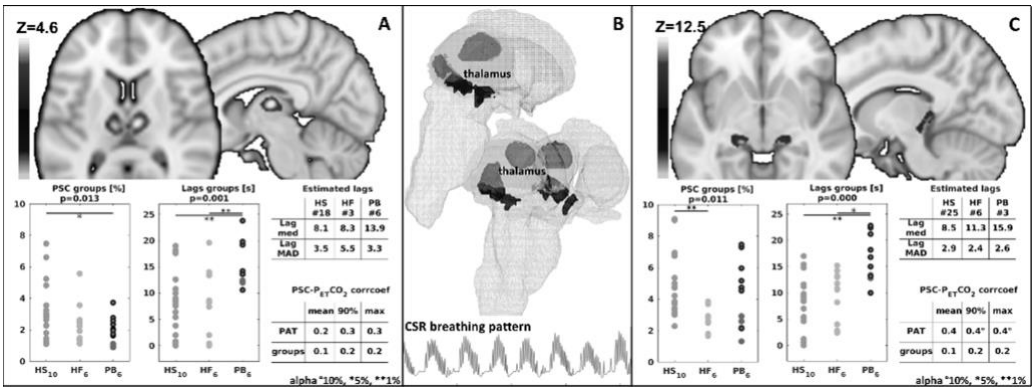

Fig. 1. A) Central Thalamic Component and C) Posterior Thalamic Component. Activation maps thresholded at $|Z|\geq 2$. For both components, we compare percental signal change (PSC) and time lags across groups; We also report median and MAD for estimated lags, and correlation of PSC with $P_{ET}CO_2$ mean, maximum and 90th percentile. B) 3D view of the thalamic components ($|Z|\geq 2$) and example of CSR respiration pattern.

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[P-18] CAVITAND-DECORATED SILICA NANOPARTICLES AS HYBRID RECEPTORS FOR THE SELECTIVE DETECTION OF N-METHYLATED AMINES

A. Cesari,[‡] D. Rosa-Gastaldo,[‡] F. Rastrelli,[‡] R. Pinalli,[†] E. Dalcanale,[†] F. Mancin[‡]

[‡]Dipartimento di Scienze Chimiche - Università di Padova, via Marzolo 1, Padova, Italia
[†]Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale - Università di Parma, Parco Area delle Scienze, 17/A, Parma, Italia
E-mail: andrea.cesari@unipd.it

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

The "nanoparticle-assisted NMR chemosensing" technique has been perfectioned in our group during the last years [1]. This protocol combines the recognition capabilities of nanoparticles with NOE-based NMR experiments to provide fingerprint detection of analytes of interest in complex mixtures. In general, the magnetization is transferred from the nanoparticles to the bound analytes through transient NOE or steady state NOE, making possible to tag and extract the analyte signals from the whole spectrum. Even if micromolar limit of detection was reached by optimization of the NMR sequences used [2], endowing the nanoreceptors with precision selectivity is still an open issue. Herein, we describe a new approach to the development of NMR nanosensors based on the spontaneous self-assembly of a cavitand equipped with four pyridinium groups (Fig. 1, left) [3] on the surface of 20 nm silica nanoparticles (Ludox) [4]. In this way, while the cavitand retains its selectivity, the perturbation of its molecular tumbling motion by the interaction with Ludox enhances the efficiency of the saturation transfer processes (Fig. 1, right). The hybrid nanoreceptors obtained in this way were capable to selectively detect *N*-methylated amines in water in the 10^{-5}-10^{-4} M^{-1} concentration range.

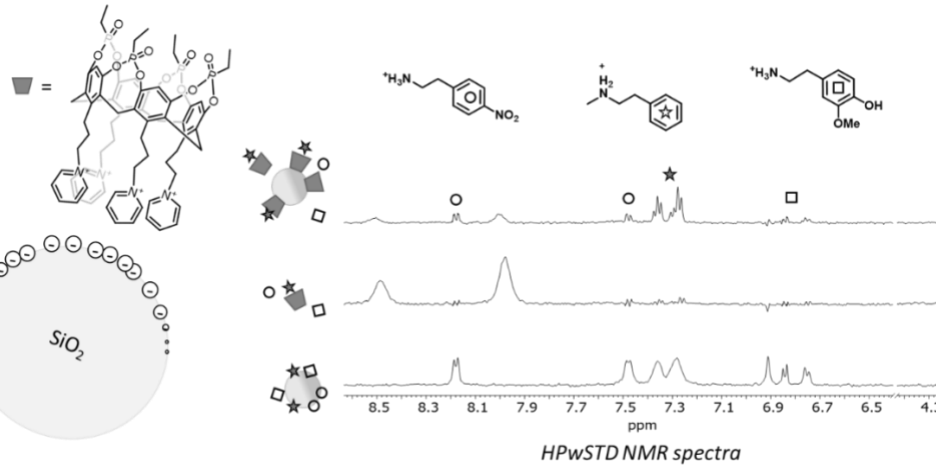

Fig. 1. Electrostatic interaction between cavitand and Ludox nanoparticle (left) and "high-power" waterSTD spectra (right) of a mixture of *N*-methylphenethylamine, 4-NO_2-phenethylamine, and 3 methoxytyramine in presence of the Ludox (low), cavitand (middle), and cavitand/Ludox (top).

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[P-19] In-depth Solid-state NMR Characterization of New Sulpiride Crystal Forms

R. Birolo,[a] F. Bravetti,[a] S. Bordignon,[a] I. d'Abbruzzo,[b] P.P. Mazzeo,[c] B. Perissutti,[b]
A. Bacchi,[c] M. R. Chierotti,[a] R. Gobetto[a]

[a]Department of Chemistry, University of Torino, Via P. Giuria 7, 10125 Torino, Italy
[b]Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.zza Europa 1, 34127 Trieste, Italy
[c]Department of Chemical, Life and Environmental Sustainability Sciences, University of Parma, Viale delle Scienze 17A, 43124 Parma, Italy
E-mail: rebecca.birolo@unito.it

Keywords: solid state NMR, small molecules.

Solid-state NMR is an effective tool for characterizing crystalline powder materials for which structural determination cannot be obtained by X-ray diffraction [1]. Salts and cocrystals are multicomponent crystal forms that have been of particular interest in recent years, especially in the pharmaceutical area for their improved properties with respect to pure drugs. The synthetic methods for achieving these new crystal forms often do not provide single crystals suitable for XRD analysis [2]. So an in-depth solid-state characterization through spectroscopic techniques is desirable. In this work, we present a thorough study that combines a multinuclear solid-state NMR (SSNMR) approach and *ab initio* calculations to determine the protonation state of new sulpiride (SULP) adducts. Twelve new crystal forms were designed to improve the solubility of SULP. The coformers chosen are mainly GRAS (Generally Recognized As Safe) molecules widely used in both food and pharmaceuticals, such as dicarboxylic acids (*e.g.* adipic acid, succinic acid, maleic acid...) or organic molecules containing an acid group (*e.g.* caffeic acid, p-aminobenzoic acid, ibuprofen…). ^{13}C and ^{15}N CPMAS SSNMR represent excellent methods for detecting proton transfers since they induce relevant shifts of the signals of the involved groups [3]. Focusing on our systems, a carboxylic acid group is present in each coformer: a high-frequency shift of its signal in the ^{13}C CPMAS spectra of the adducts indicates a salt formation. Conversely, a low-frequency shift highlights the achievement of a cocrystal (Figure 1) [4]. Using this approach, it was possible to determine the neutral or ionic character of the supramolecular synthons characterizing the newly discovered crystal forms.

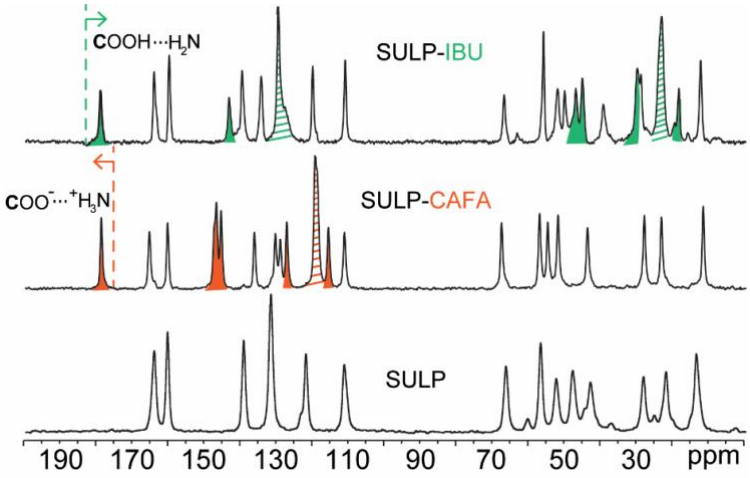

Figure 1. ^{13}C (100 MHz) CPMAS spectra of SULP, the molecular salt SULP and caffeic acid (CAFA) and the cocrystal SULP and ibuprofen (SULP-IBU). The signals of the COOH group in the pure coformers are represented by the dashed line to highlight the shift underwent upon cocrystallization. Colored peaks represent signals attributable to the coformer. Striped peaks result from the overlap of coformer signals with those of SULP.

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[P-20] LONGITUDINAL AND TRANSVERSE ^{1}H-NMRD PROFILES ANALYSIS OF LN-DOTA MRI CONTRAST AGENTS

D. Cicolari,[‡,†,*] F. Santanni,[◊] L. Grassi,[◊] F. Brero,[‡,§] M. Filibian,[*,♣] T. Recca,[♣] P. Arosio,[†,*] M. Perfetti,[◊] M. Mariani,[‡,§] R. Sessoli,[◊] A. Lascialfari[‡,§]

[‡] University of Pavia, Department of Physics, Via Bassi 6, Pavia (PV) 27100, Italy
[†] University of Milan, Department of Physics, Via Celoria 16, Milan (MI) 20133, Italy
[*] INFN, Istituto Nazionale di Fisica Nucleare – Milano Unit, Via Celoria 16, Milan (MI) 20133, Italy
[◊] University of Florence, Department of Chemistry, Sesto Fiorentino (FI) 50019, Italy
[§] INFN, Istituto Nazionale di Fisica Nucleare – Pavia Unit, Via Bassi 6, Pavia (PV) 27100, Italy
[♣] Centro Grandi Strumenti, University of Pavia, Via Bassi 21, Pavia (PV) 27100, Italy

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

The contrast enhancement in images acquired with Magnetic Resonance Imaging (MRI) techniques can be achieved by using contrast agents (CAs) [1]. The efficacy of a CA is determined with the *relaxivity*, *i.e.* the contrast enhancement in terms of the CA concentration. Amongst the ions of the lanthanide series, Gd(III)-based CA shows the highest relaxivities due to its long electronic relaxation times [2]. Non-Gd Ln(III)-based compounds has been recently suggested as potential negative CAs for high-field applications, as their paramagnetic transverse relaxation rate contribution scales quadratically with the chemical shift, which is proportional to the magnetic field [3].
In this study we analyzed by means of the models derived from the Solomon-Bloembergen-Morgan theory the Nuclear Magnetic Relaxation Dispersion (NMRD) profiles, *i.e.* the plot of the relaxivities *vs* the proton Larmor resonance frequency, of four different Ln(III)-DOTA complexes in aqueous solutions (Ln = Gd, Dy, Tb, Er; DOTA = 1,4,7,10- tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid) and of $[Mn(H_2O)^6]^{2+}$ aqua ions, for comparison, in order to study the links between the dynamic and magnetic properties characterizing the contrast enhancement at different frequencies (from 10 kHz up to 700 MHz, Fig. 1). We found that at high fields (> 7 T), the different magnetic anisotropies of non-Gd complexes influence the water exchange time so that the transverse relaxivities of Dy- and Tb-DOTA reached values comparable to the Gd-DOTA ones at clinical fields (1.5-3 T).

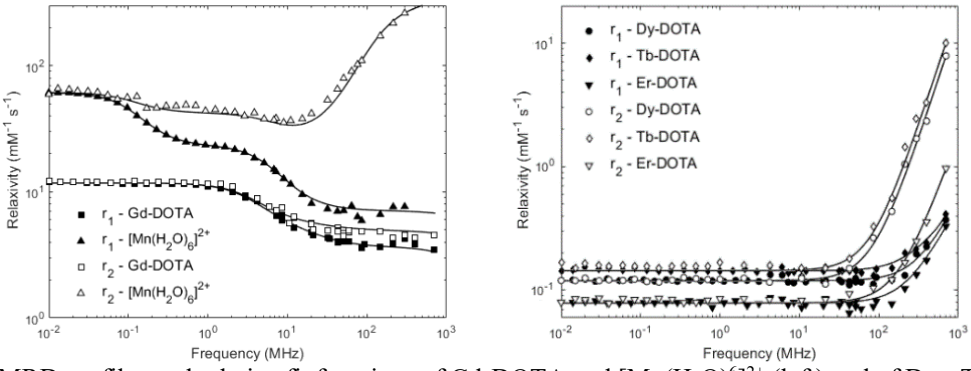

Fig. 1. NMRD profiles and relative fit functions of Gd-DOTA and $[Mn(H_2O)^6]^{2+}$ (left) and of Dy-, Tb-,and Er-DOTA (right).

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[P-21] EXPLORING THE LINK BETWEEN CELL METABOLISM AND THE RISK OF BLADDER CANCER PROGRESSION

G. Ciufolini,[‡] G. Petrella,[‡] V. Pasquale,[†§] S. Rota,[†] G. Ducci, [†] G. Campioni[†], M. Bonanomi, [§] R. Vago,[¥] E. Sacco,[†§] M. Vanoni[†§], D.O. Cicero[‡]

[‡] Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", 00133 Rome, Italy
[†] Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, 20126 Milano, Italy
[§] SYSBIO-ISBE-IT-Candidate National Node of Italy for ISBE, Research Infrastructure for Systems Biology Europe, 20126 Milan, Italy
[¥] Urological Research Institute, Division of Experimental Oncology, IRCCS San Raffaele Hospital, 20132 Milan, Italy; Università Vita-Salute San Raffaele, 20132 Milan, Italy
E-mail: ciufolini@scienze.uniroma2.it

Keywords: NMR spectroscopy, cellular metabolism, bladder cancer, progression

Bladder cancer (BC) is the 10th most commonly diagnosed cancer globally. Approximately 75% of patients are diagnosed with non-muscle invasive BC (NMIBC), with a high risk of recurrence and sometimes progression to muscle-invasive tumors (MIBC), which drastically reduces survival expectations, emphasizing the need to classify these tumor subgroups [1]. BC displays a high level of clinical and pathological heterogeneity and becomes important to identify new markers that can contribute to patients' stratification, improve prognosis, avoid relapses, provide a better quality of life, and reduce disease management costs [2]. Changes at the metabolic level are characteristic of cancer, as they allow cancer cells to satisfy their energy and biosynthetic needs to support increased cell growth and survival. Therefore, a study was conducted on six cell lines cultured in two-dimensional and three-dimensional models (monolayers and spheroids, respectively) [3,4].

The purpose is to identify metabolic targets correlated with tumor progression in bladder cancer and find potential differences between the two types of culture, considering that 3D culture can better mimic tumor conditions *in vivo*. Changes in the composition of the culture media of these cells were studied by nuclear magnetic resonance spectroscopy, evaluating the consumption of nutrients and excretion of metabolic products that reflect the metabolism of the different cell lines.

Exo-metabolomics data will be integrated with endo-metabolomics data, with the characterization of the proliferative and invasive potential of the cell lines and with their cellular bioenergetics analyzed by Seahorse XF technology.

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[P-22] DEVELOPMENT AND CHARACTERIZATION *via* NMR SPECTROSCOPY OF CHEMICAL MODIFIED STARCH-BASED BLENDS AND COMPOSITE FILMS

M.F. Colella,‡ R.A Salvino, ‡ V. Loise,‡ C. Oliviero Rossi,‡ G. De Luca‡

‡Department of Chemistry and Chemical Technology (CTC), University of Calabria, Via P. Bucci 14C Arcavacata di Rende (CS), Italy
E-mail: mariafrancesca.colella@unical.it

Keywords: solution NMR, materials, metabolomics, polymers.

Polymeric materials have always been used and produced for industrial applications for their interesting properties such as low density but high mechanical strength, corrosion resistance, electrical and thermal insulation, and relatively low costs [1]. However, to avoid environmental problems causing often by synthetic polymers derived from fossil fuel resources, the research is aimed at applying green biopolymeric alternatives such as polysaccharides like starch, cellulose, and chitosan. Starch is the second most abundant biopolymer found in nature, composed of two types of polysaccharides: amylose (linear) and amylopectin (branched) [2]. Its renewable nature, nontoxicity and low-cost are the most attractive properties that driven scientist to design, develop and produce new biodegradable substances involving starch substrates as starting material [1,3]. Nevertheless, the use of this biomaterial presents some limitation such as its poor solubility in water at room temperature, high viscosity one gelatinized, tendency to retrograde, thermal and microbial damage. To overcome these problems and improve its properties, starch could be chemical modified in different way thanks to its multiple hydroxyl groups [4,5]. In this study, still in progress, the effects and the possibility to create week interactions (with water or other polymers such PVA) or to introduce crosslinks (with epoxides or phosphate esters) to improve the performance of the starting starch material were investigated. New polymeric starch-based blend systems and film are studied in particular *via* NMR Spectroscopy that provide useful information both about the composition and molecular interactions formed into the new structure of starch systems. In particular high resolution NMR experiments have been used including 1D (^{1}H, ^{13}C, ^{31}P and ^{17}O NMR) and 2D (^{1}H DOSY and ^{1}H-^{1}H NOESY NMR). The NMR study is complementary and supported to other analytical techniques such as IR spectroscopy, to better understand the possibility of formation of new bonds, and rheological testing to explore mechanical properties of new materials and to check for their improvement.

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[P-23] MONITORING THE METABOLITES CONTENT OF SEASONED ZUCCHINI DURING SHELF LIFE BY NMR-BASED METABOLOMICS

R. Consonni[1], L. R. Cagliani[1], A. C. Boccia[1], F. Sparvoli[2].

[1] Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" - SCITEC, Lab. NMR, CNR, v. Corti 12, 20133 Milan, Italy
[2] Istituto di Biologia e Biotecnologia Agraria, IBBA, v. Corti 12, 20133 Milan, Italy

E-mail: roberto.consonni@scitec.cnr.it

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

The shelf life of a food products is the time during which food remains safe under defined storage conditions, maintaining the desired sensory, chemical, physical and biological characteristics in compliance with the label declaration [1]. Many physical factors could influence the shelf life like temperature changes, light exposure, gases transmission, humidity changes as well as contamination with microorganism and spores. Packaging plays a critical role in extending the shelf life of food products, preventing or reducing the environmental interactions. Recent EU regulations promoted a growing interest in bio-based materials production for replacement of the traditional petro-plastics, limiting the accumulation problem and reducing the environmental pollution. NMR spectroscopy represents a valid approach to evaluate the effects of packaging on the shelf life of foods and possible chemical contamination. [2]. Moreover, NMR spectroscopy has already demonstrated its pivotal role in metabolomics [3,4] allowing to monitor in a single experiment different classes of chemical compounds, and its capabilities in microstructural characterization of packaging materials. In this study the analyses of polar and organic extracts of seasoned zucchini stored at 4°C for 35 days, in plastic and compostable trays, performed by NMR in combination with chemometrics, are reported.

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[P-24] PRELIMINARY RESULTS OF A METABOHUB 2.0 INTER-LABORATORY TEST ON 1D ^{1}H NMR METABOLITE QUANTIFICATION OF A SYNTHETIC URINE.

C Deborde[1], P Giraudeau[2], E Cahoreau[3], G Da Costa[4], R Gautier[5], D Jacob[1], C Jousse[6], M Lacaze[5], I le Mao[4], E Martineau[2,7], A Moing[1], L Peyriga[3], T Richard[4], V Silvestre[2], M Traïkia[6], C Canlet[5]

[1]INRAE, UMRBFP & PF Bordeaux Metabolome – MetaboHUB, Villenave d'Ornon, Fr
[2] Nantes Université, CNRS, CEISAM, UMR 6230, Nantes, Fr
[3]TBI, Univ. Toulouse, CNRS, INRAE, INSA, MetaboHUB-Metatoul, Toulouse, Fr
[4]Univ. Bordeaux, INRAE, Bordeaux INP, UMROENO & PF Metabolome Bordeaux - MetaboHUB, Fr
[5]Toxalim, Univ Toulouse, INRAE, ENVT, INP-Purpan, & PF Metatoul-AXIOM, MetaboHUB, Toulouse, Fr
[6]Univ. Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut de Chimie de Clermont-Ferrand & Univ. Clermont Auvergne, INRAE, Pf Exploration du Métabolisme - MetaboHUB, Clermont-Ferrand, Fr
[7]CAPACITES SAS, Nantes, Fr

E-mail: catherine.deborde@inrae.fr

Keywords: solution NMR, small molecules, metabolomics.

Absolute quantification of individual metabolites in complex biological samples is of high importance in targeted metabolomics. There is a great diversity of practices in the scientific community, both in terms of data acquisition and processing methods for quantitative NMR metabolomics. Within MetaboHUB, the French national infrastructure of metabolomics and fluxomics, an inter-laboratory test was performed in order to evaluate the impact of NMR software or tools, NMR peak integration versus deconvolution approach, operator's level of expertise and know-how on quantitative results of 1D ^{1}H-NMR measurements of 32 major metabolites frequently observed in urine. For this purpose, a synthetic urine was prepared and used to compare the approach used by the 6 NMR platforms to (i) process the same set of ^{1}H-NMR spectra and quantify metabolites using commercial or in-house NMR software or tools, and (ii) share know-how between sites. One site was in charge of the preparation, NMR acquisition and distribution of the urine spectra and of five calibration range solution spectra for quantification. The synthetic urine was prepared from a "blank" stock solution supplemented with a known amount of 32 commercial compound solutions. The five calibration range solutions contained 32 compounds with concentration ranging from 0.1 to 12 mM. All samples were adjusted to pH 7.4 and supplemented with TSP for chemical shift calibration. The 600 MHz dataset was acquired and pre-processed (Fourier transform, phase and global baseline correction) with TopSpin at one site by one operator and sent to the other MetaboHUB sites. Each operator was asked to quantify the metabolites using the TopSpin integration module and external calibration factors established with the calibration range solutions, and also using their favorite in-house tool or open-access or commercial NMR software. Twenty metabolites plus the sums of creatine/creatinine or lactic acid/threonine were successfully quantified by all quantification strategies. With TopSpin, about 90% of the metabolites and metabolite sums were quantified with an accuracy below 5%. NMRProcFlow (nmrprocflow.org) allowed quantifying a few additional metabolites. The quantification accuracy improved for certain metabolites with deconvolution either with Mnova, or using a MetaboHUB in-house tool (all 20 individual metabolites quantified with an accuracy below 5%). In this poster, we present and discuss the results obtained by the different operators, software and quantification approaches. The results highlight the relevance of inter-laboratory tests to better rationalize the choice of quantification tools in targeted NMR metabolomics.

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[P-25] STUDY OF POROUS POLYMERS FOR GAS SEPARATION BY MEANS OF SOLID-STATE NMR

E. Della Latta,[‡] F. Martini,[‡, †] S. Borsacchi,[†,§] K. R. Storme,[I] T. M. Swager[I] M. Geppi[‡, †‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Giuseppe Moruzzi, 13, 56124 Pisa, Italy
[†]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), Lungarno Pacinotti 43, 56124 Pisa, Italy
[§]Istituto di Chimica dei Composti Organometallici, Consiglio Nazionale delle Ricerche (CNR-ICCOM), Via Giuseppe Moruzzi 1, 56124 Pisa, Italy
[I]Department of Chemistry Massachusetts Institute of Technology Cambridge, MA 02139, USA
E-mail: elisa.dellalatta@phd.unipi.it

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

Polymer-based membranes are very promising for gas separation, indeed they are appealing alternative systems to industrial energy-intensive processes. Polymers of intrinsic microporosity (PIMs) are a unique class of amorphous polymers, which, because of their rigid structure, cannot pack efficiently, leaving free volume, and in particular generating micropores [1]. Since these systems proved to be very effective for gas separation, the development of materials with higher performances is of current interest [2,3]. Obviously, the design of new materials requires a deeper understanding of their structure and dynamics, but also of the interaction between polymers and gases. Solid-State NMR (SSNMR) is one of the most powerful techniques for the structural and dynamic characterization of solid materials, serving as a guidance for the development of new systems with improved gas separation properties. In this work we present a study of triptycene-based polymers containing fluorinated aromatic rings (see Fig.1) by means of SSNMR.
A structural investigation was carried out by exploiting ^{1}H, ^{19}F Direct Excitation (DE)/Magic Angle Spinning (MAS), $^{13}C\{^{19}F\}$ and $^{13}C\{^{1}H\}$ Cross-Polarization (CP)/MAS experiments. To get insight into the dynamics of the polymer, ^{1}H and ^{19}F spin-spin (T_2) and spin-lattice (T_1) relaxation times were measured by low-field experiments. Moreover, spin-lattice relaxation times of carbon-13 were measured under MAS conditions and high power decoupling from either ^{1}H or ^{19}F nuclei. The analysis of the SSNMR results allowed the main structural and dynamic features of the material to be unravelled.

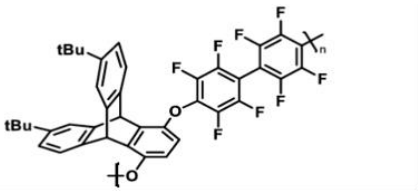

Fig. 1. Chemical structure of the repeating unit of a triptycene-based polymer

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[P-26] STRUCTURAL AND FUNCTIONAL ALTERATIONS OF HUMAN UBIQUITIN UPON EXPOSURE TO POLYSTYRENE NANO-PLASTICS

M. della Valle,[‡] G. D'Abrosca, [‡] L. Russo,[‡] C. Isernia,[‡] R. Avolio,[†] M. E. Errico,[†] M. Cocca,[†] G. Gentile,[†] G. Malgieri,[‡] and R. Fattorusso [‡]

[‡]Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania – Luigi Vanvitelli, via Vivaldi 43, 81100 Caserta, Italy
[†]Institute for Polymers, Composites and Biomaterials - CNR, via Campi Flegrei 34, 80078 Pozzuoli, Naples, Italy
E-mail: maria.dellavalle@unicampania.it

Keywords: solution NMR, materials, biomolecules, polymers.

Nowadays, the concerns about micro- and nano-plastics (MPs and NPs) pollution have been increasing constantly. Plastic micromaterials easily fragment in smaller particles that we can find in the air, water, food [1] and even in blood cells [2]. Since the causes and effects are scanty, it is very important to understand their behavior towards biological systems and (bio)macromolecules present in them.
Here, we show that polystyrene nano-plastics (23 nm) are prone to interact with the human Ubiquitin, one of the best-known proteins. Therefore, we explored structure and dynamics of the protein by performing TEM (Trasmission Electron Microscopy), CD (Circular Dichroism) and high-resolution NMR (Nuclear Magnetic Resonance) analyses. Moreover, we tested the influence of these nanoparticles on ubiquitin functions by investigating *in vitro* and *in cell* ubiquitination. Overall, our results confirm that NPs can cause potential harm and induce relevant toxicological consequences.

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[P-27] HOMOGENEOUS LIQUID-LIQUID EXTRACTION OF LITHIUM WITH THERMOMORPHIC IONIC LIQUID STUDIED BY NMR SPECTROSCOPY

Antonio DE SOUZA BRAGA,[‡] Baptiste RIGAUD,[†] Anne-Laure ROLLET, [‡] Guillaume MÉRIGUET,[‡] Juliette SIRIEIX-PLÉNET[‡]

[‡]Sorbonne Université, Physicochimie des Électrolytes et Nanosystèmes Interfaciaux, UMR 8220, 4 Place Jussieu, F-75005, Paris, France
[†]Sorbonne Université, Fédération de Chimie et Matériaux de Paris Centre (FR2482), 4 Place Jussieu, 75005, Paris, France
E-mail: *antonio.de_souza_braga_neto@sorbonne-universite.fr

Keywords: solution NMR, materials, small molecules.

The constant increase in critical metals (Li, Co, rare earths, etc.) demand promotes innovation efforts towards recycling. These metals are essential to the functionality of emerging green technologies and present a supply risk. For instance, lithium is essential for batteries production and its demand tends to increase considerably in the coming decades [1]. The recycling of critical metals is mainly carried out by a succession of stages, which are leaching, liquid-liquid extraction and recovery. The liquid-liquid extraction, which is basically the distribution of solutes between two immiscible liquids in contact with each other. The present industrial processes use large amounts of toxic volatile organic solvents and extractants. Ionic liquids (ILs) appear then as a valuable alternative to replace volatile organic solvents since they are less toxic, flammable and volatile than conventional solvents. In some cases, they improve the extraction efficiency of the process [2]. Homogeneous liquid-liquid extraction (HLLE) is an application of thermomorphic ILs. This process takes advantage of these liquids which exhibit temperature-dependent miscibility behavior with aqueous solutions [3]. In the homogeneous state, the interface disappears and there is no diffusion barrier for extraction. However, the extraction mechanisms are not fully understood. Most studies of liquid-liquid extraction process are based on analytical techniques used before and after the extraction process, and focus mainly on metal ions. Nuclear magnetic resonance (NMR) spectroscopy is a useful tool to study IL systems. This work focuses on the HLLE of lithium whit the ionic liquid choline bis(trifluoromethylsulfonyl)imide [Chol][TFSI] and extractant betaine. NMR spectroscopy is used to fill the gaps in the understanding of the process since this technique allows to observe the different species present in the system, such as, extractant, cation and anion of the IL, following different nuclei (^{1}H, ^{19}F, ^{7}Li). In particular, an NMR LOcalized SpectroscopY sequence (LOCSY), [4] is used to follow the evolution of the species (concentration in the bulk and at the interface, speciation, diffusion, etc.) during the extraction process. Quantitative NMR, through the digital ERETIC (Electronic REference To access In vivo Concentrations) method [5] is applied to access the lithium extraction efficiency of the process. To observe the dynamics in the system and the molecular environment experienced by the species involved, Diffusion ordered spectroscopy (DOSY) is applied to the measurement of diffusion coefficients. NOESY and HOESY (Nuclear and Heteronuclear Overhauser Effect SpectroscopY) are used to obtain through-space proximities between nuclei, which permits a better understanding of the mechanism of extraction.

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[P-28] NMR CHARACTERIZATION OF ROOTS, LEAVES AND FLOWERS OF GENTIANA LUTEA L. FROM MAJELLA NATIONAL PARK

G. Di Matteo,‡ M. Spano, ‡ L. Mannina‡

‡Department of Chemistry and Technology of Drugs, Laboratory of Food Chemistry,
Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
E-mail: giacomo.dimatteo@uniroma1.it

Keywords: NMR, Gentiana Lutea, metabolomics, food.

An NMR based metabolomic characterization of roots, leaves and flowers of *Gentiana Lutea* L. harvested in the "Majella National Park" (Central Italy) was carried out.
Gentiana Lutea L. is a perennial herb of 50-120 cm with an erect stem, broad lanceolate to elliptic leaves of 10–30 cm long and 4–12 cm broad and a characteristic yellow flower, diffused in the mountain of Central and Southern Europe. Unfortunately, due to the *Gentiana* roots massive collection along century there is a depletion of the natural population. The *Gentiana* roots are largely used in the world as source of many bioactive compounds with interesting pharmacological effects, reported also in the Chinese, Japanese and European Pharmacopeias and also to prepare some bitter beverages. In this scenario, it is of interest the study of the chemical profile of the Italian wild *Gentiana Lutea* L., in order to valorize the different plant parts (roots, leaves and flowers). For this purpose, the high field NMR spectroscopy has been applied to study the Blight-Dyer hydroalcoholic extracts [1] obtaining the whole chemical profile (sugars, amino acids, organic acids, etc.). It is noteworthy the identification of loganic acid (an iridoid), and sweroside (a secoiricoid). Regarding the *Gentiana* flowers (the edible part), the *in vitro* digestion and the bioavailability of different bioactive compounds were also determined through the *in vitro* intestinal epithelium model in differentiated Caco-2 cells. Moreover, the amount of iridoids, secoiridoids and xanthones, the most studied *Gentiana* bioactive compounds, was also determined by the HPLC-PDA technique.

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[P-29] Interaction of the respiratory syncytial virus non-structural protein 1 with the MED25 subunit of the mediator complex

Jiawei Dong ‡, Vincent Basse †, Marie Galloux †, Jean-Francois Eléouët †, Monika Bajorek †, Christina Sizun ‡

‡ Institut de Chimie des Substances Naturelles, CNRS UPR2301, Université Paris-Saclay, Gif-sur-Yvette, France
† Virologie et Immunologie Moléculaires, INRAE UR892, Université Paris-Saclay, Jouy-en-Josas, France
E-mail: jiawei.dong@cnrs.fr

Keywords:
Solution NMR, biomolecules, polymers

Human Respiratory Syncytial Virus (RSV) is a major cause of severe respiratory disease of the lower respiratory tract, particularly in young children. RSV was discovered 65 years ago, but there are still no effective vaccines or antivirals, although different virus-specific molecular mechanisms were tested as potential targets for antiviral therapies. Of the 11 encoded viral proteins, the non-structural proteins NS1 and NS2 play an essential role in viral escape from the host innate immune response. Here we focus on the characterization of the structure and the interactions of the RSV NS1 protein. Although the 3D structure of NS1 became available by X-ray crystallography, its behavior in solution remains puzzling. The NS1 protein appears to have a tendency to self-assemble, and NS1 was suggested to undergo conformational transitions that might favor self-assembly. In a first part, we sought for experimental conditions to stabilize NS1 in solution and to investigate its dynamics by NMR. UV CD measurements showed that under our experimental conditions, NS1 contained a mixture of a-helix, ß-sheet and random coil secondary structure, in agreement with the X-ray structure. Our NMR data gave a more contrasted view: chemical shift dispersion pointed to a structured protein, but broad lines pointed to conformational heterogeneity, suggesting a molten globule state. Under specific temperature, pH and salt conditions, HRSV-NS1 formed higher order oligomers of defined size with amyloid-like properties, like thioflavin T-binding. We therefore tested mutations to increase the spectral quality, and we obtained a deletion mutant that has lost the ability to form oligomers at low pH. In a second part, we identified the MED25 mediator complex subunit as an interactor of HRSV-NS1. We mapped the interaction to the transactivator binding domain of MED25 (MED25-ACID) and showed that both the C-terminal helix of HRSV-NS1 (NS1-a3) and the N-terminal ß-barrel domain of NS1 were involved in the interaction. ITC studies performed with MED25-ACID and full-length HRSV-NS1 revealed a high 20 nM affinity. Since the NS1-a3 region strongly reminded of a transactivation domain (TAD), we analyzed binding of the NS1-a3 peptide to MED25-ACID. Chemical shift perturbations (CSPs) in the 2D 1H-15N HSQC spectrum of 15N-labeled MED25-ACID confirmed an interaction between the peptide and MED25-ACID. Mapping of CSPs induced by NS1-a3 onto the 3D structure of MED25-ACID showed that the primary binding site is the H2 face, which is also targeted by TADs of transcription regulators such as Herpex simplex VP16. We measured lower affinity for the isolated peptide (15-20 µM), but this value was of the same order of magnitude of affinities reported for other single TAD peptides, e.g. p53 TAD2. Our next aim is to investigate the interaction between MED25-ACID and the N-terminal ß-barrel of NS1.

[P-30] EVALUATION OF THE CU(I) AND CU(II) COORDINATION BY A PROKARYOTIC ZINC FINGER ROS-87 BY NMR TECHNIQUES

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

[†] Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania Luigi Vanvitelli, via Vivaldi 43, 81100 Caserta, Italy
E-mail: martina.dragone@unicampania.it

Keywords: solution NMR, biomolecules

Prokaryotic zinc finger Ros is a protein involved in the horizontal transfer of genes from A. tumefaciens to a host plant infected by it. It acts as transcriptional repressor and was identified in 1998 by Chou et al in Agrobacterium tumefaciens [1]. Ros belongs to a family of proteins, namely Ros/MucR, whose members have been recognized in different bacteria, mostly α-proteobacteria, symbionts, and pathogens of mammals and plants [2]. The Ros/ MucR gene is very conserved in proteobacteria and is central for host-bacterium interactions [3].
The protein contains a single Cys2-His2 zinc finger domain and assumes a ββββαα fold stabilized by the presence of a 15 amino acids hydrophobic core. It was intensely studied as suitable model domain to unveil the effect of the metal ion replacement in metallo-proteins and appeared to tolerate the Zn to Cd substitution but not the replacement of the wild type metal by Ni(II), Pb(II) and Hg(II) [4]. Here, we explore the effects of the Zn to Cu(I) or Cu(II) replacement and we demonstrate that the metal, in both oxidation states, binds Ros with good affinity, but the binding does not give rise to a correct functional fold.

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[P-31] RESCUE 3: Versatile decision-making tool for NMR spectral assignments of proteins.

L. Duciel,[‡] T. Malliavin,[†] M.-A. Delsuc[*]

[‡] Casc4de, Pôle API, 300 Bd Brant 67400 Illkirch, France
[†] Laboratoire de Physique et Chimie Théorique UMR CNRS 7019. Université de Lorraine Bd des Aiguillettes 54506 Vandœuvre-lès-Nancy, France
[*] IGBMC, 1 rue Laurent Fries 67404 Illkirch, France & Casc4de, Pôle API 300 Bd Brant 67400 Illkirch, France

E-mail: laura.duciel@casc4de.eu

Keywords: biomolecules

Spectral assignments of proteins in NMR is crucial as, even today, this generally requires lots of manual work and good expertise. The development of automatic techniques would therefore greatly accelerate analyses and thus enables a higher throughput in cases where this step is required, such as for ligand interaction screening, protein-protein recognition, NMR structure determination and/or when assignment information is not obtainable (e.g non labeled proteins, methyl-based approaches).
RESCUE is a statistical approach developed in 1999 for NMR spectral assignment of proteins through a simple artificial neural network (perceptron) [1]. It allowed the type of amino acid to be determined from the observed ^{1}H chemical shift. It used as a training set the data available in the BMRB (bmrb.io) at the time. It was extended in 2004 to the whole spin set, using a "Naive Bayes" probabilistic model for each amino acid type from a set of given chemical shifts [2]. Predictions accuracy of this second version reaches up to 75%.
The recent development in Deep Learning (DL) as well as the current size of the BMRB database available today allow to get better assignment predictions. We developed a Deep Neural Network (DNN) together with a cleaned database by removing duplicates, strongly homolog entries, non-protein sequences and scarcely assign entries and by realigning chemical shift references. We obtain a final database from the BMRB including of 485264 sets of chemical shifts and a representation of the 20 classical amino acids to feed the neural network.
We developed a 7 dense layers neural network using the open-source Keras Python library. An adamax optimizer and a categorical crossentropy loss were used, with training on 25 epochs. We then built scenarios to filter the chemical shift sets according to what is acquired experimentally. This allows us to adapt the DNN to each sets of available experiments.
The newly developed algorithm has been tested in several situations reproducing possible scenarios and allowing to evaluate the efficiency of the program in different cases. In all cases the algorithm shows very good results and is able to make relevant amino acid predictions with assessment of prediction accuracy.

