UNIVERSITA
DEGLI STUDI
DI TORINO
ALMA UNIVERSITAS
TAURINENSIS
unito.it

GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XLVII National Congress on Magnetic Resonance

19-21 September 2018
UNIVERSITY OF TORINO

GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XLVII National Congress on Magnetic Resonance

Torino, 19-21 September 2018

Dept. of Molecular Biotechnology and Health Sciences

University of Torino

BOOK OF ABSTRACTS

UNDER THE AUSPICES OF

SPONSORS

BRUKER

JEOL

STELAR

RIVOIRA
INDUSTRIAL & SPECIALTY GASES

SAPIO
GRUPPO
Respirare il futuro

Chiesi
People and ideas for innovation in healthcare

Mestrelab Research
chemistry software solutions

BRACCO
LIFE FROM INSIDE

Spectra2000 Srl

SOLVAY
asking more from chemistry®

FONDAZIONE

ANTONIO
DE MARCO

ChemPubSoc
Europe

SCIENTIFIC COMMITTEE

Lucia Calucci (ICCOM-CNR, Pisa)
Donatella Capitani (IMC-CNR, Roma)
Michele Chierotti (University of Torino)
Mariapina D'Onofrio (University of Verona)
Marco Geppi (University of Pisa)
Giuseppe Pileio (University of Southampton)
Paola Turano (University of Firenze)
Vito Gallo (DICATECh, Polytechnic of Bari)

ORGANIZING COMMITTEE

Simone Bordignon (University of Torino)
Michele Chierotti (University of Torino)
Simonetta Geninatti Crich (University of Torino)
Eliana Gianolio (University of Torino)
Roberto Gobetto (University of Torino)
Enzo Terreno (University of Torino)
Alessandra Viale (University of Torino)
Mauro Botta (University of Eastern Piedmont)

GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XLVII National Congress on Magnetic Resonance

Torino 19-21 September 2018

Scientific Program

Wednesday September 19th

10:00-13:00	**Registration**	
10:30-12:30	**Bruker satellite meeting**	
12:45-14:00	**Bruker Lunch**	
14:00-14:30	**Opening**	
	Plenary session **Chair: V. Gallo**	
14:30-15:30	**GIDRM/GIRM gold medal award** **L. Mannina** - My Personal History with NMR: from the Chemical Structure of Single Compounds to the Study of Complex Mixtures	
15:30-16:15	**ChemPubSoc Plenary Lecture** **A. Kenwright** – Compressed NMR: More Information in the Same Space & Time	
16:15-17:20	**Coffee break + Poster session**	
	Parallel session A **Chair: L. Calucci**	**Parallel session B** **Chair: L. Mannina**
17:20-17:40	**L. Bianchini** – Repeatibility and Robustness of Radiomic Features Extracted from Magnetic Resonance Images: a Phantom Study	**C. Ingallina** - "Cornetto di Pontecorvo DOP" Red Sweet Pepper Characterized by a Multi-Methodological Approach
17:40-18:00	**M. R. Ruggiero** - Intracellular Water Lifetime as a Tumour Biomarker by FFC-Relaxometry	**V. Righi** – Skin Metabolomics through HR-MAS NMR: Effects of Ingenol Mebutate on Actinic Keratosis
18:00-18:30	**E. Gianolio** – Low Resolution NMR as a Powerful Tool for Diagnostic Purposes	**C. Airoldi** - NMR Interaction Studies for Identification and Development of New Bioactive Compounds
	Plenary session **Chair: M. Botta**	
18:30-19:00	**Stelar Lecture** **D. Brougham** - FFC-NMR Relaxometry Studies into the Influence of Dynamics on Function in Nano-Materials for Bio-Application	
19:00-19:45	**Plenary Lecture 2** **S. Aime** – Design and Testing of MR Molecular Imaging Reporters	

Thursday September 20th

	Plenary session Chair: P. Turano
8:45-9:30	**Plenary Lecture 3** **I. Shimada -** Developments and Applications of Novel NMR Methods to Characterize Functional Dynamics of High Molecular Weight Proteins
9:30-10:00	**Bruker Lecture** **B. Perrone -** Biosolids CryoProbe, Automation with MAS Probes, and Other Solid-State NMR News
10:00-10:30	**Under 35 GIDRM award** **E. Carignani -** Solid State Dynamics Probed by NMR Spectroscopy and Relaxometry: from Crystals to Soft Matter
10:30-11:20	**Coffee break + Poster session**

	Parallel session A Chair: J. N. Dumez	Parallel session B Chair: I. Shimada
11:20-11:50	**R. Lamanna -** ^{1}H NMR Characterization of Aging Processes in Precursor Solutions Used for YBCO Superconductors Production	**R. Pierattelli –** Un-Structural Biology by NMR Spectroscopy
11:50-12:20	**P. Cerreia Vioglio –** A Time-Resolved, in-Situ DNP NMR Approach to Gain Insights into Metastable Polymorphs of Glycine	**M. Assfalg –** Insight into Transient Protein-Nanoparticle Interactions by Solution NMR
12:20-12:40	**G. Stevanato -** An Efficient Gd^{3+} Based Complex for High Field Dynamic Nuclear Polarization	**M. Grimaldi –** Design of Peptides for the Early Diagnosis of Alzheimer Disease
12:40-13:00	**F. Paruzzo -** NMR Meets Machine Learning: Chemical Shift Predictions in Solids in Less than a Minute	**F. Favretto –** Unraveling the Role of Cis-Trans Peptidylprolyl Isomerases in Parkinson's Disease
13:00-14:20	**Lunch + Poster session**	

	Plenary session Chair: D. Capitani
14:20-15:05	**Plenary Lecture 4** **S. Mammi -** NMR Contributions to Coffee Science: a Story of Technology Transfer from Academia to Industry
15:05-15:35	**Jeol Lecture** **A. Botana –** Speeding up Structural Elucidation with NMR
15:35-16:15	**Segre Fellowships 2017** **T. Cappello –** Effects of Polystyrene Microplastics in Marine Mussels *Mytilus Galloprovincialis* by NMR-Based Metabolomics **E. Costanzi –** Structural Characterization of the Interaction Between the STAS Domain of Mammalian Prestin and Calmodulin
16:15-17:30	**Coffee break + Poster session**
17:00-17.30	**GIRM assembly**
17:30-19.30	**GIDRM assembly + announcement of poster competition winner**
19:30	**Departure for the social dinner**

Friday September 21st

	Plenary session Chair: G. Pileio
8:45-9:30	**Plenary Lecture 5** **J. N. Dumez –** Diffusion NMR of Out-of-Equilibrium Mixtures

	Parallel session A Chair: A. Kenwright	Parallel session B Chair: S. Mammi
9:30-10:00	**P. Arosio -** The Effect of Chemico-Physical Parameters of Magnetic Nanoparticles on their Fundamental and Biomedical Applicative Properties: a Few Case Studies	**G. Malgieri –** Metal Ion Recruitment Drives the Folding Mechanism and Self-Association Propensity of High Homologous Proteins
10:00-10:20	**F. Capuana -** A Novel Tetrameric Gadolinium Based Contrast Agent for MR Molecular Imaging of Tropoelastin	**F. Nardelli –** Succinimide-Based Conjugates Improve IsoDGR Cyclopeptide Affinity to AVB3 without Promoting Integrin Allosteric Activation
10:20-10:40	**F. Carniato –** NMR Determination of the Hydration Number and Exchange Rate of the Bound Water Molecule Across the Ln[AAZTA]$^{-}$ Series	**F. Munari** – Structural and Molecular Recognition Properties of UBB^{+1}, a Ubiquitin Mutant Associated with Alzheimer's Disease
10:40-11:10	**Coffee break**	

	Parallel session A Chair: M. Chierotti	Parallel session B Chair: M. D'Onofrio
11:10-11:40	**F. Martini –** Phase Separation, Dynamics and Interaction in Starch-Sucrose Amorphous Blends by Solid State NMR	**T. Pertinhez -** NMR in Transfusion Medicine
11:40-12:00	**F. Rossi –** Ultrafast MAS Proton-Detected 3D Solid State NMR Experiment to Recouple ^{15}N Chemical Shift Anisotropy	**F. Savorani -** The Human Urinary Metabolome to Assess the Risk-of-Poverty Status Across European Populations: a NMR Nutri-Metabolomic Study
12:00-12:20	**M. Mauri –** Direct and Indirect Exploration of Nanocrystal Surface	**G. Petrella -** A Comprehensive Urinary Metabolomic Approach Based on NMR and LC-HRMS to Identify Bladder Cancer

	Plenary session Chair: M. Geppi
12:20-12:50	**Poster competition winner lectures**
12:50-13:35	**Plenary Lecture 6** **M. Duer –** Heavy Mice and Light Things: Using Solid-State NMR Spectroscopy to Understand Biological Tissues in Health and Disease
13:35-13:40	**Closing**
13:40-15:00	**Lunch**

Contents

GIDRM/GIRM GOLD MEDAL AWARD

MY PERSONAL HISTORY WITH NMR: FROM THE CHEMICAL STRUCTURE OF SINGLE COMPOUNDS TO THE STUDY OF COMPLEX MIXTURES

L. Mannina,

Department of Chemistry and Technology of Drugs, Piazzale Aldo Moro 5, 00185 Rome, Italy
E-mail: luisa.mannina@uniroma1.it

My history with NMR has been strongly shaped by a series of important encounters, a bit like what happens in life. Some encounters have been transformed into collaborations that still continue today, others have been brief but all the while equally important. In this lecture, I will show you not what I did but rather what I liked doing even if obtained, sometimes, with difficulty. As Annalaura said: “you study and work hard for a few unique moments of complete satisfaction”. I experienced those moments.

The first step, the first love, was the structural study of natural or synthetic unknown compounds. 1D and 2D spectra as well as selective experiments "spoke", the answer was there, in plain sight, but it needed to be understood, imagine, putting together the different pieces like a puzzle. It was a challenge, I had fun.

Annalaura has been dealing with polymers for a long time and with great success: the group had a tradition in this field and I was able to take part in the study of polysaccharides. We studied the structure of polysaccharides in terms of composition, branching and molecular weight. This was also a challenge and I enjoyed it.

While all this happened, or better yet at the beginning of all this, it was 1992, some colleagues brought us a bottle of olive oil and asked us if we could carry out an NMR analysis. “We said yes and it was food chemistry forever". Olive oil was the first food, which we still continue to study, but it is not the only one. We studied kiwifruits, peaches, blueberries, seabass, beers, GMO products, etc. to get information regarding food geographical origin, varieties, quality and processing.

However, foods are complex matrices and it is necessary to combine different skills and methodologies to have exhaustive information. So we started collaborations with different NMR and non NMR groups to study foodstuffs. In 2015, the Italian group of Magnetic Resonance in Food Science was established in the frame of GIDRM. Together we have written three book chapters and organize, every two years, the workshop entitled “Applicazioni della Risonanza Magnetica nella Scienza degli Alimenti”. Finally, the creation of a Food-NMR database including information on foodstuffs, NMR experiments, etc is in progress. This is also a challenge, this is the future, and I like to dream.

UNDER 35 GIDRM AWARD 2018

U35GA

SOLID STATE DYNAMICS PROBED BY NMR SPECTROSCOPY AND RELAXOMETRY: FROM CRYSTALS TO SOFT MATTER

E. Carignani

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via G. Moruzzi 13, 56124 Pisa, Italy
E-mail: elisa.carignani@for.unipi.it

A large variety of molecular dynamic processes can take place in different solid samples, depending on the chemical nature of the systems as well as on their solid phases. Getting insights into molecular dynamics is of interest, not only for the improvement of the basic knowledge of condensed matter, but also in the perspective of establishing possible correlations with macroscopic properties of the materials, such as solid state stability and reactivity, solubilty, mechanical properties, or thermal and electric conductivity.
The study of molecular dynamics in solids and soft matter has characterized my research activity, which in large part has dealt with the development and application of NMR based approaches to investigate dynamics of solids with the aim of obtaining qualitative and quantitative information. In particular, our group developed an NMR approach to obtain a very detailed characterization of the dynamics by combining and simultaneously analysing a variety of NMR experimental data, including spectral properties, such as lineshapes and linewidths, and various types of relaxation times of different nuclei, measured at different frequencies and temperatures [1]. Very low temperature MAS and static NMR experiments revealed to be extremely useful in this kind of studies [2]. Among all the techiniques, NMR relaxometry, both at fixed and variable magnetic field (FFC), was extensively applied in order to obtain a quantitative characterization of several kinds of motions, from internal rotations and interconformational jumps to overall molecular reorientations and translational diffusion. In this contribution, the main results achieved will be presented, covering applications from very rigid samples, like small organic molecules in their crystalline state [1, 2, 3], to highly mobile anisotropic systems, such as small molecules in amorphous phases, rubbery polymers, plastic crystals [4], and liquid crystals [5].

References

[1] E. Carignani, S. Borsacchi, and M. Geppi *ChemPhysChem* **12**, 974-981 (2011); E. Carignani, S. Borsacchi, and M. Geppi *J. Phys. Chem. A* **115**, 8783-8790 (2011)
[2] M. Concistrè, O. G. Johannessen, E. Carignani, M. Geppi, and M. H. Levitt *Acc. Chem. Res.* **46**, 1914-1922 (2013). M. Concistrè, E. Carignani, S. Borsacchi, O. G. Johannessen, B. Mennucci, Y. Yang, M. Geppi, and M. H. Levitt. *J. Phys. Chem. Lett*. **5**, 512-516 (2014)
[3] E. Carignani, S. Borsacchi, A. Marini, B. Mennucci, and M. Geppi *J. Phys. Chem. C* **115**, 25023- 25029 (2011); E. Carignani, S. Borsacchi, M. Concistrè, O. G. Johannessen, and M. Geppi *Chem. Phys. Lett.* **706**, 107-112 (2018)

ANNALAURA SEGRE FELLOWSHIPS 2017

ASF1

EFFECTS OF POLYSTYRENE MICROPLASTICS IN MARINE MUSSEL *MYTILUS GALLOPROVINCIALIS* BY NMR-BASED METABOLOMICS

T. Cappello, M. Maisano

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy
E-mail: tiziana.cappello@unime.it

In the contemporary society, plastic has achieved a pivotal status in a myriad of applications because of its favourable properties. Despite the societal benefits, plastic represents an emerging concern as it is persistent as debris and microplastics (MPs) in seas worldwide. In order to accurately estimate the potential risk for human health posed by MPs, mainly via seafood consumption, herein the uptake of MPs and their associated toxicological effects were evaluated in marine mussel *Mytilus galloprovincialis*, a sessile and filter-feeding organism, commonly included in human diet and used as sentinel in studies of environmental risk assessment [1-3]. Mussels were exposed for 72 h to 3 μm red polystyrene MPs (50 particles/mL). At selected time points, the test solution was filtered to count MPs, and a decline in their number until 48 h and a successive increase at 72 h was detected, suggesting that mussels accumulate MPs until saturation. To verify the uptake of MPs by mussels, haemolymph samples were collected and, observed under microscope, revealed the presence of red MPs from 24 h to the entire exposure period, indicating their accumulation at the circulatory system and potential to reach and damage other mussel tissues. Moreover, gills (G), digestive glands (DG) and posterior adductor muscles (PAM) of mussels were collected and analyzed by a protonic nuclear magnetic resonance (1H NMR)-based metabolomics approach, combined with chemometrics, for a comprehensive assessment of MP effects in different mussel tissues. From NMR spectra of all tissues, several metabolites were identified, such as amino acids (i.e. alanine), osmolytes (i.e. taurine), energy metabolites (i.e. glucose), Kreb's cycle intermediates (i.e. succinate), nucleotides (i.e. inosine) and, only in G, neurotransmitters (i.e. serotonin) [4]. By principal component analysis (PCA), 1H NMR metabolic fingerprints of G, GD and PAM of MP treated mussels were clearly separated from controls, and therefore the metabolites responsible for grouping were identified and quantified for each tissue. Different metabolic pathways were found to significantly change in tissues of MP-exposed mussels compared to control, specifically osmoregulation, protein and energy metabolism and, only in G, also neurotransmission. Overall, the NMR-based metabolomics was effective in evaluating MP toxicity mechanisms in mussels and uncovering tissue-specific responses to MPs.
This study was conducted within the "GIDRM/Borse Annalaura Segre 2017".

References

[1] M. Maisano, T. Cappello, A. Natalotto, V. Vitale, V. Parrino, A. Giannetto, S. Oliva, G. Mancini, S. Cappello, A. Mauceri, and S. Fasulo *Mar. Environ. Res.* **128**, 114-123 (2017)
[2] T. Cappello, A. Mauceri, C. Corsaro, M. Maisano, V. Parrino, G. Lo Paro, G. Messina, and S. Fasulo *Mar. Pollut. Bull.* **77**, 132-139 (2013)
[3] T. Cappello, M. Maisano, A. Mauceri, and S. Fasulo *Ecotoxicol. Environ. Saf.* **142**, 417-422 (2017)
[4] T. Cappello, A. Giannetto, V. Parrino, M. Maisano, S. Oliva, G. De Marco, G. Guerriero, A. Mauceri, and S. Fasulo *Comp. Biochem. Physiol. D* **26**, 32-39 (2018)

ASF2

STRUCTURAL CHARACTERIZATION OF THE INTERACTION BETWEEN THE STAS DOMAIN OF MAMMALIAN PRESTIN AND CALMODULIN

E. Costanzi,[‡] R. Battistutta,[‡] M. Bellanda[‡]

[‡]Department of Chemical Sciences, via Marzolo 1, Padova, Italy
E-mail: elisa.costanzi@unipd.it

Prestin (SLC26A5) is the anion-dependent motor protein responsible for the outer hair cells electromotility, which is at the basis for the increased frequency selectivity and sensitivity in mammalian hearing [1]. Prestin belongs to the Sulfate Permease (SulP) also known as the Solute Carrier 26 (SLC26) family of anion transporters; the members of this family share a similar topology: a trans-membrane core with a 14-helixes topology and a C- terminal cytoplasmic portion that includes a so called "STAS" (sulfate transporter and anti-sigma factor antagonist) domain. The crystal structure of the STAS domain of rat prestin is known [2] and regarding the TM domain, an experimentally validated structural model has been recently proposed [3]. It is also known that a 30-aa long peptide of the STAS domain interacts with calmodulin (CaM) [4].
We are currently focused on the study of the interaction between the STAS domain of prestin and calmodulin from a structural point of view. In this communication we will present our recent results obtained using a combination of different, complementary techniques (SEC, DLS, SAXS, NMR).

References

[1] P. Dallos and B. Fakler *Nat. Rev. Mol. Cell Biol.* **3**, 104-111 (2002)
[2] E. Pasqualetto, R. Aiello, L. Gesiot, G. Bonetto, and M. Bellanda and R. Battistutta, *J Mol. Biol.* **400**, 448-462 (2010)
[3] D. Gorbunov, M. Sturlese, F. Nies, M. Kluge, M. Bellanda, R. Battistutta and D. Oliver, *Nat. Commun.* **5**, 3622-3634 (2014)
[4] J. Pearson Keller, K. Homma, C. Duan, J. Zheng, M.A. Cheatham, and P. Dallos, *J. Neurosci.* **34**, 1325-1332 (2014)

PLENARY LECTURES

PL1

DESIGN AND TESTING OF MR MOLECULAR IMAGING REPORTERS

S. Aime

Department of Molecular Biotechnologies and Health Sciences & Molecular Imaging Center, University of Torino (Italy)
E-mail: silvio.aime@unito.it

The possibility of carrying out Functional and Molecular Imaging protocols by means of MRI is very attractive for the superb anatomical resolution that is attainable by this technique. Most of the proposed contrast agents reliws on Gd(III) complexes. Recently, the observation of small amounts of Gd retained in brain's regions has raised some concern and has stressed the need to go for higher relaxivities and lower doses.
Besides relaxation agents much attention is currently devoted also to the design of CEST agents (CEST= Chemical Exchange Saturation Transfer). Upon applying a second rf field at the absorption frequency of an exchangeable protons pool, a net saturation effect is detected on the water signal. These are frequency encoding systems that allow multiple agents detection in the same anatomical region as well as they offer the possibility of designing innovative responsive probes that report on specific parameters of the microenvironment in which they distribute. Different routes to enhance their sensitivity have been explored essentially based on the improvement of the proton exchange rate and on the increase of the number of equivalent exchangeable protons. The design of LipoCEST and Cell-as-CEST agents played a key role in increasing CEST sensitivity.
Finally the access to hyperpolarized molecules has opened new horizons providing the possibility of investigating in vivo metabolic processes. It will be shown how hyperpolarization of molecules like pyruvate and lactate can be attained by procedures based on the use of para-Hydrogen and magnetic field cycling (PHIP-SAH).

PL2

HEAVY MICE AND LIGHT THINGS: USING SOLID-STATE NMR SPECTROSCOPY TO UNDERSTAND BIOLOGICAL TISSUES IN HEALTH AND DISEASE

M. J. Duer

Department of Chemistry, University of Cambridge, UK
e-mail: mjd13@cam.ac.uk

The extracellular matrix (ECM) forms the bulk of our structural tissues and provides them with their particular mechanical properties. At the microscopic level, it provides the scaffold which supports cells but more intriguingly, at the molecular level, it provides the communication system between the cells in the tissue and the signals that drives the individual behaviour of cells. Ultimately, if we can understand how the extracellular matrix structure dictates the behaviour of cells, then we can develop ways to treat diseases such as cancer, by changing the extracellular matrix to drive the necessary change in cell behaviour. However, understanding the molecular level properties of the extracellular matrix has been hampered by the lack of methods to study tissues at the atomic scale. In this talk, I will describe the various solid-state NMR spectroscopy approaches my group has taken over the last decade to tackle these complex questions.
The first requirement is native-like tissues in which we can control isotope labelling patterns so that we can record assignable multidimensional NMR spectra. Using multidimensional solid-state correlation NMR spectra (^{13}C-^{13}C, ^{13}C-^{15}N) as fingerprints of the underlying molecular structures in isotope-labelled native tissues has allowed us to develop laboratory-grown tissues that have very similar molecular structures to native tissues, and thus represent demonstrably good models of native tissue [1]. The refined laboratory-grown tissues can then be manipulated by growing them with isotope labels in specific components to allow detailed study of structure and function of the various extracellular matrix components. For instance, ^{13}C, ^{15}N labelling Gly and Pro in the collagen component has led to the unexpected conclusion that Gly-Pro-Hyp triplets in collagen act like springs allowing the collagen helix to flex, rather than these triplets being rigid structures as they have long been assumed to be [2].

We have now coupled this approach with NMR methods to examine calcified tissues in health, such as bone, and in diseases such as vascular calcification (hardening of the arteries). In calcified tissues, the extracellular matrix incorporates stacks of ordered nanocrystals of a complex calcium phosphate phase. Using an NMR crystallography approach, we have put forward a new model for the structure of bone mineral [3], and now have an understanding of what chemical species control bone mineral strength. In combination with the methods described above, we now also have an intriguing insight into what initiates hardening of the arteries, which leads to a potential route to prevent this condition, the major cause of cardiovascular disease worldwide.

References.

[1] W.Y. Chow, R. Rajan, K.H. Muller, D.G. Reid, J.N. Skepper, W. C. Wong, R.A. Brooks, M. Green, D. Bihan, R.W. Farndale, D.A. Slatter, C.M. Shanahan, and M.J. Duer, *Science* **344**, 742-746 (2014)
[2] W.Y. Chow, D. Bihan, C.J. Forman, D.A. Slatter, D.G. Reid, D.J. Wales, R.W. Farndale, and M.J. Duer, *Sci. Rep.* **5**, 12556 (2015)
[3] E. Davies, K.H. Muller, W.C. Wong, C. J. Pickard, D.G. Reid, J.N. Skepper, and M.J. Duer, *Proc. Nat. Acad. Sci. USA* **111**, E1354-E1363 (2014)

PL3

DIFFUSION NMR OF OUT-OF-EQUILIBRIUM MIXTURES

J.-N. Dumez

Institut de Chimie des Substances Naturelles, CNRS UPR2301, Univ. Paris Sud, Université Paris Saclay, Gif-sur-Yvette, France.
E-mail: jeannicolas.dumez@cnrs.fr

The NMR spectra of molecular species in solution mixtures can be separated with diffusion-ordered NMR spectroscopy (DOSY), a 'virtual chromatography' approach based on the measurement of translational diffusion coefficients. Classic DOSY experiments, however, require several minutes are not applicable to many important time-evolving mixtures.
Here we show that DOSY data can be collected in a single scan of less than one second for several types of out-of-equilibrium mixtures. We use the concept of spatial encoding (SPEN) of the diffusion information, as a mean to accelerate DOSY experiments by several orders of magnitude [1]. SPEN DOSY pulse sequences are developped, that compensate for convection effects and are suitable for measurements in low-viscosity organic solvents, a requirement to monitor organic chemical reactions [2]. We also show how to collect multiple consecutive scans from short-lived, non-renewable signals produced by dissolution dynamic nuclear polarisation (D-DNP) [3], which is a versatile and powerful hyperpolarisation method. These methodological developments are supported by advanced numerical simulations, based on a Fokker-Plank formalism to describe simultaneously the spin and spatial dynamics [4]. An exemple of hyperpolarised sample is given with a model mixture of small molecules, while the ability to monitor a reacting mixture is illustrated with a di-amination reaction in dichloromethane.

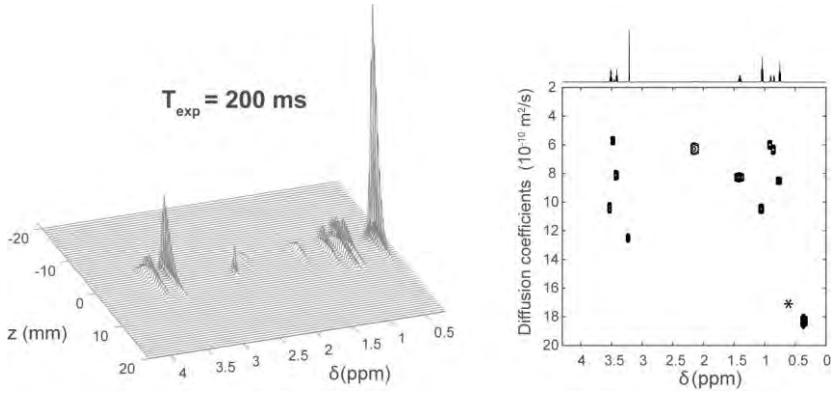


Fig. 1. SPEN DOSY: the diffusion information is encoded spatially and the experiment lasts less than 1 s.

The proposed UF DOSY methodology will contribute towards a real-time diffusion NMR analysis of mixtures, to help in the identification of a sample's components and in the analysis of molecular interactions.

References

[1] L. Guduff *et al*. *Chem. Commun.* **53**, 701-704 (2017).
[2] G. Hamdoun *et al.*, *Analyst*, in press.
[3] L. Guduff *et al. Chem. - A Eur. J.* **23**, 1-6 (2017)
[4] L. Guduff *et al*. *Phys. Chem. Chem. Phys.* **19**, 17577-17586 (2017)

PL4

COMPRESSED NMR: MORE INFORMATION IN THE SAME SPACE & TIME

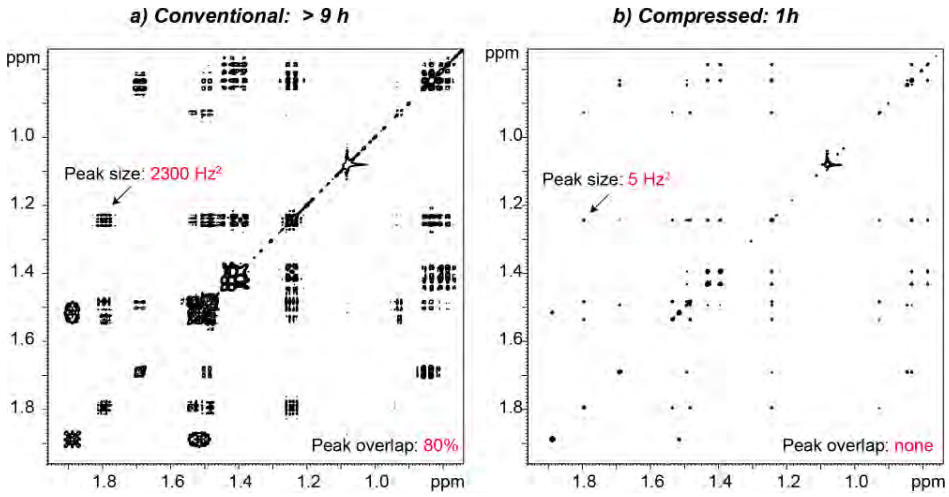
A. M. Kenwright,‡ J. A. Aguilar‡

‡Durham University, Chemistry Department, South Road, Durham DH1 3LE, UK.
E-mail: a.m.kenwright@durham.ac.uk

Resolution (the ability to effectively distinguish adjacent signals) is a key concept in many areas of scientific endeavour and is central to the ability of NMR to deal with complex molecules. It has been one of the main drivers, with sensitivity, behind the pursuit by the NMR community of ever higher magnetic fields. Unfortunately, ultra-high field magnets are very expensive and so are not generally available to the chemical community.

In this presentation we consider the problem of effective resolution in two-dimensional NMR experiments and show that the achievable effective resolution can be improved by orders of magnitude in some cases by the synergic use of Compressive Sampling and Pure Shift NMR experiments [1]. We explain why these two techniques are synergic and how, when they are appropriately combined, we can achieve much higher effective resolution without making the experiments unfeasibly long [2]. We show examples where their use reveals information that would not have been available otherwise. By applying these techniques we can achieve effective resolution at moderate magnetic fields (proton frequencies of 400 or 500 MHz) that could not be achieved using conventional techniques even at a proton frequency of 1GHz.

Finally, we present ongoing work to try to apply these ideas to possibly the most commonly used two-dimensional NMR experiment in chemistry, COSY [3].



References

[1] J. A. Aguilar, R. Belda, A. Botana, and A. M. Kenwright; *RSC Adv.* **6**, 83380-83385 (2016)
[2] J. A. Aguilar, A. M. Kenwrig andt; *Magn. Reson. Chem.* DOI: 10.1002/mrc.4705
[3] J. A. Aguilar, R. Belda, B. R. Gaunt, A. M. Kenwright,; and I. Kuprov; *Magn. Reson. Chem.* DOI: 10.1002/mrc.4727

PL5

NMR CONTRIBUTIONS TO COFFEE SCIENCE: A STORY OF TECHNOLOGY TRANSFER FROM ACADEMIA TO INDUSTRY

S. Mammi

‡Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy
E-mail: stefano.mammi@unipd.it

Coffee is a fascinating matrix from many different points of view. The exotic countries that produce coffee captivate the mind of a westerner. The complicated process of harvesting, processing, storing, roasting, and brewing coffee all the while exalting and preserving its unique flavor are challenging to different professionals, and the profits associated with this industry are also attractive.

