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Regione Siciliana
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Facoltà di Agraria

Dip. di Ingegneria e
Tecnologie Agro-
Forestali

Università degli Studi di Palermo, v.le delle Scienze 13, edificio 19

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SCIENTIFIC PROGRAM

Monday, September 21

15.00-19.00 REGISTRATION
19.00-21.00 Welcome Reception

Tuesday, September 22 - morning

8.30-9.00 OPENING REMARKS
9.00-10.30 PLENARY LECTURES, **Aula 3**. Chair: P. Conte

9.00-9.45 **O. W. Sørensen,** Technical University of Denmark, Lyngby
Heteronuclear long-range correlation, what's new and how far can it take us?

9.45-10.30 **G. E. Schaumann,** University of Koblenz-Landau, Germany
Proton nuclear magnetic resonance (NMR) relaxometry in soil science applications

10.30-10.50 **BRUKER: A. P. Minoja**, Bruker BioSpin srl, Milano
NMR and sensitivity: the never-ending story

10.50-11.20 COFFEE BREAK

11.20-12.30 PARALLEL SESSIONS

NMR OF SMALL MOLECULES
Aula 3. Chair: T. Beringhelli

11.20-11.50 **S. Chimichi**,
University of Florence
Molecular structure elucidation by NMR spectroscopy and quantum mechanical (QM) chemical shift calculations

11.50-12.10 **L. Calucci**,
University of Pisa
Orientational order of fluorinated mesogens containing the 1,3,2-dioxaborinane ring by ^{13}C and ^{19}F NMR spectroscopy

12.10-12.30 **D. Maggioni**,
University of Milano
Pulsed gradient field study of nanosized rhenium complexes

RELAXATION/LOW RESOLUTION
Aula A. Chair: G. Alonzo

11.20-11.50 **G. Fanali**,
University of Insubria
Human serum albumin: a peculiar monomeric protein

11.50-12.10 **F. Reineri**,
University of Torino
From high spin order to longitudinal hyperpolarization using parahydrogen

12.10-12.30 **C. De Pasquale**,
University of Palermo
Fast field cycling NMR for quality characterization of typical sicilian honeys

13.00 LUNCH

Tuesday, September 22 - afternoon

14.45-15.30 PLENARY LECTURE, **Aula 3**. Chair: M. Piccioli

14.45-15.30 **L. Frydman,** Weizmann Institute, Rehovot, Israel
Citius - Altius - Fortius: resonating at the EPR/NMR/MRI interface

15.30-16.40 PARALLEL SESSIONS

ENVIRONMENTAL NMR
Aula 3. Chair: S. Chimichi

15.30-16.00 **G. Casella**,
University of Palermo
Formation of metal-ethanol complexes as assessed by high and low field NMR spectroscopy

16.00-16.20 **G. Picone**,
University of Bologna
Metabolic profiling using ^{1}H NMR and chemometrics: a new powerful tool for differentiating aquaculture systems for Gilthead sea breams

16.20-16.40 **R. Sanna**,
University of Cagliari
A FTIR and solid state ^{13}C NMR study of the adsorption of bis-(2-ethylhexyl)phthalate on hydrozincite

METHODOLOGY
Aula A. Chair: H. Molinari

15.30-16.00 **R. Pierattelli**,
CERM and University of Florence
Direct ^{13}C detection for biomolecular NMR

16.00-16.20 **P. R. Vasos,**
Swiss Federal Institute of Technology, Lausanne, Switzerland
Selected spin states for line-narrowing and magnetisation storage

16.20-16.40 **M. Alecci**,
University of L'Aquila
Design and testing of high-field (3-9.4 T) double-tuned surface resonators for proton and sodium MRI

16.40-17.15 COFFEE BREAK

17.15 GIDRM AND GIRM MEETINGS, **Aula 3**.

Wednesday, September 23 - morning

9.00-10.30 PLENARY LECTURES, **Aula 3**. Chair: G. Barbato

9.00-9.45 **D. O. Cicero,** University of Rome Tor Vergata
Understanding the "love-hate relationship" between a protease and its cofactor: the NS3-NS4A Hepatitis C virus proteins

9.45-10.30 **P. Fantazzini,** University of Bologna
Magnetic resonance for fluids in porous media and cultural heritage

10.30-10.50 **VARIAN: P. Sandor,** Varian Deutschland GmbH
Practical aspects of DOSY

10.50-11.20 COFFEE BREAK

11.20-12.50 PARALLEL SESSIONS

CULTURAL HERITAGE
Aula 3. Chair: A. Maccotta

11.20-11.50 **M. Orlandi**,
University of Milano Bicocca
Characterization of archeological waterlogged woods by nuclear magnetic resonance

11.50-12.10 **A. Spinella**,
University of Palermo
Preliminary solid state NMR characterization of wooden archaeological samples

12.10-12.30 **F. Presciutti**,
INSTM and University of Perugia
Multitechnique approch to the study of ancient ceramic material

12.30-12.50 **M. Gombia**,
University of Bologna
Kinetics of water vapor absorption in polluted porous media studied by time domain NMR

BIOMEDICINE
Aula A. Chair: E. Terreno

11.20-11.50 **L. Guidoni**,
Istituto Superiore di Sanità, Rome
^{1}H MRS of tumour cells in hypoxic and hyperammonia environment

11.50-12.10 **A. Palma**,
Istituto Superiore di Sanità, Rome
Intermediates of protein glycosylation observed in tumour cells by ^{1}H MRS

12.10-12.30 **E. Gianolio**,
University of Torino
Novel probes targeted to cell surface thiols for cellular MR imaging

12.30-12.50 **A. Ceccon**,
University of Verona
Is human liver FABP a good carrier for magnetic resonance imaging contrast agents?

13.00 LUNCH

Wednesday, September 23 - afternoon

14.45-15.30 PLENARY LECTURE, **Aula 3**. Chair: M. Valentini

14.45-15.30 **M. A. Cremonini**, University of Bologna
NMR studies of meat

15.30-16.40 PARALLEL SESSIONS

FOOD SCIENCE / METABOLOMICS
Aula 3. Chair: A. Spyros

15.30-16.00 **M. Valentini**,
Agricultural Research Council,
Monterotondo (Rome)
Magnetic resonance imaging for foodstuff quality evaluation

16.00-16.20 **F. Savorani**,
University of Copenhagen, Denmark
iCOshift: a versatile tool for the rapid alignment of NMR sets

16.20-16.40 **J. Klein**,
GlaxoSmithKline, Verona
Use of NMR spectroscopy in the early determination and quantification of in vitro metabolites

BIOMEDICINE
Aula A. Chair: L. Guidoni

15.30-16.00 **L. Poggi**, Bracco Imaging SpA,
Colleretto Giacosa (TO)
MR contrast without paramagnetic ions

16.00-16.20 **E. Vescovo**, **F. di Cesare**,
University of Manchester, UK,
University of Lyon 1, France
In vivo brain temperature map using jMRUI version 4: a plugin development

16.20-16.40 **E. Cittadino**,
University of Torino
MRI assessment of the therapeutic efficacy of paramagnetic liposomes loaded with an antitumor drug

16.40-18.30 POSTER SESSION & COFFEE BREAK

18.30-19.30 ***GIDRM-GIRM 2009 Gold Medal Award Ceremony***
Aula 3. Chair: M. A. Cremonini

18.30-19.30 **M. Botta**, University of Piemonte Orientale
Lanthanide-based magnetic resonance imaging contrast agents: properties and mechanism of action

Thursday, September 24 - morning

9.00-10.30 PLENARY LECTURES, **Aula 3**. Chair: M. Geppi

9.00-9.45 **F. Babonneau**, Université Pierre et Marie Curie and CNRS, Paris, France
Exploring inorganic-organic interfaces in hybrid materials with advanced NMR tools

9.45-10.30 **D. Fushman**, University of Maryland, U.S.A.
NMR characterization of the conformation, dynamics, and binding properties of polyubiquitin as a paradigm of a multidomain system

10.30-11.00 COFFEE BREAK

11.00-12.30 PARALLEL SESSIONS

BIOMOLECULAR NMR
Aula 3. Chair: A. Spisni

11.00-11.30 **F. Arnesano**,
University of Bari
Interaction between platinum complexes and copper transport proteins

11.30-11.50 **A. M. D'Ursi**,
University of Salerno
Tuning the folding states of β-amyloid fragment Aβ(16-35) with different charge of membrane mimicking systems

11.50-12.10 **R. Anedda**,
Porto Conte Ricerche srl,
Tramariglio-Alghero (SS)
On Xenon as a neutral, independent biomolecular probe

12.10-12.30 **N. D'Amelio**,
Bracco Imaging SpA, Basovizza (TS)
NMR studies of a new potent MMP-12 inhibitor: insight into key binding interactions

MATERIALS SCIENCE
Aula A. Chair: S. Spera

11.00-11.30 **S. Borsacchi**,
University of Pisa
Understanding the molecular properties of complex sol-gel hybrid materials through solid-state NMR

11.30-11.50 **M. Mauri**,
University of Milano Bicocca
Solid state order to order transitions in block copolymers as detected by high and low field NMR

11.50-12.10 **M. L. Saladino**,
University of Palermo
Solid state NMR charactarization of a PMMA-Ce:YAG nanocomposite

12.10-12.30 **D. Donghi**,
University of Milano
NMR characterization of hydrosoluble polymers containing luminescent rhenium(I) tricarbonyl fragment

13.00 LUNCH

Thursday, September 24 - afternoon

14.45-15.30 FILIPPO CONTI MEMORIAL, **Aula 3**. Chair: S. Mammi

14.45-15.00 **S. Mammi**, University of Padova
15.00-15.30 **C. Manetti**, University of Rome La Sapienza

15.35-16.35 BEST POSTERS AWARDS, **Aula 3**. Chair: S. Mammi

16.40-17.15 COFFEE BREAK

17.15-18.30 CLOSING LECTURES, **Aula 3**. Chair: N. Niccolai

17.15-17.45 **A. Spyros**, University of Crete, Greece
Analysis of organic materials in arts and archaeology by NMR spectroscopy

17.45-18.30 **P. A. Temussi**, University of Naples and National Institute for Medical Research, London, UK
Sweet, bitter and umami receptors: a complex relationship

18.30-18.40 CONCLUDING REMARKS

19.30 SOCIAL DINNER

PLENARY LECTURES

HETERONUCLEAR LONG-RANGE CORRELATION, WHAT'S NEW AND HOW FAR CAN IT TAKE US?

O. W. Sørensen

DTU Chemistry, Kemitorvet 207, DK-2800 Kgs. Lyngby, Denmark

The lecture will give an overview of the references below with emphasis on the key features of the pulse sequences and the ideas behind them.

Heteronuclear two-bond correlation: Suppressing heteronuclear three-bond or higher NMR correlations while enhancing two-bond correlations even for vanishing $^{2}J(CH)$. Nyberg NT, Duus JØ, Sørensen OW, JOURNAL OF THE AMERICAN CHEMICAL SOCIETY Volume: 127 Issue: 17 Pages: 6154-6155 Published: MAY 4 2005

Editing of H2BC NMR spectra. Nyberg NT, Duus JØ, Sørensen OW, MAGNETIC RESONANCE IN CHEMISTRY Volume: 43 Issue: 12 Pages: 971-974 Published: DEC 2005

H2BC: a new technique for NMR analysis of complex carbohydrates. Petersen BO, Vinogradov E, Kay W, et al., CARBOHYDRATE RESEARCH Volume: 341 Issue: 4 Pages: 550-556 Published: MAR 20 2006

Improved multiplicity-editing of HMBC NMR spectra. Benie AJ, Sørensen OW, MAGNETIC RESONANCE IN CHEMISTRY Volume: 44 Issue: 8 Pages: 739-743 Published: AUG 2006

HAT HMBC: A hybrid of H2BC and HMBC overcoming shortcomings of both. Benie AJ, Sørensen OW, JOURNAL OF MAGNETIC RESONANCE Volume: 184 Issue: 2 Pages: 315-321 Published: FEB 2007

***Clean* HMBC: Suppression of strong-coupling induced artifacts in HMBC spectra**. Wurtz P, Permi P, Nielsen NC, et al., JOURNAL OF MAGNETIC RESONANCE Volume: 194 Issue: 1 Pages: 89-98 Published: SEP 2008

Adiabatic Low-Pass J Filters for Artifact Suppression in Heteronuclear NMR. Meier S, Benie AJ, Duus JØ, et al., CHEMPHYSCHEM Volume: 10 Issue: 6 Pages: 893-895 Published: APR 14 2009

3D H2BC: A novel experiment for small-molecule and biomolecular NMR at natural isotopic abundance. Sebastian Meier, Andrew J. Benie, Jens Ø. Duus and Ole W. Sørensen, Journal of Magnetic Resonance, in press, doi:10.1016/j.jmr.2009.06.017

Recent progress in heteronuclear long-range NMR of complex carbohydrates: 3D H2BC and clean HMBC. Sebastian Meier, Bent O. Petersen, Jens Ø. Duus, Ole W. Sørensen. Carbohydrate Research, in press, doi:10.1016/j.carres.2009.08.013

PROTON NUCLEAR MAGNETIC RESONANCE (NMR) RELAXOMETRY IN SOIL SCIENCE APPLICATIONS

J. V. Bayer; F. Jaeger and G. E. Schaumann

Department of Environmental and Soil Chemistry, Institute of Environmental Sciences, University of Koblenz-Landau, Fortstraße 7, 76829 Landau, Germany

Proton NMR relaxometry is a very powerful tool for investigating porous media and its interaction with water or other liquids and the mobility and interaction of organic molecules in solution. It is commonly used in material science or earth science, but only scarcely applied in soil science although it shows great potential for helping to understand water uptake into the soil matrix and processes occurring at the solid-liquid interface at soil particle surfaces. This review introduces proton NMR relaxometry in the context of soil science and discusses the most important applications of the method in this field. Relevant results from different applications of NMR relaxometry in soils are described and research gaps identified. NMR relaxometry is a sensitive, informative and promising method to study pore size distribution in soils as well as many kinds of soil physicochemical processes, among which are wetting, swelling or changes in macromolecular status. It is further a very helpful method to study interactions between molecules in soil organic matter and can serve to study the state of binding of water or organic chemicals to soil organic matter. Relaxation times determined by NMR relaxometry are sensitive to various factors that play a role in soil-water interaction which is both an advantage and shortcoming of the method: NMR relaxometry can be applied to numerous investigations in soil science, but at the same time interpretation of the results may be very difficult in such complex and heterogeneous systems like soils.

CITIUS - ALTIUS - FORTIUS: RESONATING AT THE EPR/NMR/MRI INTERFACE

L. Frydman

Department of Chemical Physics, Weizmann Institute, 76100 Rehovot, Israel.
E-mail: Lucio.Frydman@weizmann.ac.il

We have recently developed a scheme enabling the acquisition of arbitrary multidimensional NMR spectra and/or NMR images (MRI), within a single scan. This is by contrast to the hundreds or thousands of scans that are usually needed to collect this kind of data. Provided that the target molecule's signal is sufficiently strong, the acquisition time of multidimensional NMR experiments can thus be shortened by several orders of magnitude. This new «ultrafast» methodology is compatible with existing multidimensional pulse sequences (COSY, TOCSY, HSQC, solids, spectroscopic imaging) and can be implemented using conventional hardware. The manner by which the spatial encoding of the NMR interactions —which is the new principle underlying these new protocols— proceeds in these experiments, will be summarized. The protocol's performance will be exemplified with a variety of homonuclear and heteronuclear 2D and 3D NMR/MRI acquisitions on chemical, biochemical and biological systems, carried out within a ≈1 sec time scale. The incorporation into these experiments of nuclear hyperpolarization procedures capable of increasing the single-scan sensitivity of liquid state NMR by factors ranging from 10^3-10^6, will also be assessed.

UNDERSTANDING THE "LOVE-HATE RELATIONSHIP" BETWEEN A PROTEASE AND ITS COFACTOR: THE NS3-NS4A HEPATITIS C VIRUS PROTEINS

D. O. Cicero

Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, via della Ricerca Scientifica 1, Roma, Italia
E-mail: cicero@scienze.uniroma2.it

Twenty years have passed since the discovery of the Hepatitis C virus (HCV) and, among the viral proteins, the NS3 serine-protease received an immediate interest as a main target for the development of antivirals. The principal reason for this being the crucial role that NS3 plays in the viral cycle: it processes the polyprotein containing all the individual enzymes and structural elements, and in addition it deactivates the cellular response against virus attack. Almost every big pharmaceutical company tackled the discovery of chemical agents blocking NS3 activity and recently a number of compounds have reached clinical trials, leading to the hope that soon we will count on a specific antiviral treatment for hepatitis C.
The activity of the NS3 protease is enhanced by the interaction with another viral protein, NS4A, that acts as an enzymatic cofactor. During the last fifteen years more than twenty crystal structures of different complexes of the NS3-NS4A system with a large variety of inhibitors have been available for analysis. These structures suggest that the complex between NS3 and NS4A is very stable, as NS4A contributes with a β-strand to form one of the two β-barrels of the protein. NMR works, mostly done at the IRBM and lately at the University of Rome "Tor Vergata", were the only source of NS3-inhibitor complex structures lacking the NS4A cofactor. Comparison between the two sets of structures lead to some first conclusions about the role of NS4A in the activation of this protease. However, the main obstacle to study in solution this interaction is in the fact that NS3 and NS4A do not form a stable complex, as observed by NMR spectroscopy. This is confirmed by a modest micromolar affinity for an interaction that, crystal structures show, is not superficial. The lack of solution information is responsible for a yet incomplete understanding of the cofactor activation mechanism.
Our last experiments indicate that the affinity of the NS3 protease for its cofactor NS4A depends on the type of compound occupying the active site and on the protease belonging to the different viral genotypes. We will show the correlation between protein dynamics and affinity for the NS4A binding. Moreover, by stabilizing the ternary complex in solution we opened the possibility to compare features of the catalytic machinery with and without the cofactor, leading to new knowledge about the mechanism of cofactor activation. Some unexpected features of the catalytic site originated by our studies confirm that NS3 is a very peculiar serine protease. Finally, the type of interaction that we observe in solution for NS3 and NS4A represents a particular moment of the *in vivo* pathway of this crucial viral component in the HCV life cycle.

MAGNETIC RESONANCE FOR FLUIDS IN POROUS MEDIA AND CULTURAL HERITAGE

P. Fantazzini

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Developed in the last 20 years in other fields of research and for other purposes (industry, petroleum, bio-medicine, etc.), Magnetic Resonance for fluids in Porous Media (MRPM) [1] can now be profitably applied to the preservation of Cultural Heritage for both laboratory research and in situ diagnosis on porous media like stones, bricks, cements, mortars, wood.
The most important MRPM techniques that can be applied for Cultural Heritage are Imaging (MRI) and Relaxometry of ^{1}H nuclei of liquid water. What makes these techniques appealing for Cultural Heritage is the capability of probing in non-destructive and non-invasive manner water molecules inside the pore space, and it is well known that water is the main deterioration agent for porous materials, in which gases and water can diffuse from the environment. MRI can be applied to visualize and measure quantitatively the distribution of water in any virtual internal section of the sample, with the possibility of 3D reconstructions of the structure of the pore space occupied by water. Relaxometry does not give images but can provide information on the pore space structure and on the water behavior inside the pore space. Relaxometry is suited to the quantitative measurement of the moisture contained in a porous material and, under certain conditions that often apply, the distribution of a length characteristic of the pore space, that can be connected with pore dimensions. These properties can be analyzed before and after treatment with protective and/or consolidating product, and followed in time, in such a way that they can furnish information useful for diagnosis and therefore for planning the most appropriate conservation, restoration, and maintenance procedures. While imaging allows one to get information at the sample-scale, relaxation data give information at the pore-scale. Relaxometry and MRI can be combined in Relaxation-Imaging, which allows the spatially-resolved study of NMR parameters connected with the pore space. These techniques are also applicable by means of portable instruments or instruments that can in any case be transported for measurements in situ. Among the more recent possibilities is that of visualization of fractures in marble by means of MRI, not of liquid water, but of SF_6 gas [2].
In this talk some examples of application of these methods and techniques will be presented.

References

[1] P. Fantazzini *Magn. Reson. Imaging* **23**, 125-131 (2005)
[2] D.O.Kuethe, M.D. Scholz, and P. Fantazzini *Magn. Reson. Imaging* **25**, 505-508 (2007)

NMR STUDIES OF MEAT

M. A. Cremonini

Department of Food Science, University of Bologna, Piazza Goidanich 60, 47023 Cesena
E-mail: mauro.cremonini@unibo.it

Meat quality is even today assessed in the industry through experimental tests which are often time-consuming. For example, the water holding capacity trait (WHC) may be inferred in at least four different ways, whose experimental time ranges from 15 minutes to 48 hours. WHC is strictly connected to the mobility and availability of water in meat; these in turn depend on the extent of interactions between the aqueous phase and the biopolymers matrix and give rise to a multicomponent behavior of the NMR water signal that is easily revealed through CPMG experiments. CPMG experiments of meat samples are much faster than traditional WHC tests, but, before proposing them as alternative methods to obtain meat quality parameters it is necessary to carefully study what they yield and to what extent do they correlate with the traditional parameters.

In this talk we review the fundamentals of water in meat as seen by NMR and describe some of the ways - based on chemometrics - we have devised along the years for searching for correlations between the NMR signal and the traditional quality parameters of meat. We also review the problem of meat authentication of fresh *vs.* frozen/thawed meat and report an experimental proof-of-concept of discrimination between these two kinds of meat based on the joint use of diffusion tensor imaging (DTI) and discriminant analysis.

LANTHANIDE-BASED MAGNETIC RESONANCE IMAGING CONTRAST AGENTS: PROPERTIES AND MECHANISM OF ACTION

M. Botta

Dipartimento di Scienze dell'Ambiente e della Vita, Universita' del Piemonte Orientale "A. Avogadro", Viale T. Michel 11, 15121 Alessandria, Italy
E-mail: mauro.botta@mfn.unipmn.it

The magnetic properties of lanthanide(III) ions may be exploited for the development of powerful NMR probes for biomedical applications. Gd(III) complexes are in current clinical use as contrast agents for magnetic resonance imaging. Other paramagnetic lanthanide(III) complexes endowed with shift reagent capabilities are used for the separation of NMR resonances of species present in the inner and outer cellular compartments and for the measurement of pH and temperature.
The ability of the contrast agent to enhance the MR image is termed relaxivity, and it depends upon many molecular factors, including speciation, protein binding affinity, chemical structure, and dynamic processes, such as water exchange kinetics and rotational tumbling rates.
In the last 20 years we have investigated the relation between these factors and the observed relaxivities by combining relaxometric and high resolution NMR techniques. Here we summarize the most relevant results.