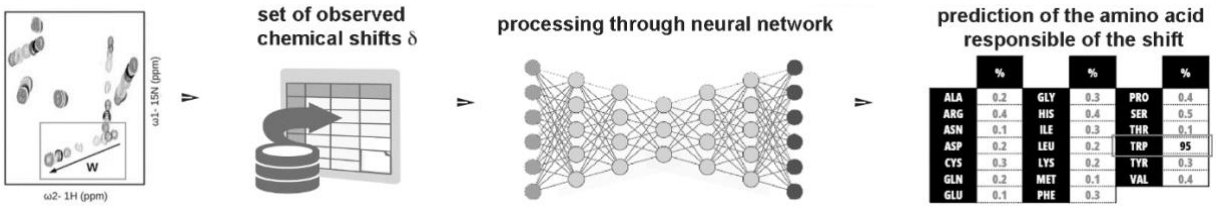

Fig. 1. RESCUE 3 approach

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[P-32] FLUOVIAL Monitoring of fluorinated emerging pollutants through ^{19}F NMR & ML

L.Duciel,[‡] A. Briot-Dietsch,[‡] M-A. Delsuc[†]

[‡] Casc4de, Pôle API, 300 Bd Brant 67400 Illkirch, France
[†] IGBMC, 1 rue Laurent Fries 67404 Illkirch, France & Casc4de, Pôle API 300 Bd Brant 67400 Illkirch, France
E-mail: laura.duciel@casc4de.eu

Keywords: solution NMR, small molecules

Fluorine is a key element more abundant than sulfur or zinc on Earth crust. It is unknown to biologist as there is nearly no fluorine metabolism in any living organism. To the chemist, it is however a favorite element, with the highest electronegativity and a really strong C-F bound that provides specific properties and extreme resistance to harsh conditions. Due to these characteristics, the use of fluorine in pharmaceutical, agrochemical and repellent or nonstick finishes industries has been extensively developed. Today ca. 30 % of drugs and 50 % of crop protection products under development contain fluorine. Indeed, it is already present within our houses in the form of drugs such as the antibiotics Ciflox, the anti-inflammatory Nifluril or the lipid lowering agent Lipitor…About 50% of agrochemicals contain a fluorine moiety. We find it also in perfluorinated polymers blockbusters (Teflon, Nafion, Scotchgard...) often used in water repellent everyday objects. Nevertheless, these molecules share a common feature: they accumulate in ecosystems and present a toxicity with confirmed impacts on human health. Therefore fluorine is considered as emergent environmental contaminants and persistent organic pollutants (POPs)[1] described e.g. as being endocrine disrupting and carcinogenic.
For now, no complete technique exists to detect, identify and quantify these new pollutants. LC-MS is classically used but although this targeted method has a high sensitivity, it also has disadvantages, in particular the need for reference standards and the specificities of time-consuming sample preparation involving treatments that may alter its content.

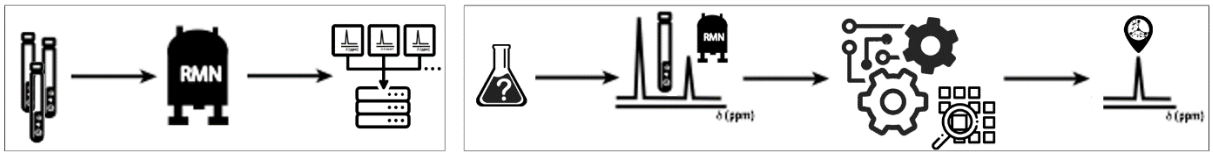

Fig. 1. FLUOVIAL approach using ^{19}F NMR and Machine Learning (

Our FLUOVIAL platform offers a novel approach for target-free, quantitative and robust monitoring of fluorinated pollutants based on ^{19}F NMR and Machine Learning (ML) techniques to improve the assignment of ^{19}F chemical shifts. It has been developed by first recording ^{19}F NMR datasets from known fluorinated compounds, and then processing them by applying the Plasmodesma algorithm generating specific information-carrying descriptors [2]. A ML approach, based on a random forest algorithm which provides explanations for the predictions made, was then trained on a in-house database and used successfully to detect and identify fluorinated compounds in polluted water and soil samples.

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[P-33] THE IMPACT OF MACROCYCLE FORMATION ON THE CONFORMATION OF TOLAASIN I AS REVEALED BY NMR SPECTROSCOPY

Durga Prasad[1], Benjámin Kovács[1], Dries Roelandt[1], Niels Geudens[1] and José C. Martins[1]

[1] *NMR & Structure Analysis Unit, Dept. of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium*
E-mail: durga.prasad@ugent.be

Keywords: Solution NMR, BIOMOLECULES.

Cyclic lipopeptides (CLiPs) are a class of secondary metabolites that consist of a peptide moiety with an N-terminal lipid tail and a macrocycle formed via an ester bond (depsi) between the C-terminal carboxyl group and a side chain hydroxyl group. They are produced by multiple genera of bacteria including *Pseudomonas* and *Bacillus*. CLiPs exhibit a multitude of biological functions in Nature such as improving bacterial motility, antibacterial activity and antifungal activities among others.[1]

Tolaasin, a CLiP produced by *Pseudomonas tolaasii*, is the causative agent for brown blotch disease in mushrooms. When tolaasin is released by the bacterium it forms pores in the membrane of epithelial mushroom cells, creating lesions which develop a brown colour, ruining the mushrooms for consumption. Recent studies have shown that a natural defence mechanism exists where the antagonistic properties of tolaasin are lost by hydrolysis of the depsi bond closing the macrocycle. This involves an enzyme released by another bacterium inhabiting the same ecological niche. [2] Mainly unstructured in water, tolaasin adopts a stable amphipathic left-handed a-helix ending in a flattened macrocycle loop when exposed to micelle forming detergents such as SDS. [3] In this project we aim to better understand why loss of the macrocycle has such a marked effect on tolaasin activity by comparing the conformation adopted by hydrolysed tolaasin with that of the native molecule, both studied in SDS micelles using NMR spectroscopy.

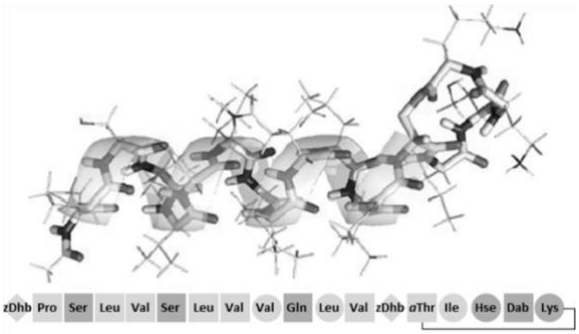

Fig. 1. The conformation and the amino-acid sequence of Tolaasin I. The fatty acid moiety is omitted for clarity.

To allow the use of more elaborate multidimensional structure assignment and analysis methods, we first produced ^{13}C and ^{15}N isotope enriched tolaasin by growing the producing bacterium on minimal medium and suitable labelled isotopically enriched precursors. Next, we produced the isotopically enriched hydrolysed form by controlled alkaline hydrolysis followed by purification. Following full resonance assignment, HNHA measurements are first used to obtain the φ torsion angle dependent $^{3}J_{HNHA}$ coupling in both forms. Next, long range HNCO measurements are recorded to identify the presence and location of long-lived hydrogen bonds through so called $^{3H}J_{HN-C'}$ scalar couplings. By comparing these coupling data in both tolaasin forms, changes in the conformation can be detected and interpreted. Experiments mapping the location and orientation of tolaasin in SDS using paramagnetic relaxation enhancement probes currently in preparation may be presented as well.

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[P-34] Towards integrated control of a chemical reactor and a high-field NMR spectrometer

N. El Sabbagh,[‡] M. Bazzoni,[‡] C. Lhoste,[‡] J. Bonnet,[‡] A. Bernard,[‡] D. Cortés-Borda,[†] P. Giraudeau,[‡] F.-X. Felpin,[‡] J.-N. Dumez[‡]

[‡]Nantes Université, CEISAM, CNRS UMR 6230, 2 rue de la Houssinière, F-44000 Nantes, France
[†]Basic Sciences Faculty, University of the Atlantic, Puerto Colombia, Colombia
E-mail: nour.elsabbagh@univ-nantes.fr

Keywords: solution NMR, small molecules, instrumentation.

Automated technologies are nowadays used to assist synthetic chemists in their daily assignments; from self-optimizing reactors [1] to whole autonomous flow optimization systems [2,3]. Different in-line analysis methods have been used, such as IR spectroscopy [4], HPLC [5] or benchtop NMR [6], to track reaction yields or conversion and seek optimal experimental conditions, while monitoring the chemical reactions on the fly. High-field NMR can also be a powerful tool for real-time monitoring, and its higher resolution and sensitivity is needed for reactions that cannot be addressed with benchtop NMR. However, to incorporate this detection method within an automated reactor, experiments have to be created, launched, monitored and their acquired spectra investigated in an automated and integrated approach.

In this work, we develop a MATLAB based graphical user interface (GUI) to control remotely a Bruker high-field NMR spectrometer. The GUI can be used to program experiments, and collect and analyse spectra. It is designed to be integrated in a software for reaction control and optimization. We will describe the design of the program and examples of applications.

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[P-35] Highly sensitive "off/on" EPR probes to monitor enzymatic activity by OMRI

S. Elkhanoufi[‡], D. Alberti[‡], E. Thiaudiere[†], R. Stefania[‡], E. Parzy[†], S. Rakhshan[‡], P. Mellet[†§], P. Massot[†], S. Aime[‡] and S. Geninatti Crich[‡]

[‡]Department of Molecular Biotechnology and Health Sciences, University of Torino, 10126 Torino
[†]Magnetic Resonance of Biological Systems, University of Bordeaux-CNRS, Bordeaux, France
[§] INSERM, Bordeaux, France
E-mail: sabrina.elkhanoufi@unito.it

Keywords: MRI, EPR, contrast agents.

OMRI (Overhauser MRI) is a double resonance experiments based on the magnetization transfer from the high electron magnetic moment of a stable radical to the water proton located close to the radicals, obtained by the saturation of the radical electronic transitions [1]. The enhancement of the MRI signal makes this technique a good reporter about the stable radicals biodistribution. To this purpose, we synthesized two nitroxide acyl esters containing a C_{12} aliphatic chain, namely Tempo-C_{12} (T-C_{12}), and Tempo-2-C_{12} (T-2-C_{12}). Both Tempo-containing esters (T-C_{12} and T-2-C_{12}) exhibit a low solubility in water and aggregate to form stable micelles. The radicals in the micellar aggregates are practically EPR silent showing a low and broad EPR signal. The two "off/on" EPR probes in micellar form were investigated as substrates for specific esterases enzymes over-expressed in pathological tissues, thus allowing to design efficient molecular imaging tests. The esterases enzymes investigated are the human Carboxylesterase (CEs) 1 and 2 and Procine Liver Esterases (PLE) [2]. The enzymatic hydrolyzation generates a narrow and intense EPR signal because of the release of the nitroxide radical from the micelle, that is proportional to the enzymatic activity [3]. Other hydrolases and proteins were also investigated without observing any detectable hydrolysing capacity on the two compounds considered in this work. The enzymatic activity was well detected in the corresponding OMRI images acquired at 0.193T (Fig. 1) and results showed that T-2-C_{12} in the micellar form does not generate any detectable DNPF (Dynamic Nuclear Polarization Factor). Upon the addition of the enzymes and the release of the nitroxide radical, the DNPF increased displaying bright images. In summary, the method has the potential to be translated to in vivo OMRI detection of specific enzymatic activities. The "off-on" transition of the probes makes the methodology extremely sensitive and quantitative avoiding the use of complex ratiometric corrections to eliminate the contribution arising from the not hydrolysed probes. In fact, the sensitivity observed is of the same order of the values achieved with optical methods.

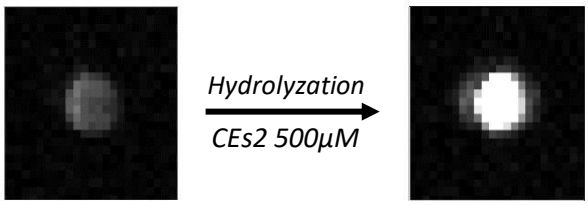

Fig. 1. OMR Image of T-2-C_{12} (1 mM) before (left) and after (right) 2 hours incubation with CEs 2 (500 nM).

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[P-36] NMR CHARACTERIZATION OF PROTEIN-PROTEIN INTERACTIONS INVOLVED IN PTP MODULATION AND RELATED DISEASES

S.Fabbian,[‡] C.Galber,[†*] V.Giorgio[†*] and M.Bellanda[‡]

[‡]Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy
[†]Institute of Neuroscience - CNR and Department of Biomedical Sciences, University of Padova, V.le G. Colombo 3, 35121 Padova, Italy
*Department of Biomedical and Neuromotor Sciences, University of Bologna, 40126 Bologna, Italy

E-mail: fabbian.simone@gmail.com

Keywords:
solution NMR, biomolecules.

The mitochondrial F-ATP synthase is emerging to be closely related to PTP (mitochondrial Permeability Transition Pore) [1]. The PTP activity was found to be dysregulated in different kind of human diseases as Alzheimer's and cancer [2]. One of the ATP synthase subunits, the OSCP (Oligomycin Sensitivity Conferral Protein), plays a crucial role into PTP modulation [3] but the biophysical analysis of this protein in solution has never been performed before for its tendency to non-specific aggregation and precipitation [4]. The OSCP is the binding site for the mitochondrial isomerase Cyclophilin D (CypD) and Bz423, which are two positive modulators of the PTP opening. The pro-apoptotic role of CypD related to the PTP is involved in Alzheimer's, ischemic reperfusion injury and aging [5]. However, the binding between OSCP and CypD has never been characterized at molecular level. The mitochondrial inhibitor IF1 (ATPase Inhibitory Factor 1) is known for its binding to the F-ATP synthase, at the F1 catalytic sector, inhibiting in this way the hydrolysis of ATP in anoxia conditions. The inhibitor is overexpressed in different kind of tumors [6] suggesting possible implications with cancer cells sustainment, likely through the modulation of PTP.
Here we describe for the first time the structural properties of the human OSCP C-terminal domain in solution by multidimensional NMR and SAXS analysis. We demonstrated that the whole OSCP specifically homo-oligomerizes through its C-terminal domain and it is present as a dimer even in diluted conditions. Furthermore, we investigated at molecular level, by chemical shift perturbation, the novel interaction between OSCP and IF1, which is related to PTP suppression and consequent apoptosis resistance in cancer cells. Finally, NMR was used to map the binding epitope on the CypD surface for the interaction with the OSCP. Altogether, our research helps to understand the molecular details of the m-PTP modulation by protein-protein interactions involving the ATP synthase subunit OSCP, for further therapeutical applications.

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[P-37] An FBDD-NMR approach to target the underdog in the Bcl-2 family, Bfl-1.

A. Favaro,[‡] F. Ferrari,[†] J. De Almeida Roger,[‡] S. Moro,[‡] M. Bellanda,[†] M. Sturlese [‡]

[‡] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, via Marzolo 5, 35131, Padova (Italy).
[†]Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131, Padova (Italy).
E-mail: annagiulia.favaro@phd.unipd.it

Keywords:
solution NMR, small molecules, biomolecules.

Bfl-1 is an antiapoptotic member of the Bcl-2 family protein and its overexpression is associated with tumorigenesis and chemotherapy resistance [1, 2]. Therefore, it is an interesting pharmacological target in anticancer drug discovery. However, there are currently no drugs that can be used in therapy to inhibit this protein and still no selective and high-affinity small molecules are available [3,4].
For this oncogenic challenging target, we performed a Fragment-Based screening by NMR to identify small molecules (MW < 300 Da) that bind to Bfl-1. These low-affinity ligands will represent the starting point for the development of a more complex molecule with a higher affinity for the target.
Specifically, a total of 1000 fragments were preliminarily screened in mixtures performing protein-based NMR experiments (^{1}H-^{15}N HMQC coupled to SOFAST pulse scheme). Mixtures containing binders were then deconvoluted using ligand-based NMR experiments (waterLOGSY) to identify which fragment showed affinity.
At the end of this screening, we selected three fragments as the most promising binders. These hits bind to Bfl-1 at the BH3 binding groove, which mediates the oncogenic protein-protein interactions, which lead to the survival of cancer cells after chemotherapy treatments [3]. Moreover, two fragments covalently bind to the unique surface-accessible cysteine, while the other one has an affinity constant in the micromolar range. These low-affinity ligands will be optimized into a larger molecule, that could inhibit Bfl-1 and its oncogenic interactions, to overcome chemotherapy resistance in tumors, where Bfl-1 overexpression determines the apoptosis escape of malignant cells after anticancer treatments.

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[P-38] EPR AND NMR CHARACTERIZATION OF NV CENTERS IN DIAMONDS

F. Ferrer [‡], F. Ziarelli [†], P. Thureau [‡], S. Viel [‡,ǂ]

[‡] *Aix Marseille Univ, CNRS, ICR, Marseille, France*
[†] *Aix Marseille Univ, CNRS, Centrale Marseille, FSCM, Marseille, France*
[ǂ] *Institut Universitaire de France, Paris, France*
E-mail: florian.ferrer@univ-amu.fr

Keywords: solid state NMR, EPR, hyperpolarization, materials, instrumentation.

The improvement of sensitivity is a major challenge in the field of Nuclear Magnetic Resonance. In order to achieve this goal, Dynamic Nuclear Polarization (DNP) is a technique already widely used but with many constraints. Other promising alternative methods are being developed. One of these is the use of nitrogen-vacancy centers (NV centers) [1,2], which are defects in diamonds used as hyperpolarising agents *via* laser radiation (532 nm). However, this method requires knowledge of the amount of NV centers present in diamonds as well as their respective spatial orientation. In this work, we have used continuous wave Electronic Paramagnetic Resonance (EPR) spectroscopy to obtain this information for a series of millimeter-sized diamonds previously irradiated with high-energy electrons beam and subsequently annealed at high temperature under specific experimental conditions. In this way we could reliably quantify the amount of NV as well as P1 centers (another paramagnetic defect in diamonds). Preliminary NMR experiments were also conducted to characterize the diamonds and evaluate the signal enhancement due to laser irradiation, and these results will be discussed with respect to the NV and P1 centers contents of the analyzed diamonds.

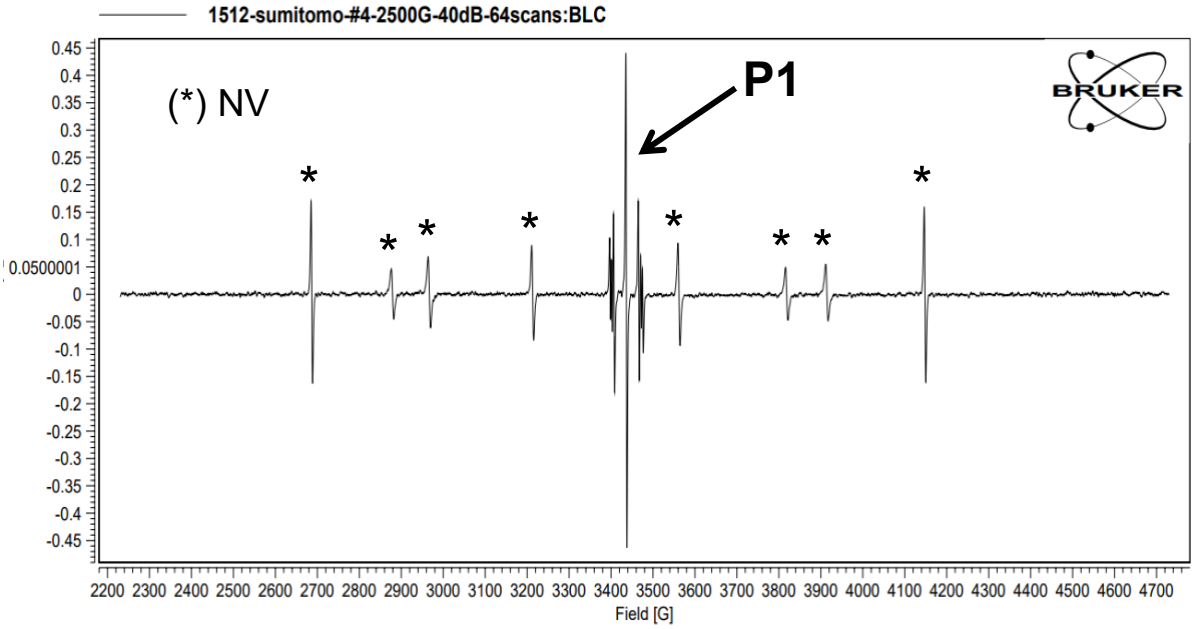

Fig.1 : EPR spectrum of a Sumitomo HPHT diamond (1.5x1.5x1.2mm) irradiated to obtain NV centers.

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[P-39] FIELD DEPENDENT EFFECTS IN PARAMAGNETIC SYSTEMS IN THEORY AND EXPERIMENTS

L. Fiorucci[† ‡], 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
E-mail: fiorucci@cerm.unifi.it

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

The application of high magnetic fields generates effects on the NMR spectra of paramagnetic systems that should be properly analyzed for a correct interpretation of the experimental data. From the preliminary results obtained for a nickel(II) complex [1], it was possible to verify how the field dependence of the magnetic susceptibility cannot be fully described by the Brillouin effect, but it rather requires inclusion of second-order corrections to the electronic energy levels [2]. More challenging, and therefore attractive, is the case of lanthanoid complexes, for which a theory on high magnetic fields effects is still at early stages. To address this problem, we chose DyDOTA and YbDOTA [3] complexes as models. For these two systems, the available theory predicts opposite shift dependencies with respect to the field, due to the different contributions of the field-dependent self-orientation effect (which increases the NMR shift with increasing the applied magnetic field) and the Brillouin effect (which decreases the shift with increasing the field). A series of spectra was acquired at different temperatures and two field values (400 MHz and 1.2 GHz ^{1}H Larmor frequencies) on both complexes. Surprisingly, the experimental data showed that the shift values decrease with the increase of magnetic field in both cases (see Fig. 1). However, on a relative scale, the variation is higher for the dysprosium(III) complex with respect to its ytterbium(III) counterpart. Considering the contact contribution as negligible, this behavior can be explained by the similar self-orientation and Brillouin contributions in the latter ion. A more detailed analysis requires the application of crystal field theory to calculate the electronic energy levels and then to calculate the field dependence of the magnetic susceptibility, on which the paramagnetic shifts depend.

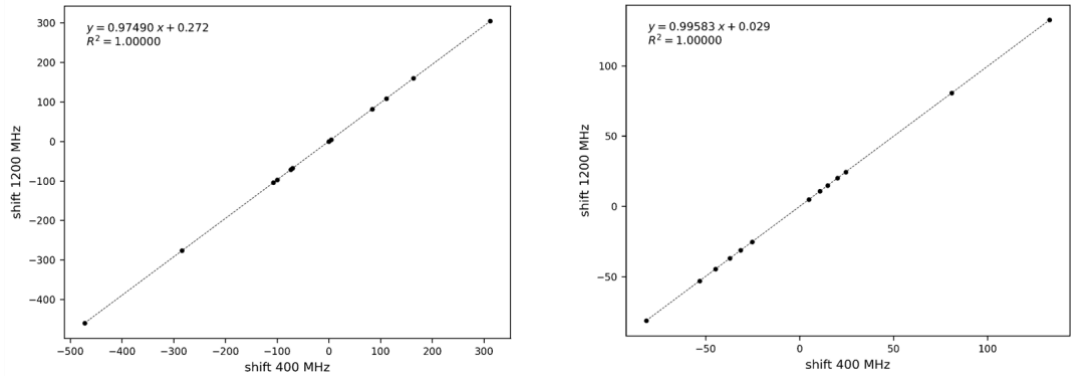

Fig. 1: Correlation plot of shifts measured at 1.2 GHz and 400 MHz, at 298 K, for DyDOTA (left) and YbDOTA (right) complexes.

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[P-40] IN VIVO INVESTIGATION OF SUSCEPTIBILITY PROPERTIES OF CEREBRAL GYRI IN AMYOTROPHIC LATERAL SCLEROSIS

Fiscone C[1], Bartiromo F[2], Vacchiano V[1,3], Mitolo M[2,4], Rizzo G[3], Bianchini C[1], Manners DN[1], Testa C[2,5], Tonon C[1,2], Liguori R[1,3], Lodi R[1,2]

[1] Department of Biomedical and NeuroMotor Sciences, University of Bologna, IT
[2] Functional and Molecular NeuroImaging Unit, IRCCS Institute of Neurological Sciences of Bologna, IT
[3] Clinica Neurologica, IRCCS Institute of Neurological Sciences of Bologna, IT
[4] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, IT
[5] Department of Physics and Astronomy, University of Bologna, IT
E-mail: cristiana.fiscone2@unibo.it

Keyword: MRI

Introduction: Quantitative Susceptibility (χ) Mapping (QSM) is an advanced MR sequence, able to detect *in vivo* iron accumulation, an undergoing process in many neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS). Previous studies exploited this technique and revealed higher χ values in the PreCentral Gyrus's (PrCG) in ALS [1]. The aim of this study was to extend the analysis to multiple cerebral gyri considering different χ properties.
Material and Methods: In the present prospective study 17 ALS patients (9F/8M; 55.7±6.4 years), assessed by ALS Functional Rating Scale and Penn Upper Motor Neuron (UMN) Score [2], were compared to 15 age- and sex- matched Healthy Controls (HC) (10F/5M; 58.0±6.1 years). The MR examinations (3T Siemens Magnetom Skyra, equipped with Siemens Head/Neck 64 Coil) provided MPRAGE (3D T_1w TR/TE = 2300/2.98 ms, 1x1x1 mm^3, 5min 21sec) and QSM (3D GRE T_2*w, nTEs=5, TE1/ΔTE/TR = 9.42/9.42/53 ms, 0.5x0.5x1.5 mm^3, 8min 45sec). A fully automatic pipeline was originally implemented. The QSM maps were linearly registered to their corresponding MPRAGE images and segmentations of ten gyri, in the frontal, parietal and temporal lobes and the cingulate cortex, were considered from the Freesurfer automatic cortical parcellation [3]. From each gyrus, different properties (median, 90th percentile, skewness, kurtosis) of χ histograms were measured and ROI-based analysis was performed, comparing their distributions between ALS and HC groups.
Results: The analysis highlighted significant differences in: 1) median and 90th percentile χ values, higher in ALS group, of all the gyri in the frontal lobe, including the PrCG, and of the supramarginal and posterior cingulate gyri and 2) skewness in the PrCG, higher in ALS group. A significant positive correlation resulted between the Penn UMN Score and median and 90th χ values of the frontal lobe gyri. Kurtosis of χ distributions of any gyrus was not significant different between ALS and HC groups.
Conclusion: These results suggest that a possible ALS neuropathological hallmark may be iron accumulation not only in the motor cortex but also in extra-motor cortical areas. This feature, for the frontal lobe gyri, was correlated to clinical scores of UMN impairment, not consistently reported in previous studies. Compared to the conventionally explored bulk χ values, expressed as median of the distribution, other properties of the χ histograms (90th percentile and skewness) resulted more sensitive for the assessment of susceptibility distribution changes in ALS cerebral gyri.

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[P-41] NMR-based approach to investigate metabolomic profile of *Citrus* hybrid Tacle before and after pressure-driven membrane operations

M. Gaglianò,[1] Serena Oliveto[1], Alfredo Cassano[2], Carmela Conidi[2], Giuseppina De Luca[1]

[1]Department of Chemistry & Chemical Technologies, University of Calabria, via P. Bucci - 87036 Rende (CS), Italy
[2]Institute on Membrane Technology, ITM-CNR, c/o University of Calabria, via P. Bucci, 17/C, I-87036 Rende (Cosenza), Italy

Keywords: metabolomics, NMR, membrane operations

Tacle is a new triploid citrus hybrid, developed in Sicily using traditional, strictly non-GMO, techniques. Both its name and flavor recall the two parents' cultivars: the Tarocco orange (*C. sinensis* L. Osbeck) and the Monreal Clementine (*C. clementina* Hort. ex Tan.).[1] This fruit, larger than a clementine and smaller than an orange, was particularly studied for its high antioxidant activity. [2,3] This work is shaped on a metabolomic study of tacle juice to get a more comprehensive view of its metabolome in order to assess its commercial value and nutraceutical properties. The method of choice for this purpose was the Nuclear Magnetic Resonance (NMR). Deuterium oxide (D_2O) and deuterated chloroform ($CDCl_3$) solvents were used to capture both polar and non-polar metabolites from the same sample. 1D (^1H and ^{13}C NMR) and 2D (COSY and HMQC) experiments were used. Moreover, tacle juice has also been subjected to pressure-driven membrane operations such as an ultrafiltration/diafiltration process followed by nanofiltration. The initial and final products obtained using integrated membrane operations can be, also, evaluated using NMR, eventually coupled with multivariate statistical analyses, to highlight possible structural changes in the metabolic composition of the tacle juice.

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[P-42] Noise Propagation in Occupational Exposure to Stray Magnetic Fields

A. Galante[a,b,c], V. Valentino[a], E. Braggio[d], R. Milanesi[d], V. Giugno[e], A. Capoccia[f], M. Alecci[a,b,c]

[a] Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy
[b] National Institute for Nuclear Physics, Gran Sasso National Laboratory, L'Aquila. Italy
[c] CNR-SPIN Institute, c/o Department of Physical and Chemical Sciences, L'Aquila, Italy
[d] Tecnorad SrL, Verona, Italy, [e]Azienda Sanitaria Locale 1 - Abruzzo, L'Aquila, Italy
[e] Azienda Sanitaria Locale 1 - Abruzzo, L'Aquila, Italy
[f] Department of Physical and Chemical Sciences, University of L'Aquila, L'Aquila, Italy

E-mail: angelo.galante@univaq.it

Keywords: MRI, instrumentation

MRI staff personnel and researchers are exposed to non-sinusoidal time-variable magnetic fields and induced electric fields, during their movements inside the scanner room. EU regulations [1] establish limits on operators' exposure which are expressed in terms of the E(t) fields induced in the body, currently not directly measurable, or by means of the Action Levels (ALs), the latter defined from measurable magnetic fields B(t) in the specific position occupied by the body.
Here, we have studied the effects of noise propagation in stray field MRI occupational exposure.
B(t) has a complex spectrum and to assess EU limits compliance we estimated the Weighted Peak (WP) function [1,2]:

$$WP(t) = \left| \sum_i \frac{B(f_i)}{B_L(f_i)\sqrt{2}} \cos\left(2\pi f_i t + \theta(f_i) + \phi(f_i)\right) \right|$$

where $B(f_i)$, $\theta(f_i)$ are the B(t) spectral amplitudes and phases at frequency f_i, $B_L(f_i)$, $\phi(f_i)$ are the frequency-dependent limits and phases as reported in [1,2], t is the observation time, and the condition WP(t)≤1 guarantees exposure compliance. Since $B_L(f_i)$ decreases with increasing frequency, higher frequencies can become dominant in WP evaluation. We measured $B(f_i)$ with wearable gaussmeters (TALETE [3] from Tecnorad) in five body districts (both hands, head, hearth, waist) of Radiology residents working with 1.5 and 3.0 T clinical MRI scanners (L'Aquila Hospital, ASL1 Abruzzo). As shown in Fig.1 (left panel), the $B(f_i)$ spectrum peaks at $f = 0$ and decreases to the sensor spectral noise at a cut-off frequency f_c (comprised between 10 and 60 Hz in our data), this value depending on the operator movements, body district under observation, scanner static field value and stray field spatial profile. Our theoretical analysis and experimental results show that if $f > f_c$ values are included in WP calculation we obtain false positive results (WP(t)>1) due to excess spectral noise propagation (Fig. 1). In conclusion, WP(t) evaluation requires care to avoid false positive events due to sensor noise propagation. The f_c values variability can be managed in *a posteriori* WP evaluation (Fourier domain approach), not in real-time computations (time domain approach) performed by means of a cascade of weighting filters [4]. The latter procedure, explicitly considered in [1,2] and adopted by currently available commercial products, could lead to false positive events if *a priori* knowledge of f_c is not available.

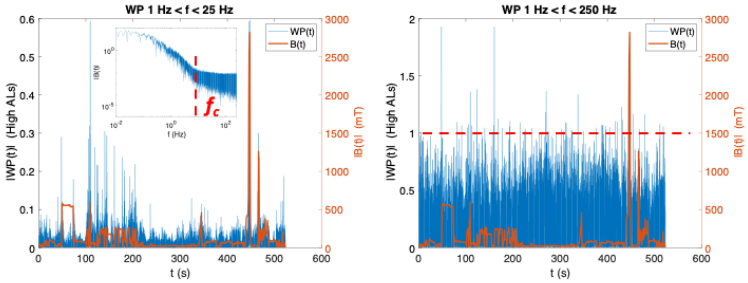

Fig. 1 WP(t), calculated for the High Action Level threshold, from 1.5 T head exposure measurement (4 h of observation, only B > 10 mT values have been considered) sampling the magnetic field every 2 ms. Left panel insert: the computed *B(f)* spectrum in log-log scale. Left panel: WP(t) calculated using the frequencies $1Hz \leq f \leq 25Hz$. WP(t) peaks correlate with *B(t)* rapid changes. Right panel: as for left panel using the frequencies $1Hz \leq f \leq 250Hz$. WP(t) peaks do not correlate with *B(t)* and several false positive events (WP>1) are present.

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[P-43] Design and Calibration of a 190 mT Point of Care Halbach Magnet

A. Sotgiu [a], P. Sebastiani [a], M. Alecci [b,c,d], A. Galante [b,c,d],

[a] Imaging Technology Abruzzo srl, SS 117, Località Pile, L'Aquila, Italy
[b] Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[c] National Institute for Nuclear Physics, Gran Sasso National Laboratory, 67100 L'Aquila. Italy
[d] CNR-SPIN Institute, c/o Department of Physical and Chemical Sciences, 67100 L'Aquila, Italy
E-mail: angelo.galante@univaq.it

Keywords: low field NMR, MRI, instrumentation

There is rising interest in low-field (10-500 mT) low-cost MRI scanners suitable for point-of-care (PoC) imaging, capable to complement high-field (1.5-3.0 T) clinical studies [1-5].
The aim of this study was the design and calibration of a novel permanent Halbach magnet suitable for PoC 190 mT MRI of the human brain.
The cylindrical magnet (Fig.1a), longitudinal axis along the z-direction, comprises six Halbach rings, each one made by an assembly of 16 magnets, with the rings radii and longitudinal positions selected to compensate finite-length effects. From the outside, the first couple of rings (internal radius R_1) are positioned symmetrically (+/-Z_1) with respect to the z-axis centre. The other four rings (internal radius R_2>R_1) are positioned symmetrically with respect to the z-axis centre (+/-Z_2, +/-Z_3, with Z_1>Z_2>Z_3). The magnet length is 46 cm with 25 cm free bore. The 96 nominally identical block magnets have hexadecagonal section (height 60 mm; diameter 60 mm; central hole 8 mm) and were fixed to an aluminium frame. The NdBFe (N45) blocks have nominal residual magnetization of 1.35 T directed perpendicularly to a lateral surface. The Halbach configuration confines the magnetic field within a restricted volume, avoiding the use of heavy iron yokes not suitable for point of care applications. The temperature dependence of the block magnetization is -1200 ppm/°C and the use of thermally insulating materials coupled to a commercial temperature controller (FEPA srl) allowed temperature stabilization to 29.00 °C. The whole magnet assembly was placed on an aluminium cart with total weight of about 170 kg.
Magnetostatic numerical routines allowed to optimise the magnetic field for maximum homogeneity over a 16 cm DSV at the magnet isocentre, with a useful volume extending to 35 % of the magnet length. Field homogeneity was further increased by means of 200 small size N45 shimming magnets with residual magnetization calculated using a recursive homogeneity optimization algorithm [2]. The spatial distribution of the magnetic field was measured with a Hall probe (Group3-DTM-151-DS) controlled by a 3-axis robotic positioning system (Zaber Technologies Inc., resolution 1 mm). The measured magnetic field B_{0x} was about 190 mT at the isocentre and the field homogeneity within the DSV was about 14000 ppm before (Fig.1b) and 300 ppm (Fig.1c) after passive shimming.
In conclusion, we have presented the design and characterization of a low field (190 mT) Halbach magnet with 300 ppm homogeneity over a 16 cm DSV, large access bore of 25 cm and reduced weight that allows easy transportation within the PoC wards. This novel magnet is well suitable for PoC human brain MRI applications.

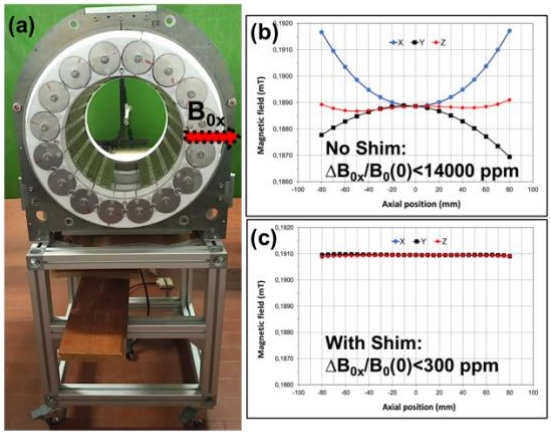

Fig. 1. (a) 190 mT Halbach prototype. (b) Magnetic field along the x,y,z axes before shimming. (c) Magnetic field after shimming.

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[P-44] Experimental Characterization of Spherical Dielectric Resonators Suitable for MR

A. Galante [a,b,c], C. Rizza [d], M. Alecci [a,b,c]

[a] Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[b] National Institute for Nuclear Physics, Gran Sasso National Laboratory, 67100 L'Aquila. Italy
[c] CNR-SPIN Institute, c/o Department of Physical and Chemical Sciences, 67100 L'Aquila, Italy
[d] University of L'Aquila, Department of Physical and Chemical Sciences, L'Aquila, Italy.

E-mail: angelo.galante@univaq.it

Keywords: MRI, theory and methods, instrumentation

High-performance dielectric materials have been previously used with several functions, including EPR [1] and MRI [2] Dielectric Resonators (DRs), as well as MRI RF shimming pads [3]. Recently, it was theoretically demonstrated that spherical DRs (SDRs) can mimic a negative permeability metamaterial, producing a significant SNR enhancement and SAR reduction compared to a standard RF surface coil [4].
This work aimed to the experimental characterization of a SDR suitable to act as a surface RF coil for EPR/NMR/MRI applications.
Following previous full-wave EM modelling [4] we focused on an SDR (diameter 40 mm) having a nominal permittivity of 156 [3]. The SDR was manufactured by HyQ Research Solutions LCC (College Station, USA). A small circular loop coil (16 mm diameter), positioned 7 mm below the bottom side of the SDR, was used to couple the RF power provided by a VNA (R&S). The S_{21} (dB) response was measured by means of a smaller (10 mm diameter) receiving inductive loop (2 turns) positioned above the SDR and coaxial with the first loop. We detected the first three SDR resonant modes (L=1, 2, 3) and mapped the external RF magnetic field above the SDR by moving the receiver coil. We performed all measurements at room temperature.
The measured S_{21}, Fig. 1, shows the first three modes of the SDR with a coupling level between -18 and -8 dB. The first mode (TE_{11}) resonates at 597 MHz (full-wave theory 593 MHz) and the measured Q was about 83. This mode could be useful for ^{1}H NMR/MRI at 14 T. As can be seen from Fig. 1, the separation between the first three resonant modes is about 250 MHz, in excellent agreement with the Mie theory and our full-wave simulations. The normalized theoretical and measured RF B_1 field distributions (not reported here) show a maximum RF field value on the upper SDR sphere pole (z=0) with a slow decrease as we move away, reaching 10% at z=35 mm. The higher modes (TE_{21}, TE_{31}) show similar RF field distributions although, as expected, the RF field decreases faster as the mode order increases.
In conclusion, we have reported, to the best of our knowledge, the first experimental characterization of a spherical dielectric resonator that can act as a surface RF coil for MR applications. To this purpose, we have mapped the external RF magnetic field distribution of the first three resonant modes. The current theoretical and experimental methods can be applied to other DRs geometries and over a wide range of frequencies.

