GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XL National Congress on Magnetic Resonance
Parma - Italy, September 26-28, 2011

Under the auspices of:

Gruppo Interdivisionale Risonanze Magnetiche
(Società Chimica Italiana)

University of Parma

Università degli Studi di Parma,
Plesso Biotecnologico Integrato, Via Volturno 39

http://www.gidrm.org/NC2011_Home.html

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Scientific Program
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SCIENTIFIC PROGRAM

Monday, September 26

10:00-14:00 REGISTRATION

14:00-14:30 OPENING REMARKS, Aula B. Chair: M. Cremonini

14:30-15:30 ***GIDRM-GIRM 2011 Gold Medal Award*** - **Stanislav Sykora**
In Spin we Trust

15:30-16:10 ***2010 Annalaura Segre Fellowship Recipients:***

15:30-15:50 **Caterina Cafiero**, CRA-RPS Tor Mancina
Characterization of Different Variety of Honey Bees with HRMAS-NMR for the Traceability and the Valorization of this Product

15:50-16:10 **Francesca Martini**, University of Pisa
Solid State NMR Investigation of the Structural, Phase and Dynamic Properties of an Anion-Exchange Membrane Used in Polymeric Fuel Cells

16:10-17:30 COFFEE BREAK & POSTER SESSION (presentation odd numbers)

17:30-18:20 PARALLEL SESSIONS

Aula B. Chair: F. Arnesano

17:30-18:00 **Giovanna Musco**,
HSR, Milano
TR-NOE on Human Cancer Cells and Metadynamics in the Design of isoDGR-Based αVβ3 Antagonists

18:00-18:20 **Valeria Righi**,
University of Modena e Reggio Emilia
HR-MAS NMR Spectroscopy for Characterisation of Steatosic Liver: Fat Quantification for a Spectroscopic Differentiation between Steatosis and Steatohepatitis

Aula E. Chair: M. Piccioli

17:30-18:00 **Noemi Proietti**,
IMC-CNR, Roma
Recent NMR Applications in Cultural Heritage

18:00-18:20 **Valeria Di Tullio**,
IMC-CNR, Roma
Unilateral NMR Depth Profiles to Probe the Penetration Depth of Hydrophobic Treatments in Porous Stones

18:25-19:10 PLENARY SESSION, Aula B. Chair: C. A. Veracini

Steven Brown, University of Warwick
Characterising Solid-State Structures Formed by Organic Molecules: What Can NMR Contribute?

Tuesday, September 27 - morning

9:00-10:10 PARALLEL SESSIONS

Aula B. Chair: E. Terreno

9.:00-9:30 **Alessandro Maiocchi**, Bracco Imaging S.p.A.
Inflammation Imaging in Atherosclerosis using Paramagnetic Nanoparticles and MRI

9:30-9:50 **Gilberto Mulas**, University of Torino
Optimized Positive Contrast for T_2 Agents: beyond Iron Oxide Nanoparticles*

9:50-10:10 **Carlo Alberto Veracini**, University of Pisa
New Functionalized Iron-Oxide Nanoparticles as MRI Contrast Agents and Molecular Imaging Precursors

Aula E. Chair: D. Cicero

9:00-9:30 **Raffaele Lamanna**, C.R. ENEA Trisaia
Identification of Complex Mixtures by NMR Profiling: Practical Aspects

9:30-9:50 **Giuseppe Pileio**, University of Southampton
Longtime Storage of Hyperpolarization via Singlet States in High Field

9:50-10:10 **Marco Tessari**, University of Nijmegen
Suppression of Multiplet Structures in 1D Proton NMR Spectra

10:10-10:40 COFFEE BREAK

10:40-11:45 PLENARY SESSION, Aula B. Chair: A. Spisni

10:40-11:00 **Manuel P. Pacheco,** MestreLab S.L.
Exploring the Chemical Space for "Special" Molecules –NMR Predictions

11:00-11:45 **Marina Bennati**, Max-Planck Institute, Göttingen
Dynamic Nuclear Polarization in Liquids

11:45-13:30 GIDRM AND GIRM MEETINGS, Aula B

13:30-14:30 LUNCH

Tuesday, September 27 - afternoon

14:00-16:00 PLENARY SESSION, Aula B. Chair: S. Mammi

ROUND TABLE DISCUSSION**:** *"Reach out for NMR"*
Chiara Perazzolo, Science & Environnement - Genève
Andrea Tapparo, University of Padova
Luisa Schenetti, University of Modena e Reggio Emilia

16:00-17:10 PARALLEL SESSIONS

Aula B. Chair: L. Franzoni

16:00-16:30 **Marilisa Leone**,
University of Napoli Federico II
NMR Studies of Heterotypic Sam-Sam Domain Associations Involving EphA2 Receptor

16:30-16:50 **Daniel Cicero**,
Fundación Instituto Leloir, Buenos Aires
Looking for Enzymes under the Ice of the Antarctic

16:50-17:10 **Daniela Valensin**,
University of Siena
Structural Characterisation of Cu(II)/Cu(I) Binding to α-Synuclein

Aula E. Chair: M. Botta

16:00-16:30 **Claudia Forte**,
ICCOM-CNR Pisa
Hydrogels, where Solution and Solid State NMR Techniques Meet

16:30-16:50 **Franca Castiglione**,
Politecnico di Milano
^{1}H HRMAS NMR: a Tool for the Study of Transport Phenomena of Drug-Mimic Molecules in Supramolecular Hydrogel Systems

16:50-17:10 **Mario Cifelli**,
University of Pisa
Translational Self-Diffusion Studies in Chiral Smectic Phases by means of Pulsed Field Gradient NMR

17:10-18:20 COFFEE BREAK & POSTER SESSION (presentation even numbers)

18:20-19:25 PLENARY SESSION, Aula B. Chair: N. Niccolai

18:20-18:40 **Rainer Kummerle**, Bruker BioSpin AG
New Cryogenically Cooled Probe Technologies for NMR

18:40-19:25 **Ivano Bertini**, University of Firenze
NMR is a Flagship in the Investigation of Biological Events

19:25-19:30 ANNOUNCEMENT OF THE POSTER COMPETITION WINNERS

20:00 SOCIAL DINNER

Wednesday, September 28 - morning

9:00-10:.55 PLENARY SESSION, Aula B. Chair: R. Gobetto

9:00-9:45 **Florence Babonneau**, Université Pierre et Marie Curie & CNRS, Paris
Structural Investigation of Nanosized Substituted Hydroxyapatites: Strengths of Solid State NMR

9:45-10:05 **Mauro A. Cremonini**, Agilent Technologies Italia
Having Fun with Push-Button Experiments

10:05-10:55 BEST POSTERS

10:55-11:15 COFFEE BREAK

11:15-12:45 PARALLEL SESSIONS

Aula B. Chair: M. Paci

11:15-11:45 **Alessandra Corazza**, University of Udine
Single-Shot NMR Measurement of Protein Unfolding Landscapes

11:45-12:05 **Simona Tomaselli**, ISMAC-CNR Milano
Structural Determinants of Site-Selective Lipid Binding Observed for Liver Bile Acid Binding Protein in the presence of a Disulphide Bridge

12:05-12:25 **Luca Simonelli**, Institute for Research in Biomedicine
Using NMR Chemical Shift Mapping to Increase the Accuracy of Computational Docking

12:25-12:45 **Daniela Donghi**, University of Zurigo
The Catalytic Core of a Group II Intron as Revealed by NMR

Aula E. Chair: M. Geppi

11:15-11:45 KLAUS MÜLLER LECTURE:
Matthias Abele, University of Trento
Application of ^{19}F Techniques in Solid-State NMR

11:45-12:05 **Maria Concistrè**, University of Southampton
Solid State NMR at Cryogenic Temperatures

12:.05-12:25 **Enrico Ravera**, University of Firenze
Solid State NMR of Proteins Sedimented by Ultracentrifugation

12:25-12:45 **Lorella Franzoni,** University of Parma
The Interactions of Retinol Carriers with Model Membranes Provide Insights into the Complex Mechanisms of Ligand Binding and Delivery

12:45-14:00 LUNCH

Wednesday, September 28 - afternoon

14:00-15:10 PARALLEL SESSIONS

Aula B. Chair: S. Chimichi

14:00-14:30 **Michele Chierotti**, University of Torino
The Polymorphism of Barbiturates: a Solid-State NMR and XRD Combined Approach

14:30-14:50 **Giulia Mollica**, CNRS, Marseille
Weak Homonuclear Dipolar Recoupling for the Detection of ^{1}H-^{1}H Proximities in Solids

14:50-15:10 **Elisa Carignani**, University of Pisa
Intermolecular Ring Current Effects in Solid Naproxen: a Combined 2D MAS NMR and ab Initio Study

Aula E. Chair: L. Mannina

14:00-14:30 **Roberto Consonni**, ISMAC-CNR, Milano
Food and Plant Metabolomic Studies

14:30-14:50 **Cristina Piras**, University of Cagliari
NMR Investigation of the Metabolic Profile of Cheese from Raw Ewes' Milk Supplemented with Indigenous and Commercial Cultures

14:50-15:10 **Leonardo Tenori**, University of Firenze
Metabolic Phenotypes and Celiac Disease Fingerprinting

15:10-16:30 PLENARY SESSION, Aula B. Chair: H. Molinari

15:10-15:55 **Vincenzo Barone**, Scuola Normale Superiore, Pisa
Computational Approaches to Resonance Spectroscopies: from Small Molecules to Nanosystems

15:55-16:30 CONCLUDING REMARKS

GIDRM-GIRM 2011 Gold Medal Award

IN SPIN WE TRUST

Stan Sykora

Extra Byte, Castano Primo, www.ebyte.it

To receive a Gold Medal from a group of good friends is a great compliment and an enormous pleasure. Allow me, therefore, to start with a deep felt *Thanks!*

I feel embarrassed regarding my presentation since, following a rigorous logic, I should speak about my personal experiences, even though limited to the Magnetic Resonance context. Having recorded my first NMR spectrum in 1964, I claim 47 years of uninterrupted faithfulness to the Spin, so to say, 38 of which in Italy and 36 as a member of GIDRM! Moreover, I worked in all branches of MR (spectroscopy, relaxometry, imaging, NMR, ESR, …) and in all imaginable guises - from a researcher to designer, programmer, producer, salesman, serviceman, promoter, and so on. Which makes me a kind of historic monument of MR in Italy (and beyond) and brings with it a vast range of memories (most of them pleasant).

I will therefore briefly review the things I have done in MR - both scientific and non - trying to convey to those much younger than me that nothing in life happens in a linear way, and usually not even in a predictable one. My life was particularly marked by highs and lows of social and economic nature and to maintain a kind of *scientific homeostasis* in the middle of the turbulent surroundings required a considerable amount of stubbornness. Though it is true, as the Poet says, that *"everybody stands alone on the heart of the earth"*, I believe that my personal experience could be nevertheless relevant to those who wish to start doing Science with a capital "S".

Another aspect I want to tackle is the frenetic evolution of the instrumentation for various types of MR and of the respective methodologies (pulse sequences and the like). As a direct witness of almost half a century of such evolution I feel that I should be able not only to sum it up briefly, but also to extrapolate it into near future, not concealing my desire to still try and influence the process a bit. In this sense there are numerous options in front of us. I believe that NMR technology is again about to change in a radical way and that - a statement which will possibly surprise many of you - Italy harbours concealed excellences that put her in the pole position in the coming revolution.

To conclude, I would like to dwell a bit on my dreams and nightmares regarding the Spin.

Among the dreams are research and technological development threads which could be pursued but require a bit of courage- Such as, for example, Magnetic Resonance on planetary and astronomical scale. Or a Large Magnetic Room where one could carry out, for example, MRI of the cetaceans. Or a truly "personal" NMR, one to carry around in the same way we carry our iPads. Or the search for new "particles" endowed with Spin of which we are presently oblivious but which are perhaps quite abundant (this one is new and I dedicate it to GIDRM!).

Among the nightmares I will present a list of the fundamental aspects of Magnetic Resonance which we still do not comprehend (or, at least, I don't). I have presented it this year already at two Meetings and I see no reason why I should not present it again. After all, they are no real nightmares, but simple "notices of ignorance". Noble ignorance, one that goes well beyond the MR, involving also quantum physics and the philosophy of nature. I hope that some of the young people present here might find fancy in some of these queries and perhaps, in one dark and tempestuous night, come up with a solution the validity of which will be immediately obvious to all!

Plenary Lectures

CHARACTERISING SOLID-STATE STRUCTURES FORMED BY ORGANIC MOLECULES: WHAT CAN NMR CONTRIBUTE?

S.P. Brown

Department of Physics, University of Warwick
www.go.warwick.ac.uk/nmr

It is shown how solid-state NMR experiments that determine ^{1}H and ^{13}C chemical shifts as well as probing proton-proton dipolar couplings and through-bond *J* couplings provide detailed atomic-level insight into the intermolecular packing of organic molecules in the solid state. Applications to pharmaceutical solids and supramolecular self assembly demonstrate the complementarity of solid-state NMR to diffraction methods (single-crystal and powder diffraction) and the advantage of combination with simulation approaches, namely both first-principles calculations of chemical shift and density-matrix simulations of the underlying quantum mechanics.

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DYNAMIC NUCLEAR POLARIZATION IN LIQUIDS

M. Bennati

Max-Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077, Göttingen, Germany
Marina.Bennati@mpibpc.mpg.de

Dynamic nuclear polarization (DNP) of nuclei coupled to paramagnetic centers has been used since the early years of magnetic resonance to obtain information about molecular motion and electron-nuclear spin relaxation. In the past few years, this technique has experienced a renaissance as it was recognized that it provides a means to overcome the sensitivity limits in solution and solid state NMR, which is particularly important for the studies of macromolecular complexes. In this contribution, I will summarize the physical and instrumental aspects of DNP in solution and discuss our recent experimental designs.

NMR IS A FLAGSHIP IN THE INVESTIGATION OF BIOLOGICAL EVENTS

I. Bertini

CERM, Universita' di Firenze, Italy

NMR, coupled with cellular biology, can provide information on the mechanisms of biological pathways, including the structure at the molecular level of the various actors even in the cell, through in-cell NMR. Examples will be presented regarding the import and maturation of proteins in the mitochondrion. NMR can provide structural information on fibrils through solid state NMR. The latter technique will tackle a number of new systems through "sedimented" NMR, which will be explained. NMR will be shown to gain structural information on proteins constituted by two flexible domains. Finally the perspective *sin metabolomis* for the future of medicine will be touched.

STRUCTURAL INVESTIGATION OF NANOSIZED SUBSTITUTED HYDROXYAPATITES: STRENGTHS OF SOLID STATE NMR

F. Babonneau

Laboratoire de Chimie de la Matière Condensée de Paris, Université Pierre et Marie Curie (UPMC-Paris 6) & CNRS, Collège de France, Paris, France.
E-mail: florence.babonneau@upmc.fr

The richness of the apatite structure is an extraordinary ability to accept substitutions and vacancies. The apatite structure can incorporate a wide variety of ions, of the same or of different charge, which affect both its cationic and anionic sub-lattices. This property is intimately related to its adaptability to the biological functions of different tissues.
Biological apatites, one of the main constituents of bones and hard tissues in mammals refer to poorly crystallized non-stoichiometric carbonate-containing hydroxyapatites (CAp). CAp is also found in pathological calcifications, such as kidney stones, and growth of CAp can be promoted by bioactive synthetic materials used as implants. In all these examples that relate to the growth of nanocrystalline substituted apatites in contact with biological tissues and/or fluids, a precise description of the mineral phase is complex due to the extreme tunability of the apatite structure. For example, carbonate ions can either substitute hydroxyl and/or phosphate groups with several possible mechanisms for charge compensation, that may induce changes in surface properties.
This presentation will illustrate how multinuclear solid-state NMR methods can be efficiently used to describe the structure of substituted hydroxyapatites. The particular cases of carbonate and silicate substitution will be presented with examples related to the development of specific absorbents for blood purification, the design of new collagen-apatite nanocomposites for tissue engineering, and the development of bioceramics with improved bioactivity.

COMPUTATIONAL APPROACHES TO RESONANCE SPECTROSCOPIES: FROM SMALL MOLECULES TO NANOSYSTEMS

V. Barone

Scuola Normale Superiore
Piazza dei Cavalieri 7, 56126 Pisa, ITALY

The interaction between computational chemistry and experimental magnetic resonance spectroscopy is increasing at a fast pace in recent years. On these grounds, the aim of this presentation is to provide an overview of current computational approaches in some specific areas of magnetic resonance spectroscopy. Apart from theoretical considerations, the focus on singlet and doublet electronic states reflects the fact that in these specific fields computational spectroscopy has nowadays reached the stage of a mature technique. Thus, for example, comparisons between measured and computed values of chemical shifts are becoming an important tool for experimental studies in structural organic chemistry. Even more demanding computational applications, for example, those involving the estimation of spin–spin coupling constants, are on their way to enter into routine use. Thus, the choice among alternative structural hypotheses can often be guided by the correspondence between measured and computed spectroscopic parameters. However, the computation of NMR parameters for organic/biochemical systems is usually feasible but rarely trivial especially when structural flexibility and environmental effects come into play. A more subtle correction related to a dynamical effect is that due to vibrational averaging of spectroscopic parameters. In this case, given the non-classical nature of nuclear vibrational motions, simple classical simulations would miss important features of the equilibrium distribution of geometric parameters, and therefore a proper quantum mechanical averaging procedure needs to be employed. Recent algorithmic improvements allow for an efficient computation of such vibrational averages, which have been shown to significantly influence spectroscopic parameters in a number of important cases. However, at room temperature and in the presence of a bath (e.g., the solvent) quantum effects are often smeared out and can be approximately described in terms of classical dynamics. From a complementary point of view, dynamical processes that occur on a time scale comparable to that of the spectroscopic transition have a direct influence on signal line-shape, and their description must rely on specific theoretical formulations (e.g., the stochastic Liouville equation).

The development of widespread user friendly codes able to take most of these effects into the proper account is allowing integrated experimental-computational studies of complex systems even by non specialists. In this spirit, the theoretical presentation has been kept at a reasonably accessible level, and a number of “case studies” have been provided in order to illustrate the potentiality of the techniques introduced. This plan should contribute to further promote the introduction of computational approaches within standard experimental studies, which, in this as in many other fields of research, allows for enhanced understanding of phenomena, faster and more efficient characterization protocols, and innovative chemical results.

Oral Communications

TR-NOE ON HUMAN CANCER CELLS AND METADYNAMICS IN THE DESIGN OF isoDGR-BASED αVβ3 ANTAGONISTS

S. Mari[1], A. Spitaleri[1], M. Ghitti[1], L. Alberici[2], C. Traversari[2], G.P. Rizzardi[2], G. Musco[1]

1. Biomolecular NMR Laboratory, Dulbecco Telethon Institute, c/o S. Raffaele Scientific Institute
2. MolMed SpA, Milan Italy

Integrin αvβ3 and the membrane-spanning surface protein aminopeptidase N (CD13) play a pivotal role in tumour growth and metastatic spread, as they are two of the major membrane bound receptors highly expressed on the surface of tumour cells during angiogenesis. RGD- and NGR-containing peptides, peptidomimetics, and conjugated compounds specifically target the tumor vasculature via αVb3 and CD13 recognition, respectively, thus originating novel direct acting vascular targeting agents. A crucial contribution to the efficacy of these approaches relies on the characterization of receptor-ligand molecular interactions in their natural membrane environment. However, this is an inherently difficult goal to achieve.

Here we show that it is possible to apply 2D-TR-NOE techniques directly on human cancer cells to prove selective binding of anti-angiogenic ligands to structurally characterized and uncharacterized receptors, such as αvβ3 and CD13, respectively [1].

In this context we will present a new computational approach based on the combination of *metadynamics* and docking simulations to evaluate in silico the αvβ3 binding properties of isoDGR, DGR and RGD-containing cyclopeptides. The computational predictions have been validated through binding experiments in vitro using conventional flow cytometry analysis and TR-NOE spectra on human cancer cells. We demonstrate for the first time that *metadynamics* performed on Gly φ and ψ angles reliably describes the Free Energy Surface of a relevant set of RGD, DGR and isoDGR-containing cyclopeptides, thus allowing the scrutiny of their intrinsic conformational equilibrium and the quantitative estimation of the population of the conformers. In addition, these metadynamics-generated conformations well agree with NMR-derived experimental data [2].

References

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HR-MAS NMR SPECTROSCOPY FOR CHARACTERIZATION OF STEATOSIC LIVER: FAT QUANTIFICATION FOR A SPECTROSCOPIC DIFFERENTIATION BETWEEN STEATOSIS AND STEATOHEPATITIS

V. Righi[a,b], C. Stentarelli[c], G. Guaraldi[c], S. Zona[c], G. Ligabue[d], G. Besutti[d], P. Loria[e], L. Losi[f], L. Nocetti[g], V. Tugnoli [b], L. Schenetti[a], A. Mucci[a]

[a] Dipartimento di Chimica, Università di Modena e Reggio Emilia, via G. Campi 183,41100, Modena, Italia.
[b] Dipartimento di Biochimica, Università di Bologna, via Belmeloro 8/2, 40126, Bologna, Italia.
[c] Clinica delle Malattie Infettive e Tropicali, Dipartimento di Medicina e Chirurgia, Università di Modena e Reggio Emilia, Modena, Italia.
[d] Unità di Radiologia, Università di Modena e Reggio Emilia, Modena, Italia.
[e] Dipartimento di Medicina Interna Endocrinologia, Metabolismo e Geriatria, Università di Modena e Reggio Emilia, Modena, Italy.
[f] Dipartimento di Anatomia Patologica, Università di Modena e Reggio Emilia, Modena, Italia.
[g] Policlinico di Modena Servizio di fisica sanitaria, Modena, Italia. E-mail:valeria.righi@unimore.it

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease. The NAFLD includes non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) [1]. The mechanisms of NAFL to NASH transition remain to be clarified [2]. NAFLD appears to originate from the dysregulation of hepatic lipid metabolism as a part of the metabolic syndrome accompanied by visceral obesity, dyslipidemia, atherosclerosis and insulin resistance. A recent study has also demonstrate that high prevalence of steatosis is correlated to HCV-related chronic hepatitis [3]. High Resolution Magic Angle Spinning (HR-MAS) NMR is a useful tool for the metabolic characterization of intact tissues [4] and can be used to support the clinical diagnosis. The first aim of this study is to characterize the NAFL and NASH metabolism using HR-MAS NMR Spectroscopy. The second one is to evaluate the possible transition from NAFLD to NASH quantifying the liver fat content (LFC), both in *ex-vivo* and *in-vivo* NMR Spectroscopy. Patients with indication for steatosis underwent *in-vivo* ^{1}H MR Spectroscopy analysis and liver biopsy. Histopathological analysis provided steatosis percentage, steatosis grade and fibrosis. A fragment of biopsy specimen was used for HR-MAS analysis, to obtain metabolic tissue characterization. Univariate linear regression analysis and Pearson r coefficient were used to study the relationship between histological steatosis percentage and LFC, estimated through HR-MAS analysis and *in-vivo* Spectroscopy. Similar high associations were found between LFC estimated by HR-MAS and histological steatosis percentage (r=0,71; p=0,006) and between LFC estimated by *in-vivo* Spectroscopy and histological steatosis percentage (r=0,79; p=0,002). HR-MAS spectra showed a high tissue metabolic heterogeneity, with particular regard to the content of free glucose, alanine, glutamine/glutamate and phospholipids. Fibrotic liver showed a higher presence of small metabolites such as choline. HR-MAS NMR Spectroscopy, well estimates the fatty infiltration of the liver. Future research on HR-MAS spectral heterogeneity may allow to find biochemical metabolic indicators of steatosis progression to be used in the differentiation between steatohepatitis and steatosis through *in-vivo* MR Spectroscopy.

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RECENT NMR APPLICATIONS IN CULTURAL HERITAGE

N. Proietti[†], V. Di Tullio[†,‡], D. Capitani[†]

[†] Laboratorio di Risonanza Magnetica " Annalaura Segre", Istituto di Metodologie Chimiche, CNR, Area della Ricerca di Roma 1, 00015 Monterotondo (Roma).
[‡] Centro di Ricerca per le Scienze Applicate alla Protezione dell'Ambiente e dei Beni Culturali c/o Dipartimento di Chimica, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma
Email: noemi.proietti@imc.cnr.it

In recent years, NMR techniques have been increasingly used to investigate Cultural Heritage materials. In fact, NMR methods are particularly suitable for studying materials of interest for cultural heritage and these methods are generally considered as non-destructive because the sample can be recovered after performing the analysis. Solid state NMR allows to characterize cellulose-based materials, clays, tuffs and hard stones. Driven by the need to study precious and unmovable artworks without compromising their integrity, non-invasive unilateral NMR technique has been developed. With this mobile NMR instrumentation the sampling can be avoid. Unilateral NMR is portable and its use is fully non-invasive, allowing the measurement of some NMR parameters which are important to establish the state of degradation of the investigated object. The sensor can be positioned near intact objects in different positions. The main advantage of the unilateral NMR technique is that it is not only portable but can also be applied directly on large objects such as *frescoes*, monuments and in general any building fully preserving the integrity and the dimension of the object under investigation. With unilateral NMR it is possible to evaluate the state of degradation of cellulose-based materials, to evaluate the performances of consolidation and cleaning treatments on wall paintings, to monitor the detachment of the painted layer from thesupport, the plaster, to quantitatively map the dampness in a wall painting and to evaluate the performances of protective and/or consolidating treatments on porous stones. The most recent development of mobile NMR allows the obtainment of depth profiles with microscopic resolution. For instance, it is possible to carefully evaluate the penetration depth of a protective treatment as a function of the time of application.
In the present work , we report on recent researches carried out in our NMR laboratory.

