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Lloyd's Baia Hotel
September 20 - 23, 2006
Vietri sul Mare - Salerno - Italy

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SCIENTIFIC PROGRAM

Wednesday, September 20 - afternoon

14.00-16.30 REGISTRATION
16.45-17.00 OPENING REMARKS

PLENARY LECTURE, Room C. Chair: **D. Cicero**

17.00-17.50 **H. Oschkinat**, Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany
Towards structure determination of integrate membrane proteins by MAS solid-state NMR

18:30- WELCOME RECEPTION

Thursday, September 21 - morning

PLENARY LECTURES, Room C. Chair: **L. Zetta**

9.00-9.45 **A. L. Segre**, IMC - CNR, Monterotondo
Polymerization mechanisms: analysis of the polymers and of the catalytic system itself

9.45-10.30 **G. Widmalm**, Stockholm University, Sweden
Structure, dynamics and interaction of carbohydrates by NMR spectroscopy

10.30-11.00 **R. Kümmerle,** Bruker BioSpin AG, Switzerland
X-nucleus detected NMR with CryoProbes

11.00-11.30 COFFEE BREAK

11.30-13.00 PARALLEL SESSIONS

NMR OF MATERIALS
Room C. Chair: **S. Spera**

11.30-12.00 **M. Geppi**,
University of Pisa
Interfaces and dynamics in polyethylene/ inorganic filler composites by solid state NMR

12.00-12.20 **A. C. Boccia**,
IMC - CNR, Monterotondo
Microstructural investigation of norbornene polymers by NMR techniques

12.20-12.40 **F. Presciutti**,
University of Perugia
A non-invasive NMR approach to the study of stratigraphy of paintings

12.40-13.00 **M. R. Chierotti**,
University of Torino
Polymorphism investigation by solid-state NMR in supramolecular adducts

NMR OF CARBOHYDRATES/ NATURAL ORGANIC SUBSTANCES
Room E. Chair: **A. Spisni**

11.30-12.00 **A. Molinaro**,
University of Napoli
Lipopolysaccharides from Gram negative bacteria

12.00-12.20 **C. De Castro**,
University of Napoli
Rhamnan polysaccharides from bacterial sources: determination of primary and secondary structure. Correlation among the 3D-structure and substitution pattern

12.20-12.40 **A. Silipo**,
University of Napoli
NMR solution structure of a dermatan derived tetrasaccharide: NMR, NOE and RDC based analysis

12.40-13.00 **C. Bassarello**,
University of Salerno
Gloriosaols A-E, novel phenolics from Yucca gloriosa (Agavaceaae): structural characterization and configurational assignment by a combined NMR-quantum mechanical strategy

13.00-14.30 LUNCH

Thursday, September 21 - afternoon

PLENARY LECTURES, Room C. Chair: **G. Barbato**

14.30-15.15 **C. Thiele,** Technische Universität Darmstadt, Germany
The use of residual dipolar couplings (RDCs) for the structure determination of organic molecules

15.15-16.00 **G. Veglia,** University of Minnesota
NMR structural dynamics and interactions of membrane proteins involved in muscle contraction and relaxation

16.00-16.20 **E. Kupce,** Varian Ltd., Oxford, UK
Hyper-Dimensional NMR Spectroscopy

16.20-16.50 COFFEE BREAK

16.50-18.20 PARALLEL SESSIONS

NMR OF PROTEINS,
Room C. Chair: **R. Fattorusso**

16.50-17.20 **L. Ragona**,
ISMAC - CNR, Milano
Interaction studies of bile acid binding proteins with different ligands: an NMR and docking approach

17.20-17.40 **M. Vacatello**,
University of Napoli
Conformational study of self-assembling peptides

17.40-18.00 **C. Alfano**,
IRBM and University of Roma, Tor Vergata
Structural studies of PRL-3: a phosphatase involved in colorectal cancer progression

18.00-18.20 **F. Prischi**,
University of Siena
Dimerization of α-bungarotoxin monitored by paramagnetic probes: a new approach for protein-protein interaction studies

NMR IN ORGANIC CHEMISTRY
Room E. Chair: **R. Riccio**
Dedicated to the memory of Luigi Gomez-Paloma

16.50-17.20 **M. Boccalini**
University of Firenze
Unambiguous structure elucidation of the reaction product of 3-acetyl-4-hydroxy- and 3-acetyl-4-methoxy-1-methylquinolinones with hydroxylamine via NMR

17.20-17.40 **G. Bifulco**,
University of Salerno
Application of STD-NMR to ligand-DNA interactions: an analysis of different binding modes

17.40-18.00 **T. Recca**,
University of Milano
^{15}N NMR of partially saturated pyrazoles

18.00-18.20 **L. Fusaro,**
University of Cagliari
NMR study of the reversible trapping of SF_6 by cucurbit[6]uril in aqueous solutions

19.00-20.30 GIDRM AND GIRM MEETINGS, Room C

21.00- Social Dinner

Friday, September 22 - morning

PLENARY LECTURES, Room C. Chair: **M. Piccioli**

9.00-9.45 **H. W. Spiess**, MPI, Mainz, Germany
Advanced solid-state NMR methods for determining structure and dynamics of functional materials

9.45-10.30 **M. Pellecchia,** The Burnham Institute, La Jolla, CA, U.S.A.
NMR and fragment-based approaches to drug discovery

10.30-11.00 COFFEE BREAK

11.20-12.50 PARALLEL SESSIONS

SOLID-STATE NMR
Room C. Chair: **A. Grassi**

11.00-11.30 **A. R. Albunia**,
University of Salerno
Solid state ^{2}H NMR molecular spectra: separation of spectral components by guest alignment in an axially oriented polymeric crystalline host phase

11.30-11.50 **M. Carravetta**,
Southampton University, UK
Solid state NMR of molecular hydrogen trapped inside open-cage fullerenes

11.50-12.10 **S. Borsacchi**,
University of Pisa
A multinuclear solid state NMR investigation of the interactions occurring at the surface of a layered silicate in its hydro- and organo-philic forms

12.10-12.30 **A. Testa**,
University of Napoli
Molecular characterization of Phyllosticta ilicina symptomatic and asymptomatic strains by advanced CPMAS ^{13}C-NMR technique and Electrolytes Leakage Assay (ELA)

NMR IN DRUG DESIGN
Room E. Chair: **A. M. D'Ursi**

11.00-11.30 **S. Davalli**,
GlaxoSmithKline, Verona
Quality Assurance project on GSK screening collection: an analytical challenge

11.30-12.00 **D. Potenza**,
University of Milano
Binding of RGD-peptide mimics to intact human platelets investigated by transferred-NOESY experiments

12.00-12.20 **C. Airoldi**,
University of Milano-Bicocca
New RAS protein inhibitors and their interaction with the target: the NMR point of view

12.30-14.00 LUNCH

Friday, September 22 - afternoon

PLENARY LECTURE, Room C. Chair: **G. Guerra**

14.00-14.45 **P. Sozzani**, University of Milano-Bicocca
NMR of gases and nanoporous materials

14.45-18.00 POSTER SESSION & COFFEE BREAK

PLENARY LECTURE, Room C. Chair: **S. Chimichi**

18.00-19.00 ***GIDRM/GIRM GOLD MEDAL:*** **C. Dalvit**, Nerviano Medical Sciences
FAXS & FABS: the use of the anisotropic and isotropic components of the ^{19}F nuclear shielding tensor in NMR-based screening

Saturday, September 23 - morning

PLENARY LECTURE, Room C. Chair: **M. Fasano**

9.00-9.45 **J. P. Hornak**, Rochester Institute of Technology, NY, USA
Overview of advanced and recent topics in MRI with a focus on Near Surface MRI

9.50-10.50 PARALLEL SESSIONS

NMR METHODOLOGY
Room C. Chair: **M. A. Cremonini**

9.50-10.10 **G. Pileio**,
Southampton University, UK
Analytic solution of γ-encoded NMR signals

10.10-10.30 **S. Viel**,
IMC - CNR, Monterotondo
Use of NMR diffusometry and HRMAS to investigate residual silanol activity in reverse chromatographic phases

10.30-10.50 **S. Sykora**,
Extra Byte, Castano Primo
Magnetic resonance in astronomy: feasibility considerations

NMR IN BIOMEDICINE
Room E. Chair: **M. Fasano**

9.50-10.20 **B. Alfano**,
IBB - CNR, Napoli
Multiparametric MRI segmentation and partial volume effect correction

10.20-10.50 **A. Brunetti**,
University of Napoli
Clinical/experimental applications of MRI

10.50-11.15 COFFEE BREAK

11.15-12.15 BEST POSTERS, Room C. Chair: **S. Mammi**

PLENARY LECTURE, Room C. Chair: **S. Mammi**

12.20-13.05 **A. Bax,** NIH, Bethesda, MD, U.S.A.
Weak alignment offers new opportunities in NMR structure determination

13.05-13.15 CONCLUDING REMARKS

13.15- FAREWELL BUFFET

PLENARY LECTURES

TOWARDS STRUCTURE DETERMINATION OF INTEGRATE MEMBRANE PROTEINS BY MAS SOLID-STATE NMR

Hiller M[1], van Rossum B[1], Krabben L[1], Flinders J[1], Lange V[1], Kühlbrandt W[2], Michel H[2], Srivastava A[2], Yildiz Ö[2], Vinothkumar KR[2], Oschkinat H[1], Becker J[1]

[1] Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Str. 10,13125 Berlin, Germany
[2] Max-Planck-Institut für Biophysik, Marie-Curie-Str. 15, 60439 Frankfurt/M

Structure determination of integral membranes by magic-angle-spinning solid-state NMR requires suitable samples, a variety of NMR techniques for achieving spectral assignments and the extraction of distance constraints, and structure calculation procedures that work preferably without manual assignment of distance-dependent cross peaks. Using the outer-membrane protein G (OmpG) as a model system, the benefits of various sample preparation techniques will be presented, including a correlation of NMR spectra and EM micrographs of various liposome preparations. An assignment concept will be presented that allows to assign this 280 residue protein, including novel pulse sequences for the selective excitation of individual amino acids or moieties, and strategies that make use of isotope labelling pattern as observed when preparing the samples with 2- and 1,3-^{13}C-labelled glycerol. Furthermore, samples are made which contain amino acids critical for the assignment in a specially labelled manner. First sequential assignments will be presented, and a new assignment strategy.

POLYMERIZATION MECHANISMS: ANALYSIS OF THE POLYMERS AND OF THE CATALYTIC SYSTEM ITSELF

A.L. Segre,[‡] V. Busico, [†] V. Van Axel Castelli[‡,†]

[‡]Institute of Chemical Methodologies CNR, M.B. 10, 00016 Monterotondo Stazione (RM), Italy.
[†]Department of Chemistry, University of Naples "Federico II", via Cintia, 80126 Naples, Italy.
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One of the most challenging target in macromolecular chemistry is the possibility to find out which specific catalyst characteristics and/or polymerization conditions can be modified in order to improve the macroscopic properties of a synthetic polymer.
A systematic screening of the thousands of possible combinations of the above factors, is practically impossible to realize, even in High-Throughput devices. In fact, NMR technique represents the most proper methodology for establish the fundamental correlation between polymerization reaction, microstructure and physical properties of polymers [1].
Nowadays highly sophisticated methods are emerging in the analysis of macromolecules and catalytic systems *via* NMR. In this presentation some example of the applications of multidimensional NMR and High Resolved-Magic Angle Spinning (HR-MAS) NMR to the polymer chemistry are reported.
The microstucture analysis of polymers requires highly resolved spectra. In many cases the possibility to spread out resonances in two or three or even more dimensions is crucial for the assignment of long monomer sequences [2].
In order to obtain highly resolved maps, 2D and 3D sequences were applied for the analysis of ethene/1-alkene/CO terpolymers. In this way the microstructure of such polymers was assessed up to hexad level.
Despite of the fact that Ziegler-Natta (Z-N) catalysis represents one of the most widely studied mechanism in the last 60 years, some aspects are still far from being clarified. One of the most debated points is the model of titanium active sites that can explain the final polymer microstructure [3].
HR-MAS technique allows a direct look to the heterogeneous catalyst, even in industrial conditions.
The innovative methodologies set up for the analysis of $MgCl_2$-supported Z-N catalysts, are reported. The role of internal and external donors, the effects of co-catalysts, such as aluminum alkyls, can be directly observed.

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STRUCTURE, DYNAMICS AND INTERACTION OF CARBOHYDRATES BY NMR SPECTROSCOPY

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E-mail: gw@organ.su.se

Prior to the study of conformation and dynamics of carbohydrate systems, the primary structure has to be determined. A large arsenal of NMR techniques for this purpose is presently available [1] and detailed analysis of polymeric systems can reveal information on biosynthetic pathways present and the biological repeating units of the polysaccharides [2]. The approaches for conformational analysis of oligosaccharides from NMR spectroscopy have for a long time been based on the nuclear Overhauser effect and more recently on heteronuclear as well as homonuclear ($^{13}C,^{13}C$) trans-glycosidic coupling constants. It is now evident that an equilibrium between conformational states should be anticipated also for small oligosaccharides (cf. Fig. 1) [3]. With the advent of residual dipolar couplings as a high-resolution NMR technique an additional robust tool has become available to determine conformation and dynamics [4]. One can then address the interplay between carbohydrate ligands and lectins or antibodies. These interactions are possible to study by transferred NOE experiments as well as by saturation transfer techniques, yielding information on the conformation of the bound carbohydrate ligand and the epitope recognized by the protein [5].

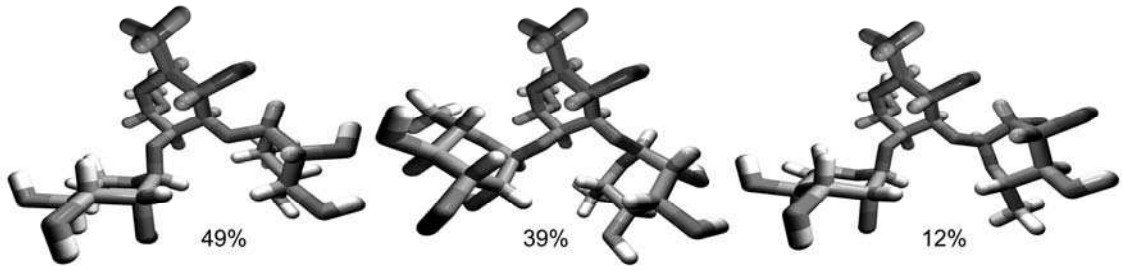

Fig. 1. The trisaccharide exhibits substantial conformational flexibility, with a high degree of correlation along the ψ glycosidic torsion angles as well as population of a non-*exo*-anomeric conformation for a ϕ glycosidic torsion angle, as observed from NMR spectroscopy and molecular dynamics simulations.

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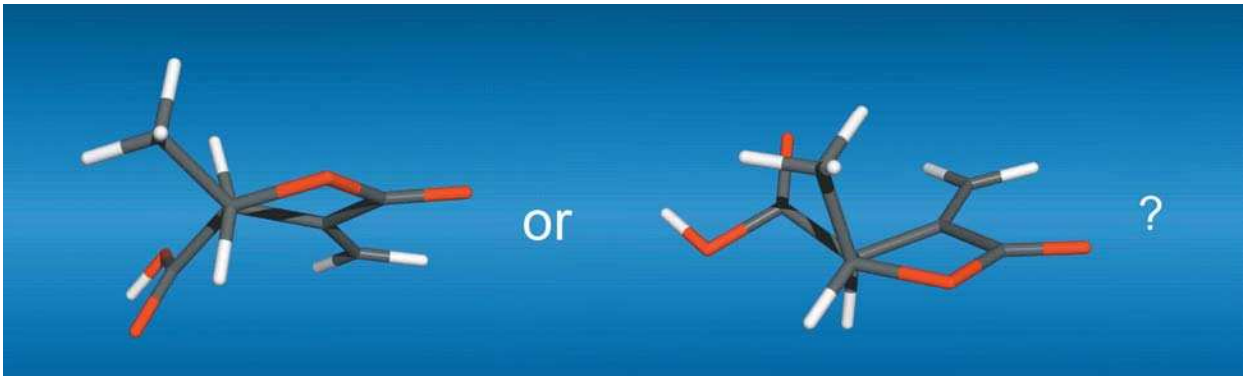
THE USE OF RDCS IN THE STRUCTURE DETERMINATION OF ORGANIC MOLECULES

C. M. Thiele

TU Darmstadt, Clemens Schöpf Institut, Petersenstr. 22, D-64287 Darmstadt

The determination of relative configuration by nuclear magnetic resonance is often complicated by either absence of NOE data and/or ^{3}J coupling data, remoteness of the stereocenters or conformational equilibria.
The recently reintroduced residual dipolar couplings[1] provide complimentary information to these convential nmr restraints. In order to demonstrate the utility of RDCs for organic structure determination (*proof of principle*) we have applied this methodology to a problem, which is comparable to the determination of relative configurations, namely the differentiation and assignment of diastereotopic protons in strychnine, which can also readily be solved by conventional methods [2].
The second application that will be shown is the determination of the relative configuration of an α-methylene-γ-butyrolactone. For this substance conventional nmr spectroscopic means fail due to the existence of structures in the conformational space of the two possible diastereoisomers, which are in line with the experimental data. Using RDCs, however, it is possible to unambiguously assign the relative configuration (in this case to be *trans*).[3]



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NMR STRUCTURAL DYNAMICS AND INTERACTIONS OF MEMBRANE PROTEINS INVOLVED IN MUSCLE CONTRACTION AND RELAXATION

G. Veglia

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Muscle contraction and relaxation are regulated by supramolecular complexes involving integral membrane proteins. In particular, in the sarco(endo)plasmic membrane SERCA/phospholamban (PLB) complex modulates the calcium flux, regulating the relaxation phase. Following β-adrenergic stimulation, PLB is phosphorylated by protein kinase A (PKA), relieving the inhibition of SERCA. We use a combination of biochemical and spectroscopic approaches (solution and solid-state NMR) to characterize the structural dynamics of the SERCA/PLB and PKA/PLB complexes with the final goal to understand both the regulatory mechanisms as well as the pathophysiologies originated from specific point mutations in those proteins.

ADVANCED SOLID-STATE NMR METHODS FOR DETERMINING STRUCTURE AND DYNAMICS OF FUNCTIONAL MATERIALS

Hans Wolfgang Spiess

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Nanostructures are in the focus of current materials science. They occur in advanced synthetic as well as in biological systems through self-assembly of carefully chosen building blocks. Secondary interactions such as hydrogen bonding, aromatic π-π interactions, electrostatic forces and attachment to surfaces are of central importance. Despite being highly ordered on a local scale, such systems often do not crystallize. Therefore, their structures cannot be determined by conventional X-ray crystallography or neutron scattering. Alternatives are needed which should provide structural and dynamic information, preferably requiring only small amounts of as-synthesized samples, without the need of isotopic labeling.

High resolution solid state NMR can meet these requirements [1], provided that sufficiently selective information can be extracted from the corresponding spectra.For that purpose solid state ^{1}H, ^{13}C, and ^{15}N NMR techniques have been developed combining fast MAS and DQ NMR spectroscopy, which make use of the homonuclear and heteronuclear dipole-dipole couplings. These techniques take advantage of the simplification of the multispin dipolar coupled network under fast MAS, where two-spin correlations predominate[2], and have provided new insight in hydrogen bonded structures in the solid state, columnar stacking and molecular dynamics of discotics, as well as organization of residues on surfaces. For full structural elucidation, the spectroscopic data have to be combined with quantum chemical calculations. The techniques will be introduced and the findings from NMR will be related to the function of such materials, such as proton conductivity, photoconductivity and catalysis [3].

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NMR AND FRAGMENT-BASED APPROACHES TO DRUG DISCOVERY

M. Pellecchia

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We have recently reported on an NMR-based approach, named SAR by ILOEs (structure activity relationships by interligand nuclear Overhauser effect) [1-5], that makes use of protein mediated ligand-ligand NOEs (ILOEs) in complex mixtures to identify initial weak hits that are converted into bi-dentate compounds with higher affinity. Combined with functional studies using the resulting ligands, the SAR by ILOEs method represents an ideal approach to reverse chemical-genetics. Reverse chemical-genetics entails selecting a protein of interest, screening for a ligand for the protein, and finally determine the eventual phenotypic alterations that the ligand induces in a cellular context. Likewise, our method enables the identification of protein's hot spots by using small molecules, regardless of the knowledge of the function of the protein, and the development of a specific assay. Subsequently, such small organic molecules can be used in cellular assays to investigate the possible role of the target. In particular, the approach was applied to the identification of the first inhibitor of the pro-apoptotic protein Bid and then used to characterize its function in cell [2, 5].

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NMR OF GASES AND NANOPOROUS MATERIALS

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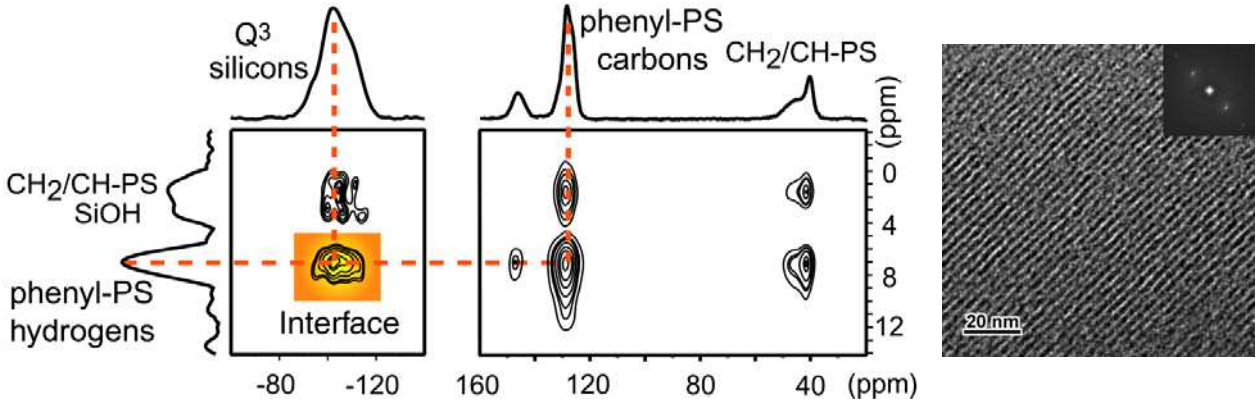
Nanoporous materials present a large interest in the recent research. Gas storage and confinement of functional molecules and macromolecules are attractive properties that can be addressed by NMR. The spectroscopy of gases, such as methane and carbon dioxide, can provide valuable information about the intermolecular distances with the host matrices and thus the specific interactions that drive the stability of adsorption. Magnetic susceptibility effects and 2D HETCOR experiments performed under fast magic angle spinning and Lee-Goldburg decoupling could provide the detailed topology of the adsorbed gases. Hyperpolarized (HP) xenon NMR demonstrates the accessibility of cavities and channels after short diffusion times and 2D exchange HP xenon experiments trace the pathway from the gas phase to the confined state of hierarchical pores. Examples of hexagonal systems with nanochannels of varied cross sections and layered organoclays containing open galleries will be addressed.
Hybrid interfaces in nanocomposite materials, especially in novel polymeric nanocomposites, are identified by ^{1}H-^{29}Si and ^{1}H-^{13}C 2D MAS NMR. Unprecedented morphologies and micrometer shapes imparted by the nanocomposite onto the polymer will be displayed.