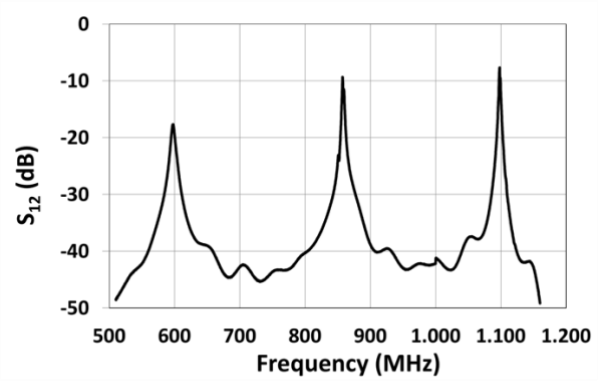

Fig. 1. Measured S_{21} response of the spherical dielectric resonator (diameter 40 mm, permittivity 156) by means of two small circular coupling loops.

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[P-45] NMR-based community-built analytical systems in food control and quantitative analysis

Vito Gallo,[1,2] Piero Mastrorilli,[1,2] Mario Latronico,[1,2] Biagia Musio,[1] Maurizio Triggiani,[1,2] Marica Antonicelli,[1] Rosa Ragone,[1] Stefano Todisco[1]

‡ Politecnico di Bari, DICATECh, via Orabona 4 – CAMPUS, I-70125, Bari, Italy
† 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, metabolomics, food

NMR spectroscopy is gaining ever-growing importance in analytical chemistry. In the last decade, quantitative NMR (qNMR) and non-targeted approaches allowed for great improvements in both quantification of molecules in complex mixtures and identification of product features in suitable sample pools. Metrological traceability of products can be successfully achieved by qNMR even when no certified reference materials are commercially available. Along with quantification and purity assessment of molecules, NMR is emerging also as a powerful tool in food chemistry, especially when searching for product features such as cultivar, geographical origin, typicality of the production process, etc.
The great application potential of NMR spectroscopy derives from the fact that, based on theory, the ratio between a signal generated by the molecule under investigation and the signal of a reference molecule depends exclusively on the corresponding mole ratio. In other words, when a given sample is analyzed by different spectrometers, the same output is obtained in terms of signal ratios independently of the hardware configuration. This offers the unique opportunity to develop community-built analytical systems capable of identifying sample features and quantifying a number of molecules.
In this presentation, based on our previous studies carried out with different NMR spectrometers (300 to 700 MHz),[1,2] the first examples of community-built analytical systems will be shown. In particular, the case study of a data-driven grape juice identification system will be presented along with the advantages and the limitations of using non-targeted NMR analyses performed at different magnetic fields. Moreover, quantification of metabolites in grape juices by using a community-built calibration tool will be also shown.[3] The feasibility of NMR spectroscopy to generate statistically equivalent NMR signal ratios from a number of different spectrometers will be demonstrated also for other complex mixtures such as aqueous extracts of wheat and flour.[4] Finally, potential use of benchtop NMR in both quantification of standard molecules and valorization of food biodiversity will be introduced.

Acknowledgements
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[P-46] HISTIDINE CONTAINING PLGA NANOPARTICLES AS NOVEL THERANOSTIC AGENTS FOR BORON NEUTRON CAPTURE THERAPY (BNCT)

Simonetta Geninatti Crich[1], Jacopo Sforzi[1], Diego Alberti[1], Valeria Bitonto[1], Rachele Stefania[1], Alberto Lanfranco[2], Stefano Parisotto[2], Polyssena Renzi[2], Annamaria Deagostino[2]

1 Department of Molecular Biotechnology and Health Sciences; University, Turin, Italy.
2 Department of Chemistry, University of Turin, via P. Giuria 7, 10125, Turin, Italy.;
E-mail: simonetta.geninatti@unito.it

Keywords: MRI, contrast agents.

BNCT is a promising option for tumoral treatment, relying on the selective delivery of boron atoms to cancer cells, followed by the irradiation with a neutron beam of the diseased organ. The innovation of this study lies on the development and test of a nanosized theranostic agent, able to maximize the selective uptake of boron atoms in tumor cells and, at the same time, to quantify (by Magnetic Resonance Imaging, (MRI) the in vivo boron biodistribution. This is crucial to determine the optimal neutron irradiation time and to calculate the delivered radiation dose. In this study, a compound containing both Gd and boron atoms in the carborane structure (AT101, Figure1) has been loaded into PLGA (Poly, lactic-co-glycolic acid) nanoparticles: these nanoparticles have been coated with DSPE-PEG-2000 and prepared with and without a 50% of a PLGA conjugated with a polyhistidine chain (n=15). PLGA-NP were incubated with AB-22 and MET-5α, a mesothelioma and healthy mesothelium cell lines, respectively. Malignant Mesothelioma is a disseminated tumor, spreading inside the whole pleura or peritoneum. Conventional radiotherapies have limited effectiveness due to the presence of several radiosensitive tissues, which limit the maximum dose deliverable to the malignant nodules. The advantage of BNCT is that it can potentially affect only tumor cells with a lethal dose of radiations, even in case of spreading and infiltrative cases. Interestingly, the nanoparticle containing polyhistidine, showed a significantly higher uptake in the mesothelioma cell line (AB-22), compared to the control one without the Poly-His feature due to the acid pH present in tumour extracellular environment. These promising insights on the possibility to selectively direct a theranostic dual agent directly into tumoral cells, thus monitoring the boron biodistribution by MRI, may be an important, versatile and new starting point for the future of BNCT technology and cancer theranostics.

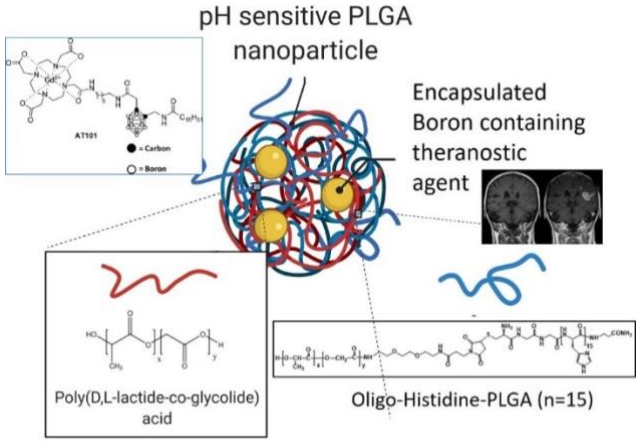

Fig. 1. Schematic representation of PLGA-NP References.

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[P-47] RAPID STRUCTURAL ELUCIDATION OF CYCLIC LIPOPEPTIDES VIA NMR FINGERPRINT MATCHING

V. De Roo[1], Y. Verleysen[1,4], B. Kovács[1], R. De Mot[2], M. Höfte[3], A. Madder[4], N. Geudens[1], J.C. Martins[1]

1 NMR & Structure Analysis Unit, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium
2 Centre of Microbial and Plant Genetics, Faculty of Bio-engineering, KU Leuven, Leuven, Belgium
3 Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
4 Organic and Biomimetic Chemistry Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium
E-mail: niels.geudens@ugent.be

Keywords: solution NMR, biomolecules.

Cyclic lipopeptides (CLiPs) are secondary metabolites that are produced and secreted by a range of bacterial genera including Pseudomonas and Bacillus. They are composed of an oligopeptide, cyclized through a lactone (depsi) bond, and capped at the N-terminus by a fatty acid moiety. Well over 100 CLiPs originating from Pseudomonas spp. have been described at varying levels of structural and biological activity details and their numbers keep rising. Structural variations are very diverse, including total amino acid sequence length, size of the macrocycle, amino acid identity and stereochemistry (e.g. D- vs. L-amino acids). In general, CLiPs are involved in several secondary functions and have been reported to display a range of antagonistic properties. The antimicrobial activities of Pseudomonas CLiPs were thoroughly reviewed. [1]
CLiPs and their producing bacteria are ubiquitous in Nature and reports detailing the discovery of novel or already characterized CLiPs from new sources appear regularly in literature. However, the lack of characterisation detail threatens to cause considerable confusion. Using solution NMR fingerprint matching, we have introduced a rapid and facile way of characterizing existing CLiPs coming from novel bacterial sources. [2] Using this approach, the identity of CLiPs can be established by simple comparison of their NMR spectral fingerprint recorded under standardized conditions.
The validation of stereochemical make-up of the CLiPs is a first crucial step in determining their 3D conformations using solution NMR spectroscopy.

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[P-48] COMPARATIVE NMR METABOLOMICS OF THE RESPONSES OF A2780 HUMAN OVARIAN CANCER CELLS TO CLINICALLY ESTABLISHED PT BASED DRUGS

V. Ghini[‡†], L. Massai[†], F. Magherini[#], L. Messori[†], P. Turano[‡†],

[‡]CERM, University of Florence, Sesto Fiorentino, Italy.
[†]Department of Chemistry, University of Florence, Sesto Fiorentino, Italy.
[#]Experimental and Clinical Biomedical Sciences, University of Florence, Firenze, Italy.
E-mail: ghini@cerm.unifi.it

Keywords: solution NMR, small molecules, metabolomics.

Cisplatin, carboplatin and oxaliplatin are the three most important platinum-based drugs approved worldwide for cancer treatment in the clinics. All these Pt drugs are characterized by the presence of a square planar platinum(II) center that is primarily responsible for the biological and pharmacological effects; in order to perform its biological actions, the platinum(II) center requires chemical activation that is achieved through the slow release of the more labile ligand(s). The activated platinum(II) center shows a relevant and broad reactivity with a variety of biomolecules, including nucleic acids and proteins inducing the cytotoxic and anticancer effects. Yet the detailed cellular and mechanistic modes of actions of this class of metallodrugs are not fully understood [1].
1H NMR metabolomics provides a powerful tool to investigate the metabolic perturbations induced by platinum-drugs in cancer cells and decipher their meaning in relation to the presumed molecular mechanisms [2]. We have carried out a systematic and comparative NMR metabolomics study to analyze the responses of A2780 human ovarian cancer cells to cisplatin, carboplatin and oxaliplatin. Notably, NMR analysis revealed some moderate and consistent changes in the metabolomic profiles of A2780 cells treated with the 3 platinum-drugs with respect to controls, but only very small differences among them. Beyond the expected alterations at the level of nucleic acid biosynthesis the observed changes can be related to ER stress. Owing to the clinical relevance of platinum resistance the behavior of a cisplatin resistant A2780 cancer cell line upon cisplatin treatment was also evaluated. Albeit starting from an altered basal metabolism, resistant cells respond to cisplatin treatment with only few but significant differences with respect to sensitive cells.

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[P-49] STRUCTURAL PROPERTIES AND DYNAMICS OF FLUORINATED METAL-ORGANIC FRAMEWORKS BY SOLID STATE NMR

A. Giovanelli,[‡] E. Della Latta,[‡] F. Martini[‡,†,§], L. Calucci[†,§], M. Taddei[‡,†], M. Geppi[‡,†,§]

[‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, 56124 Pisa, Italy;
[†]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), 56126 Pisa, Italy;
[§]Istituto di Chimica dei Composti Organo Metallici, Consiglio Nazionale delle Ricerche (CNR-ICCOM), 56124 Pisa, Italy
E-mail: andrea.giovanelli@sns.it

Keywords:
solid state NMR, low field NMR, materials

Metal-Organic Frameworks (MOFs) are a class of crystalline compounds whose scaffolding derives from metal clusters or ions that are interconnected by organic linkers. The high number of possible combinations of metals and ligands leads to high tunability of macroscopic properties and thus it is possible to employ MOFs in many fields of applications, including gas storage, gas separation, and catalysis [1,2]. In the design and development of a new MOF it is extremely important to completely understand every macroscopic property of the compound and to relate it to its microscopic origin.
Many techniques can be exploited and combined to characterize the structural and dynamic properties of a MOF. Among them, Solid State Nuclear Magnetic Resonance (SSNMR) spectroscopy is certainly one of the most important because it can shed light on many aspects of the compound at a molecular level, such as 3D structure, porosity [3], local dynamics [4], and host-guest interactions [5].
In this work, ^{1}H, ^{13}C, and ^{19}F SSNMR spectroscopy has been employed to characterize two MOFs belonging to the MIL140A class: F4-MIL140A(Ce) and F3-MIL140A(Ce). These MOFs share the same metal SBU composed by Ce^{IV}, but the organic linker has a different degree of fluorination. The former is based on tetrafluoroterephthalic acid (F4-BDC), the latter on trifluoroterephthalic acid (F3-BDC).
F4-MIL140A(Ce) is extremely relevant in the field of gas separation and storage because it presents a step-shaped adsorption isotherm for CO_2 [6]. F3-MIL140A(Ce), instead, is a novel MOF that could in principle show similar or even better adsorption properties with respect to the perfluorinated analogue.
Multinuclear SSNMR experiments and 2D correlation spectra have been used to obtain, also by comparison with powder X-ray diffraction data, a detailed characterization of the framework structure both in the presence and after removal of crystallization water. Moreover, the analysis of variable-temperature ^{19}F spin-lattice relaxation times at different magnetic fields has been applied for the investigation of the dynamic processes involving the fluorinated linkers, which are supposed to be involved in the MOF adsorption mechanism.

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[P-50] NMR STUDIES ON TRANSPORT PROPERTIES OF PROTIC IONIC LIQUIDS-BASED ELECTROLYTES

Giselle de Araujo Lima e Souza,[‡] Maria Enrica di Pietro,[‡] Franca Castiglione,[‡] Alessandro Triolo,[§] Giovanni Battista Appetecchi [†], Andrea Mele,[‡]

[‡] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, 20133 Milan, Italy

[†] ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development), Department for Sustainability (SSPT), Casaccia Research Center, Via Anguillarese 301, 00123 Rome, Italy

[§] Istituto Struttura della Materia (ISM), Consiglio Nazionale delle Ricerche (CNR), Rome, Italy E-mail: giselle.dearaujo@polimi.it

Keywords: solution NMR, materials

Developments on ionic liquids as electrolytes components have been mainly focused on the use of conventional aprotic ionic liquids (AILs). However, the complex synthesis of AILs is still hampering their mass production. As an alternative, protic ionic liquids (PILs) emerge as potential electrolyte components because they share all the characteristics of conventional AILs along with the possibility of being synthesized by a one-step neutralization reaction of Brønsted acid and Brønsted base. Then, very recently, PILs have been quoted as promising alternative electrolytes components for energy storage devices [1].

Yet, scaling the transport properties of PILs requires an in-depth understanding of the role played by the ions in the bulk system. To this end, the present work studied a set of PILs based on the 1,8-diazabicyclo-[5,4,0]-undec-7-ene ($DBUH^+$) cation and three anions obtained from very strong acids: trifluoromethanesulfonate (TFO^-), (trifluoromethanesulfonyl- nonafluorobutylsulfonyl)imide ($IM14^-$), and bis(trifluoromethanesulfonyl)imide ($TFSI^-$) (Figure 1). Both neat PILs and PILs-based electrolytes (PIL + lithium salt containing the same anion, i.e., LiTFO, LiIM14, and LiTFSI) were investigated by ^1H, ^{19}F and ^7Li temperature- dependent diffusion and relaxation NMR. These techniques allowed us to gather a comprehensive picture of the ion dynamics of these three systems. The presence of the lithium- ion unveiled a peculiar effect on each system, reflecting their distinct intermolecular network.

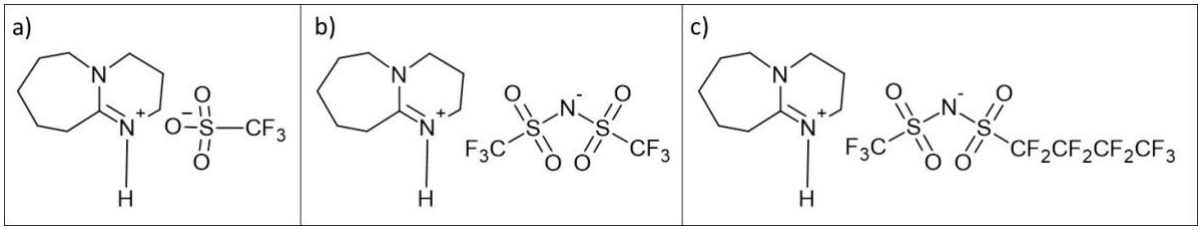

Fig. 1. Structure of the PILs studied DBUH-TFO (a), DBUH-TFSI (b), DBUH-IM14 (c).

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[P-51] NMR AS A TOOL TO STUDY THE REACTIVITY OF $CsPbBr_3$ NANOCRYSTALS TOWARDS ACID/BASE LIGANDS

Francesco Zaccaria,[†] Baowei Zhang,[†‡] Guilherme Almeida,[†] Luca Goldoni,[†] Muhammad Imran,[†] Juliette Zito,[†] Bas van Beek,[≠] Simone Lauciello,[†] Luca De Trizio,[†] Liberato Manna,[†] and Ivan Infante[†]

[†]Department of Nanochemistry, and Electron Microscopy Facility, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy
[‡]Dipartimento di Chimica e Chimica Industriale, Università degli Studi di Genova, Via Dodecaneso 31, 16146 Genova, Italy
[≠]Department of Theoretical Chemistry, Faculty of Science, Vrije Universiteit Amsterdam, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands
E-mail: luca.goldoni@iit.it

Keywords: solution NMR, materials, small molecules

The reactivity of perovskite nanocrystals (NCs) towards neutral ligands has not been investigated, so far. To fill this gap, we studied the interaction of $CsPbBr_3$ NCs passivated with didodecyldimethyl ammonium bromide (DDABr) towards a series of neutral ligands, either acids or bases, using a combined computational and experimental approach, the latter mainly based on Nuclear Magnetic Resonance (NMR). The dynamic interaction between ligands and the surface of the NCs is revealed by the ^{1}H and ^{31}P peak shift and/or such signals broadening [1] and further confirmed by Nuclear Overhauser Effect Spectroscopy (NOESY), by exploiting the differences in correlation time (τ_c) between free ligands compared to those dynamically interacting with the nanocrystal's surface [2]. Our NMR analysis indicated that DDABr-capped nanocrystals can interact with carboxylic, phosphonic and sulphonic acids. In details, dodecylbenzene sulfonic acid (pKa=-1.8) is able to completely degrade the NCs while oleic and oleylphosphonic acids (pKa 9.9 and 2, respectively) interact with surface bound DDA molecules by forming DDA-ACID couples, that are weakly bound to the nanocrystal's surface and are easily displaced upon NCs washing Fig 1. The presence and the structure of such DDA-ACID couples was elucidated by means of ^{1}H-^{13}C Hetero Single Quantum Coherence (HSQC). Our study provides not only a clear overview on the interaction between perovskite nanocrystals and neutral ligands, but also presents for the first time an effective ligand stripping strategy Fig. 1.

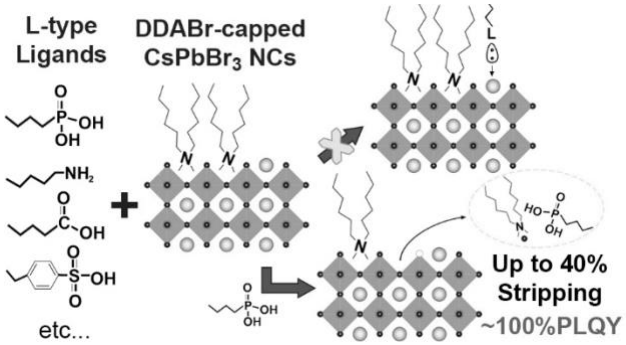

Fig. 1. Schematic representation of the interaction between DDA-Br capped CsPbBr3 NCs and acid/base ligands

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[P-52] NON-LAMELLAR WATER-LIPID PHASES AS A MEANS OF ADAPTATION TO EXTREME CONDITIONS IN ARCHAEA? A STUDY BY NMR AND X-RAY

Axelle Grélard[a,b], Josephine LoRicco[d], Brice Kauffmann[b] Laurent Soulère[c], Florence Popowycz[c], Judith Peters[e], Phil Oger[d], Erick J. Dufourc[a,b]

[a]Institute of Chemistry and Biology of Membranes and Nanoobjects, UMR5248, CNRS, University of Bordeaux, Bordeaux Polytechnic Institute. Allée Geoffroy Saint Hilaire 33600 Pessac France.
[b]Institut Européen de Chimie et Biologie, UMS3033, CNRS, Université de Bordeaux, INSERM
[c]Université Lyon 1, CNRS, INSA, CPE, UMR 5246, ICBMS, COB, 20 Avenue Albert Einstein, F-69621 Villeurbanne Cedex.
[d]INSA Lyon, Université de Lyon, CNRS, UMR5240, Villeurbanne 69621, France
[e]Institut Laue Langevin, Grenoble F-38042, France & Univ. Grenoble Alpes, Grenoble 38000, France

E-mail: a.grelard@iecb.u-bordeaux.fr & erick.dufourc@cnrs.fr

Keywords: solid state NMR, materials, biomolecules

Archaebacterial membrane lipids have been designed by nature to resist extreme conditions of temperature, pressure and pH due to the presence of unusual ether-linked branched chains (phytanyl). As archaeal lipids differ considerably from mammalian or bacterial lipids with saturated or unsaturated chains, the questions of their assembly, stability and internal dynamics in water and under extreme conditions remain to be elucidated. The main classes of such lipids were thus synthesized and their water dispersions have been studied using static and MAS solid state NMR. Phase diagrams demonstrated the presence of lamellar (L), hexagonal type II (H), cubic Pm3n (Q) phases. The sequence L>Q>H is observed when increasing the temperature and is explained by the thermal increase in phytanyl chain volume. Phytanyl chain dynamics, as explored by polarization transfer techniques, are more important than for saturated/unsaturated chains as found in eukaryotes. The combination of such high chain dynamics with a thermal change in topology is proposed as a way to withstand the extreme temperature and pH conditions that archaea face in their lives. (e.g., see Fig. 1).

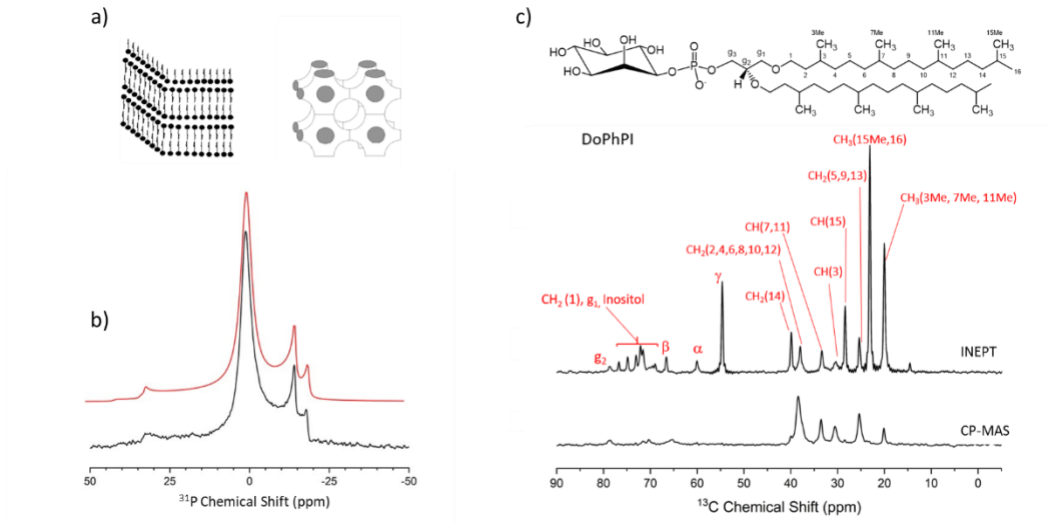

Fig. 1. a) Schematics of lamellar and cubic phases, b) ^{31}P NMR spectra (experimental in black, simulated in red), c) ^{13}C NMR spectra of DoPhPC:DoPhPI (1:1) mixture

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[P-53] NMR SPECTROSCOPY TO MONITOR THE HOS OF MABS

O. Guichard ‡, C. Leveque‡, C. Doyen ‡

‡Global CMC development (BioAnalytics), Sanofi R&D, Vitry Sur Seine, France

E-mail: olivier.guichard@sanofi.com

Keywords:
solution NMR, biomolecules.

Biologics, such as mAbs, ADC and fragments of mAb, have become an important part of the portfolio of pharmaceutical companies for the past 10 years [1]. These products have achieved outstanding success in oncology and will continue to become more significant in the next years. To assess the conservation of the protein folding as part of the stability of the product, it is mandatory to control the high-order structure of these proteins as a product quality attribute. This information is indeed required by the Health Agencies.
In recent years, the interest in NMR for such determination has sharply increased as it presents a huge potential for the monitoring of their High Order Structure (HOS) [2]. This nondestructive method, applied to samples in solution, allows the fingerprinting of the proteins and is a powerful addition to existing biophysical tools to assess the structure of the proteins from their Post Translational Modifications (PTMs) to their higher-order structural configuration.
The study presented here is a proof of concept of the application of 2D NMR with multivariate analysis for HOS determinations of mAbs.

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[P-54] UNDERSTANDING THE MECHANOCHEMICAL SYNTHESIS OF METAL-NHC COMPLEXES USING SOLID-STATE NMR SPECTROSCOPY

Lama Hamdouna,[‡ab] Gianmarco Pisanò,[‡b] Andrew G. M. Rankin,[†c] Laura Falivene,[d] Julien Trébosc,[c] Laurent Delevoye,[a] Luigi Cavallo,[e] Steven P. Nolan,[b] Catherine S. J. Cazin,[b] and Olivier Lafon[a]

a. Univ. Lille, CNRS, UMR 8181-UCCS, 59000 Lille, France
b. Ghent University, Department of Chemistry and Centre for Sustainable Chemistry, Krijgslaan 281-S3, 9000 Ghent, Belgium
c. Univ. Lille, CNRS, FR2638 □ IMEC □□Fédération Chevreul, 59000 Lille, France
d. University of Salerno, Department of Chemistry and Biology, Giovanni Paolo II 132, Fisciano, Salerno 84084, Italy
e. King Abdullah University of Science and Technology, Physical Sciences and Engineering Division, KAUST Catalysis Center, 23955-6900 Thuwal, Saudi Arabia
E-mail: lama.hamdouna@univ-lille.fr

Keywords: solid-state NMR, materials.

The use of solvents in modern synthesis has now reached enormous quantities and with the aim of reducing the footprint of synthesis, the use of solvents needs to be reduced or, even better, eliminated. Mechanochemistry is one possible route to achieve this goal. Nevertheless, several reaction mechanisms involved in mechanochemistry are still elusive. As a local characterization technique endowed with atomic resolution, solid-state NMR spectroscopy represents a promising method to unveil the mechanism of mechanosynthesis. Recently we applied solid-state NMR to study the mechanochemical synthesis of transition metal complexes bearing N-heterocyclic carbene (NHC) ligands with applications in catalysis and anti-infective coatings in medical devices [1, 2, & 3]. In particular, we investigated the effect of the successive work-up protocols for the preparation of the [Cu(Cl)(NHC)] complex. This compound was prepared under ball milling and solvent-free conditions in two different ways, (i) one-pot synthesis and (ii) step-wise synthesis (Fig. 1). Multinuclear (^{1}H, ^{13}C, ^{15}N, ^{35}Cl, and ^{63}Cu) solid-state NMR experiments at 9.4 and 18.8 T were conducted to monitor the consumption of the reactants and the formation of the intermediate and final product for the different synthetic routes and work-up protocols (Fig. 1). NMR signals were assigned with the help of 2D heteronuclear correlation as well as DFT calculations of NMR parameters. The NMR signals of ^{13}C nucleus bonded to ^{63,65}Cu isotope in [Cu(Cl)(NHC)] complex exhibits multiplet due to *J*-coupling and quadrupolar-dipolar cross-term. In this presentation, we will show the results of these experiments and the notable differences we observe between the one-pot and the stepwise processes.

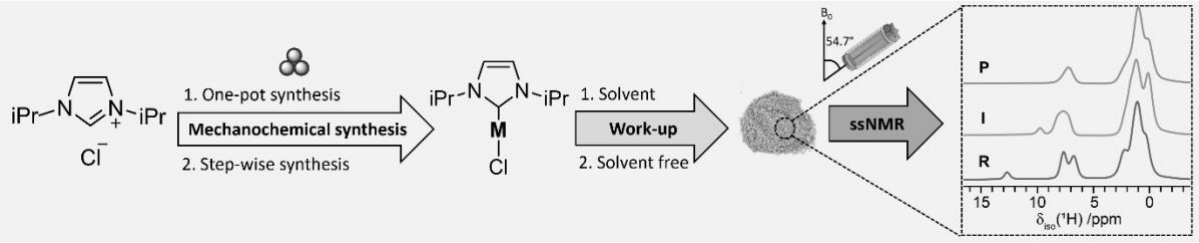

Fig. 2. Schematic representation of the mechanochemical synthetic routes used for the preparation of a metal-NHC complex followed by the different work-up protocols before being characterized by solid-state NMR spectroscopy.

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[P-55] IN VIVO AND EX VIVO LONGITUDINAL FOLLOW-UP OF RESVERATROL SUPPLEMENTATION OR DIET INTERVENTION IN FEMALE RAT HEARTS SUBMITTED TO HIGH-FAT-HIGH-SUCROSE DIET.

A. Jouenne,[‡] I. Varlet,[‡] C. Lan,[‡] K. Hamici,[‡] P. Daudé,[‡] F. Kober,[‡] M. Bernard,[‡] M. Desrois[‡]
[‡] Système cardiovasculaire, Centre de Résonance Magnétique Biologique et Médicale - UMR 7339, Marseille, France;
E-mail: alexis.JOUENNE@univ-amu.fr

Keywords: MRI, small molecules, biomolecules, food

Prediabetic women are at greater risk of cardiovascular disease than men, but first line treatments do not usually focus on the early cardiac alterations associated with prediabetes. Investigating new sex dependent therapeutic strategies is then essential to limit cardiovascular complications in prediabetic women. Resveratrol (RSV) supplementation has previously shown cardioprotective effects on type 2 diabetic female rats. We therefore aimed to compare its effects on the heart of prediabetic female rats to those induced by diet intervention, which is one of the conventional first line treatments.
72 female Wistar rats were divided into 4 groups fed for 5 months with: a standard diet (CTRL), High-Fat-High-Sucrose (HFS) diet, HFS diet supplemented with RSV (*1mg/kg/day in drinking water*) during the last 2 months (RSV) or HFS diet for 3 months followed by 2 months of standard diet (RSD). We performed a longitudinal *in vivo* study of cardiac function, morphology and perfusion by MRI at the 3rd, 4th and 5th months. Then, rats underwent an IPGTT. *Ex vivo* experiments on isolated perfused hearts were performed to simultaneously study cardiac function (Rate Pressure Product (RPP)) and energy metabolism (Phosphocreatine (PCr), ATP and Pi) along with intracellular pH with ^{31}P magnetic resonance spectroscopy during an ischemia-reperfusion injury (IR).

From 3 to 5 months of HFS diet, increased myocardial perfusion ($p<0.01$), diastolic and systolic wall thickness *((DwT) $p<0.01$ and (SwT) $p<0.05$ vs CTRL, respectively)* was found. HFS diet also induced elevated left ventricular diastolic, end diastolic volume *((LVDV) $p<0.001$; (LVEDV) $p<0.01$ vs CTRL, respectively*), diastolic left ventricular mass ((*DLVM) $p<0.05$ at the 4th month)* along with glucose intolerance ($p<0.05$). *Ex vivo,* HFS diet induced increased heart weight to tibia length ratio (*HTLR) ($p<0.01$ vs CTRL)* and alteration of myocardial tolerance to IR, as shown by impaired RPP ($p<0.001$) and lower PCr and ATP levels during reperfusion ($p<0.05$, $p<0.001$ vs *CTRL*). *In vivo,* RSD restored glucose tolerance *($p<0.001$ vs HFS*) but had no effect on the LVDV, LVEDV, DwT, LVDM and perfusion impairments *($p<0.01$ vs CTRL)* at 5 months. RSD failed to restore the elevated HTLR ($p<0.05$ vs *CTRL)* but improved tolerance to IR, characterized by increased RPP during both control and reperfusion periods *($p<0.05$ vs HFS)* along with increased PCr and ATP levels during reperfusion *($p<0.001$, $p<0.01$ vs HFS)*. At 5 months, RSV supplementation had no significant effect on perfusion and SwT impairments but decreased LVDV, LVEDV, DwT and DLVM ($p<0.05$ vs *HFS*) and improved glucose tolerance ($p<0.05$ vs *HFS)*. *Ex vivo*, RSV supplementation restored HTLR to CTRL level ($p<0.001$ vs *HFS)* but also improved myocardial tolerance to IR, characterized by higher RPP during both control and reperfusion periods ($p<0.05$ vs *HFS*) along with increased PCr levels during reperfusion ($p<0.05$ vs *HFS*).

In female rats, both therapeutic strategies improved myocardial tolerance to IR but only RSV supplementation improved LVDV, LVEDV, DwT, LVDM *in vivo* and restored HTLR *ex vivo*, exhibiting an overall better cardiac outcome, despite maintained HFS diet.

[P-56] INSIGHT INTO THE MULTISCALE DYNAMICS IN THERMOTROPIC IONIC LIQUID CRYSTALS (TILCS)

O. Karé,[†] N. Malikova,[†] A.-L. Rollet,[†] G. Mériguet,[†] Q. Berrod,[‡] S. Lyonnard[‡]

[†]Sorbonne Université, CNRS, Physico-chimie des Électrolytes et Nanosystèmes Interfaciaux (PHENIX), F-75005 Paris, France
[††]Institut Laue-Langevin, 38042 Grenoble Cedex, France

E-mail: ousmane.kare@sorbonne-universite.fr

Keywords: materials, small molecules.

Thermotropic Ionic Liquids Crystals (TILCs [1]) are a new type of solid organic electrolytes based on self-assembling ionic liquids. They exhibit valuable mechanical and conduction properties and display promising features for lithium-ion batteries. Their use in integrated systems would allow an increase in energy density and an improvement of their safety. These TILCs form different structures according to the temperature, e.g. lamellar or gyroid phases.
To understand the multi-scale dynamics in TILCs and especially the one of the mobile ions (Li^+, H^+, …), we use complementary techniques such as QENS (quasi-elastic neutron scattering) and NMR relaxometry. QENS allows us to probe the dynamics at the molecular scale and on a timescale ranging from a few picoseconds to 1 nanosecond, while NMR relaxometry probes the dynamics corresponding to slower timescale [2]. The combination of these results provides then a comprehensive view of the dynamics in TILCs.
In NMR, we determine the longitudinal relaxation time (T_1) that probes fast phenomena with characteristic times of the order of the inverse of the Larmor frequency, the transverse relaxation time (T_2) is very sensitive to slow phenomena, while the relaxation time in the rotating frame ($T_{1\rho}$) completes this range with intermediate times (0.1-10 kHz). Preliminary measurements between 20°C to 100°C for ^{7}Li at 116 MHz show very different behaviors and order of magnitude ($T_2 \sim 0.125$ ms and $T_1 \sim 2$ s). This is the signature of dynamic phenomena with intermediate characteristic times that are currently under further investigation.

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[P-57] MAX EFFECTOR PREDICTION, MODELLING AND STRUCTURE CHARACTERIZATION

M. Lahfa‡, F. Charriat†, M. Le Naour-Vernet†, J. Gracy‡, C. Roumestand‡, P Barthe‡, K deGuillen‡, F. Hoh‡, M. Raji‡, S. Cesari†, T. Kroj†, N. Declerck‡, P. Gladieux‡, A. Padilla‡

‡ Centre de Biologie Structurale, Montpellier University, CNRS UMR 5048, INSERM U 1054, France.
† PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France

E-mail: lahfa@cbs.cnrs.fr

Keywords:
solution NMR, biomolecules.

Magnaporthe ToxB-like effectors (MAX) from *Magnaporthe oryzae* constitute a superfamily of related proteins[1]. They show high evolutionary and sequence divergences, but a conserved common ß-sandwich structural fold[2,3]. Only a few MAX sequences have been structurally characterized so far and their biological functions remain unclear. Discovering significant sequence–structure–function relationships calls for the application and development of new tools and methods. As fungi effectors are involved in a large number of key roles in plant infection, they are a stimulating case study for exploring such approaches.
We selected and investigated MAX protein sequences for three-dimensional structure modeling, experimental structure determination by solution NMR and functional annotation. We developed new integrative methodologies based on several well-established bioinformatics programs including detection of conserved fold-in-sequence patterns followed by geometrical modelling, and Alphafold modeling and annotation. Their application led to an updated classification of the MAX superfamily and new functional hypotheses.
Moreover, this work allows a better representation of the diversity of fungi MAXs in terms of sequence, structure, function. Altogether this study will identify effector protein targets and provide new insights on key pathogen-host interactions.

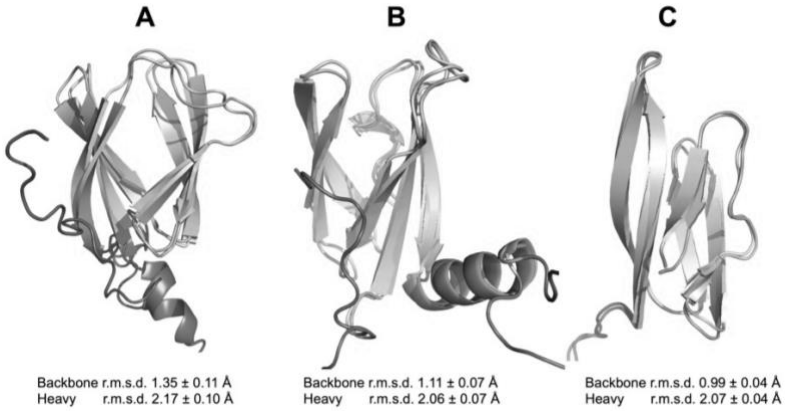

Fig. 1. Superimposition of NMR structures and AlphaFold models of (A) MAX67, (B) MAX60 and (C) MAX47.

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[P-58] MRI AS A TOOL FOR NUCLEAR SAFETY

S. Leclerc,[‡] A.V.S. Oliveira,[‡] D. Stemmelen,[‡] T. Glantz,[†] A. Labergue,[‡] G. Repetto[†], M. Gradeck,[‡] A. Archer[*]

[‡] LEMTA, Université de Lorraine, Nancy, France
[†] IRSN, Cadarache, St Paul les Durance, France
[*] EDF R&D, Chatou, France
E-mail: sebastien.leclerc@univ-lorraine.fr

Keywords:
MRI, exotica.