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UNILATERAL NMR DEPTH PROFILES TO PROBE THE PENETRATION DEPTH OF HYDROPHOBIC TREATMENTS IN POROUS STONES

V. Di Tullio[†,‡], N. Proietti[†], D. Capitani[†]

[†] Laboratorio di Risonanza Magnetica " Annalaura Segre", Istituto di Metodologie Chimiche, CNR, Area della Ricerca di Roma 1, 00015 Monterotondo (Roma).
[‡] Centro di Ricerca per le Scienze Applicate alla Protezione dell'Ambiente e dei Beni Culturali c/o Dipartimento di Chimica, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma
Email: valeria.ditullio@imc.cnr.it

Hydrophobic treatment is one of the most important interventions usually carried out in the conservation of stone artefacts and monuments. This analytical study was aimed at answering general questions such as the penetration depth of a hydrophobic treatment into a porous material, its capability to impair the water absorption, how the presence of a treatment may change the open porosity available to the water, and how a treatment may affect the diffusion of water inside a porous structure. Also, inhomogeneities in treated stones due to sharp variations of the amount of the absorbed product in the porous material were evidenced and scaled. The results of this fully non-invasive analytical study were rationalized in terms of new parameters obtained by a suitable process of nuclear magnetic resonance data. These parameters, namely the hydrophobic efficiency, the penetration depth, and angles describing changes in slope in depth profiles, gave important information in assessing the performance of a treatment.

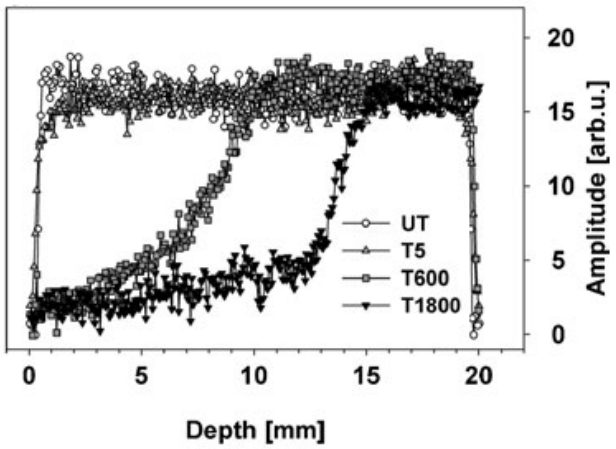

Fig. 1. NMR depth profiles of calcarenite obtained after making specimens absorb water from the untreated side. UT indicates untreated specimens; T5, T600, and T1800 indicate specimens treated for 5, 600, and 1,800 s, respectively.

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EXPLORING THE CHEMICAL SPACE FOR "SPECIAL" MOLECULES – NMR PREDICTIONS

M.P. Pacheco

MestreLab S.L.

To the present moment the accurate prediction of proton chemical shifts of small molecules remains a challenge. The task is full of obstacles, not only the quality of the predictions is affected by small changes in the experimental conditions, but also the variety of compounds to be considered is of vast proportions. Many different approaches over the last six decades have been developed to increase accuracy, from increment approaches to neural networks. But still today there is a need for improvement.

Classification of molecules defining to how much chemical space they cover on a database is of extreme importance, since the economic and time repercussion into synthesising and analysing these is considerable. The uses of proton chemical shift prediction shave a much wider applications than just elucidation or verification of chemical structures.

A directed approach describing how to identify families of molecules to enrich databases, how to test the changes introduced and new practical applications of the predictions are presented.

INFLAMMATION IMAGING IN ATHEROSCLEROSIS USING PARAMAGNETIC NANOPARTICLES AND MRI

A. Maiocchi, C. Cabella , L. Miragoli, G. Valbusa, S. Ghiani, C. Brioschi, L. Beltrami, L. Sini

Bracco Imaging spa, Via Ribes 5, Colleretto Giacosa, Turin, Italy
E-mail: alessandro.maiocchi@bracco.com

Despite the therapeutic advances over the last 20 years, cardiovascular disease is still the leading cause of death worldwide. This is mainly related to the increasing prevalence of atherosclerosis, owing to the ageing population, but above all, to the widespread under-recognition and undertreatment of individuals with risk factors for atherosclerosis.[1] The progression of atherosclerotic plaques involves the accumulation of cholesterol in the arterial wall, inflammation, leukocyte recruitment, and development of fibrotic lesions.[2] Monocyte accumulation in the intima characterizes fatty streaks, the earliest visible lesion of human and experimental atherosclerosis. In the intima, monocytes differentiate into macrophages, ingest modified lipoproteins via scavenger receptors, and secrete inflammatory mediators that can stimulate smooth muscle cell migration and proliferation. Lipid-rich macrophages, i.e., foam cells, become key constituents of the plaque's lipid core. The dynamics of macrophage accumulation in atherosclerotic lesions is an important factor in the plaque progression and its evaluation in vivo can be of great value in the assessment of therapeutic efficacy for patients under pharmacological regimen. Among the several imaging techniques which can provide information with diagnostic and prognostic value in atherosclerotic patients, Magnetic Resonance Imaging (MRI), has emerged as a versatile and non-invasive technique that can be used to assess the occurrence of track changes in arterial disease and to test the effects of therapies for atherosclerosis.[3] In addition, MRI can be used to dynamically capture the accumulation of gadolinium ion (Gd^{3+})-based contrast agents that occurs in pathological tissues characterized by altered endothelial permeability providing an additional tool for smaller plaques localization and characterization.
Indeed, in the present work, we demonstrate that using mice with targeted deletion of the gene for apolipoprotein E (ApoE -/-) , after intravenous administration of paramagnetic lipidic nanoparticles, it become possible to discriminate among several states of the atherosclerotic plaque progression following the MRI signal of the arterial wall. These findings have the potential to be translated into a diagnostic procedure to assess the effects of a pharmacological treatment on patients under statins regimen.

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OPTIMIZED POSITIVE CONTRAST FOR T_2* AGENTS: BEYOND IRON OXIDE NANOPARTICLES

G. Mulas,[‡] W. Dastrù,[‡] R. Anedda,[†] S. Figueiredo, [‡] S. Aime, [‡] E. Terreno[‡].

[‡]Department of Chemistry IFM and Molecular Imaging Center, University of Torino, Turin, Italy
[†]Porto Conte Ricerche Srl, Alghero, Italy
E-mail: gilbertomulas@tiscali.it

Superparamagnetic iron oxide (SPIO) nanoparticles have been widely used as MRI negative contrast agents for their effect on T_2 or T_2*[1]. However, it is often difficult to discriminate between SPIO induced signal loss and other sources of hypointense signal such as tissue interfaces, air or motion artifacts. Therefore new MRI techniques and sequences have been recently developed in order to obtain positive contrast from off-resonant water protons surrounding SPIO. Perhaps one of the most relevant of these is the Inversion Recovery with ON-resonant water suppression sequence (IRON) [2]. The objectives of present work were: 1) to optimize, both *in vitro* and *in cellulo*, the IRON sequence in terms of shape, RF power, length, and bandwidth of the preparatory pulse, and 2) to explore the possibility of extending this approach to other nanosized T_2 agents like paramagnetic liposomes [3]. MRI experiments were performed on a Bruker Avance 300 MHz equipped with a Micro2.5 probe. SPIO particles coated with citric acid were synthesized by the co-precipitation method. Paramagnetic lanthanide-based complex encapsulating liposomes were prepared as described elsewhere [3]. Different shaped pulses (block, sinc3 and gauss) were examined while RF power (0-12 μT), length (1-2 s), and bandwidth (5-100 Hz) were varied. Block pulses showed a better performance than the shaped pulses both in terms of positive contrast and background suppression (Fig.1). Interestingly, positive contrast was also detected for the clinically safe paramagnetic Ln-HPDO3A complex encapsulating liposomes and for SPIO labeled cells (macrophage J774). Positive contrast from SPIO was optimized using the IRON sequence, moreover it has been found that the potential of this approach may extend to paramagnetic agents accumulated in vesicles.

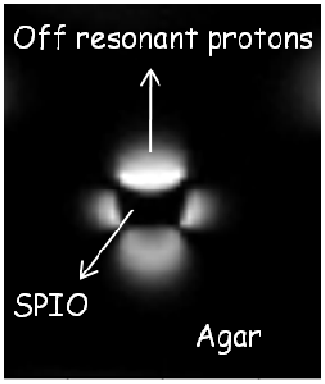

Fig. 1. In vitro IRON image of agar phantom

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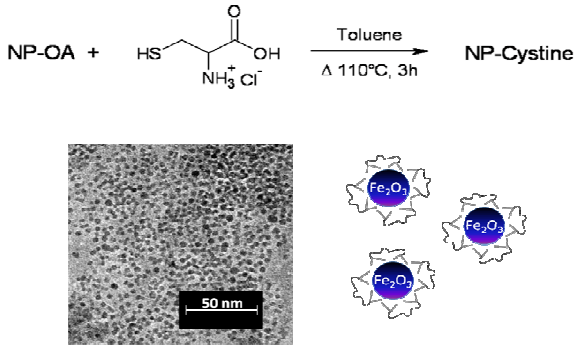
NEW FUNCTIONALIZED IRON-OXIDE NANOPARTICLES AS MRI CONTRAST AGENTS AND MOLECULAR IMAGING PRECURSORS

S. Dolci[‡], V. Domenici[‡], A. Gradisek[†], V. Ierardi[#], M. Cifelli[‡], Z. Jaglicic[§], F. Pineider[¥], T. Apih[†], G. Pampaloni[‡] and C.A. Veracini[‡]

[‡]Department of Chemistry and Industrial Chemistry, Via Risorgimento 35, 56126 Pisa, Italy;
[†]Jozef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia;
[#]Physics Department (DIFI), University of Genova and Nanobiotechnologies & (IST), Largo R. Benzi, 10 Genova, 16132 Italy;
[§]Institute of Mathematics, Physics and Mechanics, University of Ljubljana, Jadranska 19, 1000 Ljubljana, Slovenia;
[¥]LA.M.M. c/o Department of Chemistry, via della Lastruccia 3, 50019 Sesto F.no (FI), Italy.
E-mail: verax@dcci.unipi.it

Magnetic nanoparticles have high potentialities for several biomedical applications, both diagnostic and therapeutic ones[1]. Superparamagnetic iron oxide nanoparticles are used as contrast agents for magnetic resonance imaging (MRI) and their use as probes for molecular imaging is very promising [2]. The functionalization of the surface of nanoparticles is crucial to obtain water-soluble nanoparticles and offers the possibility to have contrast agents selective for different molecular or cellular targets. To this aim, we have prepared cystine-coated iron oxide nanoparticles of different sizes by reacting oleic acid coated nanoparticles with cysteine in toluene at reflux temperature, [3] which resulted in a replacement of the oleic acid on the surface of the nanoparticle with the *in situ* generated cystine. Functionalized iron-oxide nanoparticles of different sizes were fully characterized by means of FT-IR, NMR, AFM, SEM and TEM techniques. Magnetic properties were investigated by SQUID magnetometer and the relaxation rates (R_1 and R_2) of several solutions having different nanoparticles concentrations were measured as a function of the ^1H Larmor frequency by using various NMR spectrometers, a Fast Field Cycling NMR relaxometer and MRI techniques, covering a wide range of frequencies (from 5KHz to 400MHz). These data will be discussed in terms of possible applications of cystine-coated iron oxide nanoparticles as molecular imaging and MRI contrast agent precursors.



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IDENTIFICATION OF COMPLEX MIXTURES BY NMR PROFILING: PRACTICAL ASPECTS.

R. Lamanna

C.R. ENEA Trisaia, SS 106 Jonica Km 419,5 75026 Rotondella (Mt), Italy
E-mail: raffaele.lamanna@enea.it

In chemistry, a mixture is a group of different substances, mixed together, which do not react among them. In food science, some chemical mixtures have a specific identity related to their origin and final use. Each food (wine, milk, fruit juice, etc.) is actually a chemical mixture with a specific identity. Whatever two types of the same food are mixed together the final mixture (blend) retains the original specific identity, for example a mixture of two wine is still a wine. It is then clear that the identification and classification of chemical mixtures and of food blends are two different problems to be solved with different approaches.
The identification and quantification of a food blend can be addresses by NMR profiling in two steps. First the food analyzed is identified as a mixture of a determined type by solving the appropriate classification problem. Second, the relative amount of each single component in the mixture can be evaluated by solving the appropriate regression problem.
All practical aspects of the classification and quantification process will be discussed and some examples of food blends will be presented [1].

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LONGTIME STORAGE OF HYPERPOLARIZATION VIA SINGLET STATES IN HIGH FIELD

G. Pileio[1], C. Laustsen[2,3], L.J. Brown[1], M.C.D. Tayler[1], R.C.D. Brown[1], J.H. Ardenkjaer-Larsen[2,4] and M.H. Levitt[1].

[1]School of Chemistry, University of Southampton, Southampton, GB;
[2]The Technical University of Denmark, Department of Electrical Engineering, Lyngby, DK.
[3]The MR Research Centre, Aarhus University Hospital, Asrhus, DK; Danish Research Centre for Magnetic Resonance, Hvidovre Hospital, Hvidovre, DK; Center for Insoluble Protein Structures (inSPIN), Interdisciplinary Nanoscience Center (iNANO), Department of Chemistry, Aarhus University, Aarhus, DK.
[4]GE Healthcare, Park Alle 295, 2605 Broendby.
E-mail: G.Pileio@soton.ac.uk

Current nuclear spin hyperpolarization techniques are able to enhance the otherwise poor sensitivity of NMR by greatly increasing spin polarization. These techniques, however, are unable to store the hyperpolarized spin order beyond the limits imposed by the longitudinal magnetization decay constant, T_1. Recently, it was shown that the thermal nuclear polarization can be stored for longer than T_1 via the use of long-lived nuclear spin states. No need to explain how great the outcomes from the combination of these two techniques will be for many NMR and MRI applications - *in-vivo,* particularly. To accommodate these interests, successful attempts to demonstrate the feasibility of this combination have already been made and examples of hyperpolarized long-lived states have been published. However, these examples may result impractical in many situations - e.g. *in-vivo* MRI. In this contribution we show a neat methodology able to create hyperpolarized spin states that live longer than T_1 even in high magnetic fields like, for example, that of a commercial MRI scanner. To achieve this goal and to make the long-term storage of hyperpolarization practical, the use of near-equivalence spin pairs and the ability to access long-lived spin order in those pairs was fundamental. For this purpose, a new technique based on J-coupling-synchronized trains of 180° pulses was developed together with a nontrivial way to perform continuous monitoring of the long-term stored spin order.

SUPPRESSION OF MULTIPLET STRUCTURES IN 1D PROTON NMR SPECTRA

M. Tessari, A.J. Kolkman, J. Draaisma and S.S. Wijmenga

Radboud University Nijmegen, Institute for Molecules and Materials, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
E-mail: m.tessari@science.ru.nl

1D proton NMR spectra of small molecules in solution often display complex multiplet structures due to a dense network of homonuclear J-coupling interactions. The signal overlap resulting from such J-couplings can seriously hamper spectral analysis. Different approaches have been proposed to acquire 1D-^{1}H NMR spectra free from the effects of homonuclear J-couplings [1, 2]. In general, all these methods suffer from a relatively low sensitivity, which makes them unsuitable for dilute (<mM) samples. I will present a novel approach optimized for dilute samples, which allows the acquisition of proton NMR spectra without J-couplings at lower (sub-mM range) concentrations (see Fig. 1).

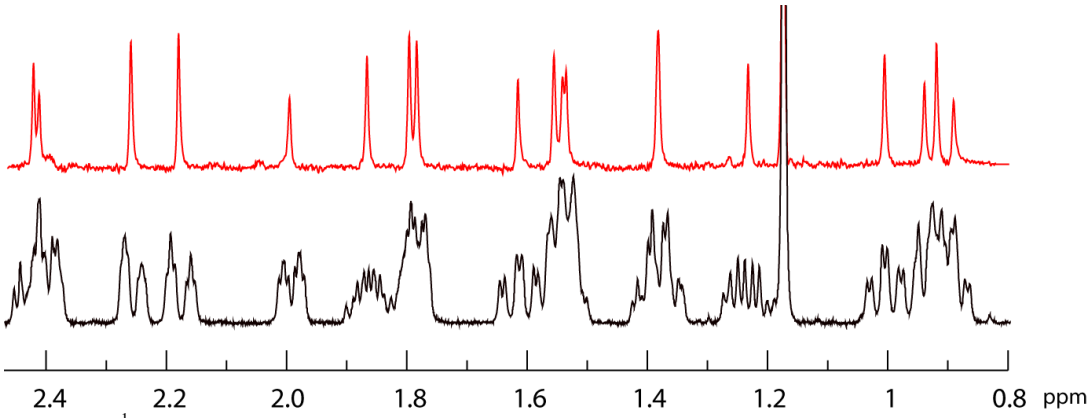

Fig. 1. 1D ^{1}H-NMR spectra of testosterone: conventional (black) and free from multiplet structures (grey). The region between 0.8 and 2.48 ppm is shown.

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NMR STUDIES OF HETEROTYPIC SAM-SAM DOMAIN ASSOCIATIONS INVOLVING EPHA2 RECEPTOR

F.A. Mercurio,[‡] D. Marasco,[‡] L. Pirone,[†] E.M. Pedone,[†] M. Pellecchia,[♯] M. Leone[†]

[‡]Department of Biological Sciences, University of Naples Federico II, Via Mezzocannone 16, Naples, Italy
[†]Institute of Biostructures and Bioimaging, CNR, Via Mezzocannone 16, Naples, Italy
[♯]Sanford|Burnham Medical Research Institute, 10901 N. Torrey Pines Rd, La Jolla, CA
E-mail: marilisa.leone@cnr.it

EphA2 is a receptor tyrosine kinase that is over-expressed in many tumors and exhibits several *pro*-cancer activities. The process of receptor endocytosis and the consequent degradation have recently raised an increasing interest as possible routes to reduce tumor malignancy [1]. In malignant breast cancer cells the lipid phosphatase Ship2 is recruited at the receptor site by means of a heterotypic Sam (Sterile alpha motif domain)-Sam association and inhibits EphA2 endocytosis [1].
NMR and ITC studies of the interaction between Ship2-Sam and EphA2-Sam have already been performed and showed that the two domains bind with a K_D value in the low micromolar range and adopt a ML (Mid Loop)/EH (End Helix) interaction model characteristic of several Sam-Sam associations [2].
Sam domains from Odin, a member of the ANKS (Ankyrin repeat and Sam domain containing) family proteins, play also a prominent inhibitory role of EphA2 endocytosis [3]. Odin contains two tandem Sam domains (Sam1 and Sam2); Odin-Sam1 shows a ~ 70% sequence homology with Ship2-Sam.
We have carried out NMR and SPR experiments and demonstrated that Odin-Sam1 tightly binds to EphA2-Sam. Besides, we have identified by means of chemical shift perturbation experiments, Odin-Sam1 and EphA2-Sam reciprocal binding interfaces and shown that Ship2-Sam and Odin-Sam1 adopt a similar binding mode to EphA2-Sam.
Our research further highlights the key structural features that are important for Sam-Sam associations and provides novel routes for the design of inhibitors of these interactions with potential ability to regulate EphA2 receptor endocytosis.

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LOOKING FOR ENZYMES UNDER THE ICE OF THE ANTARCTIC

C. Smal,[‡] E. Lanzarotti[†], M. Papouchado[¥], M. Foti[¥], M. Aran[‡], M. Marti[†], M. Gallo,[‡] S.H. Coria[§], S.C. Vazquez*, A. Bercovich[¥], W.P. Mac Cormack[§], A. Turjanski[†], D.O. Cicero[‡]

[‡]NMR Laboratory, Fundación Instituto Leloir, Buenos Aires, Argentina
[†]Department of Biological Chemistry, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina e INQUIMAE-CONICET
[¥]Bio Sidus S.A., Buenos Aires, Argentina
[§]Instituto Antártico Argentino, Dirección Nacional del Antártico, Buenos Aires, Argentina
*CONICET, Buenos Aires, Argentina
E-mail: dcicero@leloir.org.ar

The genome of the bacteria *Bizionia argentinensis*, isolated from the Antarctic marine environment [1], was recently decoded and constitutes a relevant source for the discovery of new proteins showing biological activity in extreme conditions of low temperatures. These biomolecules can help the understanding of the general mechanisms that allow biological systems to adapt themselves to particular life conditions. In addition, they can be used in biotechnological applications that can benefit from the use of enzymes showing activity at low temperatures.

As a part of the "White Genome" project in collaboration with Bio Sidus, a leading Biotech Argentinean company, the Bioinformatic Group at the University of Buenos Aires, and the Argentinean Antarctic Institute, we use Nuclear Magnetic Resonance (NMR) spectroscopy with a double purpose: i) to select a number of proteins with sequences that do not allow their functional classification, but that present characteristics of solubility and folding that make them good targets for structure determination, and ii) to determine the structure in solution of some of the targets previously selected. To this purpose we use a solid protocol already proved in a similar project dealing with proteins of *Xanthomonas axonopodis* pv. Citri [2].

In addition, comparison of proteins from this bacterium, living in extreme conditions, with corresponding analogs that work at higher temperature could shed light on the way proteins adapt their structure to very low temperature. As it is now recognized [3], most of the adaptation comes from an increased dynamics of the protein structure. For this reason, NMR represents the technique of choice to study these characteristics at atomic resolution.

In this presentation, the first results of the protein selection process using bioinformatics tools, and the evaluation of the candidates using biochemical tools and NMR will be discussed.

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STRUCTURAL CHARACTERIZATION OF Cu(II)/Cu(I) BINDING TO α-SYNUCLEIN

D. Valensin[‡], F. Camponeschi[‡], I. Tessari[†], L. Bubacco[†], S. Dell'Acqua[§], L. Casella[§], G. Valensin[‡]

[‡]Department of Chemistry, University of Siena, Via A. De Gasperi 2, I-53100 Siena, Italy
[†] Biology, University of Padova, Via U. Bassi 58b, I-35121 Padova, Italy
[§]Department of General Chemistry, University of Pavia, Via Taramelli 12 , I-27100, Pavia, Italy
E-mail: daniela.valensin@unisi.it

The aggregation of α-synuclein (αS) is a critical step in the etiology of Parkinson's disease (PD) [1]. Metal ions such as copper and iron have been shown to bind αS and enhance its fibrillation rate in vitro [2]. As reported for the Aβ-peptide and the Prion protein, αS is also susceptible to copper-catalyzed oxidation. The reaction involves the reduction of Cu(II) to Cu(I) and the conversion of molecular oxygen into reactive oxygen species (ROS), responsible for the oxidative modification of the protein, that generally leads to extensive protein oligomerization and precipitation [3,4]. This mechanism is highly selective and site-specific, and involves interactions of the protein with both oxidation states of the copper ion.
Different methods have been used to characterize Cu(II) binding to αS, suggesting the N-terminal and His50 as binding donor residues; however the presence of two independent copper binding sites [5] or the formation of a macrochelate involving the amino terminal region and the His50 imidazole is still matter of debate [6]. Moreover, it has been recently shown that αS is able to bind Cu(I) with relatively high affinity in a coordination environment involving the participation of Met-1 and Met-5 residues as the main anchoring groups [7]. In order to understand the structural basis and mechanism of the oxidative damage caused by copper-catalyzed oxidation in neurodegeneration associated with PD, we investigated the possibility to use Ag(I) as a probe for Cu(I) binding to αS. Details of Ag(I) binding to αS were explored at single-residue resolution by ^{1}H-^{15}N HSQC NMR experiments, and compared with those previously reported for Cu(I). The ^{1}H-^{15}N HSQC spectrum of αS in the presence of Ag(I) shows that the protein binds Ag(I) in a coordination environment very similar to that observed for Cu(I), with the strongest chemical shift variation effects observed for residues 3-5, 48-52, and 119-130.