The journey of a coffee bean from the plant to a cup is punctuated with an incredible number of chemical and physical transformations that require precise conditions and need to be strictly monitored. While many other analytical techniques are often more useful in this field, still there is room for NMR to contribute in a unique way.

In the past fifteen years, a fruitful collaboration with illycaffè S.p.A. has allowed our group to tackle many scientific and technological problems. In this lecture, I will describe how NMR has impacted coffee science and more specifically our involvement in this process.

Among other topics, I will discuss a method to quantify caffeine in saliva by NMR [1], the use of NMR to follow the transformation of Asn into Asp as a way to reduce the possible formation of acrylamide during roasting, the use of low-field NMR to quantify water in green beans [2], and the characterization of coffee honey [3]. I will concentrate on the quantification of 16-O-methylcafestol as a way to detect the presence of *Coffea canephora* var. robusta beans in *Coffea arabica* blends [4].

References

[1] E. Schievano, C. Finotello, L. Navarini, and S. Mammi *Talanta*, **140**, 36-41 (2015)
[2] G. Venditti, E. Schievano, L. Navarini, and S. Mammi *Food Biophys.*, **6**, 321-326 (2011)
[3] E. Schievano, C. Finotello, S. Mammi, A. Illy Belci, S. Colomban, and L. Navarini *J. Agr. Food Chem.*, **63**, 5858-5863 (2015)
[4] C. Finotello, C. Forzato, A. Gasparini, S. Mammi, L. Navarini, and E. Schievano *Food Control*, **75**, 62-69 (2017)

PL6

DEVELOPMENTS AND APPLICATIONS OF NOVEL NMR METHODS TO CHARACTERIZE FUNCTIONAL DYNAMICS OF HIGH MOLECULAR WEIGHT PROTEINS

I. Shimada

Graduate School of Pharmaceutical Sciences, the University of Tokyo, Tokyo, Japan
E-mail:shimada@iw-nmr.f.u-tokyo.ac.jp

Proteins frequently exist in an equilibrium between multiple conformations, and lowly populated conformations in the equilibrium often play critical roles in biological functions. Therefore, to reveal the mechanisms of protein functions, it is important to characterize the conformational equilibrium; i.e., the chemical exchange processes of proteins. Here, we developed novel NMR methods for characterizing chemical exchange processes utilizing multiple quantum relaxation rates of side-chain methyl groups, which can be sensitively observed in high molecular weight proteins. The methods were applied to the biologically important large proteins, which have been difficult to analyze by conventional NMR methods due to molecular size limitations.

References

[1] Y. Toyama, M. Osawa, M. Yokogawa, and I. Shimada *J. Am. Chem. Soc.* **138**, 2302-2311 (2016)
[2] Y. Toyama, H. Kano, Y. Mase, M. Yokogawa, M. Osawa, and I. Shimada *Nat. Commun.* (2017) doi: 10.1038/ncomms14523
[3] Y. Toyama, H. Kano, Y. Mase, M. Yokogawa, M. Osawa, and I. Shimada *Proc. Natl. Acad. Sci. USA*, **115**, 3858-3863 (2018)

BRUKER, JEOL AND STELAR LECTURES

SP1

SPEEDING UP STRUCTURAL ELUCIDATION WITH NMR

A. Botana

‡JEOL (U.K.) Ltd., Welwyn Garden City, United Kingdom
E-mail: adolfo.botana@jeoluk.com

NMR has been established as a leading technique for structural elucidation of unknown analytes. The information required to deduce the structure of a molecule can be obtained through a combination of different experiments such as COSY, HSQC and HMBC. This can be time consuming and there have been substantial efforts to speed up the acquisition of such experiments.

One of the approaches commonly used is the Non-Uniform Sampling of increments, which allows the acquisition of an experiment with a similar resolution in a lower amount of time. Compressive sensing processing [1] is now standard in most NMR processing software packages and this acquisition scheme is now widely used.

A different approach combines several of these pulse sequences into a nested supersequence in what has been termed as NOAH (NMR by Ordered

Acquisition using ^{1}H-detection). [2] Multiple combinations of standard pulse sequences are possible, such as an NOAH experiment combining ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H TOCSY.

Here we explore the combination of NOAH with NUS. The combination of these two techniques to reduce experimental time allows the acquisition of structural information in a very short amount of time. This has been demonstrated with an experiment of less than 2 minutes, which acquires multiplicity edited ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H COSY at once under Non-Uniform Sampling. This has been implemented in JEOL ECZ spectrometers, [3] which allow the direct acquisition of 2 (or more) separate datasets without the need of scripts to untangle the acquired data. This facilitates using the pulse sequence in open access environments using third party software for processing.

Obtaining all the information from a spectrum is not always straight-forward due to signal overlap, or baseline and phase distortions. Here we show that alternative techniques such as CRAFT can provide an advantage on obtaining information that is not easy to extract from the processed data.

Not every sample can be studied by solution state NMR. We are also presenting new solid state probes for automation, which use the same sample changers as liquid probes such as the Royal HFX [4], thus allowing full automation both in solid and in liquid state NMR.

References

[1a] F. Delaglio, G. Walker, K. Farley, R. Sharma, J. Hoch, L. Arbogast, R. Brinson, and J. Marino, *Am. Pharm. Rev.* **20** (2017)
[1b] K. Kazimierczuk,and V. Orekhov, *Magn. Reson. Chem.* **53**, 921-926 (2015)
[1c] S. Qaisar, R. M. Bilal, W. Iqbal, M. Naureen, and S. Lee, *J. Commun. Netw.* **15**, 443-456 (2013)
[2] Ē. Kupče and T. D. W. Claridge, *Angew. Chem. Int. Ed.* **56**, 11779-11783 (2017)
[3] K. Hachitani, *JEOL News* **49**, 94-98 (2014)
[4] P. J. Bowyer, J. Finnigan, B. Marsden, B. Taber, and A. T. Zens, *J. Magn. Reson.* **261**, 190-198 (2015)

SP2

FFC-NMR RELAXOMETRY STUDIES INTO THE INFLUENCE OF DYNAMICS ON FUNCTION IN NANO-MATERIALS FOR BIO-APPLICATION

D. F. Brougham,‡ J. Merlin,‡ S. Martyn,‡ E. Fox‡

‡ School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland.
E-mail: dermot.brougham@ucd.ie

Fast field-cycling nuclear magnetic resonance relaxometry is an established technique for studying dynamics on multiple timescales in complex systems. FFC-NMR provides the frequency dependence of the NMR spin lattice relaxation rate, R_1, which is known as the 'relaxation profile'. The profile maps the spectral density of the dynamic processes that drive relaxation at a given temperature. This can be interesting as for many materials microscopic dynamics determine the emergent properties.
Selected ^{1}H FFC-NMR profiles for suspension of magnetic iron-oxide nanoparticles (MNPs) will be presented. The interactions of the solvent molecules with the magnetic structure that determine the response [1] and which can be used to generate image contrast in MRI will be discussed. Finally, the process of extracting information on particle-particle interactions from the profiles and using this in developing colloids with controlled particle dispersion [2-3] and particle clustering [4-6], with the goal of improving the MRI properties of the suspensions will be described.

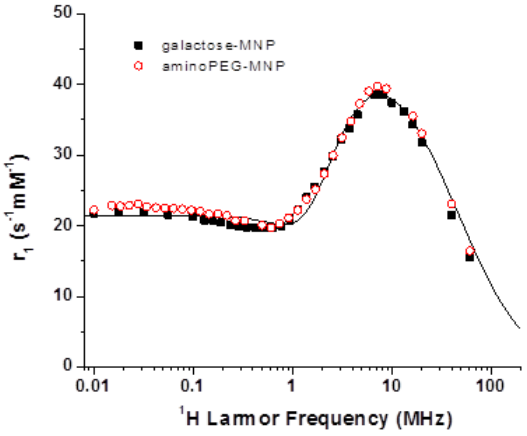


Fig. 1. FFC-NMR profiles of H_2O suspensions of dispersed magnetic γ-Fe_2O_3 nanoparticles.

References

[1] A. Roch, R. N. Muller, and P. Gillis *J. Chem. Phys.* **110**, 5403-5411 (1999)
[2] T. Borase, T. Ninjbadgar, A. Kapatenakis, S. Roche, R. O'Connor, C. Kerskens, A. Heise, and D. F. Brougham. *Angew. Chem. Int. Ed.* **52**, 3164-3167 (2013)
[3] T. Ninjbadgar, and D. F. Brougham. *Adv. Funct. Mater.*, **21**, 4769-4775 (2011)
[4] E. K. Fox, F. El Haddassi, J. Hierrezuelo, T. Ninjbagdar, J. K. Stolarczyk, J. Merlin, and D. F. Brougham. *Small*, awaiting DOI.
[5] J. K. Stolarczyk, C. J. Meledandri, S. P. Clarke, and D. F. Brougham. *Chem. Commun.* **52**, 13337-13340 (2016)
[6] J. K. Stolarczyk, A. Deak, and D. F. Brougham. *Adv. Mater.* **28**, 5400-5424 (2016)

SP3

BIOSOLIDS CRYOPROBE, AUTOMATION WITH MAS PROBE, AND OTHER NEWS IN SOLID STATE NMR

B. Perrone‡

‡Bruker Biospin AG, Industriestrasse 26, Fällanden, 8117, Switzerland
E-mail: barbara.perrone@bruker.com

In this talk we will illustrate a few new technologies and solutions related with solid state NMR spectroscopy very recently launched in the market by Bruker, which improve sensitivity and easy-of-usage, and, ultimately, the productivity.

As widely known, NMR is struggling with the lack of sensitivity when compared with other analytical techniques. This year we introduced a new technology to improve the signal-to-noise which do not require DNP, radicals nor cryogenic sample temperature, and it is based on our long experience with CryoProbes for liquids NMR and MR imaging. The ***Biosolids CryoProbe™*** represents the 3rd frontier of the high-sensitivity CryoProbe series and it allows the investigation of various biological solids, such as membrane proteins or disease aggregates at physiological temperatures, with a three-fold boost in sensitivity. Some demanding solid-state triple resonance NMR results obtained with this unique probe on biological samples will be presented.

Automation is a key factor in productivity, and we have is designed new hardware and software solutions specifically tailoring MAS probes and solid state NMR spectroscopy. Our newly introduced ***iProbe HRMAS™*** now enables full automation of high-resolution magic angle spinning (HRMAS) NMR. Built on the recently introduced iProbe platform, this new technology offers all benefits of automated tuning and matching for all RF-channels, together with accurate automated adjustment of the magic angle position, in a light and compact design with guarantees a smooth probe exchange.

For CPMAS applications, our Topsolids™ acquisition software guides the user through all the steps required to obtain the best spectrum, being on a protein or on an inorganic sample, no matter what his expertise level is. We will illustrate some of the latest experiments and extended features available in our upcoming release of Topspin™.

Once the spectra are acquired, the job of a scientist is not finished. The spectra need to be evaluated and analyzed to extract the sought information. To support our customers to perform this task we implemented an easy graphical interface which enables to setup Simpson simulations and fitting of experimental data. This feature is already included in the current version of Topspin.

ORAL COMMUNICATIONS

OC1

NMR INTERACTION STUDIES FOR IDENTIFICATION AND DEVELOPMENT OF NEW BIOACTIVE COMPOUNDS

C. Airoldi[1]

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

The versatility of NMR-based molecular recognition studies has been exploited in our laboratory for the characterization of several ligand-receptor pairs of biological and biomedical relevance.

In particular, we have characterized binding events involving amyloid species, key enzymes for the survival of pathogenic bacteria, oncoproteins, cell wall or membrane receptors [1].

In addition, we have set up new methodologies allowing the analysis of heterogeneous systems, like samples containing nanoparticle and cells [2], or complex mixtures, such as natural extracts [3].

Some selected examples will be provided in this presentation.

References

[1] selected examples: a) C. Airoldi, F. Cardona, E. Sironi, L. Colombo, M.Salmona, A Silva., F. Nicotra, B. La Ferla *Chem. Commun.* **47**, 10266–10268 (2011); b) C. Airoldi, S. Sommaruga, S. Merlo, P. Sperandeo, L. Cipolla, A. Polissi, F. Nicotra, *Chem. Eur. J.* **16**, 1897-1902 (2010); c) C. Airoldi, L. Colombo, C. Manzoni, E. Sironi, A.Natalello, S. M. Doglia, G. Forloni, F.Tagliavini, E. Del Favero, L. Cantù, F. Nicotra, M. Salmona *Org. Biomol. Chem*, **9**, 463-472 (2011); d) M. Bonanomi, C. Visentin, A. Natalello, M. Spinelli, M. Vanoni, C. Airoldi, M. E. Regonesi, P. Tortora, *Chem. Eur. J.* **21**, 50, 18383-18393 (2015); e) C. Guzzi, L. Colombo, A. De Luigi, M. Salmona, F. Nicotra, C.Airoldi, *Chem. Asian J.* **12**, 67-75 (2017); f) A. Palmioli, C. Ciaramelli, R. Tisi, M. Spinelli, G. De Sanctis, E. Sacco, C. Airoldi, *Chem. Asian J.* **12**, 2457-2466 (2017).

[2] a) C. Airoldi, S. Giovannardi, B. La Ferla, J. Jiménez-Barbero, F. Nicotra, *Chem. Eur. J.* **17**, 13395-13399 (2011); b) C. Airoldi, S. Merlo, E. Sironi, F. Nicotra, J. Jiménez-Barbero, *J. Mater. Sci. Eng. B*, **2**, 12, 618-625 (2012); c) C. Airoldi, S. Mourtas, F. Cardona, C. Zona, E. Sironi, G. D'Orazio, E. Markoutsa, F. Nicotra, S. G. Antimisiaris, B. La Ferla, *Eur. J. Med. Chem.* **85**, 43-50 (2014); d) A. Datta, A. Ghosh, C. Airoldi, P. Sperandeo, K. Mroue, J. Jimenez-Barbero, P. Kundu, A. Ramamoorthy, and A. Bhunia *Sci. Rep.* ,**5**, 11951 (**2015**) and papers in preparation.

[3] selected examples: a) C. Airoldi, E. Sironi, C. Dias, F. Marcelo, A. Martins, A. P. Rauter, F. Nicotra, J. Jimenez-Barbero, *Chem. Asian J.* **8**, 596-602 (2013); b) E. Sironi, L. Colombo, A. Lompo, M. Messa, M. Bonanomi, M. E. Regonesi, M.Salmona, C. Airoldi, *Chem. Eur. J.* **20**, 13793-13800 (2014); c) C. Ciaramelli, A. Palmioli, A. De Luigi, L. Colombo, G. Sala, C. Riva, C. P. Zoia, M. Salmona, C. Airoldi, *Food Chem.* **252**, 171-180 (2018).

OC2

THE EFFECT OF CHEMICO-PHYSICAL PARAMETERS OF MAGNETIC NANOPARTICLES ON THEIR FUNDAMENTAL AND BIOMEDICAL APPLICATIVE PROPERTIES: A FEW CASE STUDIES

P. Arosio[‡]

[‡]Physics Department, Università degli Studi di Milano, via Celoria 16, Italy
E-mail: paolo.arosio@uni.it

In the last two decades, much attention was devoted to novel multifunctional nanostructures based on magnetic nanoparticles (MNPs) useful as agents for Magnetic Resonance Imaging, Optical Imaging and Magnetic Fluid Hyperthermia, carriers for drugs and vectors for molecular targeting. Most of the MNPs systems reported in literature by a lot of research groups, have been shown to be useful as MRI contrast agents and magnetic fluid hyperthermia (MFH) mediators, displaying high nuclear relaxivity and Specific Absorption Rate (SAR). For these compounds, the possibility to collect images of the regions where the MNPs are delivered through MRI and eventually Optical Imaging (if functionalized with a luminescent molecule), is joint to their use under radio-frequency fields, with frequency of the order of 100 KHz, which causes a local release of heat directed to tumour cells (the MFH effect), possibly inducing their death. By such materials, theranostic agents can be obtained. On the other hand, in the field of drug delivery and molecular targeting, few examples of reproducible experiments using superparamagnetic nanoparticles are actually present in literature. Thus, the applications of MNPs to nanomedicine is currently of growing interest in the world.

The main objectives of my research group in the last decade was to contribute to the knowledge of physical mechanisms at the basis of MNPs used in biomedicine (especially MRI) and to propose some novel systems in strict collaboration with different research groups of chemists and biologists. I will present different case studies [1-3] where I show how the chemico-physical characteristics of MNPs are strictly correlated to their properties. Furthermore, I will discuss the failure of the most famous heuristic model [4] used in literature to interpret the NMR relaxivity profiles that does not reproduce transverse relaxivity experimental data and I will try to present which possible mechanisms are not taken into account by the theory at this time.

References

[1] L. Bordonali et al. *J. Phys.: Condens. Matter*, **25**, 066008 (2013)
[2] T. Orlando et al. *J. Appl. Phys.*, **119**, 134301 (2016)
[3] M. Basini et al. *J. Chem. Phys.*, **146**, 034703 (2017)
[4] A. Roch, R.N. Muller, P. Gillis *J. Chem. Phys.*, **110**, 5403 (1999)

OC3

INSIGHT INTO TRANSIENT PROTEIN-NANOPARTICLE INTERACTIONS BY SOLUTION NMR

M. Assfalg

Department of Biotechnology, University of Verona, 37134 Verona, Italy
E-mail: michael.assfalg@univr.it

The successful application of nanoparticles (NPs) in biosciences necessitates an in-depth understanding of how they interface with biomolecules [1]. Protein–NP interactions are also of interest for the development of nanocomposites. Thus, methodologies aimed at characterizing biomolecules associating with NPs represent an indispensable tool.
Solution NMR spectroscopy is a mature technique for the investigation of biomolecular structure and intermolecular associations, however its use in protein-NP interaction studies remains highly challenging [2]. We show that noncovalent protein-NP interactions are accessible by NMR, and we describe new approaches based on site-resolved protein signal perturbations [3,4]. We successfully used NMR methods to identify specific interaction sites that determine differential adsorption of protein structural isomers to NP surfaces [5]. Besides the mapping of binding surfaces, we also applied NMR approaches to describe the dynamics of proteins adsorbed onto NPs [6].
The developed approaches offer new opportunities for the characterization of bio-nano interfaces and should enable better control of NP bioactivity, ultimately supporting the development of NP-based drugs targeting specific biomolecular receptors.

References

[1] M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. Baldelli-Bombelli, S. Laurent, *Chem. Rev.* **111**, 5610-37 (2011)
[2] M. Assfalg, L. Ragona, K. Pagano, M. D'Onofrio, S. Zanzoni, S. Tomaselli, H. Molinari, *Biochim. Biophys. Acta* **1864**, 102-114 (2016)
[3] S. Zanzoni, A. Ceccon, M. Assfalg, R. K. Singh, D. Fushman, M. D'Onofrio, *Nanoscale* **7**, 7197-7205 (2015)
[4] S. Zanzoni, M. Pedroni, M. D'Onofrio, A. Speghini, M. Assfalg, *J. Am. Chem. Soc.* **138**, 72-75 (2016)
[5] A. Bortot, S. Zanzoni, M. D'Onofrio, M. Assfalg *Chem. Eur. J.* **24**, 5911-5919 (2018)
[6] A. Ceccon, M. Lelli, M. D'Onofrio, H. Molinari, M. Assfalg, *J. Am. Chem. Soc.* **136**, 13158-13161 (2014)

OC4

REPEATABILITY AND ROBUSTNESS OF RADIOMIC FEATURES EXTRACTED FROM MAGNETIC RESONANCE IMAGES OF PELVIS DISTRICT: A PHANTOM STUDY

L. Bianchini,[‡] F. Botta,[†] D. Origgi,[†] M. Cremonesi,[†] P. Arosio,[‡] A. Lascialfari[‡]

[‡]Dipartimento di Fisica and INSTM, Università degli Studi di Milano, Via Celoria 16, 20133 Milano, Italy
[†]Istituto Europeo di Oncologia, Via Ripamonti 435, 20141 Milano, Italy
E-mail: linda.bianchini@unimi.it

The term "*Radiomics"* refers to a process of quantitative analysis of medical images to obtain data useful for the pathology assessment and treatment. *Radiomic features* are extrapolated from the images with mathematical algorithms starting from the numerical value associated to each voxel in the image and they can be an expression of the phenotype of the imaged tissue. The features could be integrated into predictive models useful for prognosis and the choice of treatment strategy, especially in clinical oncology.
Radiomics has been applied to Magnetic Resonance (MR) images, with some promising preliminary results [1]. However, due to the quite recent development of this discipline, many aspects deserve further investigation. Aiming to identify repeatable and robust features, phantom studies are currently being performed under controlled conditions at the European Institute of Oncology (IEO) in Milan, focusing on the pelvis district.
First, images of a phantom commonly used for quality control on MRI scanner at IEO were acquired with the same protocol used in clinical practice for gynecologic imaging. To test the short- and long-term stability of the features, several acquisitions were performed; for each acquisition, radiomic features were calculated on selected Regions Of Interest (ROI). The percentage of features with r coefficient (ratio between standard deviation and mean value) less than 1% resulted around 59%, while the features with $1\% \leq r < 10\%$ were about 37% of the total. Moreover, the analysis showed that the instability of features increases by decreasing the volume of the ROI.
To mimic the acquisition conditions of real patients, a dedicated phantom is under development: an ambdomen-shape plastic container is filled with a solution of paramagnetic ions ($MnCl_2$) in order to obtain relaxation times similar to those found in literature for the muscle surrounding prostate or ovaries. Measurements are performed on a NMR spectrometer, with dedicated sequences, at the University of Milano (Physics Department) aiming to find the proper concentration to match the published values. In vivo verification of relaxation times with dedicated sequences is also under study.
To simulate prostate or ovarian cancers with different texture, cylindrical phantom inserts are prepared by mixing a jelly-like material and tiny polystyrene spheres with different diameters, in order to create different texture to be imaged. This kind of phantom will be imaged with the same sequences used for clinical acquisitions (T_2-weighted, diffusion and perfusion sequences) and radiomic features will be calculated on each insert, aiming to test the ability of radiomic features to distinguish between different patterns; this ability will be evaluated for ROI with different volumes.

References

[1] E. Scalco et al. *Physica Medica* **32**, 1672-1680 (2016).

OC5

A NOVEL TETRAMERIC GADOLINUM BASED CONTRAST AGENT FOR MR MOLECULAR IMAGING OF TROPOELASTIN

F. Capuana,[‡] R. Stefania,[‡] A. Phinikaridou,[†] S. Lacerda,[†] I. Rashid,[†] G. Digilio,[§] R. Botnar,[†] S. Aime[‡]

[‡]Dept. of Molecular Biotechnology and Health Science, University of Torino, Torino, Italy
[†]Dept. of Biomedical Engineering, King's College London, London, UK
[§]Dept. of Sciences and Technologic Innovation, University of Piemonte Orientale, Alessandria, Italy
E-mail: federico.capuana@unito.it

A large number of diseases, as myocardial infarction and stroke, are strictly related to atherosclerosis that nowadays represents one of the leading cause of death worldwide. Most of the acute manifestations of atherosclerosis share a common pathogenetic feature: the rupture of an atherosclerotic plaque. In order to prevent acute events and to provide personalized and preventive treatments the improvement of early diagnosis shall be imperative. Under pathological conditions, including atherosclerosis and aortic aneurysms, the elastogenesis (elastin is the primary component of the vessel walls) resumes with the secretion of the soluble precursor tropoelastin. Conversely, in normal condition tropoelastin is absent [1]. This study aims at developing a novel tropoelastin-binding MR contrast agent (CA) for *in vivo* imaging. The novel CA has to ensure a specific binding to tropoelastin minimizing non-specific signal from mature elastin in order to allow the detection of pathologic elastogenesis that occurs in atherosclerosis. A tri-lysine scaffold bearing four DOTA monoamide chelating cage and ending-up with a free-amino group was synthetized by a solid phase synthetic approach [2] then complexed with gadolimium (Gd4-K3-NH2). The amino group was then converted to maleimide, and the maleimide bearing tetrameric complex was finally conjugated trough Michael addition with a sixteen-residues peptide known for specific tropoelastin binding (TropoElastin Binding Peptide, TEBP). Characterization of the final Gd4-TEBP probe by NMRD confirmed the expected high flexibility of the imaging reporter that was pursued to minimize steric hindrance effects that could be detrimental to molecular recognition of the biological target (see Fig. 1). Preliminary *In vivo* MRI (3T) in ApoE$^{-/-}$ mice showed a very good contrast enhancement within atherosclerotic plaques that co-localized with TE as far as assessed by histology. Finally, TE imaging with Gd4-TEBP showed better *contrast-to-noise ratio (CNR) and a more extended imaging windows as compared to the Gd-monomer version of TEBP.*

This project has received funding from the EU's H2020 research and innovation program under the grant agreement No. 668142 (SPCCT).

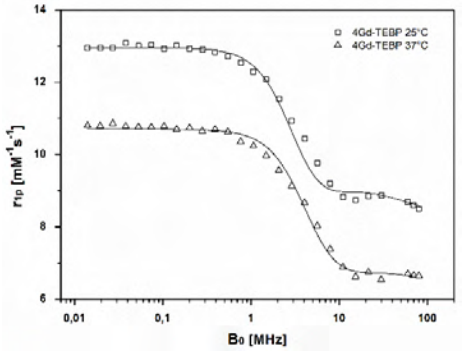


Fig. 1. NMRD profiles of Gd4-TEBP in HEPES buffer at 25°C (□) and at 37°C (Δ).

References

[1] A. Phinikaridou et al., J. *Cardiovasc. Magn. Reson.* **17**, O102 (2015).
[2] M. Tripepi et al., *Bioconjugate Chem.* **29**, 1428-1437 (2018).

OC6

NMR DETERMINATION OF THE HYDRATION NUMBER AND EXCHANGE RATE OF THE BOUND WATER MOLECULE ACROSS THE LN[AAZTA]$^-$ SERIES

F. Carniato,[1] L. Tei,[1] D. Delli Castelli,[2] S. Aime,[2] M. Botta[1]

[1] Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "A. Avogadro", Viale T. Michel 11, 15121 Alessandria, Italy.
[2] Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università di Torino, Via Nizza 52, 10126 Torino, Italy
e-mail: fabio.carniato@uniupo.it

Lanthanide complexes have been widely investigated in the last decades due to their relevance as contrast agents for Magnetic Resonance Imaging. The fine-tuning of the rate of exchange ($k_{ex} = 1/\tau_M$) of the coordinated water molecule(s) represents one of the key factors for the optimization of the performance of Gd-based T_1 probes and Ln-based ParaCEST contrast agents. Typically, T_1 agents require bound water molecules in the fast exchange regime ($k_{ex} \cong 10^8$ s^{-1}) to achieve improved efficacy (high relaxivity), whereas for ParaCEST agents, which exploit the coordinated water as source of mobile protons, optimal k_{ex} values of the bound water molecule(s) lie in the range 10^2-10^3 s^{-1}. The chemical nature of the donor groups of the chelator, hence the charge density at the Ln(III) center, greatly affects the k_{ex} of the bound water molecule. Usually negatively charged donor atoms accelerate k_{ex} in Ln(III)-chelates, therefore complexes containing only carboxylic donor groups have never been used for CEST applications.[1] Unexpectedly, it has been found that the lanthanide series with AAZTA shows a quite unusual behavior. In particular, recently, we have observed that Yb[AAZTA]$^-$ showed one coordinated water molecule with very slow k_{ex} (ca. 6.5 x 10^{-3} s^{-1}), two order of magnitude slower than bis-hydrated Gd[AAZTA]$^-$.[2] These finding, prompted us to carry out a comprehensive high- and low-resolution NMR study across the Ln(III) series. Well-established paramagnetic NMR and MRI methodologies based on ST have been used unconventionally to show the differences in the coordination sphere moving along the Ln series. This study highlights a periodic dependence on the hydration number and the exchange rate never observed previously for other complexes of *f*-elements.

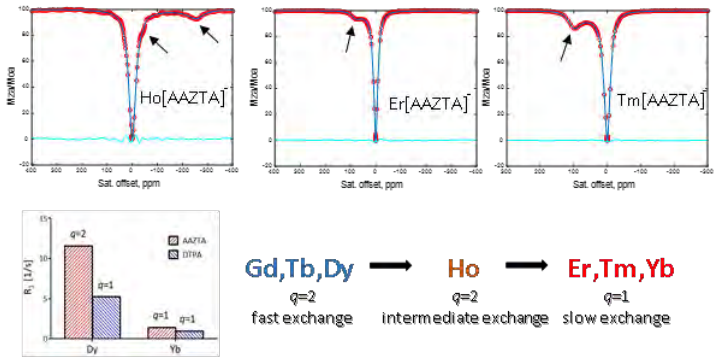


Fig. 1. above: Z-spectra of aqueous solution of different Ln(III)[AAZTA]$^-$ chelates at 7 T; below: a plot of R_1 (s^{-1}) of 52.5 mM aqueous solutions of Dy[AAZTA]$^-$, Yb[AAZTA]$^-$, Dy[DTPA]$^{2-}$ and Yb[DTPA]$^{2-}$ at 30 MHz and 298 K.

References

[1] E. Terreno, D. Delli Castelli and S. Aime, *Contrast Media Mol. Imaging* **5**, 78-98 (2010).
[2] D. Delli Castelli, L. Tei, F. Carniato, S. Aime, M. Botta, *Chem. Commun.* **54**, 2004-2007 (2018).

OC7

A TIME-RESOLVED, IN-SITU DNP NMR APPROACH TO GAIN INSIGHTS INTO METASTABLE POLYMORPHS OF GLYCINE

P. Cerreia Vioglio,[‡] G. Mollica,[‡] M. Juramy,[‡] C. E. Hughes,[†] P. A. Williams,[†] F. Ziarelli,[‡] S. Viel,[‡] P. Thureau,[‡] K. D. M. Harris[†]

[‡]Aix-Marseille Université, CNRS, ICR (UMR 7273), 13013 Marseille, France
[†]School of Chemistry, Cardiff University, Park Place, Cardiff, Wales, CF10 3AT (UK)
E-mail: paolo.cerreia-vioglio@univ-amu.fr

Polymorphism is a general phenomenon statistically occurring in one out of two molecular crystal structures [1]. Although first discussed in the scientific literature in the XIX century, polymorphism is still enigmatic: progress towards the understanding of its main chemical and physical aspects would have a huge impact in both the academic and the industry world, especially in terms of control over the solid state.
SSNMR has already proven to be a powerful technique for the study of polymorphs, representing today an established complementary technique to crystallographic methods (e.g., X-ray diffraction). The challenge now is to probe polymorphic *transitions*, which represent either an undesirable event, leading for example to product failure in the pharmaceutical industry, or an opportunity to explore the mechanisms underlying the polymorphism. In this context, the application of *in-situ* SSNMR is particular advantageous to monitor the evolution of a polymorphic transition as a function of time [2]. However, the inherent dynamic nature of these systems is a challenging factor which prevents the acquisition of 2D experiments to gain advanced structural data.
Here, we used low-temperature (150–210 K), *in-situ* SSNMR and DNP SSNMR to monitor the polymorphic phases present in flash-cooled aqueous solutions of glycine at different stages of the polymorphic transition process. Glycine was used as model system due to its known polymorphic behaviour, and to the recent discovery of a new metastable polymorph [3]. Our results demonstrate that our approach allows the acquisition of *in-situ* SSNMR 1D and 2D data of transient polymorphs of glycine, thereby providing structural insights into metastable glycine species which are elusive at room-temperature [4].

