GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XXXVIII National Congress on Magnetic Resonance

Bressanone/Brixen (Italy) September 10-13, 2008

Scientific Program
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Under the auspices of:

Gruppo Interdivisionale Risonanze Magnetiche
(Società Chimica Italiana)

Comune di Bressanone
Gemeinde Brixen

Università di Padova

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SCIENTIFIC PROGRAM

Wednesday, September 10

15.00-19.00 REGISTRATION

19.00-21.00 Welcome Reception

Thursday, September 11 - morning

8.30-8.45 OPENING REMARKS
8.45-10.15 PLENARY SESSION, Aula Magna. Chair: S. Mammi

8.45-9.30 **F. Dardel,** University of Paris 5, France
RNA as a drug target: NMR-based approaches toward new specific ligands

9.30-10.15 **D. Capitani,** IMC**,** CNR, Montelibretti **- L.Mannina**, University of Molise
Nuclear Magnetic Resonance and Annalaura Segre: a big love

10.15-10.35 **BRUKER: A. Minoja**, Bruker BioSpin srl, Milano
Developments in superconducting NMR magnet technology

10.35-11.05 COFFEE BREAK

11.05-12.35 **"ANNALAURA SEGRE" MINISYMPOSIUM**
Aula Magna. Chair: D. Capitani, L. Mannina

11.05-11.35 **B. Blümich ,** RWTH Aachen University ,Germany
Mobile NMR and Art

11.35-12.05 **V. Busico – R. Cipullo,** University of Napoli "Federico II"
Learning "Polypropylenese": at School with a Master

12.05-12.35 **A.P. Sobolev**, IMC**,** CNR, Montelibretti
NMR metabolomics of genetically modified plants: the tomato and lettuce study cases

12.35-14.10 LUNCH, Hotel Elefante

Thursday, September 11 - afternoon

14.15-15.00 PLENARY SESSION, Aula Magna. Chair: M. Piccioli

14.15-15.00 **B. Brutscher,** Institut de Biologie Structurale - J.-P. Ebel, Grenoble, France
Tools for fast multidimensional protein NMR spectroscopy

15.00-16.10 PARALLEL SESSIONS

BIOMOLECULAR NMR
Aula B. Chair: M. Piccioli

15.00-15.30 **L. Franzoni**,
University of Parma
NMR dynamics studies and line shape analysis suggested distinct mechanisms of retinol binding by two homologous cellular proteins

15.30-15.50 **M. D'Onofrio**,
University of Verona
Lipid trafficking: unfolding and binding features of liver intracellular bile acid binding proteins

15.50-16.10 **S. Scanu**,
University of Milano
Does the interaction between cL-BABP and organic compounds of pharmacological interest change its affinity to physiological ligands?

METHODOLOGY
Aula Magna. Chair: M. Cremonini

15.0-15.30 **G. Pileio**,
University of Southampton, UK
Extremely long-lived nuclear spin states of $^{15}N_2O$ and their manipulation in low magnetic fields

15.30-15.50 **M. Mori**,
CERM, University of Florence
CSM/CSM cross correlation rates observed via ^{13}C direct detection

15.50-16.10 **S. Sykora**,
Extra Byte, Castano Primo (MI).
Bayesian DOSY: a new approach to diffusion data processing

16.10-16.40 COFFEE BREAK

17.00 GIDRM AND GIRM MEETINGS, Aula Magna

Friday, September 12 - morning

9.00-10.05 PLENARY SESSION, Aula Magna. Chair: M. Geppi

9.00-9.45 **J. Brus,** Institute of Macromolecular Chemistry, Prague, Czech Republic
Arrangement and dynamics of molecular segments as seen by dipolar solid-state NMR

9.45-10.05 **VARIAN: B. Heise,** Varian Limited
A new approach to NMR: The OneNMR™ probe

10.05-10.40 COFFEE BREAK

10.40-12.10 PARALLEL SESSIONS

NMR OF SMALL MOLECULES
Aula Magna. Chair: S. Chimichi

10.40-11.10 **A. Mele**,
Politecnico di Milano
NMR study and ab initio calculations of the molecular structure of three novel pyrrolidinium based ionic Lliquids

11.10-11.30 **A. Mangoni**,
University of Napoli "Federico II"
J-coupling analysis as a means for stereochemical assignments in furanosides

11.30-11.50 **O. Tagliatela-Scafati**,
University of Napoli "Federico II"
An NMR-computational approach for the assignment of relative configuration to natural products containing medium-sized rings

11.50-12.10 **L. Fusaro**,
University of Cagliari
Sulfur hexafluoride: a powerful ^{19}F NMR spy for probing systems in solution

RELAXATION / LOW RESOLUTION
Aula B. Chair: M. Fasano

10.40-11.10 **F. Tedoldi**,
Bracco Imaging SpA, Colleretto Giacosa (TO)
Relaxation and pseudo-relaxation effects in MR-Imaging of atherosclerotic plaques

11.10-11.30 **R. Gobetto**,
University of Torino
New hyperpolarized contrastagents for ^{13}C-MRI: catalytic parahydrogenation of alkynyl substrate

11.30-11.50 **M. Mauri**,
University of Milano
Interaction of fluorinated drugs and β-lactoglobulin characterized with diffusion and relaxation ^{19}F NMR

11.50-12.10 **F. Arena**,
University of Torino
Enzyme mediated MRI probes: design, synthesis, and relaxivity behaviour

12.30-14.10 LUNCH, Hotel Elefante

Friday, September 12 - afternoon

14.15-15.00 PLENARY SESSION, Aula Magna. Chair: A. Spisni

14.15-15.00 **R. Fattorusso**, University of Napoli "Federico II"
CYS2-HIS2 Zinc Finger in Bacteria: a Novel DNA Binding Domain with Peculiar Structural Features

15.00-16.20 PARALLEL SESSIONS

BIOMOLECULAR NMR
Aula B. Chair: M. Paci

15.00-15.30 **F. Toma**,
University od Evry, France
Solution structure of the membrane proximal region of hiv-1 envelope glycoprotein gp41 in membrane mimicking environment

15.30-16.00 **F. Cantini**,
CERM, University of Florence
The NMR contribution to unravel copper homeostasis in the cell: the case of ATP7A and ATP7B proteins

16.00-16.20 **T. Eliseo**,
University of Roma, "Tor Vergata"
The structural basis of the 3A versus 1B genotype Hepatitis C virus NS3 protease inhibitors potency shift: an NMR Study

MATERIALS SCIENCE
Aula Magna. Chair: S. Spera

15.00-15.30 **M.R. Chierotti**,
University of Torino
Polymorphism and polymorph conversions: a Solid-State NMR analysis

15.30-15.50 **F. Presciutti**,
CNR-ISTM, Perugia
Study of cyclododecane effectiveness as a temporary consolidant of stones and mortars by profile NMR-Mouse

15.50-16.10 **G. Paul**,
University of Piemonte Orientale
Non-covalent interactions of a drug molecule encapsulated in a hybrid silica gel: an investigation by high resolution 2D HETCOR Solid-State NMR spectroscopy

16.20-18.15 POSTER SESSION & COFFEE BREAK

18.15-19.15 ***GIDRM-GIRM 2008 Gold Medal Award Ceremony***
Aula Magna. Chair: S. Chimichi

R. Bazzo "30 Years of NMR"

19.30 SOCIAL DINNER

Saturday, September 13 – morning

9.00-9.45 PLENARY SESSION, Aula Magna. Chair: S. Mammi

9.00-9.45 **M. Fasano**, University of Insubria
The extraordinary ligand binding properties of human serum albumin

9.45-10.45 BEST POSTER AWARD

10.45-11.15 COFFEE BREAK

11.15-13.00 PLENARY SESSION, Aula Magna. Chair: H. Molinari

11.15-11.45 **R. Simonutti**, University of Milano-Bicocca
Hyperpolarized Xenon NMR of Building Stones

11.45-12.15 **A. Mucci**, University of Modena and Reggio Emilia
Neoplasms of the Human Gastro-Intestinal Tract Characterized by HR-MAS NMR

12.15-13.00 **A. Gräslund**, University of Stockholm, Sweden
The Amyloid beta peptide involved in Alzheimer´s disease: molecular interactions and secondary structure conversions

13.00-13.10 CONCLUDING REMARKS

13.15 FAREWELL BUFFET

PLENARY LECTURES

RNA AS A DRUG TARGET: NMR-BASED APPROACHES TOWARD NEW SPECIFIC LIGANDS

F. Dardel

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Dedicated to Annalaura

NUCLEAR MAGNETIC RESONANCE AND ANNALAURA SEGRE: A BIG LOVE

D. Capitani,[‡] L. Mannina[† ‡]

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This lecture is completely dedicated to Annalaura, our beloved teacher and dear friend. Annalaura was a scientist, expert in NMR technique. She was author of more than 350 papers, so it is extremely difficult for us to summarize her scientific activity. We decided to show the major results she obtained working with us following the same criteria she used to give us a scientific thematic: Donatella, as mathematician and physicist will show the results obtained in solid state NMR, Luisa as chemist will show the results obtained in solution NMR.

TOOLS FOR FAST MULTIDIMENSIONAL PROTEIN NMR SPECTROSCOPY

Ewen Lescop, Paul Schanda, and Bernhard Brutscher

Institut de Biologie Structurale Jean-Pierre Ebel, 41 rue Jules Horowitz, F-38027 Grenoble; CEA; CNRS; Université Joseph Fourier; France

Standard multidimensional NMR techniques suffer from the long experimental times associated with the recording of hundreds to thousands of data points required to sample the indirect time domains, even if sensitivity is abundant. Here, we present new tools developed in our laboratory to speed up NMR data acquisition and processing.

A first application of these fast NMR tools concerns the rapid resonance assignment of small proteins. The BATCH (for combined BEST – ASCOM - Targeted sampling – COBRA - HADAMAC) strategy consists in time-optimized NMR data collection and analysis. In BATCH, triple resonance experiments are time-optimized by fast pulsing (BEST) and optimized ^{15}N time-domain sampling (ASCOM). The sequential connectivities for pairs of ^{1}H/^{15}N frequencies are then automatically extracted by COBRA, a computational tool that is particularly efficient for the analysis of targeted ^{13}C time-domain sampling. Amino-acid type information is retrieved from an additional amino-acid-type edited experiment (HADAMAC). Finally, complete backbone resonance assignment is achived by a dedicated computer program interfaced to the NMRView software. Fast backbone assignment is crucial in the context of structural proteomics and for the study of lifetime-limited systems.

A second application of interest is the study of molecular kinetics by off-equilibrium real-time multidimensional NMR. The SOFAST-HMQC experiment in combination with a rapid injection device provides site-resolved resolution of reaction kinetics down to a time scale of a few seconds. Applications will be shown to protein refolding transitions and hydrogen exchange measurements.

ARRANGEMENT AND DYNAMICS OF MOLECULAR SEGMENTS AS SEEN BY DIPOLAR SOLID-STATE NMR

J. Brus,[‡] M. Urbanova,[‡]

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Measurements of dipolar couplings generally yield direct information on internuclear distances. The requirement of labeled materials, however, renders application of standard solid-state ^{13}C-^{13}C (^{15}N) correlation techniques impractical for solving of many academic and industrial problems. In many cases the synthetic effort necessary to prepare selectively and/or uniformly labeled molecular systems is really daunting. The only way how to overcome this limitation is measurement of dipolar couplings involving ^{1}H atoms (e.g. ^{1}H-^{1}H, ^{13}C-^{1}H etc). Recent methodological advances in developments of homodecoupling sequences (FSLG, PMLG, DUMBO, e-DUMBO etc.) improved sensitivity and resolution in ^{1}H frequency dimension in such way that the basic concept of correlation experiments could be applied on systems at natural isotopic abundance. Subsequent analysis of the obtained highly-resolved correlation patterns provides sufficient number of dipolar contacts and distance restrains that can be used to reconstruct basic structural motifs and fragments not only for highly organized and well arranged crystals of organic solids but also for partially ordered polymer systems. Several examples of utilizations of heteronuclear correlation experiments and their analysis will be demonstrated and discussed. However, dipolar couplings provide information not only about interatomic distances. From the motional averaging of dipolar couplings valuable information about segmental dynamics – motional amplitudes – can be derived. Recently developed pulse sequences designed for the "domain-selective" recoupling of heteronuclear dipolar interactions in mobile amorphous and/or rigid crystalline phases [1] have been successfully applied to describe amplitudes of segmental motions in heterogeneous polymer nanocomposites below and above glass-transition temperature [2,3]. It will be also demonstrated that the applied dipolar recoupling experiments yield valuable data that can be related to the mechanical and thermomechanical properties of polymer nanocomposites. Finally, the analysis of segmental dynamics in crystalline active pharmaceutical ingredients (API) will be discussed with respect to the crystal disorder and to the thermodynamic stability of various polymorphs.

References

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[3] J. Brus, M. Urbanova, A. Strachota, *Macromolecules* **2008**, *41 (2),* 372.

CYS2-HIS2 ZINC FINGER IN BACTERIA: A NOVEL DNA BINDING DOMAIN WITH PECULIAR STRUCTURAL FEATURES

G. Malgieri, L. Russo, S. Esposito, I. Baglivo, M. Palmieri, Elisabetta Moroni[‡], Giorgio Colombo[†], Benedetto Di Blasio, Carla Isernia, P. V. Pedone, R. Fattorusso

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The first prokaryotic classical zinc finger containing protein was discovered in Agrobacterium tumefaciens[1]: it is a 15.5 kDa protein called Ros, and is encoded by the A. tumefaciens chromosomal gene ros. Through electrophoresis mobility shift analysis, we demonstrated that 56-146 Ros fragment (Ros87), which is soluble and contains the zinc finger domain, is still able to bind DNA[2]. We then structurally characterized through NMR spectroscopy the solution structure of Ros87 and defined its residue involved in DNA interaction [3]. The high resolution structure of Ros87, the role of the zinc ion in its folding and the modality of its interaction with DNA clearly demonstrate that the prokaryotic zinc finger domain represents a unique DNA binding domain. An analysis of structural features of Ros homologues will be also presented.

References

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[3] G. Malgieri, L. Russo, S. Esposito, I. Baglivo, L. Zaccaro, E.M. Pedone, B. Di Blasio, C. Isernia, P.V. Pedone, R. Fattorusso. *Proc. Natl. Acad. Sci. USA, **104**,* 17341-17346 (2007)

THE EXTRAORDINARY LIGAND BINDING PROPERTIES OF HUMAN SERUM ALBUMIN

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[‡]

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Human serum albumin (HSA) participates to heme scavenging, the bound heme turning out to be a reactivity center and a powerful spectroscopic probe [1]. The modular three-domain structure of HSA arises from a divergent evolution of a degenerated ancestral gene followed by fusion events and is made by three homologous domains. HSA has several binding sites for different ligands; in particular, it is able to bind up to seven equivalents of long chain fatty acids (FA) at multiple binding sites (labelled FA1 to FA7, see Figure 1) with different affinity. FA1, located in subdomain IB, hosts the heme. Recent reports suggest that FA1 structure has evolved to specifically bind the heme, FAs being secondary ligands [2] (Fig. 1).