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HYDROGELS, WHERE SOLUTION AND SOLID STATE NMR TECHNIQUES MEET

C. Forte,[‡] L. Calucci[‡]

[‡] Istituto di Chimica dei Composti OrganoMetallici, CNR – Consiglio Nazionale delle Ricerche, Via G. Moruzzi 1, Pisa, Italy
E-mail: claudia.forte@pi.iccom.cnr.it

Hydrogels are polymeric materials that find wide application in biology and biomedicine, for example as soft contact lenses, drug delivery systems, implant materials and tissue scaffolding. Hydrogel properties, such as biocompatibility and transport of small molecules, which are fundamental for their application as biomaterials, are principally determined by the status of water in these materials as well as the interactions of water with the polymer. On the other hand, the mechanical performance of hydrogels, which is of relevance for their use as implants and scaffolds, is strongly related to the structure and dynamic properties of the polymer. Due to their "soft" nature, experimental techniques normally used to characterize solids only or liquids only either are not applicable or cannot be exhaustive. In this respect Nuclear Magnetic Resonance (NMR), by the combined use of specific for solid and solution state studies has proven to be fundamental in characterizing these heterogeneous systems. Indeed, thanks to a variety of low and high resolution techniques, NMR allows both the polymeric and the water component to be observed, and, not least, water/polymer interactions to be highlighted. In fact, on the one hand, ^{1}H and ^{13}C high resolution liquid and solid state NMR experiments can be exploited to obtain site-specific information on polymer hydration and on the effects of water on polymer structure and mobility. On the other hand, ^{1}H spin-spin and spin-lattice relaxation times are highly informative on the dynamics of water molecules in the hydrogel and of its interaction with the polymeric matrix; the analysis of water proton relaxation is recognized to be highly suited to detect the presence of different states of water within the hydrogel, and highlight proton and/or water exchange processes at the polymer surface. Water/polymer interactions can be further characterized by the use of magnetization transfer experiments, which exploit the dipolar interactions between unexchangeable polymer protons and a few long-lived hydration water molecules and/or the exchange of labile polymer protons with bulk water.
The potential of the combined use of solution and solid state NMR for the characterization of hydrogels will be exemplified by representative studies on chemically cross-linked polymer systems belonging to the family of poly(amidoamine)s [1] and on physically cross-linked hydrogels formed by 8-armed poly(ethylenglycol)/poly(lactic acid) star block copolymers [2,3].

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^{1}H HRMAS NMR: A TOOL FOR THE STUDY OF TRANSPORT PHENOMENA OF DRUG-MIMIC MOLECULES IN SUPRAMOLECULAR HYDROGEL SYSTEMS

F. Castiglione, F. Rossi, M. Santoro, W. Panzeri, M. Masi, A. Mele, G. Perale

Department of Chemistry, Materials and Chemica Engineerig "G. Natta", Politecnico di Milano,
Piazza L: Da Vinci 32, 20133 Milano, Italy
E-mail: fcastiglione@chem.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. The investigation of the drug-matrix interaction and drug diffusional mobility related to the polymer network mesh size plays an important role in designing new hydrogel systems. We used HRMAS-NMR PFGSE technique to study the diffusive motion of sodium fluorescein, a drug mimetic small molecule, loaded at several concentrations in hydrogel matrices having different mesh size [1]. Experimental data clearly indicate that molecular mobility increases with concentration and is always higher than the diffusion observed in aqueous solution. This surprising effect is related to electrostatic interactions between the polymer chain and the drug-like molecules. Additionally, the diffusion behaviour of ions (both cation and anion) loaded in hydrogel polymers is studied to clarify the influence of the ions charges and concentration of the diffusing species.

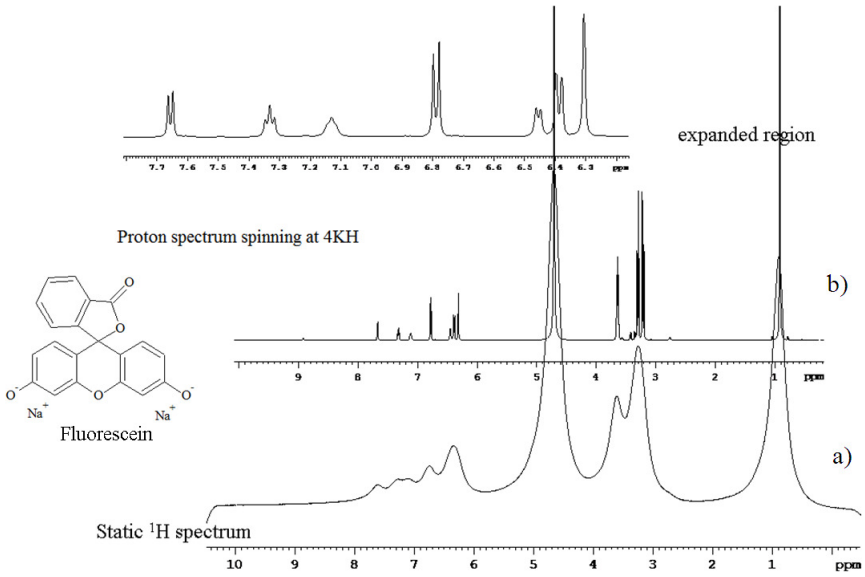

Fig. 1. Chemical structure of fluorescein, ^{1}H static spectrum (a) and spinning at 4KHz (b) of fluorescein in hydrogel.

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TRANSLATIONAL SELF-DIFFUSION STUDIES IN CHIRAL SMECTIC PHASES BY MEANS OF PULSED FIELD GRADIENT NMR

M. Cifelli,[‡] V. Domenici,[‡] S.V. Dvinskikh,[†] M. Glogarova[§], C.A. Veracini[‡]

[‡] Dipartimento di Chimica e Chimica Industriale, Via Risorgimento 35, 56126, Pisa Italy
[†] Royal Institute of Technology, Department of Chemistry, Teknikringen 36, SE-10044 Stockholm, Sweden
[§] Institute of Physics, Academy of Sciences of the Czech Republic, Na SloVance 2, 182 21 Prague, Czech Republic
E-mail: mario@dcci.unipi.it

Since the first observation of smectic liquid crystalline phases formed by chiral calamitic mesogens in the 70's [1] a strong interest raised to these systems due to their variety of structures and properties and to the possibilities of advanced technological applications. In particular, after the discovery of the chiral ferroelectric smectic C phase (SmC^*_{ferro}), where synclinic order is present, some other smectic mesogens which show instead various degrees of anticlinic order were found [2]. Among them, the SmC* antiferroelectric phase (SmC^*_{AFE}) presents a perfect anticlinic structure, where the azimuthal angle flips by 180° from one layer to the other [2].
In this communication we report recent results on studies of translational self diffusion in a variety of chiral smectic phases, performed by means of pulsed gradient NMR diffusometry [3], with particular attention to the effect on the molecular dynamics of the synclinic to anticlinic phase transformation occurring as the smectic C* phase changes from ferro to anti-ferroelectric.

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NEW CRYOGENICALLY COOLED PROBE TECHNOLOGIES FOR NMR

R. Kümmerle

Bruker BioSpin AG, Industriestrasse 26, CH-8117 Fällanden, Switzerland
E-mail: rak@bruker.ch

CryoProbes have experienced a real success story over the past decade in the field of NMR. We have used the acquired experience to further improve performance as well as to broaden the probe portfolio. Latest innovations in the area of cryoprobes will be presented and application examples will be shown.
In addition, the recently introduced CryoProbe Prodigy will be presented, a liquid nitrogen cooled probe eliminating the need for complex and costly infrastructure, such as Helium compressor and high power electrical supply lines. This cost-efficient probe offers a boost in sensitivity equivalent to additional 300 MHz of field strength compared to the best available conventional probe.

HAVING FUN WITH PUSH-BUTTON EXPERIMENTS

M.A. Cremonini[‡], P. Sándor[†]

[‡]Agilent Technologies Italia, Cernusco s/N, Milano, Italy
[†]Agilent Technologies R&D and Marketing, Darmstadt, Germany
E-mail: mauro.cremonini@agilent.com

Many years have passed from the invention of shaped pulses back in the eighties [1] and what is now well established was once in the hands of a few bright researchers who had access to the subtleties of the pulse shaping theory and to the expensive (and usually in-house made) hardware required to create a certain pulse shape. Hitherto, a countless number of useful experiments have been proposed in the literature which make use of often complex shaped pulses; it is therefore highly desirable that the NMR vendors provide the customers with software that deal with the difficult part of shaped pulse generation, thus letting the customers concentrate on the more rewarding part of data interpretation, but without loss of flexibility.
In this talk two examples will be covered where shaped pulse generation is the key step of the experiment: band-selective homonuclear 2D correlation experiments and proton broadband decoupled ^{1}H spectra (the so-called "pure shift" spectra). It will be shown that thanks to an easy-to-use, push-button, interactive interface these advanced experiments can be set up in seconds, therefore allowing even novice users to get critical information about their research sample using the most up-to-date NMR experiments available.

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SINGLE-SHOT NMR MEASUREMENT OF PROTEIN UNFOLDING LANDSCAPES

A. Corazza

Dipartimento di Scienze Mediche e Biologiche
Università di Udine

A protein experiences, in solution, a number of states that are populated according to the Boltzmann distribution, and where the stable state is the most populated. A fundamental technique for the description of the equilibrium unfolding landscape, is represented by the NMR H/D exchange measurements of amides hydrogens, that gives, at the atomic level, the kinetics and thermodynamics parameters of such a picture [1-3]. In order to achieve this goal an H/D exchange NMR experiment study can be carried out at different temperatures with the requirement of a lot of NMR time and usage of high quantities of protein. Here, we propose a new method [4] that permits in a single experiment, lasting about 20 hours, to acquire hundreds of points at different temperature while the exchange process take place during a linear temperature ramp. The method can be used to assess the unfolding landscape of a ^{15}N-labelled protein in different media and also in the presence of other unlabelled protein/cofactors.

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STRUCTURAL DETERMINANTS OF SITE-SELECTIVE LIPID BINDING OBSERVED FOR LIVER BILE ACID BINDING PROTEIN IN THE PRESENCE OF A DISULPHIDE BRIDGE

S. Tomaselli[‡], K. Pagano[‡], C. Cogliati[‡], M. Assfalg[†], S. Zanzoni[†], L. Zetta[‡], H. Molinari[†] and L. Ragona[‡]

‡ ISMAC, CNR via Bassini 15, 20133 Milano, Italy
† Dipartimento Scientifico e Tecnologico, Universita` degli Studi di Verona, 37134 Verona, Italy

Two forms of the liver bile acid binding protein have been reported in the literature, in which residue 91 is a Threonine (L-BABP) or a Cysteine (L-BABP/S-S), which can form a disulphide bridge with the conserved Cysteine 80. Both forms are able to bind in the two binding sites the two natural ligands glycochenodeoxycholic (GCDA) and glychocolic (GCA) bile acids. The two acids differ only for the presence of an additional OH group in position 12 for GCA. In the presence of either GCA or GCDA both proteins are able to form the so called homotypic complexes, where the two sites are fully occupied by the same ligand. However, when both bile acids are present, L-BABP/S-S displays a nearly complete site-selectivity towards GCA and GCDA, at variance with L-BABP whose most populated ligated state is the homotypic complex [1]. In order to understand the structural basis of the site-selectivity for the two ligands NMR structural determination of the heterotypic complex was performed trough a combination of data driven docking approaches and classical restrained molecular dynamics simulations. Analysis of protein residues involved in ligand interactions demonstrate a high hydrophobic character of the more superficial binding site, which is therefore more suited to host a dihydroxy bile salt (GCDA) rather than the more hydrophilic trihydroxy bile salt (GCA). The inner ligand is anchored to the protein through a protein-protein and protein-ligand H-bond cage involving residues H98-Q100-E109, which bind OH-12 and OH-3 on one side of the GCA molecule. Comparative analysis of L-BABP/S-S and L-BABP holo structures allows to make hypothesis on the role of disulphide bridge in ligand site selectivity and supports its functional relevance.

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USING NMR CHEMICAL SHIFT MAPPING TO INCREASE THE ACCURACY OF COMPUTATIONAL DOCKING

L. Simonelli, A. Martinelli, E. Livoti and L. Varani

Istituto di Ricerca in Biomedicina; Via Vela 6; 6500 Bellinzona, CH
[†]Department, Address, Country(Times 10)
E-mail: luca.varani@irb.unisi.ch

Computational docking, the process of predicting the structure of a complex starting from the structure of the individual partners, is emerging as a rapid, affordable and increasingly accurate method for the structural characterization of intermolecular complexes. Although docking can find accurate solutions, however, it often fails to discriminate them from inaccurate ones, due to deficiencies of the scoring functions used to evaluate the models. Using experimental data to filter and validate the accurate computational solutions is, therefore, highly desirable. Here we show how the incorporation of rapidly obtained NMR chemical shift mapping information can greatly improve the accuracy of computational docking.
We test our approach using the program Rosetta-Dock on a set of 18 protein-protein complexes with known experimental structure, add an aptly designed term derived from NMR mapping to the default scoring function and show that this modified score has an improved capacity to a) provide confirmation of positive results, b) point out incorrect computational results and c) provide positive results when the computer alone does not.

THE CATALYTIC CORE OF A GROUP II INTRON AS REVEALED BY NMR

D. Donghi, M. Pechlaner, R.K.O. Sigel

Institute of Inorganic Chemistry, University of Zurich
Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
E-mail: daniela.donghi@aci.uzh.ch

Group II intron ribozymes are catalytically active self-splicing RNAs that resemble the eukaryotic spliceosome in both structure and splicing mechanism [1]. Metal ions are essential prerequisites for their folding into a complex active 3D structure but also for their catalytic activity [2].
Their secondary structure contains six domains, each playing a specific role [3]. Biochemical studies showed that essential elements for both folding and catalysis are located in the core of domain 1 (D1), the so called κζ region [4].
Despite their biological importance, very scarce structural information is available on these molecules [5].
We are currently investigating by NMR the solution structure and the metal ion binding properties of this D1-κζ region of the mitochondrial group II intron ribozyme *Sc.*ai5γ from baker's yeast. The investigated RNA contains structural features normally associated with high mobility, making the structural study challenging. Addition of Mg^{2+} turned out to be essential to partially stabilize the highly dynamic κ region. Numerous NMR experiments were recorded under different experimental conditions and at different magnetic fields, allowing to collect restraints for the structure calculation, which is now well in progress.
We will present here the NMR solution structure of the κζ region as well as its Mg^{2+} binding properties.

Financial support by a Marie Curie Intra European Fellowship (RNABIC, 236794 to DD) and in the form of access to the Bio-NMR Research Infrastructure (FP7/2007-2013, grant agreement 261863) within the 7th European Community Framework Programme as well as by the Swiss National Science Foundation (200021-124834 to RKOS) is gratefully acknowledged.

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APPLICATION OF ^{19}F TECHNIQUES IN SOLID-STATE NMR
- IN MEMORIAM OF PROF. DR. KLAUS MÜLLER -

M. Abele[†, ‡], M. Abdellatief[‡], M. Leoni[‡] and P. Scardi[‡]

[†]University of Stuttgart, Department of Physical Chemistry, Pfaffenwaldring 55, 70569 Stuttgart, Germany
[‡]Dipartimento di Ingegneria dei Materiali e Tecnologie Industriali, Universitá degli Studi di Trento, via Mesiano 77, I-38100, Trento, Italy
E-mail: m.abele@ipc.uni-stuttgart.de

This contribution will be dedicated to the memory of Prof. Dr. Klaus Müller who passed away unexpectedly in April 2011. He devoted his scientific career for the understanding of order/disorder phenomena with Solid-State NMR. The presentation will focus on the application of ^{19}F NMR in the group of Prof. Dr. Klaus Müller.
There are many examples for the application of NMR spectroscopy to fluorinated hydrocarbons, e.g. Nafion® for the use in fuel cells, found in literature [1]. When recording the ^{13}C spectra for such materials, the use of an appropriate ^{19}F decoupling technique is essential. We will present results obtained by using the typically applied XY-16 sequence [2], as well as promising results based on the XiX decoupling technique, revealing a comparable decoupling performance at much lower spinning speeds. (see Fig. 1)
The second part of the presentation will address the use of T_1 relaxometry in structural studies of nano-crystalline CaF_2. In the presented results the combination of X-ray Diffraction (XRD) Line Profile Analysis (LPA) and T_1 data analysis proves to be a powerful tool for the understanding of microstructural features in nano-crystalline calcium fluoride, a common ionic conductor, produced by high-energy ball milling.

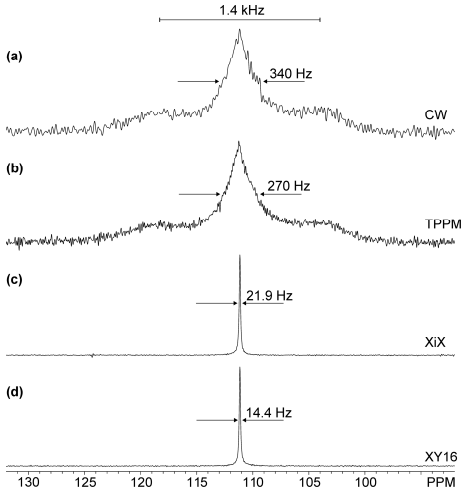

Fig. 1. ^{13}C spectra of Polyterafluoroethylene recorded under the use of different ^{19}F decoupling techniques.

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SOLID STATE NMR AT CRYOGENIC TEMPERATURES

M. Concistrè[1], R. Sarkar[1], O.G. Johannessen[1,2], M. Denning[1], P. Beckett[1], M. al-Mosawi[2], M. Carravetta[1], C. Beduz[2], Y. Yang[2] and M.H. Levitt[1].

[1]School of Chemistry, University of Southampton, Southampton, UK;
[2]School of Engineering Science, University of Southampton, Southampton, UK.
E-mail: mariac@soton.ac.uk

Solid state NMR at cryogenic temperatures offers an increase in signal, a reduction in noise, and the opportunity to study a wide range of interesting and technologically important physical phenomena. Examples include superconductivity, quantum tunnelling and quantum molecular rotation. On the biological side, cryogenic NMR has a major potential in the study of protein assembly or membrane proteins. Furthermore, cryogenic NMR coupled to DNP is expected to give rise to an enormous sensitivity gain with an even stronger impact in those applications.

We have constructed cryogenic NMR systems working at a magnetic field of 14.1 T and able to perform both static and magic-angle-spinning solid state NMR experiments at very low temperature. Our static cryoprobe is able to perform solid state NMR at temperatures down to 1.8 K. Our cryogenic MAS probe can spin a 2mm zirconia rotor at 15 kHz while keeping the real sample temperature at 13K. The sample temperature and spinning frequency may be adjusted independently, since the bearing, turbine and sample cooling lines are well separated. The cooling is done using He gas produced in a custom-made boiler by evaporating liquid He in the supercritical regime. In this contribution we will show the first results obtained with this setup run at the most extreme working conditions. We use the longitudinal relaxation constant of ^{127}I in cesium iodide as a convenient NMR thermometer for temperatures down to about 15K.

SOLID STATE NMR OF PROTEINS SEDIMENTED BY ULTRACENTRIFUGATION

I. Bertini[‡], C. Luchinat[‡], G. Parigi[‡], E. Ravera[‡], B. Reif[†], C. Rosa[‡], P. Turano[‡]

[‡] Magnetic Resonance Center (CERM) and Department of Chemistry, University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy
[†] Technische Universitaet Muenchen, Dept. Chemie, Lichtenbergstrasse 4, D85747 Garching, Germany
E-mail: ravera@cerm.unifi.it

Solid-state (SS) NMR rotors are ultracentrifuges, creating a field of force of up a few million g at their maximum speed. We have demonstrated that, under MAS, a relatively large protein in solution sediments at the internal walls of the cylinder, and its NMR signals are observable as if the protein were in the solid state.
The proof of principle of this new way of performing NMR spectroscopy is provided, showing examples based on ferritin. Ferritin is a 24-mer of 480 kDa molecular weight in the apo form (i.e. devoid of the iron oxide core). Because of the severe line broadening due to its size, it could hardly be studied in solution, and only in the perdeuterated form, despite the number of signals is only that of a 20 kDa protein. Apoferritin can be obtained in the microcrystalline state and provides high quality SS NMR spectra [1]. These spectra are used for comparison purposes.
A ferritin solution at a concentration of 60 mg/ml was sealed in a 50 µl rotor with a PTFE insert without further manipulation. CP spectra were recorded at increasing spinning rates, from the static sample to 12 kHz. Solid state signals start appearing around 3 kHz, to yield solid state-like spectra above 6 kHz [2]. When spinning is stopped, the sample reverts to solution and the SS NMR signal is lost. NMR of sedimented molecules can overcome the protein size limitations of solution NMR without the need of sample manipulation (e.g.: crystallization/precipitation) required by solid state NMR. Large protein-protein complexes, membrane proteins and soluble prefibrillar states are attractive targets for this new technique.

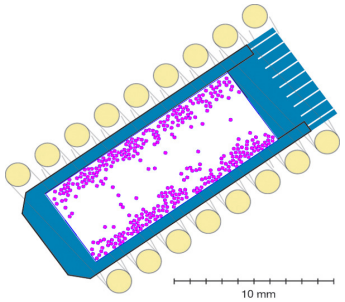

Fig. 1. Schematics of the sedimentation inside the NMR rotor.

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THE INTERACTIONS OF RETINOL CARRIERS WITH MODEL MEMBRANES PROVIDE INSIGHTS INTO THE COMPLEX MECHANISMS OFLIGAND BINDING AND DELIVERY

L. Franzoni

Department of Experimental Medicine, Sect. of Chemistry and Structural Biochemistry, University of Parma, 43100 Parma, Italy
E-mail: lorella.franzoni@unipr.it

Many natural or drug-induced cellular processes involve protein-membrane interactions. Because of the complexity of the native system, studies on such interactions are often conducted with artificial models. In the case of cellular retinol-binding proteins (CRBPs), despite what we have learned based on ^{15}N relaxation dispersion experiments, line-shape analysis as well as H/D exchange experiments [1-3], there are still numerous interesting questions that remain unanswered. Taking into account that most retinoid-processing enzymes are associated with intracellular membranes, we have performed a series of NMR and CD titration experiments on CRBP-I and CRBP-II in the presence of biomembrane mimetic systems.
The data indeed allowed us to extend the knowledge about the complex mechanisms of ligand uptake and targeted release. The understanding of these processes is of fundamental importance given the essential biological role of vitamin A.
The results will also be discussed in comparison with the ones obtained for related proteins [4, 5] in an attempt to develop a general description in the intracellular lipid binding protein (i-LBP) family.

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THE POLYMORPHISM OF BARBITURATES: A SOLID-STATE NMR AND XRD COMBINED APPROACH

M.R. Chierotti,[‡] R. Gobetto,[‡] L. Maini[†], D. Braga,[†] M.U. Schmidt[⊥]

[‡]Department of Chemistry I.F.M., V. Giuria 7 Torino, Italy
[†]Department of Chemistry "G. Ciamician", V. Selmi 2 Bologna, Italy
[⊥]Institute of Inorganic and Analytical Chemistry, Max-von-Laue-Str. 7 Frankfurt, Germany
E-mail: michele.chierotti@unito.it

Barbituric and 2-thiobarbituric acids are known to exist in the solid state only in the trioxo structure as shown by X-ray diffraction studies. However, they may give rise to many (10) possible tautomers supposed to be intermediate forms in many reactions in which the acids are involved.
Here we report the mechanical conversion of the barbituric and the 2-thiobarbituric acids into new polymorphs displaying different keto or enol character.
By milling the barbituric acid (form II) a new polymorph (form IV) has been obtained [1]. Evaluation of the N-H distance by mean of dipolar NMR methods on a not-enriched sample together with XRPD data indexing allowed to ascertain the structure of form IV for which single crystals were not available. This form has been found to be the most stable one at room temperature in agreement with theoretical calculations and consists of molecules in the enol form, as confirmed also by neutron powder diffraction [2].
On the other hand, five new polymorphs and one hydrated form of the 2-thiobarbituric acid have been isolated [3]. All the new forms have been characterized by means of single crystal X-ray diffraction, 1D and 2D (^{1}H, ^{13}C and ^{15}N) solid-state NMR experiments, DSC, and Raman spectroscopy. In both form II and hydrate form, the 2-thiobarbituric molecules are present in the enol form, whereas only the keto isomer is present in crystalline forms I [4], III, V and VI. In form IV a 50:50 ordered mixture of enol/keto molecules is present. By means of mechanochemical methods it has been also possible to induce keto-enol conversions of the different forms.

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WEAK HOMONUCLEAR DIPOLAR RECOUPLING FOR THE DETECTION OF ^{1}H-^{1}H PROXIMITIES IN SOLIDS

G. Mollica,[‡] P. Thureau,[‡] F. Ziarelli,[†] S. Viel[‡] and A. Thévand[‡]

[‡] Laboratoire Chimie Provence, Universités Aix-Marseille I, II et III – CNRS, UMR 6264, 13397 Marseille, France
[†] Fédération des Sciences Chimiques de Marseille, CNRS-FR1739, Spectropole, 13397 Marseille, France
E-mail: giulia.mollica@univ-provence.fr

The low NMR spectral resolution caused by the strong homonuclear dipolar couplings among the protons has for long time limited the detection of ^{1}H-^{1}H proximities in solids. In the last decade, enormous improvements in both hardware and pulse sequence development have allowed the observation of high-resolution 1D and 2D ^{1}H spectra of a number of different solid materials. Among the different experimental methods proposed for the detection of ^{1}H-^{1}H correlations between dipolar-coupled protons [1], "NOESY-type" spin diffusion pulse schemes allow the estimation of ^{1}H-^{1}H proximities through the detection of the z magnetization transfer between dipolar-coupled spins. In these experiments, two periods of single-quantum coherence (SQ) evolution (usually in the presence of homonuclear-decoupling schemes or fast MAS conditions) are separated by a mixing time during which spin diffusion occurs [2,3]. Here, we propose an alternative ^{1}H-^{1}H SQ-SQ correlation scheme combining windowed-PMLG homonuclear decoupling [4], DQ filtering and weak dipolar recoupling under moderate MAS conditions. The weak recoupling conditions eliminate the dipolar truncation effect [5]. The proposed method looks promising for the measurement of long-range ^{1}H-^{1}H distances in solid samples.