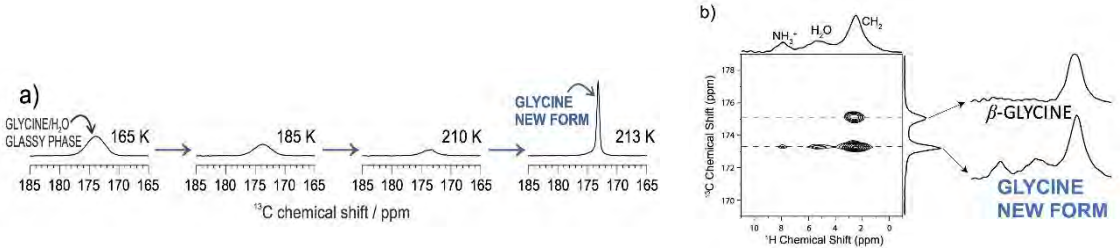


Fig. 1. (a) Monitoring the formation of a new metastable form of glycine by ^{13}C CPMAS; (b) ^{1}H-^{13}C HETCOR at 212 K of two metastable forms of glycine during a solid phase transition.

References

[1] A.J. Cruz-Cabeza, S.M. Reutzel-Edens, and J. Bernstein *Chem. Soc. Rev.* **44**, 8619-8635 (2015)
[2] K.D.M. Harris, C.E. Hughes, P.A. Williams *Solid State Nucl. Magn. Reson*. **65** 107-113 (2015)
[3] W. Xu, Q. Zhu, C. Hu *Angew. Chem., Int. Ed.* **56** 2030-2034 (2017)
[4] P. Cerreia Vioglio et al. *Angew. Chem., Int. Ed.* **57** 6619–6623 (2018)

OC8

UNRAVELING THE ROLE OF CIS-TRANS PEPTIDYLPROLYL ISOMERASES IN PARKINSON'S DISEASE

F. Favretto,[‡] S. Becker,[†] M. Zweckstetter[‡,†]

[‡]Structural Biology in Dementia (Deutsche Zentrum für Neurodegenerative Erkrankungen, DZNE), von- Siebold-Straβe 3a, 37075 Göttingen, Germany
[†]NMR-based Structural Biology (Max Plank Institute für Biophysikalische Chemie), am Faβberg 11, 37077 Göttingen, Germany
E-mail: fifa@nmr.mpibpc.mpg.de

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects dopaminergic neurons in the *substantia nigra* [1]. PD patients show characteristic symptoms such as tremors, rigidity, progressive slowing of movements and bradykinesia. Pathologically, intraneuronal cytoplasmic inclusions that are called Lewy bodies (LB) are the hallmark of PD [2,3]. LBs contain high amounts of the 140-residue protein α-Synuclein. α-Synuclein is abundantly expressed within neurons, where it constitutes up to the 1% of all neural cytoplasmic proteins and is predominantly found in presynaptic terminals [4]. In patients with PD, the natively unstructured protein α-Synuclein misfolds and aggregates into β-sheet-rich oligomers and amyloid fibrils, which combine to produce Lewy bodies [1].
Cis-trans isomerization of proline residues influences protein aggregation and might thus affect the development of neurodegenerative diseases [5]. Consistent with this hypothesis, the C-terminal domain of α-Synuclein is enriched in proline residues that play a role in the formation of toxic oligomers and amyloid fibrils [5,6]. Recently we demonstrated that cyclophilin 40, a peptidyl-prolyl isomerase (PPIase), binds to and disaggregates amyloid fibrils of α-Synuclein as well as amyloid fibrils of the protein Tau, which plays an important role in Alzheimer's disease [6].
In the present study we present new data supporting the importance of PPIases for pathogenic aggregation of α-Synuclein. Using an integrative approach based on Nuclear Magnetic Resonance (NMR) spectroscopy, we characterized the structural determinants of the interaction between the PPIase CypA – a PPIase that is similar to Cyp40 – and α-Synuclein at high resolution. In addition, we show that the PPIase activity of CypA is essential for the CypA-mediated enhancement of α-Synuclein aggregation and that α-Synuclein mutations, found in patients with early onset PD, modulate the protein's interaction with CypA. Our studies suggest that strategies based on modulation of PPIases might contribute to the development of therapeutic drugs against neurodegeneration.

References

[1] M. J. Baptista, C. O'Farrell, S. Daya, R. Ahmad, D. W. Miller, J. Hardy, M. J. Farrer, M. R. Cookson *J. Neurochem.* **85**, 957-968 (2003)
[2] E. Gomez-Tortosa, A.O. Ingraham, M. C. Irizarry, and B. T Hyman *Geriatric Bioscience* **46**, 1449-1458 (1998)
[3] L. Stefanis *Cold Spring Harb. Perspect. Med.* **4,** a009399 (2012)
[4] W. S. Kim, K. Kagedal, and G. M. Halliday *Alzheimers Res. Ther.* **6**, 73 (2014)
[5] M. Gerard, A. Deleersnijder, J. Demeulemeester, Z. Debyser, and V. Baekelandt *Mol. Neurobiol.* **44**, 13-27 (2011)
[6] J. D. Baker, L. B. Shelton, D. Zheng, F. Favretto, B. A. Nordheus, A. Darling, L. E. Sullivan, Z. Sun, P. K. Solanki, M. D. Martin, A. Suntharalingam, J. J. Sabbagh, S. Becker, E. Mandelkov, V. N. Uversky, M. Zweckstetter, C. A. Dickey, J. K. Ill, and L. J. Blair *PLOS Biology* **15**, e2001336 (2017)

OC9

LOW RESOLUTION NMR AS A POWERFUL TOOL FOR DIAGNOSTIC PURPOSES

E. Gianolio,[‡] E. Di Gregorio,[‡] G. Ferrauto,[‡] S. Aime[‡]

[‡]Department of Molecular Biotechnologies and Health Science, Via Nizza 52, Torino, Italy
E-mail: eliana.gianolio@unito.it

MR-Imaging is one of the most diffuse and powerful Imaging technique for diagnosis of a wide range of pathologies. Frequently the diagnostic power is enhanced through the administration of exogenous paramagnetic contrast agents. It is well established that the application of low resolution relaxometry is useful for the characterization of paramagnetic molecules and the study of the interactions with the microenvironment in which they are distributed. Beyond this purpose, relaxometry can be used as a valid diagnostic tool through the setup of "in vitro" bio-analytical assays based on the assessment of changes in the relaxation rate of solvent water protons. A first example will be showed in which the addition of stable Gd(III) chelates in the extracellular compartment of a cell suspension has allowed to set up a method for the determination of the water exchange rate across the cellular Red Blood Cells membrane [1]. The method has been applied to assess the changes of this parameter at different stages of malaria infection in RBCs. Moreover, changes in the transverse relaxation rate (T_2) at variable magnetic fields (in the range 20-70 MHz), have been used for analyzing infected- RBCs at different stages of malaria cycle.
We believe that further relevant information can be obtained by exploiting the T_1 dependence of protons tissue from the applied magnetic field strength (B_0). This task cannot be yet accomplished on the available MRI scanners, because they work at fixed B_0. Nevertheless, the acquisition of the so called Fast Field Cycling–Nuclear Magnetic Dispersion (FFC-NMRD) profiles [2] on *ex vivo* tissues can provide useful information on the effect of the magnetic field strength on proton T_1 values [3]. Therefore, in addition to the use of established fixed-field MRI modality, it may be of interest to explore FFC-NMRD profiles in the search of novel biomarkers for tumor early diagnosis, phenotyping and for monitoring its growth and progression.
A study will be reported which shows that FFC-NMRD profiles can provide useful insights for the detection and staging of cancer in ex vivo murine breast tissues, without the administration of exogenous contrast agents. From the acquisition of longitudinal proton relaxation time (T_1) at variable magnetic fields, two markers resulted particularly useful: i) the appearance of the ^{14}N-QPs (due to immobilized proteins) that act as an early reporter of the presence of tumor cells and ii) the difference of the relaxation rates between low-and high-magnetic fields that reports on the progression stage of the tumor.

References

[1] E. Gianolio, G. Ferrauto, E. Di Gregorio, and S. Aime *Biochim. Biophys. Acta*. **1858**, 627-631 (2016)
[2] R. M. Steele, J. P. Korb, G. Ferrante, and S. Bubici *Magn. Reson. Chem.* **54**, 502-509 (2016)
[3] M.R. Ruggiero, S. Baroni, S. Pezzana, G. Ferrante, S. Geninatti Crich, and S. Aime *Angew. Chem. Int. Ed. Engl*. **57**, 7468-7472 (2018)

OC10

DESIGN OF PEPTIDES FOR THE EARLY DIAGNOSIS OF ALZHEIMER DISEASE

M. Grimaldi[1,2], I. Stillitano[1], A. Santoro[1], M. Buonocore[1], A. M. D'Ursi[1*]

[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132 - 84084 Fisciano, Salerno, Italy.

[2]Institute of Polymers, Composites and Biomaterials, National Research Council of Italy, V.le J.F. Kennedy 54 - Pad. 20 Mostra d'Oltremare, 80125 Naples, Italy.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in adults. Senile plaques, consequence of the overproduction and/or impaired clearance of β-peptides (Aβ) are considered hallmark of AD [1,2]. The main components of amyloid plaques are Aβ(1–40) and Aβ(1–42), soluble peptides that, in response to environmental factors, aggregate forming soluble oligomers, protofibrillar oligomers and finally, insoluble fibrils. Increasing evidence show that the single phases of AD correlate with specific concentrations of soluble, oligomer or fibrillary Aβ(1–42), distributed in blood or in CSF fluid. In this context, there is an increasing interest in the development of a diagnostic tool, able to identify in these biological fluids, Aβ(1-42) peptide with a unique control in terms of both concentration and size. To this end, magnetic nanoparticles (MNP) [3] functionalized with molecules able to detect Aβ(1-42) have been investigating in view of developing an innovative diagnostic instrument endowed with high sensitivity and specificity. In the present contribution we report the design, the synthesis and the structural study of synthetic peptides selected to functionalize MNP and capable of interacting with Aβ(1-42). As several monoclonal antibodies have been experimented to target different epitopes of the Aβ peptide [4], we designed short peptides targeting Aβ(1-42) by analyzing the structural interactions between Solanezumab and Crenezumab antibodies in complex with Aβ(1-42). Accordingly, as results of molecular docking calculation seven peptides were designed. The peptide (WAibH) characterized by the lowest values of docking score was synthesized and studied for its ability to interact with Aβ(1-42). Interactions of free WAibH and MNP-WAibH with Aβ(1-42) were monitored using circular dichroism (CD) and Saturation Transfer Difference (STD) as well as Chemical Shift mapping NMR experiments.

Acknowledgement: The work has been supported by the EC HORIZON2020 project MADIA, contract number 732678.

References

[1] J. A. Hardy, and G. A. Higgins, *Science*, **256**, 184-185 (1992)
[2] J. D. Harper *et al. Chem. Biol.* **4**, 119-125 (1997)
[3] S. R. Mudshinge *et al. Saudi Pharm. J.* **19**, 129-141 (2011).
[4] A. W. Joseph *et al. Sci. Rep.* **8**, 6412 (2018).
[5] G. A. N. Crespi *et al. Sci. Rep.* **5**, 9649 (2015).
[6] M. Ultsch, *et al. Sci. Rep.* **6**, 39374 (2016).

OC11

"CORNETTO DI PONTECORVO DOP" RED SWEET PEPPER CHARACTERIZED BY A MULTI-METHODOLOGICAL APPROACH

C. Ingallina,[‡] A. P. Sobolev,[†] S. Circi,[‡] N. Proietti,[†] D. Capitani,[†] M. Locatelli,[§] S. Carradori,[§] R. Preti,[¶] G. Vinci,[¶] A. Vitalone,[*] G. Mazzanti,[*] A. Di Sotto,[*] M. E. Crestoni,[‡] B. Chiavarino,[‡] S. Fornarini,[‡] B. Botta,[‡] L. Mannina[‡†]

[‡] Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, P.le Aldo Moro 5, 00185 Rome, Italy
[†] Istituto di Metodologie Chimiche, Laboratorio di Risonanza Magnetica "Annalaura Segre", CNR, 00015 Monterotondo, Rome, Italy
[§] Dipartimento di Farmacia, Università di Chieti-Pescara "G. D'Annunzio", Via dei Vestini 31, 66100, Chieti, Italy
[¶] Dipartimento di Management, Laboratorio di Merceologia, Sapienza Università di Roma, Via del Castro Laurenziano 9, 00161 Rome, Italy
[*] Dipartimento di Fisiologia e Farmacologia "V. Ersparmer", Sapienza Università di Roma, P.le Aldo Moro 5, 00185 Rome, Italy
E-mail: cinzia.ingallina@uniroma1.it

"eALIERB: un OPEN LAB per caratterizzare e valorizzare i prodotti alimentari ed erboristici del territorio laziale" is a Regional project in which a multi –methodological approach has been developed to characterize local typical foodstuffs. This project aimed at setting up an open laboratory with different research facilities, from Sapienza University of Rome, Istituto di Metodologie Chimiche of CNR and Policlinico Umberto I, to characterize typical Italian products under chemical, biological and quality profiles. Red sweet peppers (*Capsicum annuum* L.) ecotype "Cornetto di Pontecorvo" grown in greenhouse (GH) or in open field (OF) [1] has been chosen as pilot foodstuff.

Phytochemical composition was assessed on different extracts, following three extraction procedures, on raw material. Both untargeted (NMR, MS) and targeted (HPLC and HPTLC) methods were used to provide complementary information on metabolite (primary and secondary) composition and to highlight differences between the part of the fruit (pulp, peel and seeds) and the type of cultivation (OF and GH). Quality and freshness of the fruits were evaluated also by HPLC-FLD and HPLC-MS methods, determining the biogenic amines and mycotoxins content. Last, the biological activity of extracts was assessed towards the inhibition of glucose metabolism, antifungal properties, antimutagenic and antioxidant effects [2].

The analysis of the results highlighted differences in the concentration of some metabolites between the fruits cultivated GH and OF, although the morphological analysis, required for the PDO certification, showed the preservation of the main characteristics. The comparison of peel, seed and pulp samples, suggested that has peel and seeds can be potentially useful in food industry being a good source of bioactive compounds, important from a nutritional point of view.

References

[1] A. P. Sobolev, L. Mannina, D. Capitani, G. Sanzò, C. Ingallina, B. Botta, S. Fornarini, M. E. Crestoni, B. Chiavarino, S. Carradori, M. Locatelli, A. M. Giusti, G. Simonetti, G. Vinci, R. Preti, C. Toniolo, M. Reverberi, M. Scarpari, A, Parroni, L. Abete, F. Natella and A. Di Sotto *Food Chem.*, **255**, 120-131 (2018)

[2] A. Di Sotto, M. Vecchiato, L. Abete, C. Toniolo, A. M. Giusti, L. Mannina, M. Locatelli, M. Nicoletti and S. Di Giacomo *J. Funct. Foods*, **40**, 679-691 (2018)

OC12

^{1}H NMR CHARACTERIZATION OF AGING PROCESSES IN PRECURSOR SOLUTIONS USED FOR YBCO SUPERCONDUCTORS PRODUCTION

R. Lamanna,‡ V. Pinto,† A. Vannozzi,† L. Piperno,†A. Angrisani Armenio,† G. Celentano†

‡ENEA - SSPT Department, Trisaia Research Center 75026 Rotondella (Mt), Italy
†ENEA - FSN Department, Frascati Reaeach Center 00044 Frascati (Rm), Italy
E-mail: raffaele.lamanna@enea.it

One of the methods for production of $YBa_2Cu_3O_{7-x}$ (YBCO) superconducting films is through Chemical Solution Deposition.
The stability of the precursor solutions is crucial for the quality of the films and often aged solutions give rise to poor superconducting properties.
A deep understanding of the degradation mechanisms is then important for optimizing precursors composition.
The possibility of simultaneous detection of both reagents and products in spontaneous chemical reactions, occurring during the precursors storage, makes NMR the best characterizing technique [1,2].
However, the presence of copper and the fast chemical exchange among titratable protons makes difficult compounds identification and quantification.
Actually, the impossibility to refer to literature chemical shift data (line position is determined by Cu^{++} concentration) and to perform 2D experiments (short transverse relation times) may be circumvented by spiking and model reaction studies.
On the other hand, strong spectral convolution requires innovative fitting approaches based on correlated lineshapes mimic the whole compound.
Since spontaneous reaction involve also the solvent, a simple kinetic model is needed to interpret the concentrations time evolution during storage.
Finally, paramagnetic effects are discussed through the analysis of Curie curves and relaxation measurements.

References

[1] V. Pinto, et al. *IEEE Trans. Appl. Supercond.* **26***,* 7439806 (2016)
[2] V.Pinto, et al. *IEEE Trans. Appl. Supercond.* **28**, 7500505 (2018)

OC13

METAL ION RECRUITMENT DRIVES THE FOLDING MECHANISM AND SELF-ASSOCIATION PROPENSITY OF HIGH HOMOLOGOUS PROTEINS

G. Malgieri,[‡] G. D'Abrosca,[‡] L. Pirone,[†] A. Toto,[‡‡] L. Russo,[‡] M. F. M. Sciacca,[††] V. Sivo,[‡] I. Baglivo,[‡] F. Russo,[‡] M. Coletta,[†††] P. V. Pedone,[‡] C. Isernia,[‡] M. De Stefano,[‡] S. Gianni,[‡‡] E. M. Pedone,[†] D. Milardi,[††] and R. Fattorusso[‡,†]

[‡] Department o, Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100
[†] Institute of Biostructures and Bioimaging, CNR, Via Mezzocannone 16, 80134 Naples, Italy
[‡‡] Department of Biochemical Sciences "Alessandro Rossi Fanelli", University of Rome "La Sapienza", Piazzale Aldo Moro 5, 00185, Roma, Italy
[††] Institute of Biostructures and Bioimaging, CNR, Viale A. Doria 6, 95125 Catania, Italy.
[†††] Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133, Roma, Italy
E-mail: gaetano.malgieri@unicampania.it

Using three isostructural proteins of the prokaryotic zinc finger family as model systems ($Ml1_{53-149}$ and Ros87 that bind a structural zinc ion and $Ml4_{52-151}$ that lacks it), our study is designed to contribute to the knowledge about the detailed mechanisms by which metal ions perturb proteins structure and function, folding mechanism and self-association propensities [1,2]. The prokaryotic zinc finger domain [3] shows a 58 amino acids $\beta\beta\beta\alpha\alpha$ globular fold that, stabilized by a 15 amino acids hydrophobic core, uses different combinations of amino acids to coordinate the structural metal ion when present [4]. We will discuss how the recruitment of the structural metal can modify the folding pathway of these relatively small domains, control conformational accessibility to aggregation-prone states and change aggregation kinetics. While these model domains have little direct disease-relevance, our results are certainly of broad general interest as many disease-relevant proteins bind metal ions, which could similarly influence their structures, folding pathways and aggregation.

References

[1] M. Palmieri, G. Malgieri, L. Russo, I. Baglivo, S. Esposito, F. Netti, A. Del Gatto, I. de Paola, L. Zaccaro, P.V. Pedone, C. Isernia, D. Milardi, and R. Fattorusso, *J. Am. Chem. Soc.* **135**, 5220-5228 (2013)
[2] G. Malgieri, G. D'Abrosca, L. Pirone, A. Toto, M. Palmieri, L. Russo, M. F. M. Sciacca, R. Tatè, V. Sivo, I. Baglivo, R. Majewska, M. Coletta, P. V. Pedone, C. Isernia, M. De Stefano, S. Gianni, E. M. Pedone, D. Milardi, and R. Fattorusso, *Chem. Sci.* **13**, 3290-3298 (2018)
[3] G. Malgieri, L. Russo, S. Esposito, I. Baglivo, L. Zaccaro, E.M. Pedone, B. Di Blasio, C. Isernia, P. V. Pedone, and R. Fattorusso, *PNAS* **104**, 17341-17346 (2007)
[4] G. D'Abrosca, L. Russo, M. Palmieri, I. Baglivo, F. Netti, I. de Paola, L. Zaccaro, B. Farina, R. Iacovino, P. V. Pedone, C. Isernia, R. Fattorusso and G. Malgieri, *J. Inorg. Biochem.* **161**, 91-98 (2016)

OC14

PHASE SEPARATION, DYNAMICS AND INTERACTION IN STARCH-SUCROSE AMORPHOUS BLENDS BY SOLID STATE NMR

F. Martini,[‡,§] D. Hughes,[†] G. Badolato Boenisch,[#] J. Ubbink,[ǂ] M. Geppi[‡, §]

[‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via G. Moruzzi 13, 56124 Pisa (Italy).
[§]Istituto di Chimica dei Composti Organometallici, Consiglio Nazionale delle Ricerche-CNR SS di Pisa, Via G. Moruzzi 1, 56124 Pisa (Italy).
[†]H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol BS8 1TL, United Kingdom.
[#]DSM Nutritional Products AG, Research Center Formulation & Application, P.O. Box 2676, 4002, Basel, Switzerland.
[ǂ]California Polytechnic State University, San Luis Obispo, CA, United States.
E-mail: francesca.martini@for.unipi.it

Microencapsulation is a process commonly used in the pharmaceutical, food, and agricultural industries for the protection and controlled release of active ingredients, such as drugs, flavors, or nutrients [1]. Amorphous blends of carbohydrate polymers and low molecular weight molecules in the glassy state are among the encapsulating systems mostly used for the protection of active ingredients from diffusion and oxidative degradation phenomena [2]. The barrier properties of these materials result from the interplay of multiple factors, including the proximity to T_g, the presence of free volumes and local dynamics, in a way that is still not fully understood. Moreover, a complex dependence of the properties of the glassy matrix on variables as composition, thermal history, physical aging and water content was observed [2].
In this work we present a study of glassy amorphous blends made of a hydrophobically modified starch and sucrose by means of solid state NMR spectroscopy and NMR relaxometry [3]. The phase and dynamic properties and the starch/sucrose interaction were investigated as a function of blend composition, combining ^{13}C MAS selective spectra, variable temperature ^{1}H T_2 and T_1 measurements, and Goldman-Shen experiments. NMR results were compared and integrated with those arising from DSC (Differential Scanning Calorimetry) and PALS (Positron Annihilation Lifetime Spectroscopy) measurements, providing a detailed picture of the structural and dynamic features on a nanometric and sub-nanometric scale at the origin of the barrier properties of these encapsulating matrices.

References

[1] A. G. Gaonkar, N. Vasisht, A. R. Khare, and R. Sobel *Microencapsulation in the food industry*, Elsevier (2014).
[2] J. Ubbink *Adv. Drug Deliver. Rev.* **100**, 10-26 (2016)
[3] D. Hughes, G. B. Boenisch, T. Zwick, C. Schaefer, C. Tedeschi, B. Leuenberger, F. Martini, G. Mencarini, M. Geppi, M. Alam, J. Ubbink, *Carbohyd. Polym.*, in press (2018)

OC15

DIRECT AND INDIRECT EXPLORATION OF NANOCRYSTAL SURFACE

M. Mauri,‡ M. Tawfilas,‡ R. Simonutti‡

‡Dipartimento di Scienza dei Materiali, Università di Milano-Bicocca, via R. Cozzi 55, 20125, Milano, Italia
E-mail: michele.mauri@unimib.it

The surface of nanocrystals (NCs) determines critical properties such as stability of dispersions, feasibility of nanocomposites and even cytotoxicity. Crystal size, composition and decoration can be tuned during and after the NC synthesis, and NMR can be used to characterize the resulting surface in detail. In acqueous dispersions, the fast exchange of water with the surface allows using the extremely abundant ^{1}H nuclei as probe of the surface energy. Fig. 1 (a) shows how the relaxivity of water dispersions of different titanium oxide NCs depends on their size and polymorphism. The surface was equalized by ligand stripping before the experiments to avoid any influence of chemical decoration. Relaxivity is also normalized against the specific surface measured independently through nitrogen adsorption, in order to compensate for the trivial effect of increased surface to volume ratio of smaller particles. After corrections, smaller rutile nanocrystals (Rut20, 20 nm diameter) are much more efficient than their larger Rut 50 counterparts, and even more so in respect to other polymorphs.

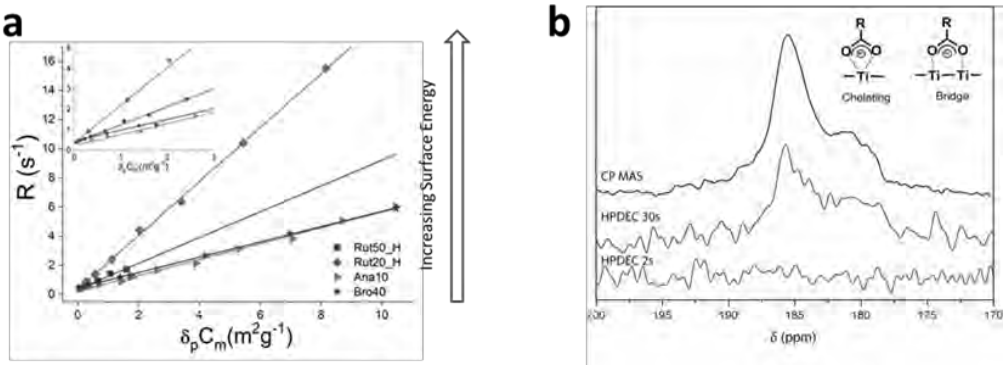


Fig. 1 (a) R_2 relaxivity of water in acqueous dispersions of various polymorphs of TiO_2 nanocrystals, including anatase (Ana10), brookite (Bro40) and two different sizes of rutile (Rut20 and Rut50) normalized against specific surface. (b) ^{13}C SSNMR spectra of anatase nanoparticles produced *via* solvothermal synthesis and capped with lauric acid with indication of the binding mechanisms.

NMR can also be used to qualitate the nature of the ligand-crystal interaction by direct investigation of the decorated nanoparticles with SSNMR, provided that systems with sufficient coverage are available. Fig. 1 (b), depicting spectra acquired on anatase TiO_2 nanocrystals covered with lauric acid. Comparison between HPDEC and CPMAS indicates that the LA chains are strongly bound to the surface, and that the binding takes places with both chelating and bridge mechanisms [2].
Together, direct and indirect NMR methods can provide a description of the surface, and more work is in progress on the study of the conformation of tethered polymers. Finally, work with ^{13}C enriched isotopes allows detecting the ligand/particle interactions in systems with low specific surface or within nanocomposites.

References

[1] M. Tawfilas, M. Mauri, L. De Trizio, R. Lorenzi, and R. Simonutti *Langmuir* **34**, 9460-9469 (2018)
[2] S. Pawsey, K. Yach, J. Halla, and L. Reven *Langmuir* **16**, 3294-3303 (2000)

OC16

STRUCTURAL AND MOLECULAR RECOGNITION PROPERTIES OF UBB^{+1}, A UBIQUITIN MUTANT ASSOCIATED WITH ALZHEIMER'S DISEASE

F. Munari,[‡] A. Bortot,[‡] M. Assfalg,[‡] M. D'Onofrio[‡]

[‡]Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy
E-mail: francesca.munari@univr.it

Ubiquitin is a small protein modifier that regulates numerous fundamental processes in eukaryotic cells, such as protein turnover via the proteasome. Dysfunction of ubiquitin-proteasome system is connected to the pathogenesis of several human diseases, including neurodegenerative diseases. Notably, Ubb^{+1}, a mutated version of ubiquitin, was found specifically accumulated in brain tissues of Alzheimer's disease patients. Ubb^{+1} originates from misreading of the Ub B gene that gives a frame-shift mutant protein where the carboxy-terminal Gly76 is replaced by a tyrosine linked to a 19-residue peptide [1]. Ubb^{+1} does not work anymore as protein modifier and terminates the elongation of polyubiquitin chains. The resulting Ubb^{+1}-capped polyubiquitin chains inhibit the proteasome [2] and specific deubiquitinating enzymes [3].
In our work, we used NMR spectroscopy to study conformational and functional differences of Ubb^{+1} with respect to the wild-type protein. In particular, we investigated structural and dynamic features of Ubb^{+1} and characterize its interactions with the UBA2 domain of the human homologue of the yeast DNA repair protein RAD23 and membrane mimics [4].

References

[1] F.W. van Leeuwen, D.P. de Kleijn, H.H. van den Hurk, A. Neubauer, M.A. Sonnemans, J.A. Sluijs, S. Köycü, R.D. Ramdjielal, A. Salehi, G.J. Martens, F.G. Grosveld, J. Peter, H. Burbach, E.M. Hol, *Science* **279**, 242-247 (1998)
[2] Y.A. Lam, C.M. Pickart, A. Alban, M. Landon, C. Jamieson, R. Ramage, R.J. Mayer, R. Layfield. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 9902-9906 (2000)
[3] D. Krutauz, N. Reis, M.A. Nakasone, P. Siman, D. Zhang, D.S. Kirkpatrick, S.P. Gygi, A. Brik, D. Fushman, M.H. Glickman, *Nat. Chem. Biol.* **10**, 664-670 (2014)
[4] F. Munari, A. Bortot, M. Assfalg, M. D'Onofrio *Int. J. Biol. Macromol.* **108,** 24-31, (2018)

OC17

SUCCINIMIDE-BASED CONJUGATES IMPROVE ISODGR CYCLOPEPTIDE AFFINITY TO AVB3 WITHOUT PROMOTING INTEGRIN ALLOSTERIC ACTIVATION

F. Nardelli,[‡] C. Paissoni,[†] G. Quilici,[‡] A. Gori,[§] C. Traversari,[*] B. Valentinis,[*] A. Sacchi,[‡] A. Corti,[‡] F. Curnis,[‡] M. Ghitti[‡] and G. Musco[‡]

[‡]IRCCS Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy
[†]Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133 Milan, Italy
[§]Istituto di Chimica del Riconoscimento Molecolare, CNR. Via Mario Bianco 9, 20131, Milan, Italy
[*]Molmed, SpA, Via Olgettina Milano, 58, 20132 Milan, Italy
E-mail: nardelli.francesca@hsr.it

The isoDGR sequence is an integrin-binding motif that has been successfully employed as a tumor-vasculature-homing molecule or for the targeted delivery of drugs and diagnostic agents to tumors [1]. In this context, we previously demonstrated that cyclopeptide 2, the product of the conjugation of c(CGisoDGRG) to 4-(N-maleimidomethyl)cyclohexane-1-carboxamide, can be successfully used as a tumor-homing ligand for nanodrug delivery to neoplastic tissues [1]. Here, combining NMR, computational, and biochemical methods, we show that the succinimide ring contained in 2 contributes to stabilizing interactions with αvβ3, an integrin overexpressed in the tumor vasculature. Furthermore, we demonstrate that various cyclopeptides containing the isoDGR sequence embedded in different molecular scaffolds do not induce αvβ3 allosteric activation and work as pure integrin antagonists. These results could be profitably exploited for the rational design of novel isoDGR-based ligands and tumor-targeting molecules with improved αvβ3-binding properties and devoid of adverse integrin-activating effects [2].