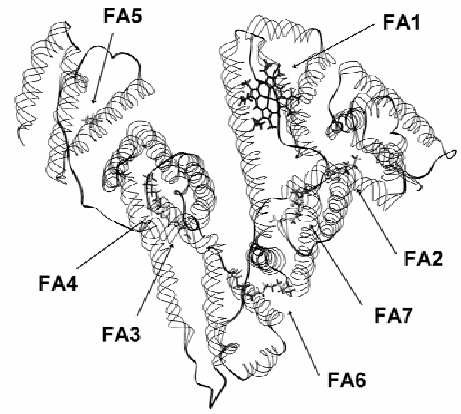

Fig. 1. HSA structure showing the seven fatty acid binding sites. FA1 is occupied by heme.

HSA shows allosteric properties, the binding properties of each site being regulated by heterotropic interactions [3]. Here, an overview of the functional links between the binding sites will be given as obtained by NMR relaxation, optical spectroscopy, and computational methods.

References

[1] M. Fasano, S. Curry, E. Terreno, M. Galliano, G. Fanali, P. Narciso, S. Notari, and P. Ascenzi *IUBMB Life* **57**, 787-796 (2005)
[2] M. Fasano, G. Fanali, L. Leboffe, and P. Ascenzi *IUBMB Life* **59**, 436-440 (2007)
[3] G. Fanali, A. Bocedi, P. Ascenzi, and M. Fasano *FEBS J* **274**, 4491-4502 (2007)

THE AMYLOID BETA PEPTIDE IN ALZHEIMER´S DISEASE: MOLECULAR INTERACTIONS AND SECONDARY STRUCTURE CONVERSIONS

A. Gräslund

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The amyloid β peptide consists of 39-43 residues and is the major component of neuritic plaques in the brain from patients suffering from Alzheimer's disease. In aqueous solution the Aβ peptide aggregates and forms β-sheet structures, finally forming solid fibrils. We study the structure conversions and aggregation properties of the Aβ(1-40) peptide using high resolution NMR spectroscopy. At low concentrations, low temperatures and low ionic conditions in an aqueous solution, Aβ(1-40) is monomeric. Although the peptide displays only weak propensities towards secondary structure, CD and NMR can be used to characterize these propensities in different segments of the peptide, also after adding metal ions like zinc or copper (1-3).

By gradually adding the detergent lithium dodecyl sulphate (LiDS) or SDS to a dilute aqueous solution of Aβ(1-40), secondary structure conversions of Aβ(1-40) can be observed (4). An initial transition after adding small amounts of LiDS involves conversion of the weakly structured peptide to β-sheet structure, concomitant with formation of large aggregates and loss of NMR signals. This structure transition may mimick the behavior of the Aβ peptide, forming oligomeric structures at a crowded membrane surface. At concentrations close to the detergent CMC or above, a second transition makes the peptide rearrange to form a partly α-helical structure, concomitant with disaggregation and formation of normal LiDS micelles which apparently partly dissolve the aggregates. This α-helical structure is similar to that previously observed by NMR at high SDS concentrations. It has two α-helical segments, Aβ(16-24) and (29-35), separated by a flexible hinge, and flexible unstructured N- and C-termini (5).

References

1. Danielsson, J., Jarvet, J., Damberg, P. and Gräslund, A. The Alzheimer β-peptide shows temperature-dependent transitions between left-handed 3_1-helix, β-strand and random coil secondary structures. FEBS J. 272 (2005) 3938-3949.
2. Danielsson, J., Andersson, A., Jarvet, J. and Gräslund, A. ^{15}N relaxation study of the amyloid β peptide: structural propensities and persistence length. Magn. Res. Chem. 44 (2006) S114-S121.
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ORAL COMMUNICATIONS

DEVELOPMENTS IN SUPERCONDUCTING NMR MAGNET TECHNOLOGY

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NMR is a leading technique for structural elucidation of biological molecules, but the contribution of NMR to structural biology is presently limited both in sensitivity and in spectral resolution. Magnetic field strength is a limiting factor.

The development of new superconducting technology (not only for NMR) is one of the core activity of the Bruker Corporation R&D group.
The development of Ultra High Field NMR magnet or of High Field MRI magnet it's really a challenging activity and also the 'core' of the NMR and MRI system.
Consequently the magnet development is becoming more and more important and Bruker has expanded a lot his activity in the basic research in such field.

The lecture will focus on the 'state of art' of the heart of the NMR system from the spectroscopist point of view.

MOBILE NMR AND ART

Bernhard Blümich

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Mobile single-sided sensors like the NMR-MOUSE [1] eliminate the need for measuring an object inside a magnet, enable nondestructive investigations of the object at its location, and are suited to acquire signals from hard materials. It was the idea of Annalaura Segre (Fig. 1) to make use of these unique features of the NMR-MOUSE to investigate objects of art and cultural heritage [2]. Recent developments of mobile NMR and the NMR-MOUSE will be reviewed [3] with particular attention to art and cultural heritage. New investigations concern a quantitative study of paintings [4], bones and mummies [5, 6], and the analysis of a series of precious violins from different ages.

Figure 1. Annalaura Segre on the occasion of the first measurements of cultural heritage at the site by mobile NMR (Rome, 2002).

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LEARNING "POLYPROPYLENESE": AT SCHOOL WITH A MASTER

Vincenzo Busico and Roberta Cipullo

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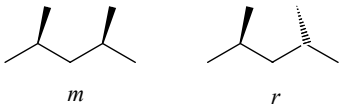
Heartily dedicated to Annalaura Segre, in memory

Stereoselective polymerization is a very special case of asymmetric synthesis. For a reaction affording low-molecular-mass products, the effectiveness of the asymmetric induction can be measured in terms of the so-called enantiomeric excess (*i.e.*, the difference between the fractional abundances of the two resulting enantiomers), but this gives no information on the reaction mechanism. For a polymerization, instead, the fact that the products of individual reaction steps (*i.e.*, the monomeric units) are permanently enchained in the form of macromolecules represents an extraordinary advantage for mechanistic purposes. In fact, from the stereochemical characterization of a polymer, in addition to the degree of stereoregularity (which is the equivalent of the enantiomeric excess) one can derive the way each reaction step affected the subsequent one(s). In this respect, a polymer chain contains its complete birth story, sequentially recorded like on a tape; of course, to know that story one must be able to read the tape.

Popular with biopolymers, the concept found limited application for synthetic polymers. For decades, we Ziegler-Natta chemists have tried with polypropylene, in order to trace the behavior of our complicated and molecularly ill-defined heterogeneous catalysts. Year after year, our reading has become more and more fluent, and our understanding lately deeper and deeper.[1] "Polypropylenese" is a rather weird language, based on two letters only – namely, "*m*" and "*r*" (see Chart) – but with a fairly high number of dialects. The isotactic version makes use predominantly of *m*'s in long strings, with just a few *r*'s usually occurring in couples (*rr* triads). The opposite holds for the syndiotactic one. The atactic form, instead, is much more varied, with an equal number of randomly distributed *m*'s and *r*'s. Learning the three is not difficult, but problems arise whenever they are intermixed, which is unfortunately the case for most polymers of industrial relevance.

Chart



m *r*

Our reading eye for "polypropylenese" is ^{13}C NMR. Routine ^{13}C NMR spectra of polypropylene give easy access to the distribution of sequences with up to four characters (pentads), but even this is usually not enough. In a 15 year long joyful collaboration with Annalaura Segre, we developed high field (150 MHz) ^{13}C NMR techniques enabling us to go far beyond – in favorable cases up to twelve characters (tridecads), more typically from six to eight (heptads to nonads). Throughout these years, we have applied such tools to a variety of polypropylene grades produced with all sorts of heterogeneous and homogeneous catalysts, and read many fascinating and often unexpected stories. In this communication, we will give an account of some of the most intriguing ones.[2]

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Dedicated to Annalaura Segre

NMR METABOLOMICS OF GENETICALLY MODIFIED PLANTS: THE TOMATO AND LETTUCE STUDY CASES

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High resolution NMR spectroscopy for the metabolome analysis of genetically modified (GM) plants is a powerful tool for the detection of primary and secondary effects of transgene expression [1, 2]. NMR gives a complete picture of complex metabolic mixtures without necessity of time consuming separation and/or derivatization of components. NMR spectra can depict "fingerprint profiles" of plant tissue extracts and, after an accurate assignment, corresponding metabolites can be identified and quantified. A proper multivariate statistics is necessary to analyze NMR data to assess the contribution of genetic modification and environmental factors into the complex metabolome changes.

This approach was used in the study of transgenic tomatoes (*Solanum lycopersicum* cv. 'Ohio') and lettuce (*Lactuca sativa* cv. 'Cortina') lines, which over-expressed the *S-adenosylmethionine decarboxylase* and the prokaryotic *asparagine sinthetase* A genes, respectively. ^{1}H NMR spectra of aqueous and methanol/chloroform extracts were assigned using 2D NMR experiments (^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) [3, 4]. The effects of transgene expressions were monitored at different phases of tomato fruit ripening and lettuce leaf development [1, 2, 5]. In both transgenic and wild type lines, the Principal component analysis (PCA) revealed that major changes of water-soluble metabolite contents were associated with organ development. In both the tomato and lettuce cases, the comparison of metabolic profiles from samples at comparable stages discriminated significantly the control from the transgenic lines. Interestingly, a transgenic lettuce line showed a dramatic increase of inulin content, which is likely not to be directly caused by the transgene activity.

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EXTREMELY LONG-LIVED NUCLEAR SPIN STATES OF $^{15}N_2O$ AND THEIR MANIPULATION IN LOW MAGNETIC FIELDS

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The existence of long-lived nuclear spin states in solution NMR has been demonstrated and their lifetime measured in many systems [1-8] and rationalized in terms of semi-classical relaxation theory [4-6]. Their lifetimes, which extend much longer than that of longitudinal magnetization, have suggested several possible applications and some of them, like the measurement of slow diffusion and chemical exchange, have already been demonstrated [7-9].
In this work we show that in some circumstances doubly ^{15}N-labeled nitrous oxide ($^{15}N_2O$) has a singlet lifetime of more than 25 minutes. This compares to longitudinal relaxation that in this case was measured to be of the order of 3 minutes. The long lifetimes are observed when a sample of $^{15}N_2O$ gas is dissolved in a liquid like DMSO-d_6, olive oil, goose fat or milk cream (to cite a few interesting examples) and the system transported into a low magnetic field region. The long singlet lifetime has been understood using a relaxation theory based on the spin-rotation mechanism.
It is easy to see the great potential of such a discovery when applied, for example, to food chemistry or to medical diagnosis, especially in combination with hyperpolarization methods (it should be noted that N_2O is completely harmless - it is already used as a food additive and as an anesthetic). However, in order to make these applications possible, it is necessary to manipulate singlet states in low magnetic fields. It is here shown a simple way to do this and its application to measure J couplings with a precision of 1 mHz. This opens the scene to a new class of experiments in low magnetic field.

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CSM/CSM CROSS CORRELATION RATES OBSERVED VIA ^{13}C DIRECT DETECTION

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The use of ^{13}C direct detection is becoming popular to obtain NMR information when ^{1}H signals are barely observable. Counter-intuitively, even structural information involving ^{1}H nuclei, such as H_NN or $H_\alpha C_\alpha$ residual dipolar couplings can be better obtained using ^{13}C direct detected experiments rather than conventional ^{1}H detection.

Within this general frame, we wanted to exploit this methodology to get more direct approaches for the measurements of relaxation rates involving heteronuclei. Cross correlation rates of zero and double quantum coherences, due to Chemical Shift Modulations (CSM), involving carbonyl C' and amide N nuclei in protein backbone provide evidences of slow motion in proteins. They may reflect dynamics on time scales in the range of micro to milliseconds and vary significantly along the protein backbone.

The sequence we present is a modified version of a CON experiment where C' spins are excited and coherence is transferred via an INEPT-type building block to obtain a C'zNz coherence. After a purging gradient, the MQ coherence is created and evolved. At the end of the evolution period, difference phase cycles may select either the auto-relaxation term or the CSM-CSM relaxation allowed coherence transfer term. Nitrogen magnetization is then evolved prior to the back transfer INEPT, which encodes an IPAP scheme to provide observable in phase C' signal during acquisition. ^{1}H spins are decoupled throughout the entire sequence while 180° $^{13}C_\alpha$ pulses prevent the evolution of undesired scalar couplings and cross correlation involving C_α spins. At the expenses of a reduction in sensitivity, the sequence is efficient in circumventing signal losses due to fast transverse relaxation arising from various sources such as exchange phenomena or paramagnetism.

To validate the sequence, we used the 75 aa protein Calbindin $D_{9k,}$ a small calcium-binding protein, in which one of the two calcium ions can be substituted by a lanthanide ion, thus giving rise to a small paramagnetic metalloprotein.

CSM/CSM experiments were performed on diamagnetic Ca_2Cb and on paramagnetic CaCeCb derivatives, in order to compare performances of the ^{1}H detected pulse sequence with those of the ^{13}C detected experiment. The two sets of data were in a very good match, thus assessing the efficiency of the new designed ^{13}C detected pulse sequence. The loss in signal intensity due to the fact that C' is the excited and observed nucleus $((\gamma_H/\gamma_C)^{5/2}=32)$ is much lower than the theoretical S/N decrease expected. This indicates that significant losses due to ^{1}H relaxation occur also in the absence of paramagnetic effects. Finally, the two experiments are fairly complementary, because spectral overlap is of course different in the two spectra.

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BAYESIAN DOSY:
A NEW APPROACH TO DIFFUSION DATA PROCESSING

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Certain types of multi-array NMR techniques present problems with the visual representation of the results in terms of the physical parameter associated with each spectral peak. This regards, in particular, the Diffusion Ordered Spectroscopy (DOSY) where the non-spectral parameter is the diffusion coefficient, and also ROSY (Relaxation Ordered Spectroscopy) for which it is the relaxation time. In such cases, the transformation of the original data set into a suitable final 2D representation (such as, for example, the DOSY transform) is *conceptually* difficult to manage.

Current approaches are often little more than graphic sketches based on the evaluation of the decays at a selected set of spectral points (usually those corresponding to peak tops). Unfortunately, the physical significance of such graphic representations does not exceed that of tabulated results and, more generally, the approaches can be criticized for treating different spectral points in a different way (trying to extend them to spectral regions with little or no signal gives rise to characteristic, noise-induced artifacts). Other, physically more acceptable approaches, such as the MaxEnt algorithm of Marc-Andre Delsuc [1] et al, unfortunately require very long processing times.

We have developed [2] a novel Bayesian approach to this problem which is computationally very efficient and physically eminently meaningful, treats all data points in the same way, and gives very satisfactory and artifact-free results. Applied specifically to DOSY data sets, it leads to what we call the **BDT** algorithm (standing for *Bayesian DOSY Transform*). It is also an excellent example of the broad class of Bayesian approaches to the evaluation of NMR data.