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FAXS & FABS: THE USE OF THE ANISOTROPIC AND ISOTROPIC COMPONENTS OF THE ^{19}F NUCLEAR SHIELDING TENSOR IN NMR-BASED SCREENING

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NMR-based binding and functional screening performed with FAXS and 3-FABS represent reliable methods for identifying ligands of a target and inhibitors of an enzymatic reaction and for measuring with high accuracy their binding constant and inhibitory activity, respectively. The high molar receptivity of the fluorine nucleus together with the 100% natural abundance of the NMR active ^{19}F isotope allows rapid screening of large proprietary compound collections. 3-FABS (three Fluorine Atoms for Biochemical Screening) utilizes the sensitivity of the isotropic component of the ^{19}F shielding tensor to small local perturbations originating from enzymatic modification of the substrate. FAXS (Fluorine chemical shift Anisotropy and eXchange for Screening) utilizes in an efficient way the large chemical shift anisotropy component of the ^{19}F shielding tensor. In addition, FAXS uses the exchange contribution to the transverse relaxation of the ^{19}F resonance of the spy molecule. The combined effect of these two terms permits the use of a spy molecule with weak affinity for the receptor thus increasing the detection threshold for small and simple molecules binding to the macromolecular target. The two methods are robust, easy to set up, versatile and applicable to all targets of interest (protein, DNA, RNA, etc.). Recent improvements in the sensitivity of these methodologies have pushed the limit of detection and have further improved the throughput.
Another strength of these methodologies resides in the quality of the generated data. This is due to the simplicity of these NMR-based assays and absence of interferences with the method of detection. In addition, a novel NMR-based quality control approach [1] called SPAM (Solubility, Purity, and Aggregation of the Molecule) Filter has been designed for the identification of false positives and false negatives. Only compounds that pass through this quality control filter are considered as bona fide ligands or inhibitors thus avoiding squandered time and resources in the pursuit of unsuitable molecules.

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AN OVERVIEW OF RECENT ADVANCES IN MRI
with an emphasis on NEAR-SURFACE MRI

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In the past 20 years, remarkable advances have been made in magnetic resonance imaging (MRI) technology, and there is no evidence to suggest that this trend will not continue. Entire new MRI sub disciplines have developed such as magnetic resonance angiography (MRA), functional MRI (fMRI), diffusion tensor imaging (DTI), hyperpolarized noble gas imaging, and smart contrast agents. Imaging time has been reduced and image quality improved, resulting in much better temporal and spatial resolution. The first part of this presentation will review some of the technology that has made these advances possible, as well as summarize some of the more recent advances in MRI and their implications.

The second part of this talk will focus on near-surface or unilateral MRI research from our laboratory. Although our primary application is geophysics [1-3], there are many possible applications for unilateral MRI system in medicine. While many methods are possible [4], our approach, based on imaging in an inhomogeneous magnetic field, is the rastered backprojection [1]. This approach utilizes a disc magnet and RF coil. (See Fig. 1.) The imaging-sensitive region is defined by a curved line along the surface of a plane perpendicular to the RF coil and magnet. When the RF coil is moved across the magnet in X, the area under the NMR signal as a function of X represents the projection of the NMR signal from within the resonance location onto the projection axis, P, or X-axis in this case. Varying the orientation of the RF coil in the XY-plane will produce projections at angles ϕ about Z. Reconstruction of the image involves warping of the result of the inverse Radon transform onto the curved surface.

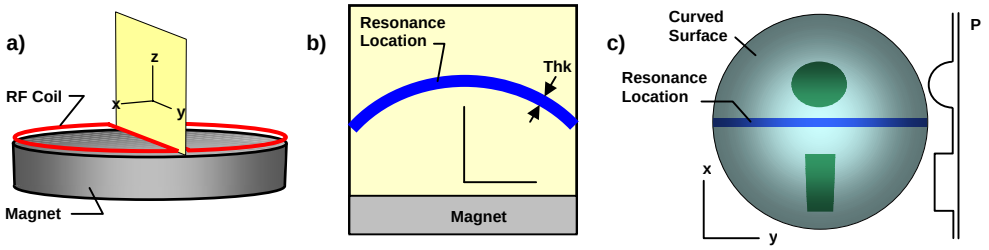

Fig. 1. Schematic representations of the a) apparatus, b) resonance location in the ZY plane, and c) projection of the signal in the resonance location as the RF coil is moved along X

Several phantoms were imaged to characterize the system. Acquisition parameters; system characteristics such as line-spread function, SNR, and penetration depth; and future system designs will be presented.

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WEAK ALIGNMENT OFFERS NEW OPPORTUNITIES IN NMR STRUCTURE DETERMINATION

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Biomolecular structure determination by NMR to date has relied mostly on local parameters, such as NOEs and J couplings. In contrast, dipolar couplings measured in weakly aligned macromolecules provide information on the orientation of individual internuclear vectors relative to the molecular alignment tensor. To date, this information has been used primarily for structure refinement as well as structure validation. However, alignment also provides access to important physical parameters, such as chemical shift anisotropy (CSA) through the residual CSA effect upon alignment. Concerted measurements of relaxation rates and residual CSA for nucleic acids yields good agreement with prior solid state NMR results on deoxy-mononucleotides, but also shows some remarkable differences, attributed to hydrogen bonding. Precise knowledge of ^{13}C CSA proves critical when refining against residual chemical shift effects, an approach that is applicable to slower tumbling systems than simple measurement of residual dipolar couplings.

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ORAL COMMUNICATIONS

X-NUCLEUS DETECTED NMR WITH CRYOPROBES

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X-nucleus detected NMR often suffers from low sensitivity due to low gyromagnetic ratios and/or low natural abundances. In addition, complex hyperfine structures typically add to the problematic of low sensitivity.
The development of new cryogenically cooled probehead types as well as the development of new methodology has pushed back significantly the limitations to implement X-detected experiments. This is reflected by a renewed interest in X-detected NMR in various fields, like trace analysis, functional screening, the study of paramagnetic or (partially) unfolded proteins or protein dynamics.
The presentation covers some of these applications and presents CryoProbes for high temperature polymer NMR. The increased sensitivity offered by the cryogenically cooled probehead and preamplifier allows studying much lower probability events in a reasonably short measurement time.

INTERFACES AND DYNAMICS IN POLYETHYLENE/INORGANIC FILLER COMPOSITES BY SOLID STATE NMR

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The use of inorganic fillers to improve the mechanical and chemico-physical properties of polymeric matrices is nowadays a common practice. For each polymeric material, the properties of the organic-inorganic composites are strongly dependent on the choice and treatment of the inorganic filler, and on the strategy followed in the preparation of the composites themselves [1,2]. Together with the knowledge of the macroscopic properties of the final composites materials, it would be very important to obtain corresponding information at a molecular level. In this sense Solid State NMR is very powerful in obtaining molecular information on both the structure and the dynamics of the polymer-filler composite [3,4]. Here we present an NMR study on Polyethylene films filled with two different inorganic fillers: Silica Gel and Laponite RD, a synthetic Na^+-Hectorite, organically modified with Dimethyldioctadecylammonium Chloride. Both the inorganic fillers have been functionalized with TSPM (trymethylsilyl propylmetacrilate) with the aim of grafting, through a photo-induced reaction, the inorganic filler to the polymeric matrix.
^{29}Si CP- and SPE-MAS, ^{29}Si-^{1}H FSLG-HETCOR, ^{13}C CP-MAS, ^{1}H MAS, ^{1}H FID analysis, as well as spin-lattice relaxation time measurements have been employed in the study of the untreated and modified fillers and of the final polymer-filler composites, in order to characterize the interactions between the inorganic filler and its organic modifiers and between the modified filler and the polymeric matrix from a structural and dynamic point of view. Moreover, we also investigated the changes in the dynamic properties of the polymer due to the treatment with the inorganic fillers, attempting a correlation with the consequent modifications observed in the macroscopic behaviour.

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MICROSTRUCTURAL INVESTIGATION OF NORBORNENE POLYMERS BY NMR TECHNIQUES

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[1]Ethylene-Norbornene (E/N) copolymers synthesized using metallocene catalysts have rather interesting material properties such as a good chemical resistance, excellent transparency and high glass transition temperatures. The design of E-N based materials with given features require a detailed description of the microstructure of copolymers as well as a complete understanding of the relationships between the microstructure and the material properties. The NMR spectroscopy is surely the most powerful analytical tool for polymer microstructural investigations, but E-N copolymers spectra are quite complex for the presence in the polymer chain of two stereogenic carbons per norbornene units and because the chemical shifts of these copolymers do not obey straightforward additive rules. Two-dimensional correlated NMR techniques can help to solve many assignment problems , e.g. Cosy ^{1}H- NMR spectrum was used to characterize the enchainment in E-N copolymer[2].
An elegant way to solve the complex microstructure of E-N copolymers come from the cooperation of different methods as RIS model, theoretical chemical shifts (*ab initio* quantomechanical model) and 1D and 2D NMR spectroscopy applied to model compounds.

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A NON-INVASIVE NMR APPROACH TO THE STUDY OF STRATIGRAPHY OF PAINTINGS

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The conservation of artworks requires a deep knowledge of materials behavior as a function of time and environment, as well as the knowledge of the distribution of different materials in the artwork. For example, in the study of paintings, it is relevant to know the execution technique of the painter in terms of the chemical nature of binders and pigments and also their distribution in terms of paint layer sequences. Usually the different layers are identified through the so-called stratigraphic studies, where cross-sections, obtained from small samples of the painting are examined by microscopy techniques, such as optical (OM) and electron microscopy (SEM), micro-FTIR and micro-Raman spectroscopy, and others. Stratigraphic studies allow to visualize the sequence of layers in the paint, identifying the primer (the so-called *imprimitura*), the painting layers, varnishes and possible repaintings. However, these studies should be avoided because the sampling damages the paintings and because the method explains properties of single points and individual results can hardly be extended to the remaining part of the artwork. Indeed, the complexity and preciousness of the understudy materials requires a *non – invasive* analytical approach.

As an example the application of the Profile NMR-MOUSE can give useful results on the stratigraphy and therefore on the painting technique in a non-invasive way and directly in the place where the artworks are conserved.

In order to carry out measurements and to check for their accuracy, first two simple easel painting models have been prepared. The two models consisted of a layer of primer made by gypsum and animal glue and a layer of pigments mixed in egg tempera. In the former panel, the pigment utilized was copper acetate [$Cu(CH_3COO)_2 \cdot H_2O$], in the latter was cobalt blue [$CoO \cdot Al_2O_3$]. As a first step, NMR depth-profile measurements have been carried out on the two models, and comparisons have been done by OM and SEM on cross-sections. As a second step, some measurements on original historical Italian Renaissance paintings have been carried out, which demonstrate the effective applicability of the device.

POLYMORPHISM INVESTIGATION BY SOLID-STATE NMR IN SUPRAMOLECULAR ADDUCTS

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Solid-state NMR spectroscopy is capable of providing valuable and detailed insight into the structure and the dynamics of a wide range of systems such us complex supramolecular architectures.
Here we report the study of hydrogen bonds networks, solid-gas reactions, intermolecular packing arrangements and dynamics of polymorphs and host-guest complex by means of solid-state NMR techniques. Several examples will be presented:
a) the structural relationship between the two crystal forms of cinchomeronic acid (3,4-dicarboxypyridine) investigated by ^{13}C and ^{15}N CPMAS and ^{1}H MAS indicates that the difference in stability can be ascribed to the strength of the hydrogen bonding patterns established by the protonated N-atom and the carboxylic/carboxylate O-atoms [1];
b) two crystal forms of ferrocene dicarboxylic acid [$Fe(\eta^5\text{-}C_5H_4COOH)_2$] behave in exactly the same way in the solid-gas reaction with gaseous amines bases (NH_3, $NH_2(CH_3)$ and $NH(CH_3)_2$) generating the same product, identified by single crystal and powder diffraction and by ^{13}C, ^{15}N CPMAS and ^{1}H MAS methods [2];
c) small organometallic molecules acting as guests within cyclodextrin host systems provide interesting examples where VT solid-state NMR techniques have allowed us to elucidate complex intramolecular dynamics. Many of the organometallic complexes we have studied by ^{13}C CP/MAS NMR have been shown to have reorientational dynamics in the CD inclusion compounds that are altered from the situation in the pure solid. The nature of the motion of the "guest" molecules within the "host" cyclodextrin cavity was shown to be dependent on the symmetry, size and orientation of guest molecule within the host cavity [3].

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LIPOPOLYSACCHARIDES FROM GRAM NEGATIVE BACTERIA

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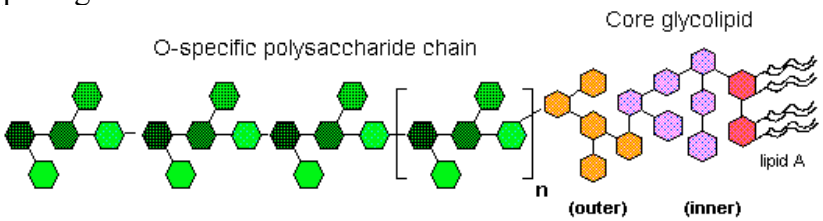
Lipopolysaccharides (LPSs) compose about 75% of the outer membrane of Gram negative bacteria and is exposed towards the external environment. LPSs are heat-stable complex amphiphilic macromolecules indispensable for the growth and the survival of Gram-negative bacteria[1]. The pro-inflammatory potential of several pathogenic bacteria is mainly due to the toxicity of LPS which often leads to septic shocks, actually, LPSs are acknowledged pathogenesis factors that have a key role in Gram-negative infections. The structural elucidation is a first essential step toward the comprehension of molecular mechanisms of pathogenesis.[1]



LPSs are built up according to a common structural architecture and are composed of a hydrophilic heteropolysaccharide (formed by a core oligosaccharide and an O-specific polysaccharide or O-chain) covalently linked to a lipophilic moiety termed lipid A, which is embedded in the outer leaflet and anchors these macromolecules to the membrane through electrostatic and hydrophobic interactions. LPS without O-chain are termed Rough LPS (R-LPS) or lipooligosaccharides (LOS). Lipid A possesses a rather conservative structure usually consisting of a β-(1→6)-glucosamine disaccharide backbone *bis*-phosphorylated and acylated with primary 3-hydroxy fatty acids at positions 2 and 3 of both GlcN residues; the hydroxyl groups of the primary fatty acids can be further acylated by secondary acyl moieties. Lipid A is the minimum part of the LPS requested for viability of the bacterium and is also the epitope region by which LPS is recognised by innate immunity cells. In the core oligosaccharide, an inner and outer region are usually distinguished: the inner core, proximal to the lipid A, consists of typical monose residues like Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid) and heptoses. Kdo is the linker between the GlcN II of lipid A backbone and the core portion. The outer core region is more variable and is usually composed by hexose residues.[2]
This lecture will focus on state-of-art NMR methodology towards the structural elucidation of LPSs.

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RHAMNAN POLYSACCHARIDES FROM BACTERIAL SOURCES: DETERMINATION OF PRIMARY AND SECONDARY STRUCTURE. CORRELATION AMONG THE 3D-STRUCTURE AND SUBSTITUTION PATTERN

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Lipopolysaccharides (LPS) or endotoxins, are amphiphilic molecules located in the external membrane of the cell wall; they play a critical role for the survival of the bacterium itself and modulate many membrane properties such as adhesion. LPS structure is divided in three regions genetically and chemically different [1]: one close to the bacterial membrane called Lipid A, a second one named Core and the more distant referred as O-chain or antigenic moiety. Lipid A moiety is integrated into the lipid bilayer of the membrane, and it is recognized from the mammalian innate immune system receptor TLR-4, due to the conserved structural feature of this portion, that is a β-(1→6) glucosamine disaccharide substituted with a variable number of fatty acids. The core region is an oligosaccharide that connects the Lipid A part with the O-chain that in S-type bacteria is the more extended part of the whole molecule, with a molecular weight in the range of 20-80 kDa. R-type bacteria differ from the S-type for the lack of the antigenic moiety.
The role of LPS in both plant and animal pathogenesis mechanisms is acquiring more and more importance. In the plant system, *PR-1* and *PR-2* genes' stimulation of *Arabidopsis thaliana* [2] with LPS from *Xanthomonas campestris* pv. *campestris* is differently modulated from the moiety employed in the test, i.e. Lipid A or core region for instance. Similarly, interesting results were obtained testing synthetic oligosaccharides constituted from Rhamnose trisaccharide unit [3].
In the present communication, the conformational behavior of these last compounds (oligomers of repeating unit **1**) will be discussed in consideration of the biological activity observed. The discussion will be extended to synthetic Rhamnan oligomers differing from the previous ones for the different type of repeating unit (structure **2**) or for the presence of glycosyl substituents (structures **3** and **4**).

1 [3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→]$_n$

2 [3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-L-Rha]$_n$
[3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-L-Rha]$_n$

3
↑
R

3: R= β-D-GlcNAc
4: R= α-D-FucNAc

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NMR SOLUTION STRUCTURE OF A DERMATAN DERIVED TETRASACCHARIDE: NMR, NOE AND RDC BASED ANALYSIS

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The dermatan sulphate is a glycosaminoglycan found mostly in skin but also in blood vessels, the heart valves, tendons, and the lungs, composed of L-iduronate and GalNAc-4-sulfate in (1→ 3) linkage [1]. The solution structure of a dermatan derived tetrasaccharide is explored by means of NMR spectroscopy, NOE based analysis and Residual Dipolar Coupling (RDC).
HexΔA-(1→ 3)-GalNAc4S-β-(1→ 4)-IdoA-α-(1→ 3)-*red*-GalNAc4S
The monosaccharide spin systems have been assigned by 1D and 2D NMR spectroscopy. The tetrasaccharide was present in four different species differing by the anomeric orientation of the *red*-GalN and the conformation of the iduronate unit. Ring conformations have been defined by the analysis of $^{3}J_{HH}$-coupling constants and *inter*-residual NOE contacts. To obtain D_{HH} and D_{CH} Residual Dipolar Couplings, the measurement of one-bond carbon-proton coupling constants ($^{1}J_{CH}$ + ($^{1}D_{CH}$) has been performed via t2 coupled and t1 coupled HSQC experiments, while proton-proton coupling constants ($^{3}J_{HH}$ + ($^{3}D_{HH}$)) have been obtained via DQF-COSY and ^{1}H NMR experiments. The oriented medium for the RDC measurements was a phage solution.
Molecular mechanics calculations have been performed to have an estimation of the conformational regions energetically accessible and to determine the low energy regions centered around the Φ and Ψ glycosidic torsion angles. Then the conformational space available for the regions was investigated *via* molecular dynamic simulations. The flexibility of Φ and Ψ torsions suggested by NOE, MM and MD studies has been investigated by residual dipolar coupling. The TRAMITE method [2-3] has been used to align the MD trajectories and then to calculate the average RDCs from them. Back-calculated RDCs have been compared with the experimental ones. The agreement between calc. and exp. RDC data has been improved when conformers with large $^{1}D_{CH}$ deviations have been filtered out.

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GLORIOSAOLS A-E, NOVEL PHENOLICS FROM *YUCCA GLORIOSA* (AGAVACEAAE): STRUCTURAL CHARACTERIZATION AND CONFIGURATIONAL ASSIGNMENT BY A COMBINED NMR-QUANTUM MECHANICAL STRATEGY

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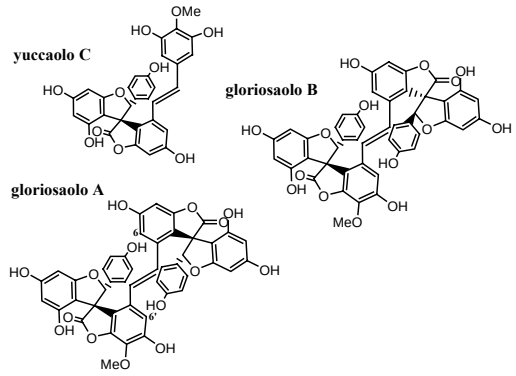
Epidemiologic studies report that the benefical effects of eating fruits and vegetables may be explained by the antioxidant activity of numerous compounds occurring in them. These studies indicate that particularly phenolic compounds play an important role in protecting against events of oxidative diseases, such as cardiovascular disorders, cancer, inflammation, and brain dysfunctions. Thus there is a lot of attention to the identification of phenolic compounds and of plants containing such compounds in high concentration. In this frame we focused on *Yucca schidigera* bark, where phenolics are present in high concentrations. At the moment two different products from the trunk of Yucca are available on the market: Yucca powder and Yucca extract. Both the products possess *GRAS* label given by FDA, which allows their use as food supplements. Our previous investigation of the bark of *Y. schidigera* resulted in the isolation of five phenolic derivatives with very unusual spirostructures, named yuccaols A – E. Yuccaols A-E are made up of two C-15 and C-14 units condensed to form a γ-lactone ring. Furthermore interesting antioxidant-, platelet activation inhibiting-, anti-inflammatory, and antiproliferative activities exerted by yuccaols have been highlighted.[1] On this basis we deemed it of interest to investigate the phenolic fraction of *Y. gloriosa.* This study led to the isolation of five new phenolic constituents named gloriosaols A-E. They resulted to be made up of two C_{15} units probably derived from a flavonoid skeleton linked via a γ-lactone ring to a central stilbenic moiety corresponding to *trans*-3,3',5,5'-tetrahydroxy-4'-methoxystilbene. Gloriosaols A and B exhibited, in correspondence of the C2-C3 stereogenic carbons, a *trans* relative configuration, gloriosaol C a *cis* relative configuration while gloriosaols D and E *trans*/*cis* relative configurations. ^{1}H and ^{13}C NMR data of gloriasols A and B were almost superimposable, some little differences could be observed for the chemical shifts of H-6′ and H-6 protons. Moreover all the NMR key correlations were identical in the two compounds. Thus two hypothesis were formulated: 1) gloriosaols A, B could be conformational isomers; 2) gloriosaols A, B could be two configurational isomers, indicating, in this case, a nonstereoselective biogenetic formation of the stereogenic centre C-2. The former hypothesis was excluded on the basis of the analysis of the QM calculated potential energy surface of gloriosaol A obtained upon variation of two dihedral angles, together with the ^{1}H NMR spectra recorded at various temperatures. Finally a strategy based on the quantum mechanical calculation of the ^{1}H NMR chemical shifts and their comparison with experimental data [2], in combination with the analysis of the ROE data allowed us to deduce a diastereomeric relation between gloriosaols A and B and to assess the relative configuration of the two diastereomers. This strategy has been successfully applied also to the relative configurational assignment of gloriosaols C, D, and E.



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HYPER-DIMENSIONAL NMR SPECTROSCOPY

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There has always been a practical limitation on multidimensional (nD) NMR spectroscopy of biomolecules because of the time factor, even though modern spectrometers now have very good sensitivity. This arises because of the erroneous assumption that evolution space must be sampled on a complete Cartesian grid. Considerable improvements in speed can be achieved by limited radial sampling -- linking the evolution variables together in a suitable ratio. Bracewell has shown that the Fourier transforms of such radial sections are projections of the frequency-domain spectrum. Consequently the full nD spectrum can be reconstructed from a small number of such projections. The time saving is roughly an order of magnitude for each new frequency dimension beyond the second. This allows unstable biomolecules to be studied, and time-dependent phenomena to be followed. Alternatively it permits higher-dimensional spectra to be recorded -- recently demonstrated for the ten-dimensional spin system of a small protein, agitoxin. Further improvements are achieved by a real-time adaptive program (OLIVIA) which uses projection-reconstruction techniques to determine connectivity between aminoacid residues of a protein. OLIVIA assesses whether the initial three-dimensional spectra are sufficient for a valid assignment, and, if not, it selects a more appropriate NMR pulse sequence and instructs the spectrometer to take further measurements until a satisfactory solution is reached.