EXPLORING INORGANIC-ORGANIC INTERFACES IN HYBRID MATERIALS WITH ADVANCED NMR TOOLS

F. Babonneau*, N. Baccile, T. Azaïs, C. Gervais, G. Laurent, C. Bonhomme

Laboratoire de Chimie de la Matière Condensée de Paris, Université Pierre et Marie Curie-UPMC and CNRS, Collège de France, 11 place Marcelin Berthelot, 75005 Paris, France
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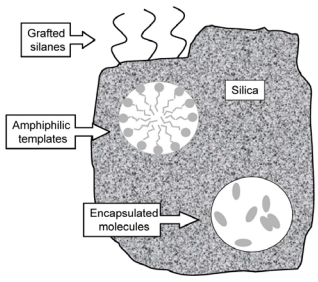
Sol-gel chemistry is a very versatile approach to design hybrid organic-inorganic materials with specific and multiple functionalities, which make them extremely attractive in a large range of applications. A large number of combinations between the organic and inorganic components exist. Organic entities can be covalently bonded to the inorganic framework, but can also interact more weakly through H-bonding, or van der Waals interactions. Very complex structures can thus be obtained with multiple organic-inorganic interfaces, for which solid state NMR appears a powerful and versatile characterization method.



In this presentation, surfactant-templated silica based materials will be used to illustrate how advanced solid state NMR techniques can address important structural issues. Such materials are now widely investigated for the possibility to design specific functionalities and porous nanostructures. Interactions at the surfactant/silica interface can be characterized using ^{1}H-^{29}Si as well as ^{1}H-^{1}H dipolar couplings; ^{1}H-^{29}Si-^{1}H double cross polarization techniques was used to selectively detect proton sites at the silica surface [1], while ^{1}H Double Quantum (DQ) NMR allowed to localize organic groups at the silica surface. This last experiment was even extended to thin film samples using the Magic Angle Coil Spinning (MACS) approach [2]. This innovative technique is very suitable for very small amount of sample (0.1 mg), and should open extremely interesting opportunities to characterize organic-inorganic thin films.

Once the structuring agent eliminated, the porous inorganic matrix can be exploited for pharmaceutical [3] or environmental applications [4]. Because of strong confinement effects, solid-state NMR sequences have to be carefully chosen to accommodate highly mobile behavior of the molecules at room temperture. In particular, NMR sequences issued from solution-state NMR can be applied.

Finally, computer modeling including DFT and VASP methods will be presented in connection with solid state NMR experiments and GIPAW method, which is now a well established tool of calculation for NMR parameters. This combined approach is currently used to get a better description of interface between an hydroxylated amorphous silica surface and organic molecules [6].

[1] N. Baccile, G. Laurent, C. Bonhomme, P. Innocenzi, F. Babonneau, Chem. Mater. 19 (2007) 1343-54.
[2] D. Sakellariou, G. Le Goff, J.F. Jacquinot, Nature 447 (2007) 694.
[3] T. Azais, C. Tourné-Péteilh, F. Aussenac, N. Baccile, C. Coelho, J.-M. Devoisselle, F. Babonneau, Chem. Mater. 18 (2006) 6382-90.
[4] N. Baccile, F. Babonneau Microporous Mesoporous Materials 110 (2008) 534-542
[5] T. Azaïs, G. Hartmeyer, S. Quignard, G. Laurent, C. Tourné-Pétheil, J.-M. Devoisselle, F. Babonneau. Pure Appl. Chem., ASAP article, doi:10.1351/PAC-CON-08-11-10.
[6] F. Tielens, C. Gervais et al., Chem. Mater., 20 (2008) 3336.

NMR CHARACTERIZATION OF THE CONFORMATION, DYNAMICS, AND BINDING PROPERTIES OF POLYUBIQUITIN AS A PARADIGM OF A MULTIDOMAIN SYSTEM

D. Fushman

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Many proteins in the cell have modular architecture, i.e. they are composed of several well-folded regions (domains). Structural organization and interdomain dynamics often play a key role in molecular recognition events and functional regulation in varous processes involving multidomain proteins. Characterization of these systems, however, presents a significant challenge, because the existing structural methods, X-ray crystallography and NMR, rest on the assumption of a unique conformation and, therefore, could be inadequate when applied to inherently flexible systems. Domain motions, naturally occurring in solution, are completely restricted in crystals, and packing forces could result in a positioning of protein domains in a crystal structure that might not represent the physiologically relevant conformation. The challenges for conventional NMR characterization of multidomain systems are due to insufficient information on the relative interdomain positioning and orientation and the absence of adequate models for interdomain mobility in these systems. I will discuss some recently developed approaches, based on spin-relaxation measurements, that address some of these problems. These methods are applied to characterize the structure, dynamics, and ligand binding properties of polyubiquitin, which serves as a signaling tag in a variety of vital events in eukaryotic cells. In particular, Lys48-linked di-ubiquitin exists in dynamic equilibrium between a closed and one or more open conformations, and the transition from the closed to an open state is critical for the protein's ability to bind various ubiquitin receptors. I will present a detailed, NMR-derived picture of the process of opening/closing in di-ubiquitin and show that using nuclear spin-relaxation measurements it is possible to characterize the interdomain dynamics and determine the structure of the interconverting states.

SWEET, BITTER AND UMAMI RECEPTORS: A COMPLEX RELATIONSHIP

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Sweet and bitter are taste qualities linked to food acceptance and rejection in humans. It was long thought that these taste sensations were closely related, but the discovery and characterization of taste receptors, revealed that mammals express a single sweet receptor and many unrelated bitter receptors. Bitter tasting chiral isomers of sweet compounds can bind the umami receptor, rather than bitter receptors, and elicit the bitter sensation through cross talk between labelled cells. In support of cross talk between labelled cells, recent findings suggest that although most receptor taste cells respond to only one taste, most presynaptic taste cells accept signals from labelled cells which respond to two or more different taste qualities.

ORAL COMMUNICATIONS

NMR AND SENSITIVITY: THE NEVER-ENDING STORY

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NMR is universally recognised as a fundamental tool for the characterization of small molecules and biological samples. However, its low sensitivity requires either amount of sample not always available or too much expensive labelled samples.
The talk will show an overview about the most recent technologies and methodologies investigated and developed by Bruker Biospin in order to overcome this limitation.

As far as the "pure" hardware developments is concerned the latest Bruker BioSoin developments are related to the improved performances of both RT probes and Cryoprobe™ and to the highest magnetic field magnets reaching the goal to energize the first 23.5 Tesla standard-bore, high homogeneity 1 GHz NMR persistent magnet. The first 1 GHz NMR spectra recorded with a 5mm triple-resonance CryoProbe™ will be shown.
As far as methodologies are concerned, the attention will be focused on two polarization methods: **DNP** (**D**ynamic **N**uclear **P**olarization) and **SABRE** (**S**ignal **A**mplification **B**y **R**eversible **E**xchange).
DNP is based on transferring the polarization of unpaired electrons spins to nuclear spins. The configuration, the applications and the further developments will be discussed in details.

Fig. 1. The world's first commercially available solid-state NMR based on DNP technique

SABRE uses hyperpolarized spins derived from parahydrogen (para-H2) to sensitize the NMR experiment. The method has been developed in York [1] and Bruker BioSpin has been one of the first collaborators in developing this technology for commercial use as it delivers a proven 1000-fold increase in sensitivity.

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MOLECULAR STRUCTURE ELUCIDATION BY NMR SPECTROSCOPY AND QUANTUM MECHANICAL (QM) CHEMICAL SHIFT CALCULATIONS

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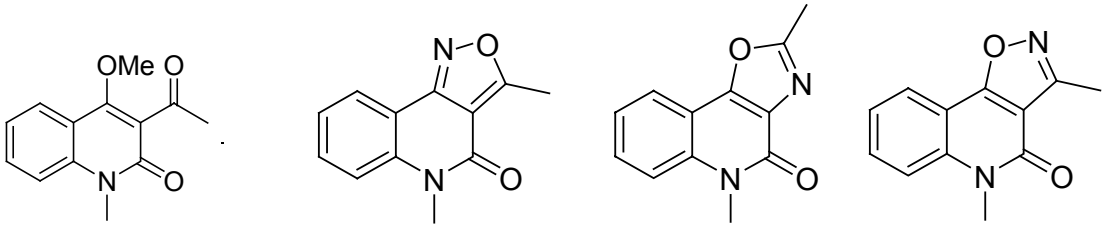
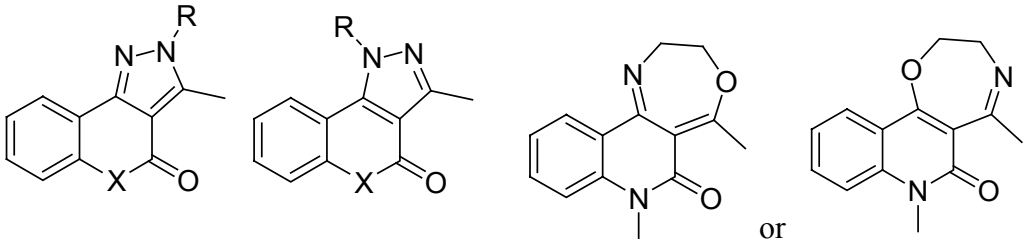
The problem of molecular structure elucidation still represents a major challenge and a fundamental problem in the fields of synthetical organic or medicinal chemistry.
A serendipity observation prompted us to investigate more deeply the simple reaction of the quinolinone derivative **A** with hydroxylamine; thus, we may prove that different regioisomers may be obtained depending on the reaction conditions [1].



A **B** **C** **D**

The combined use of 2D NMR correlation experiments and GIAO-DFT ^{13}C-NMR chemical shift calculations allowed a reliable and simple structure determination of regioisomeric heterocyclic systems originating from the above reaction of quinolinones or coumarin derivatives [2].
We will examine other examples in this field like compounds obtained employing unsymmetrical 1,2- and 1,4-bisnucleophiles as *N*-substituted hydrazines and 1,2-diaminoethanes or 2-aminoethanol:



X = N-Me, O
R = Me, Ph

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ORIENTATIONAL ORDER OF FLUORINATED MESOGENS CONTAINING THE 1,3,2-DIOXABORINANE RING BY ^{13}C AND ^{19}F NMR SPECTROSCOPY

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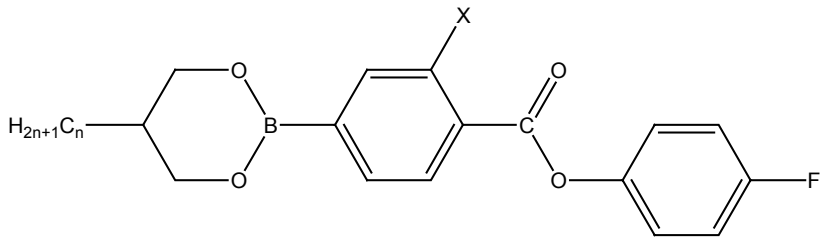
^{13}C and ^{19}F NMR techniques have been applied to obtain information on the orientational order properties of two fluorinated mesogens (**5DBF1** and **4DBF2** in Fig.1) containing the 1,3,2-dioxaborinane ring [1]. Direct excitation ^{19}F NMR spectra have been recorded in both the isotropic melt phase and at different temperatures in the mesophase(s) of **5DBF1** and **4DBF2**, whereas CP and high power proton decoupling techniques have been used to record ^{13}C spectra in the mesophases of both mesogens.



5DBF1 X=H, n=5
4DBF2 X=F, n=4

5DBF1: Cr - 348.4 K – SmA - 353.7 K – N -391.1 K – I **4DBF2**: Cr - 340.4 K – N - 356.6 K - I

Fig. 1. Molecular structure and phase transition temperatures of the investigated liquid crystals.

A rich experimental data set, containing chemical shift anisotropies for aromatic carbon and fluorine nuclei and ^{13}C-^{19}F and ^{1}H-^{19}F dipolar couplings at different temperatures, has been obtained which has been accurately analysed for determining the principal orientational order parameter and biaxiality values for the aromatic core of the mesogens in their liquid crystalline phases [2]. The geometrical parameters (bond lengths and angles) used in the analysis have been calculated from DFT geometry optimizations at the B3LYP/6-31G(d) level of theory simulating the solvent effect using the PCM model and DFT-GIAO methods, using the MPW1PW91/6-311+G(d,p) combination of hybrid functional and basis set, have been employed to calculate chemical shift tensors.
The order parameters obtained from the analysis of different experimental data sets have been crytically discussed and compared to those obtained by optical and dielectric spectroscopies.

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PULSED GRADIENT FIELD STUDY OF NANOSIZED RHENIUM COMPLEXES

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Radiopharmaceuticals incorporating ^{99m}Tc and $^{186/188}$Re have been extensively studied in the last decades [1]. Suitable nuclear properties of these metals can be exploited for both diagnosis (^{99m}Tc) and therapy ($^{186/188}$Re). Among the different fragments introduced into the radiopharmaceuticals, great interest has gained the aquo complex $[M(H_2O)_3(CO)_3]^+$ (M = Re, Tc), that contains three labile water molecules together with a stable *fac* $M(CO)_3^+$ core, suitable for bio-conjugation. To test the feasibility and stability of the bioconjugates, experiments are normally performed using the cold rhenium natural isotopic mixture as surrogate. Different chelating agents as well as different coordination routes have been proposed in order to get rhenium derivatives with high specificity and stability [2].
In this communication we show the results obtained by reacting $[Re(H_2O)_3(CO)_3]^+$ with highly hydrosoluble polymers, suitable for the use as biomedical materials.
A detailed study on the polymer size, in the presence and in the absence of rhenium, has been performed by means of longitudinal relaxation (T_1) times and pulsed gradient spin echo (PGSE) NMR experiments [3].
^{1}H PGSE NMR experiments have been performed at room temperature on diluted sample, at different ionic strength and different pH, to evaluate the influence of these parameters on polymer aggregations. Stimulated echo sequences, incorporating bipolar gradient pulses, were used, both with and without water suppression. Through these methods, diffusion coefficients have been determined and the hydrodynamic radii r_H of the random polymers, with and without rhenium, have been estimated.
Comparison with results obtained by dynamic light scattering measurements will be provided.

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HUMAN SERUM ALBUMIN: A PECULIAR MONOMERIC PROTEIN

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HSA is best known for its extraordinary ligand binding capacity. It is synthesized in the liver and exported as a single non-glycosylated chain, reaching a blood concentration of about 7.0×10^{-4} M. Human serum heme-albumin (heme-HSA) has been very recently considered as a heme-protein. Heme endows the protein with peculiar spectroscopic properties that can be used to follow functional links between the binding sites.The analysis of the solvent water proton NMR relaxation rate by nuclear magnetic relaxation dispersion (NMRD) is a powerful technique for investigating structural and dynamic aspects of heme-HSA and for analyzing allosteric properties that make HSA a peculiar monomeric protein.

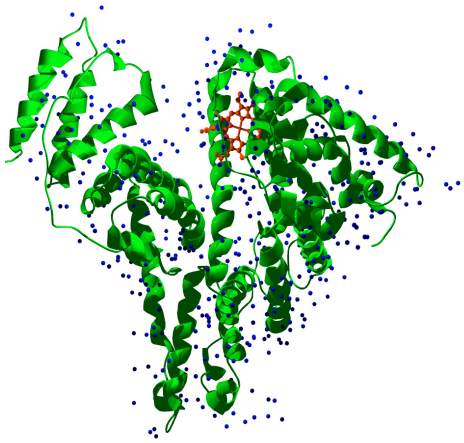

Fig. 1. Ribbon structure of heme-HSA. Heme is rendered in ball-and-sticks. Balls represent water molecules that have been localized in the protein structure obtained by X-ray diffraction (PDB Id: 1N5U)

FROM HIGH SPIN ORDER TO LONGITUDINAL HYPERPOLARIZATION USING PARAHYDROGEN

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The intrinsic low sensitivity of NMR and MRI recently promoted the development of several hyperpolarization techniques, such as DNP, optical pumping of noble gases, ParaHydrogen Induced Polarization (PHIP). Between them, the last is the cheapest and is relatively easier to manage than the other methods.
It is well known that hyperpolarization obtained from parahydrogen manifests itself, in the ^{1}H-NMR spectra, as longitudinal spin order $I_z^{H_1} I_z^{H_2}$ or longitudinal polarization difference $(I_z^{H_1} - I_z^{H_2})$, providing that a PASADENA or an ALTADENA experiment is carried out [1].
On the contrary heteronuclear hyperpolarization obtained with parahydrogen usually consists in longitudinal spin order terms ($I_z^H I_z^X$), therefore net polarization on the heteroatom is zero. As a consequence of this, the heteronuclear signal (in particular 13C hyperpolarized signal) could not be applied to MRI as it is, but spin order must be converted into longitudinal polarization. This task can be achieved using an appropriate field cycling procedure, based on two asymmetric passages, one fast (non-adiabatic) the other slow (adiabatic) from earth field to zero field [2].
In this presentation will be shown that, as far as ^{1}H PHIP is considered, both longitudinal spin order and longitudinal magnetization are *always* obtained, regardless of which kind of experiment is carried out (fig 1). This experimental finding has been explained using a theoretical model based on the density operator approach. This treatment also allows to account for the different contribution of longitudinal spin order and longitudinal polarization observed in the two different kind of experiment.
It will be also shown that the conversion of spin order to net polarization on ^{13}C nuclei by means of the field cycling procedure can be explained on the same theoretical basis developed for ^{1}H-PHIP in the ALTADENA and PASADENA experiments.

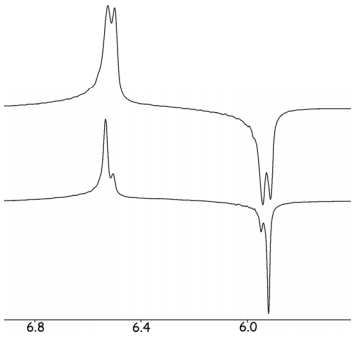

Fig.1: ^{1}H-PHIP in an ALTADENA experiment, 90° pulse (upper spectrum) and 45° pulse (lower spectrum). In the latter spectrum, lower intensity of the inner lines of the doublets is due to the superposition of longitudinal magnetization and two spin order terms.

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FAST FIELD CYCLING NMR FOR QUALITY CHARACTERIZATION OF TYPICAL SICILIAN HONEYS

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Proton NMR relaxometry with fast field cycling (FFC-NMR) setup is developing as a very promising tool for the characterization of agro-food matrices. In fact, some papers have been published reporting on differences among balsamic vinegars, porous properties of oil-water emulsions and quality of meat-products.

In the present study, quality of some typical Sicilian honey samples has been evaluated by traditional wet-chemical analyses and FFC-NMR.

Nine wet-chemical parameters have been measured such as water content, pH, acidity (free, lactonic and total acidity), fructose, glucose, saccharose, electrical conductivity, diastase activity and colour. Results showed that 84% of the samples were correctly classified, whereas about 16% of them revealed some defects primarily due to erroneous industrial production practices and/or wrong storage conditions.

In order to differentiate honey samples according to their botanical and geographical origins, correlation times were measured by FFC- NMR and related to the wet-chemical properties by statistical evaluations. The statistical analysis revealed that water content affected correlation times. Moreover, honeys having similar botanical origin showed differences in their chemical composition, mainly attributable to the geographical areas of production.

The present study reported for the first time quality evaluation of honeys by FFC-NMR, thereby confirming the great potentiality of this technique for agro-food characterization.

Acknowledgments

This work was partially funded by Ce.R.T.A. s.c.r.l. (Centri Regionali per le Tecnologie Alimentari, http://www.certa.it/default.asp)

FORMATION OF METAL-ETHANOL COMPLEXES AS ASSESSED BY HIGH AND LOW FIELD NMR SPECTROSCOPY

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Modern investigation on production of new energy sources for transport industries is addressed towards environmental impact-less processes to obtain bio-ethanol. In particular, second generation bio-ethanol appears as a very sustainable fuel since it can be produced from agricultural by-products, such as cellulose-rich biomasses coming from food industry, without impacting on food demand and costs. In this context, uptake of ethanol (EtOH) from liquid mixtures obtained from glucose fermentation is of paramount importance as an alternative procedure other than the highly-cost energy distillation technique. The aim of the present research was the study of the uptaking properties towards ethanol by metal ions such as Al(III) and Sn(IV). Solid state high field NMR and low field NMR with fast field cycling (FFC) setup were used to monitor the degree of complexation of metals by EtOH. CPMAS ^{13}C, ^{27}Al, and ^{119}Sn-NMR spectra were applied in order to reveal the coordination degree of the metals. FFC-NMR showed that Al(III) was coordinated solely with EtOH, whereas Sn(IV) was very likely coordinated with EtOH and OH^-. On these bases, early conclusions suggest that, in the operating conditions used here, Al(III) was more efficient than Sn(IV) in trapping ethanol due to its larger Lewis acidic capability.

Acknowledgments

This work was partially funded by Ce.R.T.A. s.c.r.l. (Centri Regionali per le Tecnologie Alimentari, http://www.certa.it/default.asp). The high field NMR measurements were done at Centro Grandi Apparecchiature (CGA) – Uninetlab of the Università degli Studi di Palermo.

METABOLIC PROFILING USING ^{1}H NMR AND CHEMOMETRICS: A NEW POWERFUL TOOL FOR DIFFERENTIATING AQUACULTURE SYSTEMS FOR GILTHEAD SEA BREAMS

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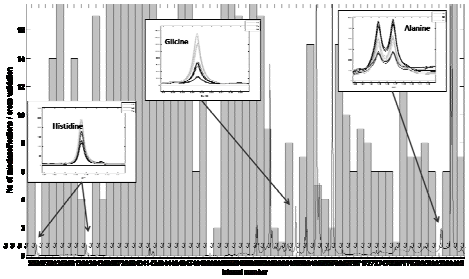
Gilthead sea bream (*Sparus aurata, Sparidae*) is an example of an economic fish [1] species whose market has rapidly expanded in the last decade. It has traditionally been cultured in Mediterranean coastal lagoons and salt water ponds or in the *lagoons* of the northern Adriatic Sea in Italy. Due to the increasing demand, gilthead sea bream is extensively farmed in lagoons, or intensively in tanks or cages [2]. This intensive production under "artificial" conditions has raised problems with the quality of the farmed fish compared with the wild fish. This work describes a metabolic profiling study of gilthead sea bream, from three different aquaculture systems, using ^{1}H NMR and chemometrics. The assignment of all major NMR signals of the perchloric extracts was performed.