The BDT algorithm is the core of the **BayDOSY** evaluation and presentation method implemented in Mnova, which is now beta-tested in practice on real experimental data. Apart from BDT, BayDOSY includes also a novel algorithm for a substantial improvement of the resolution in the diffusion-constant dimension and a contextual improvement in the alignment of peaks belonging to the same molecular species. Ongoing work aims at Bayesian handling of overlapping spectral peaks belonging to different sample components.

In this talk we will discuss in detail the BDT algorithm and illustrate on practical examples the performance of the BayDOSY method.

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NMR DYNAMICS STUDIES AND LINE SHAPE ANALYSIS SUGGESTED DISTINCT MECHANISMS OF RETINOL BINDING BY TWO HOMOLOGOUS CELLULAR PROTEINS

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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. The transfer of retinol from its plasma carrier (RBP) to the intracellular compartment is mediated by a membrane receptor [1, 2]. The molecular mechanism of the subsequent uptake by cellular retinol-binding proteins (CRBPs), however, has yet to be elucidated. Given the important role of retinoids, the characterization of protein-ligand interactions and targeted release, is of special interest. We have addressed this challenging problem by studying CRBPs type I and II, the primary carriers of retinol inside the cells. They play distinct roles in the maintenance of vitamin A homeostasis and differ in tissue distribution, while sharing the same structural topology which consists of ten antiparallel β-strands and two short α-helices.

The determination of the solution structures and backbone dynamics (*ps-ns* time scale) did not account for the access of the ligand to the binding cavity [3]. Relaxation dispersion experiments revealed more significant dynamic effects on a *μs-ms* time scale for the apo-forms of both proteins; the high degree of conformational flexibility was almost completely quenched upon ligand binding [4, 5]. These results gave rise to the question of whether the observed slow dynamics in the apo-forms are essential for retinol uptake and how they might lead to conformations that allow access to the cavity.

Interestingly, line shape analysis of ^{15}N-HSQC spectra recorded in the course of titrations with retinol suggested that the two closely homologous proteins bind the ligand by distinct mechanisms [6, 7]. A major advantage of this approach over other techniques that are used to study binding kinetics is that differential line shape changes using isotope-edited two-dimensional NMR spectra provide kinetic information on individual residues and insight into complex mechanisms of reactions.

Hydrogen/deuterium exchange experiments revealed that the two proteins also exhibit a different structural stability which seems to modulate ligand-binding affinity [8].

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LIPID TRAFFICKING: UNFOLDING AND BINDING FEATURES OF LIVER INTRACELLULAR BILE ACID BINDING PROTEINS

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A central aspect of lipid homeostasis in vertebrates is the acquisition, synthesis and metabolism of cholesterol. In particular, the majority of any excess cholesterol is degraded into bile acids (BAs).
BAs perform several functions in lipid physiology: they counterbalance the cholesterol synthesis pathway and allow homeostasis to be achieved; their detergent actions are essential within the intestine and the liver for the uptake of hydrophobic nutrients, and for the solubilization of metabolites; intermediates and endproducts of the bile acid pathway regulate the expression of genes that synthesize cholesterol, fatty acids, and bile acids themselves. It is thus clear that BAs and more generally lipid trafficking in cells is a complex and dynamic process that affects many aspects of cellular functions. In this scenario a big part is played by a group of small (14-15 KDa) proteins belonging to the family of intracellular lipid binding proteins. Little is known about their exact biological functions and mechanism of action, however it is widely accepted that they bind poorly water soluble ligands.
Although the liver is the central organ for regulation of cholesterol homeostasis it is not clear who plays the role of the bile acid chaperon in this tissue. Our group has previously proposed and validated a parallel model of bile acid transport in hepatocytes and ileocytes in non-mammalian and mammalian species [1]. In the former, a BABP protein displaying high affinity towards bile acids is found. Earlier studies on chicken liver BABP set the basis for understanding the mechanism of binding to physiological bile acids [2]. While the structural features have been now described in detail [3] [4] the determinants of binding are still to be clarified. Our current studies are addressing the possibility that folding and binding (function) may be related.
On the other hand, in human liver, the proposed bile salt carrier is a fatty acid binding protein, hl-FABP. The latter has a large hydrophobic internal cavity, capable of binding a variety of ligands, including heme, retinoic acid, fatty acids and bile acids. NMR ligand binding as well as competition experiments, currently in progress in our laboratory, aimed at elucidating the determinants of binding and, ultimately confirm the role of FABP in bile salt transport.

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DOES THE INTERACTION BETWEEN cL-BABP AND ORGANIC COMPOUNDS OF PHARMACOLOGICAL INTEREST CHANGE ITS AFFINITY TO PHYSIOLOGICAL LIGANDS?

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Chicken liver bile acid-binding protein (cL-BABP), a member of the lipocalin super family, displays a barrel fold made up of ten strands capped with a short helix-loop-helix motif called portal region. This β-barrel is the binding site for endogenous ligands of physiological interest [1]. The crystal structure of chicken L-BABP complexed with cholate showed that the protein binds two molecules of the bile salt in the interior cavity [2] like the human ileal bile acid binding protein (hI-BABP) [3]. Previous works demonstrated that cL-BABP has greater affinity towards glycochenodeoxycholic acid (GCDA) compared to glycocholic acid (GCA), but, at variance with hI-BABP, does not display site selectivity towards these conjugated bile salts [4].
In our work we employed cL-BABP, purified directly from chicken liver, as model for exploring the competition between organic compounds of pharmacological interest and bile salts in bile salt carriers. Some fluorinated drugs like flurbiprofen, (non-steroidal anti-inflammatory) or fluvastatin (HMG-CoA reductase inhibitors) were used as binding competitors of (1-^{13}C glycine)glycocholic acid (GCA) and 24-^{13}C cholic acid (AC) as endogenous ligand analogues. Combined ^{19}F- and ^{13}C-NMR measurements showed that the drugs do not interfere with bile acid binding.

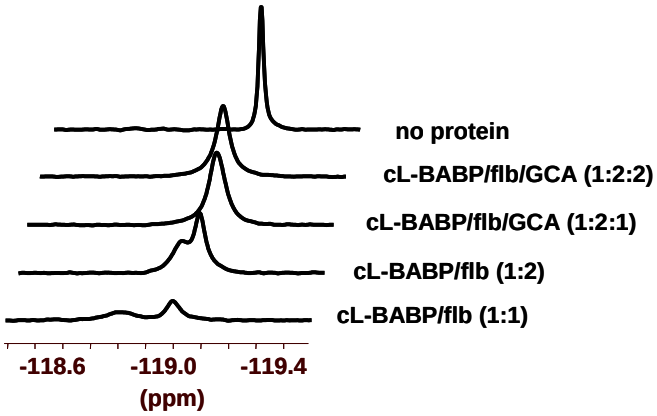

Fig. 1. ^{19}F NMR spectra of flurbiprofen (flb) after stepwise interaction with cL-BABP and competition experiments between the (1:2) protein/drug complex and GCA. Top trace: ^{19}F spectrum of flb in PBS.

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A NEW APPROACH TO NMR: THE ONENMR™ PROBE

Dr. Bert Heise, Varian Limited

In the past 30 years it has been a norm that there is no ideal probe design to accommodate equally good for all types of experiments. Traditionally people have been using direct observe (broadband) probes for experiments requiring X observe and indirect (inverse, reverse) probes for experiments requiring H1 observe. This made it mandatory for one to have two probes to address efficiently every possible situation.

In this talk we will present results towards narrowing the gap between these two traditional probe geometries with the introduction of the OneNMR probe. The results shown will indicate how does this probe compare to traditional direct and indirect probes and the features and benefits arising from this new geometry.

NMR STUDY AND AB INITIO CALCULATIONS OF THE MOLECULAR STRUCTURE OF THREE NOVEL PYRROLIDINIUM BASED IONIC LIQUIDS

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An integrated approach NMR/*ab initio* calculation was applied to novel room-temperature ionic liquids (RTILs). [1,2]. The examined compounds were based on N-butyl-N-methyl pyrrolidinium (PYR_{14}) cation with three different fluorinated anions – trifluoromethanesulfonyl nonafluorobutanesulfonyl imide (IM_{14}), bis(pentafluoroethanesulfonyl) imide (BETI) and bis(trifluoromethanesulfonyl) imide (TFSI). ^{1}H- and ^{19}F- Diffusion Ordered spectroscopy (DOSY) was used to independently measure the self-diffusion coefficients for the individual ions. Anions' diffusion coefficients followed the order TFSI> BETI>IM_{14}. The same order was found for PYR_{14} in the presence of TFSI, BETI or IM_{14} counterions, respectively. 2D NOESY provided us with detailed informations on the intermolecular interactions in the bulk liquids. Homonuclear NOESY [^{1}H-^{1}H], [^{19}F-^{19}F] data were used to determine the cation-cation (^{1}H) and anion-anion (^{19}F) intermolecular interactions, while heteronuclear HOESY [^{1}H-^{19}F] experiments gave us informations on the more complicated cation-anion interactions.

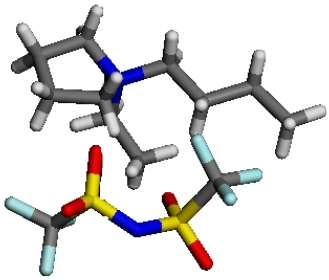

Fig. 1. Conformation of the ion-pair PYR_{14} TFSI.

Ab initio calculations aimed at understanding structural and dynamical properties of title compounds were also performed. The optimized geometries and relative conformational energies were calculated at the HF and PM3 level for all the ions. The lowest energy conformers were optimized at the B3LYP levels. The latter geometries of cation and anions were used as starting point for ion-pair calculations. Therefore combining computational and experimental results, it was possible to understand the local organization for the three samples. Interesting differences in the local ordering is observed by changing the anion with a fixed cationic species.

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J-COUPLING ANALYSIS AS A MEANS FOR STEREOCHEMICAL ASSIGNMENTS IN FURANOSIDES

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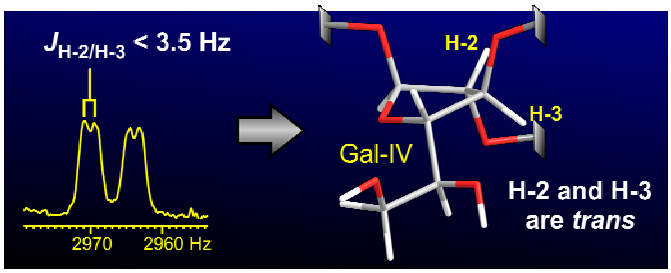
The widespread use of coupling constant information for stereochemical assignment in pyranosides is based on the conformationally rigid six-membered ring they contain. For most pyranosides, one of the two possible chair conformations is largely predominant at room temperature, and this allows an easy discrimination between the large axial-axial couplings and the small axial-equatorial and equatorial-equatorial couplings. This is not the case for furanosides, where the flexibility of the five-membered ring (leading to the so called *pseudorotation*) causes large variations in coupling constants between conformers, which prevent such an easy correlation between coupling constant values and relative configuration of adjacent carbon atoms. As a consequence, stereochemical elucidation of furanosides is usually based on NOESY/ROESY correlations and/or chemical degradation.
As a matter of fact, some correlation between vicinal couplings and relative configuration of furanosides is present in the literature. As early as in 1963, in a paper reporting the proton NMR spectra of all methyl pentofuranosides [1], it was clearly recognized that a "small" vicinal coupling in a furanoside implies that the coupled protons are *trans* oriented. However, this principle has been used in the subsequent structure elucidation work only sporadically, and, even then, in a way that resembles much more the empirical comparison with a model compound than the application of a general rule. The reason for this is probably that the threshold below which a coupling constant can be considered "small" has never been defined, making the practical application of this principle difficult.
To clarify this point, we undertook an in-depth computational study of coupling constants of furanosides, and showed that in furanosides a vicinal coupling constant < 2.0 Hz (for H-1/H-2 or H-3/H-4) or < 3.5 Hz (for H-2/H-3) can be considered a *proof* of the *trans* orientation of the relevant protons.



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AN NMR-COMPUTATIONAL APPROACH FOR THE ASSIGNMENT OF RELATIVE CONFIGURATION TO NATURAL PRODUCTS CONTAINING MEDIUM-SIZED RINGS

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A large number of naturally occurring compounds is characterized by the presence of medium-sized (8-12 membered) rings. These include terpenoids of the caryophyllane, germacrane, cembrane, jathrophane, briarane families, and many others.
The configurational assignment of natural products belonging to these classes presents unique challenges. Indeed, compared to other cyclic systems, where one or only a very limited number of conformations exist, several families of conformations are reasonably populated in medium sized-compounds, and they should all be taken into account to translate dipolar couplings into configurational relationships. Consequently, in these cases, a detailed analysis of the conformational behaviour should be considered mandatory to minimize the risks of stereostructural misassignments
The advanced computational techniques now available have made it possible also to complement the NMR analysis with quantum-mechanical prediction of ^{13}C NMR chemical shifts[1], through *ab initio* calculations of the electronic distribution. The consistency of results obtained from investigation of NOE contacts, molecular mechanics and quantum-mechanical calculation of ^{13}C NMR should be required to confidently assign the relative configuration to these molecules.
The present communication will be focused on the application of these concepts to the configurational analysis of some germacranolides (ketopelenolide D, **1**, Fig. 1) and *nor*-caryophyllanes (artarborol, **2**, Fig. 1) [2] and on their applicability to other classes of natural compounds containing medium-sized rings.

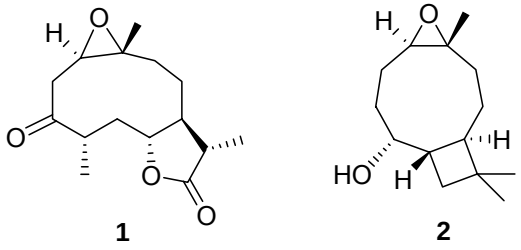

Fig. 1. The chemical structures of ketopelenolide D (**1**) and artarborol (**2**)

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SULFUR HEXAFLUORIDE: A POWERFUL ^{19}F NMR SPY FOR PROBING SYSTEMS IN SOLUTION

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The chemical shift of ^{129}Xe is exquisitely sensitive to the xenon environment and, therefore, ^{129}Xe is used as a monatomic spin-spy for characterizing systems in gas, liquid or solid states. The major drawbacks of ^{129}Xe are its extremely long longitudinal relaxation time in diamagnetic environments and its rather poor NMR sensitivity. This usually prevents quantitative interpretation of the integrals and severely limits the scope of feasible NMR experiments.
This communication reports on the interest of sulfur hexafluoride (SF_6) in the framework of the spin-spy methodology. The inclusion of SF_6 within the cavity of cucurbit[6]uril, cryptophane A and E, α-cyclodextrin and wheat nonspecific lipid transfer protein (LTP) were studied and their major results are presented.
Sulfur hexafluoride comes forward as a versatile and informative spin-spy molecule for probing systems in solution notably because (i) its detection limit by ^{19}F NMR reaches the μM range with standard equipments, (ii) integral measurements allow to quantify the amount of SF_6 in solution, and (iii) chemical shift, diffusion, longitudinal relaxation time as well as intermolecular Overhauser effect measurements can be used for characterizing the system under study.