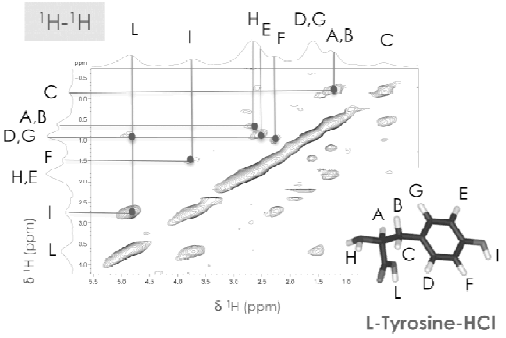

Fig. 1. ^{1}H-^{1}H 2DwPMLG spectrum of solid L-Tyrosine HCl.

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INTERMOLECULAR RING CURRENT EFFECTS IN SOLID NAPROXEN: A COMBINED 2D MAS NMR AND AB INITIO STUDY

E. Carignani[‡], S. Borsacchi[‡], J.P. Bradley[†], S.P. Brown[†] and M. Geppi[‡]

[‡] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126, Pisa, Italy
[†]Department of Physics, University of Warwick, Coventry CV4 7AL, United Kingdom
E-mail: elisa.carignani@for.unipi.it

The elucidation of local structural features of single molecules in the solid phase and of their crystal packing has a particular interest in the pharmaceutical field, since most drug formulations are solid and different solid forms can show much different properties [1]. The combination of solid state NMR and suitable computational methods is at present a very powerful approach for the characterization of crystalline solids, as proved by the development of "NMR crystallography" [2]. In this work, the anhydrous crystalline forms of Naproxen [(*S*)-(+)-2-(6-Methoxy-2-naphthyl)propionic acid] (Napro-A) and its sodium salt (Napro-S), widely used non-steroidal anti-inflammatory drugs, have been investigated by means of a combined 1D, 2D MAS NMR and *ab initio* calculations performed by using the CASTEP software package. From calculations, ^{13}C MAS and ^{1}H CRAMPS spectra, 2D ^{1}H-^{13}C MAS-J-HMQC, INEPT, FSLG-HETCOR and ^{1}H-^{1}H DQ-CRAMPS experiments, ^{1}H and ^{13}C resonances have been fully assigned for both Napro-A and Napro-S. In the case of Napro-S all the nuclei belonging to the two inequivalent molecules present in the unit cell gave rise to distinct signals, which could be completely assigned. Noticeable intermolecular ring current effects [3] on ^{1}H chemical shifts have been observed for both samples, even if involving different nuclei and with a different extent in the two cases. The ^{1}H chemical shift values experimentally measured and calculated for the crystal structures (determined by X-ray techniques [4]) showed a very good agreement for both Napro-A and Napro-S, allowing us to correlate the different ring current effects with the different crystal structures. The comparison between the ^{1}H chemical shifts calculated for the molecules in the crystal structures and in vacuum allowed us to confirm the main intermolecular character of the observed ring current effects and to quantify their extent.

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FOOD AND PLANT METABOLOMIC STUDIES

R. Consonni, L.R. Cagliani

Istituto per lo Studio delle Macromolecole, Lab. NMR, CNR, v. Bassini 15, 20133 Milano, Italy
E-mail: roberto.consonni@ismac.cnr.it ; lauraruth.cagliani@ismac.cnr.it

In the last years, NMR has largely shown its great potentiality in food and plant metabolic characterization. The capability to detect several metabolites with a single experiment, lead to increase the available information that could be used in food quality determination as well as in plant science. As the matter of fact NMR studies of foods started to grow with the aim of geographical determination as well as adulteration assessments. Conversely, metabolic changes occurring in plants upon stress conditions, could increase the production of secondary metabolites with nutraceutical or pharmacological interest. In this work, NMR techniques combined with multivariate statistical analysis were applied to different foods and plant extracts, with particular attention to geographical origin determination, fraud detection and plants characterization.

Reference

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NMR INVESTIGATION OF THE METABOLIC PROFILE OF CHEESE FROM RAW EWES' MILK SUPPLEMENTED WITH INDIGENOUS AND COMMERCIAL CULTURES

C. Piras[1], F. Savorani[2], S.B. Engelsen[2], B. Pisano[3], S. Cosentino[3], A. Lai[1] and F.C. Marincola[1]

1 Department of Chemical Sciences, Cittadella Universitaria di Monserrato, S.S. 554 Bivio Sestu, 09042 Monserrato (CA), Italy
2 Quality & Technology, Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark
3 Department of Experimental Biology, Section of Hygiene, Cittadella Universitaria di Monserrato, S.S. 554 Bivio Sestu, 09042 Monserrato (CA), Italy
E-mail: cristina.piras@unica.it

The transformation of milk into curd and then to cheese is a complex process which, for most cheeses, can be divided into two phases: manufacture (5–24 h, from the preparation of milk to salting) and ripening (2 weeks–2 years depending on variety). Microorganisms, either as natural contaminants or added as natural or industrial starter or adjunct cultures, play a fundamental role in both phases by affecting directly and indirectly most of the biochemical, chemical and physical processes that occur in milk, in curd and in cheese. Although it is widely accepted that the biodiversity of raw milk microflora is a fundamental factor for the maintenance of the typical features of traditional cheese, allowing the manufacture of high-value artisan varieties, for commercial cheeses undefined starter cultures of generic composition are widely used, resulting in a high degree of control over the fermentation process and standardisation of the final product. However, some disadvantages have to be considered. Mainly, since the biodiversity of commercial starters is limited, this often leads to a loss of the uniqueness of the original product as well as a loss of some characteristics that have made the product popular. In this context, the use of autochthonous starter cultures in the production process of artisanal cheese represents an interesting alternative to the commercial starters, since they would preserve the diversity of bacterial genera and species associated with specific products avoiding the loss of those "typical" features which characterize traditional products.

^{1}H NMR spectroscopy, joined to multivariate statistical analysis methods (chemometrics), can be successfully used for food analysis, providing information on a wide range of compounds present in the food matrix. In this work we have used this combined approach to analyze and differentiate the metabolic profile of Sardinian raw ewes' milk cheeses supplemented with different starter cultures. In particular, the aqueous extract of cheeses made with autochthonous cultures, containing strains with acidifying properties and strong anti-*E.coli* activity, has been compared to those of chesses made with commercial starters. All samples were analyzed at different ripening time points. Our findings allowed cheeses' extract profiles to be distinguished both by maturation time and by strain formulation used. The good agreement of our findings with the results of biochemical analyses carried out on same samples validates the applicability of NMR-based metabolomics for the study of metabolic processes in dairy food matrices.

METABOLIC PHENOTYPES AND CELIAC DISEASE FINGERPRINTING

M. Assfalg[1], P. Bernini[1,3], I. Bertini[1,2], A. Calabrò[4], V. De Carli[4], D. Colangiuli[1], G. la Marca[5], G. Lami[4], C. Luchinat[1,2], S. Nepi[1,3], B. Porfirio[6], D. Renzi[4], E. Saccenti[1,3], H. Schäfer[7], B. Schütz[7], M. Spraul[7], L. Tenori[1,3]

[1]Magnetic Resonance Center (CERM), University of Florence, Via L.Sacconi 6, 50019 Sesto Fiorentino, Italy;
[2]Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy;
[3]FiorGen Foundation, Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy; [4]Gastroenterology Unit, Department of Clinical Pathophysiology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy;
[5]Mass Spectrometry Lab, Clinic of Pediatric Neurology, A. Meyer University Children's Hospital, Viale Pieraccini 24, 50134 Florence, Italy;
[6]Human Genetic Unit, Department of Clinical Pathophysiology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy; [7]Bruker BioSpin GmbH, Silberstreifen, D-76287 Rheinstetten, Germany

Statistical analyses performed on NMR spectra of human urine samples reveal an invariant part characteristic of each person[1]. This individual phenotype is relatively stable over a 2-3 years period[2]. This finding provides evidence that individual metabolic phenotypes (metabotypes) exist and opens new perspectives to metabonomic studies, based on the possibility of eliminating the daily variations related to diet and lifestyle by multiple sample collection.

In phenotyping diseases such as celiac disease (CD), blood-derived samples often yield better discrimination than urine samples, probably due to the lower day-to-day variability. Using a combination of statistical techniques we are able to discriminate CD patients from healthy controls with high accuracy[3]. After 12 months of gluten free diet all but one patients were classified as healthy by the same statistical analysis, with the metabolic profile reverting to the normality. Potential CD patients largely shares the metabonomic signature of overt CD[4], allowing us to hypothesize that CD exists as such before intestinal damage occurs, so if the metabolic changes are (at least partially) independent of the bowel malabsorption, a deeper analysis of this fingerprint can be helpful to infer more information on the biochemistry of the disease.

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2010 Annalaura Segre Fellowship Recipients

CHARACTERIZATION OF DIFFERENT VARIETY OF HONEY BEES WITH HRMAS-NMR FOR THE TRACEABILITY AND THE VALORIZATION OF THIS PRODUCT

C. Cafiero,[‡*] M. Valentini,[‡] F. Savorani[†]

[‡]CRA-RPS, Instrumental Centre of Tor Mancina, Monterotondo (RM), Italy,
* Dipartimento per l'Innovazione nei Sistemi Biologici Agroalimentari e Forestali (DIBAF), University of Tuscia, Viterbo, Italy
[†]Dept. of Food Science, Quality & Technology, Faculty of Life Sciences, University of Copenhagen, DK-1958 Frederiksberg C, Denmark
E-mail: c.cafiero@unitus.it

Honey represents the most important bee's product, so that the Codex Standard for Honey gives a precise definition of what honey should be [1]. Honey quality and properties are strictly correlated to the environmental behavior of the areas where the bees live and to the production period: which are the botanical origin and the traceability. Both are fundamental information and are subject of intense researches in order to avoid frauds and to ensure high quality standards. To this aim metabolomics [2] seems to be a reliable analytical tool, and among the approaches HRMAS-NMR technique offers the almost unique opportunity of measuring honeys without any sample preparation by producing high-resolved NMR spectra [3]. The lack of any treatment minimize any possible quantitative and/or qualitative chemical modifications. The measured spectroscopic data must be analyzed with appropriate multivariate procedures in order to identify the molecular markers related to traceability and botanical origin. In the present work five different honey variety produced in Italy have been characterized: Acacia, Millefiori, Chestnut (Fig. 1), Eucaliptus and Citrus. It has also been characterized also the Acacia Honey of Lunigiana, the first Italian honey that has obtained the PDO Certification. Traceability and botanical origin are the two issues elucidated combining HRMAS-NMR spectroscopy and multivariate data analysis.

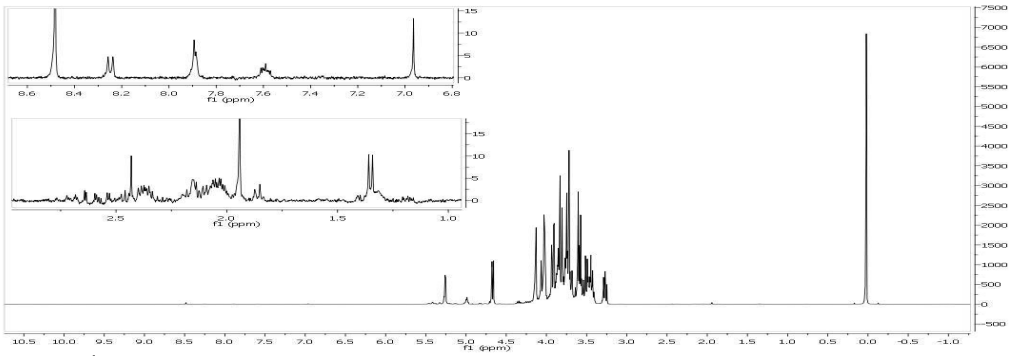

Fig. 1. ^{1}H-HRMAS NMR spctrum of chesnut honey, with the expansions of two region of interest.

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SOLID STATE NMR INVESTIGATION OF THE STRUCTURAL, PHASE AND DYNAMIC PROPERTIES OF AN ANION-EXCHANGE MEMBRANE USED IN POLYMERIC FUEL CELLS

F. Martini, S. Borsacchi, M. Geppi, A. Pucci

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Risorgimento 35, I-56126, Pisa
E-mail: francesca.martini@for.unipi.it

Polymeric materials are playing a very important role in the development of the fuel cell technology thanks to their low cost, easy processability and high flexibility [1]. In particular a great interest is now focused on the polymeric anion exchange membranes (AEM), since they provide economical advantages and better performances with respect to the proton exchange membranes widely studied in the past [1, 2]. Since the performances of these membranes are strongly dependent on their structural and dynamic properties on a nanometric and sub-nanometric scale, the study of these properties is very important for devicing and preparing optimized and improved systems. In this field solid state NMR (SSNMR) is one of the most promising techniques, even if its use is still limited [3, 4]. Indeed, a variety of SSNMR techniques can be exploited to investigate different morphological aspects of a polymeric material, such as conformational, phase and dynamic properties, the relative dispersion of different domains and, in the specific case of a composite system, interaction properties [5]. In this work the structural, phase and dynamic properties of an anion exchange membrane, in which the unit responsible for the ionic conduction, 1,4-diazabicyclo[2,2,2]octane (DABCO) containing at least one quaternary ammonium site, is grafted to a LDPE film through a 4-vinylbenzyl chloride (VBC) moiety, have been investigated and characterized by means of a multi-nuclear and multi-technique SSNMR approach. ^{13}C CP/MAS selective, ^{1}H MAS, and ^{1}H-^{13}C HETCOR experiments allowed detailed information on the structural and phase properties of LDPE, DABCO and VBC to be obtained, while the measurements of proton relaxation times, and in particular of $T_{1\rho}$, through both high- and low-resolution techniques, provided some interesting insights into the dynamic behaviour of DABCO. Moreover, the comparative study of the systems obtained at three different steps of the preparative process was very useful to understand the morphological changes, concerning in particular LDPE, that occur during the preparation of the membrane. In particular, the presence of PE quite rigid amorphous and crystalline domains has been observed, whose structural heterogeneity increases during the preparation process. Furthermore, two different DABCO fractions, experiencing different molecular mobility, have been identified.

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Posters

APPLICATION OF HIGH RESOLUTION NMR SPECTROSCOPY FOR THE STUDY OF THE CHEMICAL MODIFICATIONS OCCURRING DURING COCOA PROCESSING

D. Acquotti,[‡] A. Caligiani [†], G. Palla[†]

[‡] Centro Interdipartimentale Misure, Università di Parma, Parma, Italy
[†] Dipartimento di Chimica Organica e Industriale, Università di Parma, Parma, Italy
E-mail: domenico.acquotti@unipr.it

The chocolate and cocoa-based products are among the goods with higher added value. Recently, renewed interests has been awakened toward the established favorable healthy properties of cocoa flavan-3-ols, and many researches have been made aimed at understanding the changes that occur during the processing of cocoa beans. To obtain a product with optimal organoleptic characteristics, the cocoa beans undergo various technological treatments. Fermentation and roasting of beans are the most important steps for the development of the aroma of cocoa, which is the result of a complex series of biochemical and chemical reactions. The next transformations into cocoa paste, the conching and tempering are further essential steps to obtain a smooth-textured, structurally homogeneous and stable product. We have previously reported the assignment of ^{1}H NMR spectra of cocoa beans extracts, showing the possibility of the simultaneous detection of many classes of compounds [1]. The aim of the present work was the exploration of the possibility of using NMR to follow the modifications occurring during cocoa beans transformation to chocolate, by analyzing eight complete series of samples, each formed by fermented cocoa beans, roasted nibs, cocoa liquor and chocolate. The assignment of the ^{1}H NMR signals of roasted beans was also performed by means of homo- and hetero-correlated bidimensional experiments.
Experiments based on relaxation time and diffusion ordered spectroscopy (DOSY) were also performed to aid in the spectral assignment of such complex food matrix and, in particular, for a better comprehension of carbohydrates and flavan-3-ols distribution and transformation during processing.
A NMR-based metabolomic approach using chemometric techniques showed a clear differentiation of cocoa samples according to the processing steps.

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ADDING ANOTHER PIECE OF EVIDENCE TO THE COMPLEX MODEL OF RETINOL CELLULAR UPTAKE

F. Baroni[1], C. Lücke[2], D. Cavazzini[3], G.L. Rossi[3] and L. Franzoni[1]

[1]Department of Experimental Medicine, University of Parma, 43100 Parma, Italy;
[2]Max Planck Research Unit for Enzymology of Protein Folding, 06120 Halle (Saale), Germany;
[3]Department of Biochemistry and Molecular Biology, University of Parma, 43100 Parma, Italy.
E-mail: lorella.franzoni@unipr.it

Vitamin A and its derivatives are essential for diverse biological processes, because they are involved in the proliferation and differentiation of many cell types throughout life. During transport in the blood from liver to distant organs virtually all retinol is bound to retinol binding protein (RBP). A high-affinity cell-surface receptor for holo-RBP has been identified and termed STRA6 [1]. It is a widely expressed multi-transmembrane domain protein that mediates cellular vitamin A uptake in the target organs through a new mechanism [2]. A controlled release of retinol into cells from holo-RBP through STRA6 has an evolutionary advantage that prevents a possible toxicity deriving from an excessive accumulation of free vitamin A. There is also evidence that a specific binding site for the apo-form of the cellular carriers (CRBP) might exist on the cytoplasmic side of the membrane [3].
We have performed NMR and Circular Dichroism experiments on apo CRBP-I and apo CRBP-II in the presence of biomembrane mimetic systems, with the aim to obtain further insights about this emerging model of receptor-mediated cellular uptake of retinol.
The data demonstrate that apo CRBP-I and apo CRBP-II interact with phospholipid vesicles in a significantly different manner, despite having nearly identical backbone structures. These new evidences complement our earlier results, which had suggested that the highly homologous proteins exhibit different mechanisms of ligand uptake [4, 5]; combined with their distinct tissue distributions this may imply different roles in an intracellular context.

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UNDERSTANDING THE PROPERTIES OF THE COAGEL AND GEL PHASES: A ^{2}H AND ^{13}C NMR STUDY OF AMPHIPHILIC ASCORBIC ACID DERIVATIVES

S. Borsacchi,[a] P. Lo Nostro,[b] M. Ambrosi,[b] M. Geppi [a]

[a] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126 Pisa and I.N.S.T.M. (Consorzio Interuniversitario di Scienza e Tecnologia dei Materiali), Firenze, Italy.
[b] Dipartimento di Chimica e CSGI, Università di Firenze, Via della Lastruccia 13, 50019 Sesto Fiorentino (Firenze), Italy.
E-mail: silvi@ns.dcci.unipi.it

Ascorbic acid amphiphilic alkanoates (ASCn) combine the extraordinary radical-scavenging properties of ascorbic acid (Vitamin C) with the possibility of forming a rich variety of supramolecular assemblies in water, which can represent an ideal environment for the solubilization of hydrophobic and sensitive drugs [1]. Depending on alkanoate chain length (n), amount of water and temperature, ASCn can form liquid micellar solutions, gel and coagel, whose properties have been investigated by means of several techniques but are still not fully understood. Here we present an NMR study of the gel and coagel phases of ASC12 in water, mainly aimed at investigating the structural and dynamic properties of water and alkylic chains in the two phases, whose understanding could also help in clarifying the mechanims of the phase transitions. The NMR study was based on the analysis of ^{2}H and ^{13}C spectra, acquired in the gel and coagel phases of ASC12 in D_2O. Two different epimers, D-ASC12 and L-ASC12, were studied, in order to also clarify previously observed different behaviours related to the different ascorbic acid headgroup chirality [2]. ^{2}H-NMR spectra unambiguously showed the completely different dynamic properties of water in the two phases, while from ^{13}C spectra it was evident that the phase transition is also associated with a strong modification of the dynamic properties of the alkylic chains of ASC12. Some differences were observed between D- and L-ASC12, which were related to possible different conformational features and hydrogen-bond interactions.

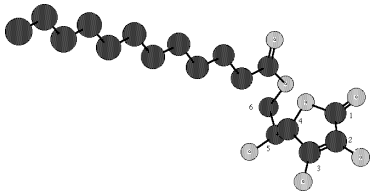

Fig. 1. Ascorbic acid ester ASC12.

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NMR METABOLOMICS STUDY OF COFFEE

L.R. Cagliani, C. Cogliati, R. Consonni

Istituto per lo Studio delle Macromolecole, Lab. NMR, CNR, v. Bassini 15, 20133 Milano, Italy
E-mail: lauraruth.cagliani@ismac.cnr.it

Coffee is one of the most popular and appreciate beverages worldwide and its particular high economical value often implies adulteration practises that could involve geographical origin. In this context the development of new analytical methods to guarantee the quality of coffee in terms of both authenticity and provenance results very important. As other food matrices, coffee represents a very complex matrix to analyze, being constituted by several class of chemical compounds. The metabolomic approach could be an useful tool to investigate all soluble chemical species at the same time. NMR spectroscopy has already demonstrated its prevalent role in metabolomic analysis [1] and in this work it is employed in combination with multivariate statistical protocols to analyze coffee beans coming from different countries with the main aim of geographical origin.

Reference

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^{1}H NMR OF BOVINE MEAT EXTRACTS COMBINED WITH CHEMOMETRICS AS A TOOL TO DISTINGUISH BETWEEN IRRADIATED AND NON-IRRADIATED MEAT

A. Caligiani,[‡] E. Zanardi, [†] M. Mariani,[§] S. Ghidini[†], G. Palla,[‡] A. Ianieri[†]

[‡] Dipartimento di Chimica Organica e Industriale, Università di Parma, Parma, Italy
[†] Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti, Università di Parma, Parma, Italy
[§] Dipartimento di Energia, Politecnico di Milano, Milan, Italy
E-mail: augusta.caligiani@unipr.it

The possibility of using high-resolution 1H NMR as a "fingerprint" in the definition of geographical origin or authentication of foods has been widely explored in the last decade [1], but only few studies regard applications of NMR to meat science [2]. The aim of this study was to assess the usefulness of NMR spectroscopy to differentiate irradiated from non-irradiated bovine meat. 1H NMR spectra of methanolic extracts of ground beef irradiated at 0, 2.5, 4.5 and 8 kGy were acquired with a 600 MHz NMR spectrometer. The data set composed by 112 integrated signals for each of 80 NMR spectra of bovine meat was statistically elaborated by multivariate analysis. Principal component analysis and stepwise discriminant analysis (Fig. 1) showed significant separation between extracts of non irradiated and irradiated meat and also among the three irradiation doses. Discriminant analysis applied to NMR spectral data correctly classified 98.8 % of originals samples according to the irradiation dose and 97.5 % of cross-validated samples.

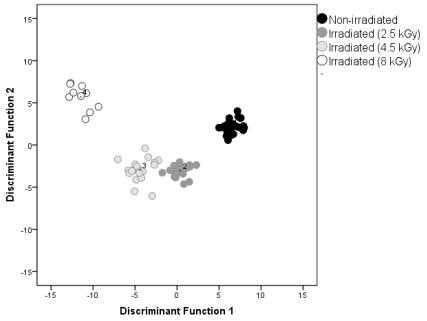

Fig. 1. Stepwise discriminant analysis: graph of sample values divided by irradiation dose

The metabolites selected by stepwise discriminant analysis, considered responsible for differentiation between irradiated and non-irradiated meat were organic acids such as succinate, lactate, acetate, various amino acids including isoleucine, valine, glutamate and finally glycerol, anserine and inosine. A one-way ANOVA was performed to statistically certify the difference in metabolite levels. The data suggest that 1H NMR coupled with chemometric is an efficient method to distinguish between non irradiated and irradiated beef samples and it could be proposed as a rapid screening method alternative to the high time consuming official methods.

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ORIENTATIONAL ORDER, MOLECULAR ORGANIZATION, AND DYNAMICS IN MIXTURES OF BENT-CORE AND ROD-SHAPED MESOGENS: A ^{2}H NMR STUDY

L. Calucci,[‡] C. Forte,[‡] M. Geppi[†]

[‡]Istituto di Chimica dei Composti OrganoMetallici, CNR-Consiglio Nazionale delle Ricerche, via G. Moruzzi 1, 56124 Pisa, Italy.
[†]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via risorgimento 35, 56126 Pisa, Italy.
E-mail: lucia.calucci@pi.iccom.cnr.it

In a recent work it has been shown that by mixing suitable bent-core and rod-shaped molecules one can form liquid crystals at room temperature that change their birefringence color at electric fields, thus opening up a path towards possible practical applications [1]. In particular, the calamitic compound 6OO8, exhibiting nematic (N) and tilted smectic C (SmC) mesophases, showed complete miscibility with the nematic bent-core mesogen ClPbis10BB; all mixtures had a N phase at relatively low temperatures and, in some concentration ranges, a Sm phase below the N one (Fig. 1).