A comprehensive multivariate data analysis proved able to distinguish the fish metabolism among the different aquaculture systems and to determine whether a fish was stored or not. The state of energy metabolism of inosine proved a robust biomarker for evaluating storage time. Thanks to a new multivariate classification tool, iECVA [3], several metabolites were found to be important biomarkers for characterizing the three different aquaculture systems: histidine, alanine and especially glycine for long storage times and mainly betaine for fresh samples. The findings represent a step forward in understanding how in vivo and postmortem processes affect the total quality of the final product.

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A FTIR AND SOLID STATE ^{13}C NMR STUDY OF THE ADSORPTION OF BIS-(2-ETHYLHEXYL)PHTHALATE ON HYDROZINCITE

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The interaction of bis-(2-ethylhexyl)Phthalate (DEHP) on hydrozincite [$Zn_5(CO_3)_2(OH)_6$] in controlled laboratory experiments was investigated by using Fourier Transform Infrared Spectroscopy (FTIR) and Solid state Nuclear Magnetic Resonance Spectroscopy (NMR). DEHP is commonly used as plasticizers worldwide and, since it is only physically bounded to the polymer chains, can be leached into food and beverages from the packaging material [1]. Environmental degradation of DEHP can occur by hydrolysis, photodegradation and biodegradation. However, the degradation rates are slow and therefore do not play an important role under typical environmental conditions.

In an attempt to investigate, in vitro, the molecular-level basis for the biomineralization of hydrozincite, in which the biological activity of organisms leads to mineral nucleation and growth, we have observed the presence in the Infrared spectra of several signals which do not pertain to hydrozincite. These signals have been characterized and identified as DEHP. The FTIR and ^{13}C Magic Angle Spinning (MAS) and Cross Polarization Magic Angle Spinning (CPMAS) NMR experiments prove that DEHP is strongly absorbed and partly integrated into the immobile hydrozincite and therefore appears as a solid constituent.

The carboxylic group and the ring carbon intensity of DEHP in the MAS and CPMAS spectra greatly decreased in comparison to the aliphatic carbons. At the same time the overlapping signals of aliphatic chains suggest the presence of structural disorder affecting the DEHP. These results are attributed to immobilization of the molecule when adsorbed on hydrozincite surface and suggest that the adsorption of DEHP may be due to a hydrogen bonding of C=O of DEHP ester polar part of the molecule with OH surface sites on the tetrahedral or octahedral planes of hydrozincite.

This study indicates the potential application of hydrozincite for molecular storage or capture of toxic compounds such as DEHP.

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DIRECT ^{13}C DETECTION FOR BIOMOLECULAR NMR

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Carbon-13 direct detection NMR provides valuable tools for the study of biological macromolecules [1]. Progresses in this area have been initially stimulated by the applications to the study of paramagnetic proteins, where the presence of a paramagnetic center supplies a wealth of additional contributions to relaxation that depend, among other factors, on the square of the gyromagnetic ratio of the observed nucleus [2]. Therefore NMR experiments based on ^{13}C direct detection that only rely on heteronuclei (*protonless* NMR) result particularly useful for the characterization of paramagnetic proteins also in regions where ^{1}H resonances are broadened beyond detection [3;4]. For similar reasons, namely the favourable heteronculear relaxation properties, ^{13}C direct detection NMR experiments can be profitably used to study large macromolecules, where ^{2}H isotopic enrichment is necessary to reduce linewidths at the expenses of the amount of information that can be obtained through ^{1}H NMR spectroscopy [5]. Carbon-13 direct detection experiments result very useful also to study systems that lack a stable 3D structure, where the contributions to signals chemical shifts are drastically reduced causing severe problems of overlap, that are less severe for heteronuclei [6]. In the latter case ^{1}H-start can be used to increase the sensitivity of the exclusively heteronuclear multidimensional experiments, still exploiting only heteronuclear chemical shifts in all the dimensions, since fast transverse ^{1}H relaxation does not constitute a limitation for these kind of systems [7]. With the exploitation of ^{1}H polarization as a starting source for ^{13}C direct detection experiments, pulse sequences can be designed to exploit the accelerated ^{1}H longitudinal relaxation through selective excitation of the attached proton in order to expedite ^{13}C direct detection experiments. Furthermore, fast methods like non-uniform sampling can be easily implemented [8]. All the examples provided demonstrate that ^{13}C NMR direct detection is a mature technique for any kind of biomolecular application.

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SELECTED SPIN STATES FOR LINE-NARROWING AND MAGNETISATION STORAGE

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Long-lived coherences (LLC's), i.e., coherent superpositions of quantum states with different permutation symmetry, are shown to have a slow oscillatory decay, and therefore to yield spectra where the homogeneous line-widths are considerably narrower than in conventional spectroscopy [1]. The effect is illustrated by proton nuclear magnetic resonance spectroscopy of proteins in isotropic solution, where the slow oscillatory decays of long-lived coherences yield spectra with considerably improved sensitivity and resolution. This opens the way to high-field magnetic resonance studies of biomolecules that are almost an order of magnitude larger than could be hitherto achieved.

Dynamic Nuclear Polarization (DNP) and the associated *dissolution* technique couple nuclear spins to the large population reservoir of electrons and make possible the detection of dilute endogenous substances in magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI). We have designed a method to preserve enhanced ('hyperpolarized') magnetization by conversion into Long-Lived States (LLS). It is shown that these enhanced long-lived states can be generated for proton spins, which afford sensitive detection. The magnetization of dilute carbon-13 nuclei in natural abundance has been enhanced by almost four orders of magnitude in the dipeptide Ala-Gly. This polarization was then transferred to a pair of protons and stored as LLS that can be sustained by radio-frequency irradiation. The detection of controlled amounts of magnetization at desired time intervals opens the way to observing slow chemical reactions and slow transport phenomena such as diffusion by enhanced magnetic resonance. In Ala-Gly, the lifetime of LLS associated with the two aliphatic glycine protons was found to be seven times longer than their spin-lattice relaxation time constant, while in the *C*-terminus of Ubiquitin it is six times longer [2].

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DESIGN AND TESTING OF HIGH-FIELD (3-9.4 T) DOUBLE-TUNED SURFACE RESONATORS FOR PROTON AND SODIUM MRI

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High field (3-9.4 T) MRI/MRS is an emerging field for research of the human brain in normal and pathological conditions [1]. One of the main hardware components is the RF resonator (coil) used for transmitting RF pulses and detecting the NMR signal. Double-tuned resonators allow detecting the signal both from protons (^{1}H), for field shimming and anatomical studies, and other nuclei (^{12}C, ^{31}P, ^{23}Na), thus adding important physiological information to the MR images [2].
In this contribution, after a brief review of state-of-art volume and surface RF resonators, we will present the design and testing of novel double-tuned (^{1}H/^{23}Na) RF surface resonators suitable for 4 T and higher field. The novel surface resonator, see Fig. 1, is made by two independent sets of strip-lines tuned to the frequency of interest [3]. The resonant modes were calculated by means of Multi-Transmission-Line theory [4]. A prototype was build and tested on the workbench using a network analyzer and suitable phantoms [5]. We report experimental 4 T MRI results with phantoms and *in vivo*, showing the feasibility of the design. This resonator should find useful applications for a range of bio-medical applications, including human musculoskeletal diseases, subcutaneous tumours and brain mapping studies.

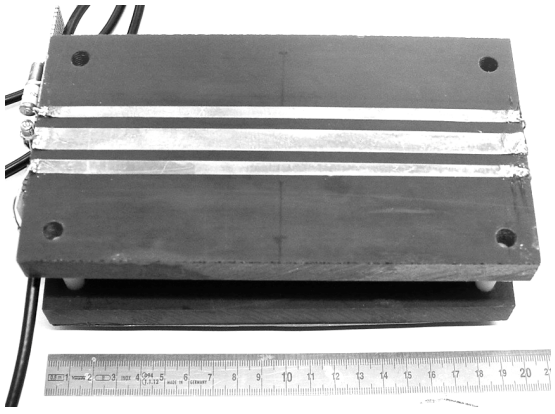

Fig. 1. Picture of the 4 T double-tuned surface micro-strip resonator composed by: two identical copper elements (length 190 mm, width 5 mm, thickness 36 μm) externally positioned and tuned to the sodium frequency; and one copper element (length 190 mm, width 10 mm, thickness 36 μm) centrally positioned and tuned at the proton frequency.

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PRACTICAL ASPECTS OF DOSY

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Diffusion sequences are among the most demanding gradient experiments in liquids NMR. For reliable results the measurements must be carried out very carefully either to avoid convections or correcting for them by proper pulse sequence design. It is equally important that data processing does not separate mathematical correctness and physical meaning. Practical aspects of both acquiring and processing diffusion NMR data will be presented.

CHARACTERIZATION OF ARCHEOLOGICAL WATERLOGGED WOODS BY NUCLEAR MAGNETIC RESONANCE

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Historical or archaeological wooden objects are generally better conserved in wet environments respect to other contexts. Nevertheless, waterlogged wood is slowly degraded by the action of anaerobic erosion bacteria, which cause the loss of cellulose and hemicellulose, leading to the formation of cavities filled with water. During this process, lignin can be also altered. The result is a porous and fragile structure, poor in polysaccharides and mainly composed of residual lignin, which can easily collapse when drying and needs specific consolidation treatments. Due to this reason, the chemical characterization of archaeological wood and lignin are aspects of primary importance in the diagnosis and conservation of waterlogged wood artifacts.
High resolution nuclear magnetic resonance (NMR) spectroscopy has been one of the most important analytical techniques for 40 years, nevertheless its impact on archaeology until recently has been minimal for some clear reasons well described by Lambert[1]. Recent developments have dramatically changed this situation and now this technique is used with good results also in the field of cultural heritage. In particular, the possibility to determine and quantify functional groups and intermonomeric bonds in lignin by NMR is demonstrated and the high-resolution nuclear magnetic resonance of ^{13}C, developed in the field of geochemistry to characterize fungal degraded wood[2] and to evaluate lignin in organic matter[3] and sediments[4], was employed to characterize archaeological wood samples from the 11th century excavation site in the lake Paladru at Charavines, France[5]. The main result observed was the preservation of the most abundant intermonomeric bond in lignin structure, so called β-O-4. The solid state ^{13}C-NMR is not enough sensible to characterize and quantify the other important intermonomeric bonds present in lignin structure, and with this technique is not possible to observe and quantify the important functional groups such as carboxylic and alcoholic moieties. In order to avoid this problem new NMR analytical tools have been adopted, such as qualitative and quantitative 2D-HSQC, quantitative ^{13}C-NMR and ^{31}P-NMR analysis which permit to achieve a complete picture of lignin chemical features.
In this study we investigate by NMR the chemical alteration of lignin and wood in lignocellulosic samples from the archaeological site of the Ancient Ships of San Rossore (Pisa Italy), where over the last ten years thirty-one Roman shipwrecks dating between 2nd century BC to 5th century AD have been discovered[6,7,8].

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PRELIMINARY SOLID STATE NMR CHARACTERIZATION OF WOODEN ARCHAEOLOGICAL SAMPLES

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This work describes the preliminary solid state NMR characterization of wooden samples of archaeological interest. These samples came from the wooden part of the roman rostrum found in the sea of Acqualadrone (Italy). The NMR analyses were performed because the assessment of the conservation state using physico-chemical techniques constitutes a preliminary step in conservative and restorative measures.
^{13}C $\{^{1}H\}$ Cross Polarization Magic Angle Spinning NMR was used to identify the wood components. Experiments of Non Quaternary Suppression (NQS) were performed in order to obtain information about the condensation degree of the lignin. Furthermore the crystallinity degree of the cellulose was obtained through the deconvolution of the C4 signals of the glucose unit.
A quantitative comparison of the holocellulose content was also performed.
The NMR analysis relating to the wooden samples are in agreement with evidences obtained through other techniques such as Fourier Transform Infrared spectroscopy (FT-IR), Gas Chromatography – Mass Spectrometry (GC-MS) and X-Ray Diffraction (XRD).
The results indicate a different state of conservation of the samples and, at least in some places, the spreading of caulk on the wood, probably using a vegetable resin.
Furthermore the crystallinity degree of the cellulose, the degree of the condensation of the lignin and the holocellulose have not homogeneous values for the samples. In particular all these properties are strictly correlated with the deep of sampling.

MULTITECHNIQUE APPROCH TO THE STUDY OF ANCIENT CERAMIC MATERIAL

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The thermal decomposition of clay is one of the most studied ceramic reaction. In fact, a complete description of these processes can be very useful both for optimizing the industrial processes and to investigate the ancient firing technologies. The study of the clay and of its thermal modifications is very interesting and very difficult due to its complexity and variability. Therefore two clays coming from Deruta, a town very famous in the Renaissance for its ceramic art, were chosen for this study. The first clay is commonly nowadays used by ceramists and the second was found during an archaeological excavation of a ceramist workshop dated between the end of the XVI and the beginning of the XVII century. The two clays differ in composition for the Ca content. The samples were fired at different temperature between 600 and 1100° C and were studied by different techniques. Mössbauer and Raman spectroscopes allow a wide characterization of the Fe-rich phases content, while ^{29}Si MAS NMR and ^{27}Al MAS and ^{27}Al 3Q MAS NMR techniques permit a detailed description of the aluminum-silicate phases. With the increasing of the firing temperature the structural modification of the clays are observed. The amount of Ca plays a determinant role in the formation of new high temperature phases and therefore in the degree of crystallinity of the resulting ceramic material.
The aim of the study is to obtain information on the ancient firing technology, therefore the spectra on the studied samples will be used as reference for the spectra of ancient ceramic shards having the same provenience.

KINETICS OF WATER VAPOR ABSORPTION IN POLLUTED POROUS MEDIA STUDIED BY TIME DOMAIN NMR

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Water vapor condensation and salt deliquescence inside the pore space are responsible for deterioration of porous materials when exposed to environmental injury by pollution in a humid atmosphere.
In this study Nuclear Magnetic Resonance relaxation of liquid water ^{1}H nuclei has been applied to study the kinetics of absorption in real porous media. Three lithotypes were selected for their similar composition (carbonate rocks) and different pore space architecture, polluted with different amounts of $Ca(NO_3)_2 \cdot 4H_2O$ and then exposed under controlled conditions to water vapor absorption. T_1 and T_2 relaxation time distributions of liquid water inside the pore space have been studied in the course of time of exposure of the samples to humid atmosphere (RH= 75-76%, T=25°C) [1].
NMR measurements of amounts of water, together with relaxation time distributions, give the possibility of information on the effect of pollution in porous materials exposed to humid atmospheres.
A difference is observed between absorption on a flat surface and in porous media that cannot be explained by the amount of absorption by the rocks in the absence of salt. The distributions show the effects of both the salt concentration and the pore space structure on the amount of water vapor condensed and its kinetics. For a given lithotype, even with different amounts of pollutant, the rate-average relaxation time T_{1ra} tends to increase monotonically with NMR signal, proportional to the amount of liquid water. This behavior suggests a trend toward the filling of larger pores as amounts of liquid water increase, but it does not indicate a strict sequential filling of pores in order of size and starting with the smallest; in fact, relaxation time distributions show that this is not the case. Any part of the fluid that was originally condensed in layers or in large pores seems likely to migrate by capillarity over time to smaller pores. The larger the amount of salt in the rock, the larger and the faster is the water vapor absorption and there are differences in the rate and amount of absorption by different rocks even with the same amounts of salt.
In conclusion NMR relaxation analysis, including total signal evaluation and relaxation time distributions, allow us to follow the absorption kinetics and show how the pore space architecture affects this very complicated phenomenon. These phenomena are of importance also in other fields, such as the exploitation of geothermal energy.

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^{1}H MRS OF TUMOUR CELLS IN HYPOXIC AND HYPERAMMONIA ENVIRONMENT

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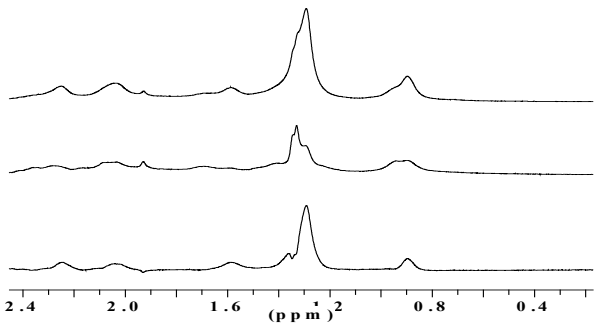
High resolution ^{1}H MRS provides information on biologically active molecules in intact cell systems, allowing the study of cell metabolism in vitro. Among the signals that can be monitored in vitro as well as in vivo, lipid signals attributed to fatty acid chains in neutral lipids, triglycerides, often referred as mobile lipids (ML), have elicited much attention, due to their occurrence in cancer cells. Many authors suggested that ML intensities could provide new tools to correlate the spectral observations to biologically relevant events, such as apoptosis [1,2] or necrosis [3]. More recently [4] it was suggested that intensities of ML signals could be related to changes in lipid synthesis and breakdown resulting in changes of the triglyceride pool. In the present study, unrelated tumor cell lines from human adenocarcinomas and gliomas are monitored to examine the effects of hyperammonia and hypoxia in tumour cells in culture in order to provide more insight on these metabolites.
Hypoxic cells were kept for 24 hours under 2% O2 before NMR experiments. ^{1}H NMR spectra were run at 400.14 MHz on a digital Avance spectrometer (Bruker, AG, Darmstadt, Germany) equipped with a 1 mm microprobe. Signals were acquired with a 90° RF pulse and a sweep width of 4006.4 Hz. Water suppression was obtained by irradiating water signal. ^{1}H NMR spectra from were run after 24 hours of mild hypoxia and compared to samples kept in normoxic conditions. The figure shows one exemplificative experiment run on HeLa cells. The lower trace is the difference between treated (upper trace) and control (intermediate trace) samples.



ML signal from methylene protons, at 1.28 ppm, increases after hypoxia: some authors showed increases in triglyceride concentration after hypoxia [5-6]. Preliminary results showed similar effects, though less marked, in cells after hyperammonia treatment.

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INTERMEDIATES OF PROTEIN GLYCOSYLATION OBSERVED IN TUMOUR CELLS BY ^{1}H MRS

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Protein glycosylation is frequently studied due to the growing importance of the dynamic changes in glycosylation. Protein glycosylation is species- and cell-specific and it is determined by the protein backbone and sugar attachment site and some aspects of sugar metabolism seem to be involved in tumour progression [1]. Moreover, the changes in glycosylation levels in response to extracellular stimuli suggest a key role in signal transduction pathways, with important effects on cell life. In principle, ^{1}H MRS can be used to observe sugar-related metabolites in intact cells. In the present study, we studied major hexosamine precursors in different tumour cell lines.
^{1}H MR spectra of cells from human adenocarcinomas (HeLa and MCF7 cells), glioma (T98G) and astrocytoma (A172) were run and compared to the spectra from solutions of UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine (UDP-GalNAc). Signals arising from the UDP portion of the molecule were detected and assigned for the first time in intact cells, in agreement with previous data from PCA extracts [2]. Signals from amide protons were also detected and assigned.

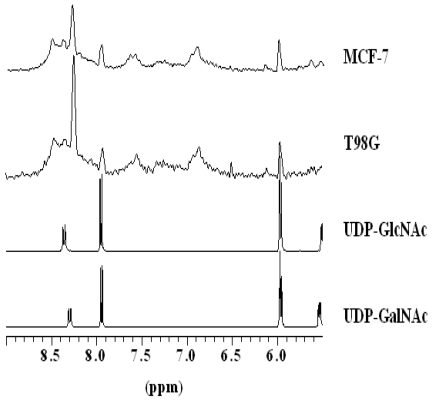

Figure: The low field spectrum of MCF7 and T98G cells compared to solutions of the UDP hexosamines

Elevated NH_4 concentration, that specifically inhibits glycosylation in glycoproteins [3], resulted in high intracellular hexosamines in MCF7 cells, less evident in the other cells. Treatment with NH_4Cl showed the specific response of cell lines of different origin with respect to of protein glycosylation.
Furthermore, free N-acetylgalactosamine was detected in HeLa cells, suggesting a role for MRS in detection of a defective or altered mucine glycosylation state.

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NOVEL PROBES TARGETED TO CELL SURFACE THIOLS FOR CELLULAR MR IMAGING

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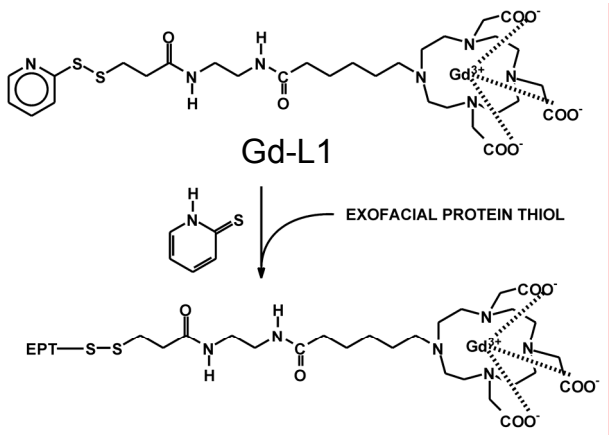
We have very recently described a new method for labeling cells with Gd(III) based CAs exploiting the chemical reactivity of protein thiols that are exposed on the outer surface of cell membranes [1,2]. The imaging probe we synthesized (Gd-L1, Figure 1) is composed of a contrastographic unit based on a Gd(III) chelate, a reactive moiety for the recognition of thiols based upon the 2-pyridyldithio function, and a flexible linker connecting them. This probe can bind to Exofacial Protein Thiols (EPTs) through a covalent disulfide bridge, leading to a very satisfactory level of cell labeling. In this work we investigated the structural determinants of the paramagnetic labels that are at the basis of a high labeling efficiency. Besides compound Gd-L1, three novel Gd(III)-complexes based either on the Gd-DO3A or the Gd-DTPA chelating cage have been synthesized and evaluated for their ability to label human myeloid leukemia K562 cells. All probes were found to promptly react with EPTs. However, the DO3A-based probes could efficiently label cells, whereas the DTPA-based ones could not. The extent of uptake of the DO3A-based probes was found to be proportional to the concentration of EPTs on the cell surface. Chemical blocking of EPTs resulted into the inhibition of the uptake. Lactate, that can coordinate the Gd(III) ion in Gd-DO3A-like probes thus making a ternary complex, was found to strongly inhibit the uptake of the DO3A-based probes. These results suggest that the labelling efficacy is related to the internalization of the probe occurring after the formation of the probe/EPTs disulfide bridged covalent adduct. Such internalization is likely triggered by the coordination of the Gd-DO3A metal ion by cell surface carboxylates. Finally, the proposed labeling procedure has been extended to cells other than K562 cells, initially chosen as a model system, to demonstrate its general applicability.