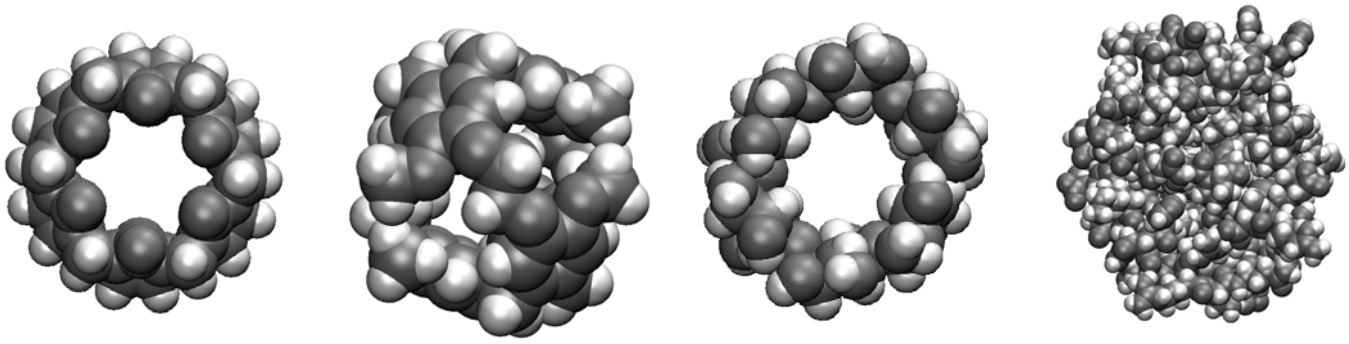

Fig. 1. Representation of cucurbit[6]uril, cryptophane-A, α-cyclodextrin and wheat nonspecific LTP.

RELAXATION AND PSEUDO-RELAXATION EFFECTS IN MR-IMAGING OF VESSEL LESIONS

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Detection and characterization of atherosclerotic plaque is a pressing medical need, being atherosclerosis a major cause of death in Western countries. Magnetic Resonance Imaging (MRI) is one of the candidate techniques that may answer this need and could become a leading diagnostic tool, when plaque-selective Contrast Agents (CA) will be available to radiologists. The peculiar position of plaques in the vessel walls makes the discrimination between a potential CA-driven enhancement of the lesion and a flux-related brightening of the arterial bounder extremely difficult, particularly in small rodents. For this reason we have simulated the Spin-Echo signal expected for a model of mouse carotid in the presence of a time-dependent CA concentration in the blood. Both flux-driven pseudo-T_2 and pseudo-T_1 contributions have been taken into account together with the 'real' magnetic terms driving the signal evolution of static nuclear spins. While the imperfect echo focalization due to spin motion across the observed slice gives rise to the so called 'black blood effect', the same motion brings unsaturated spins in the observed volume and produces signal enhancement. In slow-flux regions, such as lumen boundaries, a hyper-intense ring appears in T_1-weighted axial images, simply as a consequence of the competitive influence of spin movement on longitudinal magnetization recover and echo dephasing. The contrast of these hyperintense regions to the neighbor areas is affected by CA concentration in the blood and can hardly be distinguished by a CA uptake in a vessel lesion, as long as no dynamical analysis is performed. However, the time course behavior of the two phenomena is found to be quite different and it is thus proposed as discerning parameter. The *in vivo* test confirmed these theoretical findings. Sequence modifications, MRI parameter optimization and different ECG triggering modalities have been thus evaluated in order to avoid misinterpretation.

NEW HYPERPOLARIZED CONTRASTAGENTS FOR ^{13}C-MRI: CATALYTIC PARAHYDROGENATION OF ALKYNYL SUBSTRATES

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Many efforts have been devoted in recent years to the development of hyperpolarization procedures, mainly driven by the potential use of hyperpolarized molecules in Magnetic Resonance Imaging (MRI). In this context Para-Hydrogen Induced Polarization (PHIP) methods are currently under intense scrutiny for the preparation of ^{13}C enriched hyperpolarized substances, for which interesting applications as ^{13}C-MRI contrast agents have already been anticipated.

The main advantage of using this kind of contrast agents is due to the lack of background signal, as images are acquired directly on ^{13}C. Being the ^{13}C endogenous signal zero, contrast is simply due to the difference in signal intensity between regions reached by the hyperpolarized molecules and the other tissues.

In order to produce a ^{13}C hyperpolarized contrast agent by parahydrogenation, unsaturated substrates are needed, furthermore the hydrogenation catalyst must be removed before in vivo administration.

Examples of catalytic hydrogenation of selected unsaturated substrates, usually triple bond containing molecules with an adjacent carbonyl group will be discussed. Polarization is transferred to the carbonyl ^{13}C atom (characterized by long T_1 values which allows to reduce polarization loss) through scalar coupling with parahydrogen protons. Longitudinal enhanced magnetization on ^{13}C, necessary for MRI purposes, is achieved by applying a magnetic field cycle to parahydrogenated products.

Particular attention will be focused molecules which afford bio-compatible and water soluble parahydrogenated products. The problem of catalyst and organic solvent elimination will also be tackled.

INTERACTION OF FLUORINATED DRUGS AND β-LACTOGLOBULIN CHARACTERIZED WITH DIFFUSION AND RELAXATION ^{19}F NMR.

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Proteins of the important and diverse lipocalin superfamily display strong binding properties in respect to several small hydrophobic ligands. In the case of the structurally well known β-lactoglobulin (BLG), this interaction has been studied through a wide range of different techniques, albeit with sometimes conflicting results as to the number of binding sites and their relative affinity.[1] For its stability in low pH conditions, this protein is a potential candidate for carrying drugs through the unfavorable environment of the upper digestive tract. Since many widely used drugs contain fluorine atoms, we considered ^{19}F NMR, including pulsed field gradient techniques, as an useful tool for the characterization of their interaction with BLG.
We report the results NMR experiments during the titration of fluorinated molecules such as fluvastatin (HMG-CoA reductase inhibitor), flurbiprofen (Non Steroidal Anti-Inflammatory) and 5-fluorouracil (antimetabolite for cancer therapy) with BLG. While the chemical shift resulted almost insensitive to the presence of protein, changes in relaxation rates (R1 and R2), heteronuclear n.O.e. and diffusion coefficient indicate the formation of drug-protein complexes. In a fast exchanging system the observed properties are a weighted average of contributions relative to the bound and free state, weighted by the bound fraction.[2]
Relaxation rates are easily measured, but the values for the bound state of the ligand are not defined a priori and introduce an additional variable in the titration data fitting. The diffusion coefficient D of the bound ligand, instead, is practically identical to the one of the protein alone.[3] However, when measuring this coefficient by 19F NMR PFG, the same interaction we aim to study produces a large increase in linewidth, up to more than 100 Hz in the experiments where most of the ligand is complexed by the protein. Together with the intrinsic insensitivity of PFG techniques, this makes signal evaluation quite difficult. We simulated different titration procedures, taking into account the several constrains involved in the actual experiments, including NMR sensitivity, ligand and protein solubility limits and desirable ligand/protein ratio relative to the estimated dissociation constant. Simulation suggests that, the best experimental protocol is the addition of protein at as high as possible concentration to a ligand solution of similar concentration.
In this context the best choice among the many PFG sequences described in literature is discussed.[4]

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ENZYME MEDIATED MRI PROBES: DESIGN, SYNTHESIS, AND RELAXIVITY BEHAVIOUR

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The efficiency in accumulating the imaging reporters at the target sites is the major tasks in MRI for the visualization of biological processes at the cellular and molecular level.[1] One way to tackle this amplification route is to seek for the formation of self-assembled aggregates of Gd-chelates.[2] Bogdanov et al showed that melanin-like polymers can form when hydroxo-functionalized Gd-chelates are in the presence of the suitable enzymes (e.g. tyrosinase or myeloperoxidase).[3] Our goal is to exploit this approach in order to set-up a MRI method to assess the expression of *β*-galactosidase (*β*-gal). Therefore the tyrosine –OH functionality has been protected by a galactose moiety and the spontaneous formation of melanin-like polymer may then take place only when the tyr-gal bond is cleaved by β-gal. The in vitro relaxivity of the target molecules with and without sugar has been investigated by ^{1}H nuclear magnetic relaxation dispersion (NMRD). The Gd-DOTA derivative with galactose capped tyrosine moiety has been proven to be a *β*-galactosidase substrate that results in a system that can undergo polymerization in the presence of Tyrosinase upon cleavage of the tyr-gal.

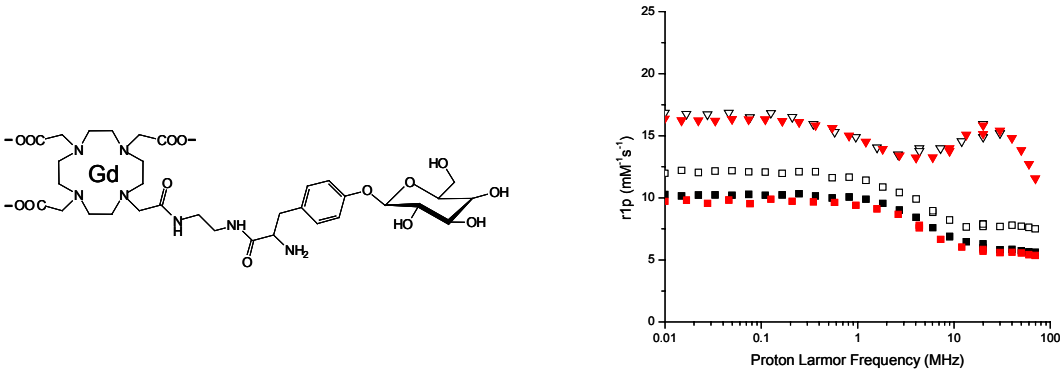

Fig. 1. Structure of the target molecule and 1/T1 NMRD profiles of the different involved species in the presence and in the absence of Tyrosinase and β-Galactosidase enzymes registered at 25°C and neutral pH.

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POLYMORPHISM AND POLYMORPH CONVERSIONS: A SOLID-STATE NMR ANALYSIS

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The phenomenon of polymorphism, namely the existence of more than one crystal structure for a given compound, is well known and widely studied.[1] The quest for, and identification and characterization of different crystal forms of the same molecule (polymorphs and solvates) or of aggregates of the same molecule with other molecules (co-crystals) is one of the most active research areas of modern solid state chemistry with relevant impact on fundamental science as well as on extremely important application areas (drugs, pigments, food industry).[2]
In this field a complete understanding of the structure-property relationships of molecules requires the combined use of complementary techniques. Indeed, when the low crystallinity of the sample does not allow benefiting from the accuracy of single crystal X-ray diffraction (XRD) experiments, the solid-state NMR technique (SS NMR) contributes to this analysis through the dependence of its parameters on the local structure.[3]
With this contribute we aim to present some organic and pharmaceutical example of polymorphism we are studying in our laboratory. Applications of multinuclear (^{1}H, ^{13}C and ^{15}N) 1D (CPMAS, spectral editing) and 2D (^{1}H DQ MAS, ^{1}H-^{13}C FSLG-HETCOR) SS NMR experiments will be presented for elucidating structure features of forms. Furthermore, examples of new technique for obtaining polymorphs (mechanochemical conversions by means of ball mill and crystallization in supercritical solvents) will be given.

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STUDY OF CYCLODODECANE EFFECTIVENESS AS A TEMPORARY CONSOLIDANT OF STONES AND MORTARS BY PROFILE NMR-MOUSE.

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As previously demonstrated in literature, the unilateral NMR relaxometric technique has been shown to be adapt for the study of porous materials such as stones and for the evaluation of the effectiveness of consolidant treatments [1]. This research has primarily focused on the study of the properties of cyclododecane (CDD) as a temporary consolidant for stones and mortars. A known volatile cyclic alkane ($C_{12}H_{24}$), CDD has been seldom adopted for use in the field of conservation in the last 15 years as a temporary consolidant, sealant and hydrophobic protective coating for fragile materials in situations of transport, excavation or handling [2]. This waxy solid at room temperature could be greatly exploited further as routine in this discipline due to its characteristic vapour pressure which permits it to sublime completely within an appropriate time [3] eliminating subsequent removal by physical or chemical operations or unnecessary treatment of residues or by-products.
CDD may be ideally utilized in this field as it has good film-forming properties, low melting point (mp 58–61°C), insolubility in water, solubility in organic solvents, and little or no toxicity [4]. Yet most importantly, its application is easily reversible. The aim of this study is to develop and establish an optimum criteria through credible scientific evaluation for the use of cyclododecane with the objective for future applications to materials of art-historic importance namely paintings, textiles, architectural buildings and sculptures that in the rare case of emergency require an immediate and safe intervention especially if fragile and/or in a dire state of conservation. Stones (carrara marble and lecce stone) and mortars with very different degree of porosity were chosen to test the effectiveness of the CDD. Different solvents were considered on the base of their affinity with the CDD and of their boiling point because this factor plays an important role in the formation of the film and in the depth of penetration of the consolidant inside the sample. Moreover two different methodologies (spray and dropping) of application were tested. Profile NMR-MOUSE [5-6] is a very suitable technique for following the formation process of the film, the depth of penetration of the cyclododecane resulting from the use of different solvents and different stone matrices and its resulting kinetics of sublimation. It is, indeed, important to underline the fact that this instrumentation is portable and all measurements are carried out in a completely non invasive manner, therefore facilitating the possibility to monitor the efficiency of treatments on actual works of art directly on site.

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NON-COVALENT INTERACTIONS OF A DRUG MOLECULE ENCAPSULATED IN A HYBRID SILICA GEL: AN INVESTIGATION BY HIGH RESOLUTION 2D HETCOR SOLID-STATE NMR SPECTROSCOPY

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The area of organic-inorganic hybrid network materials prepared by the sol-gel approach has rapidly become a fascinating new field of research interest in materials science. The non-covalent in-situ encapsulation of a molecular species in a growing sol–gel network is a complex process. A key question for an understanding of the imprinting properties of the included molecule is the nature and role of intermolecular surface interactions. In this study, a drug molecule, Propranolol, has been encapsulated by a sol–gel process in an organic–inorganic hybrid matrix derived from tetraethoxysilane and methyltriethoxysilane by in-situ self-assembly. The local molecular interaction between the drug and the host matrix has been investigated by employing two-dimensional heteronuclear correlation NMR spectroscopy. Moreover, the question as to whether the final sol–gel product is a genuine hybrid material rather than a composite of domains is investigated by 2D HETCOR solid state NMR spectroscopy. The results from the NMR studies provide deeper insights into the molecular level interactions between the host and the guest which will help us to design better materials for controlled drug release.

SOLUTION STRUCTURE OF THE MEMBRANE PROXIMAL REGION OF HIV-1 ENVELOPE GLYCOPROTEIN gp41 IN MEMBRANE MIMICKING ENVIRONMENT.

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The structure of P1, residues 649-683 of HIV-1 envelope glycoprotein gp41, has been determined for the first time from acid to nearly physiological pH, in lipid-like membrane mimicking environment (DPC micelles solution). P1 comprises the conserved membrane proximal ectodomain region MPER and is the minimal MPE region allowing interaction with the mucosal galactosyl ceramide HIV-receptor. It also contains epitopes recognized by major gp41-specific broadly neutralizing IgGs, 2F5 and 4E10, determinant in HIV fusion/infection.