The loss of coolant accident (LOCA) is one of the most dangerous failure that could happen in a nuclear reactor. It was notably the cause of the Fukushima Daiichi disaster in 2011. Even if the power plant operator is able to reflood the fuel rods after the failure in order to cool them, the water will instantly evaporate and the cooling flow will be diphasic (liquid/gas), less effective than a purely liquid flow. Moreover, the fuel rods cladding can swell due to the intense heat, and this can block the circulating channels and modify the flow distribution.
We used MRI velocimetry to visualize such phenomena, as classical (optical) velocimetry techniques are unable to image through these opaque systems. However, we faced various difficulties:

- The cooling flows are very fast, at least a few meters per seconds. This can be a problem as the spins go through the MRI probe in a few ms. Notably, we cannot use spin echo experiments or any experiments with long echo time.
- The presence of gas bubbles leads to magnetic susceptibility effects
- The experimental setup is quite complicated, with high flow rates (up to 5 L/s) and in some cases metal grids

We were able to overcome these difficulties and we obtained some significant results [1, 2] such as the effects of the mixing grids or the presence of recirculation area downstream of the swelled zone. These results will be used to benchmark a new simulation software being developed by EDF and IRSN

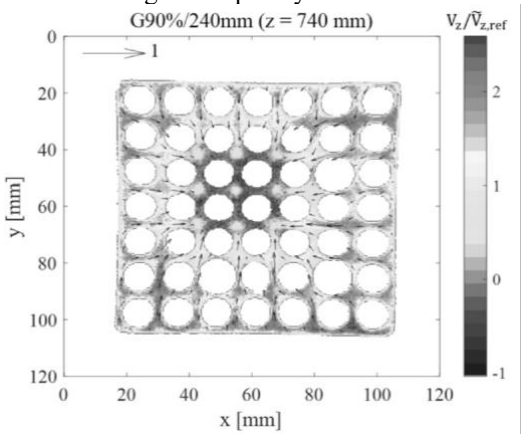

Fig. 1. Velocity map after the swelled rods. Grayscale: axial velocity. Arrows: transverse velocity

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[P-59] ANALYSIS OF CUMIN ESSENTIAL OIL BY VISCOSITY ENHANCED SPECTROSCOPY NMR EXPERIMENTS WITHOUT PHYSICAL SEPARATION

R. Leroy, ‡ J.-M. Nuzillard, P. Lameiras, ‡
‡ Université de Reims Champagne-Ardenne, CNRS ICMR UMR 7312, 51097 Reims, France
E-mail: ritchy.leroy@univ-reims.fr

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

The large majority of human activities based on chemistry (healthcare, energy, materials, etc.) obliges the identification of organic compounds in mixture. Transformation processes that lead from natural resources, either from fossil carbon industry or biomass exploitation to low-value products in large amounts or to high-value specialty products including pharmaceutical and cosmetic active ingredients, rarely produce chemically pure molecules. In the current state of knowledge, mixture analysis remains a mandatory step and effective way to carry it out remain to be established.
Individual analysis of mixture components without performing any physical separation is a recurrent problem for which only a limited number of solutions have been proposed to date:
The analysis of mixture by liquid-state NMR is an important part in dereplication of natural products. Three ways can be chosen to achieve this goal. The classical way is to physically isolate and identify each components. The fraction way with a partial separation and an analysis of each fraction like CARAMEL [1]. And the full mixture analysis directly by NMR experiments without separation. In this last way, approaches based on DOSY [2], spectral decomposition [3], concentration of analytes CORDY [4] are used. Usually DOSY NMR experiment is enough but in the case of similar molecules, this method is not sufficient.
An alternative is to use ViscY experiments, based on spin diffusion [5]. The recent use of viscous solvents has provided an exciting approach called ViscY (Viscosity-enhanced spectroscopY) for studying mixtures by lowering the molecular tumbling rate in solution. [1-3] As a result, the molecules display a negative nOe regime and their resonances can be sorted according to their ability to exchange magnetization through intramolecular spin diffusion. The 2D 1H-1H NOESY spectrum of a mixture reveals correlations between all the 1H resonances of each analyte when recorded in spin diffusion conditions, thus giving access to the individual 1H NMR spectra of the mixture components.

Application to the investigation of essential oils

for the application of our analytical methods to the investigation of essential oils mostly made of low polarity or apolar compounds. Indeed, most of them consist in their great majority of a rather complex mixture of monoterpenes, sesquiterpenes, alcohols, cetones, esters, aldehydes, oxides, etc. They correspond to a concentrated hydrophobic liquid containing volatile chemical compounds extracted from plants. Essential oils will be chosen because they constitute high added-value and increasingly widespread consumer goods with effective action of human well-being. The utilization of essential oils of plants is well-known ranging from perfumes and fragrances, to spices and medicinals.

We will preferentially consider low polarity viscous media such as chlorotrifluoroethylene polymer (CTFEP, η = 1 550 cP at 25°C)/CDCl3 or polystyrene (PS)/toluene-d8 (η = 34.8 cP, 34% w/w at 30°C), PS/THF-d8 blends due to their capability to dissolve apolar and moderate polar compounds and to induce spin diffusion under viscous conditions.

We have been assessing the effectiveness and robustness of our new analytical ViscY tools by considering different criteria such as the spin diffusion efficiency (assessment of full intramolecular magnetization exchange allowing the individualization of complex mixture components spectrum, determination of the distance of spin diffusion propagation in nm between two nuclei not close enough to present a NOE in a low viscosity medium), the spectral resolution (T2 not too lowered in particular at low temperature for maintaining spin diffusion promotion under viscous conditions), the access to J-coupling information (T2 sufficiently long for coupling together NOESY block and pulse mixing period (COSY) or isotropic mixing period (TOCSY)).
Here is the first application of ViscY NMR experiments on a natural complex mixture of small molecules without separation. The experiments are still going at this moment. The preliminary results will be compared to the standard NMR experiments of the mixture and mass spectroscopy. Finally, the validation of this ViscY method with others experiments such as NMR GEMSTONE [6] or NOAH [7] and with the limits of ViscY NMR experiments will be achieve soon.

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[P-60] Probing the coupled dynamics between lipids and membrane proteins by high-pressure NMR spectroscopy

Pozza A[1‡], Giraud F[2‡], Cece Q[1], Casiraghi M[1], Point E[1], Damian M[3], Le Bon C[1], Moncoq K[1], Banères JL[3], Lescop E[2‡], Catoire L[1†]
1 Laboratoire de Biologie Physico-Chimique des Protéines Membranaires, UMR 7099, CNRS/ Université de Paris, Institut de Biologie Physico-Chimique (IBPC, FRC 550), 13 rue Pierre et Marie Curie, Paris, France
2 Institut de Chimie des Substances Naturelles (ICSN), CNRS UPR 2301, Université Paris-Saclay, 1 avenue de la Terrasse, Gif-sur-Yvette, France
3 Institut des Biomolécules Max Mousseron (IBMM), Université de Montpellier, CNRS, ENSCM, Pôle Chimie Balard Recherche, 1919 route de Mende, Montpellier, France

‡ : Co-first author / † : Co-corresponding author
E-mail: ewen.lescop@cnrs.fr

Keywords: solution NMR, biomolecules, theory and methods.

Cell membranes represent a complex and variable environment in time and space of lipids and proteins. Their physico-chemical properties are determined by lipid components which can in turn influence the biological function of membranes. Here, we used NMR spectroscopy under hydrostatic pressure to study the close relationships between the dynamics of lipids and membrane proteins in nanodiscs [1]. We first demonstrated that nanodiscs can reversibly accommodate high pressure, in absence or in presence of embedded membrane protein. We further showed that the fluid-gel phase transition of the lipid bilayer, triggered either by temperature or pressure, can be accurately monitored in nanodiscs by ^{1}H NMR. The P/T transition phase diagram indicates that lipids have similar dynamic behavior in the highly restricted nanodiscs environment compared to infinite plan bilayers, all in absence of embedded membrane proteins. Experiments carried out on the β-barrel OmpX and the α-helical BLT2 G Protein-Coupled Receptor in nanodiscs of different lipid compositions further reveal protein conformational landscapes intimately linked to pressure and lipids. Indeed, pressure can modify the conformational landscape of the membrane protein per se, revealing differences in the molar partial volumes of protein/lipid substates, but also increases the gelation of lipids, both being monitored simultaneously at high atomic resolution by NMR. Our study also clearly shows that a membrane protein can modulate, at least locally, the fluidity of the bilayer. Notably we observed that OmpX and BLT2 prevented the gelation of the first lipids around the protein. In addition, high pressure can lead to protein dynamics modulation as revealed by the dramatic x4-5 NMR signal increase in methyl 2D ^{13}C SOFAST-HMQC spectra of the BLT2 GPCR. The strategy proposed herein establishes that high quality structural/dynamic information can be retrieved by NMR under pressure on membrane proteins embedded in lipid nanodiscs, which opens new perspectives to scrutinize the dynamic interplay between membrane proteins and their surrounding lipids.

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[P-61] From proteins to fruit juices: some insights on biological contrast agents

G. Licciardi,[‡] E. Ravera,[‡] M. Fragai,[‡] G. Parigi,[‡] C. Luchinat[‡]

[‡]Magnetic Resonance Center (CERM), University of Florence, via Sacconi 6, Sesto Fiorentino, 50019 Italy; Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3, Sesto Fiorentino, 50019 Italy
E-mail: licciardi@cerm.unifi.it

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

Paramagnetic systems are largely employed in MRI for their ability to increase the contrast in both T_1 as well as T_2 weighted images. Given the importance and the evolution of MRI, the development of low toxicity contrast agents (CAs), as well as the use of alternative compounds to those containing gadolinium(III), is highly desirable. In order to lower the amount of CA needed for the diagnostic exam, an increase of the relaxivity can be obtained through the modulation of the rotational correlation time of the paramagnetic agent by binding it to macromolecules [1]. Asparaginase is a large tetrameric protein assembly, currently used against acute lymphoblastic leukemia. A gadolinium(III)-DOTA derivative has been conjugated to asparaginase and the relaxation properties have been investigated in order to assess its efficiency as a possible theranostic agent. We observed a very large increase in the relaxivity of the paramagnetic protein with respect to small gadolinium chelates, opening the possibility of its use as MRI contrast agent (Fig. 1A).
Regarding gastrointestinal tract imaging, natural oral CAs, e.g. fruit juices, are the ideal choice (Fig. 1B). They are rich in manganese(II) ion and fulfill the requirements of non-toxicity, tolerability, and low price [2].

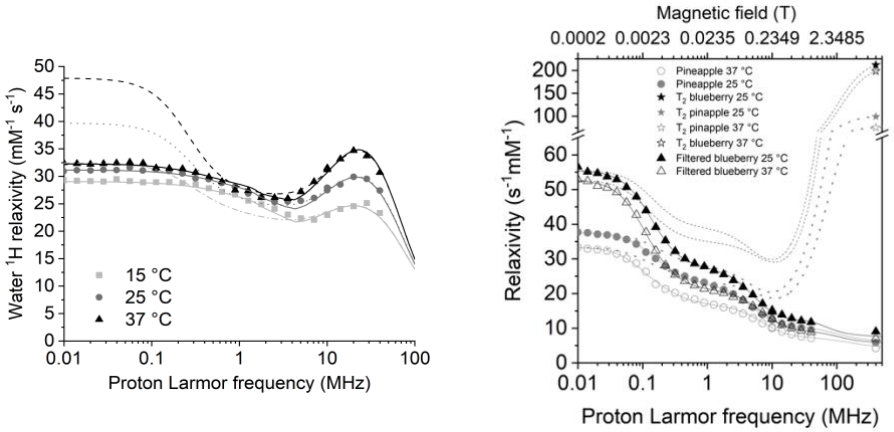

Fig. 1A ^{1}H relaxivity profiles of GdDOTA-conjugated asparaginase II at 15, 25 and 37 °C. Solid lines are the best fit profiles obtained with the Florence NMRD program and dashed lines are calculated with the Solomon-Bloembergen-Morgan (SBM) model. Fig. 1B ^{1}H relaxivity profiles of blueberry and ananas juices. Transverse relaxivity values at 400 MHz are also plotted, as well as the best fit profiles.

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[P-62] ACCOUNTING FOR GRADIENT NON-UNIFORMITY IN SPATIALLY-ENCODED DIFFUSION-ORDERED NMR SPECTROSCOPY

B. Lorandel, R. Mishra, O. Cazimajou, A. Marchand, A. Bernard, J.-N. Dumez

Nantes Université, CNRS, CEISAM UMR6230, F-44000 Nantes, France
E-mail: benjamin.lorandel@univ-nantes.fr

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

Diffusion-ordered NMR spectroscopy (DOSY) is a powerful tool for the analysis of mixtures. DOSY experiments rely on a pair of magnetic-field gradient pulses separated by a delay, that result in diffusion-weighting of the NMR signals. Conventional DOSY experiments take several minutes or more, because a series of spectra are recorded, with incremented values of the gradient pulse amplitude. This is a limitation for the analysis of mixtures that evolve in time, either due to chemical reaction or because they are hyperpolarised.

Spatially encoded (SPEN) DOSY makes it possible to collect a complete DOSY data set in a single scan, by spatial parallelisation along the gradient dimension. The SPEN DOSY experiment has notably been used for reaction monitoring and the analysis of hyperpolarised substrates. However, the accuracy of the SPEN DOSY method is still limited. One limitation of current SPEN DOSY experiment is that the data is analysed by assuming that the magnetic field generated by the gradient coil varies linearly over the sample [1, 2]. This is usually not the case for high-resolution NMR probes, and even less for triple-axis gradient probes, while triple-axis gradients have many advantages for spatial parallelisation experiment.

In this work, we have developed methods to account for gradient non-uniformity in the processing of SPEN DOSY experiment. First, we have modified an existing pulse sequence to map the field generated by the gradient coil of a triple-axis gradient probe [3]. For the processing of SPEN DOSY data, we have then modified the equation used to calculate the effective gradient pulse area for each position in the sample tube. The proposed model was validated through numerical simulation. With this model, both the trueness of the estimated diffusion coefficient and the quality of the fit are improved. Accounting for non-uniform gradients also makes it possible to use the full length of the RF coil for spatial parallelisation, rather than the reduced length over which the field gradient is approximately uniform. Finally, we also explore the potential of multivariate processing algorithms for SPEN DOSY experiment, and how they benefit from accurate gradient information.

These results are implemented in a user-friendly add-on of a powerful DOSY processing toolbox [4]. Overall, this work will increase the applicability of single-scan DOSY experiments for the analysis of out-of-equilibrium mixtures.

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[P-63] Conformational Studies of Human Prion Protein using Cell Mimicking Condition

M. Madheswaran[‡], L. Russo[‡], G. Salzano[†], G. D'Abrosca[‡], C. Isernia[‡], G. Malgieri[‡], G. Legname[†], R. Fattorusso[‡]

[‡] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DISTABiF), Università degli studi della Campania Luigi Vanvitelli, Caserta, Italy.
[†] Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy.
Email: Manoj.madheswaran@unicampania.it

Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, fatal insomnia and Kuru are a group of neurodegenerative disorders caused by prions. Investigating protein dynamics is essential for understanding protein function especially for proteins like Prion [1]. The misfolding and conversion of normal cellular prion into abnormal protease resistant pathogenic scrapie prion is believed to be the key procedure [2]. However, the functions of PrP^{c} are not completely understood and the mechanism of its structural conversion remains unclear. Human PrP^{C} is a cell surface expressed glycoprotein and has a physiological structure with a C-terminal globular domain (amino acids 122–230) and an N-terminal flexible tail (amino acids 23–121). The N-terminal tail consists of two charged clusters (CC1 and CC2), the octarepeat region (OR) and a hydrophobic domain (HD). Additionally, two N-glycosylation sites are located in the globular domain upstream of the sialylated GPI-anchor at the C-terminus. The globular domain consists of two beta-sheets and three alpha-helices. The known functions described for PrP^{C} cover a wide spectrum including ion balance homeostasis, metal ion intake (such as copper and zinc), control of cell proliferation and neural differentiation. The conformational studies of human prion proteins are widely studied in buffer solutions. However, human prion proteins form fibrils only in a physiological environment. Our primary goal is to obtain structure-related information on human prion protein under cell mimicking conditions. Very recently, we have identified that in buffer solution, a human prion intermediate involved in prion fibril formation [3]. In this study, we used Nuclear Magnetic Resonance (NMR) spectroscopy the investigate conformational equilibria of human prion protein HuPrP (residues 90-231) in cell mimicking conditions, using ficoll as a solvent, an inert crowding agent. Using CD and NMR methodologies, we carried out an atom by atom analysis of human prion thermal unfolding in the presence of ficoll. Our results will help the elucidation of structural behavior of the human prion protein in cell, providing decisive information to make clear the structural basis of its pathogenic conversion.

Keywords: NMR, Solution NMR, CD Spectroscopy, Protein dynamics, Biomolecules

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[P-64] NMR-BASED METABOLOMIC APPROACH FOR AGING DISCRIMINATION OF GRANA PADANO PDO CHEESE

V. Maestrello,[‡,†] P. Solovyev,[†] P. Franceschi,[†] F. Camin,[‡,†] A. Stroppa,[ǂ] L. Bontempo[†]

[‡]Center Agriculture Food Environment (C3A), University of Trento, Via Mach 1, 38098 San Michele all'Adige, (TN), Italy
[†]Fondazione Edmund Mach (FEM), via E. Mach 1, 38098, San Michele all'Adige (TN), Italy
[ǂ]Consorzio Tutela Grana Padano, Via XXIV Giugno 8, 25010 San Martino Della Battaglia, Desenzano del Garda (BS), Italy
E-mail: valentina.maestrello@unitn.it

Keywords:
solution NMR, metabolomics, food

Grana Padano PDO cheese is one of the most renowned Italian cheeses, thanks to its high quality and nutritional value [1]. Its aging can vary from 9 to over 20 months, and, as the aging increases, so does the price, because during storage the organoleptic characteristics can improve, giving the product a strong flavor. To prevent the mislabeling of these high-quality products, it is necessary to perform aging assessment, and this can be done with different approaches. The aging is strictly connected with the period of production related to months and years and in this study, we tried to analyze a large dataset of Grana Padano PDO cheeses produced in different periods of the year by ^{1}H NMR spectroscopy using an untargeted approach. After the analysis of the samples, we performed a multivariate statistical analysis to separate the different classes and identify the most discriminant signals. It was possible to develop a model that can distinguish samples with different aging time in a fast and reproducible way, which can be interesting also for industry.

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[P-65] NANOSIZED T_1 MRI CONTRAST AGENT BASED ON A POLYAMIDOAMINE AS MULTIDENTATE Gd LIGAND

P. Arosio,[‡] D. Cicolari,[†] A. Manfredi,[§] F. Orsini,[‡] A. Lascialfari,[†] E. Ranucci,[§] P. Ferruti,[§] D. Maggioni[§]

[‡] Dipartimento di Fisica, INFN, Istituto Nazionale di Fisica Nucleare-Milano Unit, Università degli Studi di Milano, Via Celoria 16, 20133 Milano, Italy.
[†] Dipartimento di Fisica, INFN, Istituto Nazionale di Fisica Nucleare-Milano Unit, Università degli Studi di Pavia, Via Bassi 6, 27100 Pavia, Italy.
[§] Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133 Milano, Italy.

E-mail: daniela.maggioni@unimi.it

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

Magnetic resonance imaging (MRI) is an in-clinic diagnostic modality that has received tremendous development for many decades since it allows for obtaining images of an organism in a non-invasive way. Nevertheless, in many cases, the natural contrast is not high enough and a contrast agent (CA) is needed. To date, the clinically used CA are small molecules, mostly Gd chelates that present short blood clearance times as a major drawback. Conversely, binding lanthanide chelates to macromolecules or nanoparticles can extend their blood permanence, thus improving the ability to passively reach the tumour target by the so-called enhanced permeability and retention effect. To this end, a linear polyamidoamine (PAA) named BAC-EDDS, containing metal-chelating repeat units, was prepared and interacted with Gd(III). PAA are a family of synthetic polymers obtained by Michael-type polyaddition of primary or secondary amines to bisacrylamides [1]. They are hydro-soluble, biocompatible and biodegradable and show stealth-like behaviour. BAC-EDDS was fully characterized, especially through NMR techniques. The many pK_a of the repeat unit were estimated by potentiometric titrations, using a purposely synthesized molecular ligand (Agly-EDDS) mimicking the BAC-EDDS repeat unit, and attributed to each acid site by a ^1H NMR titration. Nanoaggregates showed a mean size of 150 nm at pH 5 by dynamic light scattering (DLS) and a negative charge by ζ-potential at pH 7.4. BAC-EDDS stably chelated Gd (III) ions with a molar ratio of 0.5:1 Gd (III)/repeat unit with a log K = 17.43 for the molecular model Gd-Agly-EDDS. To understand the efficiency of Gd-BAC-EDDS as CA in MRI, the nuclear longitudinal (r_1) and transverse (r_2) relaxivities as a function of the externally applied static magnetic field were investigated and compared to the ones of commercial contrast agents. Furthermore, a model derived from the Solomon–Bloembergen–Morgan theory for the field dependence of the NMR relaxivity curves was applied and used to evaluate the rotational correlation time of the complex (τ = 0.66 ns). This relatively high value is due to the dimensions of Gd-BAC-EDDS, and the associated rotational motion causes a peak in the longitudinal relaxivity at ca. 75 MHz, which is close to the frequencies used in clinics. The good performances of Gd-BAC-EDDS as a contrast agent were also confirmed through in vitro magnetic resonance imaging experiments with a 0.2 T magnetic field [2].

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[P-66] NMR-BASED METABOLOMIC APPROACH FOR AGING DISCRIMINATION OF GRANA PADANO PDO CHEESE

V. Maestrello,[‡,†] P. Solovyev,[†] P. Franceschi,[†] F. Camin,[‡,†] A. Stroppa,[ǂ] L. Bontempo[†]

[‡]Center Agriculture Food Environment (C3A), University of Trento, Via Mach 1, 38098 San Michele all'Adige, (TN), Italy
[†]Fondazione Edmund Mach (FEM), via E. Mach 1, 38098, San Michele all'Adige (TN), Italy
[ǂ]Consorzio Tutela Grana Padano, Via XXIV Giugno 8, 25010 San Martino Della Battaglia, Desenzano del Garda (BS), Italy
E-mail: valentina.maestrello@unitn.it

Keywords:
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Grana Padano PDO cheese is one of the most renowned Italian cheeses, thanks to its high quality and nutritional value [1]. Its aging can vary from 9 to over 20 months, and, as the aging increases, so does the price, because during storage the organoleptic characteristics can improve, giving the product a strong flavor. To prevent the mislabeling of these high-quality products, it is necessary to perform aging assessment, and this can be done with different approaches. The aging is strictly connected with the period of production related to months and years and in this study, we tried to analyze a large dataset of Grana Padano PDO cheeses produced in different periods of the year by ^{1}H NMR spectroscopy using an untargeted approach. After the analysis of the samples, we performed a multivariate statistical analysis to separate the different classes and identify the most discriminant signals. It was possible to develop a model that can distinguish samples with different aging time in a fast and reproducible way, which can be interesting also for industry.

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[P-67] NANOSIZED T_1 MRI CONTRAST AGENT BASED ON A POLYAMIDOAMINE AS MULTIDENTATE Gd LIGAND

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[‡] Dipartimento di Fisica, INFN, Istituto Nazionale di Fisica Nucleare-Milano Unit, Università degli Studi di Milano, Via Celoria 16, 20133 Milano, Italy.
[†] Dipartimento di Fisica, INFN, Istituto Nazionale di Fisica Nucleare-Milano Unit, Università degli Studi di Pavia, Via Bassi 6, 27100 Pavia, Italy.
[§] Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133 Milano, Italy.

E-mail: daniela.maggioni@unimi.it

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

Magnetic resonance imaging (MRI) is an in-clinic diagnostic modality that has received tremendous development for many decades since it allows for obtaining images of an organism in a non-invasive way. Nevertheless, in many cases, the natural contrast is not high enough and a contrast agent (CA) is needed. To date, the clinically used CA are small molecules, mostly Gd chelates that present short blood clearance times as a major drawback. Conversely, binding lanthanide chelates to macromolecules or nanoparticles can extend their blood permanence, thus improving the ability to passively reach the tumour target by the so-called enhanced permeability and retention effect. To this end, a linear polyamidoamine (PAA) named BAC-EDDS, containing metal-chelating repeat units, was prepared and interacted with Gd(III). PAA are a family of synthetic polymers obtained by Michael-type polyaddition of primary or secondary amines to bisacrylamides [1]. They are hydro-soluble, biocompatible and biodegradable and show stealth-like behaviour. BAC-EDDS was fully characterized, especially through NMR techniques. The many pK_a of the repeat unit were estimated by potentiometric titrations, using a purposely synthesized molecular ligand (Agly-EDDS) mimicking the BAC-EDDS repeat unit, and attributed to each acid site by a ^1H NMR titration. Nanoaggregates showed a mean size of 150 nm at pH 5 by dynamic light scattering (DLS) and a negative charge by ζ-potential at pH 7.4. BAC-EDDS stably chelated Gd (III) ions with a molar ratio of 0.5:1 Gd (III)/repeat unit with a log K = 17.43 for the molecular model Gd-Agly-EDDS. To understand the efficiency of Gd-BAC-EDDS as CA in MRI, the nuclear longitudinal (r_1) and transverse (r_2) relaxivities as a function of the externally applied static magnetic field were investigated and compared to the ones of commercial contrast agents. Furthermore, a model derived from the Solomon–Bloembergen–Morgan theory for the field dependence of the NMR relaxivity curves was applied and used to evaluate the rotational correlation time of the complex (τ = 0.66 ns). This relatively high value is due to the dimensions of Gd-BAC-EDDS, and the associated rotational motion causes a peak in the longitudinal relaxivity at ca. 75 MHz, which is close to the frequencies used in clinics. The good performances of Gd-BAC-EDDS as a contrast agent were also confirmed through in vitro magnetic resonance imaging experiments with a 0.2 T magnetic field [2].

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[P-68] NUSINERSEN INDUCES DISEASE SEVERITY-SPECIFIC NEUROMETABOLIC EFFECTS IN SPINAL MUSCULAR ATROPHY

F. Errico[1,2*], C. Marino[3*], M. Grimaldi[3*], T. Nuzzo[1,4], C. Panicucci[5], E. Di Schiavi[6], T. Mazza[7], C. Bruno[5,8], A. D'Amico[9], A. M. D'Ursi[3,@], E. Bertini[9], L. Pellizzoni[10,11,12], A. Usiello[1,4,@]

[1]Laboratory of Translational Neuroscience, Ceinge Biotecnologie Avanzate, 80145, Naples, Italy.
[2]Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy.
[3]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132 - 84084 Fisciano, Salerno, Italy
[4]Department of Environmental, Biological and Pharmaceutical Science and Technologies, Università degli Studi della Campania "Luigi Vanvitelli", 81100, Caserta, Italy.
[5]Center of Translational and Experimental Myology, IRCCS Istituto Giannina Gaslini, Genova, Italy.
[6]Institute of Biosciences and BioResources (IBBR), CNR, Naples, Italy.
[7]IRCCS Casa Sollievo della Sofferenza, Bioinformatics Unit, Viale Cappuccini 1, 71013 San Giovanni Rotondo, Italy.
[8]Department of Neuroscience, Rehabilitation, Ophtalmology, Genetics, Maternal and Child Health - DINOGMI, University of Genova
[9]Unit of Neuromuscular and Neurodegenerative Disorders, Dept. Neurosciences, Bambino Gesu' Children's Hospital IRCCS, Roma, Italy.
[10]Center for Motor Neuron Biology and Disease, Columbia University, New York, NY, USA.
[11]Department of Pathology and Cell Biology, Columbia University, New York, NY, USA.
[12]Department of Neurology, Columbia University, New York, NY, USA.
* equal contribution
@ corresponding authors
E-mail: cmarino@unisa.it

Keywords: solution NMR, metabolomics.

Intrathecal delivery of Nusinersen – an antisense oligonucleotide that promotes SMN induction – is an approved therapy for spinal muscular atrophy (SMA). Here, we employed nuclear magnetic resonance (NMR) spectroscopy to characterize longitudinally the unknown metabolic effects of Nusinersen in the cerebrospinal fluid (CSF) of SMA patients across disease severity. Modulation of amino acid metabolism is a common denominator of biochemical changes induced by Nusinersen with distinct downstream metabolic effects according to disease severity. In severe SMA1 patients, Nusinersen stimulates energy-related glucose metabolism. In intermediate SMA2 patients, Nusinersen effects are also related to energy homeostasis but involve ketone body and fatty acid biosynthesis. In milder SMA3 patients, Nusinersen modulates mainly amino acid metabolism. Moreover, Nusinersen modifies the CSF metabolome of a more severe clinical group towards the profile of untreated SMA patients with milder disease. These findings reveal disease severity-specific neurometabolic signatures of Nusinersen treatment in SMA patients and suggest modulation of peripheral organ metabolism by this CNS-directed therapy.

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[P-69] THE NMR CHEMICAL CHARACTERIZATION OF *Acheta domesticus* FLOUR

F. Masciulli‡, D. Ambroselli‡, E. Romano‡, M. Spano‡, G. Di Matteo‡, C. Ingallina‡, and L. Mannina‡

‡Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
E-mail: fabrizio.masciulli@uniroma1.it

Keywords: solution NMR, metabolomics, food.

In the last years, several environmental, economic, and social aspects have increased the demand of alternative protein sources for both human consumption and animal feed. In this context, edible insects represent an important potential food source able to satisfy both sustainability and nutritional demands, since they represent a good source of many essential nutrients such as proteins, fatty acids, minerals, and, in some cases, vitamins [1]. Entomophagy is practiced since ancient times and today it represents a common practice in many countries and, according to FAO indications, the European Union have recently approved three insects as Novel Foods [2]. Among these, *Acheta domesticus* (house cricket) represents the last edible insect approved as Novel Food on 10th February 2022. High-resolution Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most suitable approaches to face food authenticity, traceability issues and food characterization [3]. The present study aims at characterizing the metabolite profile of organic *Acheta domesticus* (house cricket) flour using the NMR-based metabolomic approach.
The chemical composition of *A. domesticus* flour was assessed by NMR spectroscopy carried out on Bligh-Dyer extracts. The ^{1}H NMR spectra assignments of hydroalcoholic phase were carried out by means of ^{1}H and 2D experiments (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) and literature data. Compounds belonging to different classes of primary and secondary metabolites were identified and quantified in this fraction: sugars, organic acids, amino acids, including several essential ones, and other compounds such as glycerol, choline, and uracil.
NMR-based characterization allowed to obtain the metabolite profile of *A. domesticus* flour, thus contributing to define its chemical composition and representing an important tool for its implementation in food industry.

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[P-70] sCalcium binds to transthyretin with low affinity

M.C. Mimmi,[‡] C. Cantarutti,[§] G. Verona,[†] P. Mangione,[‡, †] S. Giorgetti,[‡] V. Bellotti,[‡, #, †] A. Corazza.[§]

[‡]Department of Molecular Medicine, Institute of Biochemistry, University of Pavia, Pavia, Italy.
[§]Department of Medical Area, University of Udine, UD 33100, Italy.
[†]Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, University College London, London, United Kingdom.
[#]Scientific Direction, Fondazione IRCSS Policlinico San Matteo, Pavia, Italy
E-mail: chiara.mimmi@unipv.it

Keywords:
solution NMR, biomolecules.

The plasma protein transthyretin (TTR), a transporter for thyroid hormones and retinol in plasma and cerebrospinal fluid, is responsible for the second most common form of systemic amyloidosis (ATTR). The association between free calcium ions (Ca^{2+}) and TTR is still debated, although recent work seems to suggest that calcium induces structural destabilization of TTR [1] and promotes its aggregation at non-physiologic low pH in vitro.
We applied high resolution NMR spectroscopy to investigate calcium binding to TTR, showing the formation of labile interactions that leave the native structure of TTR substantially unaltered. The binding sites deduced form chemical shift perturbation (CSP) analysis in solution are compatible with those observed in the X-ray structure of wild type TTR (PDB 4n85 [2]). In addition, CSP results reflect the electrostatic interactions of Ca^{2+} with negatively charged regions of TTR surface.
The effect of calcium binding on TTR aggregation was also assessed in physiological conditions designed to generate fibrils through the mechano-enzymatic mechanism; these assays show that fibrillogenesis is favoured by high Ca^{2+} concentration.

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[P-71] TAILORED POLARIZING AGENTS FOR MAS DNP ENABLE THE INVESTIGATION OF CRYSTALLIZATION AND POLYMORPH SELECTION IN ORGANIC SOLIDS

M. Juramy,[‡] S. F. Cousin,[‡] T. El Darai,[†] R. Chèvre,[‡] F. Ziarelli,[§] E. Besson,[‡] S. Gastaldi,[‡] S. Viel,[‡] K. D. M. Harris,[#] S. Jannin,[†] P. Thureau,[‡] G. Mollica[‡*]

[‡] *Aix Marseille Univ, CNRS, ICR UMR 7273, Marseille, France*
[†] *Univ. Lyon, CNRS, ENS Lyon, UCBL, Université de Lyon, CRMN UMR 5280, 69100 Villeurbanne, France*
[§] *Aix Marseille Univ, CNRS, Centrale Marseille, FSCM, Marseille, France*
[#] *School of Chemistry, Cardiff University, Cardiff, Wales, United Kingdom*

E-mail: giulia.mollica@univ-amu.fr

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

Crystallization plays an important role in many areas of biology, chemistry and materials science, but the underlying mechanisms that govern crystallization are still poorly understood because of experimental limitations in the analysis of such complex, evolving systems. To derive a fundamental understanding of crystallization processes, it is essential to access the sequence of solid phases produced as a function of time, with atomic-level resolution. Rationalization of crystallization processes is particularly relevant for polymorphic functional materials, for which manufacture or storage-induced, unexpected, polymorph transitions can compromise the end-use of the solid product. Interestingly, these transformations often imply the formation of metastable forms. Today, detection and accurate structural analysis of these – generally transient – forms remain challenging, essentially because of the present limitations in temporal and spatial resolution of the analysis, preventing the rationalization – and hence the control – of crystallization processes.
In this contribution, I will present some of our latest results showing that cryogenic MAS solid-state NMR [1] combined with the sensitivity enhancement provided by dynamic nuclear polarization (DNP) [2] can be an efficient way of monitoring the structural evolution of crystallizing solutions with atomic-scale resolution on a time scale of a few minutes. Specifically, I will discuss current approaches and recent developments allowing to detect, stabilize, and characterize transient, metastable phases formed at the early stages of crystallization through the use of tailored DNP polarizing agents [3].

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[P-72] PROTON AND HSQC NMR SPECTROSCOPY COMBINED WITH CHEMOMETRIC APPROACHES TO MONITORING THE QUALITY OF CRUDE HEPARIN

Elizabeth Montatixe,‡ R. Salvino,‡ L. Mauri,‡ C .Cosentino, ‡ L. Muzi, ‡ M. Guerrini‡

‡Institute for Chemical and Biochemical Research "G. Ronzoni", Via Giuseppe Colombo, 81, Milan 20133
E-mail: montatixe@ronzoni.it

Keywords: Solution NMR, chemometric, heparin, biomolecules, principal component analysis.

The combined application of NMR spectroscopy and chemometric techniques, such as principal component analysis (PCA), was demonstrated to be a valid tool in various areas of applications. In the pharmaceutical field, the control of heparin quality during manufacturing steps is essential to ensure the safety of the final active pharmaceutical ingredient (API) [1]. However, due to the intrinsic heterogeneity of heparin, a library of *bona fide* spectra of reference material is required to make the comparison with a single test sample, providing a measurement of its similarity. The construction of a library of so called *bona fid* heparin samples allows to compare samples coming from different suppliers, such as branded and generic heparin [2], monitor the treatment process of heparins from different manufacturers or production sites, detect the presence of contaminants.

Because of the complexity of the heparin supply chain, the control of heparin quality during manufacturing steps is essential to ensure the safety of the final active pharmaceutical ingredient (API). Here we show a consistent analytical method able to assess the quality of heparin in the early step of production (crude heparin) [3]. By this method, libraries of several crude heparin samples manufactured in different plants were built up and characterized by monitoring over time the characteristics of the batches within the same plant and globally among plants. The method besides to permit the monitoring of products that do not meet specifications is also an analytical tool for validations new sources of raw material.

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[P-73] MD/GIPAW calculations and ^{17}O NMR combined study of former network ability of Titanium in SiO_2-TiO_2 binary glasses.

E. Chesneau[1], V. Montouillout[1], A. Zandona[1], Joachim Deubener[2], F. Fayon[1]

[1] Conditions Extrêmes et Matériaux: Haute Température et Irradiation (CEMHTI), CNRS UPR 3079, 1D Avenue de la Recherche Scientifique, 45071 Orléans Cedex 2, France
[2] Institute of Non-Metallic Materials, Clausthal University of Technology, Zehntnerstrasse 2A, 38678 Clausthal-Zellerfeld, Germany
E-mail: montouillout@cnrs-orleans.fr

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

The Ti^{4+} ion has an intermediate role in the glass structure with respect to those of the classically defined network formers and modifiers. Contrary to crystalline phases where Titanium is typically 6-fold coordinated, low coordinated species are present in glass structures, which should indicate a glass forming ability. However, no Si-O-Ti bonds evidences can be directly detected using either ^{29}Si NMR or Raman spectra. In order to characterize the structural role of Titanium in the glass structures, classical Molecular Dynamics simulation models were created using empirical pair potentials. These models confirm the tetrahedral conformation of Titanium in binary silicate glasses with a highly predominance of Si-O-Ti bonds in comparison to Ti-O-Ti ones. GIPAW calculations performed on these models allow calculating NMR parameters of each atoms of a simulation box, in particular the chemical shift shielding. These calculations confirm the non-sensibility of ^{29}Si NMR on Si^{4+} to Ti^{4+} substitution. On the contrary, an important effect is observed on the ^{17}O NMR chemical shift, which increases of about 250ppm from Si-^{17}O-Si to Si-^{17}O-Ti. Moreover, the effect appears to be additive and then an oxygen connected to two Titanium has a predicted chemical shift shifted of 500ppm compared to an oxygen connect to two silicon. ^{17}O NMR appears then as a powerful tool to characterize unambiguously the Titanium bonding in a glassy network. Thereby, in the context of the study of incorporation of Titanium oxide in silicate glass, a ^{17}O enriched glass was synthetized by sol-gel methods. ^{17}O MAS and MQMAS NMR spectra exhibit a peak at 250ppm confirming the presence of Si-O-Ti bonds, and then the incorporation of the Titanium in the glass network. Moreover, these spectra do not highlight signal at higher chemical shift value indicating the absence of Ti clustering and then the homogeneity of the glass at least at low Titanium content.

[P-74] THE SOLUTION STRUCTURE OF RECOMBINANT PVFP-5β REVEALS NEW INSIGHTS INTO MUSSEL ADHESION

M.A. Morando[1], F. Venturella[1], M. Sollazzo[1,2], E. Monaca[1], R. Sabbatella[1], V. Vetri[3], R. Passantino[4], A. Pastore[5], C. Alfano[1]

[1]Structural Biology and Biophysics Unit, Fondazione Ri.MED, Palermo 90133, Italy
[2] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo 90128, Italy
[3]Department of Physics and Chemistry - Emilio Segrè (DiFC), Università di Palermo, Palermo 90128, Italy
[4]Biophysics Institute, National Research Council, Palermo 90143, Italy
[5]European Synchrotron Radiation Facility, Ave des Martyrs, 38000 Grenoble, France

E-mail: mamorando@fondazionerimed.com

Keywords: solution NMR, biomolecules

In the last decade, the interest in the surface adhesion ability of some marine organisms, like mussels, has attracted large attention for the wide range of potential applications in biomaterials, biotechnology and medicine, including regeneration of damaged tissues. Mussels are able to firmly adhere to wet surface thanks to the byssus plaque, a protein-base appendage which can be considered as a versatile underwater adhesive between the mussels byssal threads and the marine surface. The formation of the byssus plaque involves several processes, among which the formation of coacervates is considered one of the most important [1-2]. Our research is focused on the adhesion ability of Pvfp-5β, one of the mussel foot proteins (mfps) from the Asian green mussel *Perna viridis*. Pvfp-5β is the first protein, among the mfps secreted, to engage contact with surface and have a role in the coacervation process[3]. In nature, Pvfp-5β is rich in Dopa residues which presence is considered fundamental for the coacervation phase and for the interaction with the surface[4].
In a previous work we measured cell viability and adhesion capacity of NIH-3T3 and HeLa cell lines in presence of no DOPA-modified Pvfp-5β, revealing that it has no cytotoxic effects and despite the lacking of DOPA residues, is also able to retain adhesion properties [5]. We then moved to the characterization of the structure and dynamics in solution of Pvfp-5β and demonstrated that the protein, considered intrinsically disordered, is well folded and in agreement with the presence in the sequence of two EGF motifs. The structure is highly rigid except for a few residues affected by slow local motions in the μs-ms time scale, and differs from the model calculated by artificial intelligence methods for the relative orientation of the EGF modules. Furthermore, we were able to analyze the self-assembly process of Pvfp-5β and prove that it is able to undergo simple coacervation at neutral or basic pH without the presence of DOPA residues, and pictured the coacervation process by molecular docking. Our results provide the foundations for gaining a better understanding of the structural determinants of the adhesion properties of mussel foot proteins.