INTERACTION STUDIES OF BILE ACID BINDING PROTEINS WITH DIFFERENT LIGANDS: AN NMR AND DOCKING APPROACH

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Bile acid binding proteins belong to intracellular lipid binding proteins, a family of low-molecular mass (~15 kDa) molecules facilitating the cellular and metabolic trafficking of fatty acids, cholesterol, bile salts, retinoids and vitamins. They share a remarkably similar common fold in which 10 strands of antiparallel β-sheets surround the hydrophobic ligand binding site and two short α-helices are located between the first and second strands. We have recently characterized by NMR the structure of a member of this family, chicken bile acid binding protein (cl-BABP), which has been suggested to perform bile acid transport in non mammals liver cytosol. ^{15}N relaxation and steady state heteronuclear $^{15}N(^{1}H)$ NOE measurements of apo cl-BABP and of the protein complexed with chenodeoxycholate revealed a substantial conformational flexibility, on the microsecond to millisecond time scales, mainly localised in the C-terminal face of the beta-barrel [1]. NMR data, accompanied by MD simulations, suggested that H_{98} protonation equilibrium is the triggering event for the modulation of the motion, which is functionally important for ligand binding. A detailed study of the mechanism of binding and selectivity towards the pool of natural bile salts has been performed by NMR. Single-residue mutations were introduced and mutants tested for binding ability by NMR in order to identify the key residues in site selectivity. A docking approach (HADDOCK), which is driven by NMR and biochemical data, was used to obtain a structural model for the ternary complex of cl-BABP with two molecules of glycochenodeoxycholic acid. The efficiency of the employed docking algorithm in dealing with a multi-site interacting protein is shown and puts the basis for a thorough analysis of the binding mode with differently functionalised ligands with medical relevance. Other proteins belonging to bile acid binding protein family are actually under investigation in our laboratory in order to get new insights in enterohepatic circulation.

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CONFORMATIONAL STUDY OF SELF-ASSEMBLING PEPTIDES

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Functional behavior of bio-molecules strictly depends on their ability to dynamically interact with each other, often by forming supra-molecular aggregates with high specific competence. Recently, the interest for the molecular details of the assembling processes has largely increased. Indeed, the knowledge of these mechanisms opens to diverse and very appealing applications spanning from the opportunity to control pathologies associated with protein aggregation to the de novo design of smart materials in nanotechnology, biosensors and tissue engineering. Here we focus on a series of synthetic peptides potentially able to self-assemble and produce biocompatible membranes [1]. The peptide sequences show a typical β-strand periodicity with a repeating pattern of polar and non polar amino acids. The membranes are expected to be formed by layers of parallel (or anti-parallel) β-sheets held together on one side by hydrophobic bonding and on the other side by ionic bond between charged residues.
The peptides were analyzed in solution in order to correlate conformational properties and self-assembling propensity of the monomers in several environments. At micro- and milli-molar concentrations the peptides show, even though in different percentage along the series, typical β-strand CD profiles. We found that at milli-molar concentration the 16 residues peptides are apparently involved in aggregation equilibria. However, our NMR studies show that the peptides in free form are mainly in a random conformation while the aggregates, which are in equilibrium with the first ones, are so large that they don't contribute to the spectra. Our diagnosis, that reconciles the apparent discrepancy between CD and NMR results, was confirmed by NMR diffusion experiments and SANS analysis. The first measurements proved that the peptide which contributes to the NMR spectra is mainly in a monomeric form. The second technique proved that high molecular weight aggregates float in solution. Indeed, the very large dimensions of the floating objects and the slow kinetic of the aggregation equilibria both contribute to hide those structural details which are crucial to model the self-assembling process. By using TFE as co-solvent we also tried to stabilize some early stage of aggregation. Our feeling is that, with respect to the plain water environment, the presence of large aggregates is retained in TFE/H_2O mixture. Furthermore the free peptides result stabilized in turn/helix conformations, in spite of the β-strand design.

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STRUCTURAL STUDIES OF PRL-3: A PHOSPHATASE INVOLVED IN COLORECTAL CANCER PROGRESSION

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The role of phosphorylation in tumorigenesis is an established field of active research in the cancer area. In fact it is by now clear that kinases and phosphatases play a key role in cell growth, adhesion and migration [1-3]. PRL-3, a small PTP-ase, has been recently identified as a protein whose over-expression results in promoting cell migration and invasion, hinting thus to a major role in the metastasis process. However still unclear are both the physiological role of PRL-3 and its mechanism of action in the metastatic process [4-9]. Two NMR solution structures have been solved (1R6H.pdb [10], 1V3A.pdb [11]). Although the topology is conserved in both structures, the rmsd of the structural conserved regions is 3.3Å indicating that the structures are rather dissimilar, and a detailed check of both deposited conformations revealed that the P-loop structure (essential for activity) is different from other PTP-ases. Furthermore, evidences with Residual Dipolar Coupling (RDC) measurements show that none of the two structures is compatible with the experimental data.
Here we present a new PRL-3 NMR solution structure refined with RDC measurements. The P-loop structure is similar to other PTP-ases and our structure is able to rationalize the activity results obtained. The availability of a good solution structure opens up the possibility to perform some structural design of both substrates and inhibitors, to this respect some of the successful SAR work performed with PTP1B could be attempt also on PRL-3 [12,13].

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DIMERIZATION OF A-BUNGAROTOXIN MONITORED BY PARAMAGNETIC PROBES: A NEW APPROACH FOR PROTEIN-PROTEIN INTERACTION STUDIES

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A Nuclear Magnetic Resonace study of paramagnetic perturbation on α-bungarotoxin from snake venom has been performed by using TEMPOL, Gd(III)DTPA-BMA and the newly designed probe Gd_2L7. From the obtained paramagnetic attenuation profiles of ^{1}H-^{13}C HSQC signals, in spite of the size, shape and chemical nature of the used paramagnetic probes, a common pathway of approaching to the protein surface can be clearly identified, showing a good agreement with the known association behaviour of the toxin. Indeed, paramagnetic attenuation map, while showing an enhanced accessibility of α-bungarotoxin binding site, infers a reduced accessibility of the three probes to the surface area involved in protein-protein interaction. The surface area obtained in such way was used as restraint for molecular docking simulation of a α-bungarotoxin dimer, giving two alternative structures, both structurally similar to those obtained by X-ray crystallography. Molecular dynamics simulations showed computational and experimental models to be energetically comparable as well.

The present work has shown for the first time that the use of paramagnetic perturbation of conventional NMR spectra combined with docking simulation is a reliable method for structural study of protein interaction and a powerful tool in investigating protein aggregation related to human diseases.

UNAMBIGUOUS STRUCTURE ELUCIDATION OF THE REACTION PRODUCT OF 3-ACETYL-4-HYDROXY- AND 3-ACETYL-4-METHOXY-1-METHYLQUINOLINONES WITH HYDROXYLAMINE VIA NMR SPECTROSCOPY

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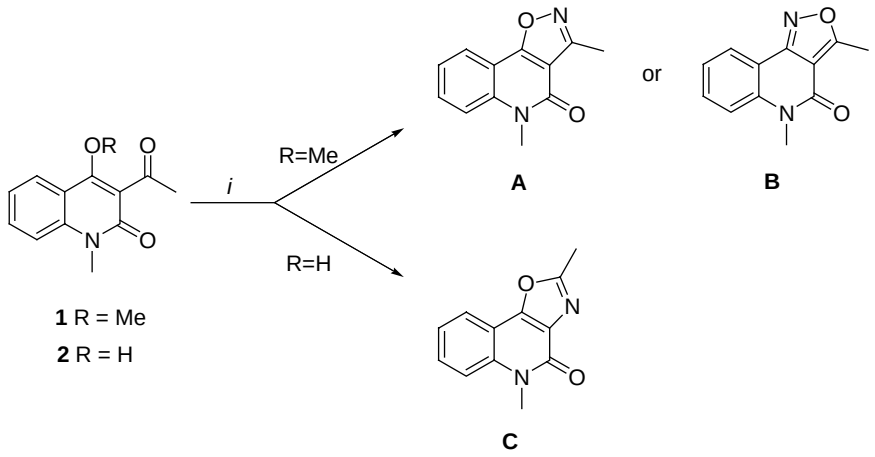
Following our research on the nature of the products deriving from the reaction of quinolinylenaminones with 1,2-bisnucleophiles,[1] we became interested in the synthesis of fused 1,3-oxazolo[4,5-*c*]quinolin-4(5*H*)ones. These tricyclic systems are attractive compounds from the pharmacological point of view as P-38 mitogen-activated protein (MAP) kinase inhibitors.[2,3]
We report here on the different chemical behaviour of compounds **1** and **2** in the reaction with hydroxylamine. Structures of the obtained compounds **A** (from **1)** or **C** (from **2)** (Fig. 1) are easily distinguished by ^{13}C NMR experiments like gHMBC and then unambiguosly determined by natural abundance ^{15}N NMR.



i) $NH_2OH{\cdot}HCl$, ethylene glycol, 200 °C, 30 min

In particular, ^{15}N chemical shifts considerations led to the distinction between isoxazolo- and oxazoloquinolinone structures **A** and **C**, whereas a gHMBCad-HN experiment allowed us to discard structure **B** owing to the presence of a significant N(2)-CH_3 coupling in the isoxazole ring.

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APPLICATION OF STD-NMR TO LIGAND-DNA INTERACTIONS: AN ANALYSIS OF DIFFERENT BINDING MODES

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In the last years several NMR methods have been developed and employed for the detection of interactions between small molecules and targets of pharmaceutical interest. Among such NMR techniques, saturation transfer difference (STD) [1], based on intermolecular magnetization transfer between ligand and receptor molecules, has enjoyed a remarkable attention from researchers operating in both academic and industrial settings.

Until now STD has been largely employed for detecting small molecules interacting with their protein targets, with just one example focusing on nucleic acid receptors. Indeed, besides a single application of an STD-based study of ligands targeting RNA [2], there are not other reported STD studies on ligands binding to a DNA system.

In the present study, we have extended the use of the STD-NMR technique to a DNA receptor, with the two-fold objective of monitoring the applicability of this method to another macromolecular system, and of verifying whether the three main ligand-DNA binding schemes (minor groove binding, external electrostatic binding, and intercalative binding) may be distinguished and characterized by this technique.

To this end, we have used a poly(dG-dC)·poly(dG-dC) copolymer as macromolecular target and five different compounds binding DNA by different modes: distamycin and netropsin (minor groove binders); spermine (external binder); thiazole orange and doxorubicin (base-pair intercalators) [3]. An additional investigation has concerned the peculiar case of ethidium bromide, previously thought to be a plain intercalator, which, on the basis of very recent acquisitions, appears rather to act by a mixed binding mode, encompassing both base-pair intercalation and external interaction to DNA phosphate backbone [4-5].

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^{15}N NMR OF PARTIALLY SATURATED PYRAZOLES

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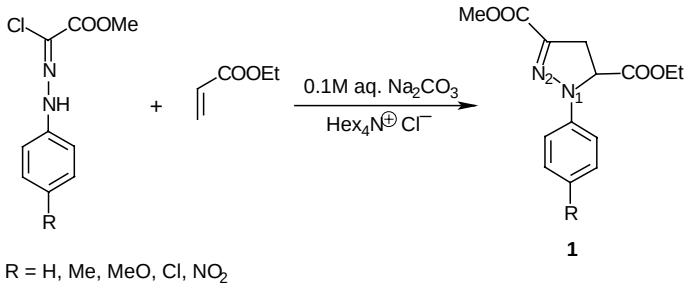
Due to its relevance in both chemical and biological research, increasing attention has been devoted to the field of ^{15}N NMR spectroscopy [1]. In particular, the chemical shifts of some azoles have been investigated through ^{15}N NMR onto ^{15}N-enriched substrates [2], but there is a lack of data concerning partially saturated systems. In order to fill this gap, we have submitted 1-(4-substituted)phenyl-3-methoxycarbonyl-5-ethoxycarbonyl-4,5-dihydropyrazoles **1**, synthesized from the corresponding hydrazonoyl chlorides as depicted in Scheme 1, to ^{15}N NMR analyses which were performed in natural abundance.



Scheme 1. Synthesis of 1-(4-substituted)phenyl-4,5-dihydropyrazoles **1**.

Chemical shifts $\delta(N_1)$ and $\delta(N_2)$ were determined through INEPT and HMBC experiments, while long range ^{15}N-^{1}H scalar couplings were elucidated through J-HMBC experiment.
Our results show that (i) both chemical shifts $\delta(N_1)$ and $\delta(N_2)$ depend upon the substituent R, (ii) the solvent exerts little or no influence upon δ, and (iii) a linear relationship between Hammett σ and ^{15}N NMR shifts were found enabling the correlation of the latter parameter with the electronic features of R.

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NMR STUDY OF THE REVERSIBLE TRAPPING OF SF_6 BY CUCURBIT[6]URIL IN AQUEOUS SOLUTIONS

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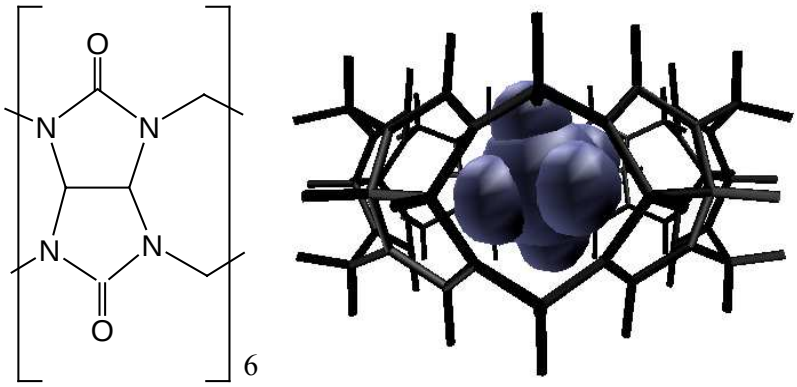
Cucurbit[6]uril (CB6) is a macrocyclic methylene-bridged glycoluril hexamer whose shape resembles a pumpkin.[1] It is a cavitand with a hollow core of approximately 5.5 Å diameter. The cavity is accessible from the exterior through two carbonyl-fringed portals of 3.9 Å diameter that may produce significant steric barriers to guest association and dissociation. The portals are cation-binding regions while the cavity is a hydrophobic binding region. CB6 can therefore bind a large variety of small chemical species including neutral guests such as xenon [2].

Sulphurhexafluoride, SF_6, is the gas with the highest greenhouse potential. The trapping of SF_6 by CB6 dissolved in aqueous solutions was investigated by ^1H and ^{19}F NMR spectroscopy at 9.4 T. The ^{19}F NMR signal of SF_6 dissolved at 25°C and 1atm in an aqueous solution of Na_2SO_4 is readily observable despite the low solubility of SF_6 in this solution. A large increase in the ^{19}F signal intensity is observed in the presence of 0.01 mol L^{-1} of CB6, suggesting that CB6 exhibits a high affinity for SF_6. At low concentrations of CB6 (~ 10^{-4} mol L^{-1}) and SF_6, both the free and bound SF_6 are observed in the ^{19}F spectrum and their relative concentrations can be measured. The ^1H spectrum also shows distinctive signals for the two species in equilibrium i.e. the inclusion complex and CB6 free of SF_6. The exchange is slow on both the ^{19}F and ^1H NMR time scales in the whole range of temperatures studied up to 85°C. The apparent affinity constant of CB6 for SF_6 was determined at various temperatures in Na_2SO_4 and D_2SO_4 aqueous solutions. ^{19}F T_1 relaxation time and diffusion data are also presented.



Cucurbit[6]uril and illustration of its inclusion complex with SF_6.

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SOLID STATE ^{2}H NMR MOLECULAR SPECTRA: SEPARATION OF SPECTRAL COMPONENTS BY GUEST ALIGNMENT IN AN AXIALLY ORIENTED POLYMERIC CRYSTALLINE HOST PHASE

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Molecular motions with time scale of 10^5-10^8 Hz are most appropriately investigated by ^{2}H NMR spectroscopy of selectively deuterated compounds. However, the ^{2}H NMR line-shape analysis gives straightforward information only for rigid guests involved in simple motions. Flexible deuterated compounds which exhibit complex reorientations often lead to solid state ^{2}H NMR spectra in which several spectral components are superimposed and the evaluation of the dynamic states can be not unique. In these cases a separation of spectral components would be helpful in the assessemnt of the dynamic states accessible to the molecule. This separation could be, in principle, achieved by confining molecules in the void space of macroscopic oriented inclusion compounds and by collecting anisotropic spectra for different sample orientations with respect to the magnetic field. However, most host systems like zeolites or metallorganic or organic frameworks cannot be easily oriented, while standard methods, involving sorption of molecules into stretched polymeric amorphous phases, produces changes in the local environment of the solute molecules, thus altering their mobility [1].
In this communication it is shown that separation of spectral components of solid-state ^{2}H NMR spectra of volatile molecules can be achieved by absorbing them in uniaxially stretched polymeric films which are able to enclose them as isolated guests in a crystalline host phase [2] and, as a consequence, high molecular orientation can be obtained without altering the local environment of the guest molecule (e.g., see Fig. 1).

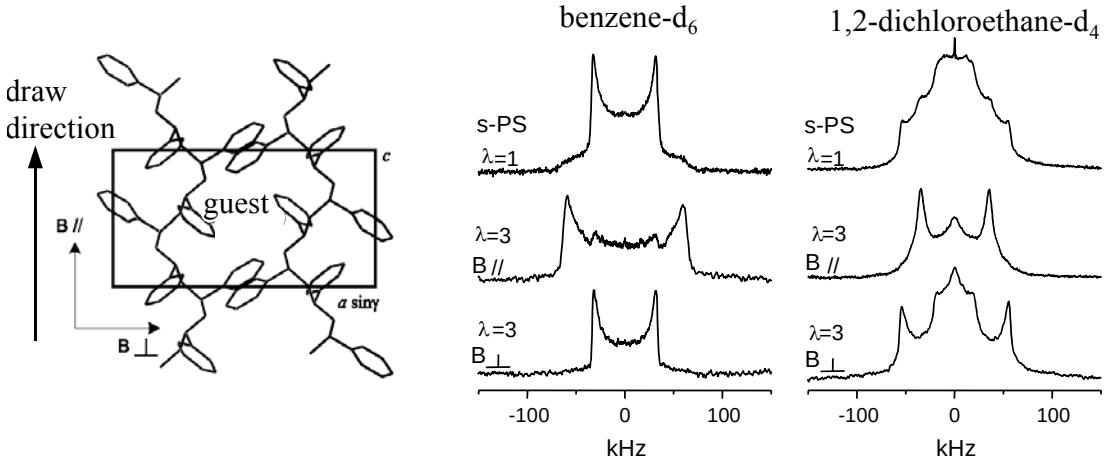

Fig. 1. Solid state ^{2}H NMR spectra of volatile molecules guests of the crystalline δ-phase s-PS films unoriented (λ=1) and uniaxially oriented (λ=1).

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SOLID STATE NMR OF MOLECULAR HYDROGEN TRAPPED INSIDE OPEN-CAGE FULLERENES

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We present a solid state ^{1}H and ^{13}C NMR study on compounds containing endohedral hydrogen molecules inside fullerenes under static and MAS conditions. Two types of fullerene cages are considered here: open cage fullerene (aza-thia-open-cage-fullerene, ATOCF) and C_{60}.

We performed T_1 measurements, static and MAS experiments as function of temperature, from room temperature down to 4K. From the MAS and static lineshape data analysis one can obtain the energy level distribution for the rotational and translational energy level of the H_2 molecule, treated as a particle in a spherical box. The highly mobile H_2 molecule gives rise to very sharp lines under MAS. For $H_2@C_{60}$, ^{13}C lines are sharp and cross-polarization from ^{1}H to ^{13}C is achievable but very narrow.

The two samples are compared, with the emphasis on the evidence of strong, T dependent intramolecular DD coupling within H_2 for H_2@ATOCF and the presence of only intermolecular H_2-H_2 interactions in the case of $H_2@C_{60}$, as indicated by spin counting experiemnts using the spherical tensor analysis approach.
More work on this materials is still in progress.

A MULTINUCLEAR SOLID STATE NMR INVESTIGATION OF THE INTERACTIONS OCCURRING AT THE SURFACE OF A LAYERED SILICATE IN ITS HYDRO- AND ORGANO-PHILIC FORMS

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The use of clay particles as fillers for many polymeric matrices is a consolidate practice in the preparation of micro- and nano-sized composites, due to the improvement of the material performances such as mechanical and gas-barrier properties. In order to enhance the compatibility between the filler and the polymer matrix, a key factor is the modification of the hydrophilic character of the clay into an organophilic one. This can be achieved by several strategies, two of the most employed being: (i) the ion-exchange among the cations originally adsorbed onto the clay platelets surface (usually sodium cations) and large organic cations (usually alkylammonium cations) [1], (ii) the direct organo-modification of the clay with alkoxysilanes, usually achieved through a grafting reaction involving the silanol groups mainly present on the clay particles edges [2, 3].
In this work we performed a multinuclear Solid State NMR study of a synthetic layered silicate, Laponite, in three different forms: the pristine hydrophilic, a C_{18}-alkylammonium cation exchanged and a double treated form, obtained by performing a grafting reaction of an organo-silane containing a polymerizable group (TSPM) onto the C_{18}-alkylammonium cation exchanged clay. Through the employment of ^{29}Si, ^{13}C, ^{1}H mono- and bi-dimensional high-resolution techniques, as well as ^{1}H FID analysis and T_2-$T_{1\rho}$ correlation low resolution experiments, it has been possible to investigate the interfacial interactions between the clay and the several species present at its platelets surface, as well as the structural and dynamic properties of both the intercalated organic cations and TSPM molecules.

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MOLECULAR CHARACTERIZATION OF *PHYLLOSTICTA ILICINA* SYMPTOMATIC AND ASYMPTOMATIC STRAINS BY ADVANCED CPMAS ^{13}C-NMR TECHNIQUE AND ELECTROLYTES LEAKAGE ASSAY (ELA)

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§. Supported by "Progetto Cervelli" MIUR, Italy

The genus *Phyllosticta* includes species pathogenic on many crops and species with a wide host range, as well as endophyte. *P.ilicina* mainly infects Holm-oak (*Quercus ilex*). Relevant attacks occur on leaves. Normally adult plants are not severely injured, while it can represent a serious problem in nurseries. Recently we trace *P.ilicina* strains both from symptomatic and a-symptomatic leaves in different Italian regions. Colonies from diverse symptomatic tissues show no differential morphological characters. We used a molecular approach to unravel possible differences related to virulence factors.

The Electrolytes Leakage Assay (ELA) involved cultural filtrates from four strain (DA60, DA72 from symptomatic leaves; DA 93, DA122 from a-symptomatic leaves) partially purified by ethyl acetate extraction. The electrolytes leakage of Holm-oak mature healthy leaves was measured in presence of the diverse cultural filtrates. The conductance changes in the solutions were measured in real-time (2-min intervals) (range 0.02-200 mS/cm; K=1.0). Data were recorded every 30 minutes for at maximum 10 hours. Statistic analysis of the ELA data-set showed the diverse behavior induced by the DA60 cultural filtrate, purified from symptomatic lesion. Conversely, statistical differences were not observed for the other three cultural filtrates and the water control.

We extended the NMR analysis to a total of 6 strains: DA60, DA72, DA90 from symptomatic leaf and DA93, DA121, DA123 from a-symptomatic leaf. The freeze-dried mycelium was produced as described for the cultural filtrates and used. Cross polarization magic angle spinning (CPMAS) ^{13}C-NMR spectra were acquired with a Bruker AVANCE™ 300 at a rotor spin rate of 13000±1 Hz. The analyses of the spectra evidenced the missing of signals attributable to aromatic carbons, whereas carbohydrates appeared to be the main constituents of the fungi. The lack of aromatic signals can be explained by the high amount of carbohydrates that prevented a precise observation of other spectral signals such as those generated from aromatic moieties.