The stability of P1 structure is pH-dependent: the single α-helix running from Q653 to I682 at pH 3.3 is partly unfolded at higher pH values. At pH 6, the N-terminal half of P1 (residues 650-666) partially overlapping the 2F5-specific epitope becomes fully disordered while the C-terminal half conserves two shorter helices (W666-W670 and W672-W680), separated by a well defined bent overlapped by the 4E10 specific epitope.

The 3D solution structures of the 2F5- and 4E10-specific epitopes of free P1 are very close to the crystal structure of the epitopes bound to the respective antibodies. This contrasts with the reported structure of shorter gp41 fragments of the same region. Moreover, the affinity of P1 for the neutralizing monoclonal antibodies in DPC micelles is higher than in aqueous solution. From these results, P1 appears an optimized MPER-derived peptide suitable to design an immunogen for inducing HIV-broadly neutralizing antibodies [1].

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THE NMR CONTRIBUTION TO UNRAVEL COPPER HOMEOSTASIS IN THE CELL: THE CASE OF ATP7A AND ATP7B PROTEINS

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ATP7A and ATP7B are two P-type ATPases involved in copper(I) homeostasis in humans. Under low copper conditions, they deliver copper to the secretory pathway of the trans Golgi network (TGN), where the metal ion is incorporated into copper-dependent enzymes; copper stimulation results in the redistribution of both proteins to the plasma membrane in order to efflux the excess copper out of the cell. The predicted topological organization of both ATP7A and ATP7B includes four major regions/domains: the transmembrane domain, the ATP-binding domain, the actuator domain, and the N-terminal copper-binding domain. The latter contains six domains that are individually folded and capable of binding one copper(I) ion. They receive copper from the cytoplasmic metallochaperone HAH1.

The exact role and interplay of the six soluble domains is still unclear. It has been suggested that the copper-regulated trafficking and catalytic cycle of ATP7A and ATP7B is mediated by protein-protein interactions. To unravel the mechanisms that regulate these processes, we first characterized the structural and dynamic properties in solution of the various soluble domains of ATP7A and ATP7B (N-terminal copper-binding tail, ATP-binding domain and actuator domain) and then subsequently studied the interactions among them.

Moreover, we investigated the entire N-terminal tails of ATP7A [1] and ATP7B (about 630 residues) in solution by NMR spectroscopy, and addressed their interactions with copper(I) and copper(I)-HAH1.

At physiological protein ratios, both the first and fourth domains of the ATP7A tail formed a metal-mediated adduct with HAH1, while the sixth domain was simultaneously able to partially remove copper(I) from HAH1. In the case of ATP7B, copper(I)-HAH1 formed detectable amounts of a macromolecular complex with domains 1, 2 and 4, while domains 3, 5 and 6 removed copper(I) from the metallochaperone, likely through formation of a similar adduct at a concentration too low for detection by NMR.

For both WLN and MNK proteins, the regulation of ATPase activity by the N-terminal tail could involve structural rearrangements of the tail itself upon interaction with copper(I)-HAH1, leading to a variation of its contacts with the other domains of the enzyme. Such events could also be important in determining the relative rates of back- and forward trafficking of the ATPase. Indeed, the present data on ATP7A and ATP7B proteins indicated that the aforementioned structural rearrangements are linked to the formation and presumably accumulation of adducts between the tail and copper(I)-HAH1 at high intracellular copper(I) concentrations.

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THE STRUCTURAL BASIS OF THE 3a VERSUS 1b GENOTYPE HEPATITIS C VIRUS NS3 PROTEASE INHIBITORS POTENCY SHIFT: AN NMR STUDY

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Hepatitis C represents a serious health problem worldwide. The ethiologic agent. Hepatitis C virus (HCV), exhibits a high degree of genetic variability, and this has to be taken into account for a drug therapy active against the different virus genotypes.. In this work we present the first structural characterization of the HCV NS3 protease belonging to the 3a genotype using NMR spectroscopy. Solubility of the protein was increased by mutating seven residues of the N-domain without changing the inhibitor potency profile with respect to the 3a wild-type enzyme. Shifts of three orders of magnitude in inhibitory potency were observed for P2-P4 macrocyclic inhibitors with respect to the 1b protease, but not for a tripeptide phenetylamide inhibitor exploiting S1' binding. The secondary structure of the complex between NS3p3aM7 and the phenetylamide inhibitor is almost identical to that of the corresponding 1b genotype. The inhibitor by its own is capable of stabilizing the active site, and interaction with the viral cofactor NS4A does not change significantly the active site conformation. The dynamics behaviour of two key residues explaining the inhibitor potency shift, Q168 and T123, were determined. The two side chains resulted flexible in the 3a NS3 protease, suggesting that inhibitors exploiting one or both of the two residues for binding, will need to pay a price in terms of a transition between a mobile side chain to a rigid side chain, leading to an extra entropic penalty.

HYPERPOLARIZED XENON NMR OF BUILDING STONES

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The development of optically pumped xenon NMR, that exploits the diffusion of highly nuclear spin-polarized xenon gas on the sample, kicked off completely new fields of applications for xenon NMR: from hyperpolarized biosensors to lung imaging, from combustion studies to the characterization of single crystal surfaces [1]. Also in the area of porous solids, where thermally polarized xenon has been applied very successfully to the study of microporous systems since many years, hyperpolarization extends the application of ^{129}Xe NMR also to systems showing low porosity or pores in the mesoscale. However, porous systems like stones and rocks are still scarcely studied by HP ^{129}Xe NMR, expect few cases regarding the characterization of permeability and porosity of reservoir rocks [2]. In fact rocks are very complex systems constituted by several minerals and characterized by a porosity ranging from the microscale to the macroscale, sometimes also containing paramagnetic systems. On the other hand, many types of rocks are used as building materials, sometimes very valuable, like travertine, granite or marble. The characterization techniques currently used for these materials, like SEM and mercury intrusion porosimetry, require a long and delicate preparation; thus, in theory, HP ^{129}Xe NMR could provide an alternative method for a quick characterization, especially if we can perform experiments directly on a macroscopic single piece of stone.

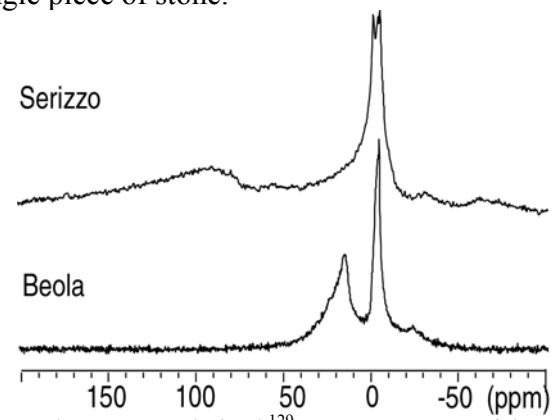

Fig.1 Continuos Flow Hyperpolarized ^{129}Xe NMR spectra of the reported stones

Here we present continuous flow HP ^{129}Xe NMR spectra of single regular large pieces (dimensions: 5.0 cm × 0.6 cm × 0.6 cm) of dimension stones. Marbles like Carrara or Candoglia cause only a broadening of the line width of the free gas resonance but no specific signals. Instead, granites (Serizzo, Beola Verde, Beola Monte Rosa) give rise to characteristic peaks at positive and negative values of chemicals shift. The different behaviours will be explained considering the texture of the stones and the presence of accessible magnetic minerals or phyllosilicates.

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NEOPLASMS OF THE HUMAN GASTRO-INTESTINAL TRACT CHARACTERIZED BY HR-MAS NMR

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The results of our studies on healthy stomach and colon mucosa, and on gastric and colon adenocarcinomas are reported. As far as the stomach tumors are concerned, the biochemical picture captured by HR-MAS is strongly different from that characteristic of the healthy mucosa, whereas intermediate situations are found for gastritis samples [1-4].

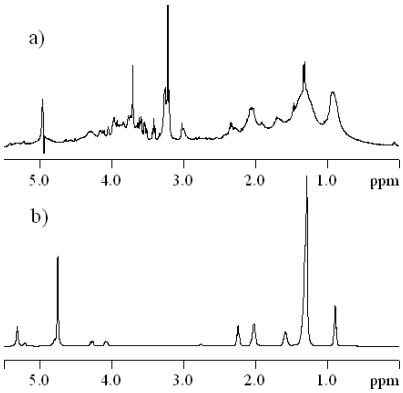

Fig. 1. ^{1}H HR-MAS NMR typical spectra of healthy (a) and neoplastic (b) stomach tissue.

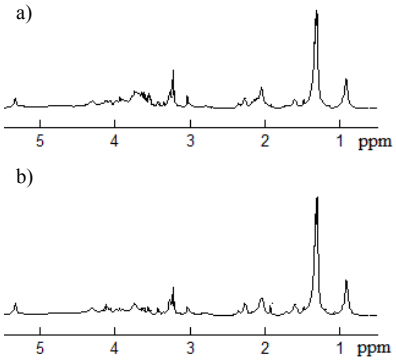

Fig 2. Mean ^{1}H HR-MAS NMR spectra of healthy (a) and neoplastic (b) colon tissue.

When colon samples are analyzed, HR-MAS highlights a marked metabolic heterogeneity within the class of the healthy specimen, as well as within that of the neoplastic ones. A statistical approach, based on the analysis of the conventional pre-saturated 1D ^{1}H spectra, was thus undertaken in order to distinguish among the two classes of healthy and neoplastic colon tissues. Interestingly, the samples taken at not less than 15 cm from the lesion, and classified as macroscopically normal by histology, are more similar to the neoplastic than to the healthy ones [5].

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POSTERS

BACKBONE NMR ASSIGNMENT AND PRELIMINARY STRUCTURAL ANALYSIS OF *T. brucei* GRX3

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Glutaredoxins (Grxs) were first discovered as glutathione-dependent reductants for ribonucleotide reductase [1] which is essential for DNA synthesis in all aerobic organisms. Today, Grxs are recognized as versatile regulatory proteins with multiple functions in health and disease [2]. The so called monothiolic Grxs belong to a recently discovered class with a CGFS consensus, in contrast to the 'classical' dithiol motif with a CPYC active site. Monothiol Grxs are found either as single domain proteins or in multidomain proteins together with thioredoxin and/or dithiol glutaredoxin entities [3]. Until now, neither the physiological reductant(s) nor substrates of monothiol Grxs are definitely proved, and their functional role is largely unknown. Nevertheless, the extent of conservation of these proteins amongst prokaryotes and eukaryotes, and the poor viability of some knock-outs, suggest a decisive importance in central processes within the cells, and a role which is not redundant with dithiol glutaredoxins.
Trypanosoma brucei is the causative agent of African sleeping sickness. African trypanosomes have a unique thiol metabolism based on the dithiol trypanothione [bis(glutathionyl)spermidine]. Enzymes of this parasite-specific redox metabolism are therefore attractive antiparasitic drug targets [4]. The genome of *T. brucei* encodes three genes for monothiol Grxs. Grx1 and Grx2 are single domain monothiol glutaredoxins, while monothiol *T. brucei* Grx3 contains an additional N-terminal thioredoxin-like domain. We determined the first and as yet only high resolution structure for a single domain Grx [5], and we have now chosen the two-domain *T. brucei* Grx3 as our next target for structure determination. We –obtained the full backbone assignment of the 24 kDa protein and a preliminary structural analysis, based on chemical shift data, will be presented here. Furthermore, we show that the cysteines in the two putative active sites behave differently with respect to pH changes and oxidation, which could be relevant for the function of the protein.

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OXAZEPINOQUINOLINONES: A NEW FUSED SEVEN-MEMBERED RING SYSTEM

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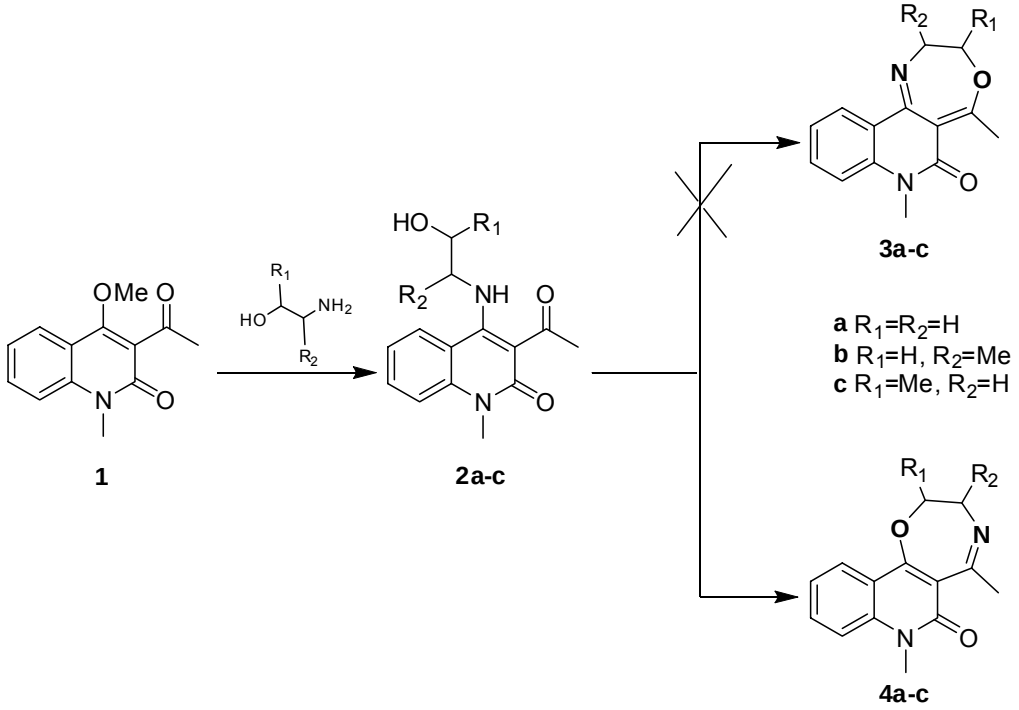
Synthesis of seven-membered heterocycles is an important tool in organic and pharmaceutical chemistry because of their interesting biological activity [1].
Following our research in this field [2-4], we report here the results of the reaction of 3-acetylquinolinone **1** with aminoalcohols carried out with the aim to obtain new biologically active oxazepinoquinolinones. Reaction of **1** with some aminoalcohols did not lead to the expected compound **3a-c** through a substitution-elimination mechanism as previously reported by reaction of the same starting material with 1,2-bisnucleophiles. The structure elucidation of the isolated compounds carried out by multinuclear MR experiments led us to assign structures **4a-c** to these compounds. The proposed reaction mechanism proceeds according to a Smiles-type rearrangement of the intermediates **2a-c**.