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IS HUMAN LIVER FABP A GOOD CARRIER FOR MAGNETIC RESONANCE IMAGING CONTRAST AGENTS?

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About one-third of MRI clinical scans are carried out in the presence of gadolinium-agents because they add relevant physiological information to the superb anatomical resolution attainable with this technique. The most important property of a paramagnetic agent is its high relaxivity, referred to the ability to enhance the relaxation rate of solvent water protons. The four commercially available MRI contrast agents are Gd(III) complexes.
The binding affinities of a selected series of Gd(III) chelates molecules to a liver cytosolic fatty acid transporter, have been determined through relaxivity measurements. Among all the ligands tested, two have been selected for further analysis given their high affinity toward the protein: $AAZTAC_{17}$ [1] and B22626 [2]. The former consists of a long aliphatic chain bound to the AAZTA coordination cage, the latter has a bile acid-like body linked to the basic unit of DTPA which chelates Gd.
A variety of NMR experiments have been carried out on the two ligands, using both diamagnetic Y(III) and paramagnetic Gd(III) complexes, bound to the human Liver Fatty Acid Binding Protein (hLFABP).
The aim of this study is the identification of the essential features of the interaction and the location of the binding site of the selected ligands on hLFABP. In particular we performed NMR titrations, competition experiments, and T1 experiments exploiting the relaxation properties of Gd complexes.
Moreover, the $AATZAC_{17}$ molecule is able to form micelles at concentrations greater than 0.1mM, so we performed an NMR analysis in order to identify spots on the protein surface involved in the binding to the micelle.
These preliminary studies will serve in a medicinal chemistry approach to the design of new Gd-based contrast agents for MRI.

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MAGNETIC RESONANCE IMAGING FOR FOODSTUFF QUALITY EVALUATION

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Magnetic Resonance Imaging (MRI), known mainly for the applications in the medical-diagnostic field,[1, 2] is becoming a popular analytical tool in food analysis.[3, 4] MRI spectroscopy is a non-destructive and non-invasive technique and offers the powerful opportunity of studying food in its wholeness without any preparative manipulation of the sample. ^{1}H-MRI provides highly spatially resolved images of internal sections or volumes of any food with molecules characterized by relatively short correlation times, e.g. water, fatty acids, sugars, etc. Images can be obtained with different weighing factors (predominantly spin density, relaxation times and diffusion coefficient) chosen depending upon the structural feature to be highlighted. The information contained in MRI images are applied in food science for the postharvest quality analysis, elucidation of internal morphology, histology, etc., of several foods, most likely fruits and vegetables.
Changes occurring during post-harvest as a function of temperature and atmosphere composition have been investigated for hazelnuts and kiwifruits.[5] For the latter also the effect on shelf-life of Plant Growth Regulators was assessed. The use of arsenic contaminated irrigation water on radish quality was studied; and variation of truffle quality during storage and fungi attack were addressed. Also the effects induced by novel bio-stimulator based on bio-available orthosilicic acid applied to grape wine, kiwifruits, tomato and strawberry was elucidated by means of MRI.

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*i*COshift: A VERSATILE TOOL FOR THE RAPID ALIGNMENT OF NMR SETS

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The increasing interest towards metabolomics and metabonomics takes advantage from the Nuclear Magnetic Resonance (NMR) spectroscopy that in many cases is able to replace laborious and time consuming chemical analysis, providing an overwhelming quantity of chemical information. However, several physical and chemical factors can affect the absolute and the relative position of an NMR signal. Though a large number of algorithms able to deal with misalignment of NMR signals have been recently published, an open source tool able to handle, in a simple but customizable way, the different cases that can occur, is still missing. As a matter of fact, methods designed for the alignment of chromatographic data such as, for instance, COW (Correlation Optimized Warping) will also affect peak shape because the alignment is achieved by local compressions/expansions of the chemical shift axis (1, 2). On the contrary, algorithms based on rigid shift models have proved to achieve the best performances for NMR data, as the line shape is safely preserved. Thanks to FFT-CC (Fast Fourier Transform Cross-Correlation) these inherently computational intensive methods are now capable of handling huge NMR datasets in a reasonable time (3, 4); as a consequence, they allow working with full spectral resolution avoiding all kind of down-sampling steps such as, for example, bucketing. Being part of the last mentioned group, the *i*COshift algorithm presented here is a comprehensive tool intended for dealing with all kind of signal aligning problems, letting the user choose among a large variety of options, from a fully automated correction ranging over the full spectrum, to a supervised and targeted intervention covering only a selected spectral region. The algorithm proved to be faster than similar methods found in the literature, taking advantage from the largeness of datasets and hence making feasible a full resolution intervention. The Matlab code and the relative documentation will be made freely available from the download section of the website: www.models.life.ku.dk

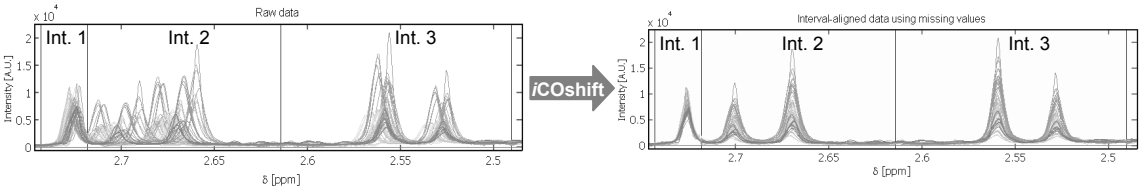

Fig. 1. An example of using *i*COshift for coping with a case of multiple misalignments.

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USE OF NMR SPECTROSCOPY IN THE EARLY DETERMINATION AND QUANTIFICATION OF IN VITRO METABOLITES

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Early in vitro metabolite identification is crucial to the drug discovery process as it can be used to predict the Phase I metabolites that are likely to be formed in vivo, to highlight differences between species in drug metabolism and characterize the major circulating metabolites of an administered drug. In addition, an early assessment of metabolic pathways provides valuable information on the major metabolic liabilities in the compounds under investigation. This information can provide guidance to chemists for synthesis of compounds, which have a greater degree of metabolic stability and, therefore, improved pharmacokinetics (1, 2).

Mass spectrometry is the preferred tool for metabolite identification thanks to its sensitivity, selectivity, reproducibility and robustness. However, this method bears some limitations in a precise identification of metabolic structure modifications and in the quantitative profiling, as the ionization efficiency of metabolites can vary greatly among each other and can be quite different from that of the parent compound. Furthermore, in order to obtain accurate quantifications, metabolite calibration curves need to be generated. This requires the knowledge of the presence of a metabolite and its chemical synthesis.

To overcome these limitations, NMR methods have been evaluated as a qualitative and quantitative tool to be used during the early stages of the drug discovery process. In this presentation, it will be shown how major non-labelled metabolites can be biologically produced in vitro in sufficient amounts for structural elucidation and how accurate concentration measurements can be carried out by NMR in the absence of synthetic standards. This information can be used in LC/MS based assays in later discovery stages.

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MR CONTRAST WITHOUT PARAMAGNETIC IONS

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The evolution of magnetic resonance imaging (MRI) has been astounding since the early 1980s and a broad range of applications has emerged. The strength of the MRI technique relies on its very high resolution, which allows anatomical details to be visualized with great accuracy, and on the variety of contrast parameters that can be exploited to differentiate soft tissues. In those cases where insufficient intrinsic contrast is observed, the use of contrast agents (CAs) is of great assistance. At present, the exogenous contrast agents available mainly fall into three categories, i.e. gadolinium chelates, manganese chelates and superparamagnetic iron oxide particles [1].
Recently, great interest has emerged toward the possibility of developing multimodal CAs, with special focus on molecules already accepted for clinical use in different techniques (X-ray, ultrasound). Iodine-containing CA (X-ray) and microbubbles (ultrasound) have chemical and magnetic properties that make them suitable also for MRI, thus providing a new class of metal-free multimodal CA. These molecules posses different properties with respect to traditional MRI agents, which in turn give rise to new potential applications. Labile protons in iodine-containing CA can be exploited as a pH responsive probe in MRI thanks to the so-called chemical exchange saturation transfer (CEST) effect [2]. The slowly exchangeable protons of the iodine-containing CA produce a pronounced contrast when saturated by an appropriate RF irradiation field. As the exchange rate of the labile protons depends on the pH of the environment, tissue pH can be imaged in vivo from the intensity of the CEST effect. Gas-filled microbubbles, on the other hand, can be used as an MR susceptibility contrast agent in vivo due to the induction of large local magnetic susceptibility differences by the gas-liquid interface. Moreover, microbubbles can serve not only as diagnostic tool but also as drug vectors in brain imaging [3].
In this presentation, the main preclinical results obtained by using iodinated compounds and gas-filled microbubbles as MRI CA will be reviewed.

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IN VIVO BRAIN TEMPERATURE MAP USING jMRUI VERSION 4: A PLUGIN DEVELOPMENT

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Introduction: Magnetic resonance spectroscopy can provide a non-invasive approach to measure the internal temperature of the brain; it relies on the linear relationship between the ^{1}H resonance frequency of water and temperature in the tissue. The absolute temperature is obtained by measuring the chemical shift of water relative to a reference compound such as N-Acetylaspartate (NAA). To convert the frequency difference between these two signals into temperature, it is necessary to apply a particular calibration curve [1].
All these procedures can be performed using a novel plugin for jMrui, a software package that allows time-domain analysis of nuclear magnetic resonance signals [2]. In this work, we used jMRUI v4.0, a new version of the software that enables the users to add their own plugins.
Method: Spectra coming from the scanner are analysed using jMrui; with the quantification algorithm AMARES. The frequencies of the water and NAA peaks are estimated. The temperature plugin reads these frequencies and calculates the difference Δ. This Δ is inserted into a suitable formula that estimates the temperature. After temperature estimation, the MRSI mode of the plugin produces a map and superimposes it on the corresponding MR anatomic image (see Fig. 1).

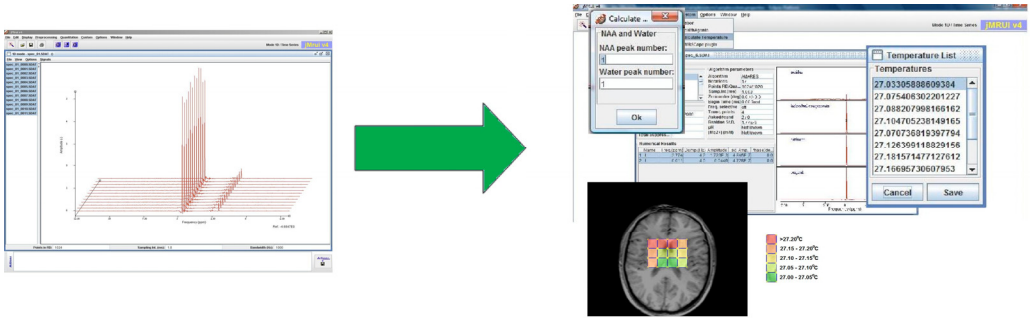

Fig. 1. Scheme of the plugin method to estimate and map brain temperature. On the left the spectra coming from the scanner are shown; on the right the result window, together with the temperature map superimposed onto the anatomic image.

Conclusion: NMR brain temperature could provide useful information for many thermal therapies such as hypo/hyperthermia and it could give us useful diagnostic information. The use of the jMRUI plugin could speed up the procedure to obtain the temperature map, so it could be possible to think to provide such a tool in the clinic and during surgery.
Acknowledgment: This work is supported by the EU Marie Curie Research Network 'FAST', MRTNCT-2006-035801.
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MRI ASSESSMENT OF THE THERAPEUTIC EFFICACY OF PARAMAGNETIC LIPOSOMES LOADED WITH AN ANTITUMOR DRUG

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The glucocorticoid prednisolone phosphate (PLP) encapsulated in long circulating liposomes (LCL) has been demonstrated to efficiently inhibit the growth of solid tumors on mice [1]. The mechanism of action of this passively targeted nanomedicine appears to involve the inhibition of pro-angiogenic/pro-inflammatory factors [2]. Recently, there has been a growing interest in the development of non (or limited) invasive *in vivo* protocols for visualizing the drug-delivery process. To this aim, Magnetic Resonance Imaging (MRI) is the technique of choice in virtue of the exquisite spatial resolution and great ability to generate contrast in soft tissues. The main scope of this work was to demonstrate the potential of this imaging modality for assessing the therapeutic effect and biodistribution of LCL-PLP on B16 melanoma xenografts on mice. The MRI visualization of the liposomal drug was possible by incorporating an amphiphilic Gd(III) complex in the liposome bilayer. The study was also supported by an *in vitro* kinetic analysis aimed at assessing the release of the drug from the paramagnetic liposomes. An amphiphilic Gd(III) complex (Gd-1) was synthesized as described in [3]. Liposomes (LCL-Gd-PLP) were formulated as DPPC/Chol/DSPE-PEG/Gd-1 (1.85/1/0.15/0.27) encapsulating 100 mg/ml of PLP. The paramagnetic liposomes were injected (doses: PLP 10 mg/kg, Gd 0.05 mmol/kg) in the tail vein of male B16 melanoma-bearing mice (the experiments were performed in triplicate). Tumor size and T_1 enhancement (in tumor, liver and spleen) were monitored at 7 T. The mice received two doses at time 0 (tumor size ca. 4mm) and 1 week after. The release of PLP from the liposomes was monitored in vitro at 37°C at pH 5.5 and 7.4.

The MR contrast observed in the tumor after each injection of LCL-Gd-PLP demonstrates a good tumor uptake. A remarkable uptake from liver and spleen was also detected. The inhibition of tumor growth was significantly higher than the control (empty liposomes) and in agreement with the previous study [1]. The in vitro kinetic study of the PLP release from the liposomes indicated a high stability of the formulation at physiological pH, whereas at pH 5.5 the presence of the incorporated MRI probe led to a PLP release of about 20%.

The possibility to design a liposomal carrier loaded with both the drug and the MRI probe allows the non invasive visualization of the biodistribution of the nanomedicine. Moreover, the high resolution of MRI considerably improves the accuracy in the assessment of the tumor growth.

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INTERACTION BETWEEN PLATINUM COMPLEXES AND COPPER TRANSPORT PROTEINS

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Cisplatin (cis-$[PtCl_2(NH_3)_2]$) and other platinum (Pt) complexes used in anticancer therapy, such as carboplatin and oxaliplatin, are able to cross the cell plasma membrane and eventually reach the nucleus, where they form adducts with DNA which are at the basis of their antitumor activity [1, 2]. Copper (Cu) transporters regulate the cellular pharmacology and sensitivity to Pt-based drugs. The high affinity Cu permease Ctr1, located on the plasma membrane, is involved in cisplatin uptake [3], while the Menkes and Wilson Cu-transporting ATPases regulate its efflux [4]. Moreover, the cytosolic Cu chaperone Atox1 has been found to translocate to the nucleus where it acts as a Cu-dependent transcription factor, thus representing a candidate nuclear carrier for Pt [5].
As a first step in understanding the chemical mechanisms of cellular transport of Pt-based drugs, we investigated the coordination properties of the Cu(I)-binding motifs of Ctr1, Atox1, and the first soluble domain of the Menkes ATPase (Mnk1) towards different Pt complexes by NMR spectroscopy and circular dichroism, and we determined the stoichiometry of the adducts by electrospray-mass spectrometry [6, 7].
Cisplatin binds to methionine-rich motifs of Ctr1 (Fig. 1) and to cysteine motifs of Atox1 and Mnk1 (Fig. 2), but only in the latter case the drug retains its ammine ligands essential for antitumor activity. A different reactivity was observed for other Pt complexes and ascribed to the diverse nature of the Pt ligands, the stability of the chelating rings, and the *trans*-labilizing effect of the Pt donor atoms.
The results are discussed in the context of current views on the mechanism of action of Pt drugs [8].

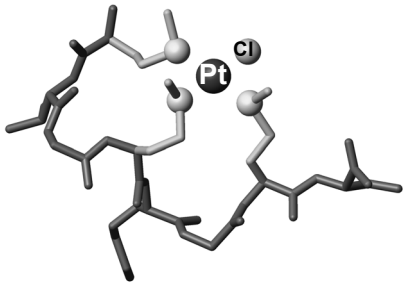

Fig. 1. Structural model of the adduct between cisplatin and the methionine-rich motifs of Ctr1.

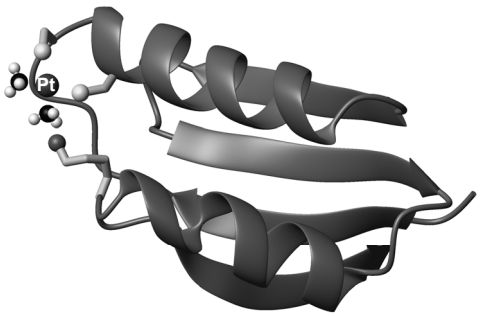

Fig. 2. Structural model of the adduct between cisplatin and Atox1.

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TUNING THE FOLDING STATES OF β-AMYLOID FRAGMENT Aβ(16-35) WITH DIFFERENT CHARGE OF MEMBRANE MIMICKING SYSTEMS

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Senile plaques composed of fibrillar aggregates of amyloid β peptide (Aβ) are a characteristic hallmark of Alzheimer's disease. β-amyloid peptides (Aβ), 39-43 amino acid long, derive from the proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases. [1] Depending on conditions, amyloid peptides undergo a conformational transition from random coil or α-helical monomers to the highly toxic β-sheet oligomers forming the mature fibrils. In view of the fact that β-amyloid peptides are enzymatic product of transmembrane protein APP, β-amyloid peptides have been extensively investigated for their ability to interact with plasma membrane and mounting evidences show that membrane components could trigger the initial events leading fibrillogenesis [2].

In the present work we present NMR and EPR conformational studies of Aβ(16-35), in membrane mimicking environments characterized by different charge contents. Aβ(16-35) represents the hydrophobic central part of β-amyloid peptide. It is proved to be implicated in aggregation/disaggregation processes, and it is focus of attention in the search for peptide or peptidomimetic inhibitors of Aβ fibrillation [3]. Moreover, in an anti-amyloid approach named anti-amyloid immunotherapy [4], this fragment is under investigation for the possibility to be specifically bound by antibodies. NMR and EPR studies show that the charge of membrane biomimics may be critical in regulating the transitions of amyloid β peptide through different folding states and the aptitude of the different portions of peptide to interact with membrane surface.

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ON XENON AS A NEUTRAL, INDEPENDENT BIOMOLECULAR PROBE

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It is by now well recognized that the Xenon atom can be used as a suitable biomolecular probe for the characterization of both solid porous materials and biomolecules in solution. Its peculiar physicochemical properties make Xenon appropriate for characterizing the structure of biological macromolecules by means of the two most widely used techniques, i.e. X-Ray crystallography and NMR. By exploiting the high sensitivity of this probe to its local environment, Xenon is often used in NMR studies aimed at finding a correlation between structural and functional properties of even large proteins. For instance, studies on protein cavities and their function as docking sites for ligand diffusion within protein networks have often exploited this biomolecular probe. In this regard, NMR is able to provide dynamical information on the interaction of this probe (guest) with the biological systems (host), while X-Ray crystallography, even if it is more frequently employed, is able to provide just a static average picture of an otherwise fluctuating molecule.
We have investigated so far several different biomolecules and biomaterials by Xenon NMR, both in solution and in the solid state. It is easily verified that the highly informative nature of this technique, which is able to provide useful hints on the size, shape and chemical composition of the local environments sampled by the probe, should now be more crytically assessed. In this regard, we believe that particular attention should be addressed to the relevant question on whether Xenon can be considered as an independent probe, as we demonstrated that it is sometimes able to interact with the host by modifying its structural characteristics and sometimes its biological function.

NMR STUDIES OF A NEW POTENT MMP-12 INHIBITOR: INSIGHT INTO KEY BINDING INTERACTIONS

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Matrix metalloproteinases (MMPs) constitute a family of more than 20 structurally and functionally related enzymes which are involved in tissue remodeling and wounds healing [1]. MMPs are zinc-dependent endopeptidases, able to degrade all the components of the extracellular matrix (ECM). Among these, macrophage metalloelastase (MMP-12) is mainly produced by macrophages and seems to be involved in acute and chronic pulmonary inflammatory diseases associated with intense airway remodelling, such as chronic obstructive pulmonary disease (COPD) and emphysema [2].

We report herein the design, synthesis and *in vitro* evaluation of a new series of compounds possessing an arylsulfonyl scaffold as potential selective MMP-12 inhibitors. Five phenylacetic hydroxamates were identified to have nanomolar IC_{50} values on MMP-12. Among them, the best compound showed an IC_{50} of 0.2 nM, with a good selectivity over MMP-1 and MMP-14.

NMR experiments on ^{15}N labeled catalytic domain of the protein and docking calculations were used to elucidate the structure of the complex. The *p*-methoxyphenoxybenzene group was found inserted into the S1' pocket. In addition, the formation of an hydrogen bond involving amide proton of A182 and one sulfonyl oxygen together with the rigidity of the inhibitor in the vicinity of the Zinc-binding group seem to be crucial for strong binding.

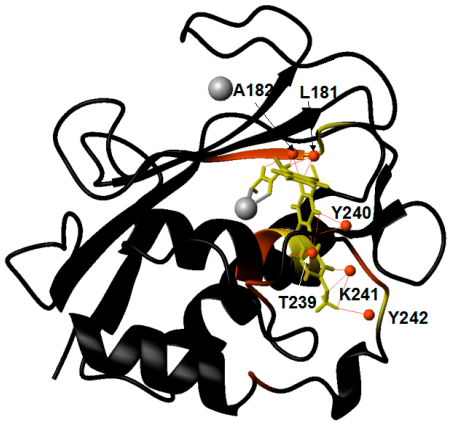

Fig. 1. MMP-12 interacting with a strong inhibitor as determined by docking and NMR.

