GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XXXIII National Congress on Magnetic Resonance

Bressanone/Brixen (Italy) - September 16-19, 2003

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Gruppo Interdivisionale Risonanze Magnetiche
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Comune di Bressanone — Gemeinde Brixen

Università di Padova

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(Pharmacia, Milano)

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(CNR, Milano)

SCIENTIFIC PROGRAM

Tuesday, September 16

10.00-19.00 REGISTRATION
19.00-21.00 Welcome Reception

Wednesday, September 17 - morning

8.30-9.00 OPENING OF THE MEETING
9.00-10.30 PLENARY LECTURES, Aula Magna. Chair: **P. Fantazzini**

9.00-9.45 **P. T. Callaghan,** Victoria University at Wellington, New Zealand
Surprising connections - the diverse world of magnetic resonance

9.45-10.30 **P. J. Cozzone**, CNRS Marseille, France
Magnetic resonance spectroscopy and spectroscopic imaging of the human brain: clinical applications

10.30-10.50 **STELAR: Lothar Helm**, Ecole Polytechnique Fédérale, Lausanne, Switzerland
NMR relaxometry applied to gadolinium based MRI contrast agents

10.50-11.20 COFFEE BREAK
11.20-12.50 PARALLEL SESSIONS

NMR IN BIOMEDICINE
Aula Magna. Chair: **F. Conti**

11.20-11.50 **A. Bifone**,
GlaxoSmithKline, Verona
Pharmacological MRI: mapping drug-induced brain activation

11.50-12.10 **N. Culeddu**,
ICB, CNR Sassari
NMR experts and MRI quality test, an application

12.10-12.30 **F. M. Cavagna**,
Bracco Imaging, Milano
Intravascular contrast agents for MR imaging: technologies and new clinical applications in cardiac, oncologic, and neurologic imaging

12.30-12.50 **S. Grande**,
ISS and INFN, Roma
Changes in ^{1}H MRS signals from mobile lipids and from lipid precursors during cell growth: a multivariate analysis study

MATERIALS SCIENCE
Aula B.Chair: **R. Simonutti**

11.20-11.50 **D. Capitani**,
IMC, CNR Montelibretti
Materials studied with a portable NMR instrumentation

11.50-12.10 **R. Gobetto**,
University of Torino
Solid state NMR investigation of hydrogen bond in supramolecular adducts

12.10-12.30 **K. Severing**,
University of Freiburg, Germany
^{2}H-NMR investigations on the biaxiality on a liquid crystalline side chain polymer

12.30-12.50 **M. Mauri**,
University of Milano-Bicocca
Continuous flow Xe-OPSE NMR applied to the characterization of materials

12.50-14.30 LUNCH, Hotel Elefante

Wednesday, September 17 - afternoon

14.30-16.00 PLENARY LECTURES, Aula Magna. Chair: **M. Piccioli**

14.30-15.15 **R. Konrat,** University of Vienna, Austria
Automated biomolecular NMR spectroscopy for large scale structural genomics

15.15-16.00 **D. O. Cicero,** University of Roma, Tor Vergata
Selectivity and flexibility in DNA-protein interactions: the E2 DNA-binding domain from human Papillomavirus

16.00-16.20 **S-IN: Brent Lefebvre,** ACD/Labs Toronto, Canada
ACD/Combi NMR: ^{1}H and ^{13}C automated verification

16.20-16.50 COFFEE BREAK

16.50-18.20 PARALLEL SESSIONS

RELAXATION/LOW RESOLUTION
Aula Magna. Chair: **M. Fasano**

16.50-17.20 **M. Botta**,
University of Piemonte Orientale
Reversible Oxy-Anion Binding in Aqueous Solution at a Gd(III) Center: an NMR Relaxometric Study

17.20-17.40 **N. Ghofraniha**,
University of Roma, La Sapienza
^{17}O-water multipole relaxation

17.40-18.00 **L. Casciani**,
University of Roma, La Sapienza
Polymeric materials characterization by application of multivariate analysis to low field nuclear magnetic resonance data

18.00-18.20 **L. Laghi**,
University of Bologna
The effect of ageing on the egg white - a water proton relaxation study

METHODOLOGY
Aula B. Chair: **M. Tatò**

16.50-17.20 **D. Carbonera**,
University of Padova
Optically Detected Magnetic Resonance of Photosynthetic Reaction Centers

17.20-17.40 **S. Bradamante**,
Institute of Molecular Science and Technology, CNR Milano
Application of a NMR-compatible microgravity-based bioreactor for on-line monitoring cell cultures

17.40-18.00 **N. Niccolai**,
University of Siena
Peptide-protein interactions studied by surface plasmon and nuclear magnetic resonances

18.00-18.20 **L. B. Pasternack,**
Scripps Research Institute, La Jolla, CA
High-resolution diffusion-ordered spectroscopy to probe the microenvironment of Jandajel and Merrifield resins