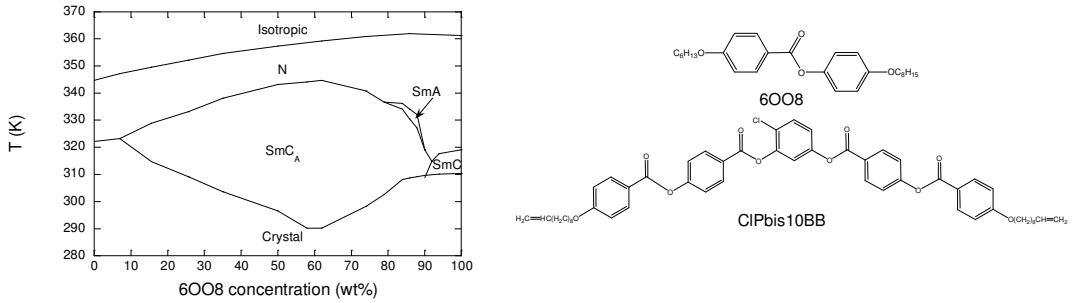

Fig. 1. Phase diagram and molecular structures of 6OO8/ClPbis10BB binary mixtures

In order to investigate structural and dynamic properties of ClPbis10BB/6OO8 mixtures, ^{2}H NMR spectroscopy techniques [2] were applied exploiting selectively deuterated isotopomers of both mesogens. The analysis of ^{2}H NMR spectra recorded in the liquid crystalline phases on several representative mixtures gave information on the orientational order properties and the molecular organization within the phases, as well as on the alignment properties upon application of a magnetic field. On the other hand, the analysis of ^{2}H longitudinal relaxation times (T_{1Z} and T_{1Q}) in 6OO8-d_2/ClPbis10BB mixtures and pure 6OO8-d_2 allowed the influence of the bent-core mesogen on the dynamics of the calamitic one to be highlighted [3].

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^{19}F FAST FIELD-CYCLING NMR RELAXOMETRY OF A NEMATIC LIQUID CRYSTAL

L. Calucci,[‡] S. Bubici,[‡] G. Ferrante,[†] M. Geppi[#]

[‡] Istituto di Chimica dei Composti OrganoMetallici, CNR-Consiglio Nazionale delle Ricerche, via G. Moruzzi 1, 56124 Pisa, Italy.
[†]Stelar s.r.l., via E. Fermi 4, Mede (PV), Italy.
[#]Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126 Pisa, Italy.
E-mail: lucia.calucci@pi.iccom.cnr.it

Fast field-cycling NMR was applied to investigate the field dependence of the ^{19}F longitudinal relaxation rate of the calamitic mesogen 4DBF2 fluorinated in two positions of the aromatic core (Fig. 1) [1]. 4DBF2 shows a nematic phase between 67 and 86 °C, with a supercooling of the mesophase down to room temperature, and its orientational order has been extensively investigated by ^{13}C, ^{19}F and ^{11}B NMR spectroscopies, as well as by dielectric and optical techniques [2, 3].

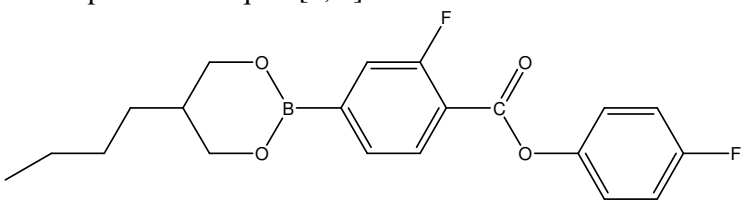

Fig. 1. Molecular structure of 4DBF2.

In the present work, ^{19}F relaxation rates (R_1) were measured at one temperature (90 °C) in the isotropic phase and at five temperatures (80, 75, 70, 65 and 60 °C) in the nematic phase of 4DBF2 using a Stelar SpinMaster FFC2000 relaxometer. The obtained dispersions, covering the Larmor frequency range between 10 kHz and 30 MHz, showed frequency dependences analogous to those previously observed in ^{1}H field-cycling measurements on nematogens [4, 5] and in the ^{19}F field-cycling measurements on C_6F_6 dissolved in a nematic solvent [6]. In particular, in the nematic phase a $\nu^{-1/2}$ dependence of R_1 was detected over a broad range, starting from the lowest frequency and ending to a plateau in the MHz region, while a smaller dispersion was observed at higher frequencies. The low frequency $\nu^{-1/2}$ dispersion disappeared in the isotropic phase, whereas comparatively small changes were found in the high frequency region. Moreover, in the nematic phase, R_1 increased by decreasing the temperature at a fixed Larmor frequency, and dips due to the presence of the quadrupolar nucleus ^{11}B were also detected. The experimental dispersions were analyzed on the basis of theoretical models for individual and collective motions in nematic liquid crystals.

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THE ROLE OF HIS 50 OF ALPHA-SYNUCLEIN IN Cu(II) BINDING

F. Camponeschi,[‡] D. Valensin,[‡] M. Luczkowski,[†] M.C. Baratto,[‡] M. Remelli,[§] H. Kozlowski,[†] G. Valensin[‡]

[‡] Department of Chemistry, University of Siena, Via A. De Gasperi 2, 53100 Siena, Italy
[†] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50383 Wrocław, Poland.
[§] Department of Chemistry, University of Ferrara, Via Luigi Borsari, 46, 44121 Ferrara, Italy
E-mail: camponeschi@unisi.it

The aggregation of α-synuclein (αS) is a critical step in the etiology of Parkinson's disease (PD) [1]. The interaction of Cu(II) with αS has been shown to enhance protein fibrillation and oligomerization rates in vitro [2], and it might represent the link between the pathological processes of protein aggregation and neuronal cell loss. Three different αS copper binding domains have been proposed: (i) a minimal copper binding site localized at the N-terminus of αS (residues 1-9), (ii) the His-50 imidazole and (iii) a low affinity binding site at the Asp- and Glu-rich C-terminus of the protein [3, 4]. The copper coordination at the N-terminus has been extensively characterized and it is generally accepted that it provides the highest affinity site. On the contrary, few studies on the second copper binding site (His-50) have been performed. In order to clarify the role played by His-50 in the binding of Cu(II) we characterized metal coordination to peptide fragments encompassing residues 45-55 of αS, including systems containing the inherited mutations E46K and A53T, as model peptides of the His-50 site. The speciation profiles at pH 6.5 and 7.5 have been determined through potentiometric titrations, and the stability constants have been used to estimate the dissociation constants of complexes corresponding to the binding modes at both pH values. Spectroscopic analyses allowed determination of the copper coordination sphere, its geometry and the structural constraints wherefrom the 3D structural models of the copper complexes could be obtained.

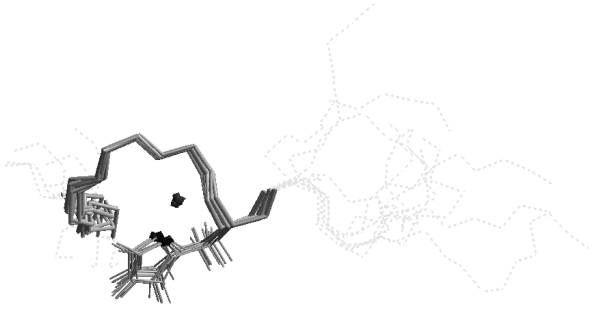

Fig. 1. The first 10 structures obtained for the Cu(II)-a$S_{45\text{-}55}$ complex.

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CP-MAS NMR DISCRIMINATION OF PURE ISOMORPHIC MANIDIPINE α/β AND OF THEIR MIXTURES

S. Capacchi,[‡] V. Mileo,[‡] S. Ghelli,[§] V. Zorin[†]

[‡] Chiesi Farmaceutici s.p.a, Corporate Preclinical Research & Development, Analytics & Early Formulations Dept., Parma, Italy: [§] Spinlab srl., Via Tamagno 3, 42048 Rubiera (RE), Italy
[†] Agilent Technologies, 10 Mead Road, Oxford Industrial Park, Yarnton, Oxford OX5 1QU, UK
E-mail: S.Capacchi@chiesigroup.com

Iperten® – Maniper® – Manivasc®– Artedil® Manidipine, a third-generation dihydropyridine calcium antagonist, is a first-line treatment, option for patients with essential mild-to-moderate hypertension [1,6]. Manidipine dihydrochloride (see Fig. 1) is able to block the entry of calcium into the arteriolar muscle cells, thus strongly counteracting the vasoconstriction always present in the hypertensive patient. This compound is an effective antihypertensive drug with a good tolerability profile and also has beneficial effects on renal function. Manidipine dihydrochloride is known in two polymorhic forms, α and β. The polymorph used for the product on the market is the β one which has a higher crystalline content than the less stable α form. As polymorphism of the active ingredients is demonstrated to be potentially involved in the bioavaiability [7], the maintenance of the β form in the tablets was in the past demonstrated via Raman spectroscopy. Recently we have decided to try a new technique such as Solid State NMR (SSNMR): the two pure isomorphs of manidipine α and β have been studied by CP-MAS NMR to discover remarkable differencies as the fingerprints in the SSNMR spectra [8,16].

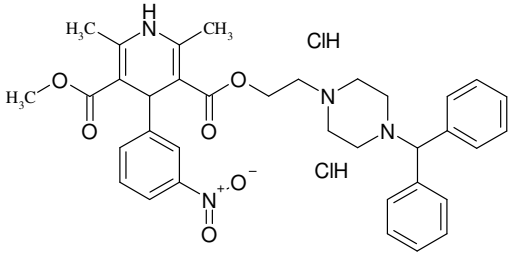

Fig. 1. Chemical structure of dihydrochloride manidipine.

The corresponding mixtures of manidipine α and β at different ratios (50-50%, 5-95%, 95-5%, 85-15%, 15-85%, 65-35%, 35-65% (w/w)) have been also investigated to try to detect and quantitate the amount of one pure form into the other.
Different regions of the CP-MAS NMR spectra, where the pure forms remarkably differed, were chosen and compared, and even small amounts (up to 5%) of one form into the other manidipine form were observed.

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VIBRATIONAL MOTIONS IN THE SOLID STATE INVESTIGATED BY NMR AND AB INITIO STUDY: EFFECTS ON ^{13}C CHEMICAL SHIFT TENSORS OF IBUPROFEN

E. Carignani, S. Borsacchi, A. Marini, B. Mennucci, M. Geppi

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, v. Risorgimento 35, 56126 Pisa (Italy)
E-mail: elisa.carignani@for.unipi.it

Large-amplitude motions, such as interconformational jumps, π-flips and internal rotations, can be characterized in detail by Solid State NMR (SSNMR) spectroscopy, determining their frequency and geometry. On the other hand, the effects on SSNMR observables of small-amplitude motions, such as vibrations and librations, due to their much higher frequency are usually quite difficult to be clearly detected and analysed and, indeed, only few studies have been reported in the literature [1]. Recently, by exploiting the measurement and the combined analysis of a variety of spectral and relaxation properties of ^{1}H and ^{13}C nuclei, we could obtain a detailed characterization of all the reorientational and interconformational motions taking place in the acid form of solid Ibuprofen [2]. Thanks to the very slow motion experienced by the phenyl ring ($\tilde{\pi}$ with characteristic frequency of about 100 Hz at room temperature), we could investigate the vibrational motions of this fragment by exploiting their effects on the ^{13}C chemical shift tensors ($\boldsymbol{\delta}$). ^{13}C $\boldsymbol{\delta}$ both measured through 2D-PASS experiment [3] and calculated, in the absence of motions, through DFT methods [4]. The observed discrepancies between experimental and calculated $\boldsymbol{\delta}$could be successfully explained taking into account the out- and in-plane CH bending and a libration of the whole phenyl ring about its para axis. By fitting the experimental $\boldsymbol{\delta}$ suitable motional models applied to the calculated tensors, the amplitudes of these motions could be quantified. The very good reproduction of the experimental data clearly showed that this approach represents a relatively simple but very effective method for the identification and characterization of the vibrational motions most affecting ^{13}C chemical shift tensors. Moreover, given the absence of internal reorientational motions, this represents an ideal case which provides the extent of the contribution of vibrational motions to $\boldsymbol{\delta}$which should be taken into account in dynamic studies based on chemical shift tensors.

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CHARACTERIZATION AND RELAXOMETRIC ANALYSIS OF GdOBETA, A NEW EXAMPLE OF STABLE q = 2

C. Cassino,[‡] M. Fekete,[‡] G.B. Giovenzana,[†] L. Tei,[‡] M. Botta[‡]

[‡]Dipartimento di Scienze dell'Ambiente e della Vita, Università degli Studi del Piemonte Orientale "A. Avogadro", V.le T. Michel 11 Alessandria, Italy .
[†]DISCAFF and DFB Center, Università degli Studi del Piemonte Orientale "A. Avogadro", via Bovio 6, Novara, Italy
E-mail: claudio.cassino@mfn.unipmn.it

Due to contrast agents sensitivity limitation of MR imaging and to the development of increasingly higher magnetic field strength MRI scanners, the synthesis of ligands capable of forming Gd^{III} complexes with two (or more) H_2O molecules bound in the first coordination sphere has been pursued for long time. To this aim, both acyclic and macrocyclic polyazapolycarboxylate ligands have been thoroughly investigated to obtain Gd^{III} complexes with q=2 without compromising thermodynamic and kinetic stabilities. In fact, a further stringent condition for new chelating ligands is that the complex formed with the gadolinium ion has to be thermodynamically and kinetically very stable (logK > 20) and for that, reducing the number of donor atoms has normally the drawback of reducing also the stability. It is therefore important to study in deep the solution thermodynamic and kinetic behavior of the Ln^{III} complexes with novel ligands in order to ascertain their possible use in clinics or in pre-clinical studies.
Thus, the ^{1}H and ^{17}O NMR relaxometric properties of the Gd^{III}-complex of OBETA (2,2'-bis-[di-(carboxymethyl)-amino]-diethyl-ether), see Fig. 1, have been investigated in aqueous media as a function of pH, temperature and magnetic field strength.

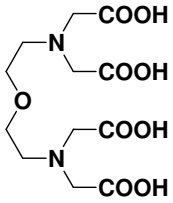

Fig. 1. OBETA (2,2'-bis-[di-(carboxymethyl)-amino]-diethyl-ether).

The Gd^{III} complex with the heptadentate ligand OBETA has been investigated in terms of solution equilibria and relaxometric properties. The decrease of the denticity of the ligand (from 8 to 7) surprisingly results in an increase of the thermodynamic stability of the complex as compared with the corresponding GdEGTA complex. In addition, preliminary results indicate that also the kinetic inertness towards the transmetallation reaction with Cu^{II} of GdOBETA is sensibly higher than for GdEGTA. The metal chelate has two water molecules in its inner coordination sphere with a rather short residence lifetime. These water molecules are not displaced by dissolved carbonate at high pH values. Future work will focus on the possibility to further increase the stability by suitable chemical modification of the ligand.

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NMR SPECTROSCOPIC STUDIES OF THE INTERACTION BETWEEN A BILE ACID BINDING PROTEIN AND MEMBRANE MIMETIC SYSTEMS

A. Ceccon, F. Favretto, S. Zanzoni, M. D'Onofrio, M. Assfalg, and H. Molinari

Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134, Verona, Italy.
E-mail: alberto.ceccon@univr.it

In the enterocytes and in the hepatocytes, bile acids transport is achieved mainly through a protein mediated mechanism that involves transmembrane and intracellular transporters. Bile acids (BA) are practically insoluble cholesterol derived molecules which, together with phospholipids, cholesterol and bilirubin, represent the principal constituents of bile. These molecules undergo, in the body, a recycling pathway between the intestine and the liver, called "enterohepatic circulation", which assure the recovery of these molecules and their subsequent reutilization. Alterations of intracellular BA transport are linked to cholestatic diseases and BA accumulation leads to liver damage and may promote the development of liver tumors.[1]
The intracellular carriers for BA are a family of soluble 14KDa proteins called BABPs that were shown to bind at most two bile salt molecules with high affinity within their internal cavity and to transfer non-polar molecules between cell compartments. The close relationship between the fatty acid binding protein (FABP) and BABP subfamilies has suggested that the uptake of BA from donor membranes by BABPs may occur with mechanisms similar to fatty acid uptake by FABPs that seems to involve a direct collision of the protein with the membrane.
BABP/membrane/BA interactions are largely unexplored. Pedò et al. show that BA gradient in hepatocytes would shift the equilibrium toward the holo free in-solution state in the proximity of the basolateral membrane and toward the apo membrane-bound state in the proximity of the apical membrane allowing the release of BAs.[2]
Using a combination of biophysical and biochemical methods Montich et al. conclude that the interaction between L-BABP and lipid membranes is driven by electrostatic forces and a protein conformational change is associated with this event.[3]
We explored the possibility to use NMR techniques to investigate the interaction between BABPs and membrane mimetic systems in order to describe the determinants and the binding mode. NMR experiments provided insight into the protein behaviour upon interaction with phospholipid vesicles and allowed us to determine binding affinities. We further explored the way by which phospholipids composition, vesicle size, curvature and ionic strength modulate such interactions and affect the protein conformational changes.

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A NEW p-H_2 HYPERPOLARISED PROBE RELATIVE TO THE PYRAZOLO[1,5-*A*]PYRIMIDINEACETAMIDE DPA-713, A VECTOR TO TARGET PERIPHERAL BENZODIAZEPINE RECEPTORS

E. Cerutti,[‡] A. Viale,[‡] A. Damont,[†] F. Dollé,[†] S. Aime[‡]

[‡]Dept. of Chemistry IFM and Molecular Biotechnology Centre, V. Nizza 52, 10126 Torino, Italy
[†] CEA, I^{2}BM, Service Hospitalier Frédéric Joliot, 4 Place du Général Leclerc, F-91406 Orsay, France
E-mail: erika.cerutti@unito.it

Much attention has been devoted in recent years to the development of hyperpolarized ^{13}C containing molecules for ^{13}C-Magnetic Resonance Imaging [1]. Two procedures can be used to prepare ^{13}C-hyperpolarized molecules, namely Dynamic Nuclear Polarization (DNP), and Para Hydrogen Induced Polarization (PHIP). The latter consists of the catalytic para-hydrogenation of an unsaturated molecule, followed by transformation of para-hydrogen spin order into net heteronuclear hyperpolarization. Unsaturated substrates, containing a ^{13}C nucleus characterized by long relaxation time scalarly coupled with the added para hydrogen atoms, which are readily hydrogenated to yield water-soluble products, are necessary and this is the major limitation of the technique.
One may think of introducing an hydrogenatable synthon containing a long T_1 carbon centre in a molecule of interest, whose biodistribution / transformation has to be assessed. We have recently proposed such an approach by conjugating glucose to an hydrogenable synthon that contains a long T_1 functionality [2]. Here, a second example of this approach is proposed. It deals with the synthesis and testing of a novel carbon-13-enriched butynoic ester derivative of DPA-713, the lead compound of a recently developed series of ligands targeting the peripheral benzodiazepine receptors [3,4]. The butynoic moiety resulted to be readily hydrogenated when reacted with para-H_2. Upon applying a field cycling procedure, the spin order of para-H_2 added hydrogens is transferred to the ^{13}C carboxylate moiety yielding a signal enhancement of about 4500 times. A ^{13}C-MR image has been acquired by using the ^{13}C RARE acquisition protocol on a 10 mM solution (Figure 1). The main drawback to the in vivo use of DPA-713 butynoate is related to its low solubility in aqueous systems.

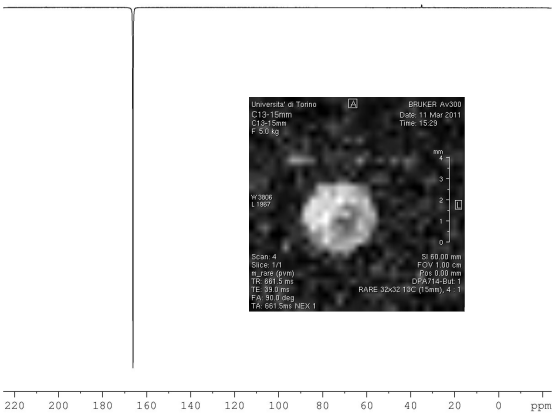

Fig. 1. ^{13}C spectrum of HP DPA-713-butenoate (14 T) and corresponding ^{13}C RARE image (7 T).

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SYNTHESIS AND STRUCTURE ELUCIDATION OF NEW TETRACYCLIC COUMARINES AND QUINOLINONES DERIVATIVES

S. Chimichi, M. Boccalini, A. Matteucci

Chemistry Department, University of Firenze, Via della Lastruccia 13, 50019 Sesto F.no, Italy
E-mail: stefano.chimichi@unifi.it

Following our research on the synthesis of coumarines and quinolinone derivatives [1-3], we report here the obtainment of new tetracyclic compounds of type reported in Fig.**1**. Seven-membered heterocycles are an important tool in organic and pharmaceutical chemistry because of their interesting biological activity [4].
Thus, reaction of 3-acyl-4-methoxy(tosyloxy)-coumarin or -quinolinone with (*E*)- or (*Z*)-1,2-cyclohexanediamine give rise in quantitative yields to racemic mixture of the required products. Structure elucidation of the latter compounds was carried out by NMR experiments (HSQC, HMBC, H2BC, and NOESY or NOESY1D).

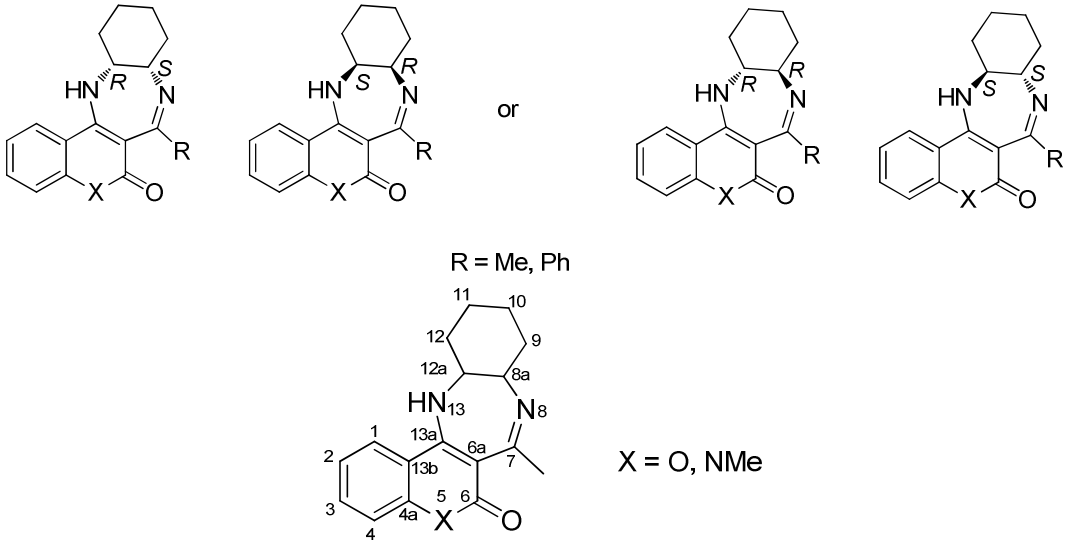

Fig. 1. The new tetracyclic compounds and the numbering system.

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SWITCHED ANGLE SPINNING OFF-MAS NMR TO PROBE INTER- AND INTRA-MOLECULAR DISTANCES IN MULTIPLE-SPIN SYSTEMS

M. Concistrè[1], A.C. Sauerwein[1], P. Thureau[2], M.H. Levitt[1]

[1]School of Chemistry, University of Southampton, Southampton, UK
[2]Université Aix-Marseille, Marseille, France
E-mail: mariac@soton.ac.uk

Solid-state NMR measurements of anisotropic interactions provide information on molecular structure. A variety of methods have been developed accordingly, and those providing structural information through dipolar couplings are among the most important and powerful. However, the determination of weak dipolar couplings in samples containing clusters of many coupled nuclei is one of the major challenges in solid-state NMR. We address this problem by developing a new solid-state NMR methodology that, combining off-MAS, frequency selective spin echoes, and multiple quantum filtering is able to give a good estimate of weak dipolar coupling even in presence of strong dipolar interactions [1,2,3]. This method provides the interesting structural information contained in long inter-nuclear distances (> 4 Å).

The technique recouples weak dipolar interaction (the frequency-selective pulses therein can be calibrated to isolate any desired internuclear distance) by spinning the sample off the magic angle value. However, large missets from the magic angle are accompanied by a loss in spectral resolution due to the simultaneous recoupling of other anisotropic interactions (CSA). To overcome this problem, the spinning sample rotor is switched back at the magic angle during data acquisition. This is performed by a switched-angle spinning probe which allows a precise mechanical switching of the rotation angle up to 20° off the magic angle, along with an independent measurement of the spinning angle via a Hall-effect device [4].

In this poster measurements of weak internuclear couplings between ^{13}C spin pairs separated by distances exceeding 6 Å are demonstrated.