A quantitative evaluation of CPMAS ^{13}C-NMR spectra revealed that the amounts of carboxylic groups (200-166 ppm) and alky carbons were comparable to each other (10 and 15%, respectively). Carboxylic and aliphatic carbons may be due to the presence of proteins, fatty acids, DNA, and RNA residues. The amount of the remaining moieties did not reveal any difference among the fungi. At this stage of our experiments, we can conclude that the symptomatic and a-symptomatic behaviour of the different fungi is not due to differences in their bio-chemical composition. The symptoms can be attributed to the stimulation effects that these fungi induce on the leaves.

QUALITY ASSURANCE PROJECT ON GSK SCREENING COLLECTION: AN ANALYTICAL CHALLENGE

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The Quality Assurance project has been undertaken in GSK to assure that our entire Automated Liquid Store for High Throughput Screening is Pure (>80%) and Sure (structures confirmed). The advantages are obvious in terms of screening costs minimised, use of negative results, reduced effort chasing uninteresting leads and impurities and so on.
To achieve that goal an extraordinary effort in terms of instruments and expert analysts was necessary. Different analytical techniques were used while automated systems were implemented not only for sample preparation and data acquisition but also for data analysis.
Experimental, logistical and practical problems involved in this task will be outlined, together with the associated software necessary for managing and interpreting the large quantity of data produced.

BINDING OF RGD-PEPTIDE MIMICS TO INTACT HUMAN PLATELETS INVESTIGATED BY TRANSFERRED-NOESY EXPERIMENTS

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The interactions of small peptides with biological membranes is central to a number of biological processes. In contrast to soluble proteins there is comparatively little information available about ligand-receptor interactions that occur at membrane surfaces. The biophysical environment of a membrane is considerably different from the isotropic extracellular medium. It is therefore desirable to investigate membrane proteins and their binding specificity directly in living cells.

NMR spectroscopic techniques are powerful tools to understand the binding process at a molecular level. Among these, trNOE[1] focus on the NMR signals of the ligand and utilizes NOE effects between protein and ligand. Moreover, tr-NOE also has the potential to offer easy detection of binding events, additionally producing information on the bound conformation of the ligands.

Herein, we show that the interactions between small ligands (pentapeptide mimics) and membrane-bound proteins (integrin $\alpha_{II}\beta_3$) can be observed by trNOE spectroscopy directly on whole human platelets, without the necessity for isolating the protein receptor.

The integrin $\alpha_{II}\beta_3$ is the most abundant platelet cell surface glycoprotein and plays a key role in adhesion of platelet to protein-coated surfaces and platelet/platelet aggregation. The ligands are cyclic pentapeptide mimics (Fig.1) incorporating stereoisomeric 5,6- and 5,7-fused bicyclic lactams and the tripeptide sequence Arg-Gly-Asp (RGD).[2]

This approach provides key information on their binding mode in natural environment and allows to deduce information on the structural requirements of the ligand in the bound state. Conformational properties of the free and bound pentapeptides are also reported.

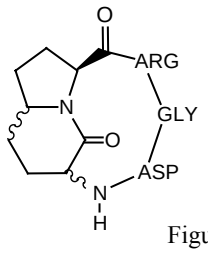

Figure 1

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NEW RAS PROTEIN INHIBITORS AND THEIR INTERACTION WITH THE TARGET: THE NMR POINT OF VIEW

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The pharmacological modulation of mutated, tumorigenic Ras protein activity could represent an efficient strategy to prevent tumour formation and development. Our goal was the development of small molecules able to bind Ras, inhibiting its activity and its ability to induce neoplastic transformation of mammalian cells.
We designed and synthesized a novel type of Ras inhibitors by inserting potential pharmacophoric groups on glycidic scaffolds derived from natural monosaccharides.
These compounds show interesting biological activity (μM range) in the *in vitro* inhibition of GDP-GTP nucleotide-exchange on human Ras (required for protein activation), and the *in vivo* inhibition of tumoral cells growth [1].
In order to elucidate the structural nature of the interaction between Ras and our compounds, we employed different NMR approaches. We obtained evidences of the binding performing NMR studies based on protein-ligand magnetisation transfer (trNOE and STD experiments, fig. 1) [2].

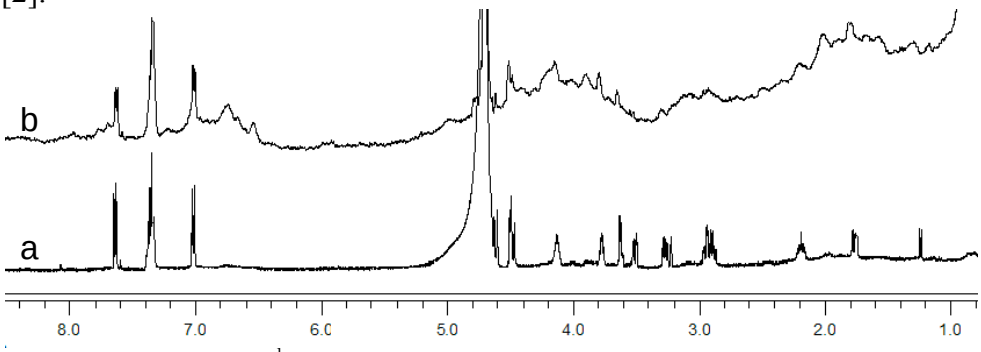

Fig. 1. a) ^{1}H inhibitor+Ras-GDP; b) 1D-STD inhibitor+Ras-GDP

In this way, the epitope mapping, that is the identification of the ligand regions involved in the interaction with the receptor, was obtained. Moreover, with ^{15}N HSQC experiments we mapped our inhibitor binding site on Ras protein surface. These results will be presented in this communication.
Further indications concerning the mechanism of action of our compounds were achieved by SPR (Surface Plasmon Resonance) experiments. In particular, our compound ability to interfere with Ras-activators/effectors (Cdc25/Raf) interaction was investigated.
Data collected suggest that the molecules presented in this communication can be considered as lead compounds for the development of new anti-tumor drugs.

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ANALYTIC SOLUTION OF γ-ENCODED NMR SIGNALS

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Several solid state NMR experiments, especially in the class of symmetry based recoupling [1], lead to a signal whose dependence on time, before the powder averaging, does not depend on γ, the Euler angle between the molecular and the rotor frame. In spite of a reduction in the number of variables, the search for an analytic solution of such an integral, fully based on theoretical argumentation, had led, in same case and up to now, only to infinite solutions. Here, we present finite solutions for all the γ-encoded sequences. The solution is also compared with infinite Bessel series previously obtained by other authors [2]. The case of double quantum filtered dipolar recoupling [3] was chosen as a probe to investigate, through comparison with both simulation and experiments, the behavior of the analytic solution by testing several effects going from CSA orientation dependence to *rf* field inhomogeneities.

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USE OF NMR DIFFUSOMETRY AND HRMAS TO INVESTIGATE RESIDUAL SILANOL ACTIVITY IN REVERSE CHROMATOGRAPHIC PHASES

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We recently introduced a novel analytical method based on NMR diffusometry and High Resolution Magic Angle Spinning (HRMAS) that combines the advantages of column chromatography separation and NMR structural analysis [1]. Specifically, we showed that, in a NMR diffusometry experiment, the separation of the NMR spectra of the components of a mixture could be enhanced by several orders of magnitude upon addition of a typical stationary phase used in HPLC. HRMAS is then required to recover high resolution NMR spectra by removing magnetic susceptibility broadenings caused by the presence in solution of the solid support. The potentialities of this technique for mixture analysis were illustrated on test mixtures with both direct (silica gel) and inverse (C18) columns.
This combined analytical method was later extended to investigate indirectly crucial steps of reverse phase liquid chromatography (RPLC), such as the partitioning of the analyte between different phases [2] or the influence of particle porosity [3], in order to contribute to a better understanding of the chromatographic process [4].
Here, we give preliminary results on how such approach can be used to investigate another fundamental aspect of RPLC, namely the activity of residual silanols on reverse phases.

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MAGNETIC RESONANCE IN ASTRONOMY: FEASIBILITY CONSIDERATIONS

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The Universe harbors many magnetic bodies, ranging from planets and stars (fractions of a Tesla) to dwarf stars (intermediate fields), pulsars (10^12 T) and magnetars (10^15 T). Since the Universe is made mostly of spinning particles endowed with magnetic moments (electrons, protons, neutrons, muons and atomic nuclides, with non-magnetic particles being a minority), it is evident that magnetic resonance (MR) phenomena should be commonplace. Yet practical astronomy so far does not seam to take the possibility of detecting such phenomena into account, nor does it strive to exploit them actively on planetary and solar-system scales. This may be due simply to a lack of faith that such phenomena can be detected in a sufficiently specific manner.

This presentation lists possible MR phenomena occurring on astronomical scale and discusses their highly specific features which might make them experimentally accessible. The hope is that such a study might help to establishing magnetic resonance astronomy (MRA) as a new branch of MR.

The considered 'sample' sizes range from sub-planetary (sections of troposphere, seas and oceans, icecaps, etc) to planetary (atmospheres, ionospheres, radiation belts) and stellar (sunspots, atmospheres of dwarf stars, pulsars and magnetars). The particles to be observed include both electrons and nuclides (plus a few more) so that no conceptual distinction is made between ESR and NMR.

Depending upon the considered special case, Larmor frequencies range from a few kHz (like protons in the Earth field) to well beyond X-rays (electrons on magnetars). There are, however, unique characteristics of MR-related radiation (be it spontaneous or stimulated) which make it quite distinct. These are (i) exceptionally narrow spectral bands, (ii) precise direction vectors (Poynting vector aligned along the magnetic field) and, above all, (iii) strict chirality (implying circular polarization).

Experimentally, since induction coupling is ruled out by the size of MRA objects, one must resort to standard techniques of emission, absorption and stimulated-emission spectroscopy, both passive (remote observation) and active (using spacecraft-mounted transmitter-receiver systems). We will discuss the instrumentation and the techniques (some of which are similar to NMR 'pulse' sequences and data-averaging) which might make it possible to isolate MR signals from non-MR background.

Low-frequency MRA might help analyze the particle composition of sunspots and, in its active version, the composition of Jovian atmosphere. On a sub-planetary scale, even the low Earth field might be sufficient, for example, to actively monitor tropospheric storms (ESR), oceans, polar icecaps and other planetary surface features.

The author discusses also the MR phenomena which might be going on in the magnetic fields of the order of 10^12 Tesla present on pulsars. Though the possibility that the observed light flashes are really MR signals is just a far-shot hypothesis but, nevertheless, it should be taken into account. It appears, in fact, that there might be ways of how the interaction between spinning particles with the extremely high fields might explain the pulsed emissions in the optical and X-ray regions.

MULTIPARAMETRIC MRI SEGMENTATION AND PARTIAL VOLUME EFFECT CORRECTION

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In Nuclear Medicine, the poor spatial resolution of the scanners (FWHM ≥ 6mm in PET and > 10mm in SPET) doesn't allow a direct measure of tracer concentration in small structures generating an apparent activity loss, described as Partial Volume Effect (PVE).
Due to PVE tracer concentrations as measured by PET in Grey Matter cannot be compared:

- Between different areas of the brain
- Between normal subjects and atrophic patients
- Between populations of different age
- Between different stages of degenerative diseases with progressive atrophy
- Between data obtained with different scanners with different spatial resolutions
- Between data from functional imaging and auto-radiographic data

Estimated error introduced in functional imaging data by PVE is up to 29% in Normal Subjects and up to 75% in Alzheimer Disease [1].
The starting point of all PVE correction techniques is the acknowledgement that the observed PET image activity I_{obs} is the result of the convolution of the real image activity I_{act} (the true activity value, i.e. without PVE) by the 3-D PSF of the scanner $h(r)$, plus some noise $b(r)$:

$$I_{obs}(r) = I_{act}(r) \otimes h(r) + b(r)$$

where r represents the position in the image space.
PVE correction is achievable when: the distribution map of the various structures within the functional images can be accurately defined and the resolution of the scanner h(r) is known. High-resolution anatomical images, such as those provided by Magnetic Resonance Imaging (MRI), contain the morphological information needed to delineate the distribution map of structures. On the other hand, the functional image resolution can either be calculated knowing the intrinsic resolution of the scanner and the reconstruction filter, or measured by scanning simple geometric phantoms.
Accordingly, different approaches have been reported in the literature for the correction of PVE.
Two types of correction techniques can be distinguished: the voxel based method [2-4], and the region of interest (ROI) based method [1, 5]. They are based on both the distribution maps, obtained segmenting MR images, and the knowledge of the resolution of the scanner.
Many brain segmentation methods have been presented in last decade and reviewed [6, 7] since segmentation accuracy is crucial in both assessment of volumetric brain data and PVE correction. Our group has developed [8] and applied to different brain diseases [9-11] a multiparametric segmentation method using proton density and relaxation rate information to classify brain tissues. The fully automated software permits an operator-independent classification and volume measurement of Gray Matter, White Matter, CSF, Muscle, connective tissue and Fat, as well as Multiple Sclerosis lesions.
This talk will focus on the multiparametric brain segmentation and on PVE correction as well as on our work in progress to improve the methods in terms of applicability and accuracy.

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CLINICAL/EXPERIMENTAL APPLICATIONS OF MRI

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Different techniques are currently used to obtain images of the structures of living beings for diagnostic purposes using different physical approaches, including Magnetic resonance imaging (MRI). MRI applications have continuously increased after the introduction of the first commercial MRI scanners in the 1980's.
MRI is now routinely used within medical diagnostics, with tens of millions investigations performed each year. With the high tissue contrast provided by MRI, using multiparametric information (in particular T1, T2 relaxation and proton density, and other properties such magnetic susceptibility and water diffusion) several invasive examination procedures have been replaced by MRI
Indeed MRI is noninvasive and harmless and has very limited contraindications (patients with pacemakers or potentially mobile metallic implants cannot be examined with MRI due to the strong magnetic field; claustrophobia may create difficulties with some patients undergoing MRI).
An additional advantage with MRI is the possibility to obtain multiplanar images (images oriented in different spatial directions) without moving the patient, by using appropriate magnetic field gradients.
Thanks to technical developments both with hardware (higher field magnets, improved circuits, higher speed computers) and software (pulse sequences, data processing) MRI can now provide high resolution images of all body structures with superb visualization of soft tissues with unrivaled results in the morphostructural characterization of Central Nervous System and of Musculo-Skeletal structures. Image processing can be used for 3D reconstruction and analysis.
MRI studies are also increasingly used in diagnosis, treatment and follow-up of cancer. In the diagnostic workup MRI data can be used to precisely define the tumor size, shape and margins providing crucial data for subsequent treatment selection (in particolar for surgery and radiation therapy
The high contrast and multiparametric characteristics of MRI can be used to extract additional information, like in tissue classification procedures known as segmentation that represent the basis for volumetric measurements of both normal and abnormal structures. Furthermore, advanced functional MRI applications (fMRI) permit the evaluation of tissue perfusion and water diffusion with many potential research applications in the cognitive neuroscience field
In addition to routine diagnostic application, MRI is also increasingly used for experimental studies, in particolar for the characterization of animal models of human disease. Since animal models of diseases are usually obtained in small sized rodents (mostly mice), dedicated ultra high field equipment is necessary to obtain high resolution information in this challenging experimental MRI application.

XXXVI National Congress on Magnetic Resonance

POSTERS

ONTOGENESIS OF PANCREATIC BETA-CELLS FROM PANCREATIC DUCT CELLS

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The diabetes mellitus is becoming an emerging problem, in particular in the western population [1]. Evidences from molecular and epidemiological studies indicate that beta-cells dysfunction is crucial in type I and type II diabetes mellitus and in both cases eems to cause the beta-cells destruction by apoptosis [2].
The regulation process of human pancreatic beta-cells mass has assumed a prominent role since very important goals have been achieved in the field of pancreatic islets transplantation [3]. Studies made in many laboratories have demonstrated that beta-cells can proliferate responding to physiological and pathophysiological stimuli and responding to genetic manipulations which lead to insulin resistance [4].
The study proposed in this project is based on the observation that pancreatic beta-cells loss occurred during diabetes mellitus is not compensated by a massive neogenesis of pancreatic islets. However, it seems that some growth factors have a biological role mediating the molecular signals in the ontogenetic program of pancreatic beta-cells development from ductal cells. Here we focus our attention on the effect of the placental lactogen hormone (hPL) that is known as one of the most potent beta-cells mitogenic agents [5-10]. Using NMR spectroscopy on cellular extracts of human pancreatic ductal cells (PANC-1), in basal conditions and treated with recombinant hPL, we are able to follow metabolic changes occurring during ontogenetic events of pancreatic beta-cells differentiation from epithelial pancreatic ductal cells.

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NMR CHARACTERIZATION OF THE INTERACTION BETWEEN A BILE ACID-BINDING PROTEIN AND A NOVEL CHOLATE-BASED ANALOGUE OF A Gd(III) COMPLEX DESIGNED AS A POTENTIAL SPECIFIC CONTRAST AGENT FOR MRI

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Gd(III) complexes are used as magnetic resonance imaging (MRI) contrast agents for diagnostic purposes (1). Indeed, they enhance the relaxation rate of water protons of tissues in which they distribute, especially if the paramagnetic complex is part of a macromolecular system. Their T1-relaxivity and their delivery/accumulation properties at the sites of interest can be well controlled by modulating the chemical properties of the paramagnetic adducts (1, 2). Structural studies on the interaction of Gd(III)-bile acid adducts, in complex with cL-BABP, the bile acid carrier protein in liver cytosol, will aid in developing contrast agents specific for liver malignancies.

Proton relaxation enhancement studies were used to screen the affinity of a number of bile acid-based Gd(III) complexes for cL-BABP. Based on the obtained data and the outcome of cellular uptake measurements, one single compound was selected for subsequent NMR characterization.For NMR studies, we used an adduct where the bound metal is the diamagnetic Y(III). Proton ligand resonances assignment was made by use of 1D, and 2D *J*-resolved, COSY, and TOCSY NMR experiments. Protein addition determines increased linewidth of signals from the steroid moiety, indicating that this portion is involved in the interaction, while the ion-coordinating portion appears to move more freely. A more detailed ligand epitope mapping was made by use of saturation transfer difference (STD) spectroscopy. Although the present system proved not ideal for such experiments, clear involvement of the methyls-bearing part of the ligand was observed. The participation of specific protein regions in binding events was studied through ^{1}H-^{15}N HSQC titration experiments. Resonances assignments and structural data for the protein were determined previously (3). Chemical shift changes indicate the occurrence of protein conformational equilibria associated with ligand binding. Although this situation prevents straightforward chemical shift mapping, careful inspection of all the resonances points out to a ligand interaction site in proximity of the helical lid at the top of the β-barrel. Residues in fast exchange were fitted to a simple one site binding model allowing the determination of the dissociation constant. Further NMR experiments are in progress to fully characterize a protein-ligand interaction which is of value in the rational design of specific contrast agents.

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^{1}H NMR REVEALS SIGNIFICANTLY DIFFERENT LEVELS OF GLUTATHIONE IN SENSITIVE OR SPONTANEOUSLY RESISTANT TO CISPLATIN CELLS FROM HUMAN OVARIAN CANCER LINES

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Cisplatin is a chemotherapeutic drug widely used to treat a variety of solid tumors. It belongs to the alkylating agent group of chemotherapeutics. Cisplatin binds to DNA base pairs creating adducts, crosslinks, and strand breaks that inhibit DNA replication. Both intrinsic and acquired resistance to cisplatin, as well as toxicity, represent a major obstacle to effective cancer therapy and limit its effectiveness in clinical use. Molecular mechanisms that underlie cisplatin resistance are poorly understood. Different molecular mechanisms may co-exist to give high levels of resistance. The plasma membrane plays an important role in the control of intracellular concentration, efflux and influx of drugs and there is convincing experimental evidence that changes in plasma membrane of tumor cells can be involved in cisplatin resistance. Biological thiols such as glutathione (GSH), present in millimolar concentrations in the cytoplasm, have the potential to interact with platinum. Because Pt-thiol adducts interact less well with DNA, formation of these complexes limits the amount of drug available to bind DNA, thus compromising its efficacy.
In this study, we have used ^{1}H-NMR to analyze two human ovarian carcinoma cell lines: one is cisplatin sensitive (2008) and the other is intrinsically cisplatin resistant (C13). The first aim of this effort was to investigate differences between wild and cisplatin resistant cell lines in order to shed some light on mechanisms of such resistance. Since previous reports have revealed differences in lipid and lipid metabolite signals during growth in culture[1], the analysis was performed on cells at different growth conditions. The two cell lines were also studied at different intervals after treatment with cisplatin. We show that proton NMR allows a clear distinction between, cisplatin sensitive (2008) and resistant (C13) human ovarian carcinoma cells. Among other differences detected between the two cell lines, the most remarkable observation relates to the significantly different levels of glutathione. The levels of this thiol are higher in the resistant cells independent of growth conditions. The levels of glutathione remain constant in C13 cells upon addition of cisplatin while they increase in sensitive cells during treatment with the platinated drug.
The two cell lines can be clearly distinguished also by the amount of mobile lipids, but this difference seems to be related to differences in cell growth rather than to resistance.
These results will be discussed here together with the approach followed to study the two tumor cell lines by NMR.

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STRUCTURAL ANALYSIS OF THE INTERACTIONS BETWEEN HUMAN α-SYNUCLEIN AND THE OXIDATION PRODUCTS OF DOPAMINE

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Parkinson's disease is a chronic and progressive disorder which involves the degeneration of dopaminergic neurons in the *substantia nigra pars compacta*. While the etiopathogenesis of Parkinson's disease remains elusive, the direct involvement of oxidative stress and α-synuclein seems clear. A possible source of oxidative stress in nigral dopaminergic neurons comes from the redox reactions which specifically involve dopamine and originate various toxic molecules. These include free radicals and quinone species. Although the physiological role of α-synuclein is still unknown, increasing evidence suggests that the protein is involved in dopamine metabolism, through interactions with proteins that regulate dopamine synthesis, uptake and synaptic vesicle recycling.

To better understand the possibility of a direct connection between oxidative stress and α-synuclein in Parkinson's disease, we structurally analyzed the interactions between α-synuclein and dopamine-derived quinones. Specifically, we identified the most reactive residues of the protein toward dopamine quinones and, by generating dopamine-derived quinones in a sample containing partially deuterated α-synuclein, we also characterized the most reactive quinone toward α-synuclein. On the basis of our results, we suggest a cytotoxic pathway which involves α-synuclein and oxidative stress.

ISOTACTIC AND SYNDIOTACTIC TRANS-1,2-POLY(3-METHYL-1,3-PENTADIENE):A COMPLETE NMR CHARACTERIZATION.