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[P-75] ON-CELL NMR SCREENING OF BACTERIAL MULTIVALENT LIGANDS

L. Moretti,[a] A. Palmioli,[a] F. Rispoli,[b] C. A. Vezzoni,[b] L. Baldini,[b] F. Sansone,[b] A. Casnati,[b] C. Airoldi[a]

[a] BioOrgNMR Lab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milano, Italy
[b] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

E-mail: luca.moretti1@unimib.it

Keywords: solution NMR, small molecules, biomolecules.

Antimicrobial resistance (AMR) is one of the major threats of the 21st century [1]. Available antibiotics target essential pathways imposing selective pressure that favors resistance development, even across distant bacterial species. This leads to the spread of resistance even to last-resort antibiotics. Further, the broad-spectrum nature of most antibiotics has long lasting detrimental effects on the healthy human microbiota. In addition to the development of novel antibacterial molecules exploiting new mechanisms of action, this alarming scenario requires:
1) diagnostic tools for a fast identification of the class of the pathogen (Gram+, Gram-, mycobacteria), thus allowing for a timely selection of the most appropriate antibiotic class to treat the patient; 2) novel drugs disarming pathogenic bacteria by interfering with their virulence mechanisms. Virulence factors are molecules produced by bacteria enabling them to: a) colonize a niche in the host, b) evade the host's immune response, c) inhibit the host's immune response, or d) scavenge nutrients from the host. Compounds targeting virulence processes will impose less evolutionary pressure than standard antibiotics for the development of resistance, will supplement conventional antibiotics to increase efficacy and will have little or no impact on the host commensal flora. To this aim, we are developing new multivalent bacteria ligands, based on calixarene or dendrimer scaffolds, targeting specific molecular patterns of the different bacteria classes, such as the terminal part of peptidoglycan (D-ala-D-ala) and teichoic acids for Gram+ bacteria, LPS for Gram- bacteria, mycolic acid, glycolipids and trehalose transporter for mycobacteria. Moreover, for a specific pathogen targeting, the adhesin FimH located at the pili end of an uropathogenic strain of Escherichia coli can be targeted through the glycoside cluster effect of carbohydrate-lectin interactions [2]. An advanced approach for the screening of these bacteria ligands, based on on-cell STD NMR experiments, has been set up and allowed for the identification of the first promising hit compounds as selective bacteria ligands [3] and anti-virulence factors [4].

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[P-76] Characterization of the BELL domain by solution NMR and X-ray diffraction

Loïc Delcourte[a,b], Estelle Morvan[b], Mélanie Berbon[a,b], Corinne Sanchez[a,b], Amandine Jalbert[a,b], Fatjona Xhango[a,b] Thierry Dakhli[b], Brice Kauffmann[b], Virginie Coustou[c], Frédérique Ness[c], Corinne Clavé[c], Alexandra Granger-Farbos[c], Sven Saupe[c] and Antoine Loquet[a,b]

[a]Institute of Chemistry and Biology of Membranes and Nanoobjects, UMR5248, CNRS, University of Bordeaux, Bordeaux Polytechnic Institute.
[b]Institut Européen de Chimie et Biologie, UAR3033, CNRS, Université de Bordeaux, INSERM
[c]Institut de Biochimie et de Génétique Cellulaire, UMR 5095 CNRS, Université de Bordeaux

E-mail: estelle.morvan@u-bordeaux.fr

Keywords: solid state NMR, solution NMR, biomolecules

All cellular life relies on immune defense mechanisms to fight off viral, prokaryotic or eukaryotic pathogens. A remarkable trend currently emergences from the comparison of the molecular pathways controlling a range of immune responses across the entire tree of life. Many of these pathways involve formation of higher-order signaling complexes (hooded under the generic term of signalosomes) and many of these pathways converge on membrane-targeting terminal pore-forming execution proteins that induce regulated immune cell death. An extensive genome mining approach has recently revealed that such signalosome machineries exist in procaryotes and specifically in species with a multicellular organisation (Cyanobacteria, Actinobacteria and Chloroflexi).

Here we present the structural investigation of the putative execution domain of signalosomes found in Streptomyces olivochromogene (B2 BELL) and in Streptomyces coelicolor (B1 BELL). The domain is termed BELL for Bacteria domain analogous to HELL, in analogy to execution motifs (called HeLo) found in fungal systems. BELL was overexpressed in e. coli and purified to obtain high-resolution solution NMR data. Using a combination of production of ^{13}C and ^{15}N labeled samples and multidimensional NMR experiments, we carried out the sequential assignment of the B2 BELL protein including 94% of the backbone nuclei and 74% of side-chain resonances. BELL forms a globular domain that consists in 5 alpha-helices and 4 loops connected. In addition, we performed ^{1}H-^{15}N relaxation experiments to characterize the mobility of the domain.

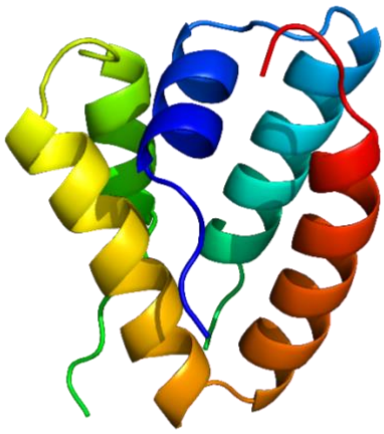

Fig. 1. Structure of B2 BELL domain by X-ray diffraction

[P-77] IN-SITU NMR & MRI CHARACTERIZATION OF PROTON EXCHANGE MEMBRANES FOR FUEL CELL

C. Mrad,[‡] J.C. Perrin,[‡] A. El Kaddouri,[‡] K. Mozet,[‡] C. Marty,[†] F. Micoud,[†] L. Guendouz,[‡] J. Dillet,[‡] O. Lottin,[‡]

[‡] Université de Lorraine, CNRS, LEMTA, F-54000 Nancy, France
[†]Université de Grenoble Alpes, CEA, LITEN, F-38054 Grenoble, France
E-mail : christine.mrad@univ-lorraine.fr

Keywords: MRI, materials, polymers, instrumentation.

While proton exchange membrane fuel cells (PEMFCs) are already well established in the industrial landscape, their large-scale deployment is still limited due to their high cost (expensive Pt catalyst) and their limited durability. This work* aims to better understand the limitations to the performance of PEMFCs due to heterogeneities in the distribution of the water produced at the cathode/membrane interface and low platinum loading of the electrodes.
The *in-situ* study of the water distribution in the thickness of the electrolyte membrane can be performed via NMR and MRI but the use of NMR techniques is usually limited by the complexity of the experimental setup which must allow the application of controlled humid gases on both sides of the thin polymeric membrane.

In the present paper we describe an experimental setup (Fig 1) that allows the generation of gases (air, hydrogen) at controlled humidity and flow rate. We demonstrate how the MRI method employed, based on the use of a surface coil, can be used to record the temporal evolution of important NMR parameters (water chemical shift, relaxation times, water profiles) and membrane properties (diffusion coefficient, thickness, water flux) when the membrane is exposed to cyclic humidity conditions at room temperature. We also present results on membranes on which platinum electrodes have been sprayed to discuss the influence of the electrode layer on the interfacial water transfer.

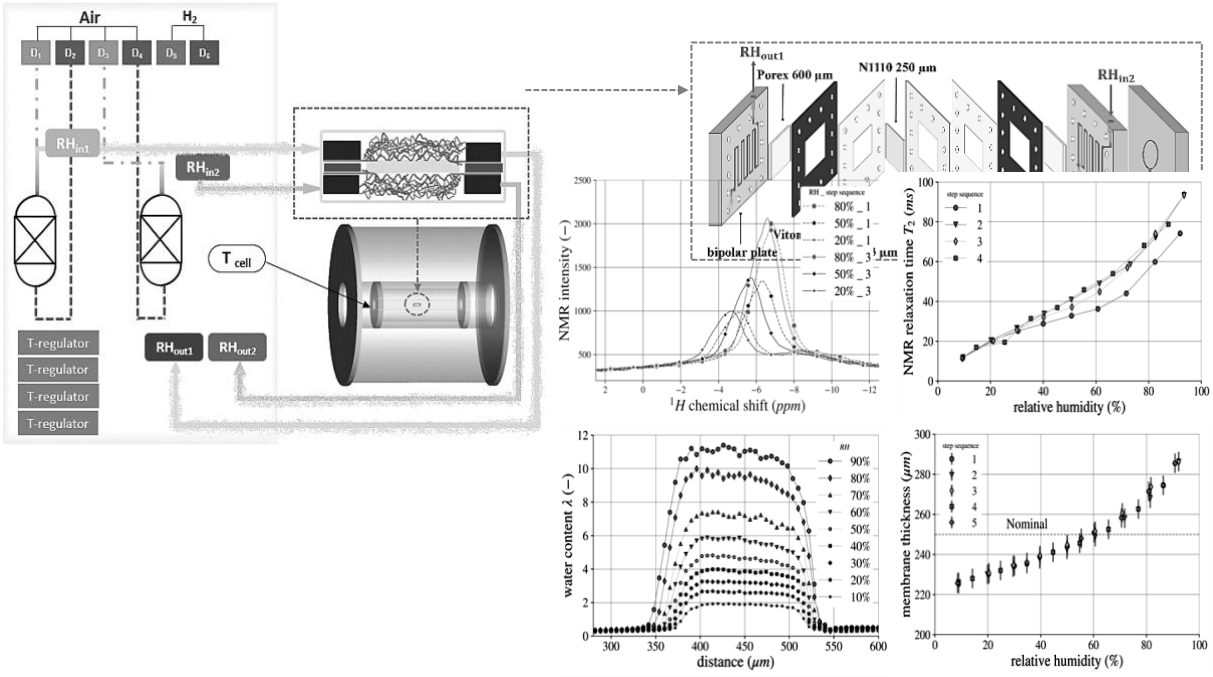

Fig 1. The experimental setup and the variation of the membrane's NMR parameters.

*This work has received funding from the European Union's EIT Raw Materials project n°19247, ALPE: "Advanced Low-Platinum hierarchical Electrocatalysts for low-T fuel cells".

[P-78] NEW SECONDARY METABOLITES IN THE AMPHINOMID FIREWORM *HERMODICE CARUNCULATA*

A. Mucci,[‡] L. Forti,[†] R. Simonini,[$] V. Ferrari, D. Prevedelli,[$] S. Righi[$]

[‡] Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy
[†] Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 213/D, 41125 Modena, Italy
[$] Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy
E-mail: adele.mucci@unimore.it

Keywords: solution NMR, small molecules, biomolecules.

Eight betaine-derived novel compounds were found in extracts of the Mediterranean stinging fireworm *Hermodice carunculata*. The identification of their structures relies on 1D and 2D NMR and HPLC-ESI/HRMS spectra (Fig. 1). Two types of terminal amine portions and a series of different alkyl chains were identified. Their matching provides the structures of uncharacterized secondary metabolites, named "carunculines", and their related isomers. These molecules differ from already known trimethylammonium inflammatory compounds (i.e. "complanines") isolated from another amphinomid species, for the structures of the terminal ammonium groups [1]. Carunculine anatomical distribution within *H. carunculata* was assessed by screening through HPLC-ESI/HRMS: their occurrence was revealed in all the body parts analyzed, both involved in predator-prey interactions [2], and mainly in the digestive apparatus. The results achieved reveal an array of different novel compounds from a chemically unknown species, improving knowledge on Marine Animal Products with chemical and biological potential for bioprospection. Overall, these data reinforce the necessity of studying poorly-investigated taxa to expand knowledge on animal venom biology, their mechanisms of action and exploitation as promising source of drug molecules.

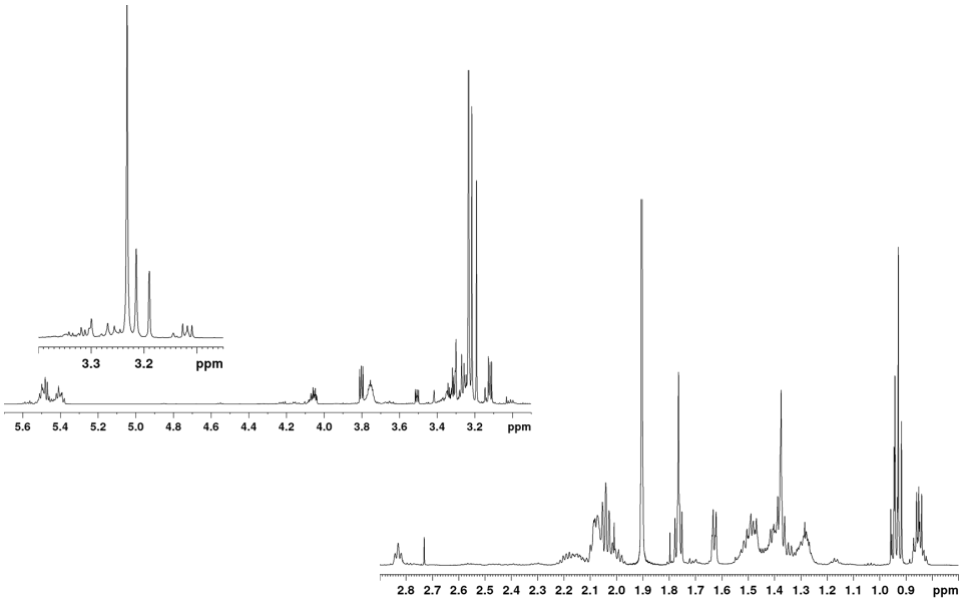

Fig. 1. ^{1}H NMR spectrum of carunculines from *Hermodice carunculata*.

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[P-79] Combinatorial readout of unmodified H3K4 and three-methylated H3K27 by the PHDvC5HCH tandem domain of NSD2

Andrea Berardi[‡], Federico Ballabio[‡], Michela Ghitti[‡], Giacomo Quilici, Charlotte Kaestner[†], Jonathan D. Licht[†], Giovanna Musco[‡]

[‡]Biomolecular NMR c/o Ospedale San Raffaele Via Olgettina 58 20132 Milano
[†]University of Florida
E-mail: musco.giovanna@hsr.it

Keywords: solution NMR, biomolecules

Overexpression of the histone methyltransferase NSD2 (also known as Multiple Myeloma SET domain containing protein) in multiple myeloma (MM) patients is one of the driving factors in the pathogenesis of this subtype of myeloma [1]. NSD2 is a multidomain protein whose uncontrolled overexpression results in de-repression of normally silenced oncogenes, herewith triggering tumorigenesis pathways [2]. Experimental evidences suggest that NSD2 transcriptional activity also depends on its non-catalytic domains, supposed to be responsible of the recruitment on chromatin via histone H3 binding. The molecular mechanisms through which these domains interact with histone H3 are still unknown. Here by NMR and ITC experiments we show that the tandem PHD domain of NSD2 ($PHDvC5HCH_{NSD2}$) preferentially recognizes histone H3K27me3 by aromatic caging. To the best of our knowledge this is the first PHD tandem cassette able to decode the methylation status of H3K27. We present here a three-dimensional model of the complex based on NMR data and molecular dynamics simulations. Importantly, $PHDvC5HCH_{NSD2}$ mutants designed to impair binding to histone H3 tail, in vivo reduce the adhesion ability of MM cells, thus suggesting a role for this domain in the regulation of cell adhesion genes.

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[P-80] PERYLENE POLYPHENYLMETHILSILOXANES: EFFECTS OF DYE CONTENT AS STUDIED BY SOLID-STATE NMR

F. Nardelli,[‡] E. Della Latta,[‡] F. Martini, [‡,†] Andrea Pucci,[‡] Guido Kickelbick,[ǂ] Svenja Pohl,[ǂ] Marco Geppi[‡,†]

[‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Giuseppe Moruzzi 13, 54126 Pisa, Italy
[†]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), Lungarno Pacinotti 43, 56126 Pisa, Italy
[ǂ]Inorganic Solid-State Chemistry, Saarland University, Campus, Building C4 1, 66123 Saarbrücken, Germany
E-mail: francesca.nardelli@dcci.unipi.it

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

Organic dyes represent one of the most desirable systems for the substitution of rare earth ions in light emitting diodes (LEDs). Furthermore, organic dye-based conversion materials are also gaining interest for the development of materials for photovoltaics, such as Luminescent Solar Concentrators (LSCs). Although these materials have a limited stability for optoelectonic applications, their decomposition processes can be minimized if they are embedded in polymeric matrices. In particular, the encapsulation in polysiloxane matrices is particularly attractive both for LEDs and LSCs applications, thanks to several properties of these polymers, including their chemical inertness, thermal stability, high transparency, and tunable refractive index by side group substitution [1,2].
In this work, we have characterized perylene-polyphenylmethylsiloxane systems containing different amounts of a perylene diimide-based dye by means of solid-state NMR spectroscopy, in order to unveil the effects of the dye content on the structure and dynamics of the polymeric matrix. In particular, a perylene diimide dye containing N,N'-diallyl substituents was previously cross-linked to a methylhydrosiloxane-phenylmethylsiloxane copolymer containing terminal and pendant Si-H group by Pt-catalysed hydrosilylation; then, the prepared perylene-polymer system was covalently integrated into a two-component polysiloxane resin containing Si-vinyl and Si-H groups, also in this case by Pt-catalysed hydrosilylation [3]. One-dimensional ^{1}H, ^{29}Si and ^{13}C Direct Excitation Magic Angle Spinning (DE-MAS) and Cross-Polarization (CP)-MAS high-resolution NMR spectra and two-dimensional ^{1}H-^{29}Si HETeronuclear CORrelation (HETCOR) experiments were acquired in order to obtain structural information and to monitor the degree of cross-linking of each sample. Furthermore, low-resolution NMR experiments were applied to measure ^{1}H transverse relaxation times (T_2), which allowed us to gain detailed information about the molecular mobility of each system.
A synergic analysis of high- and low-resolution experiments revealed a clear trend of the molecular mobility of the system in dependence of the cross-linking degree.

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[P-81] STUDY OF THE MISCIBILITY OF BIOCOMPATIBLE PLASTICIZERS IN ELASTOMERIC MATERIALS BY TIME-DOMAIN NMR TECHNIQUES

F. Nerli[1], M. Pierigé[1], F. Nardelli[1], L. Calucci[2,3], F. Martini[1,3], M. Cettolin[4], M. Geppi[1,3]

[1]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, 56124, Pisa, Italy
[2]Istituto di Chimica dei Composti Organo Metallici, Consiglio Nazionale delle Ricerche, 56124, Pisa, Italy
[3]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), 56126, Pisa, Italy
[4]Pirelli Tyre SpA, 20126 Milano, Italy
E-mail: nerli.francesca@gmail.com

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

Growing environmental and health concerns have led to a considerable attention to the formulation of sustainable elastomer blends, to be used in the tire industry. For this purpose, one aim is to replace petroleum-based resins with renewable ones, without altering the processability and the mechanical features of the final product [1]. To accomplish this task, it is important to understand what happens at the molecular level between polymer and resin in a blend and to relate this with its macroscopic behavior.
In this context, Time-Domain Nuclear Magnetic Resonance (TD-NMR) spectroscopy represents a powerful tool to characterize the molecular dynamics of polymer and resin in a vulcanizate, as well as their miscibility, as it allows to measure parameters which depend on the modulation of ^{1}H-^{1}H homonuclear dipolar interactions by molecular motions [2]. Indeed, ^{1}H T_2 relaxation times can be correlated with the dynamic behavior of protons in different molecular environments [3]. Moreover, ^{1}H-^{1}H residual dipolar couplings (D_{res}) can be exploited to evaluate the cross-link density, as they depend on the amount of topological constraints, such as entanglements and cross-links [4]. On the other side, ^{1}H T_1 relaxation times allow to assess the mixing degree of different phases in the sample: in fact, in multiphase systems with domain sizes lower than ~10 nm, the T_1's of protons in different dynamic environments tend to be averaged to a single value, due to the spin diffusion phenomenon.
In this work, several TD-NMR experiments were performed at different temperatures on vulcanized and non-vulcanized styrene-butadiene rubber samples, in the presence of petroleum-based or natural resins. In particular, Magic Sandwich Echo and Carr-Purcell-Meiboom-Gill experiments were acquired to measure ^{1}H T_2, while Inversion Recovery experiments were exploited to measure ^{1}H T_1, thus allowing to evaluate the molecular mobility of the polymeric and resin phases, as well as their miscibility. Moreover, Double-Quantum NMR experiments were acquired for the measurement of ^{1}H-^{1}H D_{res} to monitor the cross-link density in all vulcanized samples.

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[P-82] Efficient Polarizing Matrices for DNP MAS NMR at High Magnetic Fields

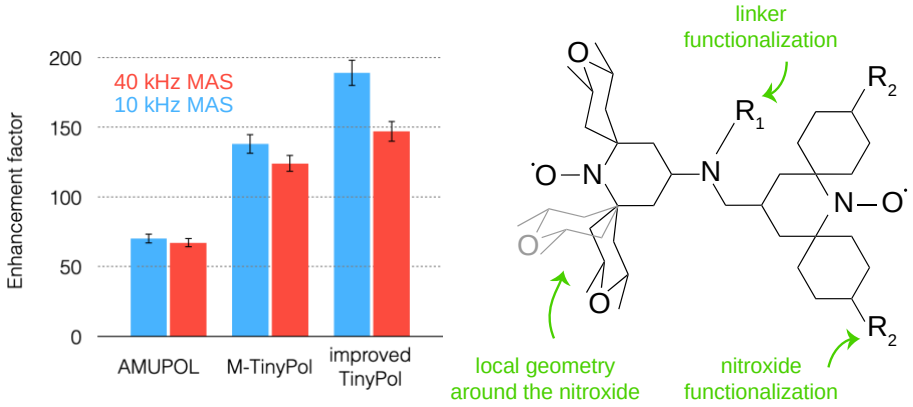
Lorenzo Niccoli (1)(2), Georges Menzildjian (1), Gilles Casano (3), Maxim Yulikov (4), Gunnar Jeschke (4), Lyndon Emsley (5), David Gajan (1), Olivier Ouari (3), Moreno Lelli (2) and Anne Lesage (1)

Author affiliations :
(1) Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCBL), 69100 Villeurbanne, France; (2) Center of Magnetic Resonance (CERM), University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy; (3) Aix Marseille Univ, CNRS, ICR, Marseille, France ; (4) Department of Chemistry and Applied Biosciences, Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich, Switzerland; (5) Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

E-mail: lorenzo.niccoli@unifi.it

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

Dynamic Nuclear Polarization (DNP) at cryogenic temperatures has proved to be a valuable technique to enhance the sensitivity of solid-state NMR spectroscopy. Over the years, sample formulations have been optimized for experiments at cryogenic temperatures. At 9.4 T, the best performing polarizing agents are dinitroxides such as AMUPol and TEKPol that lead to enhancement factors of around 250 at 100 K. However, the performance of these radicals plummets at high fields, fast MAS and/or higher temperatures.
Our group recently introduced biradicals, named TinyPols (1), that are especially suited for DNP MAS NMR at high magnetic field and fast MAS frequencies, and for the application of this spectroscopy to the structural investigation of biomolecular assemblies (2-3). Here, we will review the advances that we recently made to fine-tune the local geometry of these biradicals and improve their DNP performance in a variety of experimental conditions. We will also discuss the parameters that lead to a decrease in the efficiency of biradicals at elevated temperatures.



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[P-83] INHIBITION OF FGFR SIGNALLING BY TARGETING FGF/FGFR EXTRACELLULAR INTERACTIONS: TOWARDS THE COMPREHENSION OF THE MOLECULAR MECHANISM THROUGH NMR APPROACHES

K. Pagano,[‡] Elisa Longhi,[†] H. Molinari,[‡] G. Taraboletti,[†] L. Ragona[‡]
[‡]Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (SCITEC), via Corti 12, Milano, Italy
[†]Laboratory of Tumour Microenvironment, Department of Oncology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy
E-mail: katiuscia.pagano@scitec.cnr.it

Keywords: solution NMR, biomolecules, theory and methods.

Fibroblast growth factor (FGF2)/fibroblast growth factor receptor (FGFR) signalling is involved in several pathologies, including cancer development, metastasis formation and resistance to therapy, angiogenesis driven pathologies, vascular diseases, and viral infections. The development of small molecules, acting extracellularly to target FGF2/FGFR interactions, has the advantage of limiting the adverse effects associated with current intracellular FGFR inhibitors. We identified a few leads, either targeting FGF2 [1, 2] or FGFR-D2 domain [3], able to destabilize the FGF2/FGFR complex and inhibiting the subsequent signalling cascade.
With the aim to move a step forward on the understanding of the molecular mechanisms underpinning the inhibitory activity of small molecules acting on the extracellular portion of the FGFR/FGF2 system, and based on biological assays reported in the literature, we have built a small library of natural and synthetic molecules (Figure 1) potentially acting as inhibitors of FGF2/FGFR interactions.

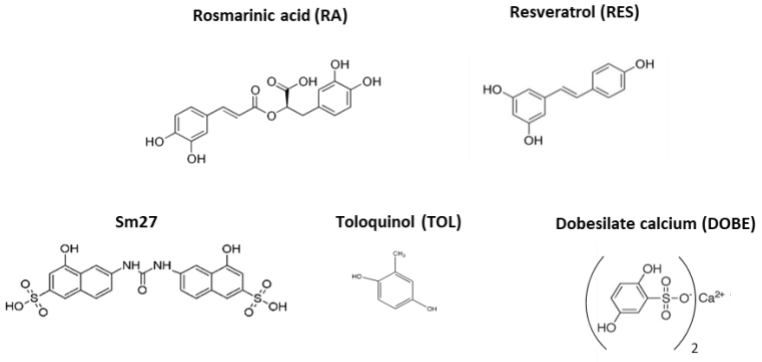

Fig. 1. Library of potential inhibitors of FGF2/FGFR interactions

Through a variety of NMR approaches (DOSY, ^{1}H-^{15}N CSP and intensity perturbations) we provided a comparative analysis of the interactions of the small molecules with FGF2/FGFR-D2 complex and the single protein domains, assessing the capability of the small molecules to interact with the extracellular portion of the receptor and highlighting the preferential binding for one of the analyzed proteins. The efficacy of the selected molecules in destabilizing the FGF2/FGFR complex, assessed by diffusion experiments, together with cell-based functional assays, allowed for the identification of two different mechanisms of inhibition of FGF2 induced cell proliferation [4].

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[P-84] A MICROFLUIDIC PHOTO-INDUCED PLATFORM TO SYNTHESIZE ULTRASMALL GLYCO GOLD NANOPARTICLES

K. Pagano,[#] P. Perez Schmidt,[‡] C. Evangelisti,[†] L. Ragona,[#] M. Marelli,[‡] L. Polito[‡]

[#]National Research Council, CNR-SCITEC, Via A. Corti 12, 20133 Milano, Italy
[‡]National Research Council, CNR-SCITEC, Via G. Fantoli 16/15, 20138 Milano, Italy
[†]National Research Council, CNR-ICCOM, Via G. Moruzzi 1,56124, Pisa, Italy

Keywords: solution NMR, small molecules, nanomaterials, biomolecules

Ultra-small gold nanoparticles (UAuNPs) are a class of gold nanoparticles with a diameter smaller than 5 nm, extremely interesting for their peculiar properties which can be exploited for bio application and nanomedicine. [1,2] In particular UAuNPs are luminescent and have longer circulation time, improved biodistribution, better tissue penetration and clearance pathways, with respect to their bigger counterpart. UAuNPs engineered with glycans (Glyco-UAuNPs) have emerged as excellent platforms for many applications.[3] In fact, the displacement of multiple copies of glycans mimic the multivalent glycoside clusters effect which can overcome the low affinity of the individual ligands towards their receptors. However, to fulfill the potentiality of these engineered UAuNPs, robust protocols for their synthesis, functionalization and characterization are needed. Herein, we propose a straightforward synthesis of a small library of Glyco-UAuNPs based on reliable microfluidic approaches.[4,5], coupled with a photo-induced reduction avoiding the use of any further chemical reductant, templating agent or co-solvent. Glyco-UAuNPs have been fully characterized by UV/vis, ICP-OES, HRTEM and XPS. The successful binding to the NP surface was confirmed by NMR and quantitative ^{1}H NMR spectroscopy allowed to estimate the loading of glycans on each NP. Diffusion coefficients, as measured through NMR DOSY experiments, afforded a clear picture of the hydrodynamic radius of the newly synthesized ultra-small gold nanoparticles [6].

Acknowledgements: KP and LR acknowledge financial support of Fondazione Antonio De Marco.

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[P-85] A PORTABLE AND UNILATERAL NMR DEVICE TO MEASURE TREE WATER CONTENT AND LOCATE CONDUCTIVE TISSUES

S. Blystone, ‡† G. Pagès, ‡ H. Cochard †, P. Conchon †

‡INRAE, UR QuaPA, F-63122 Saint-Genès Champanelle, France
‡INRAE, PROBE research infrastructure, AgroResonance facility, F-63122 Saint-Genès-Champanelle, France
†Université Clermont Auvergne, INRAE, PIAF, 63000 Clermont-Ferrand, France
E-mail : shannan.blystone@inrae.fr

Keywords: low field NMR, MRI, instrumentation, plants, trees

The use of NMR technology in the plant sciences has traditionally been limited due to the immobility of the devices, and restrictions with regard to sample size and shape. To overcome these limitations and to be able to study plants directly in their natural environment, we evaluated the NMR capacities of a portable, unilateral magnet: The Nuclear Magnetic Resonance Mobile Universal Surface Explorer (NMR-MOUSE), designed by Blümich *et al*. [1]. This device permits measuring the NMR signal in increments of up to 100-micrometers, and within a depth of approximately 25-millimeters. We tested the capacity of this device to measure tree water content, and its variation over time, by following the dehydration dynamic of cut branches from six different species and two different functional types. There was a significant linear correlation between the integral of the NMR profiles obtained and the water content of the branches. This significant correlation was present regardless of tree species or functional type. We were also able to visualize the dehydration dynamic of individual branches, over time, through the NMR profiles. We then tested the capacity of the device to differentiate the conductive tissues, i.e. the xylem and phloem fluxes, within conifer branches. The NMR profiles of the branches presented distinct peaks which corresponded to the xylem and phloem tissues, whose location was validated with x-ray microtomography imaging which allows the high-resolution visualization of the tissues within the sample (see Fig. 1). In conclusion, the NMR-MOUSE is a promising candidate for measuring plant water dynamics in the field. Future work will test the capacity of this device to measure tree water content *in-situ*, and to measure the speed of both the xylem and phloem fluxes.

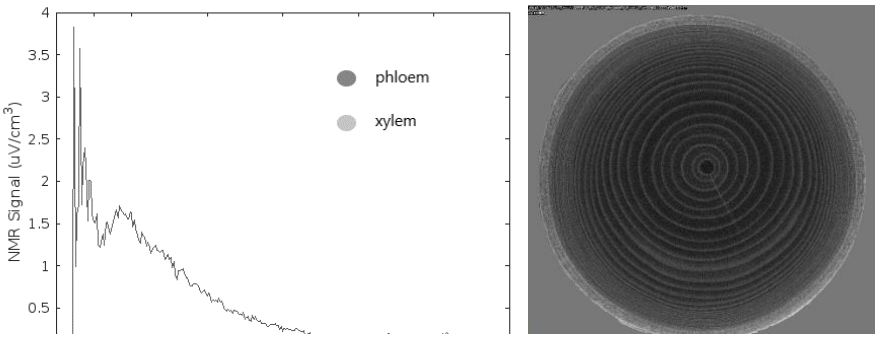

Fig. 1. The graph presents the NMR profile of a silver fir branch, showing the distribution of water within the branch. The peaks correspond to the xylem and phloem tissues. The microtomography image shows the cross-section of the branch, enabling us to precisely locate the depth of the xylem and phloem tissues.

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[P-86] NMR-DRIVEN IDENTIFICATION OF CINNAMON BUD AND BARK COMPONENTS WITH ANTI-Aβ ACTIVITY

A. Palmioli,[a,b] C. Ciaramelli,[a,b] I. Angotti,[a] L. Colombo,[c] A. De Luigi,[c] G. Sala,[b] M. Salmona,[c] C. Airoldi,[a,b]

[a] BioOrgNMR Lab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milano, Italy
[b] Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, P.zza dell'Ateneo Nuovo 1,20126 Milano, Italy
[c] Department of Molecular Biochemistry and Pharmacology, - Istituto di Ricerche Farmacologiche "Mario Negri"-IRCCS, Via G. La Masa 19, 20156 Milano, Italy
[d] School of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900 Monza

E-mail: alessandro.palmioli@unimib.it

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

The anti-Alzheimer disease (A.D.) activity reported for an aqueous cinnamon bark extract prompted us to investigate and compare the anti-amyloidogenic properties of cinnamon extracts obtained from both bark and bud, the latter being a very little explored matrix.
We prepared the extracts with different procedures (alcoholic, hydroalcoholic, or aqueous extractions). An efficient protocol for the rapid analysis of NMR spectra of cinnamon bud and bark extracts was set up, enabling the automatic identification and quantification of metabolites. Moreover, we exploited preparative reversed-phase (RP) chromatography to prepare fractions enriched in polyphenols, further characterized by UPLC-HR-MS.
Then, we combined NMR-based molecular recognition studies, atomic force microscopy, in vitro biochemical and cellular assays to investigate the anti-amyloidogenic activity of our extracts. Both bud and bark extracts showed a potent anti-amyloidogenic activity, flavanols, particularly procyanidins, and cinnamaldehydes, the chemical components of cinnamon hindering Aβ peptide on-pathway aggregation and toxicity in a human neuroblastoma SH-SY5Y cell line. Together with the previously reported ability to hinder tau aggregation and filament formation, these data indicate cinnamon polyphenols as natural products possessing multitarget anti-AD activity. Since cinnamon is a spice increasingly present in the human diet, our results support its use to prepare nutraceuticals useful in preventing A.D. through an active contrast to the biochemical processes that underlie the onset of this disease. Moreover, the structures of cinnamon components responsible for cinnamon anti-AD activities represent molecular templates for designing and synthesizing new anti-amyloidogenic drugs.[1]

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[P-87] INSIGHTS INTO THE BINDING OF GLYCOL-SPLIT HEPARIN OLIGOSACCHARIDES TO HEPARANASE BY NMR AND COMPUTATIONAL STUDIES

Michela Parafioriti[1], Stefano Elli[1], Minghong Ni[1], Maurice Petitou[1], Vito Ferro[2], Israel Vlodavsky[3], Annamaria Naggi[1], Marco Guerrini[1]

[1]Istituto di Ricerche Chimiche e Biochimiche "G. Ronzoni", Via Giuseppe Colombo 81, 20133, Milano, Italia
[2]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia
[3]Cancer and Vascular Biology Research Center, The Bruce Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel
E-mail: parafioriti@ronzoni.it

Keywords: heparanase, heparan sulfate, glycol-split heparin, NMR.

Heparanase (HPSE) is an endo-β-D-glucoronidase that cleaves heparan sulfate (HS) chains of HS proteoglycans, located on the cell surface and in the extracellular matrix. The enzyme degrades HS in both physiological conditions (HS turnover) and pathological processes in which it is often overexpressed (inflammation, metastasis and angiogenesis) [1].
The development of HPSE inhibitors is a promising strategy to face the upregulation of the HPSE enzymatic activity. Various strategies have been applied, such as sulfated polysaccharides (i.e. heparin and HS mimetics) and small molecules [2, 3, 4]. Glycol-split heparins, characterized by a significant reduction of the anticoagulant activity, were found encouraging in the HPSE inhibition [5, 6]. Moreover, the crystal structure of human HPSE in *apo* and ligand-bound forms has revealed insights into the substrate recognition providing a starting point for the design of novel inhibitors of HPSE [7]. Nevertheless, the key features, that allow HPSE to distinguish the sequence variability of HS, remain poorly understood [1].
Our study is focused on the molecular mechanism by which HPSE binds glycol-split heparins. Glycol-split heparin oligosaccharide models were synthesized and their interaction with HPSE was investigated by Saturation Transfer Difference (STD) NMR experiments and molecular modelling [8]. The epitope map of these compounds in the bound state with HPSE was detected revealing structural details of the interaction and a possible correlation with their anti-HPSE activity.

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[P-88] FAST FIELD CYCLING NMR AS A QUALITY CONTROL TOOL IN DAIRY PRODUCTS

M. Pasin,[1] R. Anedda,[2] E. Curti,[2] G. Ferrante,[1] D. Kruk,[3] P.J. Sebastião[4]

[1]Stelar srl, Mede (PV), Italy
[2]Porto Conte Ricerche s.r.l., Alghero (SS), Italy
[3]Department of Physics and Biophysics, University of Warmia & Mazury, Olsztyn, Poland
[4]Centro de Física e Engenharia de Materiais Avançados, Departamento de Física, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
E-mail: pasin@stelar.it

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

A deeper definition of structural and dynamical changes of milk and derived products following industrial dairy processes would certainly take advantage of NMR relaxometry.
Milk pasteurization, homogenization, fermentation, pH changes, renneting, curd shrinkage and syneresis, curd heating and cheese ripening are just some of the industrial processes that play a role in defining quality features of commercial dairy products. We have collected scientific evidence on the sensitivity of NMR relaxometry to several of the abovementioned treatments. As an example, this study aims at proposing Fast Field Cycling Nuclear Magnetic Resonance (FFC-NMR) [1,2] for quality evaluation of an Italian PDO cheese of which the market offer includes artisanal and industrial products with very different characteristics. FFC-NMR is able to discriminate the production chain and also potential fraudulent products which do not comply with the official cheesemaking protocol. Six cheeses (3 artisanal and 3 industrial) were measured using an FFC-NMR relaxometer in the range 0.01-10 MHz at 10°C and 20°C (room temperature). For each sample, 30 different field points were acquired. One high field T_1 measurement (500MHz) has been acquired too (Fig.1).

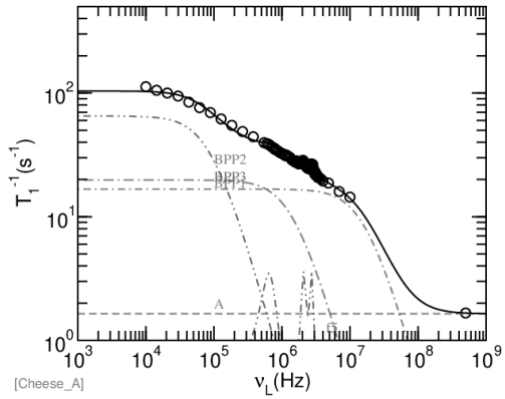

Fig. 1. Fitting of the NMRD profile of a cheese sample using Fitteia software.

NMRD profiles allowed to clearly discriminate between the PDO cheeses, especially at lower frequencies. The parameters obtained by fitting the NMRD profiles with an appropriate model [3] highlighted differences between the two cheese groups. This work confirms the capability of FFC-NMR to discriminate between dairy products produced with different processes and suggests that FFC-NMR could be used as a quality control method for the dairy industry.