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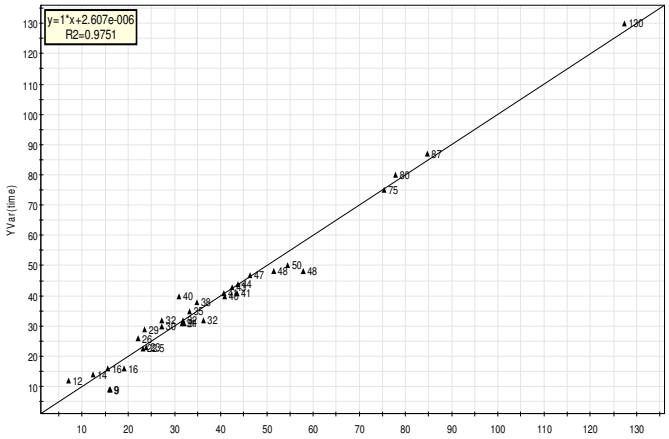
C.S.I.-NMR. APPLICATION OF NMR TO FORENSIC ANALYSIS. A PRELIMINARY RESULTS

N. Culeddu,[‡] M. Chessa[¥], V. Migaleddu[¥], L. Barberini[±], A. Faa[†] and E. D'Aloja [†]

[‡]CNR Istituto di Chimica Biomolecolare – Sezione di Sassari, Via La Crucca, 3 07040 Sassari
[†] D'Aloja Istituto di Medicina Legale e Anatomia e Istologia Patologica via Porcell n.4 - 09124 Cagliari
[¥]SMIRG (Sardinian Mediterranead Imaging Research Group) Via Stintino 2 07100 Sassari
[±]Dip.to Scienze Cardiovascolari e Neurologiche Cittadella Universitaria di Monserrato - SS Sestu KM 0,700 - CAP 09042
E-mail: nicola.culeddu@icb.cnr.it

The estimation of the time since death (Post-Mortem interval, PMI) still remains a main issue in forensic science and legal medicine. Several approaches have been so far proposed from either an analytical point of view or a qualitative-quantitative one [1]. Here we propose a new technical approach to this issue based on ^{1}H-NMR metabolomic profiling for the simultaneous determination of several metabolites represented into the vitreous humor. The rationale for the implementation of this procedure among the PMI estimation forensic tools lies on the fact that single analyte determination (such as potassium, magnesium, xanthine, hypoxanthine, lactate and more) is inclined to interindividual variations unrelated to the time since death. An ^{1}H-NMR metabolomic profiling analysis offers the opportunity to simultaneously detecting qualitative and quantitative modifications of the main products of the principal metabolic pathway, minimizing the potential effects of variables unrelated to the physiological process of decomposition. The preliminary results here reported seem to be encouraging in addressing the issue of a reproducible and robust system to estimate the time since death, but a larger sampling is needed to confirm our data.



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SEPARATION OF INTRA- AND EXTRACELLULAR HYPERPOLARIZED SIGNALS USING PARAMAGNETIC GADOLINIUM COMPLEXES

S. Aime,[‡] V. Daniele,[‡] F. Reineri,[‡] M.H. Lerche,[†] P.R. Jensen,[†] M. Karlsson[†]

[‡] Dipartimento di Chimica IFM, University of Torino, Via P. Giuria 7, 10125 Torino, Italy
[†] Albeda Research, Gamle Carlsberg Vej 10, 2500 Valby, Denmark
E-mail: valeria.daniele@unito.it

A hyperpolarized state is defined as a state in which the nuclear spin populations are altered with respect to the equilibrium value (Boltzmann equation). Hyperpolarization can lead to an increase in the NMR signal intensity up to 10^5. This increased sensitivity allows us to detect nuclei other than protons in clinically useful times, making it possible to obtain images free from background noise, because of the low natural abundance of heteronuclei such as ^{13}C and ^{15}N. A fundamental requisite for a hyperpolarized molecule to be used in magnetic resonance imaging (MRI) is the long relaxation time of the heteronucleus of interest. In fact, there must be a time long enough to acquire the image before the relaxation processes re-establish the equilibrium spin populations, thus causing loss of signal.
Dynamic nuclear polarization (DNP) is probably the most versatile hyperpolarization method [1]. The material to be polarized is doped with a stable radical species, placed into a high magnetic field, brought to very low temperature (1-2 K) and irradiated with a proper microwave frequency. After the polarization transfer, the rf is switched off, the sample is rapidly dissolved in a hot buffer, still inside the magnetic field, and injected into live animals or added to cells suspensions for MRI acquisitions.
The most challenging application of hyperpolarized probes is the assessment of metabolic pathways, since they allow direct visualization of key metabolites' formation [2]. To improve the diagnostic potential of this technique, it is highly desirable to obtain distinct signals from intra- and extracellular metabolites originating from an injected hyperpolarized agent. Paramagnetic Gd^{III} complexes can distribute only in the extracellular space and lead to a marked increase of relaxation rate of any molecule that goes into their outer and (if possible) inner coordination sphere. Therefore, Gd^{III} complexes may selectively quench the polarization of extracellular metabolites.
The effect of different Gd^{III} complexes on the relaxation rate of $^{13}C_1$ signal of butyrate was quantified by ^1H and ^{13}C relaxometry. Then the most efficient complex [Gd(DO3A)] was used to test the method: $^{13}C_1$-butyrate was hyperpolarized by means of DNP, was put into a yeast cell suspension and, after 10 seconds, a solution of [Gd(DO3A)] was added. Since DO3A acts as heptadentate ligand, leaving two coordination vacancies for the coordination of $^{13}C_1$-butyrate, the extracellular polarized signal of butyrate was instantaneously and selectively quenched.
The obtained results demonstrated that the use of paramagnetic complexes can increase the amount of information that derives from *in vivo* application of hyperpolarized probes.

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ANISOTROPIC ENHANCED WATER DIFFUSION IN SCLEROGLUCAN/BORAX SWELLED TABLETS STUDIED BY PGSE NMR

C. Di Meo[‡], T. Coviello[‡], P. Matricardi[‡], F. Alhaique[‡], D. Capitani[†] and R. Lamanna[⊥]

[‡] Department of Drug Chemistry and Technologies, University of Rome ''Sapienza'', P.le Aldo Moro 5, 00185 Rome, Italy
[†] Magnetic Resonance Laboratory Annalaura Segre, Institute of Chemical Methodologies, CNR Research Area of Rome, via Salaria Km 29.300, 00016 Monterotondo Stazione, Rome, Italy
[⊥] ENEA, Trisaia Research Center, ss 106 Jonica Km 419.5, 75026 Rotondella, MT, Italy
E-mail: chiara.dimeo@uniroma1.it

Scleroglucan (Sclg) is a branched homopolysaccharide secreted by fungi of the genus Sclerotium consisting of a main chain of (1–3)-linked β-D-glucopyranosyl units bearing, every third unit, a single β-D-glucopyranosyl unit linked (1–6). In the presence of borax Sclg is able to form a self-sustaining gel, that has been tested as a matrix for the release of model drugs with different steric hindrance. Sclg tablets, obtained by unidirectional compression of freeze dried hydrogels, show a peculiar swelling behaviour: in fact, the swelling process occurs almost exclusively in the axial direction [1]. Water molecules diffuse inside this peculiar network, which modifies their diffusion properties.
Molecular dynamic (MD) simulations, along with atomic force microscopy (AFM) analyses, evidenced the ability of borax to keep an ordered configuration of parallel-aligned triplexes, which leads to the formation of nanochannel-like structures [2].
Sclg and Sclg/borax systems were then studied by the pulsed gradient spin-echo (PGSE) NMR technique. The effects of the compression of the original polysaccharidic dry matter on the diffusion properties of water were also studied in connection with the direction of the applied stress. Actually, an anomalous and anisotropic enhanced diffusion behaviour of water molecules is observed in swollen Sclg/borax tablets. This superdiffusive process was evidenced by a power-law dependence of the mean square displacement (MSD) with diffusion time (Fig. 1). Enhanced diffusion is interpreted in the framework of a solvent mediated diffusion model [3].

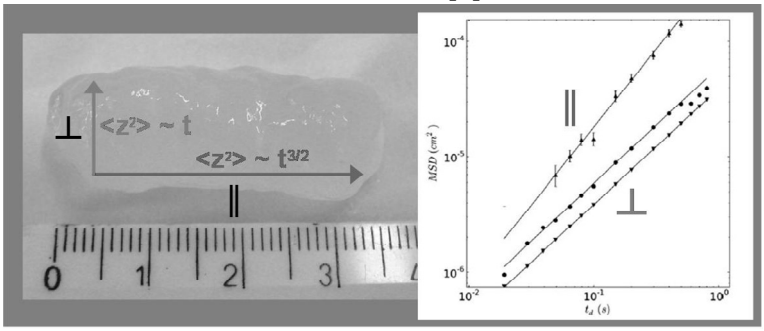

Fig. 1. Mean Square Displacement (MSD) of water in Sclg/borax tablets vs. the diffusion time. Circles and up triangles represent, respectively, the slow and fast diffusion components along the compression direction. The down triangles represent the diffusion along the direction perpendicular to the compression.

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HUMAN LIVER FABP AS TRANSPORTER OF BILE ACIDS

M. D'Onofrio, F. Favretto, A. Ceccon, S. Zanzoni, M. Assfalg, H. Molinari

Department of Biotechnology, University of Verona, Strada le Grazie 15, Verona, Italy
E-mail: mariapina.donofrio@univr.it

Liver Fatty Acid Binding Protein (LFABP) belongs to a family of 14-15kDa intracellular lipid binding proteins (iLBP) which have the ability of binding long chain fatty acids (FA) and a variety of other small hydrophobic ligands. The putative functions of LFABP, present in the hepatocytes at high concentration, include lipid uptake and transport, regulation of lipid metabolism [1] and cytoprotection against oxidative stress [2]. In addition LFABP may have a role in transporting exogenous or other endogenous ligands in addition to FAs.
A specific liver bile acids (BA) carrier is missing in mammalian species and LFABP has been proposed to play this function [3]. A number of NMR experiments have been performed using FA, BA, as well as lipid-functionalized drugs to feature the binding with human LFABP.

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FLORAL MARKERS IN HONEYS OF VARIOUS BOTANICAL ORIGINS

E. Schievano, C. Facchin, E. Morelato, M. Stocchero[#] and S. Mammi

Dipartimento di Scienze Chimiche, Università di Padova, Via Marzolo 1, 35131Padova, Italy
[#]*S-IN Soluzioni Informatiche, Via G. Salvemini 9, 36100 Vicenza, Italy*
E-mail: zanzawest@alice.it

In view of the expanding global market, authentication and characterization of the botanical origin of honey have become important issues. Many studies have been performed with the aim of searching for reliable chemical markers indicative of floral origin of honey samples [1].
In this work, we present the results of our studies carried out on six unifloral Italian honey types (acacia, linden, orange, eucalyptus, chestnut, and honeydew) provided, by either Rigoni S.p.A. or from Veneto apiaries. Using a metabolic approach [2], we analyzed the NMR spectra of chloroform extracts. These spectra, containing a high number of organic compounds, including less volatile ones, can be considered fingerprints of each floral origin. The predictive component of O2PLS-DA models (Schievano et al., submitted), was used to highlight the signals corresponding to the putative markers for the individual classes of honey. A few metabolites responsible for the discrimination in different honey types were purified by silica-gel column, using a different protocol for each honey type. An accurate analysis of two-dimensional (2D)-NMR spectra and of data from mass spectrometry, allowed us to characterize the biomarkers. Many of the compounds identified, e.g., the markers for acacia and chestnut, are bioactive molecules [3, 4].

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NMR STUDIES OF THE INTERACTION OF HLFABP WITH UIS, A PROTEIN INVOLVED IN MALARIA PARASITE DEVELOPMENT

F. Favretto, M. D'Onofrio, S. Zanzoni, A. Ceccon, M. Assfalg and H. Molinari

Department of Biotechnology, University of Verona Strada Le Grazie 15, 37134 Verona Italy.
E-mail: filippo.favretto@univr.it

Malaria infection is initiated in a human host during the blood meal of an infected *Anophles* mosquito, injecting invasive sporozoites via the skin, which represents the liver stage of malaria parasites. During this stage 25 genes are specifically up regulated and *UIS3* (Up regulated in Infective Sporozoites gene 3) is essential for sporozoite development in infected hepatocytes. This gene codifies for a protein, called UIS, localized to the sporozoite parasitophorous vacuolar membrane (PVM). It has been noted that most of the fatty acids and lipids in the parasite PVM arise from the host cell, and it has been proposed that UIS plays an important role in fatty acid/lipid import during phases of rapid parasite growth. The crystal structure of *Plasmodium falciparum* $UIS_{130-229}$ in complex with phosphatidylethanolamine has been resolved and its interaction with rat liver Fatty Acid Binding Protein (L-FABP) has been assessed via pull-down assay [1]. Further studies using the yeast-two hybrid system and coimmunoprecipitation experiments in mouse have suggested that also *Plasmodium yoelii* UIS3 interacts with rat liver Fatty Acid Binding Protein (L-FABP) and that a down regulation of the latter protein severely impaired the development of rodent malaria parasites [2].
In this work, the interaction between $UIS_{130-229}$ and hL-FABP, was investigated using NMR spectroscopy. $UIS_{130-229}$ was cloned, expressed and purified from *E. coli* cell culture. We then titrated ^{15}N-HLFABP in its apo and oleate bound form with unlabelled UIS. A series of ^{1}H-^{15}N HSQC spectra have been recorded and analyzed in order to characterize the interaction. Based on obtained data we hypothesized that $UIS_{130-229}$ can uptake the hydrophobic cargo directly from hL-FABP.
Successively we expressed and purified ^{15}N labeled $UIS_{130-229}$ that was characterized through ^{1}H-^{15}N HSQC spectra. The domain was titrated with unlabelled hL-FABP in complex with oleate and different ^{1}H-^{15}N HSQC spectra were acquired. We did not record any significant chemical shift variation in the peaks position, upon protein addition. Further investigations need to be done in order to characterize the uptake of fatty acids or other lipid molecules by $UIS_{130-229}$ in the presence of hL-FABP.

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REVERSIBLE UNFOLDING OF A LIPOCALIN PROTEIN: CONTRIBUTE OF A FREE CYSTEINE MUTANT OF THE ALLERGEN Mus m 1.0102

E. Ferrari[‡], T.A. Pertinhez[‡], E. Casali[‡], S. Burastero[†] and A. Spisni[‡]

‡ Dipartimento di Medicina Sperimentale, Sez. Chimica e Strutt. Biochimica, Università di Parma
† Fondazione San Raffaele del Monte Tabor, Milano, *elena.ferrari@unipr.it*

The mouse urinary protein Mus m1.0102 belongs to the lipocalin superfamily and is one of the most important allergens for mouse allergic patients [1,2]. It acts as a pheromone stabilizer, providing a slow release mechanism of pheromonal ligands into the environment. Being excreted into the environment, the protein can easily reach the airways, playing an important role in asthma morbidity. Exposure and sensitization to these allergens are common in occupational settings as well as in inner-city homes.
The structural, functional, and biochemical features that allergens have in common and that could explain their ability to elicit IgE antibody responses, are permanently under study. The Mus m1.0102-C138S mutant, in which the unique free Cys was substituted by a Ser has been characterized structurally and functionally. Wild type Mus m1.0102 and Mus m1.0102-C138S were expressed in *Pichia pastoris*. The structural approach combined different spectroscopic techniques: heteronuclear NMR, circular dichroism (CD) and intrinsic fluorescence. An IgE-mediated degranulation test was used to assess the allergenicity of the protein samples.
While the structural conformations of Mus m1.0102 and Mus m1.0102-C138S resulted quite comparable when evaluated by means of circular dichroism and intrinsic fluorescence spectroscopy, heteronuclear NMR measurements ($^{15}N/^{1}H$ HSQC spectra analysis) yield more detailed insights into the structural consequences of the site-directed mutagenesis: we could observe that the backbone structural alterations were mainly concentrated in the region of the mutation, involving residues nearby in space.
Equilibrium unfolding of Mus m1.0102 and Mus m1.0102-C138S, induced by GndHCl, was monitored by far-UV CD and intrinsic fluorescence spectroscopy, and showed the mutant to be more sensitive to the denaturant conditions than the native form. Successful refolding was obtained only with the mutant, which, after extensive dialysis, regained the initial elements of secondary-tertiary structures and repositioned the Trp19 residue in its native hydrophobic environment, thus recovering the native fluorescence properties.
The IgE-mediated degranulation was quantified using a RBL cell line, permanently transfected with the human receptor for IgE. C138S mutant capability to induce degranulation was higher than Mus m1.0102, being recognized with higher efficiency than the wild type form.
The mutation changed the native structure to a highly cooperative and compact folded unit, able to refold after chemical denaturation. Nevertheless, the marked destabilization observed by the replacement of cysteine with the isosteric serine residue may reflect the inability of its structural environment to tolerate short polar residues; the substitution seems to favour a partial exposure of the surrounding hydrophobic side chains that might favour protein dimerization, which is expected to stimulate the allergenic reaction [3].

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USE OF YB-HPDO3A, AN ANALOGUE OF CLINICALLY SAFE GADOTERIDOL, AS MRI-PARACEST PH SENSOR

G. Ferrauto, D. Delli Castelli, M.C. Caligara, E. Terreno and S. Aime

Department of Chemistry IFM and Molecular and Preclinical Centers, Via Nizza 52, Turin, Italy
E-mail: giuseppe.ferrauto@unito.it

MRI-CEST contrast agents (CAs) based on lanthanide are emerging as a new promising class of MRI contrast agents. They are frequency-encoding probes and this characteristic makes them useful for novel applications such as pH and temperature mapping as well as multiplex detection in cell tracking and as responsive agents. Numerous proof of concept of these properties have been reported so far, making PARACEST agents very interesting for a clinical application. Unfortunately their development appears to proceed rather slowly cause of low sensitivity and the necessity to perform overall safety MRI-CEST experiment. In order to simplify and accelerate the clinical use of these CAs, we investigated the potentiality of chemicals already approved for human use to generate CEST contrast. In particular, analogues of the clinically approved Gadoteridol (Gd-HPDO3A), in which Gd was substituted with other lanthanides, were investigated. The coordinating hydroxyl protons of HPDO3A are an interesting pool of exchangeable protons, whose exchange rate is in agreement with the $\Delta\omega \geq kex$ condition necessary for the generation of CEST contrast.

In this work different Ln-HPDO3A were studied to evaluate which could be the best lanthanide to complex to HPDO3A in order to generate a good CEST contrast. It has been shown that Ln-HPDO3A complexes are present in solution as diastereoisomers with distinct OH absorptions that can be selectively saturated in CEST experiments (See fig.1). This opened the possibility to realize a ratiometric method that make CEST effect independent from the probe concentration. The different Ln-HPDO3A complexes showed different characteristics as the chemical shift of coordinating hydroxyl protons (it spread out over a very large range of ca. 400ppm) and the response to the pH and to the temperature. Among the other Ln-HPDO3A complexes, Yb-HPDO3A appeared to be the best as pH and temperature sensor, with a sensitivity threshold as low as 2 mM. The potential of this has been evaluated *in vitro* and *in vivo*. In particular 0.2 mmol/kg of Yb-HPDO3A was injected i.v. into melanoma-bearing mice to measure pH in the kidneys and in the tumour region using the ratiometric calibration. These results have been shown that Yb-HPDO3A can be considered a promising, clinically safe paramagnetic CEST agent for simultaneous pH and temperature determination and that Ln-HPDO3A PARACEST agents are excellent candidates for multiplex detection.

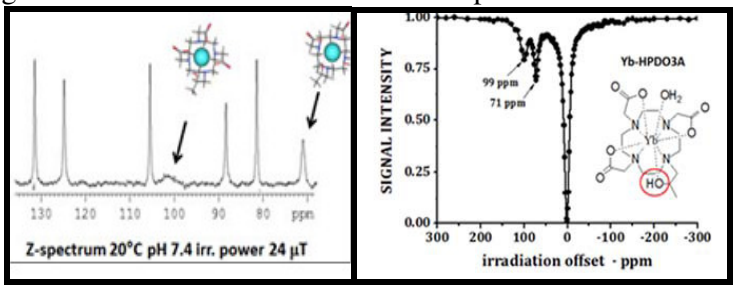

Fig. 1. Yb-HPDO3A as MRI-CEST pH sensor. Left: 1H NMR spectrum that shows the resonance of the two OH protons of the two diasteroisomers. Right: Z-spectrum that shows two different CEST absorption.

INTERACTIONS OF CELLULAR RETINOL CARRIERS WITH MODEL MEMBRANES: CLUES FOR LIGAND DELIVERY

L. Franzoni[1], F. Baroni[1], D. Cavazzini[2], G.L. Rossi[2] and C. Lücke[3]

[1]Department of Experimental Medicine, University of Parma, 43100 Parma, Italy;
[2]Department of Biochemistry and Molecular Biology, University of Parma, 43100 Parma, Italy;
[3]Max Planck Research Unit for Enzymology of Protein Folding, Halle (Saale), Germany.
E-mail: lorella.franzoni@unipr.it

Vitamin A trafficking and metabolism are regulated inside the cell primarily by specific high-affinity carriers called cellular retinol-binding proteins types I and II (CRBP-I, CRBP-II). They belong to a large family of ubiquitous intracellular lipid-binding proteins (i-LBPs) that feature a β-barrel structure with an internal water-filled cavity, where the hydrophobic ligand is bound. Expression patterns, affinities for retinoids, as well as interactions with enzymes and membranes are unique to each CRBP, although both homologs exhibit the same overall structural topology and identical retinol-binding motifs. The goal of our research is to elucidate the molecular basis of the drastic discrepancies between CRBP-I and CRBP-II.

Based on ^{15}N relaxation dispersion, line-shape analysis as well as H/D exchange, we have demonstrated major differences in the backbone dynamics of these two CRBP types, which indicated diverging mechanisms of ligand entry into the binding cavity [1-4].

Taking into account that both proteins deliver retinol to membrane-bound enzymes, either for esterification with fatty acids or for oxidation to retinaldehyde, we have performed a series of NMR and Circular Dichroism titration experiments in the presence of biomembrane mimetic systems. The data again suggest a different behaviour of holo CRBP-I with respect to holo CRBP-II. Transfer of retinol from CRBP-I seems to be mediated by interactions of the protein portal region with the phospholipid vesicles, whereas a diffusional process seems to apply for CRBP-II.

A comparison of our results with earlier ones obtained for related proteins [5, 6] will be discussed to extend the knowledge about the lipid release mechanism in the i-LBP family.

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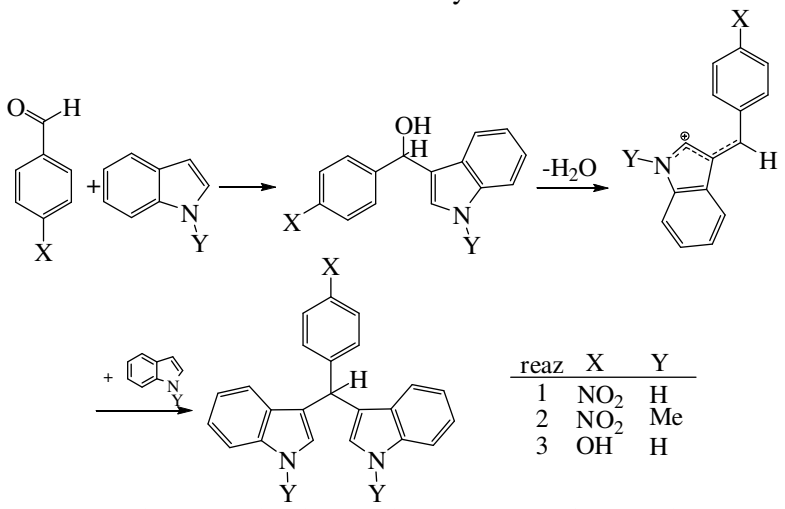
NMR STUDY OF THE SOLID-STATE REACTION BETWEEN INDOLE AND AROMATIC ALDEHYDES

K. Gaglioti,[a] M. Barbero,[b] M.R. Chierotti,[a] R. Gobetto[a]

[a]Department of Chemistry I.F.M., Via P. Giuria 7, 10125 Turin, Italy
[b]Department of General Chemistry and Organic Chemistry , Via P. Giuria 7, 10125 Turin, Italy
E-mail: katia.gaglioti@unito.it

Solvent-free organic syntheses are gathering increasing interest from the viewpoint of the green chemistry [1]. Furthermore solid-state reactions have the advantages of excellent yields, shorter reaction times, and mild reaction conditions [2].
In this work we discuss the Friedel-Crafts hydroxyalkylation reaction between indole and aromatic aldehydes. This electrophilic aromatic substitution reaction is carried out in solvent-free conditions and in the absence of catalyst.



Reaction mechanism between indole and aromatic aldehydes

Solid powdered aldehyde and indole liquefy almost immediately upon contacting each other after heating for few seconds (with a hot gun) without intervention of grinding. After mixing the reagents, the reaction proceeds through the formation of an eutectic phase melted at temperature below 25°C.
The versatility of solid-state NMR allowed the characterization of both melted and solid phases through different pulse sequences: direct excitation coupled with high power decoupling (DE) and cross polarization (CPMAS), respectively. By means of DE experiments, intermediate species in the melt could be observed and characterized providing evidences for elucidating the reaction mechanism. Furthermore, the CPMAS technique allowed the characterization of solid products that, due to solvent-free methods, are hardly obtained as single crystal suitable for the X-ray analysis [3].

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SOLID-STATE NMR AND XRD STUDIES OF WATER VAPOUR UPTAKE/RELEASE ON A WHEEL AND AXLE METALLORGANIC SYSTEM

R. Gobetto,[‡] M.R. Chierotti,[‡] A. Bacchi,[†] P. Pelagatti,[†] A. Sironi[⊥]

[‡]Department of Chemistry I.F.M., V. Giuria 7 Torino, Italy
[†]Department of Chemistry GIAF, Parco Area Scienze 17/A Parma, Italy
[⊥]Department of Chimica Strutturale e Stereochimica Inorganica, V. Venezian 21 Milano,Italy
E-mail: roberto.gobetto@unito.it

Wheel and axle systems, where two bulky groups (wheels) are connected by a rigid linear spacer (axle), have been extensively studied for their remarkable clathrating properties with hydrogen bond-acceptor solvents [1]. Here we present the Ru(II) complex [(p-cymene)Ru(κN-INA)Cl_2] (INA = isonicotinic acid) where the half-sandwich Ru(II) metallorganic moiety [(p-cymene)$RuCl_2$] as a wheel, while the linear spacer is built on the cyclic dimerization of carboxylic functionalities present on proper divergent ligands, such as isonicotinic acid (INA).