References

[1] F. Curnis, A. Sacchi, R. Longhi, B. Colombo, A. Gasparri & A. Corti *Small* **9**, 673-678 (2013)
[2] F. Nardelli, C. Paissoni, G. Quilici, A. Gori, C. Traversari, B. Valentinis, A. Sacchi, A. Corti, F. Curnis, M. Ghitti, and G. Musco *J. Med. Chem.* DOI: 10.1021/acs.jmedchem.8b00745 (2018)

OC18

NMR MEETS MACHINE LEARNING: CHEMICAL SHIFT PREDICTIONS IN SOLIDS IN LESS THAN A MINUTE

F.M. Paruzzo,[‡] A. Hofstetter,[‡] F. Musil,[†] S. De ,[†] M. Ceriotti,[†] and L. Emsley[‡]

[‡]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
[†]Institute des Sciences et Génie Matériaux, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
E-mail: federico.paruzzo@epfl.ch

Chemical shift based NMR crystallography has shown to be a powerful methods for the determination of the atomic-level structure of amorphous materials and microcrystalline solids [1]. The approach, which consists in the combination of solid-state NMR measurements (typically ^{1}H chemical shifts) and computational methods, is now widely used for both structure validation [2,3] and *de novo* crystal structure determination [4,5]. The major bottleneck lies in its high computational cost associated with the required density functional theory (DFT) chemical shift calculations. This prevents both efficient high throughput screening of potential crystalline structures and the application to larger and more complex crystals.

In the past few years, machine learning (ML) methods have shown to be a powerful tool to bridge the gap between the need for high accuracy calculations and limited computational power in many areas of chemical and physical science. Here we propose a machine learning framework to predict chemical shifts in solids, based on Gaussian Process Regression (GPR) using local SOAP fingerprints [6,7].

We train this model on DFT calculated chemical shifts of a set of 2000 molecular crystals chosen to be as diverse as possible. We then demonstrate the prediction performance on a set of 500 randomly chosen crystal structures not included in the training set (we obtain R^2 coefficients between the chemical shifts calculated with DFT and with ML of 0.97 for ^{1}H, 0.99 for ^{13}C, 0.99 for ^{15}N, and 0.99 for ^{17}O, corresponding to RMSEs of 0.49 ppm for ^{1}H, 4.5 ppm for ^{13}C, 13.3 ppm for ^{15}N, and 17.7 ppm for ^{17}O). We show that the model can be used in an NMR crystallography protocol in combination with CSP to correctly determine the structure of cocaine and AZD8329. We also show that it is possible to calculate the NMR spectrum of very large molecular crystals (>1000 atoms) in about 1 CPU minute, an acceleration of a factor 10^6 with respect to the CPU years required by DFT.

References

[1] R. K. Harris *Solid State Sci.* **6**, 1025-1037 (2004)
[2] E. Salager *et al.*, *Phys. Chem. Chem. Phys.* **11**, 2610-2621 (2009)
[3] M. Baias *et al.*, *Phys. Chem. Chem. Phys.* **15**, 17501-17507 (2013)
[4] M. Baias *et al.*, *J. Am. Chem. Soc.* **135**, 8069-8069 (2013)
[5] J. Leclaire *et al.*, *Chem. Sci.* **7**, 4379-4390 (2016)
[6] A. P. Bartók, R. Kondor, G. Csányi, *Phys. Rev. B: Condens. Matter Mater. Phys.* **87**, 1-16 (2013)
[7] S. De *et al.*, *Phys. Chem. Chem. Phys.* **18**, 13754-13769 (2016)

OC19

NMR IN TRANSFUSION MEDICINE

T.A. Pertinhez[‡,†]

[‡] Department of Medicine and Surgery, University of Parma, Parma, Italy
[†] Transfusion Medicine Unit, AUSL-IRCCS Reggio Emilia, Italy
E-mail: thelma.pertinhez@unipr.it

Translation of metabolomics data from bench to bed side is a complex task.
A hospital ward such as the "Transfusion Medicine" offers a number of possibilities to explore the products (hemocomponents), their production process, their qualification and their impact on the programmed therapeutic protocols.
Blood transfusion is a fundamental therapy in numerous pathological conditions and the main hemocomponent used is Red Blood Cells (RBCs). RBCs bags, during storage in blood bank conditions, undergo multiple biochemical alterations leading to the production of RBCs storage lesions. Up to now, many studies are being aimed to the identification of biomarkers of storage lesions and to the evaluation of the quality of RBCs.
Among the handling routinely done on RBCs bags, we chose to study by ^{1}H-NMR spectroscopy the effect of:
a) leukodepletion, a pre-storage treatment recognized to better preserve overtime the quality of RBCs;
b) aging, the bags are conserved up to 42 days at 6 °C;
c) X-ray irradiation, a protocol used to inactivate white blood cells in bags prescribed to hematological patients.
RBCs metabolic profile were evaluated by ^{1}H-NMR and the obtained data have been integrated with clinical laboratory assays, such as degree of hemolysis, electrolytes and ammonium concentration.
The use of ^{1}H NMR spectroscopy for quality control purposes in transfusion medicine was validated by using creatinine and lactate, two metabolites present in a wide concentration dynamic range and routinely quantified by standard biochemical assays. Indeed, ^{1}H NMR spectroscopy turned out to be an accurate a precise method for metabolite quantification in RBCs bag.
Around 50 metabolites were identified and quantified in the RBC bags. Among them, we found: 1) a potential biomarker of RBCs protection level against the oxidative stress: 5-oxoproline; 2) an intermediate of the purine pathway with potential toxic effect on massive transfusion: hypoxanthine; 3) a dependence of the concentration of glycine, glutamine and creatine, as a function of X-ray irradiation doses.
Overall, our results highlighted the benefits of leukodepletion and the need to adopt short storage for irradiated RBCs' bags. These contributions to the optimization of the storage protocols have been translated into operative procedures in our ward at the AUSL-IRCCS hospital.

OC20

A COMPREHENSIVE URINARY METABOLOMIC APPROACH BASED ON NMR AND LC–HRMS TO IDENTIFY BLADDER CANCER

G. Petrella,[‡] S. Lentini,[†] G. Di Pietro,[‡] L. Orsatti,[†] C. Montesano,[†] R. Speziale,[†] V. Summa,[†] A. Salonia,[†] R. Vago,[§] E.S. Monteagudo,[†] D. O. Cicero[†,‡]

[‡] Dipartimento di Scienze e Tecnologie Chimiche, University of Rome "Tor Vergata", Via della Ricerca Scientifica snc, 00133 Rome, Italy, cicero@scienze.uniroma2.it
[†] IRBM Science Park Spa,Via Pontina km 30.600, 00040 Pomezia (RM), Italy.
[§] Urological Research Institute, Division of Experimental Oncology, IRCCS "San Raffaele" Hospital, Via Olgettina 60, 20132 Milan, Italy and Università "Vita-Salute San Raffaele", Via Olgettina, 58, 20132 Milan, Italy.
E-mail: gretapet@gmail.com

Bladder cancer (BC) is among the most common types of cancer in developed countries and it has a high mortality rate. Therefore early diagnosis of BC is crucial to improve patient outcomes. Currently, cystoscopy examination coupled to histopathologic analysis remain the gold diagnostic standard for the detection of BC [1].
However cystoscopy is invasive, uncomfortable and costly [2], hence the development of non-invasive biomarkers could benefit patients. In this work we studied the metabolic profile of 52 samples of urine to evaluate the metabolic perturbations occurring in non-invasive bladder cancer compared with healthy subjects, combing NMR and LC–HRMS.
The comparison of two different methods for data fusion of the two data sets was performed and the possibilities and issues associated with data fusion will be discussed in detail. The subsequently statistical multivariate and univariate analysis of combined NMR and MS dataset lead to the discrimination between bladder cancer and healthy subjects. The significant MS-variables were detected and correlated to NMR metabolite concentrations with the aim to identify the perturbed metabolic pathways involved in bladder cancer.

References

[1] E. C. Y. Chan *et al. J. Proteome Res.* **2,** 587-602 (2015)

[2] Y. Cheng *et al. Int. J. Clin. Exp. Med.* **8,** 11052–11063 (2015)

OC21

UN-STRUCTURAL BIOLOGY BY NMR SPECTROSCOPY

R. Pierattelli

Department of Chemistry Ugo Schiff & Magnetic Resonance Center, University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino (Florence), Italy
E-mail: roberta.pierattelli@unifi.it

The importance of local flexibility in determining the function of proteins has been recognized long ago and also widely scrutinized. If the extent of local flexibility is taken to its extreme conditions it leads to completely random coil behaviour of a polypeptide chain, indicated as intrinsic disorder, through a wide variety of intermediate cases both in terms of extent of mobility or in terms of protein stretches involved.
In recent years many examples of intrinsically disordered proteins (IDPs) appeared in the literature showing how their structural plasticity and intrinsic flexibility can be key features to enable them to interact with a variety of different partners and to adapt to different conditions. These properties provide functional advantages to IDPs enabling them to play key roles in many regulatory processes and their function has also been related to several diseases.
The general properties of IDPs cannot be captured in ordered crystals, preventing them to be suitable targets for crystallographic studies. Thus, nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in their investigation, being the only method that allows a high resolution description of their structural and dynamic features in solution. The high flexibility has several consequences on the NMR spectroscopic parameters that, if properly handled, can give precious information.
We will illustrate how NMR can help in describing the importance of intrinsic disorder to encode in a relatively short polypeptide many functional modules.

References

[1] P.E. Wright, H.J. Dyson. *Nat. Rev. Mol. Cell Biol.* **16**, 18 (2015)
[2] V. Csizmok, A.V. Follis, R.W. Kriwacki, J.D. Forman-Kay. *Chem. Rev.* **116**, 6424 (2016)
[3] I.C. Felli, R. Pierattelli (Eds.) *Intrinsically disordered proteins studied by NMR spectroscopy,* Springer, Switzerland, (2015).
[4] A. Piai, E.O. Calçada, T. Tarenzi, A. del Grande, M. Varadi, P. Tompa, I.C. Felli, R. Pierattelli. *Biophys. J,* **110**, 372 (2016)
[5] S. Contreras-Martos, A. Piai, S. Kosol, M. Varadi, A. Bekesi, P. Lebrun, A. Volkov, K. Gevaert, R. Pierattelli, I.C. Felli, P. Tompa. *Sci. Rep.* **7**, 4676 (2017)
[6] M.G. Murrali, A. Piai, W. Bermel, I.C. Felli, R. Pierattelli, *ChemBioChem,* e-pub ahead of print (2018)

OC22

SKIN METABOLOMICS THROUGH HR-MAS NMR: EFFECTS OF INGENOL MEBUTATE ON ACTINIC KERATOSIS

V. Righi,* E. Tarentini,‡ C. Magnoni,‡ A. Mucci†

* Department of Life Quality Studies, University of Bologna, Campus Rimini, Corso d'Augusto 237, 47921- Rimini, Italy
‡ Surgical, Medical and Dental Department of Morphological Sciences related to Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, via del Pozzo 71, 41124 Modena, Italy;
†Department of Chemical and Geological Sciences, via Campi 103, 41125 Modena, Italy;
E-mail: valeria.righi2@unibo.it

Actinic keratosis (AK) is a cutaneous intraepithelial neoplastic lesion that typically develops on sun-damaged skin of elderly individuals. AK is accepted as the most frequent pre-malignant skin lesion in humans, and its incidence is increasing worldwide [1]. Indeed, untreated lesions may progress into squamous cell carcinoma (SCC) and even if the mechanism of development and progression of AK is still unknown, the main risk factor seems to be chronic UV exposure [2]. Ingenol mebutate gel is an approved field treatment option for AK [3], and it holds the potential to prevent the progression to SCC. However, the efficacy of ingenol mebutate has been assessed only on a clinical bases, and histological studies on its effects on skin metabolites, also over a long-term, are lacking.
Metabolomics approaches have been already successfully used in the dermatology field [4]. Nuclear Magnetic Resonance (NMR) represents an important tool for the analysis the fingerprint of tissue and has been already applied to samples from AK patients [5]. *Ex vivo* High Resolution Magic Angle Spinning (HR-MAS) NMR is able to detect the biochemical tissue composition, and identify potential biomarkers to differentiate to the ultrastructural and histopathological findings on the same specimen in order to establish the metabolic profile typical of the healthy and pathological tissue. To our knowledge this technique has never been applied specifically to AK samples. The purpose of this study was to develop a comprehensive skin metabolomics analysis for identifying potential biomarkers of AK and to monitor the effectiveness of treatment with ingenol mebutate. AK patients, when compared with healthy subjects, showed an overall increase of all the evaluated metabolites. Noteworthy, after ingenol mebutate treatment, we observed a major histological improvement and, regarding the metabolic profile, some metabolites reduced, while other continued to increase. This suggests a partial recovery of the metabolomic profile characteristic of the healthy subject following ingenol mebutate therapy.

Acknowledgments
Thanks to the "LEO Pharma" for financial support.

References

[1] T. Dirschka, G. Gupta, G. Micali et al *J. Dermatolog. Treat.* **28**, 431-442 (2017)
[2] A. Filosa, G. Filosa, *Ital. Dermatol. Venereol.* **150**, 379-84 (2015)
[3] K. Kostovic, S. J. Gulin, Z. B. Mokos, R. Ceovic, *Anticancer Agents Med. Chem.* **17**, 1304-1311 (2017)
[4] S. Jiang, T. E. Hinchliffe, T. Wu, *Genomics Proteomics Bioinformatics* **13**, 224-233 (2015)
[5] M. Mogensen, G. B. Jemec, *Dermatol. Surg*. **33**, 1158-1174 (2007)

OC23

ULTRAFAST MAS PROTON-DETECTED 3D SOLID STATE NMR EXPERIMENT TO RECOUPLE ^{15}N CHEMICAL SHIFT ANISOTROPY

F. Rossi,[a] M. R. Chierotti,[a] R. Gobetto,[a] Y. Nishiyama[b,c]

[a] Department of Chemistry and NIS Centre, University of Torino, V. P. Giuria 7, 10125, Torino Italy.
[b] RIKEN CLST-JEOL Collaboration Center, Yokohama, Kanagawa 230-0045, Japan
[c] JEOL RESONANCE Inc., Musashino, Akishima, Tokyo 196-8558, Japan
E-mail: fe.rossi@unito.it

Chemical shift anisotropy (CSA) represents a sensitive probe of electronic nuclear environment and thus, it offers deeper insights into detailed structural and dynamic properties of different chemical and biological systems. Over the years, massive efforts have been made to develop recoupling methods that reintroduce CSA interaction under MAS condition [1]. Among these techniques, one method presented by M. Levitt makes use of rotor-synchronized symmetry-based pulse sequences [2].
We present here a proton-detected 3D ^{1}H/^{15}N/^{15}N CS/CS/CSA correlation experiment which employs a γ-encoded RN_n^{ν}-symmetry based CSA recoupling scheme. The advantage of such schemes is that they can be exploited under a wide range of MAS rates within the practically attainable rf field strength. In this work, two different symmetries, i.e. $R8_7^3$ and $R10_9^4$, have been tested on [U-^{15}N]-L-histidine·HCl·H_2O as a model sample and then successfully employed to retrieve ^{15}N CSA information in natural abundance glycyl-L-alanine (GlyAla) sample under 70 kHz MAS. We demonstrate that this 3D R-symmetry based pulse sequence is robust with respect to offset wide-range mismatches and to rf inhomogeneity within mis-sets of ±10% from the theoretical value. The application of the 3D experiment on GlyAla sample results in site-resolved ^{15}N CSA lineshapes, from which CSA parameters have been retrieved by numerical fittings using SIMPSON program. The advantages of this proton-detected 3D correlation experiment are manifold: efficient and robust ^{15}N CSA recoupling is achievable; low-power rf fields can be employed to avoid sample and probe heating; the sensitivity is strongly enhanced by ^{1}H indirect detection approach which thus permits to retrieve CSA for natural abundance samples; by incorporating a ^{15}N isotropic chemical shift dimension into the 3D sequence, the experiment becomes applicable for simultaneous determination of multiple sites; the small rotor volume essential for the ultrafast MAS requires a tiny amount of sample, extending the method to systems that need isotope enrichment or for which only small amounts are feasible.

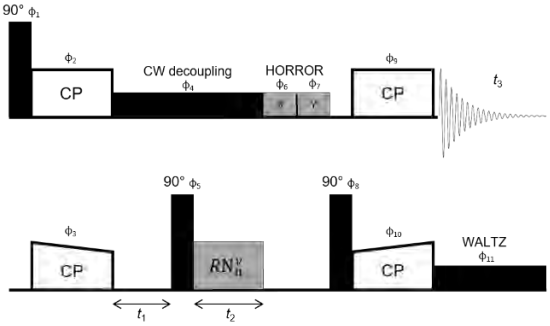


Fig. 1. Proton-detected 3D ^{1}H/^{15}N/^{15}N CS/CS/CSA pulse sequence.

References

[1] Y. Nishiyama, T. Yamazaki, T. Terao, *J. Chem. Phys.* **124**, 64304 (2006); R.M. Orr, M.J. Duer, *Solid State Nucl. Magn. Reson.* **30**, 1-8 (2006); I. Hung, Z. Gan, *J. Magn. Reson.* **213**, 196-199 (2011).
[2] M.H. Levitt, in *Encyclopedia of Nuclear Magnetic Resonance.* Wiley, Chichester, 2002, pp. 165-196.

OC24

INTRACELLULAR WATER LIFETIME AS A TUMOUR BIOMARKER BY FFC-RELAXOMETRY

M. R. Ruggiero,[‡] S. Baroni,[‡] S. Pezzana, ,[‡] S. Geninatti Crich,[‡] G. Ferrante,[†] S. Aime[‡]

[‡]University of Turin, Turin, Italy
[†]Stelar, Mede (PV) Italy
E-mail: ruggiero.mr@gmail.com

Diagnostic tools have a key role in the phenotyping of complex, heterogeneous and multifactorial diseases like cancer. They have a fundamental role also for the selection of a personalized therapy, to increase the chance of success and reduce the side effects. Magnetic resonance imaging (MRI)is one of most useful imaging modalities in the field of oncology. However, at the magnetic field strength of the currently available MRI scanners, changes in endogenous longitudinal relaxation times (T_1) do not appear sensitive enough to report on peculiar aspect of the tumour stage. The alternative diagnostic approach herein proposed is based on the in vivo measurement of endogenous T_1, in range of low magnetic fields strengths (0.01-10 MHz), using the Fast Field Cycling(FFC)relaxometer technology. Our hypothesis is that the osmosis and metabolism driven movement of free water molecules across membranes (that affects cell volume and shape), may represent an intrinsic and extremely sensitive reporter of the metabolic state. The measurement of the intracellular water lifetime (τin) may bring relevant information on the ongoing metabolism of the tumour cell. The analysis of measurements of T_1 (performed using the FFC-relaxometry) at different fields using the NMR "shutter-speed" model (model that keep in count the extra/intra-cellular compartment and the exchange water between them) allows to determine the τin. Mouse mammary adenocarcinoma cells (4T1, TS/a, 168farn) were injected in murine muscle himdlimb. In vivo measurements of endogenous T_1 were performed using the FFCrelaxometer technology. Immunofluorescence analysis of different transporters (GLUT1, AQPs, Na^+/K^+ ATPase) will be performed to better understand the biological mechanisms underlying T_1 changes measured. Longer T_1 values for all adenocarcinoma cell lines were observed at any field when compared to the healthy tissue. The observed T_1 increase was directly proportional to the tumor size increase. Moreover, significant variations among T_1 values of the different implanted tumours were also observed. The elongation of the intracellular water T_1 as well as an overall increase of the cellular volume in tumour cells could be accounted in terms of the augmented metabolic activity and the consequent increase in the local concentration of the produced metabolites. The most aggressive 4T1 cells display an overexpression of GLUT1, AQPs, Na^+ /K^+ ATPase transporters compared to other cell lines. From these preliminary results we can conclude that T_1 of tumour tissues (in particular at low magnetic fields) may act as reporter of the different water content in the tumor mass and its mobility through intraand extra- cellular compartments which change in depends of tumour grading, aggressivity and metastasis formation.

References

[1] C. S. Jr Springer et al. *NMR Biomed*. **27**, 760-773 (2014)
[2] M.R. Ruggiero et al. *Angew. Chem. Int. Ed. Engl*. **57**, 7468-7472 (2018)

OC25

THE HUMAN URINARY METABOLOME TO ASSESS THE RISK-OF-POVERTY STATUS ACROSS EUROPEAN POPULATIONS: A NMR NUTRI-METABOLOMIC STUDY.

F. Savorani,[‡] A. Trimigno[†]

[‡] Department of Applied Science and Technology – Politecnico di Torino, Corso Duca degli Abruzzi 24 – 10129 Torino, Italy
[†] Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark
E-mail: francesco.savorani@polito.it

According to a recent Eurostat report, about 119 million individuals among the European population live at risk-of-poverty [1]. This subpopulation is characterized by a low income but differs in age, culture and ethnicity among the different EU countries, and it is exposed to the risk of nutritional deficiencies due to poor nutritional habits.
This study, aimed at investigating the human urine metabolome of people at risk-of-poverty (ROP) in comparison to that of affluent (AFF) subjects, was carried out using 600 MHz ^{1}H-NMR spectroscopy with a metabolomics approach. A total of 2732 urine samples, collected from 1391 subjects recruited in five EU countries (United Kingdom, Finland, Italy, Lithuania and Serbia) were analyzed using 600 MHz NMR spectroscopy in two different analytical centers (Denmark and Italy) and the obtained data explored using multivariate data analysis. In particular, using ANOVA Simultaneous Component Analysis (ASCA) [2], it was possible to assess the effects derived from the study design factors; this approach revealed that country (ethnicity) and gender effects were responsible for most of the systematic variation. On the other hand, the effect of economic status was, as expected, rather weak, though more pronounced for the Lithuanian population. Among the observed metabolites, citrate and hippurate were the most relevant ROP biomarkers.

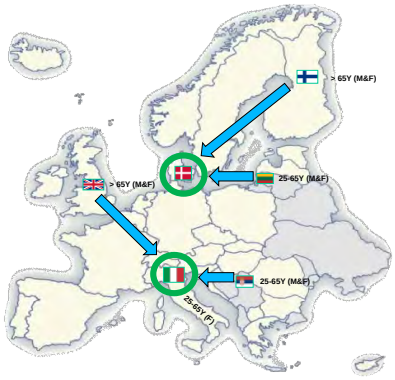


Fig. 1. The involved countries across Europe and their contribution in terms of gender and age group. The two NMR analytical centers are circled in green.

References

[1] Eurostat (2016), People at Risk of Poverty or Social Exclusion, Link: http://ec.europa.eu/eurostat/statisticsexplained/index.php/People_at_risk_of_poverty_or_social_exclusion
[2] A. K. Smilde, J. J. Jansen, H. C. J. Hoefsloot, R. A. N. Lamers, J. van der Greef, M. E. Timmerman *Bioinformatics* **21**, 3043-3048 (2005)

OC26

AN EFFICIENT GD^{3+} BASED COMPLEX FOR HIGH FIELD DYNAMIC NUCLEAR POLARIZATION

G. Stevanato,[a] D. Kubicki,[a] A.-S. Chauvin[a], M. Mazzanti,[a] L. Emsley[a]

[a] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.
E-mail: gabriele.stevanato@epfl.ch

High-spin gadolinium-based (Gd) metal complexes have been shown to act as polarizing agents (PAs) in magic-angle-spinning dynamic nuclear polarization (MAS-DNP) experiments [1-3]. Gd^{3+} is a half-integer spin characterized by a m_s=1/2 ↔ -1/2 central transition broadened by the zero-field splitting interaction. At moderate concentrations (<20 mM) the *solid effect* mechanism has been reported to induce ^{1}H enhancement of about 10 for Gd-DOTA and smaller for $GdCl_3$ and Gd-DTPA [3] up to 9.4T.
Here we report the investigation of a Gd-based metal complex [4], Gd(tpatcn)3.3H_2O, which offers *a factor >3 improvement in ^{1}H MAS-DNP signal enhancement* at 9.4 T that we correlate to the particularly narrow m_s=1/2 ↔ -1/2 central transition and the corresponding long electron transverse relaxation time.

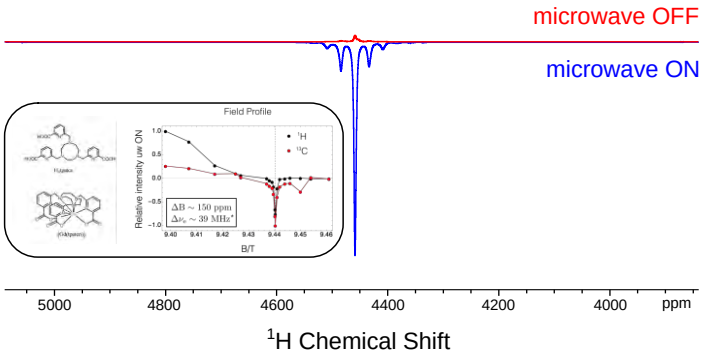


Fig.1: microwave ON/OFF ^{1}H spectra for 10 mM Gd(tpatcn)3.3H_20 in glycerol-d_8:D_2O:H_2O(6:3:1). In the inset: chemical structure and the DNP profile for the same compound with the addition of 0.7M 1-^{13}C-NaPyruvate

This observation offers a route to further optimize the complex design by improving the electron relaxation properties and corroborates the possibility to include high-spin metal systems as a viable alternative to more commonly used nitroxide mono- and bi-radicals spin probes. In addition, the compatibility of these complexes with physiological environments, as opposite to nitroxides, can broaden the scope of the presented research.

References

[1] B. Corzilius, A. A. Smith, A. B. Barnes, C. Luchinat, I. Bertini, and R. Griffin, *J. Am. Chem. Soc.* **133**, 5648-5651 (2011)
[2] M. Kaushik, M. Qi, A. Godt, and B. Corzilius, *Angew. Chem. Int. Ed.* **56**, 4295-4299 (2017)
[3] M. Kaushik et al, R. G. Griffin, and B. Corzilius, *Phys. Chem. Chem. Phys.* 18, 27205-27218 (2016).
[4] A. Borel, H. Kang, C. Gateau, M. Mazzanti, R. B. Clarkson and R. Linn Belford, *J. Phys. Chem. A* **110**, 12434-12438 (2006)

POSTERS

P1

TARGETING GRP-R EXPRESSING TUMORS: NMR INVESTIGATION OF BOMBESIN BINDING TO GRP RECEPTOR AND SYNTHESIS OF POTENTIAL MIMETICS

A. Palmioli,[a] C. Ceresa,[b] M. Rocchetti,[a] B. La Ferla,[b] C. Airoldi[a]

[a]Dept of Biotechnology and Biosciences, Unimib, P.zza della Scienza 2, Milano, Italy.
[b]Dept of Medicine and Surgery, Unimib, Via Cadore, 48, Monza, Italy
e-mail: cristina.airodi@unimib.it

Bombesin (BN) is a 14-residue peptide originally isolated from the amphibian Bombina bombina [1]. It belongs to a family of peptides showing a variety of biological activities in numerous tissues and cell types [2], exerted through their interaction with the Gastrin-Releasing Peptide Receptors (GRPR), transmembrane G-proteins coupled receptors triggering different signaling transduction pathways, resulting, among which, in the stimulation of cell proliferation. GRPRs are significantly involved in the pathogenesis of different human cancers [3], and are recently emerged as tumoral markers in early prostate and breast cancers diagnosis [4]. For these reasons, the research of new GRPR ligands as antagonists or carriers for cytotoxic and imaging molecular tools might be a promising strategy for the treatment and diagnosis of human tumoral malignancies [5]. In this scenario, structural data about BN binding to GRPR are required for the design and synthesis of high affinity receptor ligands, but, unfortunately, they are not yet available. BN conformation has been studied in various solvents demonstrating that it adopts an unordered structure in aqueous media and in dimethyl sulfoxide [6], while a partial helical structure has been observed in aqueous solutions containing TFE [7]. According to proposed models, this is the conformation that, probably, BN presents when anchored to biological membranes. With the aim to verify this hypothesis, we studied the effect of d_{25}-SDS (a biological membrane mimetic) on BN by CD and NMR spectroscopy. As for BN-GPCR interaction, the heptapeptide BN(8–14) has been shown to be the minimal carboxyl fragment interacting with the receptor, the same experiment were performed also on the BN C-terminal heptapeptide. Moreover, to discover the structural determinants of BN interaction with GRPR, the binding of both BN and BN(8-14) to human prostate carcinoma cell line (PC-3) over-expressing the receptor has been studied thought on-cell STD-NMR experiments.
Morever, we synthesized a library of ligands based on a rigid and spatially defined selected glycidic scaffold, differing for the nature of the potential pharmacophoric moieties. The biological activity of these compounds was preliminary screened by evaluating their ability to modulate the level of cytosolic Ca^{2+} (agonist or antagonist activity) in PC-3 cell.

The authors thank AIRC for funding project 17030 - Targeting of Gastrin-Releasing Peptide receptor expressing tumors: NMR characterization of Bombesin/GRP-R interaction.