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ORGANIC/INORGANIC MULTICOMPONENT MATERIALS (OIMM): THE ROLE OF SOLID-STATE FOR THE STUDY OF THIS GENERAL CATEGORY OF SYSTEMS

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Many systems currently attracting a big interest in both academic research and applications, as, for instance, polymer/filler composites, organically-modified silicates, polymer electrolytes, stationary chromatographic phases, zeolites and mesoporous silicas including small organic molecules, show the coexistence on a nano-/micrometric scale of organic and inorganic components. Focusing on the crucial information that solid-state NMR can provide for the characterization of their structural and dynamic properties, we tried to group all these different classes of systems into the general category of "organic/inorganic multicomponent materials (OIMM)", proposing a definition and highlighting both the common features and the most important variables which characterize the huge variety of materials classifiable as OIMM. The macroscopic properties of OIMM, and therefore their applications, are strongly dependent on their microscopic properties, and in particular on: nature and dimensions of organic-inorganic interfaces, mechanisms of interaction between organic and inorganic components, structural and dynamic properties of either the organic or inorganic phases. Solid-state NMR (SSNMR) has a well-recognized and growing role in the characterization of the microscopic properties of OIMM [1], but only a few reviews have been published on this subject, all of them concerning SSNMR applications to specific OIMM classes. Here we present an extract of an in press review paper [2], in which we attempted to survey the applications of SSNMR to the general category of OIMM through many examples reported in the literature, concerning a large variety of materials, which we believe representative and/or explicative of the various SSNMR techniques and approaches. In particular the contribution of solid-state NMR to the study of the following three important aspects has been considered: 1) structural properties of the organic-inorganic interface, including chemical and physical interactions between the two components; 2) structural and dynamic behavior of the organic component, including both low-molecular weight compounds and polymers; 3) organic and/or inorganic domain dimensions and their reciprocal arrangement within the OIMM.

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BOTANICAL AND GEOGRAPHICAL CHARACTERIZATION OF POLYFLORAL AND ACACIA HONEY: ^{1}H NMR AND CHEMOMETRICS

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The main aim of several research groups is the development of new analytical techniques for geographical characterization of foods and in particular search for qualitative and territorial markers. In this work ^{1}H NMR studies in combination with multivariate statistical analysis are presented to evaluate botanical and geographical characterization of honey which represents one of the largest studied foods because of its nutritional and medicinal properties in a correct diet. In particular unsupervised Principal Component Analysis resulted able to distinguish between polyfloral and acacia honey samples and for geographical characterization of the latter ones. A hierarchical PLS-DA approach was performed for the discrimination among polyfloral honey samples of different geographical origins [1].

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AN INSIGHT INTO SiO2-CdO-PbO GLASSES BY MULTINUCLEAR SOLID STATE NMR

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Silica based glasses are conventional cooled oxide melts, with an extraordinary variety of application fields. Doping or mixing metals or metal oxides with SiO2 give to the material a high variety of physical properties such as, colour, density, refractivity, and strength, chemical or scratch resistance.
In the present work ternary silicate glasses (Si-Cd- Pb oxides glasses) are taken into account, evaluating the addition of transition metal oxides with particular electrical and optical properties, but non-extensively studied from a structural point of view, to define the organization at molecular level. The contribution of every component can be evaluated in the perspective to achieve information on the role of compositional changes in the formation vs modification phenomena.
The ternary Si-Cd-Pb system appears as a good model, due to the possibility to follow the chemical behaviour of every metal with solid state NMR, to define the molecular environment caused by variable coordination of the dopants, and to correlate the data with other physical macroscopic properties, such as net charge, density and microdomain formation.
Moreover, the composition seems to be of practical interest due to the peculiar properties of the metals used to prepare the glass.
By means of 29Si, 113Cd, 207Pb NMR, XRD and IR spectroscopies, the local structure, the change in the nature of atomic linkages and possible structural evolution of those silica glasses made using silicon, cadmium and lead oxides as precursors, with different molar ratios are discussed.

NUCLEAR MAGNETIC RESONANCE OF ^{129}XE AND ^{1}H AS A PROBE FOR THE CHARACTERIZATION OF VOID SPACE IN HUMAN MYOGLOBIN ISOFORMS IN SOLUTION

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The functional activity of a biomacromolecule is strictly correlated with the structure, the slow collective as well as the internal fast local motions. In this contest, the cavities confer to the protein some characteristics, such as flexibility and thermodynamic stability, which are crucial to maintain the native structure and to control its functional activity.
The aim of this research has been addressed to characterize, from a structural and dynamical point a view, the hydrophobic cavities of the five human myoglobin (Mb) isoforms, obtained by the recombinant DNA techniques. In fact, differently from other animals, in man Mb has five isoforms: Mb I (75-80% of the total: pI= 8.57); Mb II (w.t.,15-20% of the total; pI= 7.29); Mb III, Mb IV e Mb V (in all together 5%; pI= 6.83) whose specific function is unknown. Each one differs by the substitution of a single aminoacid in the protein's surface. In particular, Mb I has the Glu54, far from the four cavities characterized by Tilton, substituted by a Lys.
The suitability of ^{129}Xe NMR to characterize voids comprised within porous materials and/or biomolecules has given to this technique a widespread diffusion during the last few years [1,2]. Among the reasons for this popularity there are the chemical inertness of the probe Xenon and the remarkable sensitivity of its NMR parameters (chemical shift, line shape and relaxation rates) toward non-bonded local environments. Moreover, the possibility to study human Mbs in the low-spin state, due to the narrow lines observable in suitably resolved ^{1}H NMR spectra, permits to achieve a better understanding of whether Xenon is able to give fine information concerning the structure of internal cavities and characterize the binding process.
Here, a detailed structural characterization of hydrophobic cavities of wild and 54K human Mbs (ciano and azide forms) are obtained by ^{1}H and ^{129}Xe NMR. Xenon-protein interactions are investigated by ^{129}Xe NMR chemical shifts in aqueous solutions as a function of the Xenon concentration. The results are complemented with ^{1}H NMR spectra to test the dependence of the ^{1}H chemical shifts on the addition of Xenon. The location of Xenon inside the cavity is deduced from 1D ^{1}H NMR and 2D COSY and NOESY experiments. Evidence is given that Xenon appears to occupy more than one type of binding sites in human Mb besides the proximal cavity.

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PRELIMINARY INVESTIGATION ON HUMAN HEPATOCELLULAR CARCINOMA USING HIGH RESOLUTION MAGIC ANGLE SPINNING ^{1}H NMR SPECTROSCOPY

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High resolution magic angle spinning (MAS) ^{1}H NMR spectroscopy is a powerful technique for investigation of metabolites within different intact tissues [1]. The determination of biochemical and metabolic profiles offers the possibilities of NMR as a medical diagnostic tool [2]. In recent studies (MAS) ^{1}H NMR technique has been employed to characterize the metabolite composition of the several kind of human tumor tissue. Human Hepatocellular Carcinoma (HCC) is the most common malignant tumor of liver and is one of the most serious human cancerous problems in the world [3].

In this preliminary investigation some needle biopsies from neoplastic liver tissue and adjacent non involved tissue were collected. Likewise, hystopathological examinations were performed for each sample and tumor grade classifications were assigned [4]. Metabolite profiles were recorded with a Bruker 600 MHz spectrometer equipped with a magic angle spinning (MAS) probe and temperature control. The aim is collecting a lot of samples and using HRMAS as a tools to understanding tumor biochemistry and liver tumor tissue classification in level grade [5].

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UNUSUAL CARBONYL CHEMICAL SHIFT IN AN HEXAIRIDIUM CARBONYL CLUSTER: A COMBINED SOLID STATE NMR AND DFT APPROACH

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Medium- to high-nuclearity carbonylmetal clusters may present terminal, double-bridging (μ_2) and triple-bridging (μ_3) CO ligands. Thanks to the different M–CO bonding order, it is possible to discriminate the three kinds of ligands by means of spectroscopic techniques such as ^{13}C NMR spectroscopy.[1]
The ^{13}C NMR spectrum of $TMBA_2[Ir_6(CO)_{15}]$ (TMBA = $(CH_3)_3N(CH_2C_6H_5)$) shows, at low temperatures, an unprecedented μ_2 bridging carbonyl low frequency shift, with the resonances of the terminal μ_1 carbonyls placed at higher frequencies.[2]
The chemical shift tensors and the shielding anisotropies of the carbonyl ligands, obtained from solid state NMR analysis, allow us to determine the nature of the M-CO interaction. The results have been compared with the ^{13}C MAS data of $Ir_6(CO)_{16}$ where μ_3-CO ligands are present. Further evidence for the assignment and for the peculiar chemical shift value of bridging carbonyls in $TMBA_2[Ir_6(CO)_{15}]$ has been obtained by the DFT calculation of the NMR parameters. The scalar and Spin-Orbit (SO) relativistic two-component zero-order regular approximation (ZORA) methods have been employed in the geometry optimization and NMR chemical shift calculations, respectively. The large SO contribution (26.6 ppm) to the μ_2 bridging COs ^{13}C chemical shifts accounts for the position of the experimentally observed resonance.

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PARAMAGNETIC NANOVESICLES LOADED WITH LANTHANIDE(III) COMPLEXES AS MR-MULTICONTRAST AGENTS

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The aim of this work is to assess the potential of paramagnetic nanovesicles loaded with lanthanide(III) complexes as multicontrast MRI probes.
Non targeted stealth liposomes and polymersomes encapsulating paramagnetic lanthanide(III) complexes were prepared and *in vitro* characterized as T_1- (Ln = Gd), T_2-susceptibility (Ln = Gd, Dy, Tm), or CEST- (Ln = Tm) MRI agents.[1] The three contrast mechanisms depends on different parameters and, therefore, may provide complementary information about the *in vivo* localization of the nanovesicles. The nanosystems were locally injected in B16 melanoma tumor xenografted on C57 mice and the temporal evolution of T_1, T_2 and CEST MR contrast (detected at 7 T) was followed until 48 h post-injection.
The temporal evolution of the three contrast mechanisms generated by the paramagnetic nanovesicles injected in the tumor was different. In particular, the CEST contrast decreased to zero within 1-2 hour, thus indicating the cell internalization of the nanovesicles. Confocal fluorescence microscopy indicated an extensive uptake by tumor associated macrophages.
Conversely, the T_2-susceptibility contrast lasted for longer time and it was still detectable after 24 hours post-injection. This contrast mechanism is much less affected by water diffusion across biological membranes and, consequently, the paramagnetic vesicles can still exert their susceptibility effect even if they are cell internalized.
As far the T_1-contrast is concerned, initially the effect decreased quite rapidly, but, interestingly, after few hours it started to increase. This observation could be the indication of the release of the Gd(III) agent from the vesicle because the T_1 contrast is strongly quenched by the presence of the liposome bilayer.
The comparison between the behaviour of liposomes (made of phospholipids) and polymersomes (made of amphiphilic di-block copolymers) confirmed the higher stability of the latter nanovesicles.
In conclusion, the MR multicontrast ability of paramagnetically loaded nanovesicles can be successfully exploited to get more insight about their *in vivo* intratumoral localization by using a non invasive technique.

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DOUBLE-QUANTUM ^{13}C-NMR OF BATHORHODOPSIN, THE FIRST PHOTOINTERMEDIATE IN MAMMALIAN VISION

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Rhodopsin, the photoreceptor responsible for dim light vision in vertebrates, is a membrane protein made by the 11-*cis*-retinal ligand bound to the opsin protein pocket. Rhodopsin bleaches in the light over a series of photointermediates; this starts the signal cascade that transmits to the brain the information caught by the eye. The process started with the isomerisation of 11-*cis*-retinal chromophore to all-trans form within 200 fs, storing instantly about two-thirds of the photon energy - 36 kcal/mol [1]. It is still unclear how the protein binding pocket stereoselectively steers and accelerates one of the fastest photoisomerisation processes known in biology. This is one of the final purposes of this work.

Here, we report measurements on bathorhodopsin, the first thermally equilibrated intermediate of the rhodopsin photocycle. It can be trapped at cryogenic temperatures (120 K) in a custom made NMR probe. Combining CPMAS with symmetry based recoupling sequences [2], double-quantum filtered solid state NMR experiments have been performed on $^{13}C_2$ labeled retinylidene samples in their native red membrane protein.

So far, the ^{13}C chemical shifts of almost all the alkyl chain carbons of retinylidene-bathorodopin have been collected and a simple model used to estimate the electrostatic contribution to the energy storage issue.

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BREAKING THE SYMMETRY OF A DIMERIC PROTEIN: NMR STUDY OF SINGLE CHAIN HPV E2 PROTEIN

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Human Papillomavirus (HPV) infection is linked to cervical cancer and represents a serious problem for women health worldwide. The E2 protein is the only transcription factor encoded by the viral genome and plays a central role in controlling the expression of all Papilomavirus genes and in regulating the virus life cycle [1]. E2 protein exerts its function through the C-terminal DNA binding domain consisting of a dimer composed by two symmetrical 80 residue monomers. In an engineered monomeric version of this protein, a short aminoacidic linker concatenates the two wild type monomers into a single chain E2 protein (sc-E2) [2].
We performed an NMR study of sc-E2 and found that the symmetry of the dimer is disrupted also at sites that are distant from the linker. In particular, amino acids at the DNA binding face, which is opposite in the β-barrel to the linker position, feel the asymmetric character of the protein. In addition, a different pH profile between the two monomers is observed for some residue and this behavior is structurally correlated to the presence of key histidines that modulate the stability of this protein and are sensitive to perturbations of the protein symmetry.

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HYPERPOLARIZATION STORAGE ON DEUTERATED MOLECULES AT DIFFERENT MAGNETIC FIELDS

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MRI applications of molecules hyperpolarized by means of parahydrogen are currently under intense scrutiny[1].
The main limit to the application of this contrast agent is the high relaxation rate that restores the equilibrium population of spin levels. Substrate deuteration is the most common way for decreasing the relaxation rate since the dipolar interaction is eliminated. Carravetta et al. introduced another more efficient method in order to store polarization for times longer than T_1: the method is based on the fact that transitions from singlet states are not allowed [2].
We studied how long polarization is kept on parahydrogenated methyl-butynoate-d_6 (**2**) at three different magnetic field strengths: 14.1 T, 50 μT (earth magnetic field) and 0.1 μT (μ-metal). We observed an exceptionally long PHIP lifetime on the perdeuterated molecule when it is maintained at earth magnetic field. Conversely, polarization decay rate increased again at very low magnetic field (0.1 μT) (fig. 1). This unexpected behaviour is due to isotropic mixing between heteroatoms that is achieved at 0.1 μT.
These experiments demonstrate that substrate deuteration is an efficient tool for polarization maintenance only if deuterium nuclei are not scalar coupled with parahydrogen protons.