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UNDERSTANDING THE MOLECULAR PROPERTIES OF COMPLEX SOL-GEL HYBRID MATERIALS THROUGH SOLID-STATE NMR

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Sol-gel process is one of the most convenient preparation method for obtaining a huge variety of hybrid materials, in which organic and inorganic components are combined at a nanometric level [1]. These materials exhibit very peculiar properties, which make them attractive for many different applications, for example as protective and functional coatings for polymeric substrates [2]. Despite their extensive use, the "microscopic" features related to their observed macroscopic properties are still often quite unclear and difficult to be experimentally investigated. Solid-state NMR (SSNMR) is at present one of the most powerful techniques for obtaining useful information on both structural and dynamic molecular properties of organic-inorganic composite materials, its applications being continuously increasing [3-6]. Here we present an extensive SSNMR study performed on a large set of two- and three-component organic-inorganic systems obtained by sol-gel process, which exhibited interesting oxygen barrier properties, strongly dependent on chemical composition. In the perspective of getting some insights into the molecular properties behind these macroscopic performances, the SSNMR study was aimed at obtaining a detailed characterization of the structural and dynamic properties of the organic and inorganic components, including inorganic domains chemical structure and condensation degree and organic oligomeric component conformational and phase properties, as well as the most important interactions occurring among the different components. To this aim a large set of high-resolution multinuclear 1D and 2D SSNMR techniques have been applied, including ^{29}Si quantitative and ^{13}C selective and quantitative experiments, ^{1}H-MAS at one of the highest spinning rate available at present (60 kHz, courtesy of Varian), 2D ^{1}H Double Quantum and ^{1}H-^{29}Si and ^{1}H-^{13}C HETCOR experiments.

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SOLID STATE ORDER TO ORDER TRANSITIONS IN BLOCK COPOLYMERS AS DETECTED BY HIGH AND LOW FIELD NMR

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Polymer self organization on nanometer scale attracted significant interest in the last decade because complex regular morphologies can be produced by selecting synthesis and thermal treatment parameters. In this work we used NMR to characterize a triblock copolymer made up of polystyrene (PS) and ethylene butene (EB) blocks with starkly different glass transition temperature T_g, comparing the results and potential with other techniques like DSC, XRD, SAXS and TEM. In Time Domain NMR (TD-NMR) performed at low field, spectral features like chemical shift are not detected but mobile and rigid fractions of a sample can be resolved by means of different relaxation.

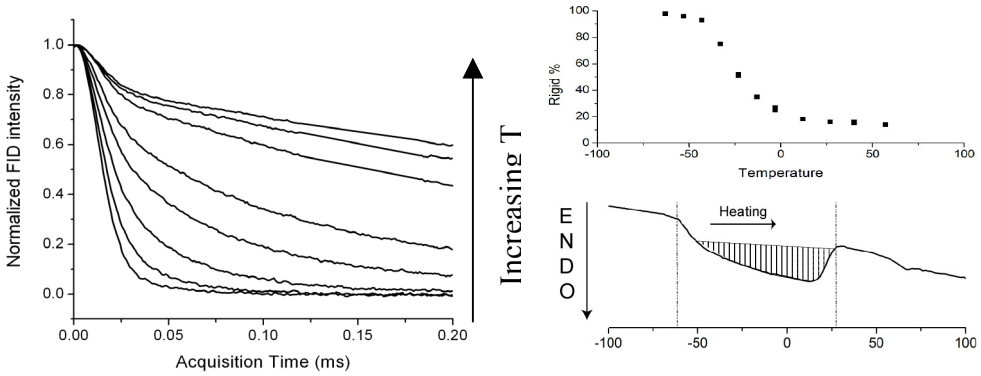

Fig. 1. On the left, FIDs for several different temperatures, on the right the resulting rigid fraction compared to the concurrent DSC trace.

FIDs like the ones in Fig.1 display bimodal relaxation and are composed by a Gaussian decay due to the rigid polymer fraction and by a stretched exponential decay due to the mobile fraction. The T_2* of the rigid phase is around 15 μs, comparable to the receiver dead time, making quantitative detection a significant problem. This is solved by insertion of an advanced Magic Sandwich Echo (MSE) block, capable of refocusing multinuclear dipolar interaction. [1] Rigid fraction measured with TD-NMR is almost 100% when both polymers are under the T_g, with a temperature dependent decrease parallel to the DSC transition between -55°C and 25 °C. Correspondingly, solid state MAS NMR at high field has shown an increase of gauche conformation in the mobile EB part. This describes in terms of crystal fusion a transition that is already detected with the much less demanding TD-NMR. Moreover, in the range where one of the phases is rigid and the other mobile, it is possible to selectively suppress the magnetization in one of the phases with appropriate sequences (dipolar or double quantum filters) and then measure the domain size by spin diffusion.[2]

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SOLID STATE NMR CHARACTARIZATION OF A PMMA-Ce:YAG NANOCOMPOSITE

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In this work the solid state NMR characterization of a transparent composite material constituted by Yttrium aluminum garnet ($Y_3Al_5O_{12}$, YAG) doped with Ce(III) nanoparticles embedded in polymethylmethacrylate (PMMA) matrix is presented together with the results obtained by the spin-lattice relaxation times (T_1H) and spin-lattice relaxation times in the rotating frame ($T_{1\rho}H$) determination.
Ce:YAG combined with the blue light emitting diodes (LED) is ideally applied for the white solid-state LED. A nanosize phosphor is useful for reducing the optical scattering loss in the optical application. Inorganic-polymer nanocomposites are of significant interest for emerging materials due to their improved properties and unique combination of properties.
In recent decades, the preparation of polymeric nanocomposites materials has been intensely studied due to their extraordinary properties and widespread potential applications. In fact, in a nanocomposite, the host matrix can maintain or increase the luminescence proprieties of materials and acts against the tendency of particles aggregation.
^{13}C $\{^1H\}$ CP-MAS NMR spectra of the polymeric matrix and of the composite were acquired to evaluate differences in the chemical shift and in the shape of signals. T_1H and $T_{1\rho}H$ relaxation times were obtained to investigate the dynamic behavior of the PMMA matrix before and after the composite formation. No differences were detected from the comparison of the two ^{13}C $\{^1H\}$ CP-MAS NMR spectra, whereas the relaxation times in the rotating frame for the composite material increase considerably. This is an evidence of an increase in the polymer rigidity which could be attributable to an order increase of the polymer chains.
Furthermore the highest increase of the $T_{1\rho}H$ value was obtained for the carbonyl carbon. This suggests that the Ce:YAG nanofiller interacts mainly with this part of the polymeric chain of the PMMA matrix.

NMR CHARACTERIZATION OF HYDROSOLUBLE POLYMERS CONTAINING LUMINESCENT RHENIUM(I) TRICARBONYL FRAGMENTS

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Biological labelling with luminescent probes is one of the most common methodologies used for diagnostic purposes [1]. However, the short-lifetime and the small Stoke's shift of the emission arising from classical organic fluorophores make often difficult to differentiate the endogenous fluorescence of biological species from that of the *bio*-probes. Therefore, many transition metal complexes have been proposed as luminescent *bio*-probes, because their emission, usually arising from metal-to-ligand-charge-transfer triplet states, display properties which allow to overcome the low signal-to-noise ratio caused by the cells autofluorescence. Several reports have recently appeared dealing with the use of iridium [2], platinum [3] and rhenium complexes [4], that show intense long-lived emission, with strong Stoke's shift, that can be easily differentiated from autofluorescence, by use of time-resolved detection.
In this communication, we show the results obtained by reacting luminescent rhenium *fac* tricarbonyl bisimine complexes with a highly hydrosoluble and biocompatible polymer.
Multinuclear and multidimensional NMR has been used to characterize rhenium complexes before and after the coordination to the polymer.
In particular, the coordination of the rhenium fragment has been proved by means of longitudinal relaxation (T_1) times and pulsed gradient spin echo (PGSE) NMR experiments [5].

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ANALYSIS OF ORGANIC MATERIALS IN ARTS AND ARCHAEOLOGY BY NMR SPECTROSCOPY

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Nuclear magnetic resonance (NMR) spectroscopy is an established, state of the art analytical tool in many areas of chemical research, especially when molecular characterization of organic compounds is imperative. Nonetheless, the use of NMR is rather under-represented in the field of arts and archaeology,[1] despite the significant advantages this technique has to offer, namely the quantitative retrieval of the sample following analysis, and the minimal treatment needed prior to analysis. In this work, we will present recent work that demonstrates the analytical power of NMR in the study of organic materials from archaeological and historical context, such as oil paintings [2], paint binders [3], varnishes, waxes, archaeological residues and contemporary works of art.

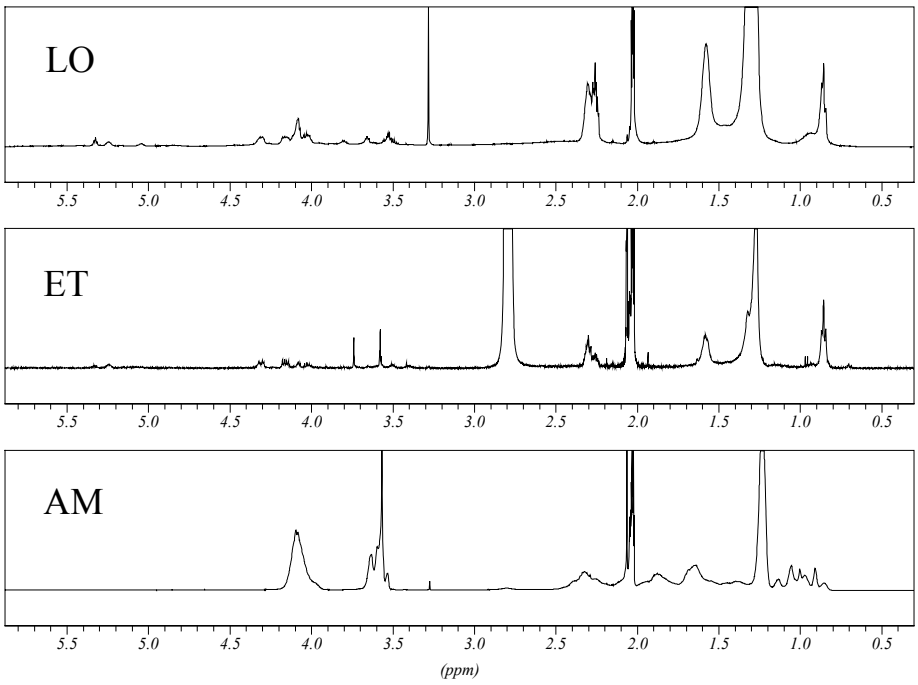

Fig. 1. ^{1}H NMR spectra of binder extracted from aged linseed (LO), egg tempera (ET) and acrylic medium (AM) paints in acetone-d_6.

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POSTERS

EFFECT OF PACKAGING IN LIPID OXIDATION - DRIVEN BROWNING OF BOTTARGA AS FOLLOWED BY COLORIMETRIC AND NMR MEASUREMENTS

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Bottarga is a typical Sardinian product obtained by salting and drying mullet roe. Currently there is a renewed scientific and technological interest on this product [1],[2]. Shelf life of bottarga is negatively affected by a browning process during the supermarket storage. In order to study the problem, freshly grated bottarga has been packaged using a plastic film in which UV-light absorbers are embedded, while a control sample has been packaged using the same film without UV-light absorbers. Samples were stored at constant temperature (30°C) for 15 days, under permanent illumination by fluorescent lamps, thus simulating storage condition in the supermarket. Colorimetric analysis and NMR measurements were done at regular time intervals aiming at correlating visible changes with the molecular composition of the product. Spectra were acquired by means of HR-MAS NMR on bottarga as such and by liquid-state NMR on lipid extracts of the samples [3]. At early stage of storage, ^{1}H NMR spectra of lipid extracts show signals due to the formation of epoxides derived from cyclization of the hydroperoxydes which are formed during the early stages of lipid oxidation. These species are supposed to be very reactive and short-lived. Signals pertaining to aldehydes, mostly saturated n-alkanals and alkenals, secondary products of lipid peroxidation, are observed at low fields (9-10 ppm), together with hydroperoxydes -OOH protons. COSY, TOCSY and ^{13}C-^{1}H HSQC spectra show connectivities of signals observable in 1D ^{1}H and ^{13}C spectra, thus providing detailed information on the involved species. The signals from saturated aldehydes (n-alkanals) generally increase with irradiation time, while those of hydroperoxydes show a decrease in intensity. These species are among those commonly associated to fish flavours and aroma, and sometimes unpleasant smell. Moreover, an unresolved very broad resonance is observed in the region between 5 and 8 ppm of ^{1}H NMR spectra of lipid extracts. This resonance is likely ascribed to the polymerization of oxidated lipids and their chemical reaction with other components like amines and/or proteins to form macromolecular aggregates which lead to non-enzymatic browning of bottarga. These observations allowed us to follow the different stages of lipid deterioration, which appears to be crucial in forming the very reactive species and promotes chain-reactions that finally determine the non-enzymatic browning of bottarga. Upon storage of bottarga packed in films containing UV-absorbers, the observed resonances more closely resemble those of freshly grated sample, thus corroborating the efficacy of packaging.

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MRI AND RAMAN INVESTIGATION OF FIRMING IN AMARETTI COOKIES AND SHELF LIFE EXTENSION

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One of the major causes of firming in baked foods is represented by inhomogeneities in water redistribution during storage, in a process which finally leads to hardening and determines the shelf life of the product. "Amaretti" are typical cookies of fine Italian bakery and represent a product of relevant economic importance for sweet factories. The product is obtained by grinding and mixing together sweet and bitter almonds, egg white and sugar. In our work, Amaretti cookies were produced by following traditional Sardinian recipe and by adding to the dough sweet whey powder, aiming to favorably change water mobility and redistribution within crust and crumb during storage, consequently delaying the firming process. Magnetic Resonance Imaging T_2 maps of the cookies prepared by following both recipes were obtained in order to spatially characterize framework mobility of the dough during the storage time and to non-destructively determine the kinetics of evolving firmness. The process of hardening usually involves the redistribution of water molecules among the different components of the mixture. According to literature, firmness of similar bakery products is due to sugar crystallization and to the spatial inhomogeneities caused by evaporation of water, in a continuous process which starts just after baking. Parametric T_2 maps were acquired just after baking and at 6, 27, 60 and 100 days of storage, and the evolution of water spin-spin relaxation rate constants R_2 give a spatial detail to the complex phenomena involving water redistribution between crumb and crust during the shelf life of the products. Further insights into the process of firming and on the final composition of amaretti at the final stages of shelf life were investigated by Raman spectroscopy. Raman spectra clearly show that the presence of sugar is confined to the crust, while mostly almond paste is contained in the inner part of the cookie. Our hypothesis concerns the diffusion of water and sugar in the first stages after baking from the crumb to the crust, water evaporation after about one month and successively the redistribution of water vapour and its equilibration between the cookie and the head space of the packaging during further storage. The diffusion of water from the crumb to the exterior is reasonable considering the hydrophobicity of the almond paste, mainly constituted by lipids. As evidenced by MR relaxation maps, this diffusion is thought to proceed through preferential channels within the cookie from crumb to crust, which make the process of water dispersion more favourable, and finally leads to dryness and cookie hardening. Moreover, we have observed that addition of sweet whey powder leads to increase in homogeneity of the mixture as it acts as water-holding component during the storage time, thus significantly increasing the shelf life of the product without significantly changing the organoleptic characteristics of the product.

MULTI-HISTIDINIC FRAGMENTS BINDING BIOLOGICAL METALS: AN NMR STUDY

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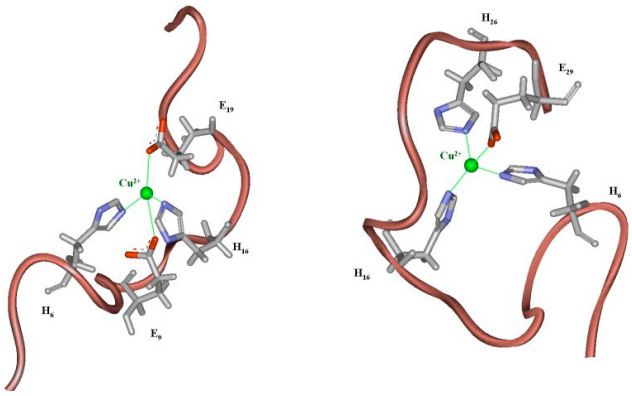
Cap43 protein can be considered as a "stress protein" since it is involved in a number of noxious events inside the cell, like hypoxia, cancerous states and metastasis suppression, and stress response just to quote some. Another interesting feature is that its expression is triggered by the rise in concentration of some metals, amongst which nickel gives the highest response [1].
We have examined the whole sequence of this protein in the search of a suitable site for metal binding, finding a remarkable aspect that prompted us to deepen our investigation. In fact, Cap43 presents in its C-terminal region a mono-histidinic decapeptide whose sequence is repeated consecutively three times (TRSRSHTSEG-TRSRSHTSEG-TRSRSHTSEG). The occurrence of such a repeated motif containing a histidine residue reminded us of neurodegenerative deseases and prions, where an octapeptide fragment bearing an histidine residue is repeated four times, and proved to be very active in binding Cu(II) ions, so that the tetra-repeat sequence is able to bind up to four metal ions. Another peptide, the β-amyloid fibril, contains a multi-histidinic sequence which seems involved in metal coordination within the process of aggregation. It is thus possible that also Cap43 could have a similar behaviour toward metals. We have used the 10-amino acid monohistidinic basic fragment (TRSRSHTSEG) and its two- and three-repeats to investigate their coordination mode towards metal ions such as Cu(II), Ni(II) and Zn(II). Multidimensional and multinuclear NMR techniques have been employed to uncover the details of metal coordination at different pH values and metal-to-ligand molar ratios. The data collected in our experiments allowed us to calculate structural models for the metal complexes, both at low and high pH-values[2-5].



A proposed $\{2N_{im},2O^-_{carbox}\},\{3N_{im}, O^-_{carbox}\}$ multi-histidinic copper models at "low" pH

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PARAMAGNETIC LIPOSOMES AS PH_RESPONSIVE AGENTS

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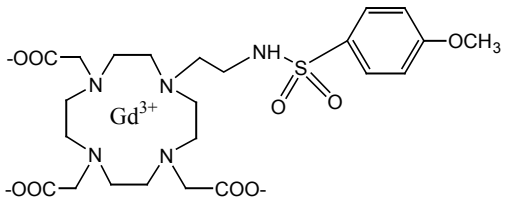
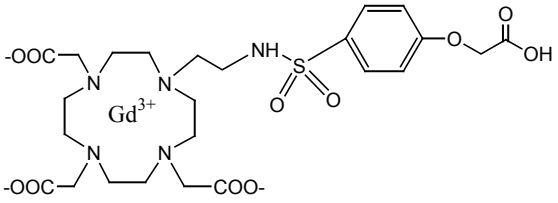
Liposomes are phospholipid-based vesicles often used as drug carries. The inclusion of MR-imaging agent [1] may allow the visualization of the drug delivery process. Moreover the upload of MRI-responsive agents may endow them with sensor reporting properties. The use of MRI pH-responsive agents [2] aims as providing systems able to show the "in vivo "mapping of this parameter. pH mapping is important in a number of pathological states ranging from tumors to stroke, infections, etc. As pH reporting systems we have used GdDO3A system functionalized with sulfonamide arms. (Fig 1).



Complex A



Complex B

Fig. 1. Structures of pH reporting systems

The presence of the aromatic moiety promotes the interaction of the Complex A with the liposome's membrane that results in a relaxation enhancement in respect to the free complex. The Complex A loaded liposomes maintain the pH dependent properties of the free complex. Complex B displays a negligible interaction with the liposome's membrane thanks to its negative residual charge. Thus it distributes essentially in the inner aqueous cavity. This peculiarity has been used to assess the rate of proton transfer across the liposome's membrane.

Liposomes loaded with pH-responsive Gd-complexes can be used to label cells in order to assess the pH characteristics of the intracellular compartments in which the liposomes are entrapped. Moreover when the liposomes are anchored on the outer cellular surface [3], they may be used to probe the pH of the extracellular environment.

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NMR SPECTROSCOPY AS A TOOL FOR RAPID ASSESSMENT OF THE STRUCTURAL INTEGRITY OF PTPASES AND RHODANESES

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Although sulfurtransferase and PTPase enzymes share a very similar catalytic signature motif, CX_4R and CX_5R respectively (which in PTPases is also referred to as P-loop), they catalyze distinct reactions: transfer of a sulphur atom from a donor to an acceptor the former, and proteic phosphate hydrolysis the latter. A plethora of structural information is available for the PTPases and for the Rhodaneses. The catalytic loop is solvent exposed, and located between the carboxy-end of a central β-strand and the N-terminal cap of an internally packed α-helix. The overall fold of the Rhodanese shows topological similarity with PTPases CDC25A and B, but low sequence similarity (<30%). The catalytic residue, in both families, is a Cys stabilized in the thiolate form. It has long been clarified that there are at least three structural factors directly influencing the stabilization of the thiolate: i) a network of six H-bonds all donated by amide groups of the catalytic loop backbone which points toward the thiolate moiety; ii) the δ+ micro-dipole orientation, of these six amide hydrogen forming the H–bonds; iii) the spatial positioning of the thiolate group at the center of N-side δ+ dipole of the helix following the P-loop. All these peculiar structural features lead to a catalytic Cys pKa of 4.5 in PTPases and about 6.0 in Rhodaneses (exposed cys pKa=8.5). In PTPases, it has also been noticed that the aminoacid preceding the catalytic cys commonly is a His. Because of this conservation often the signature motif is indicated as $(H)CX_5R$. Mutation of this His residue reduces the catalytic activity of 10-30 fold while increasing the pKa of the catalytic Cys of at least 1 pH unit [1]. The structural role of this His residue is critical, and is exerted through a network of H-bonds involving its imidazole moiety, where the δNH acts as a donor to the following catalytic Cys carbonyl, while the εN: may act as H-bond acceptor [2]. This network blocks the ψ dihedral angle, of the catalytic Cys backbone, in a conformation that projects its sidechain towards the obligated position at the center of the catalytic loop and thus positioning the thiolate moiety at the center of the network of interactions that stabilizes it. Disruption of the imidazole H-bond network results in strong de-stabilization of the loop conformation. From the NMR spectroscopy point of view, the peculiar network of H-bonding involving the His residue, results in a number of signals which could be easily and rapidly detected. We thus propose a method for fast probing the structural integrity of the catalytic site for both the PTPase and the Rhodanese. Using the minimum combination of 1D ^{1}H spectra with and without ^{15}N decoupling, and the acquisition of ^{2}J ^{1}H-^{15}N tuned HMQC and jump-return ^{1}J ^{1}H-^{15}N HMQC experiments, we show the tools to allow the fast and complete unraveling of the signals involved in the His H-bond network. The method is applied on PRL-3, a 22kDa human PTPase involved in the process of metastatization of colo-rectal cancer, and on Rhodanese A (28kDa) from *Azobacter Viinelandi* a nitrogen fixing bacteria. Both enzymes present the His residue in the signature motif $(H)CX_{4,5}R$, at the position preceding the catalytic Cys.