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3-Methyl-1,3-pentadiene (mixture of E and Z isomers) was polymerized with two catalytic systems, $FeCl_2(L)_2$/MAO (L=bipy, Phen) and $CoCl_2(PnPrPh_2)_2$/MAO obtaining two different highly crystalline poly(3-methyl-1,3-pentadiene)s.
In principle, polymerizing 3-methyl-1,3-pentadiene (3MP), ten stereoregular polymers can be obtained, depending on the type of enchainment of the monomeric units: cis-1,4-iso- and syndiotactic poly(3MP), trans-1,4-iso- and syndiotactic poly(3MP), cis-1,2-iso- and syndiotactic poly(3MP), trans-1,2-iso- and syndiotactic poly(3MP), 3,4-iso- and syndiotactic poly(3MP). Up to now only two stereoregular poly(3MP) have been described in literature: cis-1,4-isotactic obtained with the catalytic systems $AlEt_2Cl/Nd(OCOC_7H_{15})_3/Al(iBu_3)_3$[1] and cis-1,4 syndiotactic obtained by using $Co(acac)_3$/MAO and $Ni(acac)_2$/MAO[2].
In this poster a complete characterization of the two novel crystalline polymers obtained with Fe- and Co-catalysts, which were found to have a trans-1,2 syndiotactic and a trans-1,2 isotactic poly(3-methyl-1,3-pentadiene) structure respectively , has been reported.
The two polymers were extensively characterized with different techniques like IR, NMR (1D and 2D), DSC and X-ray to determine the microstructure.
The IR spectrum of the polymers is consistent with 1,2 structure, but does not give any information concerning the configuration, cis or trans, of the double bond in the side chain, and regarding the iso- or syndiotacticity of the polymers. On the contrary, a detailed NMR study allowed us to completely determine the microstructure of the two polymers.
The ^{13}C NMR spectra were assigned on the basis of the chemical shift values and of the multiplicity in the proton undecoupled spectrum; the chemical shift observed were very close to those obtained by applying the additive rules to the chemical shifts of 1,2-polybutadiene. These assignments and the 1,2 structure were also confirmed by the INADEQUATE and by the Heteronuclear Multiple Bond Correlation (HMBC) experiments of the polymers. The ^1H NMR spectra of the polymers allow us to assign a syndiotactic structure to the Fe-polymer and an isotactic structure to the Co-polymer.
Comparing the heteronuclear allylic coupling constants of different trans- and cis- 1,2 dienes, the configuration of the double bonds has also been assigned. This assignment is in agreement with the experimental evidence that only the (E) isomer, of the monomer, is polymerized while the (Z) isomer can be quantitatively recovered at the end of the polymerisations.
The results obtained have also some mechanistic implications and have contributed to elucidate the influence of the monomer and catalyst structure on the chemo- and stereo-selectivity of the polymerisation.

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INTERACTIONS BETWEEN A NANO-SIZED INORGANIC FILLER AND AN ORGANIC SURFACE MODIFIER BY SOLID STATE NMR

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It is well known that the incorporation of inorganic fillers, micro or nano-sized, in polymeric matrices, can considerably improve the mechanical properties of the polymer. At present much work is being done in the preparation of new polymeric nanocomposites, choosing and testing different fillers and filler treatments with the aim of improving specific properties of the polymer matrix [1]. Nevertheless, a systematic correlation between filler types and experimental processing techniques and the resulting properties of the composites materials is still an open challenge. In this sense, a detailed knowledge of the structure and dynamic at a molecular level of both the nanocomposites and their constituents is an important target in which Solid State NMR can give an important contribute [2,3]. Here we present an NMR characterization of a filler powder constituted by barium sulphate nanoparticles coated with silica and of the same powder treated with stearic acid as surface modifier. Structural and dynamic information on the silica coating, the stearic acid layer and their interface could be obtained by means of ^{29}Si, ^{13}C, ^{1}H high-resolution NMR techniques and wideline ^{1}H FID analysis. ^{13}C-CP MAS, ^{1}H-MAS and ^{1}H FID analysis allow us to highlight the scarce molecular mobility of the stearic acid layer and to characterize its conformational properties. From ^{29}Si, ^{13}C and ^{1}H MAS spectra and ^{1}H FID analysis, information is obtained on the nature of the silica-stearic acid interface, as well as on the modifications induced on the silica surface by the treatment with stearic acid. Useful indications are also obtained on the presence and the role of the water molecules in both the filler forms. An interpretation of the whole set of experimental data is given in terms of a possible interaction mechanism between stearic acid and silica surface.

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SOLID STATE NMR INVESTIGATION OF LDPE/SILICA DISPERSIONS OBTAINED BY PHOTO-GRAFTING REACTION

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Materials with enhanced properties, such as higher strength, better barrier toward gases, increased solvent and heat resistance and decreased flammability, are commonly obtained by filling polymers with inorganic fillers. To this regard, an important role is played by the dispersion of the filler into the polymer matrix and, consequently, by the occurrence of interphase filler-polymer interactions, which are particularly scarce in the case of apolar polymers as polyolefins [1, 2]. In this study we investigated the possibility of enhancing the compatibility between polyolefins and inorganic fillers by directly grafting the latter to the polymer matrix. To this aim, a TSPM-modified silica has been prepared and employed as filler in LDPE films, in which a photo-grafting reaction between polymerizable groups present on the functionalized filler and the polymer has been performed. Besides FT-IR, TGA and SEM characterizations, both the TSPM-modified silica and the polymer-filler blends have been extensively investigated by means of solid state NMR, through a combined analysis of several ^{29}Si, ^{1}H and ^{13}C mono- and bi-dimensional high-resolution techniques, as well as ^{1}H low-resolution FID analysis and spin-lattice relaxation times measurements. This allowed us to obtain detailed and quantitative information on the silica functionalization reaction, to characterize the silica-TSPM interface, and to get insights into the change of the dynamic properties of LDPE due to the presence of the filler. The NMR results and several macroscopic properties of the blend LDPE/silica-TSPM, such as Young's modulus and oxygen permeability, were compared with those of the same blend prepared with unfunctionalized silica and of the pristine polymer.

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NMR AND MULTIVARIATE STATISTICAL ANALYSIS IN FOOD FARMING

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The PDO (Protected Designation of Origin) are most closely linked to the concept of territory, in the sense of place discernible in the flavour of the food. PDO products must be produced, processed and prepared in a specific region using traditional production procedures. The quality and the specific characteristics of the product derive essentially from the place of origin, and in particular are affected by the climate, the nature of the soil and by the local know-how. The aim of this study is to evaluate an objective analytical method to acknowledge the PDO of products. As a matter of fact today this denomination is released by the European Community only on the basis of sensorial analysis. ^{1}H Nuclear Magnetic Resonance (NMR) is here proposed to analyze the metabolic content of some foods; NMR data were analyzed with multivariate statistical analysis protocols (PCA, PLS-DA, OPLS, Hierarchical models etc.) suitable to handle big amount of information as that obtained from NMR spectra.
This information could be used to distinguish products in relation to their geographical origin since different pedoclimatic conditions influence the metabolic content. Our previous results showed the possibility to use this approach as alternative method for assessing the quality of the PDO products analyzed [1-2].

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IS HADAMARD SPECTROSCOPY A USEFUL TOOL FOR DRUG DISCOVERY?

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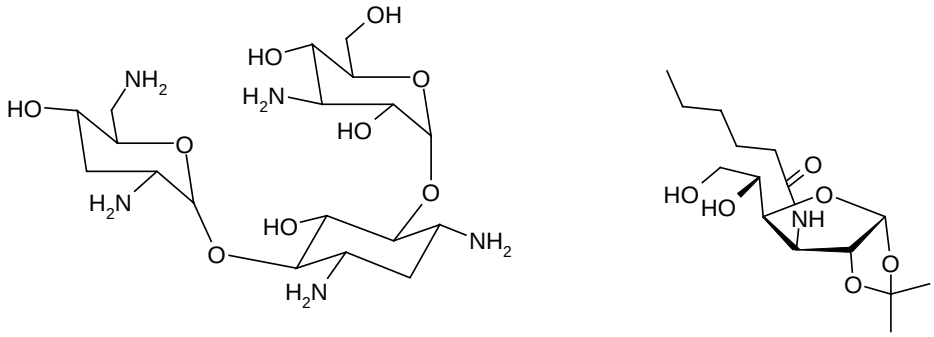
Among fast multi-dimensional NMR techniques, introduced recently, some applications of Hadamard transform spectroscopy have been published concerning 2D [1-2] and 3D spectra [3-4]. In this poster, routinely applied 2D NMR experiments such as COSY, TOCSY, NOESY and HSQC are performed on tobramycin and/or modified α-D-glucofuranose drugs (see Fig. 1) and acquired by traditional gradient techniques and by Hadamard spectroscopy.



Tobramycin **Modified α-D-glucofuranose**

Fig. 1. Tobramycin and α-D-glucofuranose drugs have been chosen as the models for Hadamard experiments.

For Drug Discovery, which needs fast screenings made on a great number of molecules in the less time possible, Hadamard would be the perfect NMR technique allowing to run 1D and 2D NMR spectra in a few minutes (and not in hours, as it is still today).
A comparative discussion of the results, obtained using Hadamard and traditional gradient techniques, will be reported to demonstrate the utility of this new technique for large scale screening of lead compounds.

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INVESTIGATING MELANOCORTIN PEPTIDE CONFORMATIONS IN DIFFERENT ENVIRONMENTS

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The melanocortin receptors are involved in many physiological functions, including pigmentation, sexual function, feeding behavior, and energy homeostasis, making them potential targets to treat obesity, sexual dysfunction, etc [1]. Understanding the conformational basis of the receptor-ligand interactions is crucial for the design of potent and selective ligands for these receptors. The conformational preferences of the cyclic melanocortin agonists and antagonists MTII, SHU9119, [Pro6]MTII, and PG911 (see below) were comprehensively investigated by solution NMR spectroscopy under different environmental situations. In particular, water and water/DMSO (8:2) solutions were used as isotropic solutions, and a 200 mM aqueous solution of DPC (dodecylphosphocholine) was used as a membrane mimetic environment.

MTII	Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-NH_2
SHU9119	Ac-Nle-c[Asp-His-DNal-Arg-Trp-Lys]-NH_2
[Pro6]MTII	Ac-Nle-c[Asp-Pro-DPhe-Arg-Trp-Lys]-NH_2
PG911	Ac-Nle-c[Asp-Hyp-DNal-Arg-Trp-Lys]-NH_2

(Nle, Norleucine; Nal, 2'-Naphtylalanine; Hyp, Hydroxyproline).

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A PRELIMINARY STUDY OF METABOLIC URINE PROFILE OF CHILDREN WITH DIABETES TYPE 1

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NMR spectroscopy of biofluids provides (1) a wealth information on the metabolic processes in human body. Spectra are very complex and in order to focuse on significant difference between a set of spectra from control and from humans with disease chemometric methods are used. NMR combined with pattern recognition has been shown to be useful in diagnosis of significant health problem (2) like coronary heart disease, Alzheimer and Parkinson disease. Diabetes type 1(Insulin dependent) is endemic in Sardinian people, with an incidence five greater of rest of Europe (3). The aim of our study is to characterize metabolic profile of urine from children affected and from controls. A PCA was performed on a ^{1}H spectra dataset obtained with 600 MHz after bucketing integration using Amix (Bruker) shown some differences between dataset (healty childrens and diabetic childrens).

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LONG-LIVED NUCLEAR SPIN STATES IN MULTIPLE-SPIN SYSTEMS

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The longitudinal relaxation time, T1, has often been assumed to determine the lifetime of coherent spin states. In recent works [1-3] we showed that in some circumstance, the lifetime of some nuclear spin states in a system of two weakly coupled protons (AX), is much longer than the spin-lattice relaxation time T1. This because, under special conditions, the intra-molecular dipole-dipole relaxation between the two sites in the spin pair, responsible for longitudinal relaxation, is inefficient to induce relaxation of the singlet state. Experimental results, on the AX spin system, demonstrate the existence of the long lifetime of nuclear spin singlet states in both low and high magnetic field.
It is easy to see that such a long-lived state can have a number of possible applications. Nevertheless, most realistic applications involve molecules that contain more than two spins.
In this contribution we will show that a singlet-like state can be created, in high magnetic fields, also for more complicated spin systems (AA'BB' and AA'XX'). We investigated their lifetimes in three biologically active substances, namely citric acid (AA'BB'), 4-aminobenzoic acid (AA'XX') and 4-hydroxybenzoic acid (AA'XX') dissolved in common isotropic solvents. Results prove the existence of spin states that live about 4 times longer than T1 in systems with more than two protons. Although their lifetimes are not as spectacularly long as in the two spins case these evidences open new perspectives for practical applications.

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RAPID MEASUREMENT OF ^{1}H 90° PULSE IN THE SOLID STATE NMR VIA CROSS POLARIZATION

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Cross polarization magic angle spinning (CPMAS) ^{13}C NMR spectroscopy is based on the excitation of carbons via proton nuclei. Namely, a 90° pulse applied on the protons is followed by a spin lock sequence on both ^{1}H and ^{13}C in order to excite the carbons. A proton decoupling sequence is then applied while the ^{13}C signals are acquired. The CPMAS ^{13}C sequence is the basis of more complicated and powerful experiments such as those based on the Lee-Goldburg decoupling. Key factors of the CPMAS ^{13}C NMR spectroscopy are: 1. the matching of the Hartmann-Hahn condition, and, 2. the precision of the proton 90° pulse. If one of the two factors are not matched, NMR artefacts may arise. The present work deals with the precise calibration of the proton 90° pulse via cross polarization on standard molecules and natural organic matter. The normal method used to calibrate the proton 90° pulse via cross polarization is the use of an optimization method on a standard system such as the simple glycine. Namely, the proton pulse length is varied until ^{13}C NMR signals are undetected. The undetectability of the carbon signals is due to the application of a proton pulse corresponding to a 180° ^{1}H magnetization inversion. The application of a 180° ^{1}H pulse prevents the ^{1}H-^{13}C cross-polarization. The main disadvantages of the standard 90° pulse measurements are related to: 1. the NMR pulse trapezoidal shapes that shorten the real pulse length to be applied in CPMAS ^{13}C-NMR experiments, 2. the use of a recycle delay that can be inadequate as the proton pulse length is increased during the 90° proton pulse measurements, 3. the use of a simple organic standard that can have a NMR behaviour different from that of the natural organic matter. In order to account for all these problems, a new method for proton 90° pulse calibration was developed. The classical CPMAS ^{13}C NMR pulse sequence was modified in order to apply a pulse train made by four 90° pulses on protons. The use of such a modified sequence minimizes the errors due to the trapezoidal pulse shapes, and allows the application of a very short recycle delay, thereby shortening the 90° pulse measuring time. In fact, the 4x90° pulse train corresponds to a rotation of 360° of the ^{1}H magnetization. Being close to the equilibrium state, an unusually short recycle delay can be applied. Moreover, the absence of ^{13}C NMR signals is a confirmation that the correct 90° ^{1}H pulse was measured. The modified 4x90° pulse sequence can be also used to rapidly measure the correct 90° ^{1}H pulse on natural organic matter (NOM), thereby preventing the use of standard organic systems that differ from NOM and can provide imprecise NOM 90° ^{1}H pulses.

STANDARD AND NON INVASIVE NMR INVESTIGATION ON DEGRADATION PROCESSES IN PAPER AND PAPYRUS

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An NMR study was carried out on paper and papyrus untreated and artificially aged samples with physical, chemical and biological treatments. To this aim ^{13}C CP-MAS NMR spectroscopy, ^{1}H pulsed low resolution and mobile unilateral NMR relaxometry were applied. ^{13}C CP-MAS NMR spectra can be considered as the fingerprint of the solid matrix of samples. A careful analysis of spectra allowed to evidence that different degradation processes are promoted by different ageing treatments. For the evaluation of the crystalline/amorphous ratio and of the amount of oligomers and carboxylic groups a full deconvolution of the spectra was performed. The extent of variation induced into the lignine structure by the degradation processes were also evidenced.
^{1}H NMR relaxometry was used to measure T_1 and T_2 relaxations times. In particular T_2 allowed to obtain information on the state of degradation of samples. In fact, in degraded samples, a shortening of T_2 relaxation time was always observed. Data obtained using standard NMR relaxometry, were compared with the corresponding ones obtained using the unilateral relaxometer. The results obtained with both techniques agree nicely.

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EFFECTS OF FREEZING ON WATER DIFFUSION PROPERTIES IN MEAT: A μDTI PRELIMINARY STUDY

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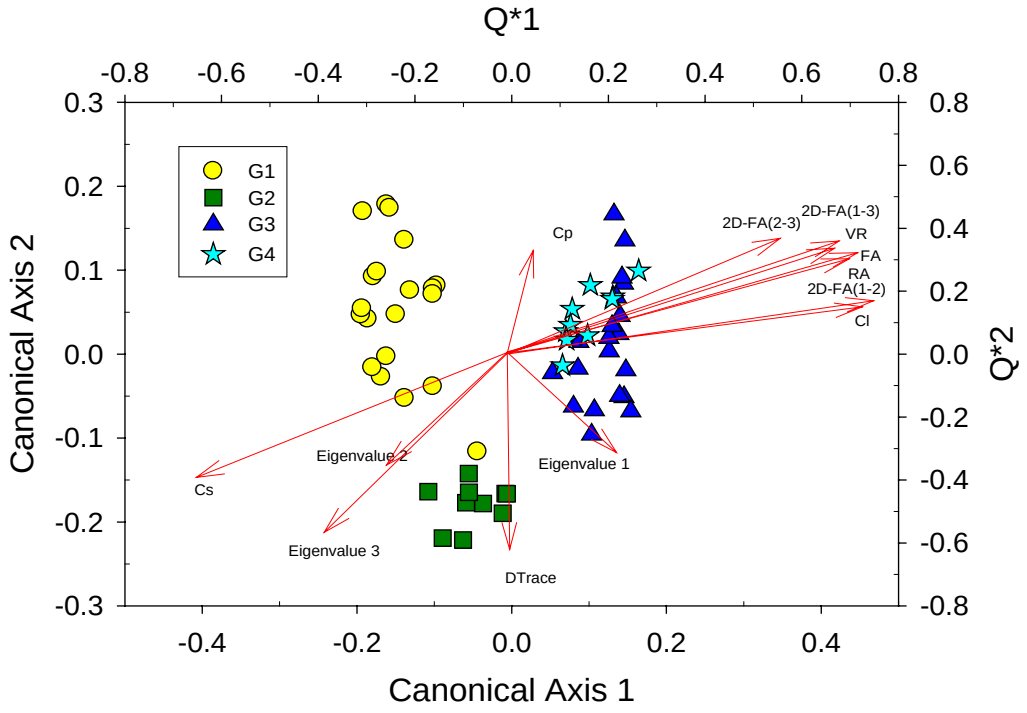
In this preliminary work fresh and frozen-thawed chicken breast samples were investigated by means of Diffusion Tensor Micro Imaging (μDTI) with the aim of looking for changes in the anisotropic diffusion properties of water upon freezing and storage at low temperature. Four groups of samples were evaluated (G1-G4), namely fresh samples (G1), samples frozen in liquid N_2 and stored at -30 °C for 1 day (G2), samples frozen in liquid N_2 and stored at -30 °C for 11 days (G3) and samples frozen at -30°C and stored for 11 days (G4). Non parametric LDA of the most important rotationally invariant parameters obtained from the μDTI data reveals a significant difference ($P < 0.001$) between fresh and frozen samples. Analysis of the LDA biplot of our data (below) suggests that, contrary to what could be expected, water anisotropy increases upon freezing, probably because of a lateral shrinkage of the meat fibers produced by ice crystals growth in the extracellular spaces.



FINITE ELEMENT MODELLING AND MRI VALIDATION OF 3D TRANSIENTS WATER PROFILES IN PEAR DURING POSTHARVEST STORAGE

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Water transport in fruit has important consequences for fruit quality as it causes shrivelling. Three mechanisms of water transport are often considered most dominant in foods in general: convection (Darcy flow), molecular diffusion and capillary diffusion. [1] They differ according to the driving force which causes the movement. This continuum approach to mass transfer is the simplest means to describe the water diffusion in fruit tissue because it avoids the necessity of modelling the microscopic pore space. It constitutes a phenomenological approach as the model parameters have to be determined experimentally. [2-4] There has been a limited development of water transport models for fruits that consider the transient water profiles in the heterogeneous fruit tissue. Veraverbeke *et al*. [5-6] developed a diffusion model which took into account the different components of the cuticle such as cutin and wax, and epidermal structures such as cracks and lenticels. This model was used to model water loss during long-term storage of different apple cultivars. Validation of the model predictions of water transport in the fruit has not been achieved up to date, especially with reference to the transient internal water profiles. A diffusion model based on Fick's second law was used to simulate water transport in pear fruit at various conditions (20 ◦C and 75% RH; 1 ◦C and 60% RH). The finite element method was used to discretise the governing differential equations over the actual 3D pear geometry. For the first time, water transport in Conference pear fruits was described at the mesoscale level by incorporating different tissues (cuticle, inner and outer cortex) with different diffusion properties. The validated model explained water transport well as validated through nuclear magnetic resonance imaging techniques and was able to predict mass loss of intact pear during postharvest conditions. It was noticed that, at high temperature conditions, the model can be improved further by taking into account respiration and shrinkage effects.

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QUANTUM MECHANICAL CALCULATION OF NMR PARAMETERS FOR STRUCTURAL CHARACTERIZATION OF THE LIGAND-RECEPTOR INTERACTIONS

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QM methods are gaining increasing popularity in the structural study of medium-large sized molecules. For istance, structure validation protocols of new natural products by QM GIAO calculation of ^{13}C NMR chemical shifts may supply a new way to sort out difficult cases in the elucidation process [1]. Also, recently theoretical prediction of ^{13}C NMR chemical shifts have also been proposed as a tool to facilitate interpretation of polymers spectra [2].
The high accuracy in the prediction of ^{1}H and ^{13}C chemical shifts, and the satisfactory results achieved at low demanding level of theory [3], has also led researchers to focus on problems inherent to the determination of the three-dimensional structure of medium and large sized molecules. In particular, calculated ^{13}C spectra have been used in the study of multiple conformer equilibria [4-6]. In addition, a combined analysis of GIAO/DFT 1H, ^{13}C and ^{15}N shieldings has been proposed for the conformational analysis of peptides, providing three dimensional ^{13}C chemical shift surfaces of the characteristic φ, ψ, and χ dihedral angles [7-9] and as a function of glycosidic bond φ,ψ dihedral angles in oligosaccharide and glycopeptide model compounds [10]. More recently, a similar analysis has shed light on the conformationally relevant structures of calixarenes [11]. We have extende for the first time the application of the QM calculations to more large and complex system like ligand-receptor. We have investigated the covalent complex between DNA undecamer and (+)-Yatakemycin by *ab initio* methods for characterizing the binding mode of the small molecule on the nucleic acid.

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SOLUTION STRUCTURE OF CHENODEOXYCHOLATE-CHICKEN LIVER BILE ACID BINDING PROTEIN TERNARYCOMPLEX

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Fatty Acid Binding Proteins constitute a large family of proteins that bind and solubilize with variable stoichiometry a diverse set of hydrophobic ligands into a large cavity located in the interior of the protein (1). In the liver two subgroups of FABs have been identified exhibiting different sequence homology and distinct binding properties: liver fatty acid binding proteins and liver basic fatty acid binding proteins. The latters, which comprise liver chicken Bile Acid Binding Protein (cl-BABP) and other basic FABs from non-mammalian species, present high affinity for bile acids rather than for fatty acids(2). Internal protein dynamics is intimately connected with ligand interaction and recognition for this class of proteins. We recently reported a dynamics analysis of apo and holo forms of cl-BABP and we discussed its implications in a probable allosteric activation mechanism upon ligand binding (3). Here we present the solution structure achieved with a conventional 3D methodology, for cl-BABP complexed to two molecules of chenodeoxycholic acid, a natural ligand for the protein. Five distinct types of one-bond and two-bond Residual Dipolar Couplings (H-N, H^{α}-C^{α}, C^{α}-C',HN-C',N-C') were measured using Philamentosus Phage as alignment medium and different experiments based on intensity J-modulation or chemical shift splitting of correlation crosspeaks. The backbone conformation results well defined by the use of such an extensive set of RDCs, in addition to hydrogen bonds and NOE information.
Constrains between the $^{13}C/^{15}N$-labeled protein and the isotopically unlabeld ligands were individuated conducting ^{13}C-filtered NOESY spectra. Assignment of the chenodeoxycholate moieties was not a trivial problem, due to the presence of two identical ligand molecules and resonance overlapping in the aliphatic region. It was obtained only for a limited set of important up-field and down-field shifted protons, driven by structural models and performing a random scrambling procedure of ambiguous resonances. Structure calculations produced a set of well-defined conformations for the ternary complex, which was compared to the apo form and analysed in terms of structure-dynamics-function relationship.