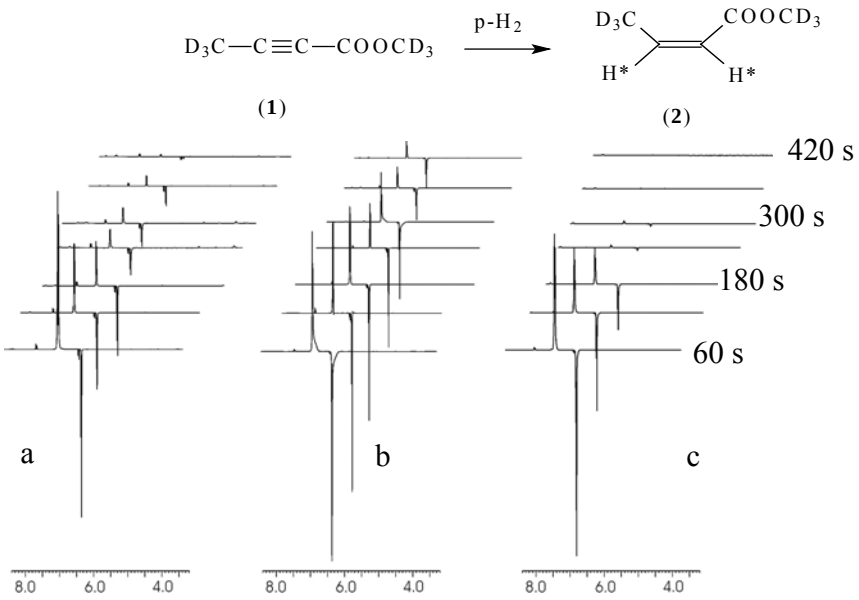

Fig. 1. Polarized signals of parahydrogenated (2) kept for increasing time intervals at a) 14.1 T (inside the magnet), b) 50 μT (earth magnetic field), c) 0.1 μT (μ-metal).

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NMR STUDIES OF BICELLES BOUND α-SYNUCLEIN FRAGMENTS

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α-Synuclein is a natively unfolded protein involved in Parkinson disease and with an unknown function, but it can interact with negatively charged membranes adopting an helical structure, already described[1] in the presence of SDS micelles. The small size of the SDS micelles compared to physiological membranes leave some structural details open to debate, such as the possible presence of breaks in the helix only in SDS.
Bicelles, described as bilayered micelles, are a very useful membrane-mimetic system for NMR structural studies in solution: they have a planar region made up of long-chain phospholipids (similar to the physiological membranes) surrounded by a rim composed of short-chain phospholipids[2]. If their size is sufficiently small ($0.5<q<1$), they can be used to study the conformation of membrane-associated biomolecules even when they are non aligned[3].
In this work, we prepared bicelles with DMPG (1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]) in the bilayer and DHPC (1,2-dihexanoyl-sn-glycero-3-phosphocholine) in the rim in 2:5 molar ratio ($q=0.4$) and we studied their stability in solution by dynamic light scattering, TEM and DOSY. We then used this system to study the structure of two fragments of α-synuclein (1-52 and 57-102), each including at least one of the controversial helix-breaks.

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BINDING OF A NON COVALENT INHIBITOR EXPLOITING THE S' REGION STABILIZES THE HEPATITIS C VIRUS NS3 PROTEASE CONFORMATION IN THE ABSENCE OF THE COFACTOR

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We present the first structure of a non covalent inhibitor bound to the protease domain of HCV NS3 protein (NS3p), solved by NMR. The inhibitor, a phenethylamide [1], exploits interactions with the S' region of NS3p to form a long-lived complex, although the absence of negative charges strongly reduces the association rate. The inhibitor stabilizes the N-terminal domain of NS3p and the substrate binding site, and correctly aligns the catalytic His-Asp residues. These actions were previously attributed exclusively to the cofactor NS4A [2], which *in vivo* interacts with the N-terminal domain of the NS3p and functions as an activator. The structure of the inhibitor/NS3p complex is very similar to that of the NS3p-4A complex, showing that binding of the NS4 cofactor is not the only event leading to a stable active site conformation.

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ORIENTATIONAL ORDER OF FLUORINATED AND BORINATED LIQUID CRYSTALS BY ^{13}C, ^{11}B, AND ^{19}F NMR TECHNIQUES

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The orientational order parameters of the molecule 4DBF2 (see Fig. 1) in its nematic phase (66-85 °C) have been obtained by means of ^{13}C, ^{19}F and ^{11}B NMR techniques.

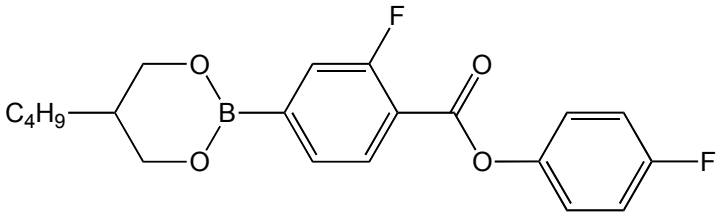

Fig. 1. Chemical structure of the liquid crystal 4DBF2.

In particular, orientational order parameters could be derived from ^{13}C and ^{19}F chemical shift anisotropy, ^{13}C-^{19}F and ^{19}F-^{1}H dipolar couplings, as well as from ^{11}B quadrupolar couplings. ^{13}C chemical shift anisotropy and ^{13}C-^{19}F dipolar couplings were obtained from ^{13}C spectra recorded under static conditions and decoupling from protons using the SPINAL-64 technique. ^{19}F chemical shift anisotropy and ^{19}F-^{1}H dipolar couplings were instead measured from ^{19}F spectra recorded using a standard solution-state apparatus. The analysis of the dipolar couplings was carried out employing strategies and approximations previously described [1, 2] using geometric parameters as obtained from suitable geometry optimization procedures. The derivation of order parameters from chemical shift anisotropies also required the calculation of the whole chemical shift tensors by means of DFT [2, 3]. ^{11}B quadrupolar splittings were measured as the distance between the peaks corresponding to the two satellite transitions in spectra recorded under static conditions using strong rf-irradiation, and the order parameters were derived making recourse to ^{11}B quadrupolar tensors calculated by DFT.
The orientational order parameters obtained by the different NMR techniques, and also by dielectric spectroscopy, were compared and discussed.

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SYNTHESIS, BIOLOGICAL EVALUATION AND NMR STUDIES OF A PEPTIDE-PACLITAXEL CONJUGATE

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Therapy of melanoma continues to be a challenge since, regardless of the treatment used, long-term survival is quite uncommon. In an attempt to improve the effectiveness and decrease the toxicity of anticancer chemotherapy, one useful approach might be to administer a prodrug that specifically releases the active cytotoxic drug at the tumor site. In this work, we describe the synthesis of a peptide conjugate of paclitaxel potentially useful in the treatment of human melanoma, and characterized by the simultaneous presence of three functional domains: a "targeting domain", an "activation sequence", and the antitumor drug paclitaxel. The "targeting domain" of the prodrug is represented by an RGD-containing cyclic peptide, able to bind selectively to αvβ3 integrin, which is known to be highly over-expressed by both metastatic human melanoma cells, and endothelial cells of tumor vessels. The "activation sequence", responsible for the selective release of the drug, is a short peptide which is cleaved specifically by cathepsin B, a protease highly up-regulated in malignant tumors. The results of NMR conformational studies, as well as those of biological experiments aimed at evaluating the plasma stability of the prodrug and its ability to inhibit αvβ3–mediated tumor cell adhesion to vitronectin, will be also presented.

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HELICAL HANDEDNESS AND STEREOCHEMICAL ASSIGNMENT OF C^{α}-TETRASUBSTITUTED CHIRAL α-AMINO ACIDS

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C^{α}-Tetrasubstituted α-amino acids induce significant constraints to the conformational freedom of the peptides that contain them. The conformational space accessible to them is narrow and includes both the α- and the 3_{10}-helix. For this reason, and for the higher stability toward degradation of their amide bonds, they are commonly used to improve the pharmacological properties of peptides, mainly by increasing enzymatic stability while preserving the chemical moieties required for functional activity. This rational is supported by the existence of a class of fungal antibiotics, termed peptaibols, characterized by the presence of a high percentage of C^{α}-tetrasubstituted α-amino acids.
In studying the conformational properties of such peptides, we observed a significant frequency separation between the protons of the βCH_2 group that replaces the C^{α} proton of protein amino acids. This separation seems to be general for all chiral C^{α}-tetrasubstituted α-amino acids with a βCH_2 group and specific for this class of residues. The examples described in this work include synthetic analogs of integramides and short segments of PTH, the parathyroid hormone.
Integramides A and B are two novel 16-mer linear peptides rich in C^{α}-tetrasubstituted α-amino acids, recently isolated from fungal extracts of *Dendrodochium sp.*, that inhibit the coupled reaction of HIV-1 integrase with IC_{50} values of 17 and 10 μM, respectively [1].
PTH is an 84-amino acid peptide hormone which regulates extracellular calcium omeostasis. The study of constrained, reduced-size PTH agonist and antagonist analogues, as short as 11 amino acids, has been the subject of extensive research [2], to develop safer and non-parenteral bone anabolic drugs.

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PEPTIDO[2]ROTAXANES: CAN AN AROMATIC TETRAMIDE MACROCYCLIC WHEEL TRAVEL BY WRAPPING UP AROUND A HELICAL PEPTIDE AXLE?

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Peptido[2]rotaxanes based on achiral, aromatic tetramide macrocycle locked onto various chiral Gly-L-Xxx dipeptide axles were first characterized by Leigh and coworkers (for a recent review-article, see [1]).

We are currently expanding this field by synthesizing and studying the properties of a new set of peptido[2]rotaxanes. In this work, we describe our results on non-symmetrical compounds involving an axle based on a central helical -$(Aib)_6$- (where Aib is α-aminoisobutyric acid) peptide linker and three stations, two of them of opposite chirality (a D-Leu-Gly-Gly tripeptide at the N-terminus and a fumaric diamide-L-Leu moiety at the C-terminus). As stoppers and macrocycle, we selected two diphenylmethyl groups and Leigh's aromatic tetramide, respectively.

As determined by NMR, the macrocycle initially positions on the fumaric diamide-L-Leu C-terminal station of the peptido[2]rotaxane. Subsequently, by using photon stimuli to induce the fumaric-maleic isomerization, we were able to switch the relative macrocycle-binding affinity in favor of the -Aib-amidoethanol ester central station. Finally, upon heating the supramolecule in acetonitrile solution at 50 ^{o}C, the macrocyclic wheel travels along the helical peptide axle to eventually reach the D-Leu-Gly-Gly N-terminal station.

This is the first example of a rotaxane where the wheel makes a journey to one of the stations by wrapping up around a helical peptide axle. Interestingly, we showed that reversibility of the process does take place by heating the 1,1,2,2-tetrachloroethane solution at 95 ^{o}C.

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THE USE OF SOLID-STATE NMR IN THE STUDY OF DRUGS

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Addressing the issue of characterizing the molecular and physico-chemical properties of a drug is particularly important from an applicative point of view, especially in the perspective of understanding their relation with its pharmacological activity. Recently, great efforts have been devoted to establishing protocols for investigating commercial drugs, the majority of which are present on the market as solids. The main concerns, usually of a practical nature, include, on one hand, revealing possible effects caused by their processing and/or storage conditions, which can in turn affect their processability or stability, as well as their release rate, bioavailability, solubility, etc. On the other hand, unravelling molecular features like the active pharmaceutical ingredient (API) physical state, which can be amorphous or crystalline, and in the latter case, possibly polymorphic, or the presence of chemical and/or physical interactions between API and other components (excipients) in the final formulation can indeed improve the comprehension of the mechanisms at the basis of the drug action. Nevertheless, the analysis of solid pharmaceutical compounds at a molecular level is made complicated by the fact that these systems can display extremely diversified characteristics for what concerns either the number and/or molecular dimensions of the components, or their chemical complexity, as well as their structural and/or dynamic properties. In fact, systems belonging to this category can range from pure APIs (usually in the form of crystalline, low molecular-weight compounds), to substances used as drug excipients (typically polymers), to solid dispersions formed by APIs and excipients, up to the actual final drug formulations. Even though a full characterization of solid drugs often requires the combined application of several techniques, solid-state NMR (SSNMR) is nowadays well-recognized as a crucial tool, especially due to its non-destructivity and flexibility, allowing several nuclear parameters to be determined and many different experiments to be performed for obtaining structural and dynamic information on a very broad space and time range, respectively. In this work, we present an extract of our recently published review paper [1], in which we tried to survey the most relevant applications of high- and low-resolution SSNMR in tackling important aspects in the pharmaceutical field, dividing them on the basis of the system investigated: pure APIs, pure excipients, and API-excipients solid dispersions. Some of the aspects reviewed are: identification of solid forms and structural properties of API in bulky samples, identification and/or quantitation of API in API mixtures or solid dispersions with excipients, stability of APIs, structural behavior of the crystalline or amorphous form of excipients in either bulky forms or solid dispersions, dynamic behavior of API and/or excipient forms, presence of API-excipient chemical and/or physical interaction, average dimensions of API and excipient domains, identification of impurities or degradation products, solid-state chemical reactivity.

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NMR STRUCTURAL CHARACTERIZATION OF H42A MUTANT OF ROS FROM AGROBACTERIUM TUMEFACIENS

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Ros, a 15,5 kDa protein encoded by the chromosomal gene *ros* [1] in *Agrobacterium Tumefaciens,* is the first classical zinc-finger containing protein discovered in the prokaryotic kingdom. Ros87, a mutant of Ros wild-type obtained by deletion of the first fifty-five amino acids, which is soluble and contains the zinc finger domain, is still able to bind DNA [2]. The NMR structure of Ros87 [3] consists of a very well defined globular domain (from Pro9 to Tyr66) of 58 amino acids, in which the zinc ion is tetrahedrally coordinated by Cys-24 and Cys-27 and by His-37 and His-42, and two disordered tails at the N- and C-terminal region. Ros87 globular fold has ββββαα topology and it is stabilized by an extended hydrophobic core of 15 amino acid. These new features define a novel fold never found in literature. The mutant H42A (in which the second coordinating histidine is mutated to alanine) of Ros87 is still able to bind the specific DNA sequence and HSQC-J18 experiment demonstrated that in H42A the zinc ion is tetrahedrally coordinated by His-37 and His-41 [2]. We report the structural characterization through NMR spectroscopy of H42A to understand the structural variation caused by this mutation and to elucidate the zinc coordination properties of this new protein fold.

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NMR STUDIES OF THE H98Q MUTANT OF A BILE ACID BINDING PROTEIN REVEAL THE FUNCTIONAL ROLE OF THE BURIED HISTIDINE

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Allosteric mechanisms are known to be at the basis of the functionality of several proteins, but the structural elements ensuring this mechanisms are often not straightforward to understand. Suitable models for the study of allosteric mechanisms are some multisites ligand binding proteins showing cooperativity binding.

The model we used is a two sites ligand binding protein, Chicken Liver Bile Acid Binding Protein (cL-BABP), which can bind simultaneously two bile acid molecules with high positive cooperativity [1].

In a previous study on cL-BABP [2] we identified the histidine at the 98 position as a crucial determinant in the bile acid binding process. In this light the protonation and tautomerization equilibria of the histidine side chains as a function of pH has been here investigated to gain more insight on the titration behaviour of H98 and its role on binding.

In order to elucidate the function of H98, the mutant H98Q was expressed in *E.coli* BL21(DE3) in ^{15}N labeled minimal media and purified by ion exchange and size exclusion chromatography.