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ADVANCES IN THE UNDERSTANDING OF p63 RECOGNITION MECHANISM BY Itch-E3 LIGASE: STRUCTURAL AND INTERACTION STUDIES

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WW domains are highly compact protein-protein interaction modules which fold into stable three-stranded antiparallel β-sheet structures, and are characterized by the presence of two conserved tryptophan residues spaced 20-22 amino acids apart. These domains are found in proteins with different important functions, such as transcription regulation, RNA processing, protein trafficking, receptor signalling, control of the cytoskeleton and ubiquitin ligation: Several signalling complexes, that these domains mediate, have been implicated in human diseases (Muscular Dystrophy, Alzheimer's Disease, Huntington Disease etc.) [1]. These domains interact with short proline-rich sequences and on the basis of their ligand-binding specificity. WW domains have been categorized into five groups. Itch E3–ligase is characterized by the presence of four WW domains considered belonging to the Group I, which binds polypeptides with PY motif characterized by a PPXY consensus sequence, where X can be any residue. Group I and IV domains structurally share a single groove formed by two highly conserved aromatic residues (typically Tyr in β2 strand and Trp in β3 strand) which interacts with the XP residues of the peptide. Recently, it has been shown that Itch mediates the degradation of TAp63 and ΔNp63 protein and that this interaction may be due to a direct interaction of WW2 domain with the PY motif of p63 [2,3]. Here, we present the expression, purification and structural characterization by fluorescence, CD and NMR spectroscopy of Itch-WW2 domain. Preliminary interaction studies *in vitro* between Itch-WW2 domain and the fragment of the p63 protein, including the PY motif, were performed. The study of WW-peptide complexes provide insights into the molecular mechanism underlying ligand selectivity of these structural modules and will facilitate the design of small molecules that could modulate the function of Itch and/or the degradation of p63 protein, with possible clinical applications.

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THE NMR STRUCTURE OF AN ANTIMICROBIAL DECAPEPTIDE

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Antimicrobial peptides are a new family of antibiotics that have stimulated research and clinical interest [1]. Most antibacterial peptides are components of the innate immunity of animals and plants against microbial infections [2,3]. Here we present a structural investigation in solution of QKKIRVRLSA, L6, a linear decapeptide which exhibits strong antibacterial activity [4,5]. As expected, the bioactive peptide is fully disordered in water at physiological conditions. Conversely, in the presence of SDS micelles the conformational equilibrium of L6 is totally shifted towards the formation of a regular α helix, as unambiguously suggested by the observed NOE network.

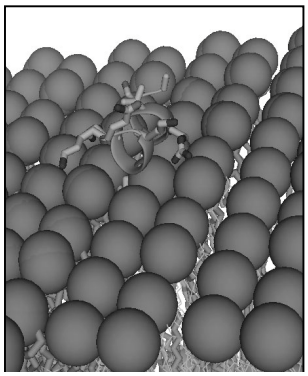

Fig. 1. The decapeptide QKKIRVRLSA laying on the surface of a SDS vesicle, as suggested by the obtained paramagnetic attenuation profile of -^{1}H-TOCSY signals.

In order to correlate L6 activity and structure, the orientation of the helix in respect to the membrane surface was investigated by analysing paramagnetic perturbation profiles of 1H-TOCSY signal attenuations obtained for L6 in the presence of Gd(III) DTPA-BMA.
The observed pattern of paramagnetic attenuations of CαH correlations is consistent only with an horizontal alignment of the decapeptide on the surface of the SDS micelle, see Fig. 1.
The sterical features of the L6 – vesicle interaction will drive the design of new analogs of increased antimicrobial activity.

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CHARACTERIZATION OF HYDROGELS BY MEANS OF HIGH AND LOW RESOLUTION NMR TECHNIQUES

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Hydrogels are polymeric materials finding wide application in biology and biomedicine, for example as soft contact lenses, drug delivery systems, implant materials and tissue scaffolding [1]. Since water represents a significant proportion of swollen hydrogels, the status of water in these materials and the iteractions of water with the polymer is fundamental in determining many of the gel properties, ranging from biocompatibility to the transport of small molecules.
Among the different experimental techniques used for the physico-chemical characterization of water in hydrogels, NMR has proven to be of relevance offering a wealth of low and high resolution techniques. In particular the dynamics of water molecules within the polymeric matrix and water-polymer interactions have been investigated through measurement of ^{1}H spin-lattice and spin-spin relaxation times, also taking into account relaxation coupling between water and macromolecular protons [2]. In some cases, ^{1}H and, most important, ^{13}C high resolution NMR techniques, both liquid and solid state, have been applied to obtain site specific information on hydration and polymer dynamics.
In this poster representative examples of the research activities on hydrogels carried out by our group will be given, with particular emphasis on ^{1}H spin-lattice relaxation and Magnetization Transfer measurements, ^{1}H spin-spin relaxation time dispersions, and analysis of ^{1}H and ^{13}C spectra. Both chemically cross-linked hydrogels of the poly(amidoamine) class [3] and physically cross-linked hydrogels made of poly(ethylenglycol) and poly(lactic acid) will be considered.

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STUDY OF DYNAMIC PROPERTIES OF ACID AND SODIUM SALT IBUPROFEN : A COMBINED SOLID-STATE NMR ANALYSIS FROM SPECTRAL FEATURES AND RELAXATION TIME MEASUREMENTS

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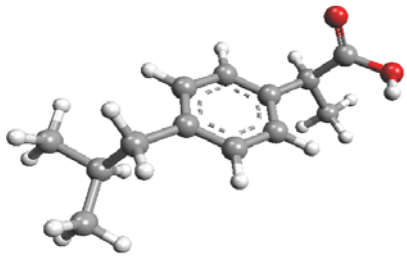
The molecular dynamics of a solid drug strongly affect its pharmaceutical properties and other important characteristics as solid state degradations. Understanding the mobility of groups in solid drugs can also lead to a deeper knowledge of the forces responsible for conformational interconversions and the factors responsible for solid state reactions [1]. Moreover, dynamics play an important role in drug-excipient interactions, which raise a wide interest because they significantly affect the release properties.

Solid state nuclear magnetic resonance (SS NMR) is an increasingly important technique for the physical characterization at molecular level of active pharmaceutical ingredients, which can be also directly investigated in their final formulation [2].

A characterization of molecular dynamic properties of two forms of ibuprofen, acid (IBU-A) and sodium salt (IBU-S), obtained through SS NMR techniques is presented.

Ibuprofen is a widely used non-steroidal analgesic and anti-inflammatory drug, recently used also as anti-pyretic in the pediatric field.

A full assignment of ^{13}C resonances and preliminary qualitative information about some motional processes were previously achieved in our research group [3].



Stucture of Ibuprofen
2-(4-Isobutylphenyl)propionic acid.

SS NMR offers several approaches to study molecular dynamics in a wide range of frequencies. In the present work we performed ^{13}C and ^{1}H longitudinal relaxation time (T_1) measurements to investigate fast motional processes, with characteristic frequencies of the order of MHz, ^{13}C and ^{1}H longitudinal relaxation time in rotating frame ($T_{1\rho}$) measurements and ^{13}C chemical shift anisotropy line shape analysis, in order to investigate intermediate motional region (frequencies of the order of kHz) while insights about the slow motional regime (frequencies of the order of 1 kHz or less) could be obtained by looking at exchange processes occurring in the ^{13}C high-resolution spectrum. Combined analysis of all the data provided either qualitative or quantitative information about the motions of the various molecular fragments (phenyl ring, methyl groups, aliphatic chains).

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NMR REINVESTIGATION OF THE CAFFEINE-CHLOROGENATE COMPLEX IN AQUEOUS SOLUTION AND IN COFFEE BREWS

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It has long been known that caffeine interacts with polyphenolic molecules in aqueous solution [1]. The complex formed between caffeine and chlorogenate, a well known major polyphenolic constituent of green coffee bean, was isolated one century ago by Gorter [1]. In the '70, Horman and Viani [2,3], on the basis of NMR chemical shift data, proposed that the caffeine-chlorogenate complex might be described as a 1:1 hydrophobically bound π-molecular complex. Their model was found similar to the crystal structure of the caffeine-potassium chlorogenate complex elucidated 15 years later by Martin et al. [4] although the orientation of the aromatic ring was not identical.
In the present work, caffeine and chlorogenic acid self-associations have been studied and self-association constants have been determined resorting to both classical isodesmic model and a recently introduced method of data analysis able to provide also the critical aggregation concentration (cac). Furthermore, caffeine-chlorogenate association constant was measured and structural features of the complex have been investigated by NOE effect.
Caffeine chemical shifts comparison (momomeric, complexed, *espresso* coffee brews) clearly indicates a significant amount of caffeine is complexed in beverage real system, being chlorogenate ions the main complexing agents.

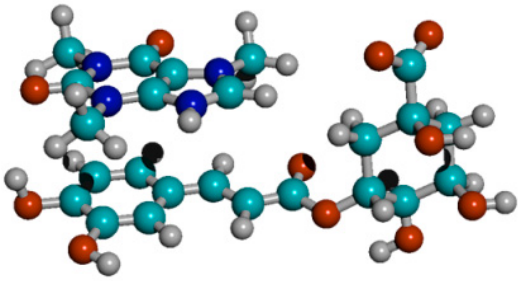

Fig. 1. The caffeine-chlorogenic acid complex as determined by NMR.

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LOW FIELD NMR SPECTROSCOPY FOR QUALITY EVALUATION OF NATURAL ORGANIC MATTER

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The effect of natural organic matter (NOM) on physical, chemical and biological soil properties is well known. In fact, NOM is widely considered as a very important system for amelioration of soil quality. As an example, NOM increases soil structure, chemical fertility, nutrient availability and soil drainage characteristics. For this reason, conformational and structural studies on NOM are of great importance. Knowledge of NOM molecular characteristics can help in understanding not only the role of NOM in affecting soil properties, but also in the comprehension of its function in pollution assessment.
Modern high field (HF) NMR spectroscopy either in the liquid (LS) or solid state (SS) has been recognized, up to now, as a very precious tool for NOM characterization. HF NMR is usually applied to monitor chemical composition of natural organic matter and to retrieve quantitative information on NOM chemical composition. However, not always HF NMR can be helpful in recognizing qualitative differences among different NOMs. In the present study, we have verified that CPMAS ^{13}C NMR spectroscopy was unable to differentiate among four humic substances extracted from a soil subjected to four different treatments. However, when low field NMR with fast field cycling (FFC) setup was applied different T1 distributions were obtained. These distributions were related to the different soil chemical treatments. This work showed for the first time that FFC NMR represents a very powerful tool for the characterization of natural organic systems.

Acknowledgments

This work was partially funded by Ce.R.T.A. s.c.r.l. (Centri Regionali per le Tecnologie Alimentari, http://www.certa.it/default.asp). The high field NMR measurements were done at Centro Grandi Apparecchiature (CGA) – Uninetlab of the Università degli Studi di Palermo.

CHEMICAL INTERROGATION OF HUMAN LIVER FABP FUNCTION

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Lipids are vital components of many biological processes and crucial in the pathogenesis of numerous common diseases, but the specific mechanisms coupling intracellular lipids to biological targets and signalling pathways are not well understood[1, 2].
Our group is focussing its research on the elucidation of the complex binding mechanisms of liver intracellular transporters of lipids, in particular bile acids[3, 4, 5].
In non-mammalian species liver bile acid binding proteins were reported and characterized, on the contrary in mammalian liver it is still not clear who plays the role of bile acid transport.
Human Liver Fatty Acid Binding Protein (hLFABP) belongs to a family of 14-15kDa intracellular lipid binding proteins that have the ability of binding long chain fatty acids and a variety of other small hydrophobic ligands, and it is present in the hepatocytes at high concentration constituting about 2-5% of the cytoplasmic proteins. The putative functions of hLFABP include lipid uptake and transport, and regulation of lipid metabolism. These characteristics suggest that hLFABP may have a role in transporting bile acids in addition to fatty acids[6].
We used a variety of NMR methods to investigate the binding properties of hLFABP as they provide site-specific and residue-specific information at the structural level. In particular our aim is distinguishing the determinants of binding depending on the differences in the ligands properties and ultimately confirm the role of hFABP in bile salt transport.

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NEW INSIGHTS ON THE PROTEIN-LIGAND INTERACTION DIFFERENCES BETWEEN THE TWO PRIMARY CELLULAR RETINOL CARRIERS

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Trafficking and metabolism of vitamin A is dependent on a number of specific proteins. The main retinol carriers in the cytosol are CRBP-I and CRBP-II, that exhibit distinct tissue distributions. They play different functional roles, while sharing the same structural topology which consists of ten antiparallel β-strands and two short α-helices.
In contrast to an earlier NMR report [1], the present study provides new evidence that in solution the global fold of the portal region in apo CRBP-II [2] is basically identical to that of CRBP-I [3]. Hence, the previously suggested structural implications for the mechanism of retinol binding to CRBP-II [1], need to be reconsidered.
Despite the fact that the ligand-binding motif as well as the fold of the entry portal are thus identical in the two homologous proteins, they feature a 100-fold difference in retinol-binding affinity, whose basis has not been identified in detail to date. To complement previous studies which indicated the role of flexibility in modulating the protein-ligand interaction [4-6], we have investigated the structural stability of CRBP-I and CRBP-II, both in the apo and holo forms, by H/D exchange experiments.
The data show that many amide protons exchange much faster in CRBP-II than in CRBP-I. While formation of the retinol complex results in a more rigid overall structure in both proteins, a significantly different intrinsic stability is observed between the two homologues. This finding appears to have a major influence on their binding affinity: the more strongly ligand binding CRBP-I displays a reduced flexibility of the backbone structure with respect to CRBP-II [2]. These differences must be due to specific amino acid substitutions in the course of evolution, which additionally stabilize the scaffold of CRBP-I: we have identified a number of potential salt-bridges on the protein surface as well as several key interactions inside the binding cavity. Our present results provide new details, as the timescale covered by NMR-based H/D-exchange measurements revealed significant differences between the two homologous proteins in the flexibility of certain secondary structure elements that were not previously addressed.

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ON THE FLUXIONAL BEHAVIOUR OF DESS-MARTIN PERIODINANE: A DFT AND ^{17}O NMR STUDY

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Following our previous studies on the structural and dynamical properties of several I(III) iodobenzenes in solution [1], we extended our investigation to the Dess Martin periodinane, a I(V) Iodobenzene compound widely used in organic synthesis as a mild oxidant [2].
By means of a combined ^{17}O NMR and DFT calculations approach we ascertained that in this $\lfloor^5$ iodane a degenerate [1,3] sigmatropic shift of iodine between the two oxygens of each of the three acyloxy groups occurs in solution. The energy barrier for this process depends on the position of the acyloxy group with respect to the iodoxolone ring, and is in all cases, much lower than that observed for $\lfloor^3$ iodanes.

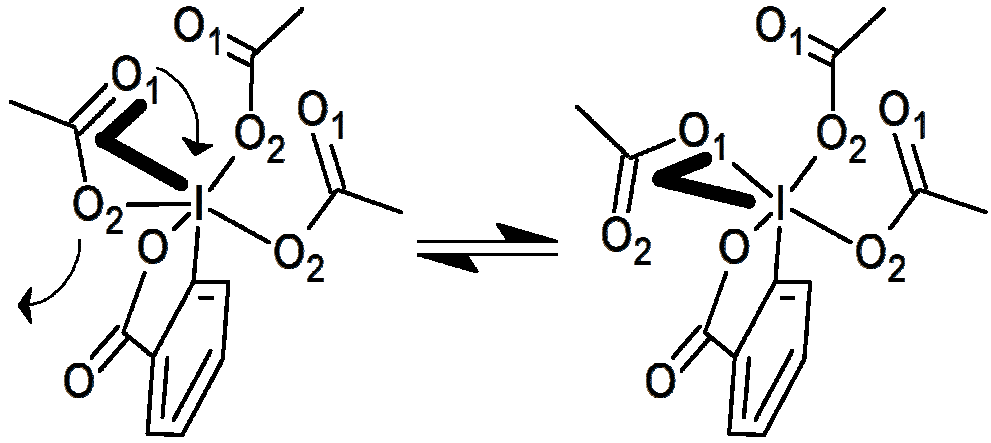

Fig. 1. Schematic representation of the [1,3] sigmatropic shift of Iodine between the two oxygens of an acyloxy group.

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CONFORMATIONAL AND STRUCTURE-FUNCTION RELATIONSHIP STUDIES OF PTH(1-11) ANALOGUES: THE ESSENTIAL ROLE OF VALINE IN POSITION 2

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The N-terminal 1-34 fragment of paratyroid hormone (PTH) is fully active both *in vitro* and *in vivo* and can reproduce all the biological responses which characterize the native intact PTH. Recent studies demonstrated that helicity-enhancing substitutions yielded potent analogues of PTH(1-11) and PTH(1-14). To further investigate the role of α-helicity on biological potency, we analyzed the conformation of a library of PTH(1-11) analogues containing hindered $C^{\alpha,\alpha}$tetrasubstituted amino acids like α-amino isobutyric acid (Aib) and α-methyl Valine (αMeVal). A rich library of analogues was generated with the goal of enhancing the α-helix content in the N-terminus, a property which seems to be determinant to activate the receptor, PTH1R. Here, we propose a complete analysis of the peptide conformations obtained by structural techniques like CD and 2D-NMR, and by Molecular Dynamics simulations. The information evinced from our data seems to demonstrate a critical role of Val in position 2, and, at the same time, suggests further pharmacophoric studies concerning the orientation of other side chains: the purpose of such a strategy could be the introduction of other constraints which could improve the activity of the peptides. In this work, we also observed the variable relative importance of 3_{10}-helix and α-helix in the different peptides which were studied.

STRUCTURAL AND DYNAMIC ASPECTS OF THE OLIGOMERIZATION OF APO SOD1 AND ITS MUTANTS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder selectively affecting motor neurons; 90% of the total cases are sporadic, but 2% are associated with mutations in the gene coding for the antioxidant enzyme copper-zinc superoxide dismutase (SOD1). The causes of motor neuron death in ALS are poorly understood in general, but for SOD1-linked familial ALS (fALS), aberrant oligomerization of SOD1 mutant proteins has been strongly implicated [1-3].

We have recently proposed a general mechanism for SOD1 oligomerization that provides a common unique picture for the behavior of the many SOD1 mutants, which are of different nature and distributed all over the protein [4,5]. Wild-type (WT) human SOD1 and its mutants, when lacking both metal ions, form large, stable, soluble protein oligomers under physiological conditions, i.e., 37 °C, pH 7.0, and 100 μM protein concentration. The oligomerization rates of the SOD1 variants are different, but they eventually give rise to the same type of soluble oligomeric species. These oligomers are formed through oxidation of the two free cysteines of SOD1 (6 and 111) and are stabilized by hydrogen bonds, between beta strands, thus forming amyloid-like structures.

Here, we present a characterization of the structural and dynamic properties of the metal-free form of WT human SOD1 and its fALS-related mutants, T54R and I113T, both in solution, through NMR, and in the crystal, through X-ray diffraction. We found that all three X-ray structures show significant structural disorder in two loop regions that are, at variance, well defined in the fully-metalated structures. Interestingly, the apo state crystallizes only at low temperatures, whereas all three proteins in the metalated form crystallize also at higher temperatures, suggesting that crystallization selects one of the most stable conformations among the many ones adopted by the apo form in solution. Indeed, NMR experiments show that the protein is highly disordered in solution, sampling a large range of conformations. The large conformational variability of the apo state allows the free reduced cysteine Cys-6 to become highly solvent accessible in solution, whereas it is essentially buried in the metalated state and in the crystal structures. Such solvent accessibility, together with that of Cys-111, accounts for the tendency of the metal-free state to oligomerize. The present results suggest that the investigation of the solution state coupled with that of the crystal state can provide major insights into SOD1 pathway toward oligomerization in relation to fALS.

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RADIO-SENSITIVITY OF TUMOUR CELLS *IN VITRO* IS PREDICTABLE BY CELLULAR GLUTATHIONE LEVELS DETECTED BY ^{1}H MRS

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Two unrelated cell lines from human carcinomas, MCF-7 and HeLa, showed association of the cellular level of reduced glutathione (GSH) - 1H MRS detected - to radiation resistance to gamma rays [1]. We then investigated whether such correlation holds also in tumour cells of different origin, namely cells from human glioma (T98G) and cells from human astrocytoma (A172), characterized by with different sensitivity to radiation with respect to cell survival after therapeutic doses. The study also aimed to detect a different role for GSH in cells from different tumours, from the study of GSH consumption to cope with the oxidative stress induced by irradiation.

Cells were irradiated with a gamma cell (60Co) at 20 Gy. 1H MR spectra were run at 400.14 MHz on a digital Avance spectrometer equipped with a 1mm microprobe. The 1H MR spectrum of cells from human glioma showed signals related to GSH metabolism with a pattern of intense GSH very similar to that of MCF-7 cells [1]. Irradiation of these two cell lines failed to induce cell killing, when observed after 48 hours. On the contrary, the same treatment induced significant killing in HeLa and A172 cells, characterised by lower initial GSH, then referred as radiation sensitive cells. These cell lines showed different radio resistance, therefore consistent with the observed initial levels of GSH.