The hydration-dehydration process of the complex [(p-cymene)Ru(κN-INA)Cl_2] (**1α**) has been followed by means X-ray powder diffraction (XRPD) and solid-state NMR analysis [2].

1α quickly converts into the hydrated form [(p-cymene)Ru(κN-INA)Cl_2]H_2O (**1·H_2O**) once exposed to water vapours through a heterogeneous solid-gas reaction. The thermally induced dehydration of **1·H_2O** leads to a not yet defined transient species (**1γ**) which finally transforms into a polymorphic form of **1α**, called **1β** (Fig. 1). The hydrogen bond network of these polymorphs, which surprisingly does not show the expected cyclic dimerization of the carboxylic functions of INA, has been ascertain by means of ^{1}H MAS and ^{13}C CPMAS experiments. The **1β** solid-state structure has been solved by a combined approach XRPD and 2D solid-state NMR experiments.

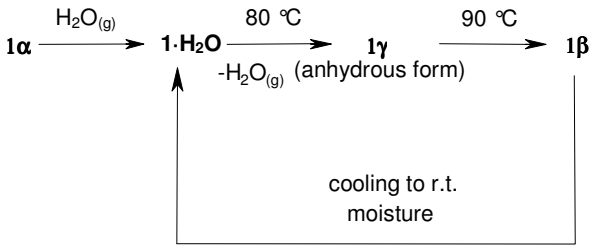

Fig. 1. Hydration-dehydration process of the complex [(p-cymene)Ru(κN-INA)Cl_2].

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STRUCTURAL BASIS FOR ENHANCEMENT OF THE CXCL12 CELLULAR ACTIVITY BY HMGB1 PROTEIN

E. Livoti, M. Schiraldi, M. Pedotti, M.G. Uguccioni, L. Varani

Istituto di Ricerca in Biomedicina; Via Vela 6; 6500 Bellinzona, CH
E-mail:elsa.livoti@irb.unisi.ch; luca.varani@irb.unisi.ch

Chemokines are a family of structurally homologues proteins that stimulate cellular movement and migration, responsible for leukocyte trafficking and homing in inflammation and other pathological conditions. The activity of individual chemokines is well characterized but much less is known about the activity of chemokines in combination to other proteins.
In particular, our collaborators have shown that the cellular activity of the chemokine CXCL12 is increased in the presence of another protein called HMGB1.
Our preliminary results suggest that HMGB1 binding induces a structural change in CXCL12, activating a conformation capable of more efficient triggering of the CXCL12 receptor which, in turn, results in an increased cellular effect.
Here we use NMR first to confirm and characterize the direct interaction of CXCL12 with HMGB1, and then plan to obtain the structure of the complex as well as its biophysical properties.
We show that one CXCL12 molecule can bind to either of the two independent domains of HMGB1 (BoxA and BoxB) via its structured region, but this is not sufficient for enhancing of the cellular activity. By contrast, interaction of two CXCL12 molecules with full HMGB1 leads to increased activity and affects also the N-terminus, which is unstructured in the free protein. Intriguingly, the N-terminus is responsible for CXCL12 activity through a direct interaction with its cellular receptor.
The N-terminus rapidly exchanges between different conformations in the free protein but our preliminary data suggest that the flexibility may be reduced upon binding to HMGB1, which might thus fix CXCL12 in a specific conformation. If this hypothesis is correct this “conformational selection” would favour the interaction between CXCL12 and its cellular receptor in two ways: i) by presenting CXCL12 in the conformation necessary for interaction with the receptor, thus facilitating interaction; ii) by lowering the entropic costs involved in receptor binding, since HMGB1, and not the receptor, would pay the energetic price of diminished flexibility at the N-terminus of CXCL12.

THE INTERACTION BETWEEN OLIVE FRUIT FLY (*Bactrocera oleae*) PHEROMONE AND PHOSPOLIPIDIC MEMBRANES: A MULTINUCLEAR INVESTIGATION

S. Macchi,[a] S. Borsacchi,[a,b] N. Peruzzi,[c] P. Lo Nostro,[c] M. Geppi[a,b]

[a] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126 Pisa
[b] I.N.S.T.M. (Consorzio Interuniversitario di Scienza e Tecnologia dei Materiali), Firenze, Italy.
[c] Dipartimento di Chimica e CSGI, Università di Firenze, Via della Lastruccia 13, 50019 Sesto Fiorentino (Firenze), Italy.
E-mail: saramacchi1@libero.it

Pheromones are key elements of the fascinating insects communication. Also due to the wide variety occurring in nature, the understanding of their chemistry and reception/transmission mechanisms is far from being complete, even if significant improvements have been achieved, as the identification of some pheromone-binding proteins and G-protein coupled receptors [1, 2]. Olive fruit fly (*Bactrocera oleae*) is one of the most serious problems for olives cultivation, especially in Mediterrean area, causing quantitative and qualitative damages to olives production. Spiroacetal pheromones are the basis of olive fruit flyes sexual attraction, and they are now exploited also in eco-sustainable traps for the protection of olive trees. To the best of our knowledge the possible direct interaction between these pheromones and phospolipid membranes has not been investigated yet through NMR. Here we present a multinuclear investigation on a cellular membrane model system, a phospholipid (DOPC)/water (D_2O) lamellar phase, neat and modified by progressive addition of fruit fly pheromone (Fig. 1). Changes in the phase, structural and dynamic properties of DOPC/water induced by the pheromone have been investigated through ^{2}H, ^{13}C and ^{1}H spectral and spin-lattice relaxation times analysis [3].

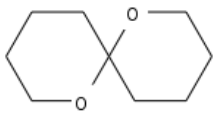

Fig. 1. Olive fruit fly pheromone.

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EFFECTS OF FREEZING TIME ON THE METABOLIC PROFILE OF UNPROCESSED AND PROCESSED MULLET ROES STUDIED BY ^{1}H-NMR AND MULTIVARIATE STATISTICAL ANALYSIS

C. Piras, E. Locci, P. Scano, F.C. Marincola

Dipartimento di Scienze Chimiche, Cittadella Universitaria di Monserrato, S.S. 554 (bivio per Sestu) 09042 Monserrato (CA)
E-mail:cristina.piras@unica.it

The eviscerated roes of striped mullet *(Mugil cephalus*) are manufactured in several countries and the salted and dried products can be found world wide under different names and typologies. The Mediterranean island of Sardinia (Italy) has a long tradition in manufacturing mullet roes to obtain a product called "bottarga". In recent years, Sardinian bottarga has become so increasingly popular in the international markets that mullets of the Mediterranean Sea are not enough to satisfy the request of this product. Then, the Sardinian producers have turned their attention to other fishing areas for roe supplies. As a result, bottarga manufactured in Sardinia is produced mainly with frozen roes coming from different parts of the globe and generally stored at -20°C for no more than one year before being processed. About 100 tons of bottarga are produced annually by the various companies in Sardinia and less than one percent comes from mullet caught in the Mediterranean Sea.

The aim of this study was to investigate the effect of freezing mullet roes on the metabolic profile of this product before and after processing. Frozen ovaries from mullets caught in the fishing area FAO 34 (Central-Eastern Atlantic) were provided by a manufacturer located in Sardinia and stored at -20°C up to one year. The low-molecular weight metabolite profile of both processed and unprocessed roes was analysed at different freezing times by ^{1}H NMR spectroscopy. Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) applied to NMR spectral data allowed us to evaluate how the modifications of the metabolic profile of unprocessed roes during freezing storage are reflected on the final product (bottarga).

NMR PETROLEOMIC ANALYSIS OF COMPLEX MIXTURES OF CRUDE OIL

A. Masili,[‡] S. Puligheddu[†], A. Lai[‡], P. Scano[‡], L. Sassu[†]

[‡]Dipartimento di Scienze Chimche, Univesità di Cagliari, Complesso Universitario di Monserrato, 09042 Cagliari, Italy
[†]Saras Ricerche e Tecnologie SpA, Traversa C, 5° Strada, Z.I. Macchiareddu, 09032 Assemini, Cagliair, Italy
E-mail: alicemasili@unica.it

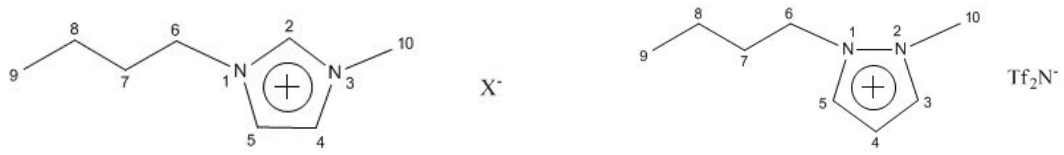
The goal of this research was to develop a strategy to classify crude oils and to predict physical-chemical properties of the petroleum samples useful for the characterization of the charges in the refinery to drastically reduce analysis time.
For these purposes we used a combined approach of ^{1}H NMR analysis and chemometric tools.
In the multivariate methods used in this research NMR spectral data have been correlated, in particular the relative intensity and location of resonance peaks, with different analytical data. The main techniques used include classification and regression of the data.
For each crude oil under study different of physical chemical parameters (density, viscosity, etc.) were collected.
A restricted number of samples has been analysed with the aim of optimise the sampling conditions (procedure for storing samples, sampling mode) NMR procedures (the solvent choice and concentration, type of NMR tubes, instrumental acquisition parameters), NMR spectral data analysis (phase, baseline correction etc.) and multivariate analytical approaches (pre-treatment of data, unsupervised, supervised techniques).
The best conditions found were then applied to the our data set and the samples were correctly classified on the base of the measured analytical parameters.

NMR STUDY OF Ni^{2+} SALTS DISSOLVED IN IONIC LIQUIDS

D. Mendola,[‡] F. Castiglione,[‡] W. Panzeri,[‡] A. Famulari,[‡] A. Mele,[‡] C. Chiappe,[†] C. Pomelli[†]

[‡] Department of Chemistry, Materials and Chemica Engineerig "G. Natta", Politecnico di Milano, Piazza L. Da Vinci 32, 20133 Milano, Italy.
[†] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Risorgimento 35, 56126 Pisa, Italy.
E-mail: daniele.mendola@mail.polimi.it; andrea.mele@polimi.it

Nickel complexes play an important role in preparative and mechanicistic chemistry and catalysis. The structure of Nickel Chloride in ionic liquids has been studied by X-ray absorption [1].
We investigated molecular interactions between Ni^{2+} salts and IL based on imidazolium (bmim) and pyrazolium (bmpy) cation (0.9 IL/0.1 Ni^{2+}). The IL chemical structure is described in Figure 1. Nuclear Magnetic Resonance homonuclear and heteronuclear NOEs correlation experiments [2] (NOESY and HOESY) techniques were used together with viscosity and conductivity measurements in order to understand the local structure of Ni^{2+}-doped IL. These results are compared with the pure compounds. Moreover, the (^{1}H-^{19}F)-HOESY experiments give information on cation-anion interactions for these new molecules. Combining all the NMR results, it is possible to propose a local organization and interaction model for this new sintetized IL doped with Ni^{2+} salts.
The influence of different anions (Cl^-, NO_3^- and Tf_2N^-) for the bmim cation is also discussed.



X = Cl^-, NO_3^-, Tf_2N^-

Fig. 1. Chemical structures of butyl-metyl-imidazolium and butyl-metyl-pyrazolium.

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NEW INSIGHTS INTO THE STRUCTURE AND DYNAMICS OF NATURAL EUMELANIN AND PHEOMELANIN BY SOLID-STATE NMR

G. Mollica,[‡] F. Ziarelli, [†] P.J. Farmer,[§] R.W. Martin,[ϒ] S. Viel, [‡] A. Thévand[‡] and P. Thureau[‡]

[‡]Laboratoire Chimie Provence, Universités Aix-Marseille I, II et III – CNRS, UMR 6264, 13397 Marseille, France
[†]Fédération des Sciences Chimiques de Marseille, CNRS-FR1739, Spectropole, 13397 Marseille, France
[§]Department of Chemistry and Biochemistry, Baylor University, 76706 (TX) USA
[ϒ]Department of Chemistry, Natural Science 1, University of California Irvine, 92697 (CA) USA
E-mail: giulia.mollica@univ-provence.fr

Melanins are a class of pigments ubiquitously found in the animal and plant kingdoms [1]. They are associated with a variety of biological functions, such as pigmentation of skin, eyes and hair, photosensitization, photoprotection, metal ion chelation, camouflage, thermoregulation and free radical quenching.
The biological functions of melanins are often attributed to their unique chemical properties. However, the exact molecular and supramolecular structures of eumelanin and pheomelanin remain, at present, not completely understood. The attempt to an intense multidisciplinary investigation of these melanins through the application of spectroscopic and microscopic techniques has shown that the main limitations to their structural characterization essentially arise from an almost complete insolubility in all solvents, the amorphous character, and the extreme molecular heterogeneity [2]. In this sense, solid-state NMR spectroscopy represents a powerful method of investigation, especially for solid samples lacking long-range translational symmetry [3]. Here we present a solid-state NMR study of natural samples of black and red melanin extracted from human hair. Several ^{1}H and ^{13}C 1D and 2D experiments have been acquired on both samples at 400 MHz and 1GHz in the attempt of revealing structural and dynamic differences between them. The results demonstrate the presence of a very high degree of disorder associated to the supramolecular structure of eumelanin, while domains characterized by a significant molecular mobility are present in the pheomelanin sample. An investigation of the dynamic properties of the two samples is supported by the quantitative analysis of the ^{1}H lineshape extracted from 2D WISE spectra [4], while a discussion of the structural features relies on ^{13}C data.

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SOLID-STATE NMR CHARACTERIZATION OF INSOLUBLE CALCIUM ALGINATES FOR BIOMEDICAL APPLICATIONS

G. Mollica,[‡] F. Ziarelli,[†] S. Lack,[§] F. Brunel[§] and S. Viel[‡]

[‡] Laboratoire Chimie Provence, Universités Aix-Marseille I, II et III – CNRS, UMR 6264, 13397 Marseille, France
[†] Fédération des Sciences Chimiques de Marseille, CNRS-FR1739, Spectropole, 13397 Marseille, France
[§] Les Laboratoires Brothier, 41, Rue de Neuilly, 92735 Nanterre Cedex, France
E-mail: giulia.mollica@univ-provence.fr

Alginates are natural polysaccharides extracted from marine brown algae (*Phaeophyceae*) [1]. They are binary, linear copolymers of (1→4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G). Because of their ability to form stable gels [2], alginates have been largely used in many biomedical and pharmaceutical applications, going from delivery vehicles for drugs, wound dressings materials, dental impression materials, or biomaterials for tissue engineering.
In this study, we present the investigation of three series of 9 insoluble calcium alginate powders with different average calcium contents (1.5, 3.5 and 8% w/w) by means of ^{13}C solid-state NMR spectroscopy. The effect of the increased calcium content on the determination of the mannuronate (M) to guluronate (G) ratio from spectral deconvolution of the ^{13}C CP/MAS spectra is discussed [3], and the variations observed are commented in function of possible structural modifications related to the interaction with the divalent cations. The possibility of using solid-state NMR spectroscopy for the quantification of the calcium content in unknown alginate samples is explored performing Principal Component Analysis (PCA) of the spectra. The results obtained show that a clear separation of alginates with slightly different calcium content is possible. The proposed method relies on the sole use of the chemical shifts of the signals corresponding to pyranose carbons, suggesting that PCA of solid-state NMR data holds promises as a rapid and undestructive method for screening the calcium content of alginate-based materials with biomedical uses.

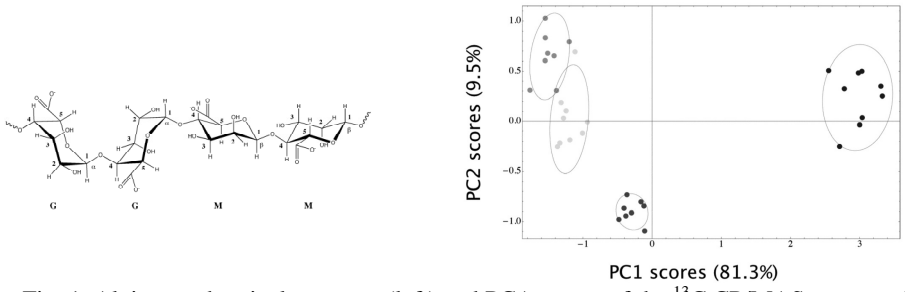

Fig. 1. Alginates chemical structure (left) and PCA scores of the ^{13}C CP/MAS spectra of 4 series of alginates with different calcium content (right).

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NMR-BASED METABOLITE FINGERPRINTING TO IDENTIFY THE BOTANICAL ORIGIN OF HONEY

E. Morelato, C. Facchin, M. Stocchero[#] S. Mammi and E. Schievano

Dipartimento di Scienze Chimiche, Università di Padova, Via Marzolo, 1, 35131, Italy,
[#]S-IN Soluzioni Informatiche, Via G. Salvemini 9,36100 Vicenza, Italy
e-mail:ely-veronica@hotmail.it

A Nuclear Magnetic Resonance (NMR)-based approach was developed for metabolic fingerprinting of 7 different unifloral honeys in the present study. In total, 353 honey samples (75 acacia, 60 chestnut, 62 linden, 40 orange, 32 eucalyptus, 36 honeydew and 48 polyfloral) were collected. All the samples were provided by Rigoni s.p.a. and from Veneto apiaries: their botanical origin was ascertained by means of sensorial analysis. The ^{1}H spectra of the chloroform extracts [1] appear very crowded and they are suitable to be considered fingerprints for each honey type. The dataset of 353 samples was investigated by performing PCA for each class of samples. Training and test sets were extracted by applying onion D-optimal design [2]. The training set was used to build *one-versus-all* O2PLS-DA [3] models where each class of honey was compared with the other classes considered together as a unique class.
By using the scores of the predictive component of the 7 individual O2PLS-DA models as descriptive variables, we built a Naïve Bayes classifier able to transform the scores of the discriminant models into the probability to belong to a specific class. For the test set, the combination of the O2PLS-DA and the Naïve Bayes classifier allowed us to obtain high percentuage of prediction. The encouraging results obtained suggest that this approach could be useful for the development of generally applicable metabolomics tools to discriminate origins of honey.

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INVESTIGATING ON THE METAL BINDING PROPERTIES OF THE C-TERMINAL REGION OF NOGO PROTEIN RTN1-C.

R. Nepravishta, T. Eliseo, M. Paci and S. Melino

Department of Sciences and Chemical Technologies, Via della Ricerca Scientifica 1, University of Rome Tor Vergata, Italy.
E-mail: melinos@uniroma2.it; ridvan.nepravishta@uniroma2.it

Reticulon 1-C (RTN1-C), is an Endoplasmatic Reticulum (ER) associated protein which present a horse-shoe-like topology with two transmembrane helices connected by a chain of 66 residues, with the N- and C-terminal regions that are supposed in the cytosolic side of ER. RTN1-C seems to be involved in several important biological functions, such as membrane vesicle trafficking and apoptosis induction but at the moment its mechanism is not completely understood [1,2]. The C-terminal region of RTN1-C is characterized by the presence of a H4 Histone consensus sequence that makes it able to interact with nucleic acids and HDAC/HAT enzymes both *in vitro* and *in vivo* [3,4]. Moreover, by analysing this region sequence we have identified a potential metal ion binding motif (HxE/D). Here we investigated on the metal binding proprieties of this motif, (synthesising the NH_2-KIPGAKRHAE-$CONH_2$ peptide corresponding to the residues 199-209 of RTN1-C), by UV-vis, CD and multidimensional NMR (^{1}H 1D, 2D ^{1}H-^{1}H TOCSY and ^{1}H-^{13}C HSQC) spectroscopy. In particular, multidimensional NMR spectroscopy was an important tool for the characterization of complexes with Fe^{2+}, Cu^{2+} and Zn^{2+} ions. NMR experiments revealed that these metal ions bind strongly to such peptide and that His and Glu residues are involved in the metal ion coordination. Finally the several metal-peptide complexes showed a different behaviour upon DNA binding and cleavage capacity. These data represent an enhancement towards the structural and functional characterization of this neuronal membrane protein suggesting a possible role of the metal binding property in its biological function.

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NMR STUDIES ON STRUCTURE AND DYNAMICS OF THE MONOMERIC DERIVATIVE OF BS-RNase

R. Spadaccini,[‡] C. Ercole,[§] M.A. Gentile,[§] D. Sanfelice,[§] A. Bernini,[#] N. Niccolai,[#] D. Picone[§]

[‡] Dipartimento di Scienze Biologiche ed Ambientali, Università del Sannio, 82100 Benevento, Italy
[§] Dipartimento di Chimica, Università degli Studi di Napoli "Federico II", 80126 Napoli, Italy
[#] Department of Biotechnology, University of Siena, 53100 Siena, Italy
E-mail: niccolai@unisi.it

Structural and dynamical properties of the monomeric form of bovine seminal RNase, mBS, have been investigated to understand if the special features of domain swapping process for this protein derive from an intrinsic property of its subunits or they are a consequence of the pre-existing dimeric structure. Thus, the structural features derived from a refined mBS NMR structure have been discussed in terms of protein dynamics by analyzing a large sets of experimental and computational results. Particular emphasis has been given to compare the results obtained here with structural and dynamical data available for pancreatic RNase A.

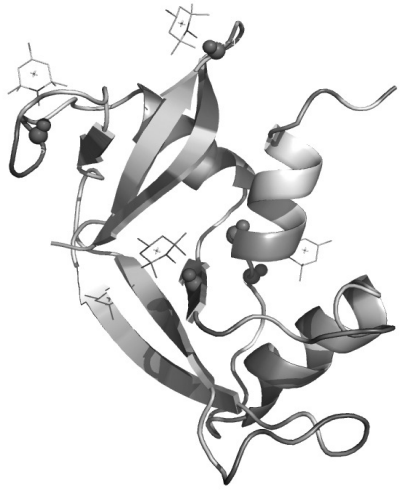

Fig. 1. Cartoon representation of BS-RNase backbone showing the most TEMPOL approached regions.

NMR dynamic parameters derived from urea denaturation, H/D exchange and paramagnetic perturbation profiles have been measured for mBS and an overall picture, consistent also with MD simulation predictions, was obtained.

H/D exchange rates, indeed, were closely related to TEMPOL accessibility, confirming that the paramagnetic probe preferably approaches solvent exposed mBS backbone amides [1].

Protein hydration has been also analyzed from 100 ns MD simulation in explicit water to correlate the stability of the hydrogen bond internal network with possible biased TEMPOL interactions. From the same MD trajectory rmsf have been calculated and a limited flexibility can be suggested for the entire mBS backbone. A close similarity of mBS and RNase A is found and new mechanisms for the domain swapping of natural BS RNase should be explored.

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NMR-BASED INTERACTION STUDIES ON THE FIBROBLAST GROWTH FACTOR-2 AND NEW DERIVED ANTIANGIOGENIC COMPOUNDS

K. Pagano,[‡] S. Tomaselli,[‡] G. Colombo,[†] G. Taraboletti,[#] L. Zetta, [‡] L. Ragona[‡]

[‡]Istituto per lo Studio delle Macromolecole, CNR, via Edoardo Bassini 15, 20133, Milan, Italy
[†]Istituto di Chimica del Riconoscimento Molecolare,CNR, viaMario Bianco 9, 20131, Milan, Italy
[#]Tumor Angiogenesis Unit, Department of Oncology, Mario Negri Institute for Pharmacological Research, via Stezzano 87, 24126 Bergamo, Italy
E-mail: katiuscia.pagano@ismac.cnr.it

Endogenous inhibitors of angiogenesis, such as thrombospondin-1 (TSP-1), are promising sources of therapeutic agents to treat angiogenesis-driven diseases, including cancer. TSP-1 regulates angiogenesis through different mechanisms, including binding and sequestration of the angiogenic fibroblast growth factor-2 (FGF2), through a site located in the calcium binding type III repeats. A FGF2 binding sequence of TSP-1 was identified in the 15-mer sequence DDDDDNDKIPDDRDN using a peptide array approach followed by binding assays with synthetic peptides and recombinant proteins [1]. The relevant residues and conformational determinants for the peptide-FGF2 interaction were identified by STD nuclear magnetic resonance and molecular dynamics simulations. The information was translated into a pharmacophore model used to screen the NCI2003 small molecule databases, leading to the identification of three small molecules that bound FGF2 with affinity in the submicromolar range. NMR titration experiments were carried out on the ^{15}N-labelled FGF2 with the most active of the identified molecules. The analysis revealed a residue specific interaction in the fast exchange regime, whereas the FGF2 structure remains overall unperturbed. The small molecule binds in a crucial position that provides specific contacts with FGF2 that are essential for the binding to heparin. A model of the FGF2-small molecule complex was calculated with HADDOCK program. The dynamics and the resident water molecules for the free and bound FGF2 protein were also characterized: long-range effects on FGF2 upon binding to the small molecule were observed, which potentially counteract FGF2/FGF receptor kinases 1 interaction.