References

[1] A. Anastasi, V. Erspamer and M. Bucci, *Experientia* **27**, 166-167 (1971)
[2] R. Bruzzone, *Eur. J. Biochem.* **179**, 323-331 (1989)
[3] I. Ramos-Álvarez, P. Moreno, S. A. Mantey, T. Nakamura, B. Nuche-Berenguer, T. W. Moody, D. H. Coy and R. T. Jensen, *Peptides* **72**, 128-144 (2015)
[4] R. Mansi, A. Fleischmann, H. R. Macke and J. C. Reubi, *Nat. Rev. Urol.* **10**, 235-244 (2013)
[5] F. Hohla and A. V. Schally, *Cell Cycle* **9**, 1738-1741 (2010)
[6] J. A. Carver, *Eur. J. Biochem.* 168, 193-199 (1987)
[7] a) J. A. Carver and J. G. Collins, *Eur. J. Biochem.* **187**, 645-650 (1990); b) M. D. Díaz, M. Fioroni, K. Burger and S. Berger, *Chemistry- Eur. J.* **8**, 1663-1669 (2002)

P2

^{1}H-NMR CHARACTERIZATION OF DRUG URINARY METABOLITES, TOWARDS THE PRECISION MEDICINE IN THE ANTI-HCV THERAPY

M. Arena,[‡] G. Capuani,[‡] A. Tomassini,[‡] F. Sciubba,[‡] E. Biliotti,[†] G. Taliani,[†] A. Miccheli[‡]

[‡]Chemistry Department "Sapienza University of Rome", 5 Aldo Moro square, Rome
[†]Clinical Medicine Department "Sapienza University of Rome", 37 University Avenue, Rome
E-mail: mattarena22@gmail.com

The discovery and characterization of the hepatitis C virus (HCV) is one of the greatest achievements of contemporary medicine, culminating with the introduction of safe and effective therapies [1]. In recent years, the introduction of more effective antiviral drugs with direct action against HCV (DAAs, Direct Acting Antivirals) allowed to heal more than 90% of patients with HCV infection. In Italy, the first DAAs, including ribavirin, were marketed in 2014 [2].

Ribavirin (RBV), also known as 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide, is a nucleoside, analogous to guanosine. Unlike guanine however, it has only one heterocyclic ring. The basic structure of ribavirin strikingly resembles the heterocyclic ring of nicotinamide, which is a component of involved coenzymes, i.e. NAD and NADP. Furthermore, the molecular structure recalls the AICAR structure (5-amino-imidazole-4-carboxamide ribonucleotide), a biosynthetic precursor of purines. As the triazole ring of ribavirin is attached to ribose, it is more similar to a ribonucleoside than a deoxynucleoside.

Although several studies have demonstrated that ribavirin has antiviral activity against both DNA and RNA viruses, the mechanism of action is still unclear. Despite scientific disagreements, ribavirin is used as a potent pro-drug for the treatment of viral infections [3].

In the present study the urinary metabolic profile of individuals with liver cirrhosis by HCV infection was analyzed by NMR spectroscopy. The study was conducted on 31 patients (19 M and 12 F) aged between 43 and 82 years, affected by HCV cirrhosis, viral genotype 1(a and b). Patients were examined at the BT_0 stage (Before Treatment t_0), before the therapy was taken, and at the EOT stage (End Of Treatment), at the end of the eight-week therapy coinciding with the ablation of the HCV.

The mono- and bi-dimensional ^{1}H-NMR analysis of the biofluid allowed the qualitative and quantitative determination of urinary metabolites of ribavirin.

Moreover, the NMR analysis allowed to identify pro-active and inactive urine metabolites of ribavirin and, therefore, to characterize the individual profiles on the basis of high and low inactivation metabolic ability. These results permit to improve the therapeutic regime and to reduce the toxic effects caused by high level of ribavirin (hemolytic anaemia) for each individual: personalized medicine.

References

[1] DP. Webster, P. Klenerman and GM. Dusheiko, *The Lancet* **385**, 1124-1135 (2015)
[2] J.M. Pawlotsky, A. Aghemo, D. Back, G. Dusheiko, X. Foms, F. Negro, M. Puoti and C. Sarrazin, *J. Hepatology* **66**, 153-194 (2017)
[3] N. Zhurilo, M. Chudinov, A. Matveev, O. Smirnova, I. Konstantinova, A. Miroshnikov, A. Prutkov, L. Grebenkina, N. Pulkova and V. Shvets, *Bioorg. Med. Chem. Lett.* **1**, 11-14 (2017)

P3

INVESTIGATING THE NATURE OF PHARMACEUTICAL COMPOUNDS THROUGH SOLID-STATE NMR: A CASE STUDY

M. Basso,[‡] M. R. Chierotti,[‡] R. Gobetto,[‡] S. Bordignon,[‡] F. Rossi[‡]

[‡]Department of Chemistry, University of Torino, Via P. Giuria 7, 10125 Torino, Italy
E-mail: mattia.basso@edu.unito.it

Nowadays, the preparation of new crystal forms of active pharmaceutical ingredients (APIs) represents a common strategy for pharmaceutical industries to improve properties such as thermal stability, hygroscopicity and dissolution rate of APIs. Indeed, pure APIs often display poor performances, which often lead to severe collateral effects. In the quest for improving the physico-chemical properties of an API, it becomes imperative to identify the obtained crystal form and thoroughly characterize it. Indeed, different crystal forms display different properties and are diversely regulated according to international laws. In this sense, solid-state NMR (SSNMR) is a powerful technique for investigating the nature and the short-range structure of pharmaceutical compounds in powder form. It can be effectively employed as an alternative to powder X-ray diffraction (PXRD) to identify the crystalline phase of a specific active pharmaceutical ingredient (API). Moreover, ^{1}H, ^{13}C and ^{15}N SSNMR spectra prove very useful to inspect the ionic or neutral nature of supramolecular adducts. This is due to the particular sensitivity of these experiments to the protonic position along the hydrogen bond (HB) axis. Additionally, here we present a real-case investigation, led to clarify the nature (salt or co-crystal) of some novel crystalline phases of a commonly employed API. ^{13}C and ^{15}N CPMAS experiments were helpful in gaining a general idea of whether the examined systems displayed a COO-H···N HB or a $COO^{-}\cdots H\text{-}N^{+}$ ionic interaction. In this challenging cases, 1D experiments were not enough to ascertain the nature of the adducts. 2D experiments, namely short- and long-range ^{1}H-^{13}C HETCOR spectra, were thus performed to reach a clear perspective of the interactions at play. Furthermore, an in-depth study of the ^{13}C CSA of the carboxylic group provide even stronger evidence.

P4

THE PHD5-C5HCH TANDEM DOMAINS OF NSD PROTEINS ARE STRUCTURAL HUBS FOR THE INTERACTIONS WITH THE CO-REPRESSOR NIZP1 AND THE HISTONE H3 TAIL.

A. Berardi,[1] G. Quilici,[1] O. Butrugnone,[2] G. Tonon,[2] S. Knapp,[3] G. Musco[1]

[1] Biomolecular NMR Unit, Division of Genetics and Cell Biology, IRCCS S. Raffaele Scientific Institute, Milan, Italy
[2]Functional Genomics of Cancer Unit, Division of Experimental Oncology, IRCCS S. Raffaele Scientific Institute, Milan, Italy
[3]Johann Wolfgang Goethe-University, Buchmann Institute, Frankfurt, Germany.
E-mail: berardi.andrea@hsr.it

The NSD transcription protein members (NSD1, NSD2, NSD3) contain several chromatin-related modules (a catalytic SET domain, two PWWP and six PHD domains), all implicated in developmental diseases and cancer [1,2]. As matter of the fact, the PHD tandem domains (PHDv-C5HCH) of these proteins have a prominent role in the transcriptional and tumorigenic activities of this protein family [2]. It is required for the recruitment of the NUP98-NSD1 fusion protein to the HoxA gene promoter in Acute Myeloid Leukaemia (AML) [3], it is essential for tumour cell proliferation induced by NSD2[4], and in NSD3 it is supposed to contribute to protein recruitment to chromatin through histone H3 interactions [5].
Here we present a systematic structural/functional investigation of the PHDv-C5HCH tandem domain of the three NSD family members. Our study shows that despite high sequence identity (~60%) [6], the NSD PHDv-C5HCH tandem domains have a divergent role in histones recognition and in protein-protein interactions. On one hand, the PHDv-C5HCH domain of NSD1 does not interact with histone H3 peptides, whereas it binds specifically with micromolar affinity to the C2HR domain of Nizp1 ($C2HR_{Nizp1}$), a corepressor regulating NSD1 transcriptional activity in AML. On one hand, the PHDv-C5HCH domain of NSD1 does not interact with histone H3 peptides [6], whereas it binds specifically with micromolar affinity to the C2HR domain of Nizp1 ($C2HR_{Nizp1}$) [6,7], a corepressor regulating NSD1 transcriptional activity in AML. On the other hand, the NSD2 and NSD3 tandem domains act as classical histone readers [5] but interact with low affinity with $C2HR_{Nizp1}$. We attribute these differences to small but crucial differences in aminoacidic sequence located on the interaction surfaces. Intriguingly, we demonstrate that NSD2 and NSD3 PHDv-C5HCH tandem domains specifically recognize H3K27me3, via the interdomain interface. Methylation of H3K27 is usually associated to repressive chromatin, we thus hypothesize that PHDv-C5HCH of both NSD2 and NSD3 contribute to recruitment of NSD2/3 to repressed chromatin, to facilitate then activation through methylation of H3K36 via the catalytic NSD-SET domain.
In conclusion, our data propose a regulative scenario in which the same NSD tandem domain can differently regulate the recruitment of cofactors/epigenetic modifications necessary for gene transcription.

References

[1] J. Douglas *et al., Am. J. Hum. Genet.* **72**, 132-143 (2003)
[2] R. L. Bennett *et al., Cold Spring Harb. Perspect Med*. **7**, a026708 (2017)
[3] G.G. Wang *et al.*, *Nature Cell Biol.* **9**,804-812 (2007)
[4] R. Popovic *et al*., *PLoS Genetics* **10**, e1004566 (2014)
[5] C. He *et al., J. Biol. Chem.* **288**, 4692-4703 (2013)
[6] A. Berardi A *et al., Nucleic Acids Res.* **44**, 3448-3463 (2016)
[7] A. L. Nielsen *et al., Mol. Cell. Biol.* **24**, 5184-5196 (2004)

P5

SOLID-STATE NMR CHARACTERIZATION OF ETHIONAMIDE SUPRAMOLECULAR ADDUCTS IN MICROCRYSTALLINE POWDERED FORM

D. Bernasconi,[1] M. R. Chierotti,[1] R. Gobetto,[1] F. Rossi,[1] S. Bordignon[1]

[1]Department of Chemistry, University of Torino, Via P. Giuria 7, 10125, Torino, Italy
E-mail: davide.bernasconi@edu.unito.it

Since growing high quality single crystals for accurate single crystal X-ray diffraction (SCXRD) is often a challenge, solid-state NMR characterization can offer a valid alternative for structure determination. Advanced mono and bidimensional experiments developed in recent years provide direct evidences of atom spatial proximity, even of the elusive hydrogen, the position of which can be very difficult to determine even with SCXRD [1].
Here, we present a solid-state NMR study to unravel the short-range structure of a series of new ethionamide supramolecular adducts for which it was not possible to grow good crystals for SCXRD. Ethionamide (2-ethylpyridine-4-carbothioamide, ETH) is a drug used, along with other antituberculosis medications, to treat active multidrug-resistant tuberculosis. It belongs to Class II of the Biopharmaceutics Classification System (BCS), comprising of poor solubility and high permeability drugs.[2] For this reason, several supramolecular adducts with GRAS (Generally Recognized as Safe) molecules citric acid, salicylic acid, alpha-resorcylic acid, cinnamic acid, and barbituric acid as coformers (see Fig. 1) were synthesized [2].
These adducts can be ionic (molecular salts) or neutral (co-crystals) and as such they fall under two different legal statuses, so it is very important to determine their nature. Specifically, the employed sequences were 1D ^{1}H MAS, ^{13}C CPMAS and ^{15}N CPMAS, and 2D ^{13}C-^{1}H HETCOR, ^{1}H DQ MAS and ^{14}N-^{1}H HMQC.

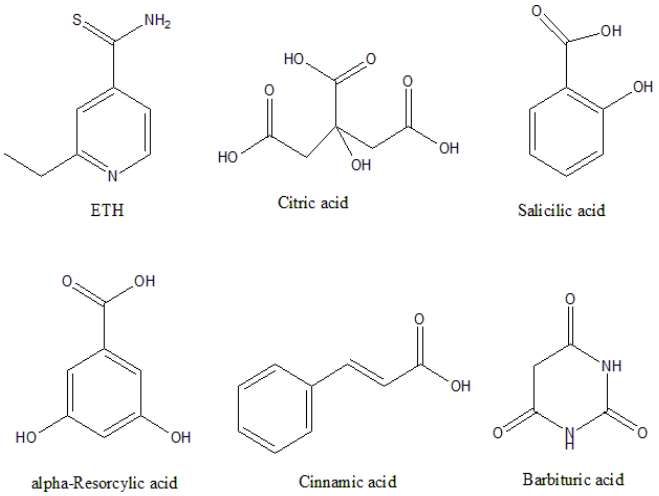


Fig. 1. Molecular structure of Ethionamide and used coformers.

References

[1] F. Rossi, P. Cerreia Vioglio, S. Bordignon, V. Giorgio, M. R. Chierotti, R. Gobetto et al., *Cryst. Growth Des*. **18**, 2225-2233 (2018)
[2] Y. Kawabata, K. Wada, M. Nakatani, S. Yamada and S. Onoue, *Int. J. Pharm.* **420**, 1-10 (2011)

P6

NMR STRUCTURE OF ANTIMICROBIAL PEPTIDE *OF-PIS1*

M. Bischetti,[‡] M. Gallo,[†] P. Punzi,[†] R. Ingenito,[†] E. Bianchi,[†] D. O. Cicero[‡†]

[‡]Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy
[†]IRBM Science Park SpA, Via Pontina km 30.600, 00040 Pomezia (RM), Italy
E-mail: martina.bischetti@uniroma2.it

Antimicrobial peptides (AMPs) are recognized as a possible source of pharmaceuticals for the treatment of antibiotic-resistant bacterial infections or septic shock. Size, sequence, charge, conformation, hydrophobicity and amphipathicity are important characteristics that affect antimicrobial activity and specificity [1], and allow AMPs to attach to and insert into membrane bilayers of microorganisms. By evaluating the interaction of AMPs with model membranes, it is possible to get an insight into their mechanism of action, whose knowledge is essential towards future therapeutic applications.
A large number of AMPs, differing for sequence, have been found in different organisms. Fish are considered to be reservoirs of distinct AMPs. The *Of-Pis1* peptide (24 residues) is a member of the *Piscidin* family, one of the major AMP families and widely distributed in fish [2].
We carried out a detailed NMR study with the aim of understanding the interaction of *Of-Pis1* with different model membranes like DPC (zwitterionic membrane model), LPS and SDS (anionic membrane model). Structural results will be analyzed in order to explain differences in the way the peptide interacts with charged or zwitterionic membranes, which can be relevant for the design of more potent and selective peptides.

References

[1] K. A. Brogden *Nat. Rev. Microbiol*. **3**, 238-250 (2005)
[2] N. Umasuthan, M.S. Mothishri, W. Shanthakumar Thulasitha, B. Nam, J. Lee *Fish Shellfish Immunol.* **48**, 154-168 (2016**)**

P7

L-FERRITIN: A NATURAL THERANOSTIC AGENT FOR MRI VISUALIZATION AND TREATMENT OF BREAST CANCER CELLS

V. Bitonto,‡ D. Alberti,‡ S. Geninatti Crich,‡ S. Aime,‡ J. C. Cutrin‡

‡Molecular Biotechnologies and Health Sciences, University of Turin, Italy
E-mail: valeria.bitonto@unito.it

The altered regulation of iron in cancerous cells compared to normal cells, along with the potential for iron misregulation to selectively cause oxidative stress and cell death, makes iron overload an attractive idea for the treatment of cancer [1]. Here we propose the use of horse spleen ferritin (HoS-Ferritin) as a natural theranostic agent that can be used for MRI visualization and treatment of breast cancer cells. Properly, due to its high percentage of L-chains (85%), HoS-Ferritin is mainly endocytosed by SCARA5 receptor-specific pathway, that appears to be up-regulated in some tumor cells [2]. Murine 4T1 and TS/A mammary carcinoma cell lines and a healthy mouse mammary gland NMuMG cell line were used. 4T1 and TS/A incubated with ferritin displayed markedly lower signal intensity when compared to untreated cells, while only small changes in signal intensity were observed in NMuMG incubated in the absence or in the presence of ferritin, respectively (Fig. 1). The T2-weighted RARE image confirmed that the ferritin-induced contrast is markedly more efficient in 4T1 cells. Internalized HoS-Ferritin triggered an iron uploading dependent cell death pathway. In all 4T1 tumour-bearing mice, contrast enhancements upon intravenous injection of HoS-Ferritin were successfully observed. Contrast enhancement in tumor, spleen and liver was already visible 3h after the injection, while the maximum signal was recorded after 6h (Fig. 1). Neoplastic cells resulted positive for the expression of the SCARA5 receptor, while in the normal tissue a mild positive reaction was visible only in some stromal cells.

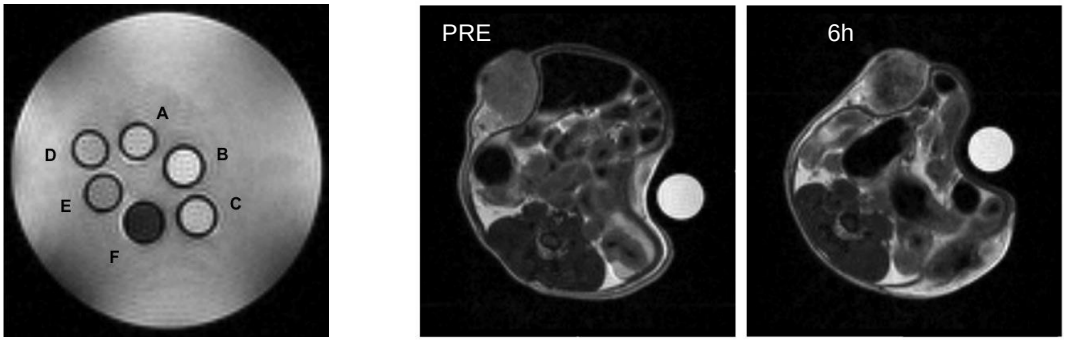


Fig.1 From left to right: T2-weighted MRI image (7T) of an agar phantom containing unlabeled NMuMG (A), TS/A (B) and 4T1 (C) cells; NMuMG (D), TS/A (E) and 4T1 (F) cells incubated for 24h with 2 μM HoS-Ferritin; *In vivo* MRI of BALB/C mice inoculated with $6x10^4$ 4T1 cells; T2-weighted image (tumor mass) acquired before and 6h after the administration of HoS-Ferritin at a Fe dose of 0.2 mmol kg^{-1}.

References

[1] S.P. Foy, V. Labhasetwar, *Biomaterials* **32**, 9155-9158 (2011)
[2] S. Geninatti Crich, M. Cadenazzi, S. Lanzardo, L. Conti, R. Ruiu, D. Alberti, F. Cavallo, J.C. Cutrin and S. Aime, *Nanoscale* **7**, 6527-6533 (2015).

P8

IDENTIFYING AND QUANTIFYING DESMOTROPES THROUGH SOLID-STATE NMR

S. Bordignon,‡ L. Lo Bello,‡ M. R. Chierotti,‡ R. Gobetto,‡ F. Rossi‡

‡Department of Chemistry, University of Torino, Via P. Giuria 7, 10125 Torino, Italy
E-mail: simone.bordignon@unito.it

The solid-state characterization of active pharmaceutical ingredients (APIs) is a central and necessary step in the development and marketing of drugs by pharmaceutical industries. In particular, this is true when an API crystallizes in more than one form (polymorphs, solvates/hydrates, salts and co-crystals), since they display often drastically different physico-chemical properties (e.g. solubility, melting point, flow properties, …) [1,2]. The quantification of the different phases (crystalline and amorphous) in a pharmaceutical preparation is fundamental as well [3]. Solid-state NMR (SSNMR) represents an advantageous approach to address these issues. In this work, we present an example of how SSNMR could identify two different desmotropic polymorphs (i.e. tautomeric forms isolated in the solid state)[4] of antihelmintic mebendazole (see Fig. 1), specifically through ^{13}C and ^{15}N CPMAS, ^{13}C-^{1}H HETCOR and ^{1}H DQ MAS spectra. Furthermore, the SSNMR quantification of the two polymorphs and of amorphous mebendazole in mixtures has been performed. It led to the definition of the LOD (0.35% m/m for the desmotropes; 2% m/m for the amorphous phase) and LOQ (1% m/m) of the technique.

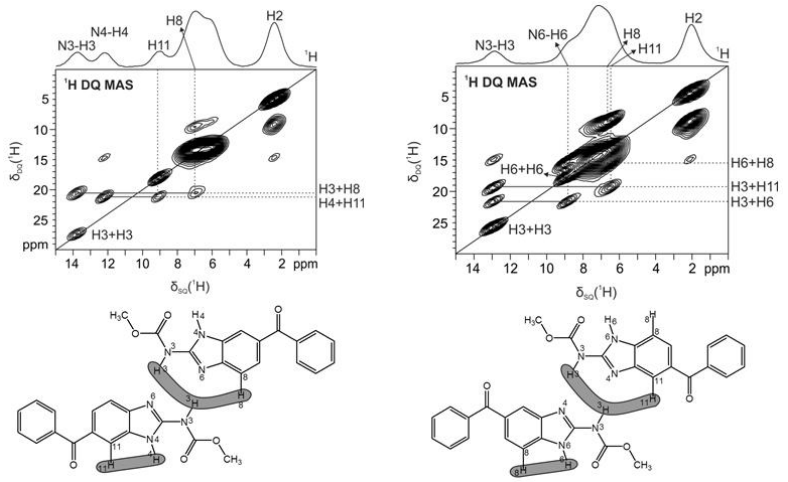


Fig. 1. Representation of the two desmotropic polymorphs of mebendazole, with their respective ^{1}H DQ MAS 2D SSNMR spectra. On the left, polymorph A; on the right, polymorph C.

References

[1] K. Gaglioti, M.R. Chierotti, F. Grifasi, R. Gobetto, U. J. Griesser, D. Hasa, D. Voinovich *Cryst. Eng. Comm*. **16**, 8252-8262 (2014)
[2] S.P. Mulye, S. A. Jamasar, P. S. Karekar, Y.V. Pore, S. C. Dhawale *Powder Technol.* **222**, 131-138 (2012)
[3] R. Chadha, S. Gupta and G. Shukla *Acta Pharm. Sin. B* **1**, 129-135 (2011)
[4] A.K. Chattah, R. Zhang, K. H. Mroue, L.Y. Pfund, M. R. Longhi, A. Ramamoorthy and C. Garnero *Mol. Pharmaceutics* **12**, 731-741 (2015)

P9

ECOSUSTAINABLE MgO-BASED CEMENTS: UNRAVELLING THE ROLE OF PHOSPHATE ADDITIVES BY MEANS OF SOLID STATE NMR SPECTROSCOPY

S. Borsacchi,[‡] F. Martini,[‡,†] M. Tonelli,[§] F. Ridi,[§] M. Geppi,[‡,†] L. Calucci[‡]

[‡] Istituto di Chimica dei Composti OrganoMetallici - CNR, Via G. Moruzzi 1, Pisa, Italy
[†] Dipartimento di Chimica e Chimica Industriale - Università di Pisa, Via G. Moruzzi 13, Pisa, Italy
[§] Dipartimento di Chimica "Ugo Schiff" & CSGI, Università di Firenze, Via della Lastruccia 3, Sesto Fiorentino (Firenze), Italy
E-mail: silvia.borsacchi@cnr.it

MgO-based cements are receiving great attention both because their production could in principle involve negligible or "negative" CO_2 emissions and especially because they are particularly promising for the containment of radioactive waste. The important binder phase in cements obtained from hydration of MgO and silica is the completely amorphous magnesium silicate hydrate (M-S-H), the structure of which has been recently deeply investigated and characterized [1]. In order to pave the way to extensive and optimized applications of these promising cements, many aspects, both fundamental and practical, need to be investigated and understood. So far Solid State NMR (SSNMR) and Relaxometry techniques have given a crucial contribution to the comprehension of these materials at the molecular and nanometric scale, providing a detailed picture of the structure of the silicate binder phase, of the dynamic properties of water included in the pores and on the pore features, also clarifying their time evolution [2,3]. In the practice of cement preparation, additives are always necessary to achieve different goals, such as: accelerate or retard setting or hardening, decrease the amount of water necessary to obtain a given degree of workability, improve the mechanical performances, entrain air, etc. In the case of MgO/silica cements, sodium hexametaphosphate (HMP) nowadays is the only additive used as plasticizer and its mechanism of action is still not understood. In this work we have carried out a wide-ranging investigation on the effects of three different phosphate salts (HMP, sodium trimetaphosphate, TMP, and sodium orthophosphate, OP) on MgO/silica cements by combining fluidity tests, calorimetric measurements, XRD and SSNMR experiments. In particular, ^{29}Si and ^{31}P SSNMR experiments clarified in detail and quantitatively the effects of the different additives on the formation kinetics and structure of M-S-H, highlighting at the same time the chemical modifications underwent by the additives and the interactions with the silicate phase. Combining these results with those of the other techniques it has been possible to propose a rationale behind the macroscopic effects observed for the different additives.

References

[1] M. Tonelli, F. Martini, L. Calucci, E. Fratini, M. Geppi, F. Ridi, S. Borsacchi, P. Baglioni. *Dalton Trans.* **45**, 3294-3304 (2016)
[2] F. Martini, S. Borsacchi, M. Geppi, M. Tonelli, F. Ridi, L. Calucci. *Micropor. Mesopor. Mater.* **269**, 26-30 (2018)
[3] F. Martini, S. Borsacchi, M. Geppi, C. Forte, L. Calucci. *J. Phys. Chem. C* **121**, 26851-26859 (2017)

P10

MICROENCAPSULATION OF PHASE CHANGE MATERIALS IN SOL-GEL ORGANOSILICA FOR THERMAL ENERGY STORAGE

E. Callone,[‡] G. Fredi,[†] A. Dorigato,[†] L. Fambri,[†] A. Pegoretti,[†] and S. Dirè[‡]

[‡] "K. Müller" Magnetic Resonances Lab., Department of Industrial Engineering, University of Trento, via Sommarive 9, 38123 Trento - Italy
[†] Department of Industrial Engineering, University of Trento, via Sommarive 9, 38123 Trento - Italy
E-mail: emanuela.callone@unitn.it

Nowadays the need of a more effective use of energy in both domestic and industrial activities has become a priority. Thus, the phase change materials (PCMs) capable to store and release thermal energy have attracted much interest. The main features of these materials are the possibility to undergo reversible solid/solid or solid/liquid phase transitions storing a large amount of thermal energy per unit mass, at a nearly constant temperature, with a small change in volume [1]. Among all, paraffin waxes are the most widely used due to their properties, such as high energy density, small volume change over phase transition, narrow working temperature range, tunable operating temperature, low density and cheapness [2,3]. Nevertheless, PCM shows some weaknesses, in particular low thermal conductivity and leakage above the melting temperature. Actually, the latter issue has been taken into account, considering both the encapsulation of the PCMs in organic or inorganic micro- or nano shells, and the confinement in useful materials.
The present activity is focused on the encapsulation of docosane, $CH_3(CH_2)_{20}CH_3$ in organosilica microcapsules, obtained by the sol-gel route in oil-in-water emulsion, in order to protect the wax from the environment and prevent its leakage when the particles are employed as fillers in composites for thermal energy storage [4].
Changing the synthesis conditions gives the possibility to tuning both size and shell thickness of microcapsules. According to the ^{29}Si CPMAS spectra, the development of the organosilica network does not result affected by the different amount of paraffin used in the processing. On the contrary, the ^{13}C solid state NMR analyses point out that the organosilica shell size induces conformation differences and chain mobility restrictions on the confined paraffin. The observed confinement effects are due to the extent of paraffin-organosilica interface interactions, and influence the thermal behavior of the phase change material. The obtained results suggest the possibility to tune the processing parameters in order to obtain the desired thermal features of the final composites.

References

[1] G. Fredi, A. Dorigato, and A. Pegoretti, *eXPRESS Polymer Letters* **12**, 349-364 (2018)
[2] S-G. Jeong, S. Kim, and W. Huh, *J. Adh. Sci. Technol*., **28**, 711-721 (2014)
[3] M. Akgun, O. Aydın, and K. Kaygusuz, *Energy Conversion & Management*, **48**, 669– 678 (2007)
[4] G. Fredi, S. Dirè, A. Dorigato, L. Fambri and A. Pegoretti, *ECCM18 - 18th European Conference on Composite Materials* Athens, Greece, 24-28th June 2018.

P11

^{1}H and ^{17}O NMR RELAXOMETRIC CHARACTERIZATION of Mn^{2+}-PICOLINATES CHELATES

F. Carniato,[1] D. Esteban-Gómez,[2] C. Platas-Iglesias,[2] M. Botta[1]

[1] Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "A. Avogadro", Viale T. Michel 11, 15121 Alessandria, Italy.
[2] Universidade da Coruña, Centro de Investigacións Científicas Avanzadas (CICA) and Departamento de Química, Facultade de Ciencias, 15071, A Coruña, Galicia, Spain.
E-mail: fabio.carniato@uniupo.it

In recent years, high-spin Mn^{II}-chelates, have attracted growing interest as potential MRI probes alternative to the commercially available Gd^{III}-complexes, due to their intrinsically lower toxicity.[1] In particular, acyclic polydentate ligands containing picolinate groups have been extensively studied for Mn^{2+} complexation.[2] In this work, we extend this family of ligands with a new pentadentate $PAADA^{3-}$ chelating agent (Fig. 1). ^{1}H and ^{17}O NMR relaxometric characterization was carried out to determine the molecular parameters that influence the relaxing efficiency (relaxivity, r_1) of Mn^{II}-PAADA. The relaxivity value for the complex at 25 °C and 0.5 T is 4.0 mM^{-1} s^{-1}, higher than those reported for other monohydrated Mn^{II}-chelates, indicating the presence of two inner sphere water molecules in the fast exchange regime. The rotational correlation time (τ_R) and the electronic parameters of the chelate were extrapolated by the simultaneous best-fitting of the ^{1}H nuclear magnetic relaxation dispersion profiles (NMRD) and ^{17}O NMR relaxation and shift data.

A lipophilic derivative of $PAADA^{3-}$, bearing in the structure a dodecyloxo group attached to the pyridyl unit, ($H_3C_{12}OPAADA$, Fig. 1) was also synthesized and compared to the previous one. The complex, below the critical micellar concentration (cmc = 0.31 mM) shows enhanced relaxivity (5.4 mM^{-1} s^{-1} at 25°C and 0.5 T) compared to Mn^{II}-PAADA that can be associated with the reduced mobility of the complex in aqueous solution. Furthermore, the complex exhibits the ability to self-aggregate in aqueous solution (above the cmc) and to interact strongly with bovine serum albumin (BSA), leading to supramolecular structures with enhanced r_1 values at high magnetic fields.