Radiation resistant cells were then irradiated after Buthionine sulfoximine (BSO) treatment to decrease the concentration of GSH in cells, preventing its radio protective effects, in order to increase cell killing. Though the initial level of GSH was lowered in both cell lines at the same extent, relevant apoptosis and cell killing were observed only in MCF-7 treated cells, while T98G cells were not affected .

We then compared the 1H MR spectra to detect differences in GSH recovery kinetics. After 2 hours from the end of BSO treatment, both cell lines displayed lower glu-GSH and higher free glu due to GSH synthesis block. Then, T98G cells quickly recovered GSH, while MCF-7 cells did not. After 24 hours from the end of BSO treatment GSH signals were in fact still low in MCF-7, while they were almost completely restored in T98G cells. The protective effect of GSH is testified by its consumption in the corresponding irradiated samples. Fast GSH restoration may be ascribed to highly efficient recovery systems of glial cells, to protect them against an oxidative stress by producing glutathione.

Present data show that GSH levels, 1H MRS detected, can provide a method to foresee cell sensitivity to radiation treatment. Moreover, the experimental data also show that GSH metabolism of cell lines from different origin can be differently regulated, thus allowing further hypotheses on cell resistance to possible antitumor treatments.

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PRELIMINARY STUDIES ON TREATED WATERLOGGED BY LOW FIELD NMR SPECTROSCOPY

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Waterlogged wooden objects, that is wooden artefacts coming from wet soil, sea or lakes, are very fragile because combined chemical, physical and biological deterioration. As a result of the loss of cell wall components (especially water-soluble substances and holocellulose, through hydrolysis), spaces between the cells and molecules increase, and the wood becomes more porous and permeable to water. All of the deteriorated elements of the wood, including cell cavities, are filled with water. The remaining lignin and the absorbed water preserves the shape of the wood and a waterlogged wooden object will retain its shape as long as it is kept wet.

After its recovery and removal from original archaeological site, the problem of its handling and conserving rises. In fact, artifact, if directly dried in air, risk a structural collapse, loss of original form and dimension and it cannot be used for study and museum exhibits.

The progressive substitution of water with proper strengthening materials can preserve the characteristic of the wood finds. Among various natural and synthetic substances suitable for this porpoise, colophony was chosen for the treatment of some wooden samples coming from the excavations of the ancient port of Pisa.

Colophony, also called rosin or Greek pitch, is a solid form of resin obtained from pines and other conifers. In order to evaluate its performance as hydrophobic treatment, low field 1H-NMR relaxation rates measurements on samples from different wooden taxa were performed. In fact, it is well experimented that NMR allows the determination of moisture content of wood [1]. Preliminary results seem to indicate that colophony treatment shows a good hydrophobic performance, also in high moisture contexts.

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Acknowledgments

This work was partially funded by Ce.R.T.A. s.c.r.l. (Centri Regionali per le Tecnologie Alimentari, http://www.certa.it/default.asp)

MULTINUCLEAR SOLID-STATE NMR CHARACTERIZATION OF PE-PEG-Si/PHS/SILICA SOL-GEL HYBRID MATERIALS

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It is known that hybrid materials in which organic and inorganic components coexist on a nanometric scale, as those obtainable through sol-gel processes, can exhibit interesting unique macroscopic properties. The important relationship between macroscopic and "molecular" properties is still often unknown. Solid-State NMR (SSNMR) is a very powerful technique for obtaining a molecular characterization of materials, thanks to the huge variety of available experiments and to the possibility of observing different nuclei [1, 2].
In this work sol-gel hybrid materials constituted by poly(hydroxystyrene) (PHS), α-triethoxysilane terminated poly(ethylene)-block-poly(ethyleneglycol) (PE-PEG-Si) and silica have been studied by SSNMR in order to try to find a correlation between the oxygen barrier properties shown by these systems and their microscopic features [3]. In particular, it has been seen that the introduction of PHS determines a significant improvement of the barrier properties with respect to the two-component materials constituted by silica and PE-PEG-Si, already studied in a previous work [4, 5], and a strong dependence of the barrier properties on composition has been observed [3]. High resolution ^{13}C selective experiments have been used to investigate the dynamic and structural properties of the organic component; in particular the number of different phases and the crystalline/amorphous ratio for systems with different composition have been determined for both PEG and PE blocks. Through ^{29}Si-CP- and SPE-MAS spectra, the degree of condensation of silica has been evaluated. Interesting indications on the nature of the interface and in particular of the interaction between the organic and inorganic components have been found by means of ^{1}H-MAS spectra at high spinning rate.

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DIFFUSION MEASUREMENTS IN AGAR GELS BY TIME DOMAIN NMR

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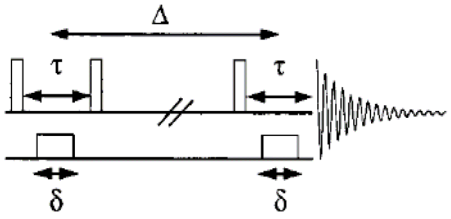
Diffusion properties in gels are relevant to many food and biotechnological applications, including the design of compounds for delivering active molecules according to specific release patterns. Physical resistance to diffusion is due to complex interaction between gel strands, solute and solvent and a measure of this resistance is not completely defined. Physical polysaccharide gels of agar in water were prepared with agar concentration of up to 10% weight and investigated by ^{1}H Time Domain NMR. The study of relaxation times, accessible with relatively simple and low cost 20 MHz instrument, gave evidence of decreasing T_1 and T_2 of water contained in gels with increasing polysaccharide content. In the same context, more information is acquired by measurement of the diffusion coefficient D with the pulsed field gradient technique. [1] Motion of water or other probe molecules in the gel is a constrained Brownian motion. The supporting polysaccharide network acts as a barrier to diffusion, segmenting the volume in cells and thus limiting the time dependent decay of the self-correlation function of solute molecules at long times.



$$\ln\left(\frac{I(g,\Delta)}{I_0}\right) = -\gamma^2 g^2 \delta^2 \left(\Delta - \delta/3\right) D$$

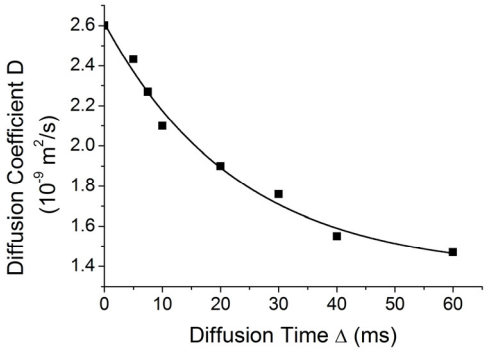


Fig. 1. On the left, the Stimulated Echo Pulsed Field Gradient sequence, with the relevant equation and the most relevant parameters highlighted. On the right, values of D as a function of Δ in 10% agar gel at 20 °C. The value for zero diffusion time is the value of water self diffusion at 25 °C.

D depends on the timescale of diffusion and the function D(Δ) containins relevant information on the network topology. Given the inherent insensitivity of PFG measurements and a T_2 which can be as low as 19 ms in the agar gel, we are limited to very short diffusion times. In order to solve this sensitivity problem and obtain results for Δ>50 ms, we have successfully implemented for the first time the stimulated echo sequence [2] (Fig. 1) to the low field environment. With this sequence, signal loss is not dictated by T_2 but rather by the much slower T_1. Preliminary results, reported in Fig. 1, demonstrate the possibility of using water as a probe molecule for gel structure.

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APPLICATION OF NMR-MOUSE AND LOW-FIELD NMR TO SOFT MATTER CHARACTERIZATION

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The NMR-MOUSE (MObile Universal Surface Explorer) is a small and portable LF-NMR system with one-sided magnet layout that is used to replace the conventional magnet and equipped probe on a LF-NMR instrument. The high magnetic field gradients associated with the one-sided MOUSE magnet result in NMR signal decays being dominated by molecular diffusion effects. The aim of our investigation is to obtain information about the T_2 of the water in soft matter, such as hydrogels, meat and to establish if some information can be achieved concerning *in vivo* skin. In the literature T_2 measurements performed on hydrogels[1] and meat[2],[3] carried out by using LF-NMR are already reported. Thus we wanted to repeat the same determination in order to test the available instrument (Bruker Minispec MQ180) and compare the obtained data with those recorded by means of the Bruker NMR-MOUSE. In the literature, at our knowledge, only one investigation on oil-water emulsion has been reported with both a NMR-MOUSE and a conventional low-field NMR (LF-NMR) instrument. [4] The authors observed that: (a) the signal/noise (S/N) ratio is approximately 10 times lower for the NMR-MOUSE than for the bench-top LF-NMR; (b) the trend in relaxation behaviour with increasing oil content measured using the NMR-MOUSE is the reverse of that obtained using bench-top LF-NMR. The large reduction in the S/N ratio is primarily due to the signal only being acquired from a thin slice through the sample, whereas it originates from a larger sample volume when bench-top LF-NMR is used. The reversing of the trend in the decay rates with decreasing oil contents is also expected as a result of the strong magnetic field gradient which greatly decreases the apparent relaxation time of the water due to its rapid diffusion rate. We confirmed the same behavior in all our samples: selected data obtained for samples of poly(acrylic acid) partial sodium salts with different amounts of water are reported in Table 1 and Figures 1a and 1b show two typical bi-exponential T_2 distributions (calculated with UPEN software) for LF-NMR and Mouse determinations.

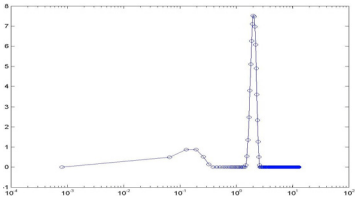

Figure 1a (LF-NMR)

Poly(acrylic acid) partial sodium salts/H_2O			
LF-NMR		NMR-MOUSE	
Amp	T_2 [ms]	Amp	T_2 [ms]
5.50	200	50.00	12.50
35.0	2100	25.80	85.50

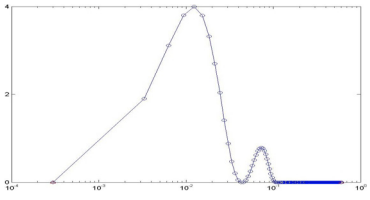

Figure 1b : NMR-MOUSE

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DIFFERENT BEHAVIOR OF CHEMICAL SHIFT MODULATION IN THE SECONDARY STRUCTURE ELEMENTS

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Recent spectroscopic developments have focused on the extraction of exchange contributions from ΔR relaxation rates[1]. Fluctuations of isotropic chemical shifts caused by slow exchange between energetically accessible conformations contribute to the transverse relaxation of nuclei like ^{15}N and ^{13}C. These fluctuations contribute to the differential line broadening between zero- and double-quantum coherences (ZQ and DQ) involving in the selected pair of nuclei[2].
Cross correlation rates of ZQ and DQ coherences, due to Chemical Shift Modulations (CSM), involving carbonyl C' and amide N nuclei in protein backbone provide evidences of slow motion in proteins. They may reflect dynamics on time scales in the range of micro to milliseconds and vary significantly along the protein backbone. Chemical shift may be modulated by conformational dynamics on the μs-ms timescales due to several factors including the formation or disruption of hydrogen bonds, change in local geometry by alteration of dihedral angles or changes in local chemical environmental by repositioning of neighboring aromatic rings.
CSM/CSM cross correlation rates were measured on three proteins with different folding: Calbindin D9k (a EF-hand type system), CuZn-SOD (a β-barrel system), and MMP12 (5β-sheet, 2α-helix system).
Correlations were found between CSM values and the secondary structure of different proteins. Anti-correlated motions are observed in α-helix regions whereas β-sheet are dominated by correlated motions. Interestingly, a Karplus type distribution of CSM/CSM relaxation rates with the backbone dihedral angle psi was found. Although data exhibit an intrinsically large scattering that prevents one to parameterize $R^{CSM/CSM}$ values as a function of the ψ angle, the observed trend is unambiguous. To best our knowledge, this is the first case where a relaxation rate could be correlated with a backbone dihedral angle.

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APPLICATIONS OF QUANTITATIVE NMR SPECTROSCOPY TO LEAD OPTIMISATION STAGES IN THE DRUG DISCOVERY PROCESS

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Although chromatographic methods are still first choice as they are well established and documented in analytical standards, pharmacopoeia and drug master files, quantitative NMR (qNMR) methods have gained increasing acceptance and widespread use in recent years.
As the ratio of substances in a mixture can be determined directly from the NMR spectrum, NMR spectroscopy can be considered as primary relative quantification method. Absolute amounts of substances have long been determined using internal or external reference substances; however that implies contamination of the analyte or cumbersome sample preparation, respectively. The introduction of ERETIC 1 (electronic reference to access in-vivo concentrations) techniques resolved these issues, however, required extra hardware components.
Using techniques such as PULCON 2 (pulse length based concentration determination) that are related to the principle of reciprocity, absolute, unknown concentrations of a sample can, for a given coil, be determined based on the known concentration of a reference sample.
The poster presents applications of the PULCON technique to different analytical or physico-chemical sets of problems that frequently recur during lead optimization stages.

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IN VITRO ^{1}H AND ^{31}P NMR SPECTROSCOPY AS A TOOL FOR INVESTIGATING MUSCLE ENERGY STATE IN FACIOCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD) MOUSE MODEL

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Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary neuromuscular disorder characterized by progressive weakness and atrophy of the facial, shoulder, abdominal and pelvic girdle muscles. We proposed that its pathogenesis could be associated with the over-expression of genes mapped at chromosome 4q35, ANT1, FRG1 and FRG2 [1]. Consistently, transgenic mice over-expressing FRG1 develop a progressive muscular dystrophy with features of the human disorder and can be considered a reliable mice model to study FSHD [2]. FSHD mouse model shows reduced tolerance to exercise and muscle weakness which can be related to an alteration of the muscular energetic systems.
To investigate this possibility we have applied ^{1}H and ^{31}P NMR spectroscopy (Fig. 1) to wild-type and dystrophic vastus muscle PCA extracts to evaluate the concentration of muscular energetic metabolites (ATP, ADP, AMP, Pi, Cr and PCr) and measure energetic parameters [3].

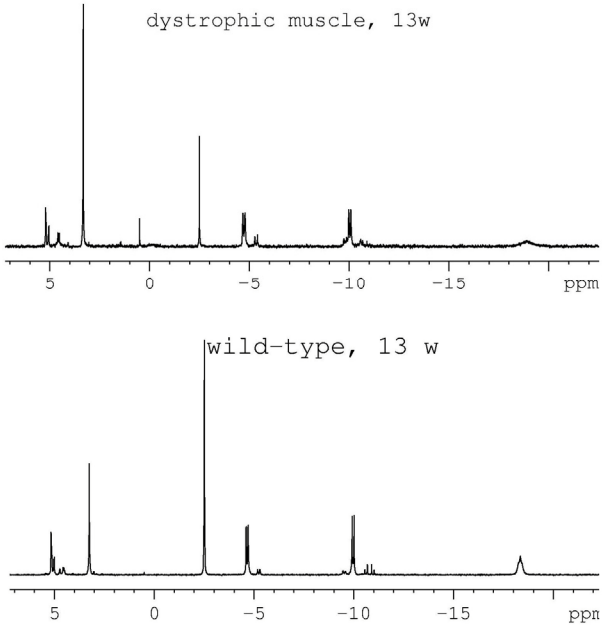

Fig. 1. ^{31}P NMR spectra of wild-type and dystrophic vastus muscle PCA extracts

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NMR METABOLIC PROFILING OF A CLONAL SELECTION OF *VERMENTINO* GRAPES DURING BERRY DEVELOPMENT

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Vermentino is a late-ripening white grape variety mostly cultivated for winemaking. In the North of Sardinia (Gallura region), represent the only Sardinian wines that obtained the DOCG denomination (Denominazione di Origine Controllata e Garantita). It is characterized by having a very fruity and rich taste: fresh, with white flowers fragrances, with remarkable complexity and surprising sapidity. *Vermentino* is a relevant product for Italian enology, as it represents the fifth wine most sold in 2008 in Italian large retail stores [1] and shows a continuous increase in the average selling price.

Surprisingly, despite the great commercial interest, very little is reported about molecular composition of *Vermentino* grapes in the scientific literature. However, a detailed molecular characterization appears as a major issue, as many flavour compounds and enologically relevant molecules are derived from the metabolism of the starting grape berries, and on how it is influenced by pedoclimatic conditions (terroir) and agronomic practices.

We sought in our work to identify and quantify the principal metabolites of Sardinian *Vermentino* grape berry, which are the first determinants of wine aromas, by means of Nuclear Magnetic Resonance Spectroscopy (NMR). This analytical technique is in fact a powerful tool for qualitative and quantitative characterization of metabolites present in grape berry. Our attention is focused in particular on the evaluation of metabolic profiling variability among different clones as a function of phenological phases. Seven different clones (CAPVS3, CAPVS12, 640, RENZO, SN, VCR1, VCR2) were sampled from the same vineyard at four different phases of grape berry development. Sample preparation protocol for NMR samples involved use of perchloric acid (PCA) [2] to extract polar metabolites (mainly amino acids, organic acids and sugars) while preventing enzymatic degradation and removing proteins and acidic macromolecules. One-dimensional ^{1}H NMR, 2D ^{1}H-^{1}H COSY, 2D ^{1}H-^{1}H Clean-TOCSY, 2D ^{13}C -^{1}H HSQC spectra were acquired [3] and more than 30 molecules have been identified. The analysis of results was divided in three parts. The first one is focused on the study of evolution and changes of grape composition during vegetative development and ripening; the second one concerns the investigation of mature grapes (vintage period) from different zones of the same vineyard, and finally the last part involves statistical analysis of collected data in order to look at differences and correlations between samples.

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POLY-β-CYCLODEXTRIN BASED PLATFORM FOR pH MAPPING VIA RATIOMETRIC $^{19}F/^{1}H$ MRI METHOD

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Mapping pH is an important task in Medical Imaging as changes in pH usually accompany the development of various pathologies including tumors, stroke, infection, etc. Several paramagnetic metal complexes whose relaxivity is pH-dependent have been reported. However none of them have been succesfully applied *in vivo* because in order to have images reporting the pH map it is necessary to transform the observed changes in relaxation rates (R_1) in changes of relaxivities (r_1). This transformation requires the knowledge of the local concentration of the metal complex. A route to acquire this information may be pursued through the acquisition of the MR image of a heteronuclear signal originated from a molecule that displays the same *in vivo* biodistribution of the paramagnetic complex. Herein we report a supramolecular construct formed by: i) a Polycyclodextrin substrate that hosts ii) a suitably functionalized pH responsive Gd(III)complex and iii) an analogously functionalized ^{19}F-containing molecule (Fig. 1). The binding to the PolyCD substrate is pursued through the introduction of an adamantane group on both Gd and F containing systems, because adamantane is known to have a high binding affinity to β-CD cavities. The proof of concept of this approach has been obtained by acquiring the ^{1}H and ^{19}F-MRI images of a phantom consisting of four tubes filled with solutions of Gd/F/PolyCD adduct at different values of concentration and pH. As shown in Figure 1A the ^{1}H-MR image does not account for a proportional change in contrast with pH because the observed R_1 is dependent on both pH and concentration. Through the acquisition of the ^{19}F-MR image it has been possible to assess the concentration of the adduct in the four tubes thus allowing the $R_1 \rightarrow r_1$ transformation (Fig. 1B). The method proved to work well with a small (1-2%) error in the pH assessment. Finally the Poly-CD/F/Gd adduct can be endowed with targeting properties by hosting in one of the empty β-CD cavities an adamantane functionalized moiety able to provide the system with the proper recognition towards the target of interest.

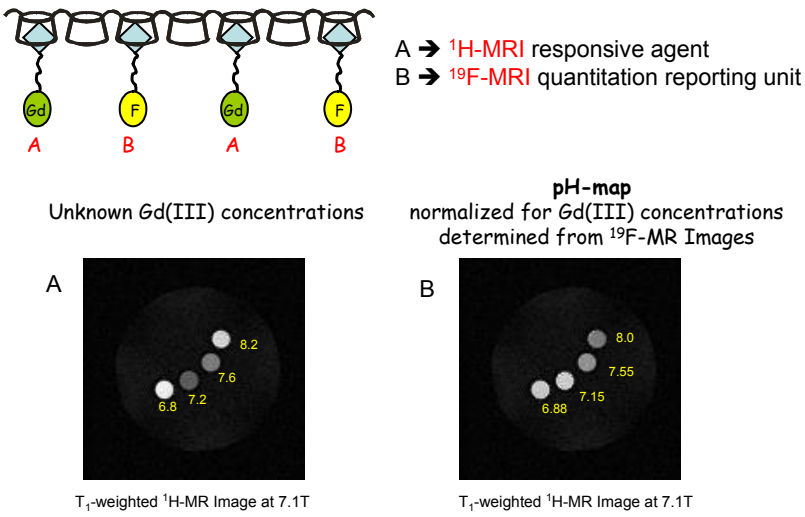

Fig. 1: Scheme of the Polycyclodextrin adduct and ^{1}H-MR images.

NMR-BASED STRUCTURAL REFINEMENT OF THE N- AND C-TERMINAL DOMAINS THE MONOTHIOL GLUTAREDOXIN GRX3 FROM *TRYPANOSOMA BRUCEI*

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Glutaredoxins (Grxs) are glutathione-dependent oxidoreductases that belong to the Thioredoxin super-family (Trxs). They are ubiquitous enzymes, being conserved throughout all the kingdoms of life from viruses to eukaryotes, and take part in a wide variety of biological processes: Grxs have been described as partners of ribonucleotide reductase, a protein essential for the biosynthesis of deoxyribonucleotides, as well as modulators of the reactivity of Cysteine-containing substrates through the formation or the reduction of disulfides bonds. Moreover, the central role of some glutaredoxins in iron metabolism and omeostasis has been demonstrated [1].
From a structural point of view, glutaredoxins are generally small proteins (9-14 kDa), and are thus suitable for routinary NMR experiments aimed at the determination of their solution structures. Depending on the active site's amino acid composition, Grxs can be divided into two subclasses, i.e., monothiol and dithiol Grxs [2]. Up to now, 40 structures of dithiol glutaredoxins have been solved either by NMR and/or X rays diffraction, while only one monothiol glutaredoxin structure has been deposited in the PDB [3]. In this work, we focus our attention on Grx3 from *Trypanosoma brucei*, a pathogenic protozoon responsible of human African trypanosomiasis, also known as sleeping sickness. A preliminary homology modeling study along with a chemical shift analysis shows that this protein consists of a monothiol glutaredoxin moiety coupled with a dithiol thioredoxin domain. The models of the two domains have been refined applying a simulated annealing protocol improved by the introduction of empirical restraints available from NMR experiments, such as chemical shift data, residual dipolar couplings from partially aligned samples, NOEs and hydrogen-deuterium exchange kinetics parameters.