20.30-22.00 GIDRM AND GIRM MEETINGS, Aula Magna
(**with after-dinner drinks**)

Thursday, September 18 - morning

9.00-10.30 PLENARY LECTURES, Aula Magna. Chair: **P. A. Temussi**

9.00-9.45 **M. Llinas**, Carnegie Mellon University, USA, "Lincei Lecturer"
The CLOUDS approach to biomolecular NMR structure computation

9.45-10.30 **P. Fantazzini,** University of Bologna
More than seventeen years of NMR in porous media at the University of Bologna

10.30-10.50 **VARIAN: E. Kupce,** Varian, Oxford, UK
Fast multi-dimensional NMR spectroscopy

10.50-11.20 COFFEE BREAK

11.20-12.50 PARALLEL SESSIONS

NMR AND BIOINFORMATICS
Aula Magna. Chair: **N. Niccolai**

11.20-11.50 **R. H. Fogh**,
University of Cambridge, U.K.
CCPN - New NMR analysis software and an NMR data standard

11.50-12.10 **F. Capozzi**,
University of Bologna
NMR folding investigations of recombinant EF-hand calcium-binding proteins

12.10-12.30 **A. Rosato**,
University of Firenze
Bioinformatic and NMR tools for the characterization of molecular features of metalloproteins

12.30-12.50 **S. C. E. Tosatto,**
University of Padova
A divide & conquer approach to fast loop modeling

"GIULIO CESARE BORGIA" MINISYMPOSIUM ON POROUS MEDIA AND MATERIALS
Aula B. Chair: **P. T. Callaghan**

11.20-11.50 **J.-P. Korb**,
CNRS Palaiseau, France
Surface dynamics of liquids in porous media: a field cycling approach

11.50-12.10 **M. Balzarini**,
ENI Divisione E&P, San Donato (MI)
NMR diffusion measurements and MRI image analysis in reservoir rocks

12.10-12.30 **R. Toffanin**,
PROTOS Trieste
High resolution NMR imaging for the quantitative analysis of trabecular bone architecture

12.30-12.50 **M. Gussoni**,
University of Milano
Modeling biological and material porous systems by ^1H NMR T_1 and T_2 relaxation and imaging

12.50-14.30 LUNCH, Hotel Elefante

Thursday, September 18 - afternoon

14.30-16.00 PARALLEL SESSIONS

FUNCTIONAL PROTEOMICS
Aula Magna. Chair: **M. Paci**

14.30-15.00 **M. Casu**,
University of Cagliari
Solution Structural Investigation of Pig Metmyoglobin cavities and surface by ^{129}Xe NMR Spectroscopy

15.00-15.20 **R. Ugolini**,
University of Verona
Folding studies on liver basic fatty acid binding protein

15.20-15.40 **A. M. D'Ursi**,
University of Salerno
Conformational behaviour of Aβ(25-35) in different environmental conditions

15.40-16.00 **M. Valerio**,
University of Roma, La Sapienza
Study of aggregation and folding of proteins by NMR and molecular dynamics: 1-40 and 1-28 β-amyloids

"GIULIO CESARE BORGIA" MINISYMPOSIUM ON POROUS MEDIA AND MATERIALS
Aula B. Chair: **P. T. Callaghan**

14.30-15.00 **G. Maddinelli**,
EniTecnologie Milano
Application of NMR techniques in petrochemical industrial research

15.00-15.20 **C. Casieri**,
University of L'Aquila
Use of model systems to interpret NMR in-situ measurements for cultural heritage materials

15.20-15.40 **M. Alesiani**,
University of Roma, La Sapienza
Porous structure properties determination by NMR imaging and relaxation

15.40-16.00 **S. Spera**,
Istituto Donegani, Novara
A solid-state NMR study of β-zeolite, carried out with the help of probe molecules

16.00-18.00 POSTER SESSION & COFFEE BREAK

18.00-19.00 PLENARY LECTURE, Aula Magna. Chair: **C. Marchioro**

18.00-19.00 ***GIRM GOLD MEDAL:*** **A. Spisni**, University of Parma
My first 30 years of acquaintance with the spins

Friday, September 19 - morning

9.00-9.45 PLENARY LECTURE, Aula Magna. Chair: **L. Zetta**

9.00-9.45 **G. Esposito**, University of Udine
Near the borderline of the structural and functional information that can be obtained by NMR spectroscopy

9.45-10.45 BEST POSTERS

10.45-11.15 COFFEE BREAK

11.15-12.55 PARALLEL SESSIONS

FUNCTIONAL PROTEOMICS
Aula Magna. Chair: **H. Molinari**

11.15-11.45 **R. Fattorusso**,
Second University of Napoli
Solution structure and dynamic behaviour of the K18G/R82E Alicyclobacillus acidocaldarius thioredoxin mutant: a molecular analysis of its reduced thermal stability