The backbone amides of H98Q cL-BABP have been assigned through 3D NOESY and TOCSY ^{1}H-^{15}N HSQC experiments and ^{15}N relaxation measurements have been performed on the apo form. The relaxation analysis of apo H98Q reveals a marked reduction of flexibility for those residues showing a conformational exchange contribution in the wt protein. This same effect was observed for the wt protein upon ligand binding.

A titration of the mutant protein with the putative ligand ^{15}N glicochenodeoxicolic acid (^{15}NGCDA) has been followed to investigate the effect of H98 substitution on the bile acid binding mechanism. The main effects due to H98 substitution involve the binding of ligand in site 1.

The present results indicate a clear involvement of H98 in the stabilization of the ligand in site 1, possibly confirming the role of this residue in determining the binding mechanism. Measurements, currently in progress, on the dynamics of the holo mutant will complete the set of data and possibly elucidate the structural basis of allostery in cL-BABP .

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STABILITY IMPROVEMENT OF THE FATTY ACID BINDING PROTEIN SM14 FROM *S. MANSONI* BY SUBSTITUTION OF THE SINGLE CYS62 RESIDUE: STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A POTENTIAL VACCINE CANDIDATE.

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The *Schistosoma mansoni* fatty acid binding protein Sm14, is a vaccine candidate against two helminths, *S. mansoni* and *F. hepatica*. In a previous work [1] we showed the importance of a correct folding in order to achieve protection in immunized animals after cercariae challenge. We also suggested that the non satisfactory effectiveness of the vaccine could be due to structural instability of Sm14 during storage at 4°C. Here we show that the free cysteine residue (C62) is responsible for protein dimerization and subsequent aggregation. Site directed mutagenesis was used to produce Sm14-M20(C62S) and Sm14-M20(C62V). SDS page proved the absence of dimerization. Molecular dynamics (MD) calculations and thermal and urea unfolding experiments highlighted a higher stability of these mutants with respect to the wild-type Sm14-M20(C62). In addition, the mutated proteins, after thermal denaturation, refolded almost completely and maintained their fatty acid binding ability, thus confirming their ability to recover the active native architecture: a feature essential to ensure efficacy as a vaccine. Among those mutants, Sm14-M20(C62V) turned out to be the more stable one. Its enhanced structural stability over time allowed to obtain, for the first time, the 3D solution structure of a Sm14 isoform in its apo form. Combining the NMR data with MD calculations we could ascribe that extra-stability, to the onset of a hydrophobic cluster, inside the binding pocket, involving the residue in position 62. Finally, our results indicate that, in experimental animals, Sm14-M20(C62V) induces a protection against *S. mansoni* cercariae infections comparable to the one obtained with the wild type protein. Overall Sm14-M20(C62V) appears to be a good candidate for large-scale production and for exploring its use as the prototype of an anti-helminth vaccine.

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EVALUATION OF KIWIFRUITS RIPENING BY UNILATERAL NMR

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In this work the evaluation of ripening of kiwifruits was monitored using the unilateral NMR. This instrument, portable and fully non-invasive, allows the measurement directly on the plant, avoiding the sampling, to be performed.
In the three measurement campaigns, performed between October and November, T_1 spin-lattice relaxation times, and T_2 spin-transverse relaxation times, were performed on three different cultivar namely, Zespri Gold, Hayward and Cigi. A 5mm probe-head was used to perform the measurements at a 5 mm depth inside the fruit, fully disregarding the signal of the peel.
The results, obtained during the three measurement campaigns, showed that the T_2 values were significantly affected by the ripening: ripe fruits shows longer T_2 values with respect to unripe fruits. Therefore, T_2 values might be used as ripening parameters to determine the better harvesting period for each specific cultivar.
Moreover, a high resolution NMR study on aqueous extracts of kiwifruits was performed. The variation of metabolic profile at different ripening times and for the three different cultivar was investigated.

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TOWARD AND ASSESSMENT OF REAL STRUCTURAL PROPERTIES OF BIOLOGICALLY CONTROLLED HYDROZINCITE

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During the last years, interaction between microbes and minerals has attracted interest of the scientific community. Almost all biominerals are composite materials comprised of both mineral and organic components. Furthermore, the crystals of biominerals are nanosized, their surface energy is often biologically controlled via the chemical and physical properties that are typical of biological activity, and this effect nucleation and growth, and lead to surface features significantly different from the inorganically formed contropart [1].

Much less studied is a zinc carbonate, hydrozincite [$Zn_5(CO_3)_2(OH)_6$], that has been found in a mine environment at Naracauli creek (Sardinia, Italy) in association with cyanobacteria (Scytonema sp.) and algae (Chlorella)[2].

This work aim at investigation the differences between the structure of hydrozincite crystals grown under the control of bacteria, geologically sample and synthetic hydrozincite. The sample are investigated by both spectroscopy and microscopy techniques among which Solid State Nuclear Magnetic Resonance Spectroscopy, Scanning Electron Microscopy and High Resolution Transmission Electron Microscopy.

^{13}C MAS NMR and CPMAS NMR spectra show for all the investigated sample more than one peak despite that carbon atoms have a unique crystallographic position in the hydrozincite structure. The additional peaks reflect the presence of lattice defects typical of nanocrystals. The NMR technique is well known to be sensitive to crystal order and to the presence of lattice defects such as stacking faults. The presence of additional peaks is confirm by the HR-TEM images where high concentration of line, plane and surface defects can be observed especially in the nanocrystals both in the natural and synthesized hydrozincite.

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MULTINUCLEAR NMR SPECTROSCOPY IN SILICA NANOTECHNOLOGIES

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A well established method for the preparation of SiO_2 nanoparticles is the NH_3 catalyzed hydrolysis of tetraethyl orthosilicate (TEOS) and successive polymerization in alcoholic media, known as Stöber synthesis. High resolution multinuclear NMR, namely of ^{29}Si, ^{13}C and ^{1}H, had been successfully employed to shed light onto intermediate species and reaction course [1]. A particularly well suited environment for the control of particles nucleation and growth is provided by W/O microemulsions, where nanodroplets of water behave as nanoreactors [2]. The particles size and shape can be controlled simply by changing the microemulsion parameters. Our aim was to asses the effect of the confinement on the kinetics, the chemical species involved and the characteristics of the product.

Here we report the investigations by ^{29}Si, ^{13}C, ^{1}H NMR, SAXS and TEM carried out in a NP-5/cyclohexane/aqueous ammonia system (NP-5: polyoxyethylene(5)-nonylphenyl-ether).
Due to the low silicon concentration (initial [TEOS] = 0.04 M) and to the absence of paramagnetic relaxation agents, not to affect both the reaction and the microemulsion stability, the ^{29}Si spectra were acquired through INEPT and displayed only the TEOS signal. The ^{13}C NMR allowed to follow easily both the decay of the TEOS signals and the increase of the EtOH ones, as they resonate well apart and separated from those of the other components of the microemulsion. In the ^{1}H NMR spectra the methyl triplets of TEOS and EtOH appear at lower frequencies with respect to the very intense signal of cyclohexane. Both the ^{13}C and ^{1}H intensities fit a first-order kinetic law in [TEOS]. Also the SiO_2 volume fraction, determined by means of SAXS [3], varies in time in line with the above first-order kinetics. Thus the hydrolysis of TEOS results to be the rate determining step of the overall reaction. Meaningful amounts of intermediate species were not detected. The size of the particles obtained in this kind of microemulsion was determined by TEM and is on the order of 20 nm. By using more diluted NH_3 solutions the rate decreases drastically and the ^{1}H peak deriving from the exchangeable NH and OH protons moves to higher frequencies.
For the heterogeneous system occurring in the nanotechnological syntheses it results extremely profitable the joint use of TEM and SAXS, techniques able to provide information about solid phase, and high-resolution NMR, which provides information about liquid phase.

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FAST DETERMINATION OF HISTAMINE IN CHEESE BY NMR

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Cheeses are among those high-protein-containing foodstuffs in which enzymatic and microbial activities cause the formation of biogenic amines (BA) from amino acids decarboxylation [1]. High levels of histamine in foods can have important vasoactive effects in human [2].
Several analytical techniques have been proposed for the determination of BA in various foods [3]. The large majority of assays employs fluorimetric detection with precolumn or postcolumn derivatization techniques. Here we present a new rapid method based on ^{1}H NMR to quantify histamine in cheeses. The method is simple because the acid extract is analyzed directly, without any need for further filtration, derivatization, or other manipulation. The specificity of the method was demonstrated by 2D TOCSY and HMQC NMR experiments. The properties of the method, i.e., linearity, accuracy, recovery, repeatability and detection limit were evaluated.
The developed method was successful applied to the determination of histamine in cheeses, but it can be easily transferred to other kinds of food, such as yogurt, beer, fish and ripened-fish products, as well as cooked and fermented sausages.

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IDENTIFICATION OF THE PRODUCTION CHAIN OF "ASIAGO D'ALLEVO" CHEESE THROUGH PCA OF NMR DATA

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In the present work, a rapid and simple NMR method to discriminate Asiago d'Allevo cheese samples from different production chains is described. A fast and reproducible extraction of the organic fraction was employed. Applying chemometric analysis to NMR data, PDO Asiago cheese produced in alpine farms can be differentiated from that produced in lowland and mountain industrialized factories. PCA of both ^{1}H and ^{13}C NMR spectra showed a good separation of alpine farm products from the other ones, while the lowland and mountain industrialized cheeses are undistinguishable. The samples were differentiated on the basis of a higher content of unsaturated fatty acids, principally oleic, linoleic, linolenic and conjugated linoleic acids for the alpine farm cheeses and a higher content of saturated fatty acids for the industrialized products. Conjugated linoleic acid and 1-pentene are also discriminating components.

STUDY OF THE MORPHOLOGY AND PHASE TRANSITIONS IN A TRIBLOCK COPOLYMER USING ADVANCED SOLID STATE NMR TECHNIQUES AT 700 MHZ.

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Block copolymers have received considerable attention due to the intriguing periodic ordered morphologies and interesting physico-mechanical properties they exhibit. Among all the possible copolymer structures, ABA triblock copolymers are quite relevant, in fact many thermoplastic elastomers, an important family of industrial materials, are triblock copolymers. In this case the A blocks, characterized by a high glass transition temperature, behave as crosslinks in the rubbery phase constituted by the B block. Polystyrene-*block*-poly(ethylene-co-but-1-ene)-*block*-polystyrene triblock copolymer (SEBS) is one of the most used thermoplastic elastomers. SEBS presents several morphologies depending upon the thermal history of the sample. As-cast samples show well-ordered lamellar microdomains (LAM), a non-equilibrium morphology. The annealing of the material at a temperature above the glass transition of the polystyrene blocks causes a transition to hexagonally packed cylinders (HEX).
The lamellar structure of SEBS copolymer is constituted by two nanometric domains, one is the styrenic block and it is rigid at room temperature, in fact its glass transition temperature (Tg) is about 373 K, the other is the rubbery aliphatic part (poly(ethylene-co-but-1-ene)) and it is extremely mobile, Tg about 213 K. At 700 MHz and spinning speeds of about 22 kHz, this difference in molecular mobility allowed us to acquire ^{13}C MAS spectra using the INEPT polarization transfer from the protons, showing only the resonances due to the rubbery phase. Other quite relevant results came from low temperature measurements. In fact at 248 K, together with an expected overall increase of the peak line width, there is a dramatic change in the carbon resonances around the 30 ppm. In fact a new strong peak comes out at 33.83 ppm, due to the change from gauche-rich to all-trans conformations in the ethylene homosequences (EEE) of the poly(ethylene-co-but-1-ene)-block. The appearance of all-trans conformations at a temperature above the glass transition can be explained considering the low-temperature crystallization of relatively long sequences of ethylene homosequences, as vaguely suggested by Bates et al. [1] on the basis of calorimetric data. It should be noticed that low temperature crystallization takes place in the confined environment of a lamellar morphology.
Exploiting high magnetic field, high spinning speeds and specifically designed decoupling schemes, as for example e-DUMBO sequences [2], 2D ^{1}H-^{1}H spin diffusion NMR spectra were performed in order to measure ^{1}H-^{1}H spin-diffusion build up curves. Lamellar width derived from spin-diffusion curves will be compared with data obtained from SAXS and TEM measurements.

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STATISTICAL ANALYSIS OF HUMAN PATHOLOGICAL PLEURAL EFFUSIONS BY NMR AND ICP

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Serous effusions are a frequently encountered clinical manifestation of metastatic disease, with breast, ovarian, and lung carcinomas and malignant mesothelioma leading the list. Tumor metastasis to the peritoneal and/or pleural cavity is evident in two-thirds of cases at diagnosis and relapse is most often detected at this anatomic site.

The aim of this work is to obtain a fingerprint of metabolites in pleural effusions, through different approaches, independent methods and analytical chemical techniques. NMR spectroscopy has the unique ability to allow the simultaneous quantification of a large number of metabolic components present in biological fluids with equal sensitivity, without prior knowledge about the nature of the constituents and a very limited manipulation of the sample prior to analysis. The presence of trace elements was determined by ICP-MS.

Sixty-six effusions were analysed, 38 of which were pleural effusions and 28 were ascitic effusions; 13 samples were from patients diagnosed with some neoplastic pathology.

The ^{1}H-NMR spectra and the ICP data were subjected to Principal Component Analysis. In the best cases, 75% of the variance was explained. The clustering of the data was not very good, most likely because of the limited number of samples analyzed.

NMR SOLUTION STRUCTURE OF THE SUPRAMOLECULAR ADDUCT BETWEEN A LIVER CYTOSOLIC BILE ACID BINDING PROTEIN AND A BILE ACID-BASED GD(III)-CHELATE, A POTENTIAL HEPATOSPECIFIC MRI CONTRAST AGENT

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Recently, scientific interest has grown in MRI contrast agents (CA) which enter hepatocytes by means of active transport mechanisms. Active molecular transport in hepatocytes may be conveniently realized by exploiting the bile acid enterohepatic circulation machinery. In this frame, bile acid binding proteins are considered the intracellular carriers of these amphipatic molecules [1]. A number of potential CA were synthesized by conjugating bile acid moieties to gadolinium chelating units such as DTPA or DOTA, and tested in terms of pharmacokinetic properties and toxicology [2]. While a few studies have addressed the cellular uptake and excretion of bile salt-CA, little is known about the mechanisms of intracellular transport. A recent study from our group identified a promising bile acid-based gadolinium CA which showed high levels of hepatocyte internalization as well as good affinity to liver BABP [3]. We determined the first solution structure of the adduct between a liver bile acid binding protein and a bile acid-based gadolinium chelate, a model for a new hepatospecific contrast agent. The identification of unambiguous intermolecular distance constraints, made difficult by the intrinsically poor chemical shift dispersion of the steroid moiety of the ligand and by the unfavourable binding affinity, was possible through F_1-[^{13}C]-filtered, F_2-[^{13}C]-separated, F_3-[^{13}C]-edited NOESY-HSQC 3D experiments, together with distance information derived from paramagnetic relaxation enhancements. These intermolecular contacts were used, together with experimental data derived from a variety of other NMR experiments, for the structure determination of the complex, using the HADDOCK software. The obtained structure represents the starting point for the design of new and more efficient contrast agents.

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