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NMR STUDIES REVEAL THE ROLE OF BIOMEMBRANES IN MODULATING BILE ACID BINDING AND RELEASE FROM INTRACELLULAR BILE ACID BINDING PROTEINS

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Transport mechanisms are essential biological activities that assure material movement and communication between distant sites and different cellular environments. One very important transport system of the body is represented by the enterohepatic circulation, where liver and ileal bile acid binding proteins acts as cytosolic transporters of bile acids. [1] We present here NMR data on the behavior of bile acid binding proteins in cell and in the presence of membrane mimetic systems, such as DMPG anionic liposomes. These studies give clear evidence that avian apo Liver Bile Acid Binding protein (L-BABP), a model for the investigation of the intracellular trafficking mechanism of bile acids, undergoes partial unfolding and association in the presence of the anionic membrane. Interestingly the addition of the physiological ligand to the protein-liposome mixture is capable of modulating this interaction, shifting the equilibrium towards the monomeric folded holo-protein. Different NMR titration experiments, performed by combining ^{15}N protein and ligand observation, confirmed that the membrane and the ligand establish competing binding equilibria as depicted in the following scheme:

$$P + DMPG \rightleftharpoons P–DMPG$$

$$P–DMPG + GCDA \rightleftharpoons P–GCDA + DMPG$$

where P = L-BABP, DMPG = 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) liposomes and GCDA = glychochenodeoxycholic.
The indicated equilibrium could have a functional relevance possibly modulating the cytoplasmatic permeability of bile acids and support a mechanism of ligand binding and release controlled by the onset of a bile salt concentration gradient within the polar cell. The location of a specific protein region interacting with liposomes has been highlighted.

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NEW POLYMORPH OF DIDANOSINE OBTAINED BY SUPERCRITICAL ANTISOLVENT PROCESS

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Didanosine (2'-3'-dideoxyinosine, DDI) is a reverse transcriptase inhibitor, effective against HIV and used in combination with other antiretroviral drugs as part of a highly active antiretroviral therapy.
The aim of this work was to investigate the formation of new polymorphs of didanosine re-crystallized from a dimethylsulfoxide (DMSO) solution using supercritical CO_2 as an antisolvent (SAS process) and to study their solid-state properties and the solubility in water in comparison with the commercially available drug product. Structural differences between the commercial product and the SAS re-crystallized DDI are underlined by X-ray diffractometry and well described by solid-state NMR. We applied modern solid-state NMR techniques, namely 2D ^{1}H DQ CRAMPS (Combined Rotation And Multiple Pulse Spectroscopy) and ^{1}H-^{13}C on- and off-resonance CP (cross polarization) FSLG-HETCOR experiments, known for providing reliable information about ^{1}H-^{1}H [1] and ^{1}H-^{13}C [2] intra- and intermolecular proximities. These pulse sequences allowed comparisons and considerations about the crystal packing and hydrogen bonds since X-ray structures are not available. The analysis of homo- and heteronuclear proximities obtained by means of 2D NMR experiments shows that commercial and re-crystallized samples possess very similar conformation and hydrogen bond network, but different packing.
The new polymorph proved to be a metastable form showing higher solubility in water and lower stability to mechanical stress.
The particle size of the new crystal phase can be reduced by varying the antisolvent density through a pressure increase.

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CHARACTERIZATION OF THE MONOMERIC STATE OF CERATO-ULMIN, A PHYTOTOXIC HYDROPHOBIN.

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Hydrophobins are secreted as monomers by filamentous fungi. The formation of hydrophobin films at the air-water interface is a key mechanism which plays an important role in different stages of fungal development. Cerato-ulmin (CU) is a fungal toxin involved in Dutch elm disease that belongs to the class II hydrophobin family. Interestingly, CU shows a 40% homology with cerato-platanin (CP), a fungal secreted protein involved in the canker stain of plane. Here we present the NMR characterization of CU in its monomeric form.

Recombinant ^{15}N CU has been cloned and expressed in *Pichia pastoris* and purified by RP-HPLC. To confirm its monomeric state, CU has been solubilised in 30% deuterated acetonitrile and analyzed by NMR diffusion experiments.

Combining backbone and side chain NMR assignments with dynamic data from hydrogen-deuterium exchange experiments we derived information on the protein secondary structure.

Using the WHAT IF software a model of CU has been generated by homology modelling of hydrophobin II (PDB 1r2m), which shares 30% identity. The model was validated using experimentally obtained D_{HN} residual dipolar couplings.

Relaxation measurements revealed that both N-terminus and the loop connecting β1 and β2 strands, a region which contains hydrophobic residues involved in the protein dimerization process, have a high degree of mobility.

Interestingly, the 38-52 helix is homologous to the 33-47 helix of the hydrophobin II that is expected to be involved in host interaction.

Recognizing that CU is one of the few examples of phytotoxic hydrophobins, the determination of its structure not only will help to understand the mechanism of fungus-host interaction, but also comparison with the CP structure may contribute to define a general model to explain how proteins of different fungal families interact with their hosts.

^{1}H AND ^{13}C NMR INVESTIGATION OF THE 1-ALKYL-3-METHYLIMIDAZOLIUM BROMIDE IONIC LIQUIDS

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Ionic liquids (ILs) are room-temperature molten salts, typically composed of an organic cation and a non-coordinating inorganic anion. Due to their characteristic physicochemical property, ILs represent a new type of solvent with relatively low melting points and low vapor pressures. Because of these peculiarities, they can serve as a 'green' recyclable alternative to the noxious and volatile compounds regularly used as industrial solvents.

ILs owe many of their properties to their amphiphilic nature, as they are built up by an oily alkyl tail (e.g. butyl), a positively charged head (e.g. methylimidazolium) and an anion, typically fluorinated such as $[BF_4]^-$or $[PF_6]^-$. The presence of water in ILs, as a contaminant or deliberately added, can strongly affect their physical and chemical properties, such as conductivity, viscosity, and polarity. As a consequence, information on the structures of IL and their interaction with water are important to understand the relation between molecular and macroscopic properties of these systems.

Here we present the preliminary results of a ^{1}H and ^{13}C NMR study performed on ILs, namely 1-alkyl-3-methylimidazolium bromide ([C*n*MIM]Br, where *n=6, 8 or 10* is the alkyl chain length). The reorientational dynamics of samples was studied over a wide range of temperatures by measuring ^{13}C spin-lattice relaxation times and NOE factors. Cation-cation interactions were investigated by homonuclear NOEs in the rotating frame (ROEs). The results of the pure liquid were compared with those of samples containing known amounts of added water.

METABOLIC PROFLING OF SWEET PEPPER (*Capsicuum annum* L.) BY MEANS OF HRMAS-NMR SPECTROSCOPY

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HRMAS-NMR (High Resolution Magic Angle Spinning Nuclear Magnetic Resonance) is an innovative tool that offers the almost unique opportunity of measuring gel-like samples without any chemical and/or physical preparation, by producing highly resolved NMR spectra. This approach is of particular use in the food science, most likely in the characterization of several foodstuff: cheese,[1, 2] meat,[3, 4] wheat[5] and bread and flour,[6] among all.

In this work HRMAS-NMR spectroscopy has been used to assess the metabolic profile of sweet pepper (*Capsicum Annum* L.). 1D- (^{1}H and ^{13}C) and 2D- (TOCSY, COSY, HSQC and HMBC) NMR spectra (see Fig. 1), performed directly on few mg of sweet pepper, allowed the recognition of the major metabolites, such as carbohydrates, e.g. glucose and fructose and their isomers; organic acids; 13 amino acids; saturated and unsaturated fatty acids, and minor components, such as trigonelline, C4-substituted pyridine, choline and cinnamic derivatives.[7]

The results suggest that HRMAS-NMR is a very powerful tool for sweet pepper characterization, especially when statistical analysis is needed, since quantitative determination of many compounds could be obtained with a single experiment.

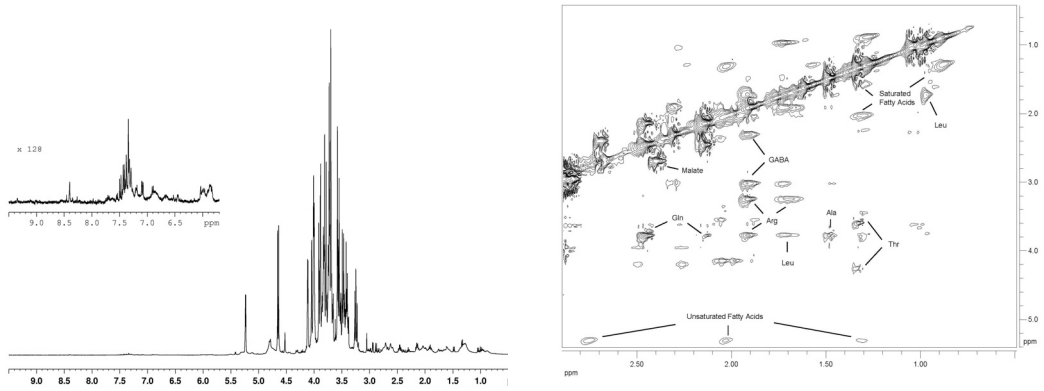

Fig. 1. On the left: ^{1}H HRMAS NMR spectrum of sweet red pepper in phosphate/D2O buffer with 0.5% of 3-(trimethylsilyl)propionic-2,2,3,3-*d*4 acid sodium salt (TSP). On the right: TOCSY spectrum expansion of the high field region.

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THERMO-INDUCED LIPID OXIDATION OF CULINARY OILS: A KINETIC STUDY OF THE OXIDATION PRODUCTS BY MAGNETIC RESONANCE SPECTROSCOPIES

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Deep fried foods introduce in the human diet some by-products that originate from the thermal stress of lipids. The main reaction involved in the oxidative degradation of lipids is the thermally induced, radical-mediated auto-oxidation of polyunsaturated fatty acids. This process mainly generates conjugated hydroperoxydienes that are unstable at the standard frying temperatures (180 °C) and are degraded to a variety of by-products including saturated and unsaturated aldehydes. This class of compounds, in particular, is known for its ability to exert toxicological effects in vivo because of its high reactivity with most biomolecules.[1,2] In this work we employ both EPR spectroscopy and enhanced-sensitivity ^{1}H NMR [3] techniques to explore the kinetics of peroxides and aldehydes buildup during episodes of thermal stress on culinary oils and fats,[4] using peanut oil as model substrate.

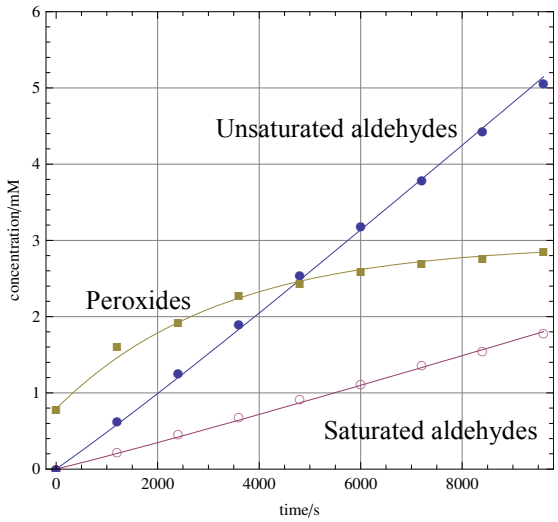

Figure 1 Aldehydes and peroxides buildup during an episode of thermal stress at 180 °C.

The obtained concentration profiles indicate that the secondary oxidation products (saturated and unsaturated aldehydes) may not be formed only via a direct degradation of primary oxidation products (hydroperoxides). [5]

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LANTHANIDE LUMINESCENT ORGANIC COMPLEXES: EFFECT OF N-BASED CO-LIGANDS AS REVEALED BY NMR PARAMAGNETIC SHIFTS AND PHOTOPHYSICAL PROPERTIES

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The unique photophysical properties of lanthanide ions have encouraged wide research activities in view of photonic application such as tuneable lasers, components of the emitting materials in multilayer organic light emitting diodes (OLED) and light conversion molecular devices. It is well-known that the use of suitable ligands as absorbing components is required to improve the efficiency of the lanthanide metal complexes. The β-diketone ligand can form stable and strong adducts with all the metals of the lanthanide series and it is one of the most important "antenna" from which the energy can be effectively transferred to Ln(III) ions. Moreover, ancillary co-ligands such as substituted bipyridines and phenanthrolines can influence the emission intensity of these complexes.The above considerations prompted us to synthesize various octa-coordinated lanthanide β-diketonate complexes of general formula: $Ln(acac)_3(L)$ [where Ln= Eu, Sm, Tb, Dy]. These Ln(III) complexes were characterized by NMR and their photophysical properties were studied by UV-Vis absorption and by photoluminescence.

For what concerns the NMR characterization of these complexes, they are affected by strong paramagnetic shifts, typical of the lanthanide metal ions.

The paramagnetic shifts can change in intensity and sign according to the geometry of the complex, but, assuming that the geometry is almost the same in a series of complexes where only the ancillary ligand has been changed, the entity of the paramagnetic shifts can be correlated to the coordination strength between metal ion and ligands, that is with the basicity of the ligands.

Similar considerations can be done by looking at a series of complexes with the same general formula but different Ln(III) metal ion. The results obtained for the paramagnetic lanthanide ions have been compared with those found for the analogous diamagnetic yttrium derivatives.

These NMR results can be correlated with the photophysical properties of the complexes: the results up to now obtained lead to the conclusion that the overall quantum yields seem not only to be directly related to N-based ligand basicity but other physical features appear to be involved and further investigation will be necessary to elucidate these evidences.

STRUCTURAL AND FUNCTIONAL MODIFICATION INDUCED ON DJ1 BY DOPAMINOQUINONES (DAQ)

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Parkinson disease (PD) is a multifactorial neurodegenerative condition characterized by the progressive loss of dopaminergic neurons in the substantia nigra and by the presence of intracellular inclusions, composed predominantly of fibrillar alphasynuclein (aS). Postmortem studies indicate the presence of oxidative damage in the nigral neurons of PD patients. Dopamine oxidation, which leads to the formation of highly reactive quinones (DAQ), may account for the specificity of dopaminergic neuron degeneration in PD. DAQ can modify many potential protein targets. Among them, we focused our attention on DJ1, a protein that has gained great interest on in different forms of PD and recently it has been identified as DAQ target. Various functions have been ascribed to DJ1, such as a protective role against oxidative stress, either as a redox sensor or as an antioxidant protein. Another function is its redox-dependent chaperone activity that could prevent aS aggregation and fibril formation.
In the present work, we analyzed the structural and functional modification induced on DJ1 by DAQ. Several NMR experiments on DJ1 were recorded in the presence of different amounts of DAQ and chemical shift perturbations were used to identify the DJ1 residues target of DAQ and their relative reactivity. Molecular Dynamics Simulation, aggregation and thermal stability assays were also performed to assess the effects of DAQ modification on the chaperone activity of DJ1.

STUDY OF SiO_2-TSPM / PMMA COMPOSITES BY MULTINUCLEAR SOLID-STATE NMR TECHNIQUES

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The unique performance of an organic-inorganic hybrid material is strongly related to its structural features [1]. In polymer encapsulated nano-sized inorganic particles, the surface characteristics of the inorganic particles are modified by coating with a polymer layer. Polymer encapsulation may be applied to reduce particle toxicity, mask taste and odour, to enhance stability and to improve dispersibility in organic media [2]. Polymer encapsulated inorganic particles offer potential properties for use in bio-medical or cosmetic fields, textiles, paints, optics & electronics [3].
The samples we have studied are prepared by a simple method for the encapsulation of silica particles by a shell coating of poly (methyl methacrylate) (PMMA). The silica particles are modified in situ with the surface grafting of the silane coupling agent, 3-(trimethoxysilyl) propyl methacrylate (TSPM) and the modified particles are coated with PMMA by a seeding method and thus yielding the SiO_2-TSPM/PMMA hybrid materials whose morphology was investigated by TEM analysis [4]. Solid-State NMR is a key tool of investigation, allowing chemical structural and molecular dynamic information to be obtained on the organic and the inorganic components and at their interfaces [5].
A SiO_2-TSPM/PMMA composite, prepared under static conditions, was extensively studied by Solid State NMR techniques, along with unmodified SiO_2, SiO_2-TSPM and PMMA samples, in order to characterize the structural and dynamic properties of the single components, as well as the interactions occurring among them, like the condensation degree of the silica moieties. The main techniques involved were CP, Delayed CP, NQS and DEPTH experiments, all applied under MAS conditions. We have also studied another SiO_2-TSPM/PMMA composite, which was prepared by the same reaction procedures as the previous composite but under stirring. This composite exhibited a different morphology, as seen by TEM experiments. The NMR results obtained for these two composites have been compared and discussed.

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MAGNETIC RESONANCE IMAGING FOR KIWIFRUIT QUALITY EVALUATION: SHELF-LIFE AND PGR DETECTION

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Magnetic Resonance Imaging (MRI) is a technique known predominantly for its medical and diagnostic applications [1,2], is capable of producing in non invasive fashion high quality images of any internal volume or section of the analyzed sample. MRI has been widely applied in food science in the last decade, mostly for studying fruits and vegetables. Several papers are present in literature, mainly concerning the determination of the internal morphology, the evolution of tissues during post-harvest ripening/storage, and the evaluation of the overall quality by measuring quality related parameters.
Kiwifruit has been largely investigated [3], we have recently considered the variation of the internal structure as a function of the post-harvest storage conditions, i.e. temperature and atmosphere composition, for *cv.* Hayward and for new cultivated varieties, which might be appealing for the market in the next future.
We have also considered the effects of the use of plant growth regulators (PGR) on the shelf-life. We observed that MRI is capable of distinguishing kiwifruits treated with PGR from those non treated, as well as of discriminating the PGR used, i.e. auxin or cytoquinine.
Comparison of T_2-weighted MRI images of untreated and treated (with two different PGR) samples showed that the use of PGR can be determined, both at harvesting and commercialization step. MRI images revealed large differences in terms of tissues' organization of the external pericarpus, with water arrangement and cellular wateriness playing a fundamental role. We observed that T_2-weighted MRI images of untreated kiwifruits are characterized by four regular concentric spherical crowns. Auxin-treated samples showed several 2-3 mm thick channels dark in color, while T_2-weighted MRI images of cytoquinine-treated kiwifruits have darker stains in the outer zones (Figure 1).

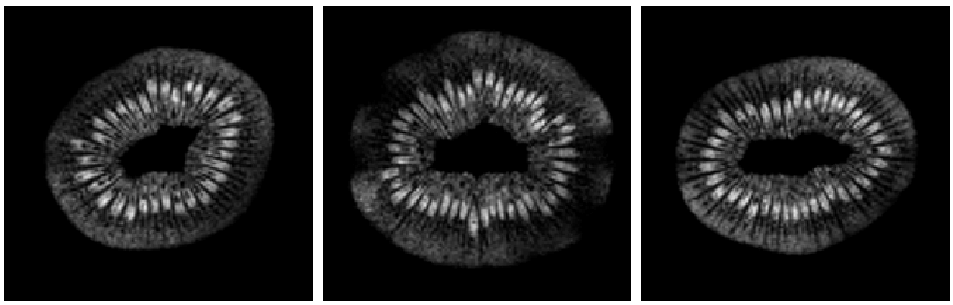

Fig. 1. T_2-weighted MRI images of untreated, cytoquinine-treated and auxine-treated kiwifruits (from left to right).

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AN IMPROVED METHOD FOR THE SIMULTANEOUS DETERMINATION OF OIL AND MOISTURE CONTENT IN DIFFERENT KINDS OF SEEDS BY PULSED NUCLEAR MAGNETIC RESONANCE

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The industrial interest in the correlation between oil and moisture content of seeds and their commercial quality has been increasing.
Because of the large volume of commercial lots of grains of different type and quality, the determination of these parameters requires a suitable technique for quick and reliable estimates.
Some works performed in the past years on different kinds of commercial oilseeds [1, 2] and olive husks [3] have already established the utility of time-domain Nuclear Magnetic Resonance spectroscopy as a valid tool to provide a rapid simultaneous evaluation of oil and water contents, and also the superiority of this technique compared to other ones conventionally used in this field.
In the present communication, a recently developed improvement on the rapid and non-destructive method for the simultaneous determination of oil and moisture contents in seeds is described.
The oil and moisture content of unknown samples were obtained by using two different calibration lines built with the standard addition method for oil and with weight loss for moisture.
The application of the proposed method was successful on seeds with low oil content, such as green coffee and other kinds of seeds.

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CHICKEN ILEAL BILE ACID BINDING PROTEIN: THE PROTOTYPE OF AN EFFICIENT LIPID CHAPERONE?

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Bile acids are potent detergents essential for efficient digestion and absorption of dietary fat. They also play an important role as signaling molecules, with diverse endocrine and paracrine functions, and have a role in carcinogenesis. In this study we invwstigated the mechanism for the complex multi-site interaction of bile acids with a model protein carrier, the newly identified chicken ileal bile acid binding protein (cI-BABP), belonging to the intracellular lipid binding protein (ILBP) family. ILBPs from different species display high structural similarity and have been shown to bind multiple bile acid ligands with different stoichiometry, selectivity and cooperativity. A thoroughly NMR investigation, including ligand-based and protein-based NMR titration experiments together with ligand binding competition assays, mass spectrometry analysis and calorimetric measurements, is presented here. The obtained data suggest that chemical shift changes upon binding are governed by conformational changes of cI-BABP in response to binding rather than to local effects caused by direct contact with the ligands. The results, analyzed within the framework of the ensemble of binding parameters emerging for the studied ILBPs, point to the presence of a protein scaffold which is able to establish long-range communication networks. The detection of a singly-ligated intermediate sets the molecular basis for the comprehension of the cooperativity observed for the liver paralog and human ortholog of cI-BABP. Thus, we have identified an allosteric system with an imperfect, but already encoded, predisposition to positive binding cooperativity.

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