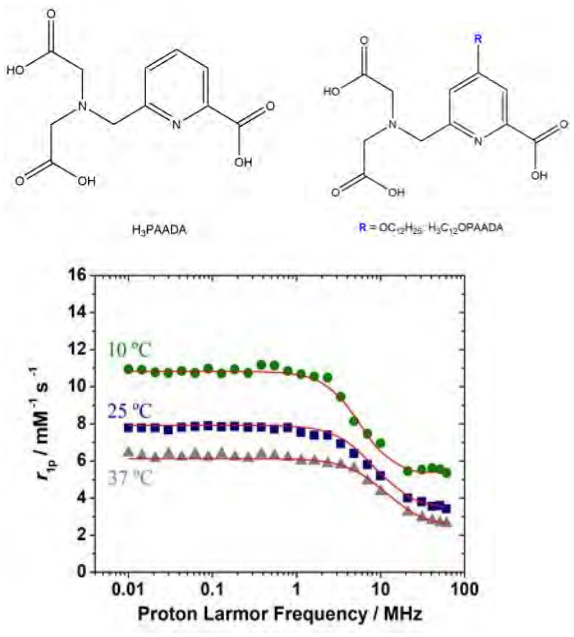


Fig. 1. Chemical structure of $PAADA^{3-}$ and its lipophilic derivative and ^{1}H NMRD profiles of Mn^{II}-PAADA.

References

[1] E.M. Gale, I.P. Atanasova, F. Blasi, I. Ay, P. Caravan *J. Am. Chem. Soc.* **137**, 15548-15557 (2015)
[2] A. Forgács, R. Pujales-Paradela, M. Regueiro-Figueroa, L. Valencia, D. Esteban-Gómez, M. Botta and C. Platas-Iglesias *Dalton Trans.* **46**, 1546-1558 (2017).

P12

EFFECT OF COFFEE EXTRACTS AND THEIR MAIN POLYPHENOLIC COMPONENT 5-CQA ON ONCOGENIC RAS PROTEINS

C. Ciaramelli,[‡] A. Palmioli,[‡] M. Spinelli,[‡] G. De Sanctis,[‡] R. Tisi,[‡] E. Sacco,[‡] C. Airoldi[‡]

[‡] Dipartimento di Biotecnologie e Bioscienze, Università degli studi di Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
E-mail: carlotta.ciaramelli@unimib.it

Recent epidemiological studies demonstrate that consumption of healthy foods, especially rich in polyphenols content, might reduce the incidence of cancer and degenerative diseases [1]. In particular, chlorogenic acids (CGAs), esters formed between hydroxycinnamic acids (mainly caffeic and ferulic) and quinic acid occur ubiquitously in food, being 5-caffeoylquinic acid (5-CQA) the most abundant polyphenols in the human diet [2]. A number of beneficial biological effects, including anti-inflammatory activity, anti-carcinogenic activity and protection against neurodegenerative diseases have been described for CGAs [3]. However, the molecular mechanisms at the basis of these biological activities have not yet been investigated in depth. Here we report our contribute to the elucidation of the molecular mechanism through which 5-CQA carries out its potential as chemoprotective supplement against carcinogenesis. In particular, we evaluated: 1) the molecular interaction between 5-CQA and the proto-oncogenic human protein h-Ras by mean of molecular docking and STD-NMR spectroscopy; 2) the effect of 5-CQA binding on Ras ability to switch-on the proliferative signaling; 3) the biological effects of 5-CQA in *ex vivo* assays using MDA-MB-231 (Breast cancer, RasG13D) cell lines; 4) the biological effects of enriched CGAs natural extracts obtained from green and roasted coffee beans [4].

This work was supported by AIRC (MFAG-17030 Targeting of Gastrin-Releasing Peptide receptor expressing tumors: NMR characterization of Bombesin/GRP-R interaction)

Fig. 1. Natural compounds in cancer prevention.

References

[1] C. J. Weng, G. C. Yen, *Cancer Treat. Rev.* **38**, 76-87 (2012).
[2] M. N. Clifford, *J. Sci. Food Agric.* **80**, 1033-1043 (2000).
[3] a) C. Ciaramelli, A. Palmioli, A. De Luigi, L. Colombo, G. Sala, C. Riva, C. P. Zoia, M. Salmona, C. Airoldi, *Food Chem.* **252**, 171–180 (2018); b) H. S. Shin, H. Satsu, M.-J. Bae, Z. Zhao, H. Ogiwara, M. Totsuka, M. Shimizu, *Food Chem.* **168**, 167-175 (2015); c) L. Amigoni, M. Stuknytė, C. Ciaramelli, C. Magoni, I. Bruni, I. De Noni, C. Airoldi, M. E. Regonesi, A. Palmioli, *J. Funct. Foods* **33**, 297-306 (2017); d) G. Oboh, O. Agunloye, A. Akinyemi, A. Ademiluyi, S. Adefegha *Neurochem. Res.* **38**, 413-419 (2013).
[4] A. Palmioli, C. Ciaramelli, R. Tisi, M. Spinelli, G. De Sanctis, E. Sacco, C. Airoldi, *Chemistry - an Asian Journal* **12**, 2457-2466 (2017).

P13

^{1}H-NMR TO EVALUATE THE INFLUENCE OF VARIETY, ORIGIN AND EXTRACTION PROCEDURE ON COFFEE BENEFICIAL EFFECTS FOR HUMAN HEALTH

C. Ciaramelli, A. Palmioli, C. Airoldi

Dipartimento di Biotecnologie e Bioscienze, Università degli studi di Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
E-mail: carlotta.ciaramelli@unimib.it

An NMR-based protocol for the rapid and semi-automatic identification and quantification of metabolites present in both green and roasted coffee extracts has been developed, using the Simple Mixture Analysis (SMA) tool of MestReNova software [1].
The methodology was applied to the analysis of green and roasted coffee extracts differing for species (Arabica and Robusta), bean geographical origin (from ten different countries) and prepared according to three extraction methods (hydroalcoholic extraction, espresso and moka), and allowed revealing the influence of these variables on their potential beneficial effect on human health.
In addition, for each coffee we compared the content of metabolites with proved positive biological activities for human health (e.g. chlorogenic acids, trigonelline and choline) to that of compounds with negative implications (e.g. caffeine). These data were correlated to the coffee species, the geographical origin, the extraction procedure employed for extract preparation and the antioxidant activity.
Our results could provide fundamental information for the identification of coffees to be used for the preparation of coffee-based dietary supplements and nutraceuticals or for their use as functional foods.

Acknowledgments
This work was supported by Fondazione CARIPLO and Regione Lombardia, project n° 2015-0763. Authors acknowledge Beyers Koffie (Belgium) for providing coffee samples.

References

[1] C. Ciaramelli, A. Palmioli, C. Airoldi, *submitted*

P14

NMR-BASED APPROACH TO CHARACTERIZE TOMATOES FROM LAZIO REGION

S. Circi,[1] C. Ingallina,[1] D. Capitani,[2] A. P. Sobolev,[2] L. Mannina[1,2]

[1]Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Piazzale Aldo Moro, 5, 00185 Rome, Italy
[2]Istituto di Metodologie Chimiche, Laboratorio di Risonanza Magnetica "Annalaura Segre", CNR, Monterotondo, Km 29500 Via Salaria, Rome, Italy
E-mail: simone.circi@uniroma1.it

High field NMR methodology was used to study seven different varieties of tomato (San Marzano, Torpedino, Fiaschetta, Bamano, Dolce Miele, Confettino Rosso and King Creole). This study was carried out in the frame of *e*-ALIERB OpenLab Project* funded by "Regione Lazio" and aimed at setting up and making available research facilities of "Sapienza OpenLab" to small and medium farms of Lazio region to chracterize local and traditional products [1] and to promote their products on the market in a more competitive way. NMR results will be included in a food-based database*.
Metabolites of different classes (carbohydrates, amino acids, organic acids, nucleosides, carotenoids, lipids, sterols) in water-soluble and liposoluble extracts were identified and quantified. Some differences were observed by comparing metabolite profiles of the seven tomatoes cultivar. San Marzano, Torpedino and Fiaschetta varieties showed a higher level of aminoacids than the others, whereas in Bamano and Dolce Miele varieties a higher content of carbohydrates was observed. The knowledge of these differences can be useful to suggest the best variety for specific uses (fresh consumption or use for preserves and sauces). The comparison of unripe and mature tometoes of the same variety (San Marzano, Torpedino and Fiaschetta) showed that almost all metabolites are present in higher level in ripe fruits with the exception of organic acids, namely citric acid and malic acid which decrease during the ripening.

* "e-ALIERB: an OPEN LAB to characterize and valorise foodstuffs and botanicals of Lazio region" (FILAS-RU-2014-1157, Codice CUP B82I15003570002)

References

[1] A.P. Sobolev, L. Mannina, D. Capitani, et al. A multi-methodological approach in the study of Italian PDO "Cornetto di Pontecorvo" red sweet pepper. *Food Chem.* **255**, 120-131 (2018)

P15

^{1}H NMR-BASED METABOLOMIC STUDY OF A MOTHER-INFANT DYAD ON THE EVOLUTION OF GUT MICROBIOTA DURING THE BREASTFEEDING AND WEANING

G. Conta,‡ G. Capuani,‡ A. Tomassini,‡ F. Sciubba,‡ A. Miccheli‡

‡Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro, 5, 00185, Rome, Italy
E-mail: conta.giorgia@gmail.com

Human milk is a buffet of fats, proteins and sugars, designed to be easily digestible and to provide an adequate supply of nutrients to support healthy growth and development of the infant. In addition, milk is rich of non-nutrient bioactive factors, such as cells, anti-infectious and anti-inflammatory agents, growth factors, and prebiotics, providing protection to the newborn. Among this plethora of components, human milk oligosaccharides (HMOs) were supposed to have anti-adhesive and antimicrobials properties, thus preventing pathogen attachment to infant mucosal surfaces and lowering the risk for viral, bacterial and protozoan parasite infections [1]. Furthermore, HMOs were recognized as a substrate to promote the growth of desired bacteria, such as bifidobacteria, in the infant's intestine ("bifidogenic" or prebiotic effects) [2]. Early microbial colonization is essential for healthy intestinal and immunological development in neonates. The development of gut microflora during infancy consists in a complex succession of bacterial species, modulated by breastfeeding, perinatal antibiotic use and environmental factors. In a recent study, we have compared the changing fecal microbial composition with the fecal metabolome of newborns in the first thirty days of the breastfeeding [3]. The results showed a relation between microbiota and fecal biochemical composition, probably linked to the changes of breast milk composition during the maturation process.In this study, we have investigated, by ^{1}H NMR spectroscopy, the changes of the neonatal fecal metabolome in relation to diet: breastfeeding and weaning. We have examined breast milk and newborn faeces of a single mother-infant dyad for 6 months, starting from the 4th month after the birth, in order to evaluate the changes in microbial metabolome during the diet evolution. The NMR spectroscopy of the breast milk allowed to recognize the mother's phenotype as Secretor (FUT2 gene positivity) on the basis of the structure of fucosyl-HMO, and to confirm the stability of milk biochemical composition during the considered period of lactation. On the other hand, the NMR analysis of fecal samples showed that the microbiota HMO metabolism changed as a function of time, and furthermore the daily variability increased during the weaning period. Therefore, important experimental aspects are evidenced in order to obtain representative samples of faeces from newborns to compare metabolomics with metagenomics data. The results display the evolution of the infant gut microbiota metabolism in relation to changes of the diet composition.

References

[1] L. Bode *Glycobiology* **22**, 1147-1162 (2012)
[2] D. A. Sela, D A Mills *Trends Microbiol.* **18**, 298-307 (2010)
[3] Del Chierico et al. *Plos One* **10**, e0137347 (2015)

P16

NMR CHARACTERIZATION OF INDUCED ACCUMULATION OF AROMATIC BIOACTIVE COMPOUNDS IN FLAX CELL CULTURES

E. Cusano,[‡] M. Mattana,[†] I. Mascheretti,[†] S. Mapelli,[†] M. Lauria,[†] F. Locatelli,[†] A. Genga,[†] R. Consonni[‡]

[‡] NMR laboratory, Institute for Macromolecular Studies (ISMAC), CNR, v. Corti 12, 20133 Milan, Italy
[†]Institute of Agricultural Biology and Biotechnology(IBBA), CNR, v. Corti 12, 20133 Milan, Italy
E-mail: erica.cusano@ismac.cnr.it

Flax is one of the oldest and highly cultivated oil seed crop in Europe and is regarded as a functional food due to the presence of high quality omega-3 fatty acids (α-linolenic acid), proteins, lignin, dietary fibers and phenolic derivatives (lignans) [1]. In particular, lignans exhibit antioxidant, cytotoxic, antifungal, antiviral and phytoestrogenic and cancer chemopreventive properties [2,3]. In this work, two different flax species have been exploited to obtain in vitro cell cultures for the improvement of lignans production. The species have been selected considering that lignan profile correlate with the genus systematic division of flax. Moreover, several elicitors such as methyl jasmonate (Me-JA) were used to increase metabolites production. NMR based metabolomics was performed to evaluate the metabolic profiling of selected flax extracts. Stable cell lines were established from leaf explants of *Linum usitatissimum* (cv Valoal) and *Linum austriacum*. The extracts (control and the 4 days Me-JA treatment) of the cell suspensions obtained from the two flax species were investigated by 1H NMR to evaluate differences in their total phenolic content. The same samples were analyzed by Folin-ciocalteu method to evaluate the total phenolic content. The metabolite profiles of flax cell suspensions were obtained by recording 1H-NMR spectra at 14.09 T. The aromatic regions of proton spectra highlighted different profiles between the two species showing that *L. austriacum* samples were more rich of aromatic compounds most likely belonging to the lignan class and that the two species are able to synthesize different molecules. The results suggest that the cell suspension technology combined with the elicitation could represent a promising technology for industrial production of bioactive compounds.

References

[1] B. D. Oomah, *J. Sci. Food Agric*. **81**, 889-894 (2001)
[2] J. K. Mason and L. U. Thompson, *Appl. Physiol. Nutr. Metab*. **39**, 663-678 (2014)
[3] A. Goyal, V. Sharma, N. Upadhyay, S. Gill, and M. Sihag, *J. Food Sci. Technol*. **51**, 1633-1653 (2014)

Acknowledgements: This work has been supported by Fondazione Cariplo, Milan, Italy, grant 2016-0700, InFlaMe project.

P17

MONITORING OF APPLE JUICE FERMENTATION PROCESS BY NMR SPECTROSCOPY

E. Cusano,[‡] B. Simonato,[†] R. Consonni[‡]

[‡] NMR laboratory, Institute for Macromolecular Studies (ISMAC), CNR, v. Corti 12, 20133 Milan, Italy
[†] Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy
E-mail: Erica.cusano@ismac.cnr.it

Cider is a slightly alcoholic beverage obtained from the fermentation of apple juice, largely popular in Europe, North America, and Australia [1,2], constituting an important and promising segment of the fruit industry. In the presented study, a simultaneous characterization of bioactive compounds in apple juice, in the intermediate products of the cidermaking process and in still cider was performed for the first time using Nuclear Magnetic Resonance spectroscopy.
Alongside sugar consumption, a progressive increase in different chemical compounds like trigonelline, chalcone, fumarate, caffeic acid, uracil, tyrosol and xanthine content was detected during the alcoholic fermentation process. Interestingly the presence of a relatively high tyrosol content was observed as well, thus conferring additional health benefits to cider [3].
The concomitant changes in phenolic compounds, amino acid and organic acid (malic, lactic, quinic, pyruvic, citric and succinic) content, represent an important task when monitoring the fermentation process in order to assessing the quality of the final product.

References

[1] H. Zhang, F. Ji. B. Zhou, R. M. Nout, Q. Fang, and Z. Yang, *Eur. Food Res. Technol*. **227**, 1183-1190 (2008)
[2] M. Ye, T.,Yue, and Y. A. Yuan, *Eur. Food Res. Technol*. **239**, 67-77 (2014)
[3] A. Zuriarrain, J. Zuriarrain, A. I. Puertas, M. T. Dueñas, M. Ostra, and I. Berregi, *J. Sci. Food Agric*. **95**, 2931-2943. (2015)

P18

ACQUISITION OF NMRD PROFILES FOR EARLY DIAGNOSIS AND PHENOTYPING OF BREAST CANCER IN NeuT MURINE MODELS

E. Di Gregorio,[†] G. Ferrauto,[†] S. Lanzardo,[†] E. Gianolio,[†] S. Aime[†]

[†]Department of Molecular Biotechnologies and Healthy Sciences-University of Turin, Turin, Italy
E-mail: enza.digregorio@unito.it

Breast Cancer is a multifactorial disease, considered a major public-health issue worldwide. It is the most diffuse cancer among women and the treatment outcome is influenced by the possibility to detect it at a very early development stage. Quick and detailed diagnostic tests able to provide a detailed characterization of tumor is still needed in order to further improve the chances of curing this disease. It typically evolves through a multistep progression process, starting from epithelial simple hyperplasia, to atypical hyperplasia, to carcinoma in situ (CIS) and finally to metastatic carcinomas.
Herein, Balb-NeuT mice at different ages (7, 15, 21 and 30 weeks) have been used. They are transgenic mice in which breast cancer spontaneously develop in all mammary glands and closely recapitulate human breast cancer development. The onset of cancer is triggered by the overexpression of the activated form of the rat ErbB2 (Her/2-neu) oncogene, whose amplification is typically observed in 20–30% of human breast.
In this work, for the first time, it has been reported that Fast Field Cycling Nuclear Magnetic Resonance Dispersion (FFC-NMRD) profiles can be used for the detection of cancer in murine breast tissues biopsies. In particular, from the analysis of longitudinal water relaxation time (T_1) at variable magnetic field (FFC relaxometry), it has been possible to detect the presence of tumor in NeuT mice at a very early stage (7-weeks) when the disease is not detectable by common high resolution MRI and shows minimal and not diffuse histological modifications. Tumor progression is strongly correlated with significant changes in T_1 values and of the overall shape of NMRD profiles (Fig.1A). By fitting with a line the log/log NMRD profiles, it has been possible to quantify the slope of the curve. This parameter is strongly correlated to the tumor stage (Fig.1B). In particular, the slope is small in healthy control (ca. 0.1) and it increases at late stages of tumor (up to *ca.* 0.35 for 30weeks NeuT mice). In addition, ^{14}N-quadrupolar peaks (^{14}N-QPs) have been analyzed. They are not present in healthy mammary tissue (Fig.1A) but are clearly detectable in presence of the tumor. Therefore, the presence of ^{14}N-QPs is an early biomarker of tumor onset. In such a way, NMRD profiles can be suitable for i) making detectable breast tumor at a very early stage (by investigating the presence of ^{14}N-QPs) and ii) making possible to assess tumor stage (by investigating the overall NMRD profile shape). Importantly, both the information can be gained without the administration of exogenous agents.

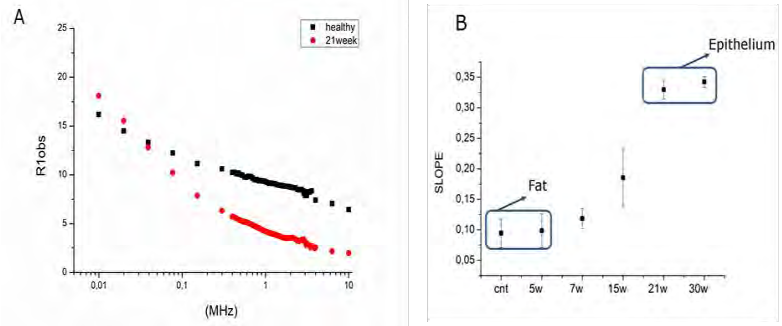


Fig. 1. (A) NMRD profiles of *ex vivo* breast tissue of NeuT at different stages (7, 15, 21 and 30 weeks) compared with healthy mice. (B) Slope of NMRD profiles at different stages of NeuT.

P19

^{1}H HRMAS NMR: A TOOL FOR THE STUDY OF DRUG DYNAMIC IN SUPRAMOLECULAR HYDROGEL SYSTEMS

M. E. Di Pietro, F. Castiglione, M. Ferro, M. Casalegno, F. Rossi, M. Masi and A. Mele

Department of Chemistry, Materials and Chemical Engineering "G. Natta", Via L. Mancinelli, 7 20131 Milano, Italy
E-mail: mariaenrica.dipietro@polimi.it

Controlled and targeted drug delivery using hydrogel matrices has become a major research topic in the pharmaceutical field. Hydrogels are macromolecular systems that strongly swell in water, building an open polymer network in which drug molecules can be stored and subsequently released. The description of the translational dynamic of small molecules dissolved in hydrogel systems is a key step for drug-delivery applications. In particular, the mutual contributions of solute-solute and polymer-solute interactions on the drug diffusional mobility, related to the polymer network mesh size, play an important role in designing new systems [1,2]. We use Pulse Gradient Spin Echo (PGSE) techniques under Magic Angle Spinning (MAS) for the direct investigation of the dynamic regime of a drug-like molecule, sodium fluorescein, and two drugs, ethosuximide and ibuprofen sodium salt, loaded at several concentrations in hydrogel matrices having different mesh size (Fig. 1). Experiments performed at several observation times allowed us to determine the molecular mean square displacement as function of time. The results indicate that both drugs exhibit a peculiar motion regime in both fine-mesh and coarse-mesh hydrogels compared with water solution. This surprising effect is related to drug-anionic polymer chain chemical interactions and hydrogel network physical barrier for drug motion.

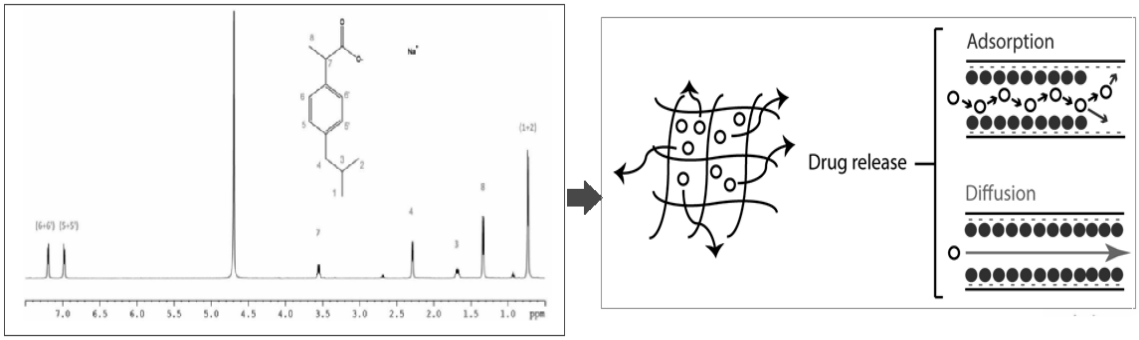


Fig. 1. ^{1}H HRMAS spectrum of ibuprofen sodium salt encapsulated in hydrogel and pictorial representation of the partitioning model.

References

[1] F. Rossi, F. Castiglione, M. Ferro, M. Moioli, A. Mele and M. Masi *Chem. Phys. Chem*. **16**, 2818-2825 (2015)
[2] F. Rossi, F. Castiglione, M. Ferro, M. Moioli, A. Mele, and M. Masi *Chem. Phys. Chem.* **17**, 1-9 (2016)

P20

DEVELOPMENT OF INNOVATIVE ON/OFF COPPER AND ZINC RESPONSIVE PARACEST MRI CONTRAST AGENTS

G. Ferrauto,[‡] K. Srivastava,[†] S. M. Harris,[†] D. Longo,[‡] M. Botta,[§] S. Aime,[‡] and V. C. Pierre[†]

[‡]Department of Molecular Biotechnolgoies and Health Sciences, University of Torino, Via Nizza 52, Torino (It)
[†]Department of Chemistry, University of Minnesota, Minneapolis MN55455, USA
[§] Dipartimento di Scienze e innvoazione tecnologica, Univ del Piemonte Orientale "A.Avogadro",15121 Alessandria (It)
E-mail: giuseppe.ferrauto@unito.it

Zinc and copper are essential to several biological pathways. Disruption of their homeostasis is implicated in the onset of many diseases (Alzheimer's, Parkinson's, several cancers, etc…) [1]. In the last decade, a number of MRI contrast agents responsive to copper and zinc have been investigated, mainly based on relaxometric-based agents [2].

Herein, two new thulium-based ParaCEST responsive contrast agents, Tm-DOTAm-py and Tm-DOTAm-βAla-py, have been synthesized (Chemical structure in Fig.1) and evaluated for imaging of copper and zinc [3]. Unusual for responsive MRI contrast agents, both agents display a complete *on/off* response in the presence of the transition metals. Both complexes function as paraCEST agents in the absence of copper and zinc, with the positively charged Tm-DOTAm-py being more sensitive than the neutrally charged Tm-DOTAm-βAla-py. In each case, the CEST signal arises from the amides' protons rather than from a water molecule coordinated to the Tm^{3+} ion. Upon binding to Cu^{I}, Cu^{II}, or Zn^{II}, the exchange rate of the amide protons increases substantially, resulting in a complete loss of the CEST signal (Fig.1B). This efficient mode of action along with the lack of inner-sphere water molecule both in the presence and absence of transition metals was confirmed by $1/T_1$ NMRD profiles, ^{17}O NMR measurements, and molecular modelling simulations. Neither complex is selective for copper over zinc. Both form either a 1:1 $TmL:Cu^{+}$ or a 2:1 $TmL:Cu^{2+}$ and $TmL:Zn^{2+}$ complexes with binding affinities comparable to that of other responsive MRI contrast agents and sensitivity comparable to other CEST contrast agents

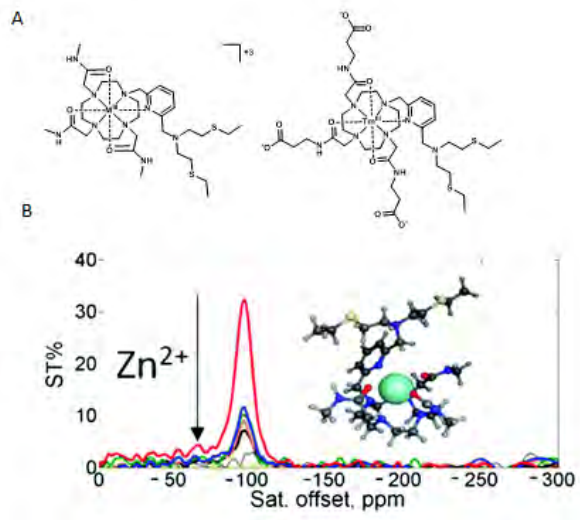


Fig. 1. (A) Chemical structure of Tm-DOTAm-py (left) and Tm-DOTAm-βAla-py (right) complexes; (B) ST% effect of Tm-DOTAm-py paraCEST agent in presence of increasing concentrations of Zinc.

References

[1] K. J. Barnham and A. I. Bush, *Curr. Opin. Chem. Biol*. **12**, 222-228 (2008)
[2] J. L. Major, et al., *Proc. Natl. Acad. Sci. U. S. A.*, **104**, 13881-13886 (2007)
[3] K. Srivastava, G. Ferrauto *et al*., *Dalton Trans*. **47**, 11346-11357 (2018)

P21

ANALYSIS OF COMPLEX LINE SHAPES IN TIME-DOMAIN NMR: A NOVEL APPROACH

R. Francischello,* M. Francischello,‡ M. Geppi†

*Istituto di Fisiologia Clinica CNR, Via Giuseppe Moruzzi 1, Pisa, Italia
‡Department of Mathematics, Imperial College, London, England
†Dipartimento Chimica e Chimica Industriale, Via Giuseppe Moruzzi 13, Pisa, Italia
E-mail: roberto.francischello@gmail.com

In spite of the fact that Time Domain (TD) NMR techniques are as old as NMR itself, their application in various NMR fields is still very popular, bringing to very important information and revealing fully complementary to high-resolution techniques. In particular, in the last years there has been an increasing attention to those applications of TD-NMR on materials generating complex line shapes. These bring into the Free Induction Decay non exponential components, which need to be treated accordingly. From a mathematical point of view we need to generalize the problem of the Inverse Laplace Transform to an integral equation, which is related to the inhomogeneous Fredholm integral equation of the first kind.

$S(t) = \sum_{i=1}^{n} \int K_i(t, \tau_i)\rho_i(\tau_i)d\tau_i + \psi(t, q) + \epsilon$ eq. 1

Here S(t) is the measured FID signal, ψ(t,q) is the FID of a complex line shape spin population. The integral transform represents the signal generated by a population of spin, $\rho_i(\tau)$, with the same line shape function $K_i(t,\tau)$, but with different parameter τ, e.g. for a lorentzian line shape K_i is an exponential function with a decay factor $\tau=T_2$.

For Free Induction Decay signals which are generated by a distribution of gaussian and exponential decay, we propose the use of a linearization of the Fredholm equation using the Nyström method and a Tikhonov regularization with multiple regularization parameters for solving the linear system. We tested different methods for the selection of regularization parameters and here we point at a couple of open questions from a mathematical point of view. We also tested the limits of this strategy when the FID includes complex line shapes like Pake, originating from a pair of dipolar coupled spin-1/2 nuclei.

For more complex line shapes we explored the use of a Nonlinear Least Squares (NLS) approach for solving the equation that we obtain by the Nyström method from eq. 1.
Although the model contains a large number of fitting parameters, it exhibits a linear dependence on the majority of them. This characteristic can be used to simplify the solution of the NLS problem. Therefore, it is possible to use a hybrid approach which combines the classical “continue" and "discrete" methods of FID analysis to investigate complex systems, which are difficult to investigate under a more common framework.

From an NMR point of view this kind of analysis could be useful in the studies of many materials, such as, for instance, low-cure level epoxy resins, heterogeneous organic materials like wood or silk, porous materials, tight sand oil and every system where phases or domains with significantly different dynamic properties (from solid-like to liquid-like) coexist.
From a more general point of view this work is related to some interesting questions concerning the inversion problem and could have applications in a very large number of disciplines.

P22

EFFECTS OF THE USE OF HIGH QUALITY COMPOST IN THE PRODUCTION OF TABLE GRAPES, TOMATO AND WHEAT BY THE NMR-BASED SUPPORTING DECISION SYSTEM P.A.S.C.Qua.

V. Gallo,[a,b] L. Miccolis,[c] M. Latronico,[a,b] P. Mastrorilli,[a,b] S. Todisco,[b] A. Rizzuti,[b] R. Ragone,[b] S. Pontrelli,[b] N. Intini,[b,d] M. Triggiani,[b] M. Mongelli,[c] F. Lo Greco[a,e]

[a]Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica, Politecnico di Bari, Bari, Italy; [b]Innovative Solutions S.r.l. – Spin Off del Politecnico di Bari, Noci (BA), Italy; [c]Progeva S.r.l., S.C. 14 Madonna delle Grazie – Caione, 74014 Laterza (TA) – Italia; [d]Agenzia Regionale per la Prevenzione e la Protezione dell'Ambiente, ARPA Puglia, Bari, Italy; [e]Dipartimento di Chimica, Università degli Studi di Bari, Bari, Italy
E-mail: vito.gallo@poliba.it

The use of high quality compost in sustainable and integrated agricultural practices is gaining an ever growing interest because of the beneficial effects on both environment and production process. Much of the inedible food waste can be converted into high quality compost by suitable industrial processes and the resulting nutrient-rich brown matter can be returned back to the earth and not to the landfill. The effects of compost application on the quality of food productions have been extensively studied under agronomical viewpoint. Great attention has been paid to safety issues by analysis of possible contaminants in the food products resulting from compost based farming practices. Few studies are reported on the effects of compost on metabolic features of the food productions, especially if NMR is considered as analytical detection tool. Recently, NMR spectroscopies were applied to identify the changes of metabolome, morphology, and structural properties induced in seeds (caryopses) of maize plants grown at field level under either mineral or compost fertilization [1].
In the framework of our studies aimed to discriminate agronomical practices by metabolomic approach [2-4] and to develop supporting decision systems based on NMR data input,[5] we have designed *P.A.S.C.Qua.* system.
P.A.S.C.Qua. is a decision supporting system capable to recognize whether an agri-food sample has been produced by sustainable agricultural practices involving high quality compost. The products considered in the project were table grapes, tomato and wheat produced by Apulian farmers under controlled conditions.
In this presentation, the NMR study allowing for development of *P.A.S.C.Qua.* will be presented moving from the NMR characterization of the three products to discrimination of samples by multivariate statistical analysis.