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[P-89] FAST FIELD CYCLING APPLIED TO POLYMERS: TACTICITY AND MOLECULAR WEIGHT

M. Pasin,[1] G. Ferrante[2]

[1]Stelar srl, Mede (PV), Italy
E-mail: pasin@stelar.it

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

In this study, we show the power of the Fast Field Cycling [1,2] NMR relaxometry (FFC) technique by addressing one of the key properties of polymers, that is tacticity, through a case study on the most widely used and well-known industrial polymer: polypropylene. "Tacticity" of a polymer refers to the relative stereochemistry of adjacent chiral centres within the macromolecule (polymer). This has important implications on its physical and mechanical properties as the regularity of the polymer structure will influence its degree of rigidity (crystalline long-range order) and flexibility (amorphous long-range disorder), as well as its melting point and solubility. In this study, we investigate the potential of the FFC technique to discriminate commercially available isotactic polypropylene (PP) from atactic PP. We considered the following samples: isotactic PP with molecular weight 250K, atactic PP (amorphous) and a mixture of two PP's composed of 20% amorphous and 80% isotactic PP's melted together at over 260°C.

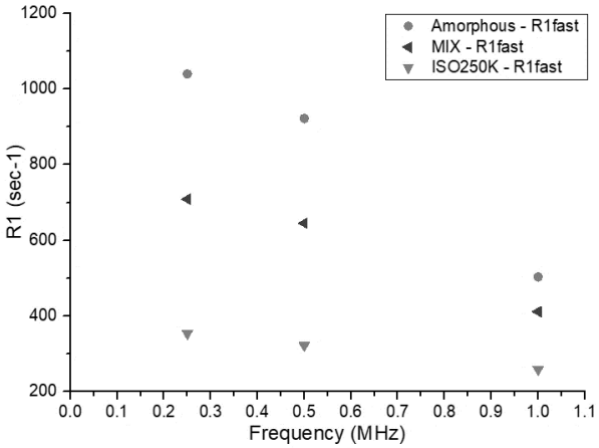

Fig.1: NMRD profiles for the fast-relaxing longitudinal relaxation component of the samples.

Results (Fig.1) show the capability of the FFC technique to distinguish an isotactic polymer from both an atactic and a 20% adulterated mixture of isotactic polymer at room temperature (25°C). It has been proved that important information can be revealed only at low magnetic fields and it has been confirmed that FFC can provide a unique fingerprint for a polymer. Polymer characterization could be developed to become a standard analytical tool and FFC could provide a quality control method for the polymer and plastic industries, with the clear advantage that FFC is a non-destructive method and there is no need to heat the sample to carry out the analysis and no requirement for deuterated solvents.

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[P-90] A NOVEL FAST FIELD CYCLING APPROACH TO OBTAIN FIELD DEPENDENT T_1-T_2 2D MAPS

M. Pasin,[1] V. Bortolotti,[2] P. Conte,[3] A. Nagmutdinova, [2] L. Brizi,[4] G. Landi,[5] P. Lo Meo,[6] D.F. Chillura Martino,[6] G. Ferrante[1]

[1]Stelar srl, Mede (PV), Italy
[2]University of Bologna, DICAM, Bologna, Italy
[3]University of Palermo, Department of Agriculture, Food, and Forestry Science (SAAF), Palermo, Italy
[4]University of Bologna, DIFA, Bologna (Italy)
[5]University of Bologna, Mathematical Department, Bologna (Italy)
[6]University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Palermo (Italy)
E-mail: pasin@stelar.it

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

Fast field cycling (FFC) NMR relaxometry [1] is a very valuable tool for studying molecular dynamics of many different chemical systems such as soils, food, environment, organic systems and a number of different porous materials [2]. Up to now FFC NMR relaxometry has been used to describe the dependence of longitudinal relaxation time T_1 from the relaxation field. So far, no information has never been obtained on the behavior of the transversal relaxation time T_2 as affected by the modulation of T_1 when the proton Larmor frequency of the applied magnetic field is changed. In the past, the main reason for such a lack of information was due to some hardware limitations. However, some recent developments of the electronics, allow us to produce FFC NMR instruments which can be used also to measure T_2 relaxation values.

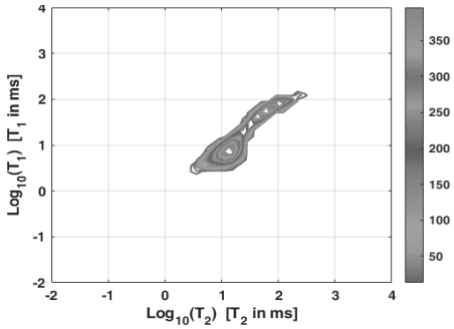

Fig. 1. T_1-T_2 map acquired at the acquisition field of 7.2 MHz and at the relaxation field of 0.07 MHz.

In the present study, an attempt to monitor the behaviour of T_2 as affected by T_1 modulation at different magnetic fields was given by computing the experimental data with a new robust 2D NMR inversion algorithm that uses a locally adapted multi-penalty regolarization approach [3].
This study highlights a novel method for obtaining 2D maps at different magnetic fields (Fig.1) and shows the usefulness of these maps for discriminating between different motion components in only a few shots.

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[P-91] MRI-BASED PREDICTION OF THERAPEUTIC OUTCOMES OF US-BOOSTED REDUCED-DOSE CHEMOTHERAPY

D. Patrucco, C. Furlan, F. Garello, E. Terreno

University of Turin, Department of Molecular Biotechnology and Health Sciences, via Nizza 52, 10121, Italy

E-mail: deyssy.patrucco@unito.it

Keywords: **MRI**, **contrast agents**

The adverse effect of chemotherapeutics is one of the major limits in the treatment successful. Recently, ultrasound (US) in combination with nanomedicines has been shown to have great potential to enhance the cancer treatment [1]. The aim of this study is to develop a MRI-based protocol to predict the therapeutic effect of chemotherapy based on sonosensible theranostic liposomes (STL) loaded with a reduced dose of doxorubicin and an MRI contrast agent.
Xenograft mouse model of human ovarian A2780 cancer cells was enrolled. After STL administration the tumor uptake was improved through two US stimuli i) sonoporation, the temporally permeabilization of tumor cell membrane ii) release from liposomes of their content. The US insonation of the tumor mass caused a T_1-contrast enhancement in the tumour and in the kidney calix, confirming the effective intratumor release of Gadoteridol and drug from the liposomes and internalization in the tumor. Indeed, there is a correlation between the contrast enhancement and the tumor volume at the end point (Fig.1). It's noteworthy that in mice treated with US and liposomes loaded with Doxorubicin 2.5 mg/kg the tumor growth was significantly reduced compared both the same treatment without ultrasound and treatment performed with a liposome administrated 5 mg/kg. These results suggest that it could be possible lower the Doxorubicin dose and obtain the same therapeutic outcome of standard treatment.

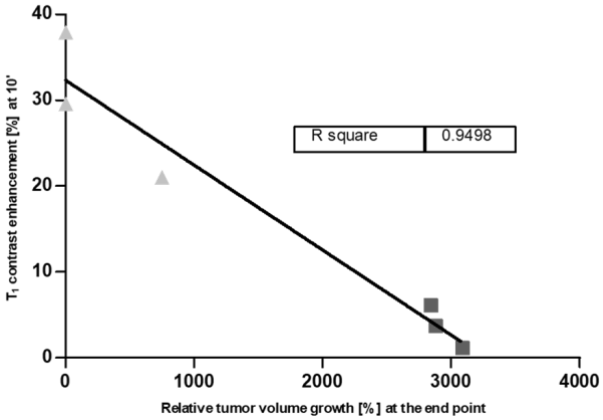

Fig. 1. Correlation between the contrast enhancement and the tumor volume at the end point (light grey triangle: tumor of mice treated with STL and US; dark grey triangle: tumor of mice treated with STL.).

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[P-92] PROFILING METABOLITES AND LIPOPROTEINS IN COMETA, AN ITALIAN COHORT OF COVID-19 PATIENTS

Valentina Pecchioli[a,b], *Veronica Ghini*[a,b], *Paola Turano*[a,b]

[a] CERM, University of Florence, via Luigi Sacconi 6, 50019, Sesto Fioretino, Italy;
[b] Department of Chemistry, University of Florence, via della Lastruccia 3, 50019, Sesto Fiorentino, Italy.

E-mail: valentina.pecchioli@stud.unifi.it
Keywords: solution NMR, small molecules, metabolomics.

This work arises from the COMETA project (funded by Regione Toscana) which is aimed at the metabolomic characterization of the biochemical alterations induced by COVID-19 disease [1]. In this framework, ^{1}H NMR spectra were acquired on the EDTA-plasma samples from 510 hospitalized COVID-19 positive patients infected with the different variants of the virus (360 wt/α/β, 106 δ, 44 o) and with various disease severity; among all the patients, 76 were previously vaccinated. These spectra were compared with those of 95 recovered subjects sampled after 3-6 months from SARS-CoV-2 eradication. The whole NMR spectrum, which contains the metabolome and lipoproteome information, has a high discriminatory power between the two groups, with an accuracy higher than 90%. The COVID-19 metabolic fingerprint was independent from the different variants as well as from the vaccination status of the subjects.
The differences between COVID-19 positive patients and recovered subjects originate from significant alterations in the concentrations of 15 metabolites and 74 lipoprotein components. Characteristic trends in metabolite levels are observed as a function of the disease severity. The metabolites found altered in COVID-19 patients with respect to recovered individuals coincide with the acute infection biomarkers identified in the comparison with healthy subjects [2], indicating the substantial metabolic healing of recovered COVID-19 subjects.

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

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[P-93] INFLUENCE OF SULFUR-CURING CONDITIONS ON THE DYNAMICS AND CROSSLINKING OF RUBBER NETWORKS: A TIME-DOMAIN NMR STUDY

M. Pierigé,[‡] F. Nardelli,[‡] F. Nerli,[‡] L. Calucci,[†,§] E. Carignani,[†,§] S. Borsacchi,[†,§] M. Cettolin,[*] M. Arimondi,[*] L. Giannini,[*] F. Martini,[‡,§] M. Geppi[‡,§]

[‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, 56124 Pisa, Italy
[†]Istituto di Chimica dei Composti Organo Metallici, Consiglio Nazionale delle Ricerche, 56124 Pisa, Italy
[§]Centro per l'Integrazione della Strumentazione Scientifica dell'Università di Pisa (CISUP), 56126 Pisa, Italy
[*]Pirelli Tyre SpA, 20126 Milano, Italy
E-mail: michele.pierige@phd.unipi.it

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

Elastomers are polymeric materials extensively used in the tire industry. These materials are obtained by vulcanization of one (or more) polydiene polymer(s) in the presence of sulfur and other additives (accelerators, activators, plasticizers, fillers, *etc*.). During this process, chemical crosslinks are formed between the polymeric chains, providing the final product's elasticity and durability. Depending on the formulation and the vulcanization conditions, other mechanical properties can be obtained. Importantly, such properties are strongly related to the microscopic structure of the polymeric network [1]. Consequently, the investigation of microscopic and macroscopic properties giving access to information on the network structure in relation to the vulcanization conditions is fundamental for the design of optimized elastomeric materials.
In this context, ^{1}H time-domain NMR (TD-NMR) represents a valuable tool to gain insights into the molecular dynamics of the polymeric chains. This technique allows to measure NMR observables (^{1}H T_1 and T_2 relaxation times and ^{1}H-^{1}H residual dipolar couplings (D_{res})), which depend on the modulation of ^{1}H-^{1}H dipolar couplings by segmental motions. These motions are quite fast in elastomers above the glass transition temperature, but are anisotropic, resulting in residual ^{1}H-^{1}H dipolar interactions, which depend on the amount and distribution of topological constraints in the polymeric network [2]. In this work, natural and isoprene rubbers vulcanized at different curing temperatures and different sulfur contents have been investigated by exploiting ^{1}H TD-NMR techniques, including ^{1}H multiple-quantum experiments for the measurements of D_{res}, Carr–Purcell–Meiboom–Gill pulse sequence for the evaluation of ^{1}H T_2 relaxation times, and field cycling NMR relaxometry for the measurements of ^{1}H T_1 relaxation times on a wide range of Larmor frequencies (10 kHz-35 MHz). The NMR observables were compared with the crosslink density or macroscopic properties of the material that depend on this quantity, obtained using routinely employed methods in industrial analyses, allowing to gain insight into the effects of the formulation and the vulcanization conditions on the structure and dynamics of the polymeric networks [3].

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[P-94] **EUTECTOGELS AS DRUG DELIVERY SYSTEMS:**

AN HR-MAS NMR STUDY

J. Pietrowska, V. Vanoli, M. E. Di Pietro, A. Mele, F. Castiglione

Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Italy.

Email: joanna.pietrowska@mail.polimi.it

Keywords: HR-MAS NMR, small molecules.

Physically crosslinked gels are formed due to non-covalent interactions, such as hydrogen bond, electrostatic forces and van der Waals interactions. There has been growing attention in designing drug delivery systems based on physical gels as the gelation process often occurs under mild conditions and in absence of chemical crosslinking agents. As materials, Deep Eutectic Solvents (DESs) are an emerging class of sustainable materials formed by two or more components linked by a strong hydrogen bonding network and used in different applications [1]. The use of DESs as drug delivery systems is related to the possibility of developing gel-based DESs "eutectogels" able to deliver the loaded drugs in a controlled way [2]. Here we present the synthesis and characterization of supramolecular gels based on DESs and dissolve some active pharmaceutical ingredients in them at different concentrations. The final goal is to study the propertiesof these systems from a microscopic point of view, mainly exploiting ^{1}H High-Resolution Magic Angle Spinning (HR-MAS) NMR technique to investigate drug dynamics in eutectogels. The gels prepared arebased on nonionic type V DESs consisting of thymol as hydrogen bond donor and menthol as hydrogenbond acceptor. In our study, we have chosen two model drugs: 1) ethosuximide a water-soluble anticonvulsant drug, and 2) dimethyl fumarate, which has low water solubility, used for the treatmentof multiple sclerosis. Experimental data clearly indicate that molecular mobility in gel systems is not influenced by concentration changes and is always lower than the diffusion observed in aqueous solution.

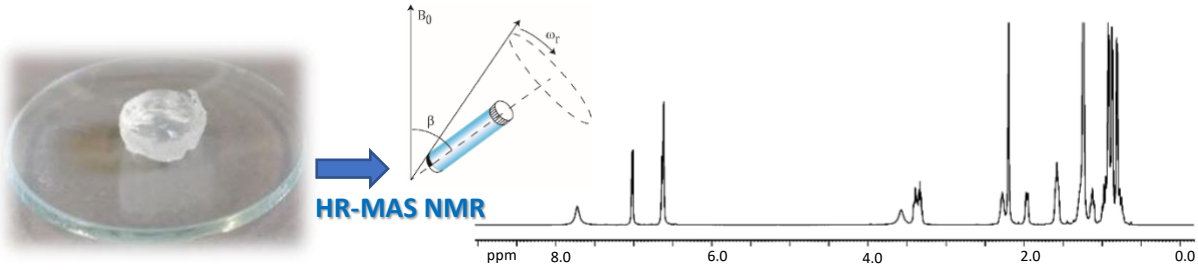

Fig. 1. Menthol/Thymol 1:1 eutectogel; schematic HR-MAS functioning concept; ^{1}H HR-MASspectrum of menthol/thymol eutectogel.

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[P-95] HYPERPOLARIZED ^{129}Xe NMR: AN INVALUABLE TOOL TO EXPLORE NANOPOROUS CRYSTALLINE STRUCTURES

S. Piva‡, J. Perego ‡, C. X. Bezuidenhout‡, S. Bracco‡, P. Sozzani‡, and A. Comotti‡

‡Department of Materials Science, University of Milano-Bicocca, via R. Cozzi 55, 20125 Milano, Italy
E-mail: s.piva7@campus.unimib.it

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

Solid-state NMR (ssNMR) is an essential tool for the characterization of metal-organic frameworks (MOFs), a new and emerging class of porous hybrid materials constructed by metal-based nodes and organic ligands. While the crystal structure is commonly determined by diffraction techniques, ssNMR is the technique of choice for elucidating the dynamics of the molecular moieties comprising the material and gases diffusing inside it. High resolution ssNMR techniques are used to characterize new crystal structures. In fact, ^{1}H, ^{19}F and ^{13}C MAS spectra (1D and 2D) can demonstrate the purity of the materials and monitor changes in the structural symmetry. By changing contact times, the distances and connectivity between nuclei can be evaluated, thus realizing a sort of NMR crystallography.

In addition, we can investigate the porosity of MOFs using continuous-flow hyperpolarized (HP) ^{129}Xe ssNMR, which is a laser-assisted technique that exploits ^{129}Xe nuclei as an invaluable probe to explore the shape and size of the cavities in the porous materials. [1] Due to the very high sensitivity of this technique, we adopt extremely diluted conditions (2% Xe in He/N_2 gas mixture), thus preventing room temperature Xe-Xe interactions. A neat chemical shift anisotropy (CSA) is observed for MOFs with narrow channels, while for larger cavities only the isotropic signals are collected, which give information on xenon confinement. [2] An interesting result was observed in the HP ^{129}Xe spectra of the hetero-ligand Zr-DPT:DPA-8% MOF (DPT = 5,12-diphenyl-tetracenedicarboxylate; DPA = 9.10-diphenyl-antracenedicarboxylate), which exhibits a single peak with a chemical shift corresponding precisely to the expected weighted average of the two chemical shifts of the homo-ligand samples (see Fig. 1). The absence of any residual peak of the homo-ligand MOFs demonstrates the excellent structural homogeneity of the co-assembled frameworks. [3]

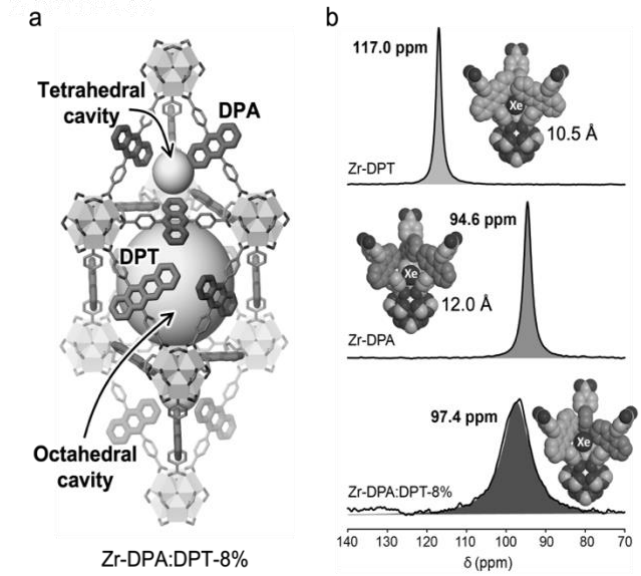

Fig. 1. a) Crystal structure of Zr-DPT:DPA MOF highlighting the tetrahedral and octahedral cavities; b) Hyperpolarized laser-assisted ^{129}Xe NMR spectra of hetero-ligand nanocrystals compared with homo-ligand MOFs based on DPA and DPT.

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[P-96] ^{1}H-NMR RELAXATION PROPERTIES OF IRON-OXIDE BASED MNPs: THE POSSIBLE ROLE OF COATING

Margherita Porru[‡], Francesca Brero[†], Davide Cicolari[‡], Martin Albino[χ], Francesco Orsini[†], Claudio Sangregorio[χ], Claudia Innocenti[χ], Manuel Mariani[‡], Paolo Arosio[†], Alessandro Lascialfari[‡]

[‡] Department of Physics, INFN and INSTM, University of Pavia, Italy
[†] INFN- Pavia, Italy
[χ] Department of Chemistry, ICCOM-CNR and INSTM, University of Florence, Italy
[†] Department of Physics, INFN and INSTM, University of Milan, Italy
E-mail: margherita.porru01@universitadipavia.it

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

Iron oxide based magnetic nanoparticles (MNPs) play an important role in the biomedical field [1], for diagnostic and therapeutic applications. Their magnetic nature and high magnetization values induce sizable inhomogeneities in the local magnetic field, perturbing the surrounding hydrogen nuclei present in the biological tissues of a particular region of interest (ROI). The perturbations at nuclear sites shorten the *spin-lattice* (T_1) and *spin-spin* (T_2) nuclear relaxation times, enhancing the contrast in the MRI images, an effect quantified by the nuclear longitudinal and transverse relaxivity ($r_{1,2}$) i.e., the change of relaxation rates normalized to 1 mM of CA concentration. The MNPs are normally coated with organic moieties that have a major role in the biocompatibility and biodistribution of these systems. This work is based on the study of the effect that different polymeric coatings might induce on the longitudinal and transverse relaxivity, bringing the attention to a feature of nanoparticles that might be interesting to be tailored for MRI applications.
We studied two sets of colloidal solution of spherical superparamagnetic iron oxide MNPs with different core size: the first set (core diameter $d_{S1} = 8.9 \pm 0.9$ nm) was coated with APPA or DMSA, whereas the second one ($d_{S2} = 4.4 \pm 0.7$ nm), with DMSA, PAA or TMA. Each set has been morpho-structurally characterized by XRD and TEM acquisitions, and magnetically investigated through ZFC/FC and hysteresis curves measurements. The NMRD profiles (longitudinal r_1 and transverse r_2 relaxivity vs frequency) have been acquired in the 0.01 MHz-298 MHz range. The relaxation times below 7.2 MHz have been determined using the Fast-Field-Cycling (FFC) technique, that resorts to Pre-Polarized (PP) acquisition sequencies (SR and SE), while at higher frequencies classical NMR sequences SR and CPMG were used. The experimental data have been fitted to the Roch-Müller-Gillis heuristic model [2]. As expected, discrepancies between the NMRD profiles of particles with different core size, but presenting the same polymeric coating (DMSA), were observed. Longitudinal NMRD profiles of the smaller MNPs family with different coatings show different relaxation intensities and dispersion levels (i.e., anisotropy energy) at low frequencies.
As the particles with the same magnetic core are expected to have very similar physical properties, we suggest that possible interactions between the polymeric coating and the surface spins of the core might influence the fundamental relaxometric properties of the compounds. Hence, we can interpret the organic coating as an element with a major role in finely tuning the nuclear relaxation mechanisms of nanometric systems.

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[P-97] CHARACTERIZATION OF BIODEGRADABLE POLYBUTYLENE SUCCINATE MODIFIED BY SEQUENTIAL INFILTRATION OF AL_2O_3

A. Pulvirenti[1], A.C. Boccia[1], R. Consonni[1], A. Motta[2,5], M. Perego[2], P. Cerruti[3], C. Wiemer[2], F. Sparvoli[4].

[1] Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" CNR-SCITEC, Lab. NMR, v. Corti 12, 20133 Milan, Italy
[2] Istituto per la Microelettronica e Microsistemi, CNR-IMM, v. C. Olivetti 2, I-20864 Agrate Brianza, Italy
[3] Istituto per i Polimeri, Compositi e Biomateriali, CNR-IPCB, v. G. Previati 1/E, 23900 Lecco, Italy
[4] Istituto di Biologia e Biotecnologia Agraria, CNR-IBBA, v. Corti 12, 20133 Milan, Italy
[5] Department of Energy, Politecnico di Milano, Via Ponzio 34/3, 20133 Milan, Italy

E-mail: alfio.pulvirenti@scitec.cnr.it; roberto.consonni@scitec.cnr.it

Keywords: solution NMR, food, polymers.

Polybutylene succinate (PBS) is one of the most important and commercially available biodegradable polyesters obtained by polycondensation between succinic acid and butanediol [1]. It is endowed with high mechanical properties, comparable to polyethylene (PE) and polypropylene (PP); high thermal, chemical resistance and high heat deflection temperature. However, its applications suffer of some limitations due to softness, gas barrier properties and melt viscosity, limiting its practical use. To overcome these problems, the introduction of inorganic materials into biopolymers has been envisioned as a viable option to improve the structural properties of PBS and promote the exploitation in different application fields. The sequential infiltration synthesis (SIS), based on atomic deposition of Al_2O_3 on PBS, via trimethylaluminum (TMA) and H_2O precursors, provides an attractive option for the production of a polymer−inorganic hybrid material. After the growth of Al_2O_3 in freestanding ~30 □m thick PBS films by SIS process at 70°C, changes on PBS microstructure and mass uptake in the films were evaluated as a function of the number of SIS cycles [2]. Results evidenced that mass uptake in the PBS films was much higher than in standard polymethylmethacrylate (PMMA) films, at the same process conditions.
This study evaluates changes on PBS microstructure *vs* mass uptake and explores the reactivity of the PBS functional groups, (ester and ether) after TMA treatment, through solution Nuclear Magnetic Resonance spectroscopy (NMR) thus suggesting a plausible reaction mechanism able to justify structural changes in PBS[3].

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Acknowledgements

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[P-98] Structural and Dynamical Characterization of a Intrinsically Disordered Polipeptide working as inhibitor of DUX4 in FSHD

G.Quilici,[‡] A. Berardi,[‡] P. Ghezzi,[†] G.Musco[‡], D.Gabellini[†]

[‡]Biomolecular NMR laboratory at San Raffaele Hospital, Via Olgettina, 58 Milan, Italy
[†]Gene expression and muscular dystrophy Unit at San Raffaele Hospital, Via Olgettina, 58 Milan, Italy
E-mail: quilici.giacomo@hsr.it

Keywords: solution NMR, biomolecules.

In facioscapulohumeral muscular dystrophy (FSHD) aberrant expression of the transcription factor double homeobox 4 (DUX4, 424aa) leads to impaired muscle differentiation and activation of cell death via apoptosis eventually leading to muscle wasting [1]. A natural intrinsecally disordered polypeptide (IDP) was identified that directly binds and inhibits the toxic activity of DUX4.
The aim of the work is to characterize the interaction and identify the key residues of the IDP responsible of the binding in order to design drug-like DUX4 inhibitor molecules mimicking the natural interaction.
The complete assignment of IDP's resonances (^{1}H, ^{15}N and ^{13}C) was carried out in solution NMR spectroscopy through both canonical and direct ^{13}C acquisition experiments. Due to the high level of superimposition in the ^{15}N HSQC we individuate C_CON (^{13}C direct acquisition) as "fingerprint" of the IDP. We performed C_CON experiments in presence of increasing concentrations of DUX4 in order to individuate the most affected residue in terms of chemical shift perturbation. We further performed ^{15}N relaxation rate analysis on both free and bound IDP.
These collected observations are an important starting point to define the key residues on the IDP sequence that play a fundamental role in the interaction and to build a model of interaction with DUX4.

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[P-99] SILK FIBROIN: EXPLOITING A FUNCTIONAL AMYLOID TO INVESTIGATE AGGRETATION MODULATORS AND CROSS SEEDING EFFECTS

K. Pagano,[‡] S. Tomaselli,[‡] L. De Rosa,[#] L. D'Andrea,[‡] A. M. Mammedzade, [†] O. K. Gasymov, [†] H. Molinari,[‡] L. Ragona[‡]

[‡] Istituto di Scienze Chimiche e Tecnologiche (SCITEC), CNR, via Corti 12, Milano, Italy
[#] Istituto di Biomolecole e Bioimmagini (IBB), CNR, Via Pietro Castellino 111, 80131 Naples, Italy
[†] Institute of Biophysics of ANAS, 117 Khalilov, AZ-1141 Baku, Azerbaijan
E-mail: henriettemolinari0@gmail.com

Keywords: solution NMR, small molecules, biomolecules

Aberrant protein aggregation, such as amyloidogenesis, is a hallmark of several human neurodegenerative disorders such as Alzheimer's and Parkinson's diseases but is also a common feature of some functional protein assemblies, such as silk fibroin. In both cases, the protein backbone can adopt β-strand conformations, capable of assembling into elongated unbranched fibrils, a self-recognition process, characterized by homotypic interactions. Silk fibroin (SF) is an appropriate model to investigate amyloid aggregation [1] and screen aggregation inhibitors [2]. We show here, employing different biophysical approaches (turbidity, Congo Red assays, CD, DLS and fluorescence), that fusidic acid (FA), a well-known antibiotic, acts on SF as an anti-aggregating agent, being also able to revert SF aggregation. We further provide the proof of principle that FA displays anti-aggregation properties also on lens material derived from cataract surgery [3].
In the pathophysiological cascade of neurodegenerative diseases, heterotypic interactions between amyloids and other proteins have come into focus as they occur in a wide range of amyloid processes, modifying fundamental aspects of amyloid aggregation. Along this line, the issue of cross-talk between pathological and non-pathological amyloidogenic proteins is under investigation in our lab, focusing on NMR interaction studies between SF and Aβ42.
We have previously investigated by NMR the aggregation kinetics of Aβ42 peptide and the role of small molecules and other proteins as aggregation inhibitors [4-5]. We have shown that the aggregation state of the starting Aβ42 sample is a critical determinant that influences both Aβ kinetics and its interactions with other proteins [5]. We have thus synthesized an Aβ depsipeptide [6], which is pure and stable, and, once in solution, converts within few minutes to Aβ1-40 monomeric sample.
Aβ aggregation mechanism in the presence of SF is now under investigation in our laboratory.

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Acknowledgements

The authors acknowledge financial support of CNR-ANAS bilateral project (2021-2022) and Fondazione Antonio De Marco.

[P-100] SOLID STATE NMR STUDY OF SOME DOUBLE PEROVSKITE FLUORIDES A_3XF_6 (A = K, Rb, Cs; X = Al, Sc) WITH NON-COOPERATIVE OCTAHEDRAL TILTING

A. Rakhmatullin,[‡] G. King,[†] I. B. Polovov,[§] K. V. Maksimtsev,[§] F. Šimko,[‖,⊥] M. Korenko,[‖,⊥] R. Bakirov,[◊] C. Bessada,[‡] and M. Allix[‡]

[‡]Conditions Extrêmes et Materiaux: Haute Température et Irradiation, CEMHTI, UPR 3079 -CNRS Univ Orleans 45071 Orléans, France
[†]Material and Chemical Sciences, Canadian Light Source, 44 Innovation Blvd, Saskatoon, SK S7N 2V3, Canada
[§]Department of Rare Metals and Nanomaterials, Institute of Physics and Technology Ural Federal University, 19, Mira str. 620002 Ekaterinburg, Russia
[‖]Department of Molten Systems, Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 36 Bratislava, Slovakia
[⊥]Centre of Excellence for Advanced Materials Application - CEMEA, Slovak Academy of Sciences, Dúbravská cesta 5807/9, 845 11 Bratislava, Slovakia
[◊]Department of Technology of mechanical engineering and instrument making, Votkinsk branch of Kalashnikov Izhevsk State Technical University, 1, Shuvalova str. 427000 Votkinsk, Russia
E-mail: rakhmat@cnrs-orleans.fr

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

A crystallographic approach at room and high temperature incorporating multinuclear high field solid state NMR; synchrotron, laboratory X-ray, and neutron powder diffraction; electron diffraction; and density functional theory (DFT) was used to characterize the polymorphs of K_3AlF_6, Rb_3AlF_6, Cs_3AlF_6, Rb_3ScF_6, and Cs_3ScF_6. The structures of all these compounds are a small part of the elpasolite or double perovskite type, which show non-cooperative octahedral tilting (NCOT). In these phases, one of the octahedra is rotated by ~45° while the other remains untilted. Some of the elpasolite-type materials are suitable for scintilators, showing desirable isotropic optical and mechanical properties. The cryolite systems of alkali metals could also have other industrial applications.
Comparison of NMR measurements and computational results revealed the dynamic rotations of the (Al or Sc)F_6 octahedra. Using in-situ variable temperature MAS NMR measurements, the chemical exchange between rubidium and cesium sites was observed.

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[P-101] EVALUATION OF THE ROBUSTNESS OF OPTIMAL CONTROL-BASED RF PULSES IN THE FRAMEWORK OF MAGNETIC RESONANCE ELASTOGRAPHY

P. Sango-Solanas[‡], K. Tse Ve Koon[‡], H. Ratiney[‡], E. Van Reeth[‡], C. Caussy[†‡] and O. Beuf[‡].

[‡]Univ Lyon, INSA-Lyon, Université Claude Bernard Lyon 1, UJM-Saint Etienne, CNRS, Inserm, CREATIS UMR 5220, U1206, F-69616, Lyon, France
[†]Univ Lyon, Laboratoire CarMen, Inserm, INRAe, INSA Lyon, Université Claude Bernard Lyon 1, Pierre-Bénite, France
[‡]Hospices Civils de Lyon, Département Endocrinologie, Diabète et Nutrition, Hôpital Lyon Sud, Pierre-Bénite, France

E-mail: pilar.sango@creatis.insa-lyon.fr

Keywords: MRI, theory and methods.

Magnetic Resonance Elastography (MRE) quantifies the mechanical properties of tissues based on the properties of shear wave propagation [1]. Classically, MRE applies oscillating motion encoding gradients (MEGs) to encode the external induced motion. Their presence involves long echo times, limiting the technique to very short relaxation times (T2) tissues and high excitation frequencies. RF pulses designed with an optimal control (OC) algorithm applied with a constant gradient demonstrated their ability to simultaneously perform slice selection and motion encoding enabling short echo times independently of the excitation frequency [2,3]. However, a mismatch between experimental and optimization parameters could affect the performance of the OC pulses. A prior knowledge of the acquisition parameters is thus required to compute the OC pulse. In this study, we evaluated the robustness of the OC pulses to the variability of two critical parameters: the T2 and the shear wave amplitude. From simulations, we found that motion is still encoded when T2 is bigger or slightly smaller than the optimized one and when the excitation amplitudes are smaller than the amplitudes fixed for the optimization (Figure 1). Experimental acquisitions carried out on a T2-heterogeneous phantom are in agreement with the results found by simulation (Figure 2). In conclusion, we validated the robustness of OC pulses to the sample T2 variability. This means that an OC pulse could be used in a wide range of different T2s. Also, we demonstrated that OC pulses can encode the motion attenuation along the sample. This would facilitate the use of the OC-based MRE strategy on clinical setups since just a few pulses could be sufficient for different applications. Further studies are needed to confirm the applicability of OC pulses *in vivo*.

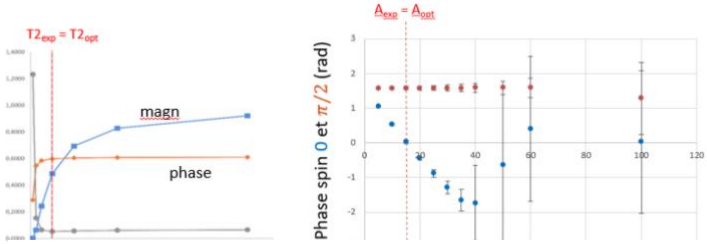

Figure 1. Numerical evaluation of the robustness of an OC pulse. Left: simulated transverse magnetization amplitude and phase variation with respect to sample T2-value. Right: simulated accumulated phase variation with respect to excitation amplitude.]

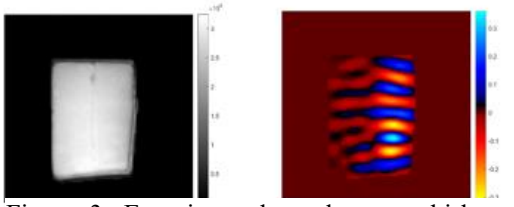

Figure 3. Experimental results on a bi-layer phantom with T2 values of 1ms (left-layer) and 5 ms (right-layer): magnitude (left) and phase (right) images.

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[P-102] NMR INVESTIGATION OF FLUORESCENT ANTHRACENE-SUBSTITUTED CYCLOPENTAISOXAZOLINE SHORT PEPTIDES AS □-TURN MIMICS

T. Recca‡ M. Mella†, M. Leusciatti†, P. Quadrelli†

‡Centro Grandi Strumenti (CGS), Università di Pavia, Via Bassi 21, Pavia
†Dipartimento di Chimica, Università di Pavia, Via Taramelli 12, Pavia
E-mail: teresa.recca@unipv.it

Keywords: solution NMR, small molecules, contrast agents.

Due to the important role of the turn structures in peptides and proteins [1], many efforts in this area are directed to the designing and developing novel structures for specific applications and using classical and uncommon scaffolds for promising new synthetic targets. In this context, three-dimensional interactions, spatial orientation determination derived from a conformational analysis, based on spectroscopic techniques and molecular modeling, are essential for the design of analogues in the drug discovery process. Recently, we presented and developed our original approach towards the design of non-peptidic turn-inducers [2] based on easily synthesized cyclopenta[d]isoxazoline aminols through the nitrosocarbonyl intermediates chemistry. On pursuing our research work on the synthesis of novel turn motifs (see Fig. 1), we developed this topic in order to verify the influence of the two side chains on the type of turn [3]. Therefore, we present here the peptide chain effect on the turn formation through the characterizations of the diasteroisomeric turn structures through NMR and circular dichroism (CD) techniques. Furthermore, the fluorescence properties of the synthesized turns will be finalized to design proper fluorescent tags, for imaging studies at the protein turn points and activity-based protein profiling (ABPP) investigations [4].

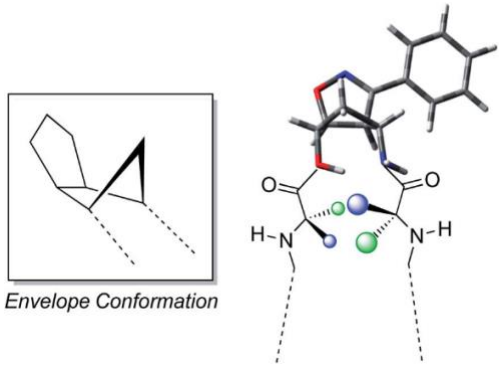

Fig. 1 Envelope conformation of the isoxazoline aminol structure and development of the turn conformation.

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[P-103] EXTENT OF STRUCTURAL DISORDER IN PHASE SEPARATED GLASS AND GLASS CERAMIC FROM ADVANCED SOLID-STATE NMR SPECTROSCOPY

Amandine Ridouard, ‡ Cécile Genevois, ‡ Michael J. Pitcher, ‡ Mathieu Allix, ‡ Dominique Massiot, ‡ and Franck Fayon ‡

‡ CNRS, CEMHTI UPR3079, Univ. Orléans, F-45071 Orléans, France
E-mail : amandine.ridouard@cnrs-orleans.fr

Keywords: solid state NMR, materials.

Characterizing the structure of disordered systems lacking of long-range 3D periodicity, like glasses, remains a challenging experimental task. Over the last years, various solid-state NMR methods have been proposed to obtain detailed short and longer-range structural information in disordered systems [1]. In this work, we have used this NMR approach to probe the structure of novel gallium-niobium-silicate glasses. Indeed, homogeneous glasses or nucleation-growth phase-separated glasses with different droplet size can be obtained depending of the amount of Na_2O in the investigated compositional range. Moreover, crystallization of phase-separated glasses leads to transparent glass-ceramics with $GaNbO_4$ nano-crystals.
The challenge here is to understand and characterize the mechanism of phase separation and crystallization in this complex disordered system. In that case, ^{29}Si, ^{71}Ga, ^{23}Na, ^{95}Nb solid-state NMR experiments at different magnetic fields, ^{29}Si-{^{71}Ga/^{93}Nb/^{23}Na} double-resonance methods complemented with DFT calculation, TEM microscopy and X-ray diffraction have been employed to study the nature of disorder in homogeneous and nanostructured glasses having potential applications in optics [2], [3].