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SINGLE SCAN TROSY AS A SIMPLE AND EFFICIENT METHOD FOR MEASUREMENT OF ^{1}H-^{15}N RESIDUAL DIPOLAR COUPLINGS

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The use of Residual Dipolar Couplings (RDCs) in the analysis of biomolecular structure and dynamics has rapidly expanded (1). Accurate measures of one bond RDCs can be obtained from so-called quantitative *J*-correlation experiments using intensity analysis (2), which is unfortunately time consuming. Alternative approaches involve the measurement of the two doublet components splitting in the F1-coupled HSQC spectra, such as in the IPAP method (3). Drawbacks are increased degree of peak overlapping and presence of artefacts upon spectra editing. Here we present a clean and sensitive method that uses the spin-state selection of TROSY experiment to accurately determine RDCs without problems associated with the previously mentioned methodologies. Product operator analysis demonstrates that it is possible to select the most slowly relaxing component of the ^{1}H-^{15}N cross-peak multiplet (TROSY component) with a single scan per FID without necessity of phase cycling. In general, any of the four components of the multiplet can be selected only by changing the polarity of the encoding gradient pulse and/or changing the phase of a specific ^{1}H pulse in the sequence. In this way, the TROSY and AntiTROSY peaks are obtained in distinct spectra and result separated along both the F1 and F2 dimensions exactly by the one-bond ^{1}H-^{15}N coupling. Analogously one can select the two E-COSY components.
Effects of acquisition and processing parameters such as acquisition time, quadrature detection schemes, zero filling and phase correction on the accuracy of RDCs measurements are analysed. Artefacts can be easily controlled and suppressed optimizing a specific delay in the sequence. A difference is noticed when measuring the splitting along the F1 indirect dimension or the F2 direct dimension, where pasive couplings and cross-correlation effects are present. Advantages and drawbacks with respect to IPAP and quantitative *J*-modulation methods are discussed.

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RELAXOMETRIC CHARACTERIZATION OF ANTI-HIV DRUG INTERACTION WITH HUMAN SERUM ALBUMIN

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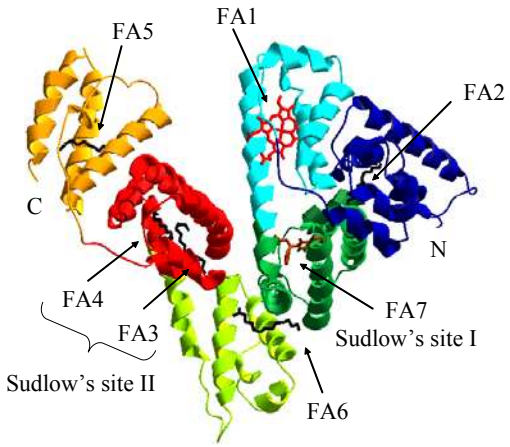
Human serum albumin (HSA), the most prominent protein in plasma, is best known for its exceptional capacity to bind ligands (e.g. heme and drugs). It acts as a volume expander, allowing endogenous and exogenous compounds to be available in quantities well beyond their solubility in plasma and determining the pharmacokinetic behaviour of many drugs. HSA can hold some ligands in a strained orientation, allowing their metabolic modification and renders potential toxins harmless, transporting them to disposal site [(1)]. The heme-HSA complex is obtained by binding of Mn(III)heme or Fe(III)heme to HSA fatty acid-free. The metal-heme binding to HSA endows the protein with peculiar spectroscopic properties and it is used as spectroscopic probe to follow a number of events involving the conformational of the protein. Variation of relaxivity values of metal-heme-HSA in the presence of anti-HIV drugs abacavir, nevirapine and nelfinavir is investigated by NMR spectroscopy. NMRD profiles are recorded at different fatty acid to protein molar ratio. Results obtained here seem to confirm fluorescence analysis[(2)] that shows abacavir binding to Sudlow's site I (FA7) such as the most of therapeutic agents do. On the contrary, nevirapine shows a different effect on the affinity of the Mn(III)heme probe to FA1 with respect to most FA7 ligands, thus suggesting binding to fatty acid site FA6. Remarkably, FA6 and FA7 sites could not be distinguished by fluorescence analysis[(2)], because the occupancy of these sites produces the same quenching of Trp214 that is located between two drug binding sites.
As a general remark, the paramagnetic contribution to the NMR rate of solvent water protons is useful for better characterization of the molecular environment of the metal binding site and of its dynamics.



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DEVELOPMENT OF MULTIDIMENSIONAL FAST FIELD-CYCLING RELAXOMETRY

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2-dimensional cross-correlation T_1-T_2 relaxometry is finding increasing application in both the study of porous materials and food. In this poster we show how the T_1-T_2 pulse sequence can be implemented on the latest generation of fast field cycling relaxometer being developed by Stelar s.r.l. This permits measurement of a three-dimensional stacked-plot of T_1-T_2 spectra where the spectrometer frequency forms the third dimension. This permits the simultaneous measurement of the T_1 frequency-dispersion of each of the T_1-T_2 relaxation peaks between spectrometer frequencies of 10kHz and 100MHz, which not only assists in peak assignment but also helps resolve peaks that overlap at high frequency. The protocol will be illustrated with measurements on aqueous sucrose solutions.

PROBING THE ROLE OF DYNAMICS IN PROTEIN-LIGAND INTERACTIONS: THE CASE OF CRBP

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Over the past several years, new NMR methods have been developed to provide site-specific information about protein motions spanning a wide range of time scales [1]. The insights obtained from dynamics studies have important implications for the understanding of biological function.

We have investigated the dynamics of cellular retinol-binding protein type I (CRBP) using different NMR experiments in order to resolve an apparent paradox. Differently to other members of the intracellular lipid-binding proteins (iLBP) family, the solution structures and *ps-ns* mobility of apo and holo-CRBP are highly similar [2]. The closed conformations observed in both states seemingly offer no access to ligand, yet the protein binds retinol rapidly and with high affinity. More significant dynamic effects were found on a *μs-ms* time scale for apo-CRBP [3], with particularly strong exchange phenomena in a region previously described as a putative portal within the iLBPs. The high degree of conformational flexibility observed in the apo-protein was almost completely quenched upon binding of retinol. These results gave rise to the question of whether the observed *ms*-dynamics in the apo-form are essential for ligand uptake and how they can lead to conformations that allow access to the cavity.

We have used NMR line-shape analysis to shed new light on the role of protein dynamics for retinol binding by CRBP. The results suggested an unexpected view of ligand uptake by its carrier, with a complex mechanism [4]. In an initial step, the retinol molecules interact non-specifically with the protein surface. This initial interaction which is characterized by a high off-rate, induces the formation of long-lived conformers in the portal region that allow the ligand to access the cavity.

Further investigations on hydrogen/deuterium exchange showed that the overall structure of CRBP is less flexible with respect to CRBP type II. The main cause for this difference appears to reside in certain amino acid substitutions, which stabilize the protein fold either via a hydrogen-bonded network of structural water molecules inside the binding cavity or through additional salt bridges on the protein surface. This divergence in structural stability correlates with different binding affinities between the two CRBP types.

By combining several NMR methods on members of the iLBP family we have confirmed that the characterization of protein dynamics in different time scales is a key for understanding protein function.

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DETERMINATION OF THE SYNTHETIC ORIGIN OF METHAMPHETAMINE SAMPLES BY NATURAL ABUNDANCE ^{2}H NMR SPECTROSCOPY

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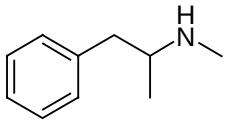
Each seizure of illicit drugs generally undergoes a careful physical and chemical examination to obtain evidence of its probable origin for use in the criminal investigation. All the information is combined to identify the source of the product, in order to establish whether or not products from different seizures are from a common origin. Isotopic analysis has been recently introduced as an additional step in the systematic methodology of this origin assignment.

Stable isotopic ratios, such as ^{2}H/^{1}H, ^{13}C/^{12}C, ^{18}O/^{16}O *etc.,* are influenced by environmental factors and synthetic routes and they can be used to trace back the origin of a certain compound. The simultaneous determination of ^{2}H/^{1}H ratios at the various sites of a molecule by natural abundance ^{2}H NMR allows multisite isotopic analysis to be performed, and greatly enhances the discriminating power of isotopic ratios.

Methamphetamine **1** is a synthetic drug with a high potential for abuse and dependence which is sold in tablets with the street names of *ice, speed*, *crank or quartz* and others.



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We performed a ^{2}H NMR study on several samples of this drug [1] prepared according to the main known synthetic routes, and starting from precursors of different known origin, mainly ephedrine. We could establish that the deuterium content at the various sites of the molecule bears memory of the corresponding synthetic procedure. The technique provides a chemical fingerprint of these substances which can help to trace back the starting materials and the synthetic pathway employed in the preparation of the samples.

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METABOLIC PROFILING OF WILD TYPE AND *OSMYB4* TRANSGENIC MAIZE AND OSTEOSPERMUM PLANTS USING ^{1}H NMR COUPLED WITH MULTIVARIATE STATISTICAL ANALYSIS

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In the "omics" era, several rapid, reproducible and stable analytical techniques have been used in metabolite investigation for genotype discrimination. Among these techniques, high resolution NMR has been largely used because it allows the direct comparison of the quantities of the compounds present. A further improvement in the metabolic determination is the use of multivariate statistical analysis in combination with NMR techniques, as a powerful method for large data treatment and interpretation. In this respect, we present a ^{1}H NMR analysis and statistical methods (PCA, PLS etc) study applied to maize and osteospermum wild type and *Osmyb4* transgenic plants.
The rice *Osmyb4* gene encodes a Myb transcription factor involved in stress tolerance. As previously reported *Osmyb4* constitutive expression in *Arabidopsis thaliana* plants results in the activation of many genes involved in stress tolerance and in the accumulation of metabolites essential for stress response [1]. Here, we present the metabolic profile of maize and osteospermum either for wild-type and for transgenic plants, under normal growth condition, in order to study the effect of *Osmyb4* in metabolites accumulation. The comparison among the maize and osteospermum ^{1}H NMR spectra indicates that improved the production of metabolites in the two species examined. However, *Osmyb4* in both maize and osteospermum, increase the accumulation of some compounds such as chlorogenic acid.
On the basis of the differences in metabolites accumulation, the multivariate statistical analysis of the obtained data allowed to cluster the samples according to the species (maize and osteospermum) and the genotypes (WT and transgenic).

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STRUCTURAL AND DYNAMIC STUDIES OF XACb0070 FROM *XANTHOMONAS* PV. *CITRI* REVEAL IT IS A DNA BINDING PROTEIN BELONGING TO THE ARC/METJ SUPER-FAMILY

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Xanthomonas axonopodis pv *citri* (*Xac*) is the agent of the citrus canker, which produces important economical losses in the world's citrus production. The elucidation of the molecular processes associated to this pathology depends upon the understanding of the biology of that organism. The complete *Xac* genome sequencing [1] has revealed a number of proteins of unknown function. A *Xac* structural genomics effort has been initiated to describe their structural features with the final goal to disclose their function. Among these proteins, the protein expressed by the ORF *XACb0070* has been selected using a high throughput strategy devised for NMR proteomics studies [2]. Here we present the solution structure of the protein XACb0070 and we show it is a member of the Arc/MetJ bacterial transcriptional repressors: a family of β-sheet DNA binding proteins of conserved 3D fold but low sequence homology [3]. As all known members of the Arc/MetJ super-family, XACb0070 forms a homodimeric structure that gives rise to a rigid hydrophobic core. Each monomer presents a ribbon-helix-helix motif. In our experimental conditions, XACb0070 is characterized also by two long unstructured tails corresponding to the C-terminal region of each monomer. Interestingly, unstructured regions are frequent in this family of proteins and, when present, these supplementary N- or C-terminal stretches are responsible for additional functions. Analogously, in XACb0070, we suggest that, while the ribbon-helix-helix motif is involved in DNA binding, the unstructured C-terminal regions may serve for other purposes. Backbone dynamics and hydrodynamic properties of XACb0070 will be analysed and discussed. The effect of the unstructured tail on the dynamics of the protein will be investigated. In conclusion, this study has provided hints not only to disclose the function of the protein but also to progress in the understanding of the role of the unstructured regions that characterize this repressors' family.

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CONFORMATIONAL BEHAVIOUR OF SMALL CYCLIC AND LINEAR PEPTIDOMIMETICS CONTAINING A 5,6- FUSED BICYCLIC LACTAM

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In the field of peptidomimetics, major efforts have been focused on the design and synthesis of conformationally constrained compounds that mimic or induce certain secondary structural features of peptides and proteins. One common motif in protein structure is the reverse-turn, which is defined as a site where the peptide backbone reverses the direction of propagation by adopting a U-shaped conformation.

In the course of our studies we have synthesized several 6,5- and 7,5-fused 1-aza-2-oxobicyclo[X.3.0]alkane amino acids.[1] These lactams can be viewed as conformationally restricted X-Pro dipeptide units, and they might be used as synthetic replacements for the *i+1* and *i+2* elements of the four consecutive residues of β-turn motifs. In particular, the compound **1** (Fig.1) showed marked properties as reverse turn inductor.

Now, we report on the conformational analysis of a series of small cyclic and linear peptide sequences incorporating the 5,6-fused bicyclic lactam **1**.(Fig.2)

The conformational properties of these peptidomimetics have been investigated by a combination of computer modelling and ^{1}H NMR spectroscopy. The linear peptide showed the desired properties in term of β-hairpin inducing propensity: a tendency to form an inverse γ-turn or a type II' β-turn through intramolecular hydrogen bonding with varying degree of β-hairpin formation was observed.

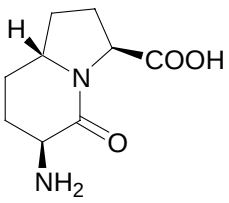

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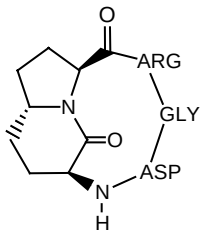
Figure 1



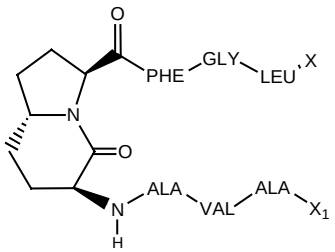


Figure 2

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ORDER PARAMETERS IN FLUORINATED LIQUID CRYSTALS BY ^{13}C NMR

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Fluorinated liquid crystals represent an important class of materials for their possible application in active matrix liquid crystal displays, especially for their wide nematic ranges, low rotational viscosity and high dielectric anisotropy.
Structural and orientational order properties of the two nematogens (trans, trans) 1-fluoro-4-(4-(4-propylcyclohexyl)cyclohex-1-enil)benzene (**3CyHeBF**) and (trans, trans) 1,2-difluoro-4-(4-(4-propylcyclohexyl) cyclohexyl)benzene (**3CyCyBF2**), exhibiting a nematic range of 91 and 78 °C, respectively, have been investigated by means of ^{13}C NMR and dielectric spectroscopy methods.
^{13}C NMR spectra have been recorded in the whole nematic ranges by means of the Linear-Ramped Cross-Polarization technique, applied under static or Magic Angle Spinning (MAS) conditions, by decoupling from ^{1}H nuclei by means of the SPINAL-64 technique [1]. The assignment of the ^{13}C resonances have been carried out thanks to the comparison with solution state and ^{13}C CPPI spectra, as well as to the analysis of the trends of ^{19}F-^{13}C dipolar couplings and of the anisotropic component of chemical shift. From the experimental splittings, obtained from the spectra, the ^{19}F-^{13}C dipolar couplings ($D_{C\text{-}F}$) have been extracted taking into account the contribution of the scalar coupling, determined from solution state spectra. The order parameters have been calculated analyzing $D_{C\text{-}F}$ for the various ^{13}C-^{19}F couples by means of a least-square fitting procedure, including corrections due to vibrations and anisotropic contribution to scalar coupling for each carbon nucleus [2], and using geometrical parameters determined by DFT methods [3].
The dielectric permittivity tensor components, $\varepsilon_{\parallel}$ and $\varepsilon_{\perp}$, as well as the optical anisotropy Δn in the nematic phase of both compounds were measured. Using commonly accepted approximations the nematic order parameter was determined. The order parameters obtained by three different experimental techniques are compared and discussed.

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^{13}C NMR AND DFT STUDIES OF CHEMICAL SHIELDING TENSORS IN FLUORINATED LIQUID CRYSTALS

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The chemical shift in nuclear magnetic resonance is closely linked to the environment of the nuclei. In anisotropic media the chemical shift interaction is described by the chemical shielding tensor (σ) over the whole space of an arbitrary coordinate system, for each interested nucleus. Recent achievements in a density functional theory (DFT) calculations have made it possible for the σ tensor components of many molecular systems to be accurately evaluated. On the other hand, experimental improvements allow the determination of the principal values of ^{13}C chemical shielding tensors in complex molecules. Thus, the measurement and theoretical calculation of the chemical shielding tensors can provide useful information on the electronic and molecular structure of molecules.
In this work a ^{13}C NMR and a DFT comparative study has been carried out on the two fluorinated nematogens (trans, trans) 1-fluoro-4-(4-(4-propylcyclohexyl)cyclohex-1-enil)benzene (**3CyHeBF**) and (trans, trans) 1,2-difluoro-4-(4-(4-propylcyclohexyl) cyclohexyl)benzene (**3CyCyBF2**), exhibiting a nematic range of 91 and 78 °C, respectively.
Tensorial properties of ^{13}C chemical shielding have been experimentally obtained either from the analysis of anisotropic chemical shifts or from the application of dedicated bidimensional solid state techiniques. To this aim we have exploited the accurate determination of orientational order and geometrical properties achieved by the analysis of ^{13}C-^{19}F dipolar couplings.
The experimental chemical shielding values have been compared with those obtained by means of quantum-mechanical calculations, using Gaussian'03 [1]. Geometries and carbon nuclear shielding tensors of the different conformers have been determined at the DFT level of theory using the B3LYP/6-31G(d) and MPW1PW91/6-311+G(d,p) combination of hybrid functional and basis set, respectively.
NMR chemical shielding tensors have been calculated by the method of gauge-including atomic orbitals, GIAO [2]. In order to rationalize the effects of the presence of fluorine atoms on ^{13}C chemical shielding, we have also carried out some DFT calculations on probe-molecules, containing fluorine atoms in different position of the aromatic core. On these model systems we have performed an analysis in terms of paramagnetic and diamagnetic contributions as given by the Natural Chemical Shielding (NCS) approach [3], which can partition the results into individual magnetic contributions from chemical bonds and lone pairs.

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DYNAMIC BEHAVIOUR OF SILKWORM COCOON SILKS BY MEANS OF NUCLEAR RELAXATION PROPERTIES IN THE SOLID STATE

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The understanding of the molecular structural and dynamic properties of silk, and of their link with the macroscopic mechanical properties is an important task because of the extensive applications of this material in fields ranging from textile industry to biotechnologies [1]. In particular, the influence of some variables, such as the origin of the silkworm and its alimentary regimen on the properties of Bombyx Mori silk may represent an important piece of knowledge for future technological improvements or in the optimization of industrial processes.

Here we present a ^{1}H and ^{13}C low- and high-resolution solid state NMR study of several cocoons arising either from different origins (Turkish, Chinese, Japanese) or from silkworms raised with a natural (mulberry-tree leaves) or an artificial diet.

The results obtained for both "natural" or dried samples from ^{13}C and ^{1}H spectra recorded by different techniques, ^{1}H FID analysis, spin-lattice relaxation times and $T_{1\rho}$-T_2 correlation experiments [2], have been discussed in terms of structural and dynamic properties. Most of these properties result to be common to all the cocoons, while some differences can be highlighted either for cocoons arising from silkworms of different origin [3] or having undergone different diets.

Some of the aspects that are here discussed concern in particular the side-chain dynamics, the role of water, its interaction with silk and its influence on the dynamic and nuclear properties of the two protein fractions (sericin and fibroin), the influence of silkworm individual variability, as well as the determination of spin-lattice relaxation sinks and the presence of dynamic heterogeneity.

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EXPRESSION AND NMR CHARACTERIZATION OF A BILE ACID-BINDING PROTEIN IDENTIFIED IN CHICKEN ILEUM

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Bile acids play an important role in efficient digestion and absorption of dietary fats and undergo enterohepatic circulation, which is important for maintenance of bile acid and cholesterol homoeostasis. This transcellular trafficking is mediated by a machinery of carrier proteins for active transport of the bile acids across the membranes and the intracellular cytosolic milieu. Bile acid binding proteins (BABPs) are small proteins (14–15 kDa) belonging to the fatty acid-binding protein (FABP) family [1].
We have recently reported the structural characterization of a BABP from chicken liver (cl-BABP) [2,3]. In chicken, as in mammals, chenodeoxycholic acid is the predominant primary bile acid deriving from cholesterol catabolism, followed by cholic acid, both conjugated with taurine. In bile acid enterohepatic circulation the key steps are mediated by i) a receptor system, that binds bile salts on one surface and translocates them into the cell ii) a cellular bile salt binding protein, that moves them across the cell and iii) an exit system, which moves bile salts out of the other side of the cell. We have previously suggested that chicken liver-BABP, characterized in our laboratory, is the cytosolic protein carrying bile salts in liver and we have now identified, through the analysis of chicken genome, the corresponding ileal protein.
In the present poster we report on the cloning, expression and NMR characterization of chicken ileal BABP. Up to now, ileal BABPs have been characterized only in mammals, while liver BABPs are likely to be present only in non mammalian species. This is, to our knowledge, one of the two examples of a liver and ileal bile acid binding protein identified in the same organism.

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THE ROLE OF MYOGLOBIN IN HUMAN MUSCLE: RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND FUNCTION OF THE DIFFERENT ISOFORMS

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As is well known, myoglobin (Mb) is a cytosolic metalloprotein found in relatively high concentration in the myocardium and in the skeletal muscle of man and other mammals. Its functional structure is that of a globin and a porphyrin ring containing an atom of iron (Fe), i.e. the heme. Fe is the linkage site of Mb with O_2 and other ligands of functional interest such as NO. Its role, besides O_2 storage and molecule facilitating O_2 diffusion is that of scavenger of NO and O_2 free radicals. Differently from other animals, in man Mb has five isoforms: Mb I (75-80% of the total: pI= 8.57); Mb II (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. The observation that Andean populations [1] and high altitude Tibetan natives [2] are characterized by a greater concentration of muscle Mb (+ 20%), totally attributable to the increase of isoform Mb II (+ 100%), together with the observation that high altitude natives are characterized by higher efficiency of locomotion, support the hypothesis that there might be a cause-effect relationship between these two experimental findings. It goes without saying that in order to verify the validity of such hypothesis the preliminary step is to study the structure of the different isoforms, particularly Mb I, the more expressed, and Mb II that increased in chronic hypoxia.
To this aim, the experimental steps were:
1) clonation of the Mb gene by techniques of recombinant DNA for "in vitro" production of large amounts of the protein not available from human muscle;
2) NMR validation of recombinant Mb isoforms, by comparing ^{1}H spectra from the literature [3] on human Mb protein;
3) ^{1}H NMR structural analysis of the myoglobin isoforms in different linkage states (met, deoxy, azide and cyanoMb);
4) ^{129}XeNMR study of the structure to function relationship of hydrophobic Mb cavities.