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REFINEMENT OF STRUCTURAL HOMOLOGY MODELS AND CONFORMATIONAL ARRANGEMENT OF THE TWO DOMAINS OF GRX3 FROM *Trypanosoma brucei* EXPLOITING RESIDUAL DIPOLAR COUPLINGS

C. Pavan[‡], M. Sturlese[‡], C. Farès[§], Y. Lu[†], B. Manta[*], M. Comini[*], M. Sunnerhagen[å], S. Mammi[‡], M. Bellanda[‡]

[‡] Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131, Padova, Italy
[§] Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, D-45470, Mülheim an der Ruhr, Germany
[†] School of Molecular Bioscience, University of Sydney, NSW 2006, Australia
[*] Redox Biology of Trypanosomes Laboratory, Institut Pasteur Montevideo, Calle Mataojo 2020,CP11400, Montevideo, Uruguay
[å] Division of Molecular Biotechnology, Dept. of Physics, Chemistry and Biology, Linköping University, SE-58183 Linköping, Sweden
Email: carlo.pavan@unipd.it

Glutaredoxins (Grxs) are ubiquitous enzymes conserved throughout all the kingdoms of life and were first described as glutathione-dependent oxidoreductases [1]. So far, Glutaredoxins have been found to take part in a plethora of biological processes, the most important being the maintenance of the redox status of cysteine-containing proteins through the reduction of both intramolecular and mixed disulphide bridges [2]. Quite recently, it was demonstrated that some Grxs are involved in the biosynthesis and binding of [4Fe-4S] Iron Sulfur clusters [3]. In this work, we focused our attention on Grx3 from *Trypanosoma brucei*, a pathogenic protozoan responsible for human African trypanosomiasis, also known as sleeping sickness. These parasites have developed a unique antioxidant system based on bis(glutathionyl)spermidine rather than glutathione. This peculiarity, together with the pivotal role of glutaredoxins in the neutralization of reactive oxygen species (also produced by the host immune system), make these proteins potential candidates as drug targets [4]. Different from the vast majority of glutaredoxins, which are classically 9-14 kDa, single domain proteins, *Tb* Grx3 is a 24 kDa two-domain protein in which an N-terminal Thioredoxin moiety is coupled to the C-terminal monothiol glutaredoxin through a twelve residue-long bridge. Because of its size and multi-domain pattern, the determination of the NMR solution structure can be cumbersome if the well-known, routinary NOE-based techniques are used. Most of the times, these short-range restraints are not able to produce the mutual position, orientation and dynamics between domains. An unconventional method based on the refinement of homology models using secondary chemical shift, residual dipolar couplings (RDCs) [5], small angle X-ray scattering (S.A.X.S.) [6] was used for this purpose.

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INSIGHTS ON CHANNEL SELECTIVITY FROM THE STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE Kv1.3 CHANNEL BLOCKER Tc32.

T.A. Pertinhez[1], E. Polverini[2], E.G. Stehling[3], M.L. Sforça[4], N.I.T. Zanchin[4], A. Pignatelli[5], O. Belluzzi[5], and A. Spisni[1]

[1]Dept. Experimental Medicine, Chemistry and Structural Biochemistry Unit, and [2]Dept. Physics, University of Parma, Parma, Italy; [3]Dept. Toxicological and Bromatologic Clinical Analyses, USP, Ribeirão Preto, Brazil; [4]National Laboratory of Biosciences,Campinas, Brazil; [5]Dept. Biology and Evolution, Physiology and Biophysics Unit, University of Ferrara, Ferrara, Italy.
E-mail: *thelma@unipr.it*

The utility of toxins in biomedical research, diagnosis, and therapy is widely recognized. Unfortunately their use is limited by an inadequate target discrimination. Thus, the search for target-specific toxins is of primary relevance. The fact that despite the incredible number of toxins present in the animal kingdom, only a limited number of molecular scaffolds has been selected, is a clear evidence of the importance of the nature and spatial orientation the side chains. The description and understanding of the contact surface between the toxin and the channel entrance appears to be the target for the rationale design of selective and high affinity drugs.
Tc32 toxin from the scorpion *Tityus cambridgei* has been reported to have a clear inhibitory effect on Kv1.3 K^+ channel [1]. This channel, member of the *Shaker* family [2], carries a large proportion of the outward current not only in leucocytes [3] but also in a variety of neuronal cells [4].
In the present work, Tc32 has been cloned and expressed in a soluble and active form for the first time, employing a new protocol we devised [5]. Tc32 activity has been characterized by electrophysiological assays on a distinct subpopulation of periglomerular cells of olfactory bulb and its 3D solution structure determined by ^{1}H-NMR spectroscopy. The structure reveals it exhibits an α/β scaffold typical of the members of the α-KTx family. A structural comparison with the other members of α-KTx 18 subfamily is presented following molecular modeling calculations, and docking simulations to Kv1.1 and Kv1.3 channels.
Our data point out Tc32 as a good lead molecule for the development of new molecules suited for research, diagnosis and therapy.

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A MEAN FIELD BROWNIAN DYNAMICS SIMULATION TO EXPLORE INTERMOLECULAR NUCLEAR SPIN RELAXATION MECHANISMS IN SOLUTION

P. Håkansson[1], M.H. Levitt[2] and G. Pileio[2]

[1] School of Engineering Science, University of Southampton, SO17 1BJ, Southampton
[2] School of Chemistry, University of Southampton, SO17 1BJ, Southampton
E-mail: *p.hakansson@soton.ac.uk* ***and*** *g.pileio@soton.ac.uk*

The study of solvent effects on nuclear spin relaxation have a long history and have been explored, mainly, with analytical models and numerical solution of Smoluchowski equation to compute spectral densities [1]. For instance, the effects of translational motions have been studied using either force free diffusion models or radial mean field potential approaches. The effects of spins that are eccentric in molecules have been accounted for in these calculations but only in an approximate way [2].

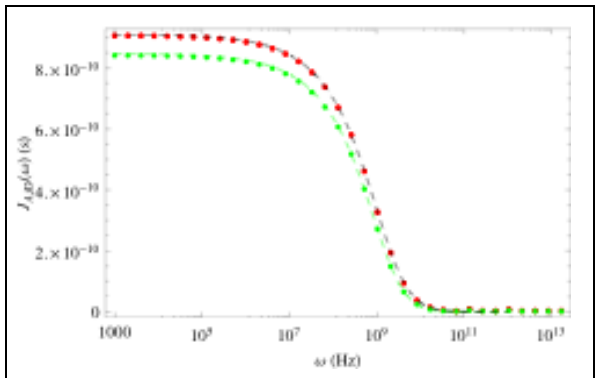

Fig. 1. Spectral densities for force free translational diffusion (green) and with rotational diffusion (red) obtained using analytical model (dashed line) and simulation method (filled circles).

We introduce a mean field Brownian dynamics simulation method significantly cheaper, in computational cost, than atomistic molecular dynamics simulations. This simulation technique allows us to explore spin eccentricity and rotational diffusion in an exact form (see fig. 1) and under different dynamical regimes. The method is flexible in allowing for higher dimensions and therefore a wider range of relaxation effects become accessible. So far, we have exploited these potentialities exploring nuclear spin relaxation, out of the spherical molecule approximation and using mean field results from atomistic molecular dynamics.

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IN VIVO MRI VISUALIZATION OF DIFFERENTIAL RELEASE FROM WATER-FILLED LIPOSOMES TRIGGERED BY LOW INTENSITY NON-FOCUSED ULTRASOUND

S. Rizzitelli, P. Giustetto, D. Delli Castelli, C. Boffa, S. Aime, E. Terreno

Department of Chemistry IFM and Molecular & Preclinical Imaging Centers, University of Torino, Italy
E-mail: silvia.rizzitelli@unito.it

The development of anticancer combination therapies is a hot topic. Moreover, there is a significant interest to design new therapeutic schemes where different drugs are differentially released from a single delivery system to control release sequence, timing, and dose. Among drug-delivery systems used in medicine, liposomes are likely the most suitable carrier because the release can be triggered by different stimuli for controlling a differential release. Acoustic radiations are a safe tool to be considered for this purpose. We have explored the local application of low intensity non-focused US, able to stimulate mechanic drug release from water-filled liposomes. US transducers operating between 27 kHz and 3MHz were fabricated and tested. The encapsulation into liposomes of MRI agent Gadoteridol gave the opportunity to study its release thanks to the relaxivity enhancement associated with the probe leakage. The attention was focused on two liposome stealth formulations differing in the length of the alkylic chain of the main phospholipid component: DPPC and DSPC and the two formulations displayed a differential release in vitro. The most probable release mechanism relies on the formation of transient pores on the liposome bilayer generated by the mechanic effect on the acoustic pressure, whereas heating and cavitation showed a minor role. The very promising results obtained in vitro were validated in vivo. Stealth liposomes were injected in the tail vein of mice bearing subcutaneous melanoma B16 tumors. Just after injection, the tumor was locally insonated using pulsed 3MHz US waves. MR imaging sessions were performed before and after US exposure. The results confirmed that only DPPC-based liposomes released their content as demonstrated by the large T1 contrast enhancement detected. As liposomes take quite a long time passively accumulate in the tumor, the released occurred in the tumor vasculature. The differential released was also confirmed by measuring the biodistribution of Gadoteridol after US exposure. In case of DPPC-based vesicles the agent was mostly detected in the kidneys and bladder, whereas in case of DSPC-based liposomes it was mainly detected in liver and spleen. In conclusion, low intensity US trigger a differential release from water-filled liposomes that could improve anticancer therapies.

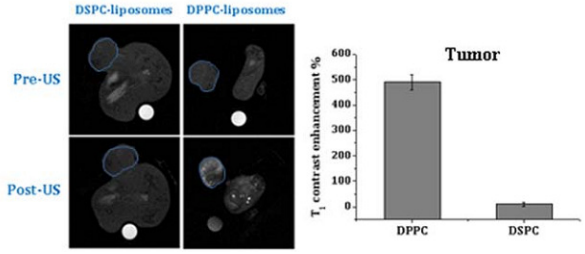

Fig. 1. Differential release from liposomes encapsulating Gadoteridol triggered by pulsed low intensity US. Left: MR T_1w axial images (7T) of mice bearing subcutaneous B16 melanoma. Images were taken: i) after the i.v. injection of stealth liposomes made of DPPC (top left) or DSPC (top right), and ii) 30 min after tumor application of pulsed 3MHz US (2min, T_{on-off}0.5 sec, duty cycle 50%) (bottom row). Right: corresponding T1 contrast enhancement in the tumor after US exposure.

DOSY ANALYSIS OF POLYCYCLIS AROMATIC HYDROCARBONS FROM TYRES AND TARS

C. Schillaci, F. Bertocchi, T. Eliseo, M. Paci

Dipartimento di Scienze e Tecnologie Chimiche, Università Tor Vergata, Via della Ricerca Scientifica 1, Roma, Italia (carlo.schillaci@hotmail.it).

Polycyclic aromatic hydrocarbons are ubiquitous environmental agents commonly believed to significantly contribute to human cancers. PAHs are formed in the process of incomplete combustion of organic material and are found widely in the environment, for example, in engine exhaust, cigarette smoke, soil, water and food [1], and human exposure to PAHs is therefore unavoidable. Like many other carcinogens, polycyclic aromatic hydrocarbons are metabolized enzymatically to various metabolites, of which some are reactive. In the large group of enzymes involved in carcinogenic metabolism, cytochrome P450 enzymes CYP1A1, 1A2, 1B1, and 3A4 are the most important enzymes in the metabolism of PAHs[2].
The characteristic of NMR spectra are the signals assigned to the so called " bay protons" which are separated from other resonance in the NMR spectra. This is formalized in the procedure ISO/DIS 21461. This is the usual methods for the quantitation of these compounds in many samples like foods, tyres oil , tars. But this method is totally irrespective of the dimension of the condensated rings involved in the molecular structure.
The problem is the assessment of a method for the determination in these mixture of the molecular weight distribution as to better characterize the danger associated with these distributions, particularly for their capacity to interact with DNA.
Application of Diffusion ordered spectroscopy (DOSY) firstly applied to this determination gave good results which are promising to correlate biological effects with molecular features.

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IDENTIFICATION OF A NEW PEPTIDE INHIBITOR OF PROTEIN-PROTEIN INTERACTIONS LEADING TO APOPTOSIS THOUGHT MCL-1 BINDING

M. Sturlese[‡], W.J. Placzek[†], B. Wu[†], J.F. Cellitti[†], J. Wei[†] and M. Pellecchia[†]

[‡]Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy
[†]Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Rd. La Jolla, CA 92037
E-mail: mattia.sturlese@unipd.it

Mcl-1 is an anti-apoptotic member of the B-cell lymphoma-2 (Bcl-2) family, and is a highly expressed pro-survival protein in several cancer cell lines. Mcl-1 plays its anti-apoptotic role interacting with BAK and BAX, pro-apoptotic members of the Bcl-2 family, and the inhibition of this interaction promotes cell death in cancer cells. To identify new small peptides able to bind the BH3 cleft of Mcl-1 and displace the pro-apoptotic binder, we performed a screening of a 10^9 different 12-mer peptides using the phage display technique. NMR was used as the technique of choice to validate the binding while Isothermal Titration Calorimetry (ITC) and fluorescence polarization assays (FPA) were used to measure the affinity. Three peptides with affinity in the low micromolar range were identified. The binding mode of these peptides was investigated *in silico* mixing the information harvested during the NMR studies. BLAST analysis of the identified sequences against the human genome identifies this characteristic pattern in a selection of interesting proteins including glucokinase, hexokinase, and a number of tumor suppressors, among others. A short peptide sequence derived from glucokinase exhibits binding to Mcl-1 comparable to that seen for a 12-residue endogenous peptide. These peptides are the shortest sequences ever observed to bind Mcl-1 and they may warrant development into improved Mcl-1-specific small molecules and peptide-based therapeutics. Further, their identification may provide the basis for increased understanding of possible cross-talk taking place between a number of divergent cellular signaling and homeostatic processes and the regulation of apoptosis.

A SOLID-STATE NMR STUDY ON A TRIS(DIBENZOYLMETHANIDO) (*o* – PHENANTHROLINE) – YTTRIUM(III) COMPLEX INCORPORATED INTO SILICA NANOPARTICLES – A MODEL COMPLEX FOR LUMINESCENT EUROPIUM COMPOUNDS

U.P. Sudhakaran[‡], S. Borsacchi[‡], M. Geppi[‡], C.M. Malba[†], I. Freris[†], A. Benedetti[†]

[‡] Department of Chemistry and Industrial Chemistry, University of Pisa, v. Risorgimento 35, 56126 Pisa, Italy
[†] Department of Chemical physics, Università Cà Foscari di Venezia and INSTM, via Torino 155/b, 30170 Venezia-Mestre, Italy
E-mail: umayal@ns.dcci.unipi.it

Inorganic nanoparticles doped with rare-earth compounds have been widely applied for biological labeling, imaging and sensing applications [1]. Incorporation of such rare earth complexes into silica nanoparticles can provide significant advantages such as high optical emission intensity, low toxicity, efficient surface functionalization for molecular recognition and cheap, fast and reliable production process [2].

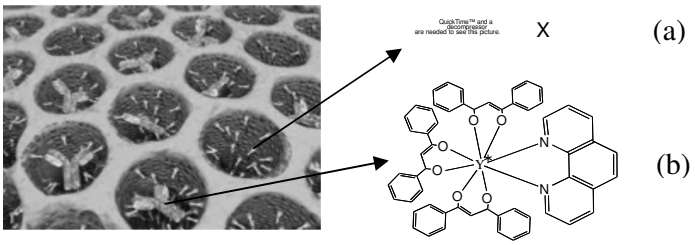

Fig. 1. Incorporation of $Y(DBM)_3Phen$ into the Mesoporous silica nanoparticles (MSN). (a) – Functionalized MSN (X – Functional groups); (b) – Yttrium (III) complex

In this work we present a solid-state NMR study of a tris(dibenzoylmethanido) (*o* – phenanthroline) – yttrium(III) complex, $[Y(DBM)_3(Phen)] \bullet xH_2O$, which has been physically incorporated into mesoporous silica nanoparticles (MSN), functionalized with alkyl-alkoxysilanes, which should improve physical interactions between the complex and the nanoparticles. The yttrium complex was chosen instead of the luminescent europium(III) one in order to avoid paramagnetism without changing the molecular properties (Y(III) and Eu(III) have very similar ionic radius). With the aim of characterizing the main molecular structural and dynamic properties of the complex incorporated into the silicas, as well their mutual interactions, ^{13}C and ^{1}H-MAS spectra have been exploited together with the analysis of ^{1}H and ^{13}C T_1 and T_{1r} relaxation times measured at variable temperature for the composites, the neat functionalized silicas and complex [3]. Additional detailed structural information concerning the functionalization of the silicas have been obtained from ^{29}Si high-resolution spectra.

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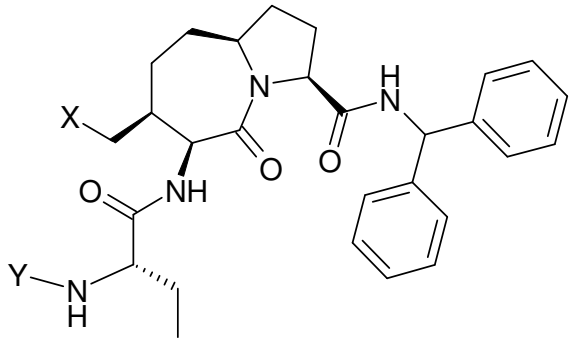
A NMR STUDY OF SMAC MIMICS TARGETING L-BIR2-BIR3 DOMAIN OF XIAP PROTEIN

F. Vasile, D. Potenza, L. Belvisi, C. Drago and P.F. Seneci

Dipartimento di Chimica Organica e Industriale, University of Milano, via Venezian 21 and Centro Interdisciplinare di Studi Biomolecolari e Applicazioni Industriali (CISI), Milano, Italy
E-mail: francesca.vasile@unimi.it

XIAP is a central apoptosis regulator that inhibits apoptosis by binding to and inhibiting the effectors caspase-3/-7 and an initiator caspase-9 through its BIR2 and BIR3 domains, respectively. Smac protein in its dimeric form effectively antagonizes XIAP via its N-terminal four residues (AVPI) in a way similar to the interaction between caspase-9 and XIAP. Smac protein concurrently targeting both XIAP BIR2 and BIR3 domains [1]. Smac mimetics may have great therapeutic potential as a new class of anticancer drugs
Novel proapoptotic Smac mimics/XIAP inhibitors have been designed, synthesized and characterized in our group [2]. These pseudopeptides, containing the 1-aza-2-oxobicyclo[4.3.0]nonane scaffold (Figure 1), were tested in presence of the linker-BIR2-BIR3 construct by tr-NOE and STD-NMR experiments [3].
Here, we show evidences of binding and the epitope mapping of new monomeric and dimeric compounds with respect to linker-BIR2-BIR3 fragment of the XIAP protein. STD spectra of the selected Smac-mimics highlight the role of each group involved in the binding and guide the design of new compounds with higher affinity.



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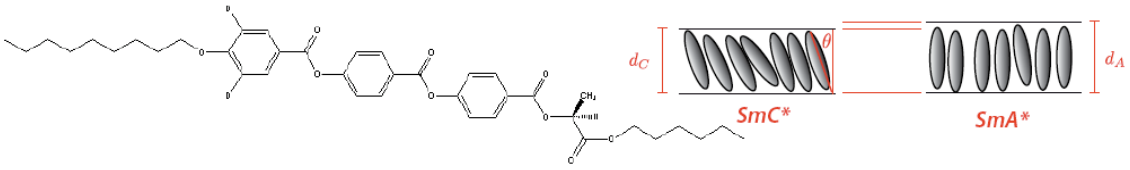
NEW PHYSICAL INSIGHTS ON 'DE VRIES' LIQUID CRYSTALS BY COMBINED ^{2}H, ^{13}C, ^{13}C-^{1}H 2D SOLID STATE NMR METHODS

M. Cifelli[‡], V. Domenici[‡], A. Marchetti[†#], M. Lelli[†], V. Novotna[§], A. Lesage[†], A. Pell[†], and C.A. Veracini[‡]

[‡]Dipartimento di Chimica e Chimica Industriale, via Risorgimento 35, 56126 PISA (Italy)
[†] Centre de RMN à Très Hauts Champs (FRE 3008 CNRS / ENS Lyon / UCB Lyon 1), 5 rue de la Doua, 69100 Villeurbanne (France)
[#] Scuola Normale Superiore di Pisa, Piazza dei Cavalieri 7, I-56126 Pisa (Italy)
[§] Institute of Physics, Academy of Sciences of Czech Republic, Na Slovance 2, 180 Prague (Cezch Republic).
E-mail: mario@dcci.unipi.it / verax@dcci.unipi.it

In the last decades the combination of several experimental NMR techniques has provided valuable information about molecular structure, order and dynamics of various liquid crystalline mesophases [1]. In this work, we show how these techniques can play a key role in the understanding of the peculiar structure and behaviour of the so-called smectic "de Vries mesophases". Those encompass the *SmA* phases that show no smectic layer shrinkage on cooling from the *SmA** into the *SmC** phase, explained as an effect of a distribution of the azimuthal orientational angle of the smectogens composing their layers (see Scheme) [2]. The target of our analysis is the smectogen 9HL, studied both in ^{13}C natural abundance and in its deuterated form on one aromatic moiety, 9HL-d_2 [3,4]. ^{2}H NMR analysis at four values of the magnetic fields of this system confirms the presence of a short-range ordered structure within the smectic planes and allows the calculation of the tilt angle in both SmA and SmC* phases. These data are compared with the tilt angle as obtained from electro-optical measurements [5]. Moreover, the analysis of 1D ^{1}H-^{13}C CP and 2D experiments (HETCOR and PDLF[6]) allowed a self-consistent determination of local and molecular orientational order and structural properties of the mesogen, together with a characterization of its conformational changes as a function of the temperature. Interestingly, the analysis of this large amount of data gives important clues in the understanding of the 'de Vries' SmA structure. A discussion of the theoretical models proposed to describe the SmA-SmC* transition in 'de Vries' systems in view of our results will be presented.



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STRUCTURAL REQUIREMENTS FOR COOPERATIVITY IN BILE ACID BINDING PROTEINS

S. Zanzoni, M. Assfalg, A. Giorgetti, M. D'Onofrio, H. Molinari

Dipartimento di Biotecnologie, Università di Verona, Strada Le Grazie 15, 37134 Verona,Italy.
E-mail: serena.zanzoni@univr.it

Ileal bile acid binding proteins (I-BABP), belonging to the family of intracellular lipid binding proteins, control bile acid trafficking in enterocytes and participate in regulating the homeostasis of these cholesterol-derived metabolites [1,2]. Proteins of the I-BABP family found in different animal species have been previously shown to display different levels of binding cooperativity for the two internally bound ligands, with two extremes being set by human I-BABP (maximum cooperativity) and chicken I-BABP (lowest cooperativity) [3]. This behavior prompted us to undertake a structural chemistry approach, exploiting the combined results of NMR, mutation, and molecular simulation data, to provide a deeper understanding of the molecular requirements for cooperativity. We envisaged chicken I-BABP (cI-BABP) as the most suited system for this investigation, as it undergoes a final conformational transition without establishing efficient energetic coupling to the second binding event. The question here addressed relates to whether it is possible to introduce a function (cooperativity) in this non-optimally functioning system. The here determined high resolution structure of cI-BABP in complex with two molecules of glycochenodeoxycholate, in comparison with reported experimental data on homologous proteins, provided the basis to design a double mutant (H99Q/A101S cI-BABP) capable of restoring a cooperative binding mechanism. Analysis of extended MD simulations helped clarifying the structural basis of an "extended conformational selection model" of binding.

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IDENTIFICATION OF MILK MIXTURES BY ^{1}H-NMR PROFILING

R. Lamanna, [‡] A. Braca, [†] E. Di Paolo, [†] G. Imparato [†]

[‡]ENEA Research Center of Trisaia, SS 106 Jonica Km 419.5, 75026 Rotondella (Mt), Italy
[†]CO.T.IR., ss 16 nord 240 - 66054 Vasto (Ch), Italy
E-mail: raffaele.lamanna@trisaia.enea.it

Nuclear magnetic resonance profiling, combined with a single layer artificial neural network (ANN), is used for the evaluation of the content of mixtures of different milks [1]. In particular, aqueous fractions of cow and sheep milk mixtures are analyzed by ^{1}H NMR. The spectral differences are highlighted by analysis of the variance (ANOVA) [2-3] and principal component analysis (PCA).
The species classification problem is solved by linear discriminant analysis (LDA): a reduced set of NMR discriminant variables have been selected according to their F of Fisher by setting a suitable threshold. Having two categorial variables (cow and sheep) LDA provides only one linear discriminant, which is plotted against the sample identification number. All the validation samples were correctly assigned.
To build a regression model, for the evaluation of the content of milk of different species in a mixture, a set of few variables has to be selected from the spectrum.
The analysis of the variance was performed, on all the sample mixtures, by assuming six groups according to the percentage of sheep milk into the cow base.
In total 22 variables have been selected and used to train an ANN [4]. Average ANN predicted values with relative standard deviation are obtained by leave-one-out validation test and compared with the real amount of the second component in the mixture. A linear relationship is observed and a simple regression gives a slope of
1.006 ± 0.006 and an intercept of -0.1 ± 0.4. The correspondence between the actual mixture content and the ANN predicted value is thus very high (correlation coefficient $R > 0.9999$).

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