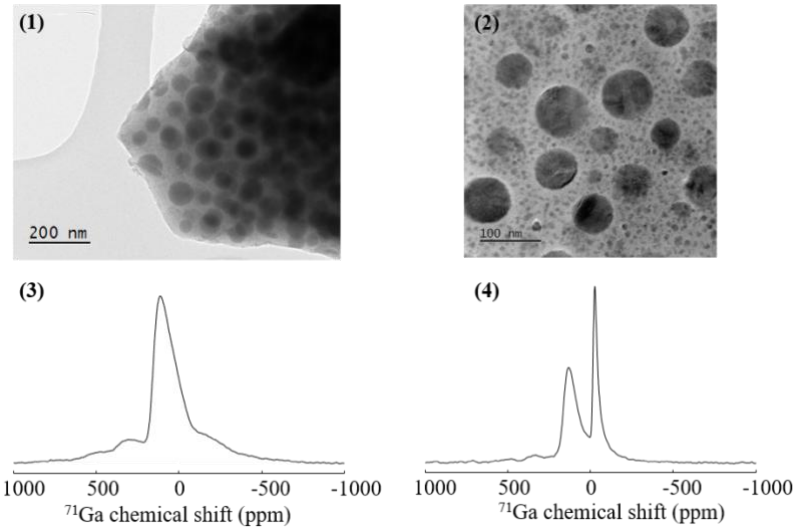

Fig. 1. TEM picture of phase separated (1) glass x=4 (2) glass x=4 crystalized at 800°C for 2h; ^{71}Ga high field NMR echomas spectra (20T, 60kHz) (3) glass x=4, (4) glass x=4 crystalized at 800°C for 2h.

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[P-104] INVESTIGATING PHYSICAL PROPERTIES OF BIOPOLYMERS IN HAIRS

V. Righi1,‡ F.D. Koufi2,‡ A.Mucci3†
‡Department Life Quality Studies, University of Bologna, Campus Rimini, Italy;
†Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy
E-mail: valeria.righi2@unibo.it

Keywords: hair, keratin protein, solid state NMR, solution NMR, biomolecules.

Hair is of outmost importance for the human body. Hair is composed of 1–8% external hydrophobic lipid epidermis, 80–90% α-helix or β-sheet conformation of parallel polypeptide chains to form water-insoluble keratin, less than 3% melanin pigment, and 0.6–1.0% trace elements, 10–15% moisture [1]. Keratin proteins are insoluble in water and play a protective role. They possess a heterogeneous morphology that classifies them to the fibrous structural proteins.
We received samples of hair that had undergone treatment with a hair mask prepared by a cosmetic company. The samples were taken in different timelines to assess not only the reconstruction of keratin induced by the cosmetic formulation but also the possibility of a long-term effect on the hair. Our main goal was the qualitative and quantitative assessment of the reconstructive effect on keratin. For the qualitative analysis, we employed the SEM-FEG technique and obtained a complete visualization of the keratin integrity. Moreover, liquid NMR was used to analyze the lipids extracted from the hair samples in a chloroform: methanol solution.

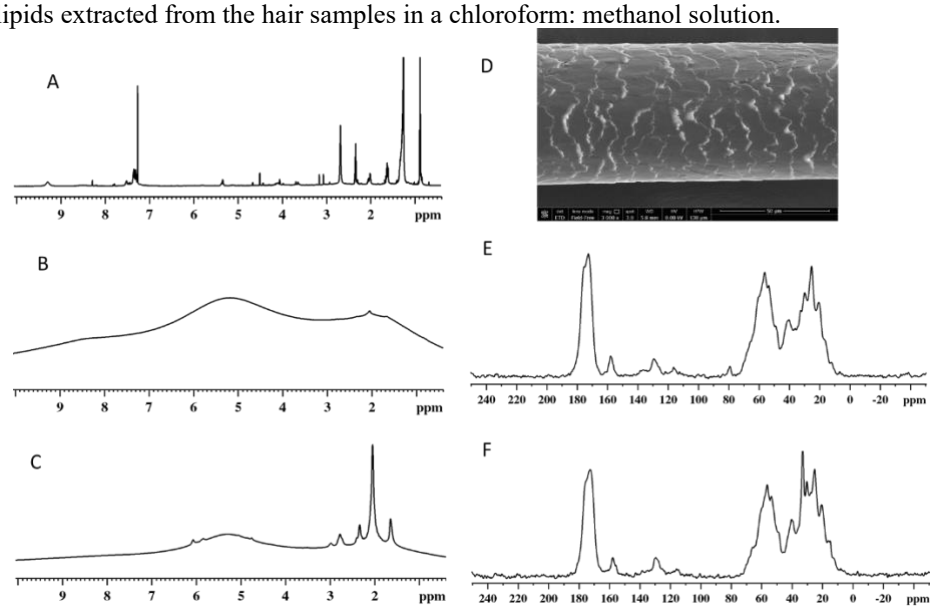

Figure 1. Measurements of hairs. Hair samples were subjected to lipid hair extraction: (A)1H NMR on lipid extracts, (B) 1H MAS NMR on hair after extraction, (C) 1H MAS NMR on hair before extraction, (D) SEM images of hair, (E)13C CP-MAS spectra on hair before extraction, (F)13C CP-MAS spectra on hair after extraction.

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[P-105] A two magnetic core NMR system with RF and 3D-gradient capabilities in low-field

T. B. R. Robertson,[‡] T. A. A. Cartlidge,[‡] T. Hugger,[†] S. Breham, [†] K. Zick, [†] F. Engelke, [†] M. Utz[‡] and G. Pileio[‡]

[‡]School of Chemistry, University of Southampton, SO17 1BJ, Southampton, UK
[†]Bruker BioSpin GmbH, Rheinstetten 76287, Germany
E-mail: T.B.R.Robertson@soton.ac.uk

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

Many materials of key interest for industrial applications, such as porous media and battery electrodes, possess large internal heterogenous magnetic fields originating from magnetic susceptibility mismatches between the medium and imbibed solution [1]. This results in gradients which drastically reduce the lifetime of transverse magnetization and make diffusion measurements over distances of interest very challenging at high field.

This instrumental development seeks to address this challenge to enable the investigation of structural parameters such as porosity, pore size distributions and tortuosity in porous media. The approach taken herein is to combine singlet-assisted diffusion NMR methodology (SAD-NMR [2]) with field-cycling methods.

This is practically accomplished through a dual probe system and a sample shuttle able to move the NMR sample between high field (7.05 T) and low field (~12mT) where RF coils and a 3D- axis gradient system can be used to perform diffusion NMR.

Applications such as access to long lived singlet lifetimes and diffusion tensor imaging (DTI) are of particular interest as these will enable unique insights into diffusion within porous media otherwise inaccessible without the hardware described herein [2].

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[P-106] FAST-FIELD NMR RELAXOMETRY STUDY OF WATER MULTISCALE DYNAMICS IN HYDROGEL-CLAY COMPOSITE

Claire Hotton[a], Natalie Malikova[a], Juliette Sirieix-Plenet[a], Guylaine Ducouret[b], Guillaume Mériguet[a], Laurent J. Michot[a], Pierre E. Levitz[a], A.-L. Rollet[a]

[a] PHENIX laboratory, Sorbonne University, CNRS, Paris, France
[b] SIMM laboratory, ESPCI Paris, Université PSL, Sorbonne University, CNRS, Paris, France
E-mail: anne-laure.rollet@sorbonne-university.fr

Keywords: solution NMR, low field NMR, materials.

Hydrogels are macromolecular materials made up of polymer chains linked together to form a three-dimensional network, that presents appealing properties for various domains as biomedicine, biotechnology, pharmaceutical industry, or even the food industry [1]. For instance, they make it possible to develop biomimetic artificial tissues, biosensors, new dressings, even super-absorbent materials or underwater adhesive materials [2].
There are two kinds of hydrogels depending on the interactions into play between polymer chains: chemical hydrogels and physical or supramolecular hydrogels. Unlike chemical hydrogels where the polymer chains are connected by covalent bonds, supramolecular hydrogels exhibit only physical interactions. The very interesting properties of these supramolecular hydrogels lies in the reversibility of their bonds, allowing tunable applications such as the controlled release of active substances, by passing simultaneously from the solution to the gel phase and conversely. The counterpart is that most physical hydrogels possess inferior mechanical properties to chemical hydrogels. In order to be able to combine reversibility and resistance of the material, nanoparticles are added in these hydrogels.
In this work, we combined cationic polyelectrolytes (ionene) and clay nanoparticles to formulate anisotropic hydrogels and we investigated the dynamics of water using Fast Field Cycling NMR relaxometry. The NMR Dispersion profiles obtained with the composite hydrogels were compared to those obtained with the individual components, i.e. the solutions of cationic polyelectrolytes and the dispersions of clays nanoparticles (Fig. 1). It reveals the way the polyelectrolyte impedes the interaction of water with clay platelets.

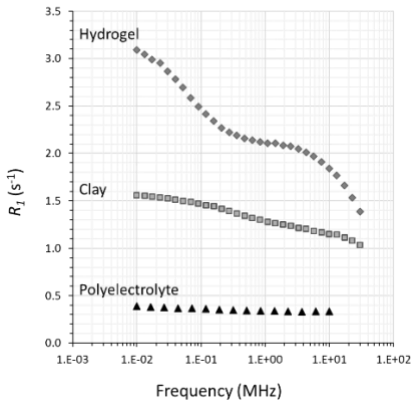

Fig. 1. ^{1}H NMRD profiles of cationic polyelectrolyte solution, montmorillonite aqueous dispersion and of the composite hydrogel made with the two previous components.

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[P-107] METABOLOMIC CHARACTERISATION OF *Arctium lappa L. BY* UNTARGETED NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

*Enrico Romano[1], Fabrizio Masciulli[1], Donatella Ambroselli[1], Cinzia Ingallina[1], Mattia Spano[1], Giacomo Di Matteo[1], Luisa Mannina[1].
[1]Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Pizzale Aldo Moro 5, 00185 Roma (Italy)
*Presenting Author: e.romano@uniroma1.it

Keywords: solution NMR, metabolomics, food.

Arctium lappa L., also called Burdock, is a genus of dicotyledonous angiosperm plants of the Asteraceae family. Roots are the most widely used part of this plant. According to the composition table of the United States Department of Agriculture (USDA, 2015) [1], burdock root contains 80 % moisture, 1.5 % protein, 0.9 % ash (a measure of the total amount of minerals present), 0.1 % lipids and 17.5 % total carbohydrates. Burdock root also has antioxidant compounds with health benefits, such as caffeine, flavonoids and lignans.
The present study aims to characterise the metabolimonic profile of Burdock roots, and to assess the different amounts of metabolites in the spontaneous and organic ecotypes, by the application of untargeted Nuclear Magnetic Resonance Spectroscopy (NMR).
Burdock roots were extracted by the Bligh-Dyer [2] protocol in order to obtain two different fractions with different polarity compounds. The hydroalcoholic phase was prepared for the NMR analysis. Both one-dimensional proton (^{1}H NMR) and two-dimensional (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) experiments were performed, using previously reported experimental conditions [3], in order to achieve a complete peak assignment. Sugars, organic acids, amino acids, nucleoside and other metabolites, such as coline and trigonelline, were quantified in all the analysed samples.
Chemical characterisation by NMR spectroscopy has made it possible to obtain the metabolomic profile of *Arctium lappa L.* and to diversify, in quantitative terms, the spontaneous ecotypes and the ecotypes cultivated with organic methods, in order to subline useful differences for health and nutraceutical application.

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[P-108] NMR STUDIES OF THE ROLE OF WATER IN THE STRUCTURING AND HYGROSCOPIC BEHAVIOUR OF CELLULOSE-HEMICELLULOSE ASSEMBLIES

Corinne Rondeau-Mouro[†], Xavier Falourd[‡], Laurena Rutin[‡], Alice Besserer[†], Cassandre Guinet*, Brigitte Chabbert*, Véronique Aguié-Béghin[*]

[‡] INRAE, UR1268 BIA, F-44316, Nantes, France
[†] INRAE, UR1466 OPAALE, 17 Avenue de Cucillé, CS 64427, F-35044 Rennes, France
[*] Université de Reims Champagne Ardenne, INRAE, FARE, UMR A614, 51097 Reims, France.

E-mail: corinne.rondeau@inrae.fr
Keywords: solid state NMR, low field NMR, materials, biomolecules, polymers

The functional properties of biosourced and innovative materials based on cellulose and hemicellulose can be modulated according to their composition and the interactions established between the polymer chains and the different structural domains thus formed. In terms of applications, the role of water in these assemblies and their hygroscopic properties represent essential characteristics to study. The use of high-field solid-state NMR and low-field NMR relaxometry approaches allows characterization of these macromolecular systems organized under variable moisture conditions.

Measurement of water relaxation times (T_2) and dipolar second moments (M_2) of polysaccharides constituting micrometric films, i.e. cellulose (nanocrystals or nanofibrils) and hemicellulose (Konjac glucomanan) allow quantifying more or less strong molecular interactions and to access inter-chain distances which can be correlated to the mechanical properties modulated by the composition and the hydration of the films [1, 2, 3, 4]. The methodologies developed in solid-state NMR, meanwhile, make it possible to investigate a wide range of molecular movements ranging from millisecond to picosecond, and to discriminate structural domains by their stiffness [5] or homogeneity [6]. Thus, recent work on the study of proton-carbon polarization transfer kinetics [7,8] has made it possible to access, among other things, $T_{1\rho}^{H}$ times which give information on the molecular order in a volume of 2 to 30 nm.

Various films based on cellulose, glucomannan, alone or in mixtures, were studied by solid-state NMR at 400 MHz and by low-field NMR (20MHz) with three water contents (relative humidity 33, 59 and 85%). The structuring of the systems were first analysed through the determination of the $T_{1\rho}^{H}$ of the polymer chains. Interactions of polymers with water were investigated through the determination of several spin diffusion times (T_{HH}) and relaxation time T_1 and T_2. The interpretation of the data and their links with the physico-chemical properties of the assemblies were achieved through complementary measurements in Dynamic vapour sorption (DVS) and Dynamic Mechanical Analysis (DMA).

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[P-109] TRIPLE STACK OF A VIOLOGEN-PHENYLENE-IMIDAZOLE GUEST IN A CB[10] PAIR: A NMR STUDY

Roselyne Rosas,[‡] Fengbo Liu,[†] Simin Liu,[†] Shagor Chowdhury,[§] Valérie Monnier,[‡] Hakim Karoui,[‡] Christophe Bucher,[§] Anthony Kermagoret,[‡] David Bardelang[‡]

[‡]Aix Marseille Univ, CNRS, Marseille, France
[†]Wuhan University of Science and Technology, Wuhan, 430081, People's Republic of China
[§]École Normale Supérieure de Lyon, CNRS UMR 5182, Laboratoire de Chimie, F69364 Lyon, France
E-mail: r.rosas@univ-amu.fr

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

Controlling the organization of building blocks in self-assembled oligomers is thus fundamental to tune their properties.[1] Cucurbit[n]urils (CB[n]) allow an large diversity of oligomeric host:guest complexes in water.[2] Here, a viologen-phenylene-imidazole (VPI) guest forms antiparallel triple stacks in water with cucurbit[10]uril (CB[10]), pairwise complexing the guest trimer (Fig. 1a).[3] The quinary host:guest 2:3 complex showed features assignable to charge-transfer interactions.
NMR study, including NOESY and DOSY, permitted to characterize the host:guest complex. Moreover, the exchange rates of the opposite VPI guests in the complex were evaluated by Exchange spectroscopy (2D EXSY),[4] realized from ROESY experiments recorded at various mixing times (0−700 ms and 300 K, Fig. 1b).[3]

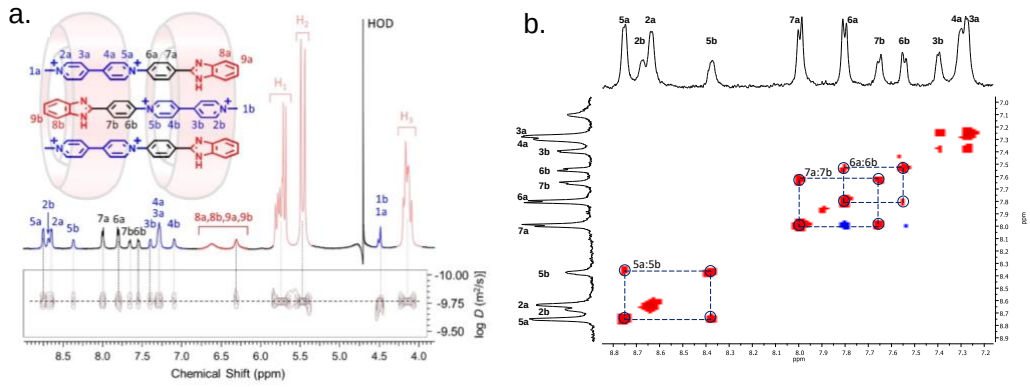

Fig. 1. 500 MHz NMR spectrum of the $VPI^{2+}_3 \cdot CB[10]_2$ complex, (a) corresponding DOSY spectrum and (b) 600 MHz ROESY spectrum (9-7 ppm region, mixing time: 400 ms, 300 K).

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[P-110]

[P-111] KINETIC LIMITATIONS IN LI-ION BATTERIES FOLLOWED BY OPERANDO ^{7}LI NMR SPECTROSCOPY

E. Salager[1,3], L. Afonso de Araujo,[1,2,3] R. Praud,[1,2,3] D. Sicsic,[2,3] V. Sarou-Kanian,[1,3] M. Deschamps,[1,3]

[1] CNRS, CEMHTI UPR3079, Université d'Orléans, Orléans, France
[2] Technocentre Renault, Guyancourt, France
[3] Réseau sur le Stockage Electrochimique de l'Energie (RS2E), CNRS FR3459, Amiens, France

E-mail: elodie.salager@cnrs-orleans.fr

Keywords:
materials, instrumentation, exotica

Li-ion batteries are powerful and light devices that have been at the heart of portable electronics revolution. At the heart of energy transition, transport electrification and renewable energy storage are demanding applications in terms of kinetics. For electric cars the ultimate goal is to charge the battery in 15 minutes with high efficiency and even at low temperature.
We are interested in *operando* NMR of functioning Li-ion batteries to detect *in real time* parasitic reactions arising from kinetic limitations, in harsh conditions such as low temperature or fast charge.
We will present the *in situ* setup that was developed to reach temperatures as low as -20°C and to reduce the noise introduced by the electrochemical connections. The processing was also carefully devised to enhance the detection and measure the parasitic products as early as possible and in real time [1]. This approach was applied to batteries made of commercial electrodes to study the deposition of metallic lithium in the negative electrode on charge. Metallic plating competes with lithium insertion in the negative electrode is one of the parasitic reactions that contribute to accelerated ageing of the batteries.

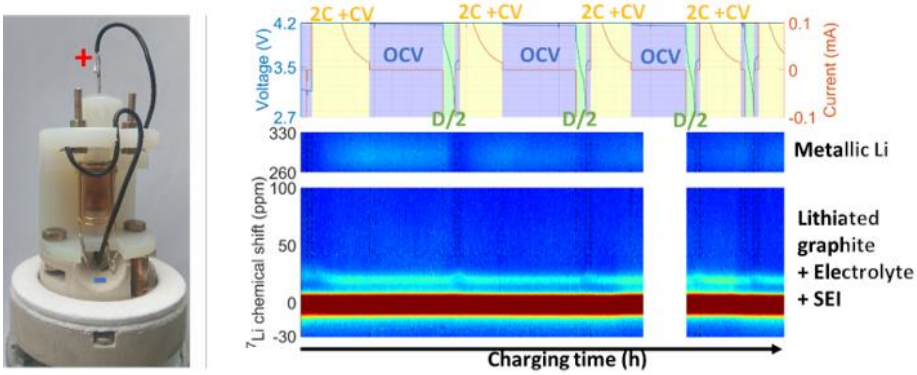

Fig. 1. (left) Electrochemical NMR cell in the RF coil of the NMR probe and (right) simultaneous electrochemical profiles and ^{7}Li NMR spectra recorded during 5 cycles of charge (yellow) and discharge (green). Open circuit measurements are shown in blue.

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[P-112] STRUCTURAL ANALYSIS OF A SIMPLIFIED MODEL REPRODUCING SARS-CoV-2 S RBD/ACE2 BINDING SITE

M. Buonocore[1§], A. Santoro[1§], M. Grimaldi[1], V. Covelli[1], M. Firoznezhad[1], M. Rodriquez[1], M. Santin[2], A.M. D'Ursi[1*]

[1]University of Salerno, Department of Pharmacy, Via Giovanni Paolo II, 132 - 84084 Fisciano, Salerno, Italy
[2]Centre for Regenerative Medicine and Devices, School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, BN2 4GJ, UK
§ equal contribution
E-mail: asantoro@unisa.it

Keywords: solution NMR, biomolecules.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a RNA virus identified as the cause of the coronavirus outbreak in December 2019 (COVID-19). Like all the RNA viruses, SARS-CoV-2 constantly evolves through mutations in its genome, accumulating 1-2 nucleotide changes every month, giving the virus a selective advantage through enhanced transmissibility, greater pathogenicity, and the possibility of circumventing immunity previously acquired by an individual either by natural infection or by vaccination. Several SARS-CoV-2 variants of concern (VoC) have been identified, among which we find Alpha (Lineage B.1.1.7)[1], Beta (Lineage B.1.351), and Gamma (Lineage P.1) variants[2,3]. Most of the mutations occur in the spike (S) protein, a surface glycoprotein that plays a crucial role in viral infection; S protein binds the host cell receptor, the angiotensin-converting enzyme of type 2 (ACE2) via the receptor-binding domain (RBD) and catalyzes the fusion of the viral membrane with the host cell. In this work, we present the development of a simplified system that would afford to study the change in the SARS-CoV-2 S RBD/ACE2 binding related to the frequent mutations. In particular, we synthesized and studied the structure of short amino acid sequences, mimicking the two proteins' critical portions. Variations in the residues were easily managed through the one-point alteration of the sequences. NMR and CD spectroscopy allowed us to gain insights into ACE2 and SARS-CoV-2 S RBD structure with its related three variants (Alpha, Beta and Gamma). Spectroscopy data supported by molecular dynamics lead to the description of ACE2/RBD binding model, pointing out information on the structural impact of the single amino acid mutations, which are determinant in differently influencing the binding with the macromolecular cognate and can define a valuable strategy in the perspective of building a model for the rapid development of molecules targeting SARS-CoV-2 S and its variants.

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[P-113] NMR investigation of an aptamer-small molecule complex, the case of the testosterone binding TESS.1 aptamer

Sofie Schellinck[1], Dieter Buyst[2], Annemieke Madder[3], Karolien De Wael[4], José C. Martins[1,2]

[1] NMR and Structure Analysis Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent 9000, Belgium.

[2] Department of Organic and Macromolecular Chemistry, NMR Centre of Expertise, Ghent University, Ghent, Oost-Vlaanderen, 9000, Belgium.

[3] Organic and Biomimetic Chemistry Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent 9000, Belgium.

[4] A-Sense Lab, Department of Bioscience Engineering, and NANOlab Center of Excellence, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.

E-mail: Sofie.Schellinck@UGent.be

Keywords: solution NMR, small molecules, biomolecules.

Since their discovery in 1990 [1,2], DNA aptamers have shown to be promising bioreceptors for small molecule detection. Numerous articles describing newly raised aptamers capable of binding with their target with high affinity and selectivity, are turned into a biosensor using a fairly simple and logical design based on the target induced conformational change of the aptamer. Although a number of these biosensors appear successful, there is still a general lack of knowledge about the underlying molecular events taking place during an aptamer-target interaction. Such knowledge could aid in further optimising towards real-world applications. We describe our efforts to apply NMR based strategies to this end, using the structure-switching testosterone binding TESS.1 DNA aptamer, developed and extensively characterized by the Stojanovic group, as model system [3]. While NMR spectroscopy is a uniquely suited technique to acquire molecular level information about conformational changes and intermolecular interactions, the TESS.1-testosterone model system provides two important complications. First, at 51 nucleotides, the impact of size and flexibility on spectral complexity and line-broadening effects prevents straight forwards analysis using NMR. Thus, trimming of the duplex stem and introduction of some nucleotide substitutions was used to generate a more 'NMR optimal' sequence that shows interaction with the target in a fashion similar to the original sequence, affording improved analysis and interpretation of the aptamer-target interaction. Second, the low sensitivity and concomitant need for sufficiently concentrated samples is at odds with the low solubility of steroids (~80 µM) such as testosterone. We devised a sample preparation protocol that could handle this challenge, and allowed to demonstrate the occurrence of conformational changes upon interaction with the target. Through both approaches, first molecular insights into the testosterone-TESS.1 complex will be presented.

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[P-114] EXPLORING COVID-19 DRUGGABILITY: THE INTERACTION BETWEEN HEPARIN AND THE HIGHLY DISORDERED NUCLEOCAPSID PROTEIN

M. Schiavina,‡ L. Pontoriero,‡ G. Tagliaferro,‡ R. Pierattelli,‡ I.C. Felli,‡

‡ Magnetic Resonance Center (CERM) and Department of Chemistry "Ugo Schiff", University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino (Florence), Italy

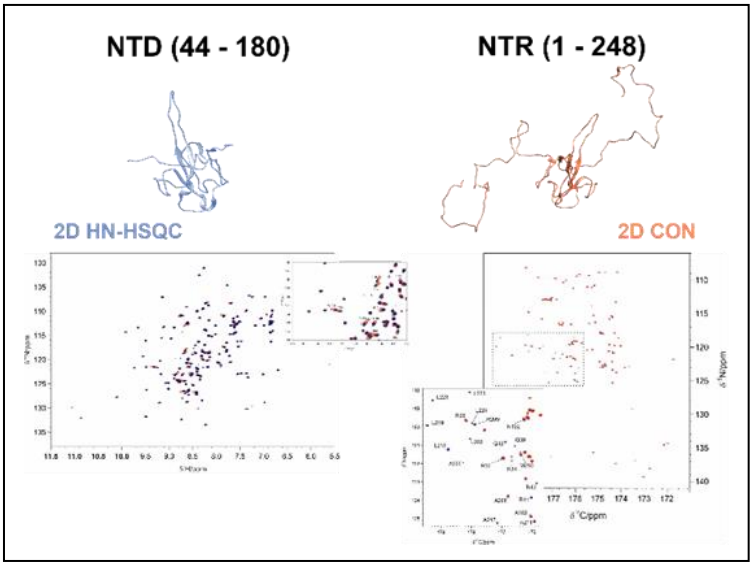
E-mail: schiavina@cerm.unifi.it

Keywords:

solution NMR, biomolecules

Since the Covid-19 pandemic impacted our lives, the development of robust pharmacological strategies to contrast the SARS-COV-2 virus became a priority. One of the most promising targets is the Nucleocapsid protein (N), the viral structural protein whose main function is to package genomic RNA. N is composed of two folded domains and three intrinsically disordered regions, which were found to be relevant for the biological functions of N. Both the globular RNA binding domain (NTD) and the tethered IDRs are rich in positively charged residues which might drive the interaction between the N protein and its partners. The study of the interaction of N with polyanions can thus help to elucidate one of the key driving forces responsible for its function, i.e. the electrostatic contribution.
Several recent studies point to the use of heparin, a highly negatively charged glycosaminoglycan, to contrast serious cases of Covid-19 infection and we decided to study its interaction with N at the molecular level. We focused on the NTR construct [1], which comprises IDR1-NTD-IDR2, and on the NTD construct in isolation. We characterized the interaction using different NMR approaches and we complemented the analysis with Isothermal Titration Calorimetry. The high-resolution mapping of the binding obtained in this work could help the design of tailored polyelectrolytes able to interfere with N protein function.

Figure 1. HN and CON spectra recorded on the two constructs used in this study, represented by the two structural models drawn on the upper panels. The HN spectrum ensures enough resolution for the NTD globular domain while the CON spectrum was recorded on the NTR construct giving insights on the flexible linkers comprised in this latter construct.
The enlargements show some of the most perturbed residues in the two different protein constructs.



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[P-115] Quantification of polyols in sugar-free foodstuffs by qNMR

A. Scettri, E. Schievano

Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy
E-mail: elisabetta.schievano@unipd.it

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

Polyols are valuable food additive thanks to their properties, as cooling agents or sweet-tasting products [1, 2]. In food industry, the control of polyols amounts in sugar-free foods is essential in terms of nutritional information and quality control [3].
Polyols detection and quantification in mixtures, employing analytical methods based on chromatographic separation, pose several challenges due to their structural similarity, the lack of chromophores and their high boiling point and low volatility.
We present a qNMR method for the determination of low calories sweeteners (erythritol, mannitol, maltitol, sorbitol, isomalt and xylitol) in sugar-free foodstuff. The structural similarities of these compounds determine often a severe spectral overlap that hampers their quantification via conventional 1D and 2D NMR spectra. This problem is here overcome by exploiting the resolving capabilities of the CSSF-TOCSY experiment (Fig.1), allowing the quantification of all six polyols, with satisfactory results in terms of LoQ (2.8–7.4 mg/L for xylitol, mannitol, sorbitol, 15 mg/L for erythritol, 38 mg/L for maltitol and 91 mg/L for isomalt), precision (RSD% 0.40–4.03), trueness (bias% 0.15–4.81), and recovery (98–104%). Polyol's quantification in different sugar-free confectionary products was performed after a simple water extraction without any additional sample treatment [4]. While these results demonstrate the robustness of the proposed method for polyols quantification in low calories foods, its applicability can be further extended to other food matrices or biofluids.

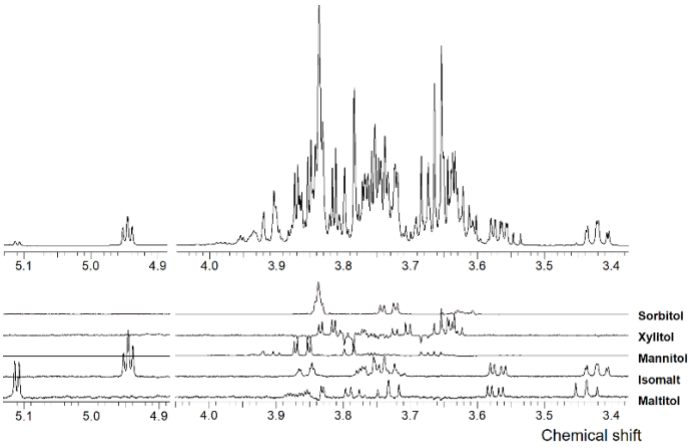

Fig. 1. ^{1}H NMR spectrum of a sugar-free chewing-gum sample (top)
Selective spectra of the contained polyols (bottom).

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[P-116] NMR-BASED AUTOMATIC PROTOCOL FOR THE ASSESSMENT OF HONEY AUTHENTICITY

E. Schievano,[‡] M. Tessari[†]

[‡] Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy
[†]Magnetic Resonance Research Center, Radboud University, Nijmegen, the Netherlands
E-mail: elisabetta.schievano@unipd.it

Keywords:
solution NMR, metabolomics, food.

Chloroform extracts of honey contain plant nectar metabolites and compounds secreted by the bees that can provide information about its botanical, geographical, and entomological origin [1,2,3]. The ^{1}H NMR spectrum of such an organic extract can, therefore, be considered as a fingerprint of honey to confirm or disprove its authenticity [4]. Here we present a protocol to check honey authenticity based on the automatic analysis of the NMR spectrum of its organic extract. From the integrals of specific regions of the spectrum we demonstrate that it is possible to reveal the presence of honey manipulations or confirm its genuineness. In the case of non-genuine samples, the values of these integrals allow to formulate some hypotheses about the corresponding type of adulteration. This approach has proved able to unmask adulterations that had escaped official methods such as pollen analysis and Isotopic Ratio Mass Spectrometry. Importantly, the method requires only a single NMR measurement on a low amount of material, with reduced sample preparation time and can, in principle, be applied to high-throughput analysis of honey.

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[P-117] NMR METABOLOMIC CHARACETRIZATION OF DANDELION AND LEMON BALM PLANTS GROWN WITH DIFFERENT CONDITIONS

M. Spano,‡ G. Di Matteo,‡ F. Masciulli,‡ D. Ambroselli, ‡ E. Romano,‡ L. Mannina‡

‡ Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

E-mail: mattia.spano@uniroma1.it

Keywords: solution NMR, metabolomics, food

The plant world has always represented a source of products from which obtain natural molecules useful in the pharmaceutical field. However, the whole set of secondary metabolites present in different plant matrices can represent a very useful source of products for the food and nutraceutical fields.
The nutraceutical properties of natural products have increased the interest in the field of medicinal plants. Among them, Dandelion (*Tarassacum officinale*) and Lemon Balm (*Melissa officinalis*), are largely used in Italy for obtaining products for human health [1].
The chemical profile, and consequently, the biological properties of a plant product are sensitive to various factors such as light, temperature, humidity, altitude, nature of the soil, and cultivation. It is therefore necessary to evaluate the difference, in qualitative and quantitative terms, of the metabolomic profile of these plant products when they are subjected to different growth conditions.
The present study is aimed at the characterization of the NMR metabolomic profile of *Tarassacum officinale* and *Melissa Officinalis* cultivated in Lazio region and the evaluation of chemical variations due to different growth factors.
The Bligh-Dyer hydroalcoholic extracts of root and aerial part of *Tarassacum officinale* and *Melissa officinalis* were characterized through the application of ^{1}H and 2D experiments (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC). In the hydroalcoholic phase, several classes of secondary metabolites namely sugars, organic acids, amino acids, including essential ones, and other compounds were identified and quantified.
The NMR characterization of the considered samples allowed to obtain the metabolomic profile of *Tarassacum officinale* and *Melissa officinalis* and, in particular, to diversify, in qualitative and quantitative terms, the ecotypes cultivated with different methodologies, allowing to delineate differences for both health and nutraceutical uses and to support agronomic research in order to develop the most profitable production technique.

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[P-118] PRELIMINARY DEVELOPMENT OF A MAGNETIC RESONANCE FINGERPRINTING FRAMEWORK FOR FAST MULTIPARAMETRIC LOW-FIELD NMR RELAXOMETRY

G.V. Spinelli[1], F. Zama[1], G. Landi[1], L. Brizi[2,3], M. Barbieri[4], V. Bortolotti[5], D. Remondini[2,3], and C. Testa[2,3].

[1]Department of Mathematics, University of Bologna, Bologna, Italy
[2]Department of Physics and Astronomy, University of Bologna, Bologna, Italy
[3]INFN, Istituto Nazionale di Fisica Nucleare, Sezione di Bologna, Bologna, Italy
[4]Department of Radiology, Stanford University, Stanford, CA, United States
[5]Department of Civil, Chemical, Environmental and Materials Engineering, University of Bologna, Bologna, Italy

E-mail: giovanni.spinelli4@unibo.it

Keywords: low field NMR.

Magnetic Resonance Fingerprinting (MRF) is an MRI methodology that allows multiparametric mapping in a one-shot measurement [1] exploiting a pulse sequence designed to make the magnetization evolution simultaneously dependent from multi MR parameters (the so-called fingerprint). A pattern recognition method is used to match the experimental signal with a dictionary of simulated fingerprints.
In this preliminary study, we investigate the feasibility of developing a MRF framework with a low-field NMR device.
The application of MRF to compact low-field devices is challenged by static (B_0) and excitation (B_1) field inhomogeneities, which are more severe than those found in high-field MRI scanners.
Different samples of CuEDTA-doped distilled water at various concentration were scanned with a low-field NMR customized Jeol C-60 electromagnet using the KEAII spectrometer.
The acquisition protocol included a 2D-ANGLE-FID sequence for B_0-B_1 characterization (in fig. 1) [2], a novel IR-bSSFP-like sequence to acquire the MRF, and standard CPMG and IR sequences for ground-truth T_1 and T_2 characterization.
The reference T_1 and T_2 values were used to verify the ability to simulate the real MRF signals (Bloch simulations using in-house Matlab toolbox, MARSS [3]). The root-mean-squared-error between real and simulated MRF signals were used as similarity metric.
The full MRF framework (fig. 1) was tested by training a fully connected Deep Neural Network [4] (fc-NN). The pre-trained fc-NN was used to predict the CuEDTA-doped water (T_1,T_2) values and compared with the reference values.
Simulated and acquired MRF signals of the CuEDTA sample are shown in Fig. 2. The synthetic signal was generated using the T_1 and T_2 values predicted by the fc-NN algorithm. A good match was obtained (RMSE $=3\cdot10^{-4}$ a.u.).
This preliminary study showed the feasibility of applying the MRF framework using low-field instruments since the simulated MRF signal and the experimental MRF data were in excellent agreement. This was achieved using an accurate characterization of the B_0-B_1 correlation map.

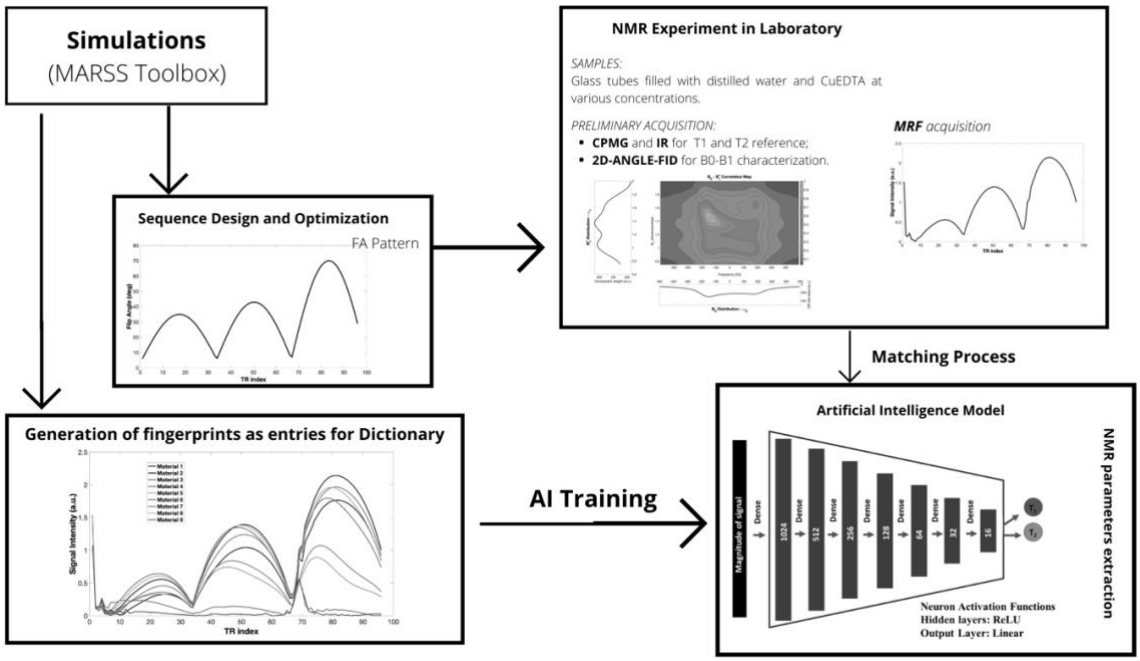

Figure 4: Framework of the proposed method.

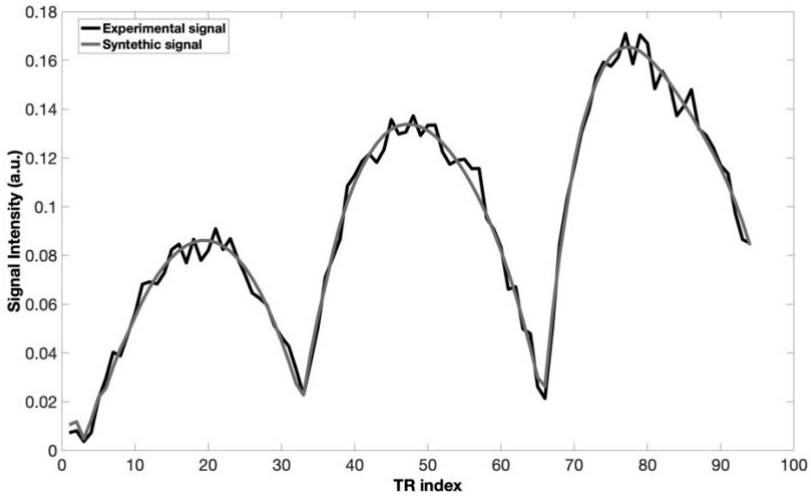

Figure 5: Comparison between experimental and simulated signal. Sample with T1 = 2.2 ms and T2 = 1.9 ms. The relaxation times extracted by the network were T1 = 3.2 ms and T2 = 2.1 ms. The RMSE was 0.0003.