References

[1] P. Mazzei, *et al.*, *J. Agric. Food Chem.* **66**, 2580-2588 (2018)
[2] V. Gallo, *et al.*, *J. Food Comp. Anal.* **35**, 44-52 (2014)
[3] A. Rizzuti, *et al.*, *Food Chem*. **171**, 341-350 (2015)
[4] G. Ferrara, *et al.*, *Am. J. Enol. Viticul*. **65**, 381-387 (2014)
[5] V. Bevilacqua, *et al.*, *Lecture Notes in Artificial Intelligence LNAI* (Subseries of Lecture Notes in Computer Science), **7390**, 95-102 (2012).

Acknowledgments:
Regione Puglia (Dipartimento Agricoltura, Sviluppo Rurale e Ambientale) is gratefully acknowledged for financial support [Project P.A.S.C.Qua. under the call "Presentazione di proposte di ricerca e sperimentazione in agricoltura" det. Dirigente Servizio Agricoltura n.175/agr del 15/04/2013]

P23

IDENTIFICATION BY NMR SPECTROSCOPY OF URINARY BIOMARKERS OF HCV CIRRHOSIS AND OF SYSTEMIC METABOLIC CHANGES DUE TO ANTI-VIRAL HCV THERAPY

O. Giampaoli,[‡] A. Tomassini,[‡] G. Capuani,[‡] E. Biliotti,[†] D. Palazzo,[†] G. Taliani,[†] A. Miccheli[‡]

[‡]Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro, 5, 00185, Rome, Italy
[†]Department of Clinical Medicine, Sapienza University of Rome, P.le Aldo Moro, 5, 00185, Rome, Italy
E-mail: ottaviagiampaoli@gmail.com

Hepatitis C is a hepatic infection caused by the hepatitis C virus (HCV). Globally, an estimated 71 million people have chronic hepatitis C infection and approximately 399,000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma. Recent NMR-based metabolomics studies have been conducted on the serum of patients affected by hepatitis B related cirrhosis [1], and hepatitis C related fibrosis [2]. In both works the NMR technique proved to be crucial in differentiating the metabolic patterns of healthy individuals from those of cirrhotic and fibrotic patients. NMR-based metabolomics was also applied to urine of patients with HBV (hepatitis B), who developed hepatocellular carcinoma, successfully highlighting the differences between the urinary metabolic profile of cirrhotic patients and that of healthy controls [3]. In the present study the urinary metabolic profile of subjects with HCV-induced cirrhosis was analyzed by using NMR spectroscopy. The aim of this work was to define the characteristic metabolic profile of HCV-induced cirrhosis and to evaluate the metabolic changes due to viral replication inhibitor drugs. For this purpose, study on the urines of 43 fasting male subjects, aged between 43 and 82 years, was conducted, including 20 healthy subjects (Ctrl) and 23 affected by HCV cirrhosis. Patients were examined at the BT0 stage (Baseline Time 0) before treatment, at the EOT stage (End Of Treatment) at the end of the eight-week therapy, and at the FU1 and FU3 stage (Follow Up 1 and Follow Up 3 one month and three months after the end of treatment, respectively). The qualitative and quantitative determination of metabolites was carried out by mono- and bi-dimensional ^{1}H-NMR analysis of urine. The comparison between the urinary metabolic profile of patients with liver cirrhosis HCV (genotype 1a and 1b) and healthy subjects was performed on 45 metabolites, 38 of which were identified, while 6 were classified as unassigned signals. Data from the Ctrl, BT0, EOT and FU experimental groups were analyzed by multivariate PLS-DA and univariate statistical analysis, that allowed to define the metabolic changes induced by cirrhosis from those related to the active phase of HCV infection. In addition, urinary metabolic biomarkers, linked to the systemic effect of the anti-HCV drugs (side effects) and long-term drug metabolic effects, were identified. This study represents the first investigation by NMR-based metabolomics on the metabolic changes occurring in cirrhosis patients with HCV over time during and after anti-viral therapy.

References

[1] H. Zheng, M. Chen, S. Lu et al., *Metabolomics* **13**, 121 (2017)
[2] T. Gabbani, M. Marsico, P. Bernini, E. Lorefice, C. Grappone, M. R. Biagini, S. Milanie and V. Annese, *Dig. Liver Dis.* **49**, 1338–1344 (2017)
[3] I. J. Cox, A. E. Aliev, M. M. E. Crossey, M. Dawood, M. Al-Mahtab, S. M. Akbar, S. Rahman, A. Riva, R. Williams and S. D. Taylor-Robinson, *World J. Gastroenterol.* **22,** 4191-4200 (2016)

P24

H^1-NMR BASED METABOLIC PROFILING OF PURPLE AND ORANGE CARROTS DURING RIPENING

G. Giorgi,[‡] A. Tomassini,[‡] M. E. Di Cocco,[‡] F. Sciubba,[‡] W. Aureli,[†] A. Miccheli[‡]

[‡] Department of Chemistry, University of Rome "La Sapienza", P.le Aldo Moro 5, 00185 Rome, Italy
[†] R&D, Aureli Mario S.S. Agricola, Via Mario Aureli 7, 67050 Ortucchio (Aq), Italy
E-mail: james16@hotmail.it

The carrot (Daucus carota L.), a root of the Apiaceae family, is an economically important horticultural crop that can be consumed both fresh or as transformed products, like juices. Carrots do not provide a significant amount of calories to the human diet (43 kcal to 100g), but they contain numerous chemical species with documented anti-inflammatory, antioxidant, antimicrobial, antiviral and anticancer properties. There are different types of carrots that can be generally distinguished by the color [1]. The orange carrot mainly contains carotenoids as coloring agents, while the purple carrots (*Daucus carota* ssp. *Sativus* var. *Atrorubens*) contains anthocyanins and other biologically active compounds about 9 times more abundant than in the orange carrot [2]. These differences are very important both for the consumers and the agri-food industry as foods with a high content of flavonoids and antioxidants have been accepted by the scientific community as a good starting point for diets to prevent the rise of various diseases. In this regard, the knowledge of the detailed chemical composition in relation to agronomic conditions is of paramount importance and has made the carrots the subject of several spectroscopic studies [3].
Similar to the case of fruit ripening, the development of roots is a process that, can have a significant influence on the phytochemical composition depending on different speeds of maturation and the harvesting time [4, 5].
In the present study, high-resolution ^{1}H NMR spectroscopy coupled with univariate and multivariate statistical analyses has been applied to unravel the changes in the biochemical composition during the maturation of two carrot cultivars, purple and orange, from September to November. Moreover, as the over maturation of the purple variety, can give rise to a bitter taste, the development of this cultivar has been extended to December to analyze the chemical processes that can cause this phenomenon. The results showed, besides the known differences in carotenoids and anthocyanins, a higher content of free amino acids in the purple carrot. Furthermore, potential chemical biomarkers of full maturation have been identified for both orange and purple carrots. Finally, the increase in falcarinol and falcarindiol has been linked to the bitter taste in the over maturation of the purple root.

References

[1] S. A. Arscott, S. A. Tanumihardjo, *CRFSFS* **9**, 223-239 (2010)
[2] A. Smeriglio, M. Denaro, D. Barreca, V. D'Angelo, M.P. Germanò, D. Trombetta, *Fitoterapia* **24**, 49-57 (2018)
[3] A. Tomassini, F. Sciubba, M. E. Di Cocco, G. Capuani, M. Delfini, W. Aureli, A. Miccheli, *J. Agric. Food Chem.* **64**, 5284-5291 (2016)
[4] D. Capitani, L. Mannina, N. Proietti, A. P. Sobolev, A. Tomassini, A. Miccheli, M. E. Di Cocco, G. Capuani, R. De Salvador, M. Delfini, *Talanta* **82**, 1826-1838 (2010)
[5] F. Sciubba, D. Avanzato, A. Vaccaro, G. Capuani, M. Spagnoli, M. E. Di Cocco, I. N. Tzareva, M. Delfini, *Nat. Prod. Res.* **31**, 765-772 (2017).

P25

NMR RELAXOMETRIC STUDIES ON GD^{III} COMPLEXES OF DO3A-HYDROXYPROPIONAMIDES FUNCTIONALIZED LIGANDS

L. Leone,[†] G. Ferrauto,[‡] M. Botta,[†] L. Tei[†]

[†] Dipartimento di Scienze ed Innovazione Tecnologica, Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Viale Teresa Michel 11, 15121 Alessandria, Italy
[‡] Department of Molecular Biotechnology and Health Sciences, Molecular Imaging Centre, University of Torino, Torino, Italy
E-mail: loredana.leone@uniupo.it

Gd^{III}-complexes act on the relaxation rates of the tissue water protons in Magnetic Resonance Imaging T_1-weighted images yielding hyperintensity. Among the Gd^{III} based contrast agents, Gd(HP-DO3A) (Fig.1) differs since it contains two sources of protons for transferring the paramagnetism of Gd^{III} to the bulk water, namely the coordinated water molecule and the proton from the coordinated hydroxyl group [1]. In this work, we explored the effect caused by changing the methyl group in the ordinary hydroxypropyl arm of HP-DO3A with electron-withdrawing amide groups. The presence of an amide group has three effects: 1) it makes the –OH group in the hydroxypropyl arm more acidic; 2) it introduces an acid-catalyzed prototropic effect and 3) it makes possible the presence of water molecules in the second sphere of hydration, thus enhancing the relaxivity. In particular, we have synthesized (Fig.1) three HPA-DO3A ligands bearing, respectively, a primary, a secondary and a tertiary amide (**1**-**3**) and a dimeric $(HPA\text{-}DO3A)_2$ system, **4** [2]. A pH responsive ^1H NMR relaxometric behavior was observed in case of the mononuclear Gd-complexes, more pronounced for Gd(HPA-DO3A). A ^1H and ^{17}O NMR relaxometric study on the Gd^{III} complexes and a Chemical Exchange Saturation Transfer (CEST) study on Eu^{III} and/or Yb^{III} complexes were carried out to evaluate the parameters that govern the relaxivity associated with these complexes.

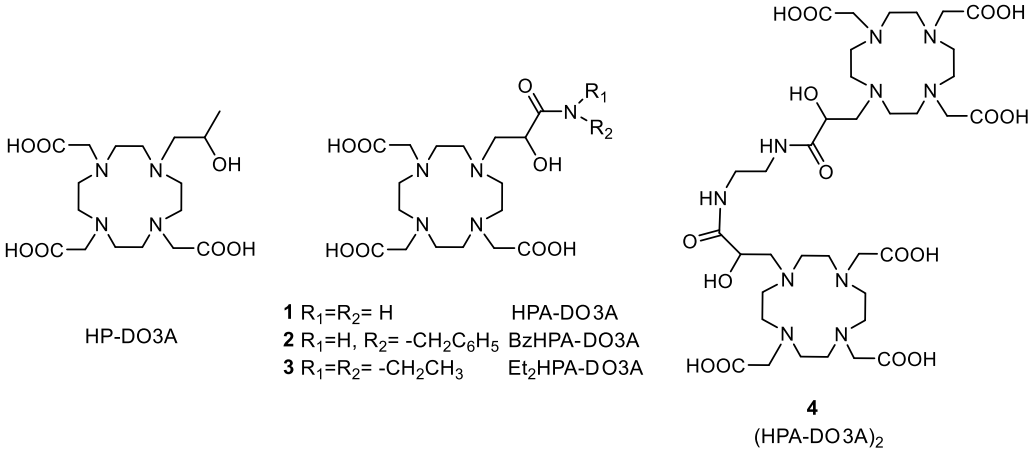


Fig. 1. Structures of the ligands synthesized

References

[1] D. Delli Castelli et al. *Inorg. Chem.* **52**, 7130 (2013); S. Aime et al. *Inorg. Chem.*, **57**, 5567 (2018).
[2] L. Leone, G. Ferrauto, M. Cossi, M. Botta and L. Tei, *Front. Chem.* **6**, 58 (2018).

P26

APPLICATION OF THE NMR TECHNIQUE FOR THE ANALYSIS OF SERA AND URINES OF SUBJECTS WITH DIET ENRICHED IN DHA. *

C. Licari,[‡] V. Ghini,[‡] L. Tenori,[‡,†] C. Luchinat[‡,#]

[‡]Center for Magnetic Resonance (CERM), University of Florence, via Luigi Sacconi 6, 50019 Sesto Fiorentino (Florence), Italy.
†Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla 3, Florence, Italy.
#Department of Chemistry "Ugo Schiff", University of Florence, via della Lastruccia 3-13, 50019, Sesto Fiorentino (Florence), Italy.
[*]on behalf of the whole Pathway-27 Consortium (www.pathway27.eu)
E-mail: licari@cerm.unifi.it

Nuclear Magnetic Resonance (NMR)-based metabolomics is an efficient and highly reproducible approach to investigate biofluids which can be collected non-invasively [1].
In the present study, this technique is used to analyze serum and urine samples from volunteers subjected for a period of twelve weeks to a diet enriched in DHA, in the context of the research activity sponsored by the pan-European PATHWAY-27 project.
It is well known that docosahexanoic acid (DHA) has a positive effect on a great variety of diet-related disease risks, including the metabolic syndrome (MetS) [2]. Indeed, in this study, it has been evaluated how different food matrices (dairy, bakery and eggs-based food) enriched with different combination between DHA and oat β-glucan (OBG) or anthocyanins (AC) influence the serum and urine metabolome of the volunteers.
Blood serum and urine samples taken from each subject at the beginning (T0) and at the end of BEFs consumption (T1) have been analyzed in order to explore the change induced by the dietary treatment. Furthermore, a placebo group has been considered to have a more critical analysis of the effect of bioactive-food matrix.
In particular, for each serum and urine samples, ^{1}H-NMR spectra have been acquired using different types of experiments (NOESY, CPMG, and DIFFUSION for sera and NOESY for urines) and they have been analyzed using multivariate statistical techniques.
In this preliminary study is described the assignment of more than 40 metabolites in urine and 30 in serum. Further, a tool for lipidomic analysis of NMR spectra has been applied to serum spectra providing information about lipoprotein subclasses (e.g. Chylomicrons, VLDL, IDL, LDL and HDL) [3].

References

[1] J. K. Nicholson and J. C. Lindon *Nature* **455**, 1054-1056 (2008)
[2] X. F. Guo, X. Li, M. Shi and D. Li *Nutrients* **9**, 703 (2017)
[3] V. Aru et al. *TrAC Trends Anal. Chem.* **94**, 210-219 (2017)

P27

TOWARDS SINGLET-ASSISTED DIFFUSION TENSOR IMAGING (SAD-TI)

G. Melchiorre,[1,2] M. C. Tourell,[1] G. Pileio[1]

[1] Department of Chemistry, University of Southampton, Southampton SO17 1BJ, United Kingdom
[2] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Giuseppe Moruzzi, 13 - 56124 Pisa, Italia
E-mail: giuliamelchiorre@hotmail.it

Diffusion NMR methodology includes a range of techniques that allow to investigate the microstructure of a medium, starting from measurements of molecular diffusion. In one of its popular variants (diffusion tensor imaging, DTI) the whole diffusion tensor is reconstructed and used to explore the anisotropy of the medium itself. In conventional DTI it is possible to either reconstruct a single tensor representative of the entire sample or to couple the diffusion measurement with 3D imaging techniques, where one tensor for every voxel is reconstructed.
These techniques have been successfully used for biological systems, where the diffusion of water is measured to probe the architecture of a tissue in a non-invasive way. Nevertheless, the longitudinal relaxation time T_1 of water of the order of some seconds at best, is a limit that makes impossible to highlight the anisotropy of all those systems where no restriction to diffusion occurs within a few tens of micrometres.
Recently in our group, we introduced a further variant of diffusion NMR, namely SAD-NMR (singlet-assisted diffusion NMR) where the very slow relaxation decay of singlet nuclear spin order enables diffusion measurements over several minutes and thus the possibility to gain insights into much larger structures of up to a few millimetres [1].
We are now working towards the development of SAD-TI a singlet-assisted diffusion tensor imaging technique that allows the reconstruction of the diffusion tensors in larger compartments than currently possible. This contribution aims to compare the outcomes of all these strategies, showing how it is possible to gradually improve the description of an organised structure. Two 3D printed phantoms with a known inner architecture are compared, the one characterised by cylindrical channels of 1mm cross section and the other with helical channels of same cross section. At first, the diffusion tensors of water in both systems are derived with conventional DTI techniques, to verify the unrestricted motion and investigate any contingent causes of apparent anisotropy (e.g. convection). At a second stage, the diffusion sensitising gradients are combined with singlet-order preparation [2] pulse sequence, in order to highlight the structural differences between the two phantoms, information not accessible with conventional DTI.

References

[1] G. Pileio, J-N. Dumez, I-A. Pop, J.T. Hill-Cousins, R.C.D. Brown, *J. Magn. Reson.* **252**, 130-134 (2015),
[2] G. Pileio, M. Carravetta, M.H. Levitt, *Proc. Natl. Acad. Sci. U.S.A* **107**,17135-17139 (2010)

P28

RHODAMINE BINDS TO SILK FIBROIN AND INHIBITS ITS SELF-AGGREGATION

H. Molinari,[1] K. Pagano,[1] S. Tomaselli,[1] O. Gasymov,[2] S. Zanzoni,[3] L. Ragona[1]

[1] Istituto per lo Studio delle Macromolecole, CNR, Via Corti 12, 20133 Milano
[2] Institute of Biophysics of ANAS, 117 Khalilov, AZ-1141 Baku, Azerbaijan
[3] Centro Piattaforme Tecnologiche, Università di Verona; Strada Le Grazie 15, Verona
E-mail: henriettemolinari0@gmail.com

Amyloid structures are universal structures, widely diffuse in nature. Silk, capable of forming some of the strongest tensile materials on earth represents an important example of formation of functional amyloid fibrils, a process reminiscent of the oligomerization of peptides involved in neurodegenerative diseases. The stability of silk fibroin solutions in different conditions and its transition from alpha-helix/random coil to beta-sheet structures, at the basis of gelation processes and fibril formation, have been here investigated and monitored employing different biophysical approaches [1]. Silk fibroin aggregation state as a function of concentration, pH and aging has been characterized employing NMR ordered diffusion spectroscopy. The change of silk fibroin diffusion coefficient over time, which reflects the progress of oligomerization, has been monitored for silk fibroin alone and in the presence of a polycondensed aromatic dye, namely rhodamine 6G. NMR, UV and DLS measurements indicated that rhodamine specifically binds to silk fibroin with a micromolar K_D. The reported data reveal, for the first time, that RHD is capable of inhibiting fibroin self-association, thus controlling beta-conformational transition at the basis of fibril formation. The described approach could be extended to other amyloid forming proteins, allowing a better control of the oligomerization process.
The development of nanoparticles as inhibitors of amyloid protein aggregation has recently gained attention [2]. In this line, further investigations are presented on the capability of 5 nm anionic citrate-coated gold nanoparticles to modulate, at low substoichiometric ratios, silk fibroin beta-sheet driven oligomerisation.

References

[1] L. Ragona, O. Gasymov, A.Y. Guliyeva, R.B. Aslanov, S. Zanzoni, C. Botta, H. Molinari, *Biochim. Biophys. Acta*, **1866**, 661-667 (2018).
[2] M. Zaman, E. Ahmad, A. Qadeer, G. Rabbani, R.H. Khan, *Int. J. Nanomedicine*, **9**, 899-912 (2014)

Acknowledgements
LR, KP, ST and HM acknowledge financial support of University of Verona (Bando JOINT Projects 2017, Prof. Michael Assfalg - Dr. Laura Ragona) and Fondazione Antonio De Marco

P29

AN EFFICIENT MRI AGENT TARGETING EXTRACELLULAR MARKERS IN PROSTATE ADENOCARCINOMA

A. Pagoto,[†] M. Tripepi,[†] R. Stefania,[†] S. Lanzardo,[†] F. Garello,[†] D. L. Longo,[†] S. Aime,[†] E. Terreno[†]

[†] Molecular & Preclinical Imaging Centers, Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza 52, 10126 – Torino, Italy
E-mail: amerigo.pagoto@unito.it

Purpose: Prostate Cancer (PCa) is the most widespread male tumour in western countries [1]. Herein a novel MRI molecular tetrameric probe based on the heptadentate Gd-AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid) able to *in vivo* detect PCa through the recognition of the fibrin/fibronectin complex is proposed.
Methods: The peptide CREKA (Cys-Arg-Glu-Lys-Ala) [2], targeting the fibrin/fibronectin complex in the reactive stroma of the tumour, was synthesized by Solid Phase Peptide Synthesis (SPPS) and conjugated to the tetramer dL-(Gd-AAZTA)$_4$. The resulting probe was characterized by ^{1}H relaxometry, tested *in vitro* on fibrin clots, and *in vivo* on an orthotopic mouse model of PCa.
Results: CREKA-dL-(Gd-AAZTA)$_4$ showed a remarkable relaxivity of 18.2 $mM_{Gd}^{-1}s^{-1}$ (0.47 T, 25°C), 70% higher than already reported probe with DOTA derivaties [3]. Due to the presence of two water molecules (q=2) in the inner coordination sphere of each Gd^{3+} ion, whose rotational motion (τ_R) is lengthened as the results of the relatively high molecular weight. The probe displayed a detectable affinity for plasma-derived fibrin clots. Upon the i.v. injection of the probe in an orthotopic mouse model of PCa, a significant increase in the prostate T_1 contrast (ca. 40%) was observed (Fig 1). It appears statistically higher either with respect to the one observed for the control probes and to the one detected when CREKA-dL-(Gd-AAZTA)$_4$ was administered to healthy animals.

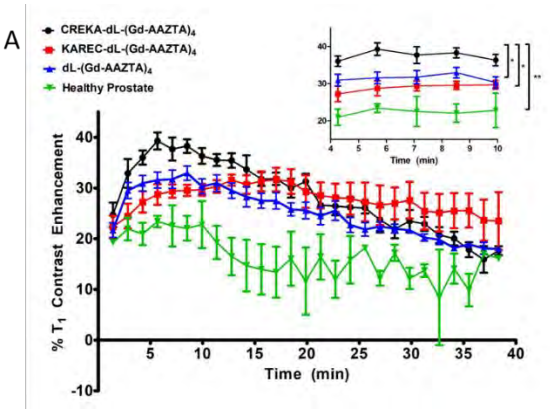


Fig 1. % T_1 contrast enhancement followed in time after injection of CREKA-dL-(Gd-AAZTA)$_4$, control probes and healthy tissue.

Conclusions: This study demonstrated the ability of the CREKA-dL-(Gd-AAZTA)$_4$ probe to specifically localize in prostate tumour after the injection. The high relaxivity of the probe allows the reduction of the injected dose to 20 μmol_{Gd}/kg, yielding a good *in vivo* contrast enhancement in the region of prostate tumour.

References
[1] Siegel R. et al. CA *Cancer J. Clin*. **67**, 7-30 (2017)
[2] Simberg D. et al. *Proc. Natl. Acad. Sci. U S A.* **104**, 932-936 (2007)
[3] Wu X. et al. *Am. J. Nucl. Med. Mol. Imaging*. **4**, 525-536 (2014)

P30

SALIVARY METABOLIC FINGERPRINT OF PHYSICAL EXERCISE IN PROFESSIONAL FEMALE SOCCER PLAYERS

E. Pitti,[‡] S. Di Marino,[†] V. Summa,[†] S. Bernardini,[‡] D.O. Cicero[†§]

[‡] Dipartimento di Medicina Sperimentale e Chirurgia, University of Rome "Tor Vergata", Rome, Italy
[§] Dipartimento di Scienze e Tecnologie Chimiche, University of Rome "Tor Vergata", via della Ricerca Scientifica snc, 00133, Rome, Italy
[†] IRBM Science Park Spa, Via Pontina km 30.600, 00040 Pomezia (RM) Italy
E-mail: ericapitti27@gmail.com

Physical exercise induces changes in metabolic profile of human bio-fluids [1-6]. The use of saliva for monitoring metabolic variations in physical exercise gained ground in recent years being its collection not invasive and very quick [7]. At the same time, metabolic profiling of saliva is only partially explored as potential biomarker due to its propensity to be affected by dietary habits, presence of oral bacteria and contaminant agents.
In this preliminary study we want to investigate the metabolic changes induced by physical exercise in saliva samples. For this pilot study, we collected saliva samples of eleven professional female soccer players before and after a football match.
The samples were analyzed by ^{1}H NMR spectroscopy. For each spectrum, 56 metabolites were identified and quantified. Those metabolite concentrations constitute the dataset for the statistical analysis using a synergic combination of multivariate and univariate analysis.
These preliminary results underline a metabolic variation in saliva after exercise and open to the use of saliva as potential bio-fluid for monitoring athletic performance and physical state. Further analysis will be focused both on validation of these results and to increment the dataset size.

References

[1] C. Morris, C. Grada, M. Ryan, and L. Brennan, *Mol. Nutr. Food Res*. **57**, 1246-1254 (2013)
[2] G. D. Lewis, L. Farrell, M. J. Wood and R. E. Gerszten, *Sci. Trans. Med*. **33**, 33-37 (2010)
[3] C. Santone, V. Dinallo, M. Paci, S. D'Ottavio, S. Bernardini, *J. Pharm. Biomed. Anal*. **88**, 441-446 (2014)
[4] A. Bassini, L. C. Cameron, *Biochem. Biophys. Res. Commun*. **4**, 708-716 (2014)
[5] A. Pechlivanis, G. A. Theodoridis, *J. Proteome. Res*. **12**, 470-480 (2013)
[6] G. M. Kirwan, M. J. Adams, *Anal. Chim. Acta.* **652**, 173-179 (2009)
[7] D.O. Cicero, S. Di Marino, V. Dinallo, M. Pero, V. Summa, Desideri, A., et al., *Biomed. Spectrosc. Imaging* **5**, 55-70 (2016)

P31

NMR CHARACTERIZATION OF NOVEL SOLID FORMS OF NSAIDs: THE CASE OF IBUPROFEN ARGINATE

A. Pucci,[‡] E. Carignani,[‡] P. Rossi,[†] E. Macedi,[†] L. Bernazzani,[‡] R. Carosi,[‡] P. Paoli,[†] M. Geppi[‡]

[‡]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via G. Moruzzi 13, 56124 Pisa, Italy
[†]Dipartimento di Ingegneria Industriale, Università degli Studi di Firenze, via S. Marta 3, 50139 Florence, Italy
E-mail: a.pucci11@studenti.unipi.it

The exploration of new solid forms of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with higher onset of action and reduced side effects maintains a big interest in the pharmaceutical field. The development of drugs in the form of salts and co-crystals usually results in higher solubility in water and more rapid absorption in the body compared to the corresponding acidic forms. Pharmaceutical products in the amorphous state generally show improved release properties and high dissolution rates. Solid state NMR revealed particularly useful to characterize the structural and dynamic properties of many pharmaceutical systems, including salts, co-crystals and amorphous forms [1, 2].
Among the commonly used ibuprofen salts, the L-Arginine one (IBU-Arg) shows interesting analgesic, antipyretic and anti-inflammatory effects, in addition to a considerable reduction in the problems of gastrolesivity, typical of this type of drugs [3]. However, no characterizations of pure IBU-Arg solid forms have been reported so far in the literature.
In this work, the crystalline (synthesized following ref. [4]) and amorphous (obtained by quench cooling of the melt) IBU-Arg forms were prepared and, together with the commercially available product, investigated by Powder X-Ray Diffraction, Differential Scanning Calorimetry, Thermogravimetric Analysis, and high resolution Solid-State NMR.
In particular, the structural properties of the synthesized crystalline form were investigated by ^{13}C CP- and DE-MAS, ^{1}H MAS, 2D ^{1}H-^{13}C MAS-J-HMQC and HETCOR experiments. On the other hand, the synthesized amorphous form was studied particularly focusing on its stability and dynamic properties, also exploiting variable temperature experiments. Moreover, the commercial product revealed an amorphous nature, showing similar spectral properties, but a different calorimetric behaviour with respect to the synthesized amorphous form.

References

[1] P. Cerreia Vioglio, M. R. Chierotti, R. Gobetto, *Adv. Drug Deliver. Rev.* **117**, 86-110 (2017)
[2] A. Paudel, M. Geppi, G. Van den Mooter, *J. Pharm. Sci.* **103**, 2635-2662 (2014)
[3] P. Desjardins, P. Black, M. Papageorge, T. Norwood, D. D. Shen, L. Norris, A. Ardia, *Eur. J. Clin. Pharmacol.* **58**, 387-394 (2002)
[4] I. Vayá, M. C. Jiménez, M. A. Miranda, *Tetrahedron Asymmetry* **16**, 2167-2171 (2005)

P32

DYNAMICAL PROCESSES IN CRYSTALLINE SOLID SOLUTIONS OF IONIC ROTORS: A STRUCTURAL AND SPECTROSCOPIC STUDY

F. Rossi,[a] S. D'Agostino,[b] L. Fornasari,[b] F. Grepioni,[b] M. R. Chierotti,[a] R. Gobetto,[a] D. Braga[b]

[a] Department of Chemistry and NIS Centre, University of Torino, V. P. Giuria 7, 10125, Torino Italy.
[b] Department of Chemistry Ciamician, University of Bologna, V. F. Selmi 2, 40126, Bologna, Italy.
E-mail: fe.rossi@unito.it

Dynamic processes, such as rotation and libration of molecules within crystals, have long attracted the attention of researchers. Indeed, the understanding, control and exploitation of such movements can give access to new properties in functional materials [1-4].
We report on the synthesis and characterization of two supramolecular salts of general formula [(12-crown-4)·(DABCOH$_2$)]X$_2$ (X = Cl$^-$ or Br$^-$) and of their solid solutions [(12-crown-4)·(DABCOH$_2$)]Cl$_{2x}$Br$_{2(1-x)}$ in the whole composition range ($0 < x < 1$). The mixed crystals have been investigated by a combination of solid-state techniques including variable temperature single crystal and powder X-ray diffraction (XRD), and solid-state NMR spectroscopy. The combined use of these methods has made possible the rationalization of the correlation between the dynamical processes taking place within the crystalline materials and the solid solution composition. VT solid-state NMR measurements allowed to describe an uncommon thermally activated molecular motion based on a precession of the DABCO unit within the cage created in the crystal structure by the crown ether and the halide packing (Figure 1). To the best of the authors' knowledge, this is the first observation of a RT precession motion (a "precession within a cage") that is frozen as the hosting cage is made tighter by decreasing the temperature.