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PHOSPHOLIPID BILAYER INTERACTION OF DYNORPHINS INVESTIGATED WITH SATURATION TRANSFER DIFFERENCE NMR SPECTROSCOPY

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Dynorphins are endogenous ligands to the opioid receptors. They are primarily ligands to the κ-opioid receptor but through enzymatic conversion also have the ability to interact with the the δ- and μ- receptors. The peptides can be found in several areas in the brain and the central nervous system and are mainly connected with pain regulation and analgesic effects. In addition to the opioid receptor mediated mechanisms, experiments with the opioid receptor blocking substance naloxone have revealed numerous of Dynorphin originated non-opioid effects, e.g. limb paralysis, hyperalgesia and chronic pain. Dynorphins is also present in neurotoxic concentrations in spinal cord injuries which aggravate the damage, and is hypothesized to be linked to Alzheimer disease. In addition to this versatility Dynorphins are rich in Arg and Lys residues, which is a characteristic feature of the so called cell-penetrating peptides (CPPs). These peptides have the capability to internalize into cells with high efficiency, low toxicity and seemingly without any receptor, while carrying large hydrophilic cargoes.
A suggested pathway for the Dynorphin receptor binding begins with a preadsorption of the peptide to the membrane, followed by the receptor interaction. This would not only simplify the interaction process between the signal substance and the receptor, but can also induce structure and orientation of the peptide necessary for receptor interaction. Thus the first step towards understanding the dynorphin-receptor interactions is to investigate the peptide-membrane interaction. In this project the endogenous peptides Dynorphin A (YGGFLRRIRPKLKWDNQ) and Dynorphin B (YGGFLRRDFKVVT) are studied in the presence of phospholipid bicelles, disc-shaped lipid aggregates which provide a flat bilayer surface of a size suitable for NMR experiments. During these studies we have examined the differences in the membrane interactions with a variety of NMR methods, as well as CD and fluorescence spectroscopy, in order to characterize the structure and more importantly the position of the peptides in the phospholipid bilayer. With Saturation Transfer Difference (STD) experiments it was possible to very elegantly determine the surroundings of single residues by irradiating specific regions of the bilayer phospholipids and detecting signal enhancements of the peptide protons. Together with methods such as analysis of H^N secondary shifts and additions of spinlabeled lipids, a very consistent picture of the peptide orientation could be made. The combined results from all methods support a conclusion that Dynorphin A is bound slightly tilted to the surface of the bilayer with the N-terminal residues inserted into the hydrophobic bilayer region and the C-terminal residues more loosely attached to the surface. Also Dynorphin B binds parallel to the bilayer surface, but there are no indications of any N-terminal trends different from the rest of the peptide. Although the seven first residues of the two peptides are identical, only Dynorphin A situates its N-terminal region deeper inside the lipid layer. This detected difference can explain previous observations that Dynorphin A has membrane perturbing effects, causes calcein leakage from large unilamellar phospholipid vesicles and is cytotoxic, while dynorphin B lacks these effects.

SOLID-STATE ^{13}C NMR STUDY ON THE THERMAL DEGRADATION OF THE *QUERCUS SUBER*, *EUCALYPTUS GLOBULUS*, AND *PINUS LARICIO* BARKS

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Thermal treatment affects the chemical structure of wood and, consequently, its chemical, physical, and mechanical properties. While more and more emphasis has been placed on the study of thermally modified whole-woods, very little is known on the thermal modification of outer barks.
In the present study, ^{13}C CPMAS and DDMAS NMR were used to investigate the changes induced by thermal treatment in the molecular structure of the barks of three species from Mediterranean vegetation: *Quercus suber* (Cork oak), *Eucalyptus globulus* (Tasmanian blue gum) and *Pinus nigra* subsp. *laricio* (Corsican pine). The results of this investigation show that untreated pine and eucalyptus barks are mainly composed by O-alkyl and di-O-alkyl structures (holocellulose), while cork presents more alkyl structures (suberin). Furthermore, the samples differ in the composition of aromatic and phenolic structures (lignin and condensed tannins). Thermal treatment under air at 250, 300, 320, and 350°C induces removal of waxes and extractives, decrease in amorphous polysaccharide content, condensation and demethoxylation of lignin.

NMR TECHNIQUE AND STATISTICAL ANALYSIS IN THE CLASSIFICATION OF PDO OLIVE OILS

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An NMR and statistical protocol is proposed to classify olive oils according to their PDOs: the sample preparation, the NMR experimental and processing parameters as well as the statistical approach are described. The Liguria PDO has been chosen as "PDO reference" and the corresponding olive oils have been compared to olive oils coming from Italian, Spanish, Greek, French PDOs. The results obtained comparing Liguria PDO with the other PDOs are good and extremely promising. This study is carried out within the European TRACE project (FP6-2003-FOOD-2-A;contract number:0060942) funded by the Commission of the European Community.

NMR STRUCTURAL STUDIES OF THE 29.6 KDA THIOSULFATE SULFUR TRANSFERASE RHODANESE FROM *AZOTOBACTER VINELANDII*

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Rhodanese is a cyanide:thiosulfate sulfur transferase widely distributed in both plants and animal species. Rhodanese displays, *in vitro,* the formation of thiocyanate from cyanide and thiosulfate or other suitable sulphur donors. The physiological role of this enzyme is still controversial and it may play distinct biological roles starting from transport mechanisms of sulphur/selenium in a biological available form to the modulation of general detoxification processes, the restoration of iron-sulphur centres in Fe-S proteins, as ferredoxin and could be also involved in selenium trafficking.

Rhodanese from *Azotobacter vinelandii* (RhdA) (1) is a protein of 29.6 kDa that transfers the sulfane sulfur atom from thiosulfate to cyanide, producing thiocyanate by a double displacement mechanism. During the transfer of the sulfane sulphur this enzyme cycles between two stable intermediates, a sulfur-loaded (ES) and a sulfur free form (E). The three dimensional structure of the ES form has been elucidated by X-ray crystallography (2). RhdA is a monomeric protein of 271 residues arranged in two well-folded domains connected trough a long linker. Both domains, each one about 120 amino acid long, reveal the same fold and display α/β topology. The only essential Cys residue, located in the C-terminal domain, is the first residue of a five amino acid loop (the active-site loop) that folds in a cradle-like structure defining the catalytic pocket. The catalytic site of RhdA is located close to a cleft between the two domains and all side chains essential for the catalysis are provided by the C-terminal domain only. The presence of interactions between some structural elements of the N-terminal domain and the catalytic one close to the active site indicates that the first domain could be involved in the stability of the active site and in the substrate selectivity of the enzyme and moreover in protein/protein interactions.

We obtained the almost complete NMR backbone assignment of ES form RhdA in solution. 3D heteronuclear NMR experiments with uniformly ^{2}H, ^{13}C, ^{15}N-labelled sulfur-loaded RhdA were recorded to assign the backbone and C_β atoms This information can be useful to elucidate the biological function of this protein and also can be of great utility in interaction studies of RhdA with other proteins or molecules involved in the sulfur or selenium metabolism. In addition we have compared the dynamic properties of the E and ES forms, using ^{15}N T1 and T2 and 1H-^{15}N NOEs measurements.

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PYRROLIDIN-2-ONE-BASED β-AMINO ACIDS AS A HELICAL BACKBONE

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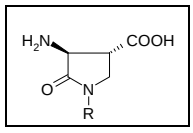
The ability of β-peptides to mimic natural features can be exploited as an effective tool in biological and medicinal chemistry[1]. Efforts in this field of research are driven by the advantages of this class of compounds. They can act as peptidomimetics, and at the same time they have higher biostability (being non degradable by enzymes such as proteases) and can be prepared in enantiomerically pure form, a particularly important aspect in modern medicinal chemistry. Some β-peptides, and specifically cyclic ones with five-membered rings, adopt well-defined conformations such as helixes with 12-membered ring C=O(i)···H–N(i+3) hydrogen bonds[2], which are structurally similar to the naturally occurring α-helix and represent a source of potential α-helix mimics. Side-chain modification of this basic scaffold can result in a variety of biologically useful peptidomimetics.



In this work, we studied the conformational features of an hexamer of the β-amino acid (3S, 4R, 1'S)-3-amino-4-carboxyl-1-[1'-(4-methoxyphenyl)ethyl]pyrrolidin-2-one[3] (see figure) in chloroform by 2D-NMR and IR. The results of the NMR studies were used as input for molecular dynamics simulations. The peptide adopts a 12-helix in our experimental condition, which is in line with previous studies on pyrrolidine-based β-peptides. This peptide could be the basis to introduce different side chains in the pyrrolidine ring and therefore it is promising for manifold applications.

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SOLUTION STRUCTURE OF SOME λ^3 IODANES

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Despite the increasing attention to the λ^3 Iodanes class of organic compounds, motivated not only by the pure chemical interest but also by the application of many compounds of this class as green oxidants, it appears that a detailed knowledge of their structure in solution is still missing. The present work attempts to address this subject by studying several acyloxyiodobenzenes and related compounds combining the use of DFT calculation and ^{17}O NMR spectroscopy. This approach allowed us to enlighten that the I-O bond can have a fluxional character depending both on the structure of the compound, and on the nature of the oxygenated group; furthermore a good evaluation of the energy involved in the dynamical phenomenon has been obtained.

DYNAMICS OF AMORPHOUS POLYMERS THROUGH A MULTI-NUCLEAR AND MULTI-FREQUENCY UNIFIED ANALYSIS OF SPIN-LATTICE RELAXATION TIMES

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NMR has been extensively used in order to characterize the dynamics in polymeric systems, since it allows motional processes in different ranges of frequencies to be revealed and a variety of information on the mechanism of the motions, their rate, and distributions of activation energies, to be obtained. Nevertheless, when the experimental data set is not enough wide and/or various, the fitting could turn out to be scarcely sensitive to the chosen theoretical model, avoiding a unique and reliable characterization of the dynamics to be obtained. A new approach to extract reliable quantitative dynamic information from NMR relaxation data of amorphous polymers has been recently presented [1], consisting of the simultaneous fitting of ^{1}H and ^{13}C T_1 *vs* temperature curves, obtained at different frequencies, by means of unified motional models. The reliability of the dynamic parameters obtained by this approach is substantially increased with respect to the single curve and/or single nucleus analysis because of the possibility of both investigating motions over a wide frequency range and combining relaxation times carrying either global (^{1}H) or local (^{13}C) dynamic information.
Here, the dynamics of three ethylene-propylene amorphous random copolymer at different ethylene/propylene ratios, was investigated by analyzing proton relaxation times measured by wideline NMR techniques at three Larmor frequencies (25, 300 and 400 MHz), and carbon T_1's measured by high-resolutions techniques (MAS and High-power proton decoupling) at two Larmor frequencies (75 and 100 MHz). The measurements were carried out as a function of the temperature in the temperature range 238-358 K, just above the glass-transition temperature.
The experimental data were analyzed in terms of segmental main-chain motion, rotation of the methyl groups about their ternary symmetry axes, and libration of C-H bonds, described by suitable models.

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STRUCTURAL AND DYNAMIC BEHAVIOUR OF FLURBIPROFEN AND ITS SOLID DISPERSIONS WITH EUDRAGIT RL100 BY MEANS OF HIGH AND LOW RESOLUTION SOLID STATE NMR

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The formulation of oral controlled-release delivery systems formed by solid dispersions of non-steroidal anti-inflammatory drugs (NSAIDs) and suitable carriers represents a very important task in order to improve the pharmacokinetics and reduce drug side-effects. Solid State NMR has recently been recognized as one of the more useful techniques in order to characterize the morphology of drug solid formulation forms and to ultimately correlate it to the pharmacological properties of the system [1]. To this aim, the conformational and molecular dynamic properties of the acidic and sodium salt forms of the NSAID Flurbiprofen in two different forms (acid and Na-salt), as well as their solid dispersions with the carrier Eudragit RL100, obtained by two different preparation methods (physical mixtures and coevaporates), have been investigated through several solid state high and low resolution NMR techniques [2]. A deep characterization of the two pure drugs was performed in order to observe the modifications induced by the presence of the carrier and understand their nature: both the assignment of the different resonances in the ^{13}C spectra for the sodium-salt form of Flurbiprofen and information on conformational properties of the two drug forms were extracted from the 2D-LG-HETCOR [3] experiment. Conformational, molecular packing and dynamic differences were observed between the two pure forms of Flurbiprofen, as well as between the pure drugs and the corresponding coevaporates, as shown by the ^{13}C spectra and confirmed by the low resolution ^{1}H FID analysis. The degree of mixing between both the acidic and Na-salt form and Eudragit RL100 in their solid dispersions at the hundreds of Å level was estimated by investigating the ^{1}H spin diffusion process through the indirect measurement of ^{1}H T_1's exploiting the high resolution of the ^{13}C spectra at 400 MHz. A comparison between both the relaxation behaviour and ^{1}H spectra of the two coevaporates and the corresponding physical mixtures suggests the presence of a more intimate mixing in the acidic form of the NSAID. The *in vitro* drug release profiles given by the solid dispersions and the results for the coevaporates are in agreement with the presence of significant interactions between drug and carrier [2].

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3D STRUCTURE OF THE PHYTOTOXIN CERATO-PLATANIN FROM THE ASCOMICETE FUNGUS *CERATOCYSTIS FIMBRIATA*.

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One of the most challenging problems facing plant pathology is to understand the molecular basis of the interaction between fungal plant pathogens and their host. Often, plant-pathogen interaction involves the production of a host-specific toxin by the fungus. Cerato-platanin (CP) is a phytotoxin produced by the ascomicete fungus *Ceratocystis fimbriata*, which is the causative agent of the canker stain, a severe disease with incidence in a great number of plants, such as *Platanus acerifolia* and *Theobroma cacao* among others. CP is 120 amino acids in length, has 4 cysteins residues forming two disulfide bridges and has 40% hydrophobic residues. CP has been indicated as the founder of the "Cerato-Platanin Family" that, at the moment, includes seven other secreted fungal proteins involved in a variety of phytopathological phenomena and/or immunological reactions [1]. CP has been cloned and expressed in eukaryotic systems [2] in its natural and ^{13}C, ^{15}N isotopically labelled form. Multinuclear and multidimensional NMR has been used to determine its 3D solution structure. The NMR experiments have been recorded, at 20°C, pH 5.8, on a Varian Inova 500AS spectrometer operating at 11.7T. The full assignment of CP has been obtained by means of standard triple-resonance NMR experiments [3]. 100% of the backbone atoms and, for the side-chain, 92% and 95% of ^{13}C and ^{1}H chemical shifts, respectively, have been assigned. CSI analysis indicates the presence of seven β-strands and two α-helices. The CP backbone dynamics has been determined by NMR measurements of ^{15}N T_1, T_2, ^{1}H-^{15}N NOE and H/D exchange of the amide protons. The experimental data reveal that CP has not a variable motional regime along the backbone, instead it presents a rather rigid structure with motions in the ms time scale. Only the C- and N- terminal regions exhibit values of ^{15}N T_1, T_2 and ^{1}H-^{15}N NOE significantly lower than the average value. Exception is the stretch Ser^{105}-Arg^{110} that present a small increase of the ^{15}N T_2 and ^{1}H-^{15}N NOE values, indicating these residues are less restricted. The H/D exchange experiments showed that the backbone NH protons of Ser^{13}-Ala^{19}, Leu^{43}-Val^{46}, Val^{52}-Ile^{55}, Trp^{70}-Ile^{74}, Ile^{80}-Asp^{86}, Asn^{93}-Asn^{95} and Asn^{115}-Val^{119} are slowly exchanging. Even one month after CP solubilization in D_2O those protons, that are mainly located in secondary structure elements, were inaccessible to the solvent. The refinement of the 3D solution structure is now in progress and it will be presented. It is expected it will provide new insights to disclose not only the biological function of this phytotoxin but also of other proteins belonging to the "Cerato-Platanin Family".

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STRUCTURAL BASIS FOR THE INTERACTION OF THE MYOSIN LIGHT CHAIN MLC1P AND THE YEAST CALMODULIN CMD1 WITH THE MYOSIN V MYO2P IQ MOTIFS

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The yeast *S. cerevisiae* Ml1cp is a member of the calmodulin superfamily of EF hand proteins that does not binds calcium. Mlc1p acts as essential light chain for the class V myosin Myo2p and, together with the calmodulin Cmd1, regulates the function of Myo2p in transporting intracellular cargo and organelles to the site of polarised delivery. The fact that Mlc1p and CaM do not behave exactly in the same way when they interact with Myo2p was already evident from the first works on Mlc1p (Stevens and Davis, 1998). It was shown that Cmd1 but not Mlc1p can interact with the IQ motifs when they are attached to a globular tail domain lacking a C-terminal region. This was recently corroborated by further experiments (Caroli Casavola et al., submitted). Moreover, although localization of Cmd1 to sites of polarized growth is dependent on the six IQ sites of Myo2p, Mlc1p is the only light chain that can stabilize the yeast myosin by interaction to the neck (Stevens and Davis, 1998). For this reason, a fixed ratio between Mlc1p and Myo2p copies is necessary to confer normal growth, but this requirement is not evident for Cmd1. Finally, Mlc1p can interact with the IQGAP protein (Stevens and Davis, 1998; Shannon and Li, 2000) but Cmd1 seems to have a low affinity for this protein that displays five IQ motifs (Stevens and Davis, 1998). Binding of Mlc1p to the IQ motifs of Myo2p regulate the association of vesicles to the myosin via the formation of a complex with Rab/Ypt proteins (Wagner et al., 2003). This function, which is essential for the correct vesicle targeting to specific location, is not shared by Cmd1 Altogether, these results show that, although very similar in sequence, the role of these two proteins diverge significantly.

To gain further insights into the mechanism of selective interaction between this class of myosin light chains and the class V myosin IQ motifs, we have performed a series of interaction experiments between chemically synthesized peptides spanning different IQ motifs present in Myo2p and 13C/15N labelled Mlc1p and Cmd1. We have found that there is a major difference in the interaction of the two proteins with IQ1, the first of the six repeated motifs of Myo2p. The result will be analyzed in terms of subtle but significant structural and dynamics differences between the two light chains. This differential behaviour opens new ways of interpreting the diverse role that these two proteins play in the myosin regulation, despite the substantial homology in aminoacidic sequence and structure.

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USE OF NMR FOR A STRUCTURAL PROTEOMICS STUDY ON THE PHYTOPATHOGEN *Xanthomonas axonopodis pv. citri* STRUCTURE-BASED INVESTIGATION OF THE FUNCTION OF UNKNOWN PROTEINS

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Xanthomonas axonopodis pv. *citri* (*Xac*) is the agent of citrus canker, a disease with great impact in Brazilian citric culture. Approximately one quarter of *Xac* genome presents several ORFs encoding proteins of unknown function and/or structure [1]. Since the structural characterization of a protein may provide information regarding its cellular function we have determined the 3D structure of three of those proteins, selected on the basis of a previous NMR assay that assessed their folded state in bacterial lysates [2]. The ORF *XAC0862*, encoding the protein ApaG, is located in a multifunctional operon and reveals a fibronectin-3 fold [3]. The ORF *XAC1516*, encodes an outer membrane lipoprotein A (OmlA). The *omlA* genes are often found adjacent to the *fur* gene, the most important transcriptional regulator of intracellular iron levels [4]. The ORF *XAC2000* encodes the protein ClpS that shows 76% similarity with *E. coli* ClpS involved in the recognition of aggregated substrates for the ClpAP proteosome. These proteins were ^{13}C and ^{15}N double labelled for NMR studies. Backbone and side chains atoms resonances have been assigned and heteronuclear relaxation experiments have been carried out. The description of the 3D structural features as well of the internal backbone dynamics of these proteins will be discussed aiming to highlight their biological function. Final goal is to discover potential targets for the design of new selective agents against this phytopathogen.

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STRUCTURES AND LIPID INTERACTIONS OF MONOMERIC AND DIMERIC VARIANTS OF MAGAININ ANTIMICROBIAL PEPTIDES BY NMR SPECTROSCOPY

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Magainins are antimicrobial peptides that strongly interact with cell membranes. Magainin activity is modulated by oligomerization within the membrane bilayers. Here, we present the structures and lipid interactions of two synthetic magainin analogues (MSI-78, MSI-594) designed by the Genaera Corporation. Using solution and solid-state NMR on these peptides solubilized in dodecylphosphocholine micelles or reconstituted in lipid membranes, we found that while MSI-594 forms a distorted α-helical conformation and does not dimerize, MSI-78 folds into an anti-parallel dimer with a "phenylalanine zipper" holding together two helical protomers. This report contrasts with previous NMR studies of magainins solubilized in detergent micelles and underscores the importance of selecting the "right" membrane-mimetic system to study the structures and the dynamics of antimicrobial membrane peptides.

STATE OF CONSERVATION OF FRESCO PAINTINGS STUDIED BY UNILATERAL NON-INVASIVE NMR

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Unilateral non-invasive NMR has been used to monitor the state of conservation of frescoes in a church in Rome, Chiesa di Nostra Signora del Sacro Cuore.
The causes of deterioration of ancient frescoes are varied, which result in the detachment and crumbling of the painted film from the supporting plaster and in the outcropping of salts. Unilateral measurements of Hahn echo performed on such frescoes have allowed the identification of the detachment of the painted film from the plaster. The presence of salts on the pictorial film affects the spin-spin relaxation time T_2. It is then possible to characterize the effect of chemical treatments, of cleansing and consolidation procedures using the distributions of T_2.
Moreover, with this technique it has been possible to monitor the effect of previous restoration treatments performed for reducing the high amount of water in the walls of the frescoes.

INTERACTIONS BETWEEN A PRION PROTEIN AND SOIL-LIKE COMPONENTS AS EVIDENCED BY CPMAS ^{13}C-NMR

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Prion proteins (PrP) are regarded as the main agents of Transmissible Spongiform Encephalopathies (TSE). Some studies showed that PrP may remain in soil in an active form, thereby making the soil a potential reservoir of TSE infectivity. The polymerisation of single phenolic molecules, catalysed by abiotic oxidative catalysts, is one of the processes naturally occurring in soils and possibly involving biomacromolecules such as proteins.
The aim of the present work was to study the interaction between a prion protein and a model soil system done by a birnessite (δ-MnO_2) coated with a polymerised catechol. The protein used here was a recombinant, non pathogenic ovine protein, recPrP.
CPMAS ^{13}C-NMR was applied to follow the complexation of the protein to the soil-like system.
The NMR spectrum of the prion protein interacting directly with birnessite revealed disappearance of the signals due to the paramagnetic nature of manganese oxide. Conversely, the signal pattern of the protein re-appeared as it was mixed to the soil-like system either during or after the catechol polymerisation process.
Results suggested that the possible interactions of the prion protein with soil systems can be mediated by natural organic matter. However, deeper studies on more complex real soil systems are needed to definitely confirm such hypothesis.

STRUCTURAL CHARACTERIZATION OF AN ENDOSTATIN-DERIVED PEPTIDE BY NMR AND MOLECULAR DYNAMICS

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Angiogenesis, the formation of new blood vessels from pre-existing ones, plays an important role in tumor progression and metastasis [1]. The inhibition of this process, or antiangiogenesis, is a promising new therapeutic anticancer strategy [2]. Endostatin (hE), a C-terminal proteolytic fragment of type XVIII collagen, have been shown to inhibit or reduce tumor growth in several experimental animal models [3]. However, its mechanism of action remains unknown.
It was recently discovered that the antiangiogenic activity is typical not only of the full length protein but also of some of its fragments obtained by chemical synthesis [4-7].
In particular, it has been found that an arginine-rich peptide of 11 amino acids (termed ES-2 and corresponding to the sequence 60-70) was a potent inhibitor of endothelial cell migration and proliferation as well as of tubular morphogenesis of microvascular endothelial cells [6]. However, no activity was observed for the 42-amino acid peptide covering amino acids 50-92, which contains the sequence of the ES-2 peptide [4].
Through molecular dynamics simulations of human endostatin and some of its synthetic fragments, we have been able to rationalize the experimental findings. In particular, we identified a pattern consisting of six amino acids, namely R-R(or G)-A-D-R-A, which appears to be an active epitope when properly exposed to the solvent. This occurs in ES-2 and 6-49 isolated fragments, but not in that corresponding to the 50-92 sequence [8].
We report here a conformational study of the ES-2 fragment (IVRRADRAAVP) by NMR spectroscopy in a wide range of solution conditions.