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[P-119] REX: A NEW COMMUNITY-ORIENTED SOFTWARE FOR NMR RELAXOMETRY

G. Selva, E. M. Vasini, S. Sykora,

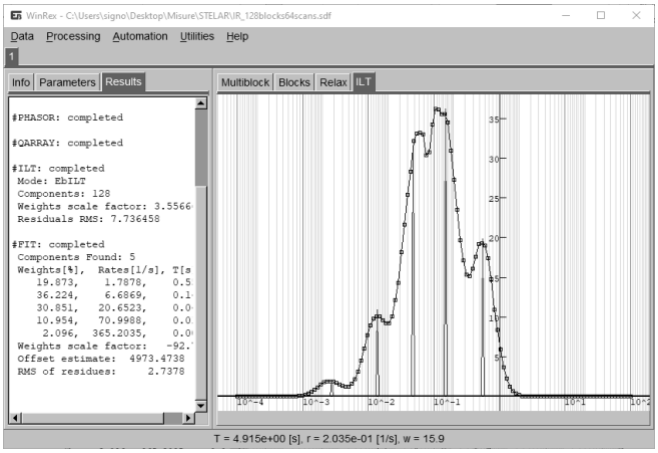
Extra Byte, p.zza Mazzini 80, 20022 Castano Primo (MI) Italy
E-mail: gselva@ebyte.it

Keywords:
NMR, time-domain, relaxometry, spectroscopy, diffusometry, data evaluation, software, methods, algorithms, tool, applications development

REX was conceived as a low-cost commercial software platform for evaluation of relaxometry data of all kinds. It currently supports low-field time-domain NMR data, but the plan is to extend it also to high-resolution relaxometry, as well as to more complex multi-dimensional techniques based on various combinations of relaxometry, spectroscopy, and diffusometry.

After loading raw experimental (or even simulated) data, REX permits a wide series of operations that are useful to most researchers using NMR relaxation and/or to developers of NMR applications involving relaxation. Such operation include: data sub-selections, denoising, TD-phasing, FID's fitting and quantifying, building of relaxation curves of any type, fitting and/or ILT (inverse Laplace transforms) of the latter, building & fitting relaxation profiles (in case of FFC), building & fitting temperature profiles of relaxation curves, as well as FFT and subsequent frequency-domain processing (such as apodization, phasing, baseline correction, integration).

Example of relaxation times distributions obtained with REX on a sample of common building stone.
Discrete and continuous ILT results are plotted and compared.



Note: one problem we have encountered is that in NMR relaxometry, there does not exist any standard data format, while there is a growing need to share raw data, make them more persistent in time, and more accessible to a wider audience. As of today, REX supports only a limited number of data formats such as plain text, Stelar (including FFC), Resonance Systems, some Bruker, some Oxford Instruments, … However, since REX is a community-oriented software, we guarantee that we will happily adapt it to any data format, once we get an actual example of the data.

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[P-120] DETERMINATION OF TOTAL CONTENT OF ANY NUCLIDE FROM EITHER HIGH- OR LOW-FIELD TD- NMR SPECTRA

G. Selva[1], M. Abele[2], S. Falkenstein[2], E. M. Vasini[1], S.Sykora[1]

[1]Extra Byte, P.zza Mazzini 80, 20022 Castano Primo (MI) Italy
[2] Evonik Operations GmbH, Smart Materials, Untere Kanalstrasse 3, 79618 Rheinfelden, Germany
e-mail: g.selva@ebyte.it

Keywords:
NMR, qNMR, quantitation, assay, time-domain, high-resolution, low-resolution, calibration, sample components, canonical subfractions

The fact that NMR is an intrinsically quantitative method is well known, even though the exact definition of the term is not as trivial as it might look. Simplifying a bit, we can say that it means that the signal amplitude just after the pulse is proportional to the number of nuclei present in the sensitive sample volume.

Upon a closer look, however, one notices further complications in converting the generic statement about NMR linearity into actual practice. Complications like:

- The local NMR sensitivity is not uniform over the whole sample volume. This must be taken into account since it makes the total number of X-nuclei assays very different from the total X-nuclei concentration assays.
- The initial parts of FID's are distorted by probe ringing, and by receiver recovery effects, which obscure the physically true shape of an FID just after the pulse.
- With recent digital receiver models (>1995), the initial parts of an FID are further very heavily distorted by the artefact known as 'group delay'.
- If we desire a method that quantifies the nuclide X regardless of its chemical contexts inside the sample (chemical shifts and/or coupling constants), additional complications arise on high-resolution instruments since the evolution of the FID a certain time after the pulse is affected much more by the chemical and chemico-physical nature of the sample than just by the total content of the nuclide. On the other hand, use of high-field instruments is desirable since they have much higher sensitivity than their low-field counterparts, and because their high-resolution versions are widely available.

In this presentation we will illustrate and discuss the experimental procedures, supported by a related software utility TotalXMR, whose primary aim is the best possible estimate of the absolute amount of a nuclide X in the sample (or, alternatively, its volume concentration), immune, or almost immune, to all the above-listed complications. It is a time-domain based method that can be used with both low-resolution and high-resolution instruments. However there is a set of optimal assay parameters specific for each nuclide (1H, 13C, 29Si, 19F, 7Li, …) and possibly also each type of instrument, and a calibration procedure for each case (the software keeps a record of all these data)

While developing the method, we have realized that we can easily build-in all the usual ("canonical") differentiations between possible sample components ("mobile" 29Si versus the "immobile" one in the sample tube, aromatic 13C versus aliphatic, 1H or 13C in amorphous parts of polymers versus the crustalline fractions, etc.). At the writing of this abstract, all these possibilities are still in development, but some reults are likely to become available at the time of the meeting.

[P-121] DISCRIMINATION AND QUANTIFICATION OF ENANTIOMERS VIA NON-HYDROGENATIVE PARAHYDROGEN INDUCED POLARIZATION

M. Tessari‡

‡Magnetic Resonance Research Center, Radboud University, Nijmegen, the Netherlands
E-mail: m.tessari@science.ru.nl

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

Reversible association of small molecules and parahydrogen to an iridium catalyst forms the basis of SABRE techniques for nuclear spin hyperpolarization in solution [1]. Under suitable conditions, hyperpolarization of the transient complex resulting from this reversible association can be exploited to detect specific analytes at concentrations much lower than routinely observed in thermal NMR measurements. In this respect the iridium catalyst employed in SABRE can act as an *NMR chemosensor*, allowing the selective detection of dilute analytes in complex mixtures, while removing the large signal background originating from other species in solution [2,3]. Here, the most recent NMR applications concerning the detection and discrimination of enantiomers by non-hydrogenative Parahydrogen Induced Polarization (nhPHIP) will be presented. Our results demonstrate that it is possible to quantitatively discriminate enantiomers directly in biofluids or natural extracts without any prior functionalization or separation.

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[P-122] NMR INVESTIGATION OF INTERACTION BETWEEN TAU AND NATURAL COFFEE COMPOUNDS AND THEIR EFFECTS ON AGGREGATION

R. Tira,[‡] G. Viola, [‡] C. G. Barracchia, [‡] F. Floriani, [‡] D. Trivellato, [‡] F. Munari, [‡] M. Assfalg, [‡] M. D'Onofrio.[‡]

[‡]Department of Biotechnology, University of Verona, Italy
E-mail: roberto.tira@univr.it

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

Neurodegenerative diseases (NDs) are an ever-increasing threat to human life. An early event in NDs is the accumulation of specific proteins in neuronal cells, leading to cellular dysfunction, loss of synaptic connections, and brain damage.
In Alzheimer's disease, one of the pathological hallmarks is the presence of intracellular neurofibrillary tangles composed of "paired helical filaments" of hyper-phosphorylated Tau protein.
Mounting evidence suggests the possibility to perturb the formation of aggregates species of Tau using food derived small molecules, macromolecules, and nanoparticles. Coffee and coffee compounds are attracting the interest in the field of neuro-inflammation and neuro-protection against oxidative-stress because of their bioavailability and ability to cross the Blood Brain Barrier [1]. Recent studies showed that brewed-coffee and molecules such as phenylindanes and other flavonoids, have the ability to inhibit Aβ and Tau protein aggregation [2].
In our work we investigate the effects of Italian espresso coffee extract and a selection of coffee-derived bioactive molecules towards mitigation of Tau aggregation. We first studied the kinetics of aggregation and the conformational transitions of Tau in the presence of Italian espresso coffee, caffeine, trigonelline, theobromine, and genistein.
To obtain further insight on the mechanism of the modulation of Tau aggregation, we employed NMR. Specifically, we acquired NMR spectra to characterize the composition of the coffee extract. We then performed titration experiments of 15N-Tau with the selected compounds to verify possible conformational changes of the protein in their presence. Moreover, we employed STD and WaterLOGSY spectra to determine if coffee extract, caffeine and genistein are able to interact with Tau in its aggregated state. We believe that this approach based on natural and readily available molecules could open new possibilities in the Alzheimer's disease treatment.

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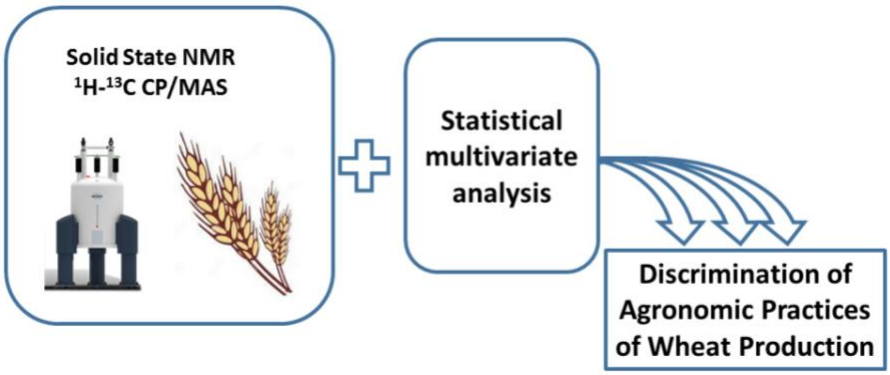
[P-123] EVALUATION OF FARMING PRACTICES IN WHEAT PRODUCTION BY ^{1}H-^{13}C CP-MAS SPECTRA

S. Todisco,[‡] B. Musio,[‡,†] R. Ragone,[‡] M. Antonicelli,[‡] M. Triggiani,[‡,†] G. D'Orazio,[‡] P. Mastrorilli,[‡,†] V. Gallo[‡,†]

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

Keywords: solid state NMR, metabolomics, food.

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



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[P-124] REAL TIME NMR ANALYSIS OF BACTERIA METABOLISM DETECTS EFFECTIVE CONCENTRATIONS OF BACTERICIDAL MOLECULES RELEASED FROM MEDICAL DEVICES

Simona Tomaselli[‡], Mariacecilia Pasini [‡], Erika Kozma [‡], Umberto Giovanella [‡], Guido Scavia[‡], Katiuscia Pagano [‡], Salvatore Iannace [‡], Laura Ragona [‡]

[‡] Istituto di Scienze e Tecnologie Chimiche (SCITEC), Consiglio Nazionale delle Ricerche – CNR, Milan, Italy
E-mail: simona.tomaselli@scitec.cnr.it

Keywords: solution NMR, materials, exotica.

Bacterial colonization of biomedical devices surfaces is one of the most important causes of infections during hospitalization. Many different molecules with antibacterial activity have been incorporated into devices and the active molecule release is a crucial issue in light of the final device biocompatibility. We report here a NMR-based approach to detect drug effective concentration released in solution and guide the optimization of low releasing silicon catheters including an anti-microbial agent. The newly proposed strategy is based on real time detection of metabolism of bacteria grown in the presence of the functionalized material. The kinetics of nutrients consumption and metabolites production is translated in effective concentration of released active molecule. The use of bacteria as sensors lowers the detection limit of two orders of magnitude with respect to the direct NMR observation of the released molecule.
The method is here employed to optimize the protocol of material impregnation and to obtain low releasing silicon tubes active against planktonic and sessile E. coli and S. aureus.
The proposed NMR approach, detecting sub-lethal amounts of released bactericidal molecules, can be easily extended to other materials to support the development of new highly biocompatible medical devices.

Acknowledgements
This work was carried out with the financial support of Regione Lombardia Project “Piattaforma tecnologica per lo sviluppo di sonde innovative in ambito biomedicale” (ID 244356). SIDAM srl (Mirandola, Italy) is acknowledged for the supply of catheter silicon tubes and for collaboration in the aforementioned project. Fondazione Antonio De Marco is acknowledged for financial support.

[P-125] STRUCTURAL CHARACTERISATION OF NOVEL NATURAL PRODUCTS FROM RARE ACTINOMYCETES *DACTYLOSPORANGIUM VINACEUM* AND *FULVUM* USING SOLUTION NMR, MS, AND ANALYSIS OF THEIR BIOSYNTHESIS

X. Trivelli,[‡] T. Caradec,[†] S. Moureu,[†] H. Drobecq,[†] B. Villemagne,[#] A. Herledan,[#] N. Willand,[#] R. Hartkoorn[†]

[‡] Institut Michel-Eugène Chevreul-FR2638, Univ. Lille, CNRS, INRAE, Centrale Lille, Univ. Artois, Villeneuve d'Ascq, France
[†] Center for Infection and Immunity of Lille-U1019-UMR9017, Univ. Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, Lille, France
[#] Drugs and Molecules for Living Systems-U1177, Univ. Lille, INSERM, Institut Pasteur de Lille, Lille, France
E-mail: xavier.trivelli@univ-lille.fr

Keywords:
solution NMR, **biomolecules**.

Natural products make up the backbone of anti-infective therapy, as well as being a major contributor to the treatment of non-infectious diseases. With increasing levels of drug resistance emerging, it is important that novel bio-active molecules are discovered, researched and developed to feed the pipeline of potential future drugs, and novel natural products are a rich potential source of such molecules. Here, we investigated two novel natural products produced by the rare actinomycetes of the genus *Dactylosporangium*, characterised their structure, biosynthesis and potential function.
Firstly, we investigated the chemical identity and function of a deep wine red-coloured diffusible pigment produced by *Dactylosporangium vinaceum* using a combination of LC-MS and solution NMR [1]. In parallel we performed whole genome sequencing and transcriptional analysis of the genome in the absence and presence of a chemical modulator found to inhibited pigment production, to identify the associates PKS based biosynthetic gene cluster. This data identified the pigment to belong to the chemical family of Rubrolones, and subsequent analysis of their potential environmental role found them to react with a number of amine-containing antibiotics, antimicrobial peptides and siderophores as shown by MS then solution NMR pointing to them acting as potential "minesweepers" of xenobiotic molecules in the bacterial environment. NMR was crucial to determine which amine function had reacted with prerubrolone.
The second class of natural products that were identified and characterised were from *Dactylosporangium fulvum* where a novel series of multi-glycosylated 22-membered polyene macrolides were identified, here named Dactylosporolides A-C [2]. These molecules were isolated and structurally characterised by solution NMR. Again, whole genome sequencing was used to assemble the genome of the producing bacterium, and genetic gene invalidation was used to pin point the associated biosynthetic gene cluster. Dactylosporolide C was found to be active against S. *pneumoniae* and *P. falciparum*.

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[P-126] DRUG DIFFUSION IN BICONTINUOUS POLYMERICSYSTEMS

V. Vanoli, F. Pizzetti, G. Massobrio F. Rossi, A. Mele, F. Castiglione[1]

Department of Chemistry, Materials and Chemical Engineering “Giulio Natta”, Politecnico di Milano, Italy.
Email: valeria.vanoli@polimi.it
Keywords: HR-MAS NMR, small molecules, polymers.

Bicontinuous jammed emulsions, known as bijels, are particular Pickering emulsions where oil and water are both continuous phases. Due to the presence of biphasic domains, hydrophilic and hydrophobic drugs can be loaded simultaneously so to have a system suitable for drugs codelivery. Drug delivery systems have become increasingly interesting in different fields, from medicine to materials science, thanks to the possibility to deliver drugs in a more controlled and targeted way [1]. One on the key parameters to consider in this context is the diffusion of drugs in the scaffold matrix, as it helps to understand how the diffusive mechanisms governing solute transport at the nanoscale work. One way of the most popular and commonly accepted procedure to investigate the dynamics of heterogeneous soft-materials and polymers [1] is the Pulse Gradient Spin Echo (PGSE) NMR techniques and the analysis of the experimental data based on Gaussian diffusion approximation, which yield the diffusion coefficient. These approaches can be applied to drug delivery studies, helping to understand how the diffusive mechanisms governing solute transport at the nanoscale work.

The bijel-like structures studied in this work is prepared using a hydrophobic monomer (caprolactone) able to polymerize in bulk, resulting in a bicontinuous system with polymer and water as immiscible phases. The emulsion is stabilized by colloidal nanoparticles made of hydroxyapatite (inorganic) or nanogel nanoparticles (organic) [2].

Samples loaded with different concentrations (25-100 mg/ml) of a hydrophilic drug, ethosuximide, and bijels loaded with dimethyl fumarate (12.5-25 mg/ml), as hydrophobic drug, were prepared. The diffusion coefficient of the drugs was investigated by ^{1}H High Resolution Magic Angle Spinning (HR-MAS) NMR Spectroscopy. The diffusion coefficient of ethosuximide and dimethyl fumarate were analyzed in comparison with the dynamics of the drugs in water and caprolactone solutions respectively.

The results show a slower diffusion of both drugs in bijels compared to the solution. Moreover, the diffusion coefficient of the ethosuximide shows a decreasing trend with the increase of the concentration both in bijels and in solution. Further studies will be conducted on the co-delivery of the two drugs.

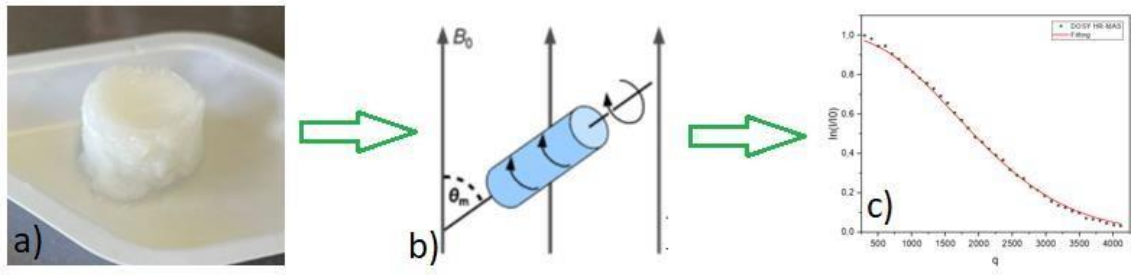

Fig.1 – a) Polymer-water bijel; b) HR MAS schematic principle; c) example of Gaussian curve obtained withHR-MAS experiment that yields the diffusion coefficient of the drug.

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[P-127] ^{31}ParaCEST: ^{31}P MR-CEST Imaging based on the formation of ternary adduct between $HPO4^{2-}$ and Eu(III)DO3A complexes

G. Vassallo‡, F. Garello‡, S. Aime‡, E. Terreno‡ and D. Delli Castelli‡

‡Department of Molecular Biotechnology and Health Science, Via Nizza 52 Turin, Italy E-mail: giulia.vassallo@unito.it

Keywords: solution NMR, MRI, contrast agents.

Chemical Exchange Saturation Transfer, better known as CEST, is a technique that allows the detection of molecules in low concentration exploiting the amplification effect of their NMR signal through its indirect visualization over a much intense signal. As far as ^{1}H MRI is concern the minimum concentration of molecules able to provide a detectable saturation transfer on bulk water is in the millimolar range. It has recently been reported that by using nuclei different from protons it is possible to lower the concentration of detectable molecule by reducing the concentration of the bulk signal [1]. Unfortunately, this studies are poorly translatable because they lack of an endogenous bulk signal. In this work we have explored the efficacy of performing 31P CEST using as endogenous bulk molecule inorganic phosphate (Pi) and Pi bound to a paramagnetic complex as exchanging pool. Pi is quite abundant in vivo (1-10 mM depending on the district), nevertheless its concentration is three order of magnitude lower than the concentration of water, thus it is expected that micromolar concentration of CEST agents could be detected. The chosen complex is Eu(III)DO3A that is known to strongly bind Pi thus shifting its NMR signal. ^{31}P Z-spectrum of this system display a saturation Transfer centred at-134 ppm from the signal of diamagnetic Pi (Fig 1). The sensitivity threshold was evaluated and the minum detectable amount of the bound substrate was 5µM. Owing to the encouraging results, we have tested the feasibility of cellular labelling. TS/A cells were loaded with EuDO3A by the hypotonic swelling method [2] and a ^{31}P saturation transfer of 20% could be observed in the labelled cell pellet.

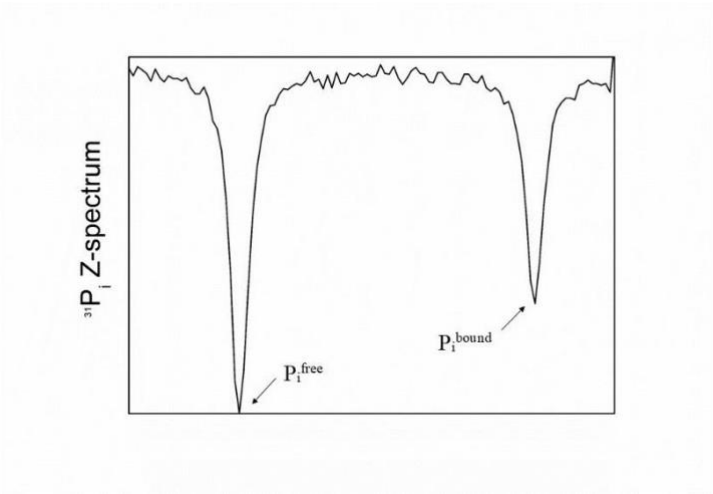

Fig 1. ^{31}P Z-Spectrum of a solution containing EuDO3A 40µM and KH_2PO_4 10 mM, pH 7.

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[P-128] ^{19}F-NMR Fragment Screening & Biophysical Assays for the Identification of Novel Selective GSK-3β Inhibitors

Marina Veronesi[‡§], Beatrice Balboni[†‡], Shailesh Kumar Tripathi[†], Debora Russo[‡], Ilaria Penna[‡∫], Barbara Giabbai[ł], Tiziano Bandiera[‡], Paola Storici[ł], Stefania Girotto[†§] and Andrea Cavalli[†]

[‡] D3 PharmaChemistry, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy;
[§] Structural Biophysics and Translational Pharmacology Facility, Via Morego 30, 16163 Genova, Italy;
[†] Computational and Chemical Biology, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy;
[‡] Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna,Italy;
[∫] Medicinal Chemistry And Technologies for Drug Discovery and Delivery, Via Morego 30, 16163 Genova, Italy;
[ł] Structural Biology Laboratory, Elettra Sincrotrone Trieste S.C.p.A., Basovizza, 34149 Trieste, Italy
E-mail: marina.veronesi@iit.it

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

In the last decades, ^{19}F-NMR has become the leading technique for hit identification in the Fragment-Based Drug Discovery (FBDD) approach [1], thanks to its ability to detect ligands with low affinity [2]. The success of the technique is confirmed by the development of specific fluorinated chemical libraries [3] and optimized ^{19}F-NMR screening methods [4, 5]. Glycogen synthase kinase 3 beta (GSK-3β) is a serine-threonine kinase, constitutively active, that plays a critical role in many intracellular pathways [6] and in regulating transcription factors controlling the expression of different genes. GSK-3β is dysregulated in many pathologies such as cancer, type II diabetes, and Alzheimer's disease, and is a validated target of pharmaceutical interest [7]. Unfortunately, most of the identified GSK-3β inhibitors lack selectivity versus other kinases and show toxicity, mainly because a strong GSK-3β inhibition affects a large number of pathways. The aim of this work was the identification of new GSK-3β inhibitors able to bind protein hotspots other than the ATP binding pocket, or to the ATP binding pocket, but with an affinity comparable to that of ATP. To this purpose, we conducted ^{19}F-NMR competition binding screening, using two different ATP concentration. To select the most promising hits, showing selectivity for GSK-3β and modulating its activity without complete inhibition, we set up several biophysical assays and a phosphorylation activity assay on a panel of 58 kinases. We managed to identify two small molecules that appears to overcome the most common flaws of potent GSK-3β inhibitors [8].

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[P-129] ULTRASMALL GOLD NANOPARTICLES: POSSIBLE CANDIDATES TO MITIGATE AMYLOIYDOGENIC PROTEIN AGGREGATION

G.Viola,‡ C.G. Barracchia,‡ R. Tira,‡ F. Munari,‡ M. D'Onofrio,‡ M. Assfalg,‡

‡ Department of Biotechnology, University of Verona, 37134 Verona, Italy
E-mail: giovanna.viola@univr.it

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

Amyloidogenic intrinsically disordered proteins (IDPs) have been attracting considerable scientific interest. These proteins are found to undergo aberrant aggregation under defined conditions and are associated with irreversible neurodegenerative diseases. The protein tau, an IDP, is strongly associated with Alzheimer's disease. [1]
Nanomaterials are increasingly investigated for the design and development of biotechnological and biomedical applications. [2,3] In particular, nanoparticles can be produced in a variety of diverse formulations and can be optimized for interaction with a specific biological target. Among NPs, ultrasmall NPs (usNPs), usually defined as particles with core size in the range of 1–3 nm, have drawn increasing attention in recent years due to their distinctive physicochemical properties and unique biological behaviors. [4,5]
In this context, elucidating the interactions of fibrillogenic proteins with NPs and the associated conformational rearrangements could provide the molecular basis for developing new treatments. [6]
In our work we synthetized and characterized ultrasmall Gold Nanoparticles (usGNPs) and we investigated their interaction with the protein tau. We used different biophysical techniques, including Nuclear Magnetic Resonance (NMR), to obtain thermodynamical and chemical information about their interaction. We performed titration experiments by acquiring 2D ^{1}H-^{15}N HSQC spectra to gain insight into the adsorption mechanisms of tau onto usGNPs, at single-residue resolution. The intensity changes of peaks in the HSQC spectra allowed us to define the interaction region. To gain insight into the conformational dynamics of tau in the presence of usGNPs, we carried out ^{15}N-spin Carr–Purcell Meiboom–Gill Relaxation Dispersion (CPMG-RD) experiments. [7] The analysis of R_2^{obs} vs ν_{CPMG} dispersion curves provides information on the underlying dynamic process. The protein alone displayed virtually no relaxation dispersion, by contrast small but clear-cut RD effects for some residues were apparent in the presence of usGNPs.
Finally, we explored the activity of usGNP in different aggregation assays. Based on transmission electron microscopy images we found, that, usGNP influenced aggregation and, at the higher concentration we tested, they were able to inhibit the formation of the fibrils.

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[P-130] CHEMICAL CHARACTERIZATION OF ZINC GRANULES IN THE MALPIGHIAN TUBULES OF *DROSOPHILA MELANOGASTER*

D. Vitone,[‡] A. Barbanente,[‡] E. Garay,[†] N. Schuth,[§] L. Quintanar,[§] F. Missirlis,[†] F. Arnesano[‡]

[‡]Department of Chemistry, University of Bari, 70125 Bari, Italy.
[†]Department of Physiology, Biophysics and Neuroscience, Cinvestav, 07360 Mexico City, Mexico.
[§]Department of Chemistry, Cinvestav, 07360 Mexico City, Mexico.
E-mail: daniele.vitone@uniba.it

Keywords:
solution NMR, small molecules, biomolecules.

The storage of zinc (Zn) in Drosophila melanogaster depends on the consumption of tryptophan [1]. Specifically, the tryptophan metabolite kynurenine (kyn) is released from insect fat bodies and induces the formation of Zn storage granules in the Malpighian tubules, where 3-hydroxykynurenine (3HK) and xanthurenic acid (XA) act as endogenous Zn chelators (see Fig. 1). Confocal microscopy and Zn K-edge X-ray absorption spectroscopy (XAS) were performed on the Malpighian tubules to assess Zn coordination in situ. Moreover, the chemical characterization of Zn complexes with kyn metabolites was carried out in solution by nuclear magnetic resonance (NMR), UV-vis and fluorescence spectroscopy and by electrospray ionization mass spectrometry (ESI-MS). Finally, ab initio density functional theory (DFT) calculations yielded the structure of Zn complexes with metal-ligand distances consistent with those determined experimentally by XAS analysis. The discovery that the tryptophan metabolites 3HK and XA are the major Zn-binding ligands in insect cells establishes the kynurenine pathway as a regulator of systemic Zn homeostasis. This novel direct molecular link will allow the elucidation of many biological processes modulated by Zn and the kynurenine pathway, such as immunity, blood pressure, aging and neurodegeneration [2-7].

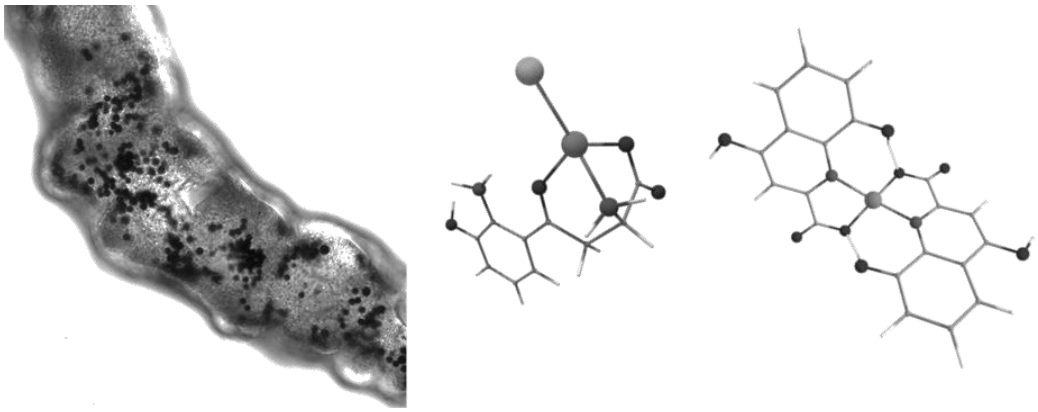

Fig. 1. Confocal image of zinc granules within *Drosophila* Malpighian tubules and structural models of the identified complexes, 3HK-Zn-Cl and XA-Zn-XA.

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[P-131] SPECIATION AND TRANSPORT PROPERTIES IN MOLTEN CARBONATES AT HIGH TEMPERATURE

A. Zhadan[1],‡ A. Carof [2], L. Del Campo[1], L. Cosson[1], V. Sarou-Kanian[1], M. Malki[1], C. Bessada[1]
[1] CEMHTI-CNRS, 45100 Orleans, France
[2] LPCT-CNRS, 54506 Vandoeuvre-les-Nancy, France
E-mail: antonii.zhadan@cnrs-orleans.fr

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

The capture of CO_2 and its exploitation have become a significant worldwide issue because of environmental reasons. Among different methods proposed to take advantage of this energy source, the electrolysis of CO_2 in molten carbonate batteries, the MCFCs (molten carbonate fuel cells), is one of the most efficient solution. Actually, the stability of CO_2 is not efficient in most of solvents except in molten carbonates. These systems are liquids in large temperature and pressure ranges. They have low viscosity and a significant capability to dissolve volatile compounds such as water and CO_2. To better understand speciation and physical properties of molten carbonates in fuel cells we need to combine various methodological developments.
This work deals with the structural and transport properties study of different mixtures of molten alkali carbonates. The HT NMR [1] (see Fig. 1), HT PFG NMR [2] and NMR MAS were used to study different compositions of carbonates and carbonate glasses synthetized at high temperatures and pressures. The high temperature experiments were conducted under CO_2 atmosphere up to 800°C. We proposed some relation between electrical conductivity and self-diffusion coefficients, using theoretical calculations [3], [4], and the construction of a consistent model describing the physical properties of these melts.

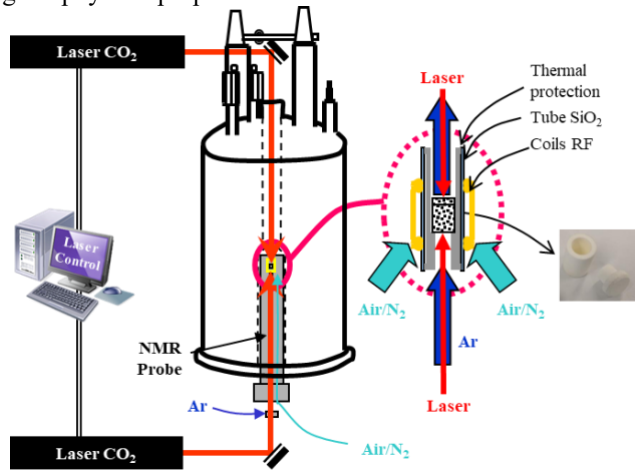

Fig. 1. High temperature NMR experimental setup [1]

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[P-132] MOLECULAR UNDERSTANDING OF THE MECHANISM OF ACTION OF AN ANTI-PRION PORPHYRIN

Chiara Zucchelli[a], Giacomo Quilici[a], Federica De Leo[a], Antonio Masone[b], Giada Lavigna[b], Enrico Caruso[c], Alfredo Cagnotto[b], Giuseppe Ciossani[d], Valentina Cecatiello[e], Sebastiano Pasqualato[e], Mario Salmona[b], Jesús R. Requena[f], Gabriele Giachin[g], Roberto Chiesa[b], Giovanna Musco[a]

[a] Biomolecular NMR Unit, Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy.
[b] Mario Negri Institute for Pharmacological Research, Via Giuseppe La Masa 19, 20156 Milan, Italy.
[c] Department of Biotechnology and Life Science, University of Insubria, Via Ravasi 2, 21100 Varese, Italy.
[d] Biochemistry and Structural Biology Unit, IEO, Via Adamello, 16, 20139 Milan, Italy
[e] Structural Biology Research Centre, Human Technopole, V.le Rita Levi-Montalcini, 1, 20157 Milan, Italy
[f] CIMUS Biomedical Research Institute, University of Santiago de Compostela-IDIS, Avenida de Barcelona s/n 15782 Santiago de Compostela, Spain.
[g] Department of Chemical Sciences (DiSC), University of Padua, Italy
E-mail: zucchelli.chiara@hsr.it

Keywords: solution NMR, small molecules, biomolecules

Porphyrins are organic compounds coordinating metal cations and consisting of four pyrrole rings linked by methine groups. Prion protein (PrP^C) is a mammalian, highly conserved GPI anchored glycoprotein, expressed predominantly in the central nervous system and possibly involved in copper metabolism. It folds into a C-terminal globular domain and a long, unstructured N-terminal region responsible for pH-dependent copper binding. Prion diseases (hereditary, sporadic or acquired) are neurodegenerative disorders of mammals, currently untreatable and thus invariably fatal. They arise from the conversion of PrP^C structure into a β-sheet rich, pathogenic and infectious form (PrP^{Sc}), which propagates by inducing misfolding of native PrP^C. PrP^{Sc} rapidly accumulates in the brain causing neuronal dysfunction and degeneration. We applied NMR spectroscopy and other biophysical techniques to investigate the mechanism of action of a metal-prophyrin (VA01) that inhibits prion propagation:
i) we proved direct VA01 interaction to human PrP^C, we measured binding affinity by ITC experiments and we characterized the protein binding pocket by NMR chemical shift mapping;
ii) we investigated whether different VA01 functionalizations and coordinated metal cations affect PrP^C interaction;
iii) we used circular dichroism melting experiments and SAXS to study changes in PrP^C stability and shape upon VA01 binding.
Our results indicate that VA01 interaction promotes opening of PrP^C structure and destabilization of the globular domain. Collectively our structural data allow for a molecular understanding of the mechanism of action of this antiprion ligand and prelude for the design of optimized metal-porphyrins with increased affinity.

[P-133] MUSCLE-WISE PREDICTION OF QUANTITATIVE MUSCLE MRI BIOMARKERS IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY THROUGH RADIOMICS AND MACHINE LEARNING

F. Brero[‡], L. Barzaghi[†,⍏], G. Colelli[†,⍏], I. Postuma[‡], P.F. Felisaz[†,⍏], M. Paoletti[⍏], G. Manco[⍏], X. Deligianni[⁜,ⅆ] F. Santini[⁜,ⅆ], M. Monforte[ⅼ], G. Tasca[ⅼ], N. Bergsland[ⱺ], A. Lascialfari[†,‡], A. Pichiecchio[†,⍏]

‡ INFN-Pavia, Italy
† University of Pavia, Italy
⍏ IRCCS Mondino Foundation, Pavia, Italy
⁜ University Hospital Basel, Basel, Switzerland
ⅆ University of Basel, Basel, Switzerland,
ⅼ IRCCS Fondazione Policlinico Universitario A. Gemelli, Rome, Italy
ⱺ University of Buffalo, Buffalo, NY, USA.
E-mail: francesca.brero01@universitadipavia.it

Keywords: MRI

Muscle MRI has become a supportive tool for the diagnosis and the assessment of disease progression in neuromuscular disorders. A muscle-by-muscle type of involvement occurs in the progression of facioscapulohumeral muscular dystrophy (FSHD), in which an early stage of muscle damage precedes fat replacement of individual muscles. Quantitative MRI (qMRI) is useful for the identification and quantification of several biomarkers, *i.e.* quantitative features providing information regarding muscle atrophicity, the extent of active and chronic degenerative changes. The more widespread are the percentage of fat replacement of the muscle (Fat Fraction, FF) and the muscle water T_2 relaxation time (wT_2), as expression of intramuscular edema and therefore as in vivo indicator of ongoing disease activity. Up to date qMRI methods are unavailable in many clinical centers, mainly due to their complexity/cost; on the other hand, conventional MRI with semiquantitative scales is still used in the everyday clinical practice. In the last years, Radiomics has shown a great potential in the field of quantitative imaging. Within this framework, this study aims at exploiting radiomic texture analysis of multi-echo gradient echo sequences (MEGE) and machine learning algorithms (ML) as a processing pipeline to quantify wT_2 and FF parameters. Twenty-four FSHD patients were scanned on a 3T MRI scanner (3D 6-point Multi-Echo Gradient Echo -MEGE-, Multi-echo Spin Echo -MESE- with 17 echoes) at the level of the thighs. We applied an automatic segmentation toolbox to draw twelve regions of interest (ROI) for the principal thigh muscles. After pre-processing of the dataset, texture analysis was performed on MEGE images, using the LIFEx software. Forty-two radiomic features were obtained with 2D extraction from each of the 12 ROI. We compared the performance of a set of ML models in predicting muscle-wise FF and wT_2. For each thigh muscle we implemented the parametric linear, R and Lasso regression and the non-parametric K-nearest neighbor, Support Vector Machine, tree, and Random Forest algorithms. The ML ground truth for FF and wT_2 values were calculated using Fatty Riot algorithm from MEGE sequence and by EPG signal simulation from MESE sequence respectively. The performance of ML algorithms is quantified by the Mean Absolute Error metric. FF mean algorithms accuracy is around 85% whereas the mean wT_2 accuracy is about 88%. The mean prediction accuracy for both biomarkers depend on neither muscles size nor muscles shape. These results show that the combination of texture analysis and ML algorithms guarantees good accuracy in predicting FF and wT_2 in patients with FSHD. This result appears as promising, and we hypothesize that a larger dataset may eventually increase the performance of the algorithms or that deep learning algorithms and neural networks may even better perform. Further studies are needed to confirm such data.