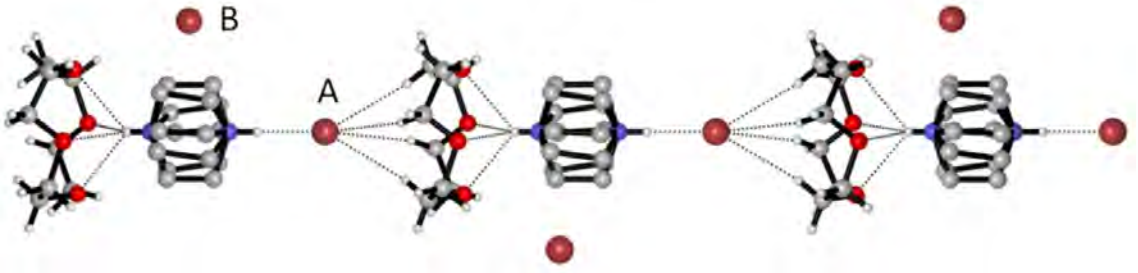


Fig. 1. Ball and stick representation of hydrogen-bonded chains detected within crystalline [(12-crown-4)·(DABCOH$_2$)]Br$_2$.

References

[1] W. Setaka, K. Yamaguchi, *Proc. Natl. Acad. Sci.* **109**, 9271-9275 (2012)
[2] Z. Sun, J. Luo, S. Zhang, C. Ji, L. Zhou, S. Li, F. Deng, M. Hong, *Adv. Mater.* **25**, 4159-4163 (2013)
[3] S. Bracco, M. Beretta, A. Cattaneo, A. Comotti, A. Falqui, K. Zhao, C. Rogers, P. Sozzani, *Angew. Chem. Int. Ed.* **54**, 4773–4777 (2015)
[4] S. Bracco, F. Castiglioni, A. Comotti, S. Galli, M. Negroni, A. Maspero, P. Sozzani, *Chem. - A Eur. J.* **23**, 11210-11215 (2017)

P33

SALIVA OF PATIENTS AFFECTED BY PAROTID TUMOR: AN NMR METABOLOMICS ANALYSIS

A. Santoro,[‡] M. Grimaldi,[‡] A. Palisi,[‡] I. Stillitano,[‡] P. Montoro,[‡] R. Romano,[‡] M. Rodriquez,[‡] R. Palladino,[†] A.M. D'Ursi[‡]

[‡]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132 - 84084 Fisciano, Salerno, Italy
[†]U.O.C. Clinical Pathology D.E.A. III Umberto I - Nocera Inferiore – Salerno, Italy
E-mail: dursi@unisa.it

Human saliva is secreted from three pairs of major salivary glands namely parotid gland, submandibular gland and sublingual gland.[1] Salivary gland cancer (SGC) is not detected until it reaches an advanced stage, due to the difficulty in distinguishing benign from malignant tumors.[2] Therefore, there is an increasing interest in the development of new diagnostic approaches enabling early detection as well as the screening of high risk populations with parotid precancerous lesions remains an unmet medical need.
In the present work, we present a NMR-based metabolomic study of saliva of patients suffering from parotid tumours. Our data, were analyzed using a combined approach based on PRICONA quantitative analysis and statistical multivariate analysis.[3] Interestingly, both the analytical methods indicate that individuals affected by parotid tumor have a characteristic metabolomic profile, suggestive of alteration in aminoacid pathways. Our data reveal a preliminary metabolomics fingerprint of parotid tumour that, in contrast to a single metabolic biomarker, reflect the multifactorial nature of oncogenesis and the heterogeneity of oncogenic pathways.

References

[1] O.R. Beirne, *J. Am. Dental Assoc*. **121**, 672 (1990).
[2] V.S.H. To, J.Y.W. Chan , R.K.Y. Tsang , W.I. Wei. *ISRN Otolaryngology*. **2012**, 872982 (2012).
[3] R. Romano, F. Acernese, R. Canonico, G. Giordano, F. Barone, *Int. J. Biomed. Eng.Technol*. **13**, 357-369 (2013).

P34

EFFECT OF PHOSPHORYLATION ON THE µs-ms DYNAMICS OF EIN

V. Sivo,[‡] V. Venditti[†]

[‡]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania 'L Vanvitelli', via Vivaldi 43, 81100 Casertae, Italy
[†]Department of Chemistry, Iowa State University of Science and Technology, Ames IA 50011-1015, USA
E-mail: valeria.sivo@unicampania.it

The bacterial phosphotransferase system (PTS) is a signal transduction pathway that couples phosphoryl transfer to active sugar transport across the cell membrane. The PTS is initiated by phosphorylation of enzyme I (EI) by phosphoenolpyruvate (PEP) [1]. EI is a multidomain protein comprising a N-terminal domain (EIN) that contains the phosphorylation site (His^{189}) and the binding site for HPr (histidine-containing phosphocarrier protein) and a C-terminal domain (EIC) that is responsible for protein dimerization and contains the binding site for PEP and the competitive inhibitor αKG. The EIN and EIC domains are connected by a short helical linker [2]. EI is an ideal model for investigating the interplay between local and interdomain dynamics in proteins. Indeed, EI is a 128 kDa dimer whose enzymatic activity depends on four conformational equilibria: (i) a monomer-dimer equilibrium [3], (ii) a compact-to-expanded equilibrium within EIC [4], (iii) a state A-to state B equilibrium within EIN, and (iv) an open-to close equilibrium describing a reorientation of EIN relative to EIC [5-6]. Binding of PEP to EIC promotes transition to the dimer/compact/state B/closed form and activates the enzyme for catalysis [5].

We are using NMR to characterize the structures, kinetics and thermodynamics of conformational equilibria in isolated EIN. Our working hypothesis is that the intradomain conformational equilibria that concur in regulation of the activity of the full-length protein are at play in the isolated EIN. We expect the data acquired here will unambiguously inform on the effect of EIN phosphorylation on the kinetics and thermodynamics of the state A-to state B equilibrium.

References

[1] V. Venditti, G.M. Clore, *J. Biol. Chem.* **287**, 26989-26998 (2012)
[2] V. Venditti, R. Ghirlando, G.M. Clore, *ACS Chem. Biol.* **8**, 1232–1240 (2013)
[3] H.V. Patel, K.A. Vyas, R. Savtchenko, S. Roseman, *J. Biol. Chem.* **281**, 17570–17578 (2006)
[4] V. Venditti, G.M. Clore, *J. Biol. Chem.* **287**, 26989-26998 (2012)
[5] V. Venditti, V. Tugarinov, C.D. Schwieters, A. Grishaev, G.M. Clore, *Nat. Commun.* **6**, 5960 (2015)
[6] V. Venditti, C.D. Schwieters, A. Grishaev, G.M. Clore, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 11565-11570 (2015)

P35

THE EFFECT OF DROUGHT ON THE METABOLITE PROFILE OF POPULUS NIGRA LEAVES: AN NMR STUDY

A. P. Sobolev,[1] D. Capitani,[1] C. Brunetti,[2] M. Centritto[2]

[1]Istituto di Metodologie Chimiche, Laboratorio di Risonanza Magnetica "Annalaura Segre", Consiglio Nazionale delle Ricerche, Via Salaria Km 29.300, 00015 Monterotondo (Roma), Italy
[2]Istituto per la Valorizzazione del Legno e delle Specie Arboree, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy
E-mail: anatoly.sobolev@cnr.it

The ideal biomass crop is not only fast growing, but also drought tolerant. Plant responses to drought are regulated by a complex interaction of hydraulic and chemical signals [1]. The elucidation of the biochemical/physiological responses elicited by drought stress are critical to enhance drought tolerance in fast growing biomass species. In the present study black poplar (*Populus nigra*) plants subjected to water deficit were used as the model system to elucidate the effect of drought on the plant metabolite profile. Water stress was imposed by withholding water for 3-4 (moderate water stress) and 7-8 days (severe water stress), whereas control well-watered plants were irrigated to pot capacity each day during the experimental period. Water soluble metabolites were extracted from black poplar leaves and analyzed by NMR spectroscopy. The quantitative data from NMR spectra obtained using bucketing or integration of selected resonances were subjected to chemometric analysis to reveal the changes in metabolite profile associated with drought.

References

[1] J. P. Comstock *J. Exp. Botany* **53**, 195-200 (2002)

P36

EVIDENCE OF THE PRESENCE OF STRUCTURED WATER AT THE WATER-NAFION INTERFACE FROM T1-WEIGHTED MRI MEASUREMENTS

G. Spatola,[†] S. Aime,[†] A. Viale,[†] R. Binetti,[§] E. Brussolo[§]

[†]Department of Molecular Biotechnology and Health Sciences, University of Turin, Via Nizza 52, 10126 Turin, Italy
[§]1Research Center, Società Metropolitana Acque Torino S.p.A., corso Unità d'Italia 235/3, 10127 Turin, Italy
E-mail: giulia.spatola@unito.it

Phenomena at the interface between water and different types of surfaces have raised interest, since several years, for their importance both in biological and in chemical processes. Recently, the attention has been focused on the investigation of the phenomenon known as Exclusion Zone (EZ): different types of colloidal particles (e.g. microspheres with various functional groups) and a variety of solutes (e.g. some proteins and dyes) are extensively excluded from a region adjacent to hydrophilic surfaces, such as for example hydrophilic polymers, hydrogels, ion-exchange beads. The size of this exclusion zone varies between 100 and 200 μm depending on the surface, on the excluded molecules and on the experimental conditions [1]. Different hypotheses about the nature of the forces that cause the exclusion have been proposed [2]; in particular, G. Pollack and co-workers proposed that the exclusion zone is the result of the ordering of tightly bound water molecules in the proximity of surfaces, yielding a structured layer of water molecules with lower mobility and diffusivity with respect to bulk water, and intermediate properties between liquid and solid water (the so-called third state of water) [3]. We used Magnetic Resonance Imaging (MRI) and Nuclear Magnetic Resonance (NMR) to investigate further the nature of water in the exclusion zone. High resolution NMR spectra allow to differentiate bulk and interstitial water, which give raise to two separate peaks in the proton spectrum of Nafion (a perfluorionated sulphonated polymer which is known to afford wide and stable EZs) immerse in water, but do not show any direct evidence of this third state of water. On the other hand, in T_1 weighted MR images, the lower mobility of water in this layer produces a signal intensity which differs from that of both bulk (more mobile water) and interstitial water (more constrained water). The thickness of this low water mobility layer is lower than the EZ as observed by optical microscopy, indicating that the EZ extends beyond it and it is most probably caused both by a structuring effect of the surface on the water molecules and by diffusion processes.

References

[1] J.M. Zheng, G.H. Pollack, *Phys. Rev. E* **68**, 031408 (2003)
[2] J.M Schurr, B.S. Fujimoto, L. Huynh, D.T. Chiu, *J. Phys. Chem. B.* **117**, 7626-7652 (2013)
[3] J.M. Zheng W. ChunChin, E. Khijniak, E. Khijniak Jr., G.H. Pollack, *Adv. Colloid Interface Sci.* **127**, 19-27 (2006)

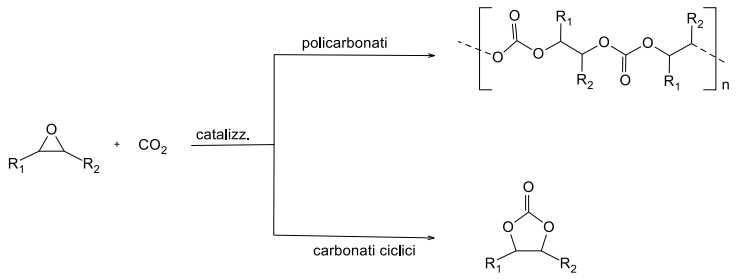
P37

CO_2 UTILIZATION FOR THE PRODUCTION OF "GREEN" COPOLYMERS WITH EPOXIDES

S. Spera, ‡A. Savoini,‡ F. Melchiorre,‡ P. Biagini,‡ L. Veronese,† and M. Brivio†

‡ CENTR - Renewable Energy & Environmental R&D, Via Fauser, 4 - 28100 Novara (NO) - Italy
†ISMAC_CNR National Research Council, via E. Bassini 15, 20133 Milano
E-mail: silvia.spera@eni.com

Carbon dioxide can be considered an important carbon resource: its catalytic transformation into chemical products has attracted a lot of attention because it is widely available, nontoxic and, most importantly, renewable. Nowadays it also constitutes a "green" route to produce chemicals, not only because it provides "green" chemicals but also because CO_2 is a waste product of many chemical processes and its consumption may contribute to the reduction of the greenhouse effect.
One of the most promising "green" reactions in this area is the alternating copolymerization of CO_2 with epoxides to generate biodegradable polycarbonates.
The high thermal stability of CO_2, however, hinders its widespread application as a reagent in chemical processes, unless a highly efficient and selective catalyst system is used, even in the interactions with very reactive organic substrates
For this reason, continuous research efforts have been devoted to the development of new copolymerization catalysts, resulting in a significant improvement of the catalytic activity and selectivity (polymer - cyclic carbonate, carbonate - ether linkages and so on ...).
Recently, we have also been involved in the optimization of the alternating copolymerization of CO_2 with epoxides monomers such as cyclohexenoxide and cyclopropylenoxide, with different catalytic systems and cocatalysts, and in the physicochemical characterization of the so obtained copolymers.
In this poster we report some of the aspects of the physicochemical characterization of the copolymers CO_2-epoxide performed with NMR analysis in solution and FT-IR, mainly related to polycarbonates/cyclic carbonate ratio, carbonate/ether linkages ratio, regioselectivity, and stereoselectivity.



P38

MEMBRANOTROPIC ACTIVITY OF C8 PEPTIDE AND ITS DERIVATIVES

I. Stillitano,[‡] M. Grimaldi,[‡] G. Amodio,[†] A. Santoro,[‡] O. Moltedo.[†] P. Remondelli,[†] A. M. D'Ursi[‡]

[‡]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 134, I-84084 Fisciano (SA) Italy
[†]Department of Medicine and Surgery, University of Salerno, Via S. Allende, 84081 Baronissi (SA) Italy
E-mail: istillitano@unisa.it

Feline immunodeficiency virus, a naturally occurring Lentivirus [1], enters cells via a mechanism involving a surface glycoprotein named gp36 [2-4]. C8 is a short synthetic peptide corresponding to the residues ^{770}WEDWVGWI^{777} of gp36 MPER [5]. It elicits antiviral activity by inhibiting the fusion of the virus and cell membrane, and in biophysical experiments, it exhibits fusogenic activity, altering the phospholipid bilayer organization. Tryptophan residues are important for antiviral activity. The C8 derivative C6a was derived by truncating the ^{770}WE^{771} N-terminal residues and exhibits a partially conserved antiviral activity, while the C8 derivative C6b originated from the truncation of the C-terminal ^{776}WI^{777} and is nearly inactive. To investigate the structural factors of C6a and C6b that are responsible for the different activity profiles, despite their high similarity, we studied the conformation of the peptides by confocal microscopy in DOPC/DOPG 90:10 M:M liposomes, and by CD and NMR spectroscopy in a DPC/SDS 90:10 M:M micelle solution.

References

[1] S. Giannecchini, *et al., Virology* **320**, 156-166 (2004)
[2] G. Pancino, L. Camoin, and P. Sonigo, *J Virol* **69**, 2110-2118 (1995)
[3] P. F. Serres, *C R Acad Sci III* **323**, 1019-1029, (2000)
[4] S. C. Frey, E. A. Hoover, and J. I. Mullins, *J Virol* **75**, 5433-5440 (2001)
[5] S. Giannecchini *et al., Antivir. Ther.* **10**, 671-680 (2005).

P39

PROGRESS IN MULTI-SPECTRA AUTOMATIC STRUCTURE VERIFICATION (MS-ASV)

S. Sykora,[1] C. Cobas,[2] F. Seoane,[2] V. Zorin,[2] I. Lopez,[2] E. M. Vasini[1]

[1] Extra Byte snc, Castano Primo, Italy
[2] Mestrelab Research, Santiago de Compostela, Spain
E-mail: sykora@extrabyte.eu

We have developed software for automatic structure verification (ASV) using any combination of various NMR spectra, presently covering 1H, 13C, HSQC, COSY, TOCSY, HMBC, and NOESY/ROESY, but with a possibility to include any other types. This task is well known for its extreme complexity. Mathematically, it involves combinatorial NP algorithms, a fact which implies the existence of many approaches, none of which can be proved to be best. Moreover, in order to be usable, the algorithms must be tolerant to the many imperfections and artifacts in real-world routine samples and spectra. This means that the problem, in addition to its NP character, is also quite "fuzzy" and requires sophisticated probabilistic scoring methods.

It is therefore not surprising that the degree of complexity of MS-ASV is comparable to that of an AI for autonomous-driving cars. We are definitely talking about an AI wizard which will keep developing for many decades to come. Nevertheless, we have reached a state where we can deploy in practice a truly operational MS-ASV which, on the average, performs better than a chemist after a standard training in NMR spectroscopy. Typical application areas for the product are pharmaceutical compounds and, more in general, structural analysis of both synthetic and natural compounds of moderate size (below about 1000 Daltons).

To achieve this result, we have overcome an overwhelming number of mathematical and computational challenges, many of which are still of proprietary nature - though that will not last too long, especially considering that new challenges are continuously arising from the field deployment and usage of the package.

In this presentation, we illustrate on many examples the current state-of-the-arts of Mestrelab's MS-ASV art and discuss, to the extent to which it is feasible, some of the involved problematic issues.

P40

METABOLOMIC EXPLORATION OF PSORIATIC SKIN TISSUE BY HR-MAS NMR SPECTROSCOPY

E. Tarentini,[‡] A. Mucci,[†] C. Magnoni,[‡] V. Righi*

[‡] Surgical, Medical and Dental Department of Morphological Sciences related to Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, via del Pozzo 71, 41124 Modena, Italy;
[†] Department of Geological and Chemical Sciences, via Campi 103, 41125 Modena, Italy;
* Department of Life Quality Studies, University of Bologna, Campus Rimini, Corso d'Augusto 237, 47921- Rimini, Italy
E-mail: elisabetta.tarentini@unimore.it

Psoriasis is a complex and multifactorial disease that affect skin after the interplay of predisposing genetic factors, dysregulated immune system response and environmental stimuli [1, 2]. The researchers work hard for understanding the pathophysiology of psoriasis to develop efficient therapies [3]. At that time, there is no definitive cure for psoriasis, and available treatment is only to decrease disease activity and improve symptoms. Metabolomics is an emerging field in the study of psoriasis, and only few works on this pathology have been published. Some of these papers are conducted on free-circulating amino acids in biological liquids [4], whereas only one paper reported a study performed directly on psoriatic tissues [5].
The purposes of the present study is the characterization of the metabolic patterns in psoriatic patients and to monitor the biochemical changes in psoriatic skin compare to the healthy tissues. We used HR-MAS (High Resolution – Magic Angle Spinning) NMR (Nuclear Magnetic Resonance) spectroscopy to characterized and to compare psoriatic with healthy cutaneous tissues. 10 healthy persons and 8 psoriatic patients were enrolled (by informed consent) and their skin tissues were collected after routine dermatological surgery. The resulting spectra were also analyzed by peak area calculations to obtain relative measures of tissue metabolite contents. Furthermore on the NMR data we carried out statistical analysis to define representative metabolites biomarkers of the disease.

Acknowledgments
Thanks to the "Fondazione Natalino Corazza Psoriasi & Co" for financial support.

References

[1] P. Di Meglio, F. Villanova, and F. O. Nestle, *Cold Spring Harb. Perspect. Med.* **4**, a015354 (2014)
[2] W.-H. Boehncke, *Rheum. Dis. Clin. N. Am.* **41**, 665-675 (2015)
[3] J. E. Hawkes, T. C. Chan, and J. G. Krueger, *J. Allergy Clin. Immunol.* **140**, 645-653 (2017)
[4] M. A. Kamleh, S. G. Snowden, D. Grapov, G. J. Blackburn, D. G. Watson, N. Xu, M. Stahle, and C. E. Wheelock, *J. Proteome Res.* **14**, 557-566 (2015)
[5] B. Sitter, M. K. Johnsson, J. Halgunset, and T. F. Bathen, *BMC Dermatology* **13**, 1-7 (2013)

P41

AMINO ACID-LEVEL INVESTIGATION OF ALPHA-SYNUCLEIN ADSORPTION ONTO NANOPARTICLE SURFACES

R. Tira,[‡] F. Munari,[‡] M. D'Onofrio,[‡] M. Assfalg[‡]

[‡]Department of Biotechnology, University of Verona, Italy
E-mail: roberto.tira@studenti.univr.it

Continuous progress in nanotechnological research has aroused tremendous interest in the interactions between biomacromolecules and nanoscale objects [1-3]. Noncovalent protein–nanoparticle (P–NP) interactions play fundamental roles in numerous recent technological developments, particularly in the fields of biomedicine and sensing [1-3]. Previous work has shown that protein orientation on NP surfaces can modulate protein activity and supports the possibility to develop NP systems targeted to amyloidogenic proteins implicated in neurodegenerative disorders [4].
In recent studies, we demonstrated that P-NP interactions are accessible by solution NMR spectroscopy under certain conditions, providing invaluable details about the adsorption mechanisms of folded proteins [1-8]. Here, we address the investigation of alpha-synuclein, an intrinsically disordered protein, binding to nanoscale surfaces.
Alpha-synuclein exists in solution as a heterogeneous and dynamic conformational ensemble, which was shown to be perturbed in the presence of silica NPs. In our work, we aimed at an improved understanding of the molecular determinants of alpha-synuclein binding to silica NPs. HSQC-type experiments were acquired on protein samples in the presence of NPs and under a variety of conditions to determine the polypeptide regions contacting the NPs. We also performed complementary investigations with gel electrophoresis, fluorescence spectroscopy, and dynamic light scattering.
Our preliminary findings contribute to an improved description of bio-nano interfaces that is an integral part of predicting the impact of NPs on aggregation-prone biomolecular systems.

References

[1] M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. B. Bombelli, S. Laurent, *Chem. Rev*. **111**, 5610-5637 (2011).
[2] I. Lynch, K. A. Dawson, *Nano Today* **3**, 40-47 (2008)
[3] D. F. Moyano, V. M. Rotello, *Langmuir* **27**, 10376-10385 (2011)
[4] F. Liu, L. Wang, H. Wang, L. Yuan, J. Li, J. L. Brash, H. Chen, *ACS Appl. Mater. Interfaces* **7**, 3717-3724 (2015)
[5] M. Assfalg, L. Ragona, K. Pagano, M. D'Onofrio, S. Zanzoni, S. Tomaselli, H. Molinari, *Biochim. Biophys. Acta* **1864**, 102-114 (2016)
[6] S. Zanzoni, A. Ceccon, M. Assfalg, R. K. Singh, D. Fushman, M. D'Onofrio, *Nanoscale* **7**, 7197-7205 (2015)
[7] S. Zanzoni, M. Pedroni, M. D'Onofrio, A. Speghini, M. Assfalg, *J. Am. Chem. Soc*. **138**, 72-75 (2016)
[8] A. Ceccon, M. Lelli, M. D'Onofrio, H. Molinari, M. Assfalg, *J. Am. Chem. Soc*. **136**, 13158-13161 (2014)

P42

NMR SPECTROSCOPY FOR DETECTION OF SAFFRON ADULTERATION

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

[‡]Innovative Solution S.r.l., Spin Off del Politecnico di Bari, zona H, 150/B – 70015 Noci (BA)
[†]DICATECh, Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica, Politecnico di Bari, via Orabona, 4 – 70125 Bari
E-mail: stefano.todisco@poliba.it

Saffron is among the most expensive spices and its authenticity assessment is an important issue which draws attention not only to consumers but also control agencies.
Recently, a study has been reported in which NMR spectroscopy combined to multivariate data analysis has been used to reveal the presence of adulterants in saffron at a minimum level of 20% (w/w) [1-3].
Given the large price difference between saffron and adulterants even low concentrations of adulterants can make big profits. For this reason, it is important reveal the presence of adulterants also in low concentrations.
In this contribution, the efficacy of a NMR method in adulterants identification has been evaluated using a training set of 50 saffron samples and a test set of 18 spiked samples with safflower and turmeric powder at three different concentration levels.

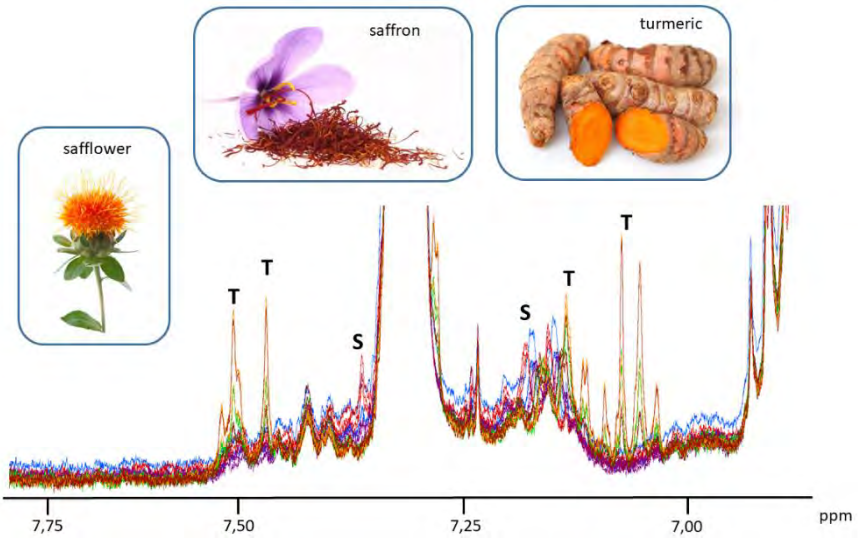


Fig. 1. Portion of aromatic region of ^{1}H-NOESY 1d spectra of saffron samples in DMSO-d_6 which reveals the presence of safflower powder (S) and turmeric powder (T).

References

[1] L. R. Cagliani, N. Culeddu, M. Chessa, R. Consonni, *Food Control* **51**, 342-348 (2015)
[2] E. A. Petrakis, L. R. Cagliani, M. G. Polissiou, R. Consonni, *Food Chem.* **173**, 890-896,(2015)
[3] S.A. Ordoudi, L.R. Cagliani, D. Melidou, M.Z. Tsimidou, R. Consonni, *Food Control* **81**, 147-155 (2017)

P43

GIANT LIPOSOMES AS HIGHLY SENSITIVE VERSATILE MRI CONTRAST AGENTS

M. Tripepi, G. Ferrauto, P. Bennardi, D. Delli Castelli, S. Aime

Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza 52, 10126 Torino, Italy
E-mail: mtripepi@unito.it

The last ten years have witnessed the development of a large variety of nanosystems for applications in MRI. This trend has been spread from the need to overcome the intrinsic low sensitivity of the MRI technique in Molecular Imaging protocols.
Pushing further the same reasoning, in this work we have explored the potential of microsystems such as giant liposomes (GUVs) as T_1, T_2, CEST and ^{19}F MRI agents. In particular, we optimized a procedure reported in literature [1] in order to design the most suitable probe for Molecular Imaging purposes. The newly developed vesicles, loaded with Gd complexes, have been characterized in terms of dimensions and millimolar relaxivity (both r1p and r2p) at different magnetic fields. Their NMRD profiles have been analyzed and the parameters have been compared with the analogues nano-sized liposome reported in literature [2] and with iron oxide nanoparticles [3].
As the mean diameter of GUVs is 2 μm, the amount of Gd-complexes that can be loaded in the inner cavity is three order of magnitude larger than nanosized liposomes whose typical diameter is about 200 nm. It follows that the sensitivity per particle has been increased accordingly.
Moreover, GUVs have been loaded with SRs to obtain LipoCEST agents with very large sensitivity for multiple detection imaging applications. [4] These Giant LipoCEST have been characterized by Z-spectra at 300 MHz and 600 MHz. Finally, GUVs loaded with $NaPF_6$ have been prepared with the aim of developing quantitative *in vitro* diagnostic protocols based on ^{19}F-MRI.
In cellulo and *in vivo* toxicity have been performed and the same very low toxicity as nanosized liposomes was found. Targeting experiment are currently being performed. For the best of our knowledge this is the first time that GUVs have been developed as MRI agents.

References

[1] J. P. Reeves, R. M. Dowben, *J. Cell. Physiol.* **73**, 49-60 (1969)
[2] F. Kielar, L. Tei, M. Botta, *J. Am. Chem. Soc.* **132**, 7836-7837 (2010)
[3] S. Zheyu, W. Aiguo, C. Xiaoyuan, *Mol. Pharmaceutics* **14**, 1352-1364 (2017)
[4] G. Ferrauto, D. Delli Castelli, E. Di Gregorio, E. Terreno, S. Aime, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **8**, 602-618 (2016)

P44

SOLID-STATE NMR SPECTRA PRECONDITIONING USING PcBc

E. M. Vasini,[1] S. Sykora,[1] C. Cobas,[2] M. Chierotti[3]

[1]Extra Byte snc di Sykora e Vasini, Castano Primo (MI), Italy
[2]Mestrelab research, Santiago de Compostela, Spain
[3]Department of Chemistry, University of Torino, Italy
E-mail: em.vasini@extrabyte.eu

PcBc is an algorithm designed to automatic correct the phase and baseline of a spectrum. The algorithm optimizes a quality function based on the histogram of the spectrum, and the corrections are applied on both the in-phase and the out-of-phase parts of a spectrum. The automatic feature of the algorithm ensures objectivity and reproducibility of the results, making it suitable for objective conditioning of large datasets of spectra avoiding subjective distortions arising from manual procedure [1,2].

We describe the algorithm we have developed, with the latest updates. We also illustrate the results achieved on a number of solid-state NMR spectra.

[1] E. M.Vasini, C. Cobas, and S. Sykora, PcBc - A Novel Automatic Phase and Baseline Correction Algorithm, Presentation at XLVI GIDRM, Fisciano (Italy), September 27-29, 2017. DOI: 10.3247/SL6Nmr17.005.
[2] E.M. Vasini, C. Cobas, and S. Sykora, Automatic Concurrent Phase & Baseline Correction in 1D NMR Spectra, Poster at SMASH 2017, Baveno (Italy), September 17-20, 2017. DOI: 10.3247/SL6Nmr17.004.

Author Index

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Z