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THE CHOLINE METABOLITE PATTERN DETECTED BY 1 H HR MAS ACCURATELY DISCRIMINATES BETWEEN HIGH AND LOW HUMAN GLIOMA GRADE

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Introduction/Purpose. *In vivo* ^{1}H MRS has been widely used to identify the different metabolites present in brain tumors. However, due to the poor resolution of the *in vivo* spectra, it only allows the detection and the accurate quantification of very few. The HR-MAS (High Resolution Magic Angle Spinning) Magnetic Resonance Spectroscopy has proved to be a useful tool to overcome this problem. The purpose of this work is the study of the relative contributions of the main choline containing compounds (Cho, PC and GPC) to the total choline (tCho) signal and the evaluation of the potential role of these data in the discrimination between low and high grade glioma.

Methods. Human biopsies of grade II (low grade) and grades III and IV (high grade) astrocytomas were obtained from the neurosurgery department at "La Paz" Hospital. The HR-MAS spectra of the tumor biopsies were acquired on a 500 MHz Bruker AVANCE Spectrometer, at 4ºC and 4 kHz spinning rate. Data were acquired with a classical CPMG with an effective echo time of 144 ms. Metabolite assignments were confirmed by 2D-COSY. The relative contributions of Cho, GPC and PC to the tCho were calculated by fitting lorentzian peaks to the corresponding resonances. The statistical analysis was performed using SPSS (SPSS Inc., Chicago, Illinois).

Results and Discussion. The HR-MAS spectra showed a very high resolution (see Fig. 1a), allowing for the individual analysis of Cho, PC and GPC. The PC/tCho and the GPC/tCho ratios were significantly different between low grade and high grade gliomas, but did not help to discriminate between grade III and grade IV (Fig. 1b). Also, the scatter plot of all the data showed that no overlapping at all exists for these two ratios between low and high grade (Fig. 1c), which implies that no false positives would occur when using this variables as markers for diagnosis. We also calculated the tCho/Cr ratio, which is conventionally used in the *in vivo* spectra analysis since Cho, PC and GPC cannot be distinguished under these conditions. No statistically significant differences were found for the tCho/Cr ratio between low and high grade gliomas, indicating that the individual contributions of PC and GPC to the tCho, rather than the tCho itself, are the specific markers of tumor malignancy.

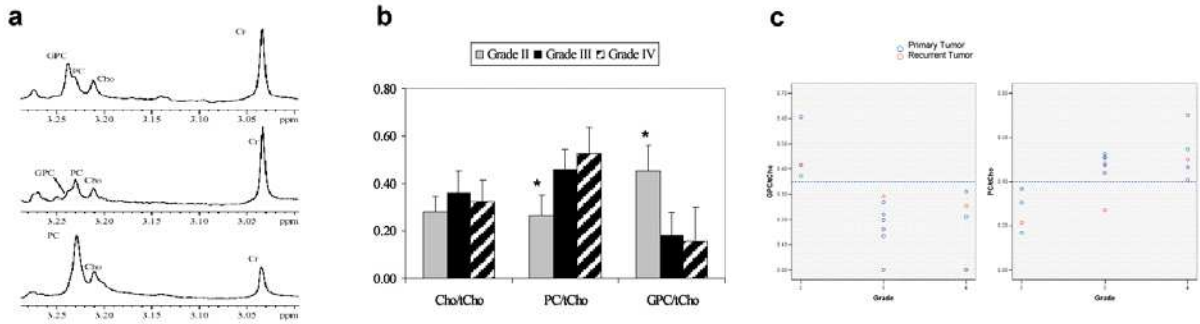

Figure 1. a) HR-MAS spectra of grades II, III and IV (from top to bottom), b) Statistical analysis of the relative contributions of Cho, PC and GPC to the total Choline, c) Scatter plot of the PC/tCho and GPC/tCho ratios for all gliomas investigated.

NMR STRUCTURE OF THE DNA BINDING ROS PROTEIN FROM AGROBACTERIUM TUMEFACIENS: FIRST STRUCTURAL CHARACTERIZATION OF A PROKARYOTIC CYS2-HIS2 ZINC FINGER DOMAIN

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The first prokaryotic classical (or Cys2-His2) zinc finger containing protein was discovered in Agrobacterium tumefaciens it is a 15.5 kDa protein called Ros, 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. We then structurally characterized through NMR spectroscopy the solution structure of Ros87 and defined its residue involved in DNA interaction. 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 prokaryotic zinc finger domain represents a unique DNA binding domain.

NMR AND HPLC COUPLING IN CHEMOMETRICS: TOWARD THE IDENTIFICATION OF MOLECULAR MARKERS

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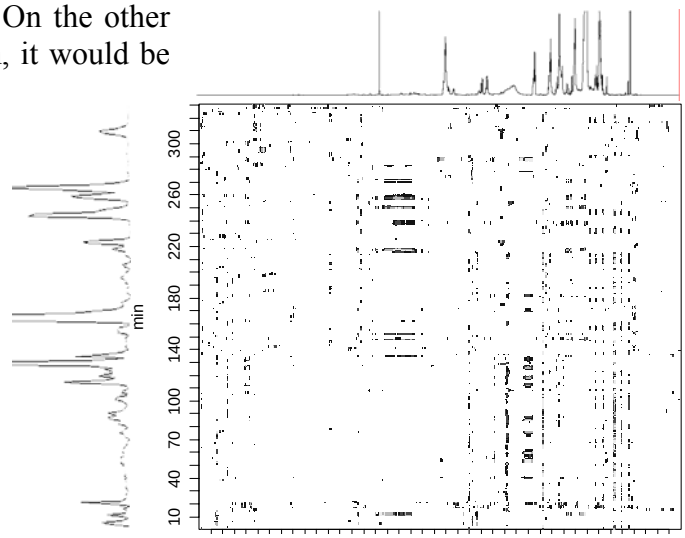
In the 1D 1H-NMR spectrum of a complex matrix, such as foods, it is difficult to assign every group of signals to specific substances because of their substantial overlap. However, this technique offers the advantage to show more than one signal for each substance, with the chance that at least one of them is, in good approximation and for a significant number of substances, not overlap-ped to others. On the other hand, also in the HPLC chromatogram, it would be much unlikely that each peak corresponds to only one single substance, being much frequent the presence of interferences. This holds especially for generic elution conditions, e.g. those ones not aimed at separating a particular class of molecules. The coupling between HPLC and NMR data, obtained from the same samples, can be useful in order to find self-consistent information, which is strengthened from the existence of correlation in the spectrum/chromatogram pair of every sample. Both chromatograms and NMR spectra can be subdivided in a high number of bins. The correlation coefficient can be determined, within the samples series, between each chromatographic and each spectral bin. The analysis of correlation matrices (Figure) allows to characterize which spectral regions is combinable with each chromatographic region. In alternative, once some spectral regions have been selected, by chemometrics, as the ones helping to discriminate between samples belonging to different classes, it is possible to find those chromatographic peaks which are highly correlated with such spectral regions. Thus, we decided to use NMR spectroscopy in order to select molecular markers able to identify the class to which a product belongs and to determine in which chromatographic peak/s such molecular markers are eluted. This work presents some results obtained within a research project aimed at assessing the geographic origin of tomatoes through the identification of molecular markers.



Linear correlation plot between the NMR spectra and the chromatograms @375 nm

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BACKBONE DYNAMICS OF A 21-mer MINI-PROTEIN THAT ADOPTS A STABLE TRP-CAGE FOLD

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In 2001, Andersen *et al.* described a remarkable 39-mer peptide from *Gila monster saliva* that adopts a small but well-defined globular shape [1]. Although other short peptides with stable tertiary structure had been previously reported, this was the first example of a peptide containing all-natural amino acids with no cross-linking *via* disulfide formation, metal ion chelation or stabilization through oligomerization to form a stable tertiary structure. Truncation and mutation of the 39-residue peptide produced 20-mer constructs that are >95% folded in water at physiological pH. These constructs optimized a new fold, defined as the "Trp-cage" motif [2].
Folding of the Trp-cage is cooperative and hydrophobically driven by encapsulation of a Trp side chain in a sheath of Pro rings. Sequence-remote hydrogens located above and below the indole ring of Trp display large chemical shift deviations due to ring current shielding effects.
In the present work, ^{15}N relaxation parameters of the backbone amides of one of the most stable mutants were determined in aqueous buffer.

G N A Y A Q W L A D G G P A S G R P P P S

The relaxation parameters were analyzed using the reduced spectral density functions approach [3].

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CONFORMATIONAL ANALYSIS OF THE NEW OBESITY RELATED PEPTIDE OBESTATIN

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Obestatin is a new endogenous ghrelin associate peptide, involved in the regulation of food intake and weight gain. Although obestatin and ghrelin originate from a common precursor of 117 residues, named propre-ghrelin, they are reported to exert opposing physiological roles by binding distinct receptors belonged to subgroup of type A GPCRs.
Obestatin is anorexigenic, decreases food intake, gastric emptying and jejunal motility. It was found to be the natural ligand of the orphan GPR39 receptor. GPR39 is meanly expressed in jejunum, duodenum, stomach, pituitary and hypothalamus and has a peculiar constitutive activity, probably induced by an aromatic cluster on the inner face of the extracellular ends of TMs VI and VII. Elucidation on the mechanism of action of obestatin could clarify its potential in metabolic diseases leading to develop new specific drugs against obesity. On this way, we synthesized mouse obestatin (FNAPFDVGIKLSGAQYQQHGRALNH_2) and its related C-terminal sub-fragment (LSGAQYQQHGRALNH_2), and investigated their conformational behaviour in aqueous solution and in membrane mimicking environments, by means of Nuclear Magnetic Resonance (NMR) and Circular Dichroism (CD). The data indicate that the C-terminal portion of obestatin has a peculiar ordered structure, suggesting its involvement in the biological activity of the peptide.

NMR CHARACTERIZATION OF ALKOXY-SUBSTITUTED GUAR GUM DERIVATIVES

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Guar gum is a natural polysaccharide derived from the Guar plant (*Cyamopsis tetragonoloba*) native to NW India and Pakistan, with a backbone consisting of β-(1→4)-D-mannose residues with α-(1→6)-D-galactopyranosyl units randomly bound as side groups.
The properties of the Guar gum can be finely tuned for industrial application modifying the guar structure by introducing suitable substituents to obtain alkoxy-derivatives such as the methoxy-Guar (MG) and the hydroxy-propyl-Guar (HPG).
In the present paper we will show that through a careful analysis of suitable 1D spectra and 2D maps it is possible to obtain a reasonable assignment of substituted guar gums.
The following experiments have been performed:
^{1}H and ^{13}C spectra at 343K and 363K in buffered D_2O solution, and ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC-DEPT. From all these experiments a full assignment was obtained for MG derivative with a substitution degree spanning from 0.6 to 1.8, while only partial assignment was achieved for HPG derivative.
It is important to observe that in all these polymers the position 2 of the mannose backbone is never substituted, while multiple substitution is eventually present on all galactopyranose positions.
It must be noted that for both classes of polymers a full characterization is needed either for their industrial application and for the improvement of these important materials.

STRUCTURAL NMR CHARACTERIZATION OF STERICALLY CROWDED HYDRIDOTRIS(PYRAZOLYL)BORATO COMPLEXES: UNUSUAL DOUBLE 1,2-BOROTROPIC SHIFT AT A TITANIUM CENTRE

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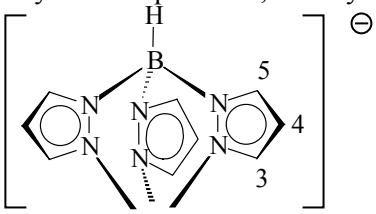
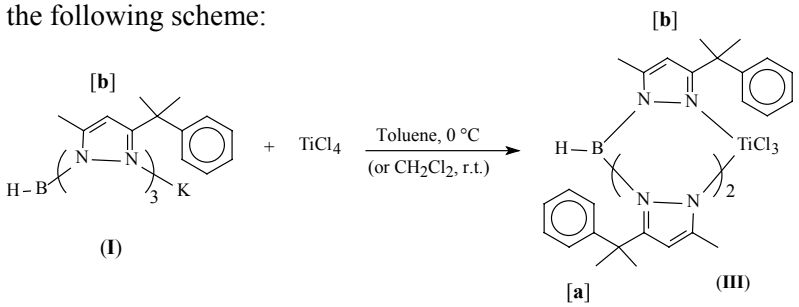
Hydrido–tris(pyrazol–1–yl)borato (Tp') ligands have been extensively used in coordination and organometallic chemistry and the corresponding metal complexes (i.e. Mt=Ti) show a certain catalytic activity, which is strongly influenced by the Tp' steric properties. In fact Tp' ligands offer the possibility of an efficient three–dimensional control around the metal center and the regulation of the electronic effects, because as many as three positions, namely 3, 4 and 5, are available for substitution.

Tp' = 

It has recently been observed that Tp' ligands, bearing bulky substituents in position 3 or 5, generate more efficient olefin polymerization catalysts.
In some cases the presence of three bulky groups in position 3 of the pyrazolyl ring produces high steric crowding around the metal centre, leading, under particular conditions, to the formation of isomeric complexes, through a 1,2–borotropic shift which exchanges the positions 3 and 5 of the pyrazolyl ring.
These observations prompted us to investigate the behaviour of sterically hindered Tp' ligands.
In this work we report a complete NMR characterization of the potassium and thallium(I) derivatives of the new hydrido–tris(3–Me_2Bz,5–Me–pyrazol–1–yl)borato ligand, K[$Tp^{Me}2^{Bz,Me}$] (**I**) and Tl[$Tp^{Me}2^{Bz,Me}$] (**II**). The ^{1}H and ^{13}C NMR spectra of **I** and **II** are in agreement with a C_{3v} symmetry, showing all pyrazolyl groups to be equivalent. The thallium complex shows ^{205}Tl-^{13}C coupling costants related to its molecular structure, because they decrease going away from the ^{205}Tl center.
Besides, we observed that the reaction of **I** or **II** with 1 equivalent of $TiCl_4$ affords, in CH_2Cl_2 or toluene at 0 °C a Ti(IV) trichloride complex, showing a spectral pattern different from the one observed as in the related K or Tl compounds, as in the product of a single isomerization process: in this case a double isomerization process has been observed that leads to a complex whose structure is reported in the following scheme:



The geometrical structure of complex (**III**) has been demonstrated through NMR spectroscopy. Particularly useful in this context has been the observation of the H-B NMR signals in the ^{1}H{^{11}B}NMR spectra, not observable in the normal ^{1}H spectra because of the large broadening coming from the quadrupolar effect of the boron atom, and the measurements of NOE cross-peaks between H-B and the surrounding groups in ^{1}H{^{11}B} gradient NOESY spectra. The molecular structure of **III** has been confirmed in the solid-state by an X-ray diffraction experiment showing that the titanium centre has a pseudo-octahedral coordination, with the nitrogen and chloride ligands in a *fac* geometry. A possible mechanism of formation of **III** is proposed.

NEW UNIVERSAL SEQUENCES: *PERFIDI* and *LAPSR*

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This Poster presents two new families of sequences developed within two different projects at the University of Bologna. The two families are related by a common feature, which is the use of 180° inversion pulses as a preamble to a classical NMR technique. The exclusive use of inversion pulses confers them a remarkable insensitivity to offset-related artifacts, a property particularly appreciated in NMR relaxometry of large, complex samples and in MR imaging. The final aims of the two families, however, are somewhat different:

PERFIDI (Parametrically Enabled Relaxation Filters with Double and multiple Inversion) is a family of relaxation filters applicable as a preamble to almost any NMR pulse sequence. As such, it will find a wide range of applications in all branches of NMR, including spectroscopy, relaxometry and imaging. It has been designed keeping in mind complex, chemically and/or physically heterogeneous systems such as untreated body fluids, biological tissues and whole organs, porous media, etc. The patented sequences (patent BO2005A000445, University of Bologna), comprised of n inversion pulses (n = 2,3,4,...) are crafted to provide (borrowing electronics terminology) high-pass, low-pass and band-pass T_1-filters of various shapes and cut-off sharpness.

LAPSR (Logarithmically distributed A-Periodic Saturation Recovery) is one of a family of **SMS** (Sample Magnetization Suppression) sequences whose aim is to suppress as fast as possible the nuclear magnetization of all components of a sample. A particular attention is paid to samples with wide distributions of relaxation times (e.g., from 0.3 ms to 3 s), offsets, and nutation angles (B_1 inhomogeneity). The development of SMS sequences started from the observation that:

(i) Classical methods of NMR relaxometry such as inversion recovery (IR) are rather slow because they require reaching the equilibrium magnetization before every scan. In addition, they fail in situations where sample complexity combines with severe and unavoidable instrumental imperfections (ex-situ NMR, large samples and coils, severe B_1 inhomogeneity, insufficient transmitter power, etc).

(ii) The alternative is to use the saturation recovery sequence (SR) or the APSR sequence (one composed of 90° pulses with linearly decreasing delays), possibly in combination with gradient pulses. The goal is to achieve the zero-magnetization starting state and do so in a fast and reproducible way. The results are in fact often better than using IR but, nevertheless, still far from being free of artifacts.

These observation prompted us to start an extensive series of theoretical simulations trying to answer the question of *how fast and how well can one suppress the magnetization of complex samples using standard pulse sequences*. The theoretical results, by themselves very interesting, were then compared with experiments. It turns out that the best sequences in this category are composed of a large number (15-20) of inversion pulses (nominally 180°) with logarithmically decreasing delays. Relaxation curves can be obtained about 3 times faster than using IR and they remain meaningful even in experimental conditions which, from the NMR point of view, appear quite unsatisfactory.

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COOPERATIVITY AND SPECIFICITY OF CHICKEN BILE ACID BINDING PROTEIN (CL-BABP): NMR AND DATA DRIVEN DOCKING APPROACH

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Chicken bile acid binding protein (cl-BABP) belongs to a class of protein involved in the metabolic trafficking of fatty acids, cholesterol, bile salts, retinoids and vitamins and it has been proposed that it is involved in the bile acid transport in non mammals liver cytosol. All the proteins belonging to the family of the fatty acid binding proteins share a common fold: 10 strands of antiparallel β-sheets and two short α-helices are located between the first and second strands. It has been reported that human ileal bile acid binding protein (hI-BABP) shows a very high degree of positive cooperativity and site selectivity in its interactions with glycholate (GCA) and glycochenodeoxycholate (GCDA), the two major bile salts in humans. We performed interactions studies on cl-BABP in presence of GCDA, GCA and a combination of the two bile salts. NMR studies performed in presence of ^{15}N-GCDA and ^{13}C-GCA revealed that the protein binds bile acids with a 1:2, protein:ligand stoichiometry and the binding is highly cooperative. We also demonstrated that cl-BABP has a higher level of affinity for GCDA compared to GCA and, differently form hI-BABP, doesn't show any site selectivity for the two ligands.
Experimental data derived from different biochemical and biophysical techniques were employed to perform data driven docking studies with the software HADDOCK (1). In particular the following data were used as input to obtain a model of the ternary complex of cl-BABP with two molecules of GCDA: i) NMR chemical shift perturbation in the presence of ligand; ii) ^{15}N-relaxation data on apo and holo protein; iii) hydrogen deuterium exchange data recorded on the apo cl-BABP used as a threshold of solvent exposition for each binding involved residue; iv) ligand/protein NOE data; v) NMR saturation transfer difference (STD) data; vi) limited proteolysis data. This docking approach puts the basis for the analysis of the binding mode of other functionalised ligands with medical relevance.

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3-FABS: RELIABLE NMR-BASED FUNCTIONAL SCREENING ON PROTEINS OF PHARMACEUTICAL INTEREST

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The NMR-based functional screening 3-FABS (Three Fluorine Atoms for Biochemical Screening) has emerged in the last years as a powerful and reliable approach for the identification of potential drug-candidates and for the accurate measurement of their inhibitory potency. The substrate of an enzymatic reaction is tagged with one or more CF_3 moieties. The modification of the substrate through the enzymatic reaction induces a change in the electronic cloud of the CF_3 group and this alteration results in distinct chemical shifts for the product and substrate CF_3 signals, even when the CF_3 moiety is inserted far from the reaction site of the molecule. The method requires an easy set-up and its versatility allows application to many different enzymes. Recent improvements in the sensitivity of the 3-FABS thanks to the use of probes optimized for ^{19}F detection with cryogenically cooled RF-coils and preamplifiers and the use of substrates tagged with magnetically equivalent multiple CF_3 moieties allow rapid functional screening with very low enzyme consumption. One of the main advantages of 3-FABS when compared to other HTS techniques is the reduced number of generated artefacts. In addition the direct NMR characterization of the screened compound achieved by recording the ^1H spectrum after the ^{19}F spectrum allows the identification of false positives and false negatives. The use of 3-FABS is having a major impact in the discovery process aimed at identifying potential drug candidates for curing or alleviating diseases or pathological situations. A comprehensive insight into 3-FABS and some applications of this methodology to the screening against different enzymes of pharmaceutical interest will be presented.

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ISOLATION AND IDENTIFICATION OF ELBURZENSOSIDE C1/C2 AND D1/D2 FROM *ALLIUM ELBURZENSE*

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Introduction: Walak (*Allium elburzense* Wendelbo) is a endemic plant in Iran that used as food, due to its sweet taste, typical of the plant species belonging to the onion species. In Iranian folk medicine it has also been used as an antirheumatic, aphrodisiac, antiduretic, and anthelminthic herb. A phytochemical investigation of the bulbs of *Allium elburzense* Wendelbo has been undertaken, leading to the isolation of some new saponins.
Methods: The bulbs were air dried, powdered and extracted with hexane, $CHCl_3$, $CHCl_3$–MeOH (9:1), and MeOH. The MeOH extracts were partitioned between butanol and water phases. The $CHCl_3$–MeOH (9:1) and butanolic extracts were chromatographed by CC or MPLC on a RP_{18} Silica gel, using a linear gradient solvent system from H_2O to MeOH as mobile phases. Interested fractions with saponins contents were selected, base on preliminary ^{1}H NMR analysis. The selected fractions were purified by preparative HPLC with the suitable mobile phases (mixture of MeOH–H_2O). ^{1}H NMR, ^{13}C NMR, 2D NMR, FABMS and IR, techniques were used to elucidate of isolated compounds.
Results: On the basis of spectroscopic analysis, mainly 2D NMR and mass spectrometry, and chemical methods, the structures of the compounds were determined as furost-2α,3β,5α,22α-tetrol 3-*O*-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside (**EC1**), and furost-2α,3β,5α,22α-tetrol 3-*O*-[β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranosyl] 26-*O*-β-D-glucopyranoside (**ED1**), and the corresponding epimers at position 22, and the corresponding epimers at position 22 (**EC2**–**ED2**).
Conclusion: It seems that high concentration of new saponins in the edible investigated plant indicates that further pharmacological and SAR (structure activity relationship) studies are needed. Additionally phytochemical investigations of other *Allium* species growing in Iran is recommended.

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