11.45-12.15 **D. Picone**,
University of Napoli
The swapping of terminal arms in ribonucleases

12.15-12.35 **R. Pierattelli**,
University of Firenze
Study of transient intermediates in biomolecular processes by NMR

12.35-12.55 **A. Bernini**,
University of Siena
Surface accessibility of HEW lysozyme

MOLECULAR REACTIVITY AND DESIGN
Aula B. Chair: **S. Chimichi**

11.15-11.45 **M. Peruzzini**,
ICCOM, CNR Firenze
^{31}P NMR spectroscopy as an useful tool to evaluate the solution structure of transition metal complexes incorporating fragments and units derived from white phosphorus

11.45-12.15 **G. Cerioni**,
University of Cagliari
^{17}O NMR: a quasi-exotic tool for the organic chemist

12.15-12.35 **F. Sancassan**,
University of Genova
Lanthanide-Induced Shifts (L.I.S.) in structural and conformational analysis

12.35-12.55 **S. Ripoli**,
University of Pisa
Solution molecular structure determinations: paramagnetic NMR of Ytterbium complexes

12.55-14.30 LUNCH, Hotel Elefante

Friday, September 19 - afternoon

14.30-16.00 PLENARY LECTURES, Aula Magna. Chair: **S. Bradamante**

14.30-15.15 **A. Watts,** University of Oxford, United Kingdom
Solid state NMR to resolve intimate structural and dynamic details about ligand-receptor interactions

15.15-16.00 **R. Riccio,** University of Salerno
Relative stereochemical analysis in chiral organic compounds with conformational mobility by quantum mechanical calculation of ^{13}C NMR parameter

16.00-16.20 **BRUKER: R. Kerssebaum,** Bruker BioSpin GmbH, Germany
DOSY – Applications and limitations

16.20-16.50 COFFEE BREAK

16.50-18.20 PARALLEL SESSIONS

INTERACTOMICS
Aula Magna. Chair: **G. Esposito**

16.50-17.20 **D. Bentrop**,
University of Freiburg, Germany
Cytoplasmic domains of potassium ion channels and their protein-protein interactions

17.20-17.40 **D. A. Middleton**,
University of Manchester Institute of Science and Technology, U.K.
Insights into protein-protein interactions within biomembranes by solid-state NMR

17.40-18.00 **N. D'Amelio**,
University of Siena
NMR structural model of the interaction of acifluorfen with the photosynthetic reaction center from Rhodopseudomonas viridis

18.00-18.20 **G. M. Contessa,**
University of Roma, Tor Vergata
Calmodulin modulation of the olfactory CNG channel: solution structure by NMR of a complex between a fragment of the N-terminal domain and calmodulin

MOLECULAR REACTIVITY AND DESIGN
Aula B.Chair: **M. Cremonini**

16.50-17.20 **S. Provera**,
GlaxoSmithKline, Verona
Use of HPLC-NMR for speeding up the structure elucidation process in pharmaceutical industry

17.20-17.40 **A. Mele**,
Polytechnic University of Milano
NMR study of the structure of some imidazolium-based room temperature ionic liquids

17.40-18.00 **D. Maggioni**,
University of Milano
Association equilibria involving $(C_6F_5)_2B(OH)$ species in toluene-d_8 and CD_2Cl_2 solutions

18.00-18.20 **S. Chimichi**,
University of Firenze
Selective monoalkylation of dihydroxycoumarins via Mitsunobu dehydroalkylation: a regiochemical problem solved by NMR

18.20-18.30 CONCLUDING REMARKS

19.30-22.30 SOCIAL DINNER

PLENARY LECTURES

SURPRISING CONNECTIONS-THE DIVERSE WORLD OF MAGNETIC RESONANCE

Paul Callaghan

Alan MacDiarmid Professor of Physical Sciences, School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington, New Zealand

When Rutherford discovered the atomic nucleus he could not possibly have imagined that it might be a window to understanding molecular biology, or how the brain works. And yet so it has come to pass. It is the through the magnetism of the nucleus that these insights, and so much more, are possible. The phenomenon of "Nuclear Magnetic Resonance" has proven an essential tool in physics, it has revolutionised chemistry and biochemistry, it has made astonishing contributions to medicine, and is now making an impact in geophysics, chemical engineering and food technology. It is even finding applications in new security technologies and in testing fundamental ideas concerning quantum computing. This talk will explain why the nuclear spin is so ubiquitous and interdisciplinary, and will illustrate how unpredictable and surprising are the consequences of a major scientific discovery.

MAGNETIC RESONANCE SPECTROSCOPY AND SPECTROSCOPIC IMAGING OF THE HUMAN BRAIN: CLINICAL APPLICATIONS

Patrick. J. Cozzone

Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS 6612, Faculté de Médecine, 27 Bd J.Moulin,13005 Marseille (FRANCE)

Proton localized magnetic resonance spectroscopy (MRS) is a method of noninvasive exploration of human neurochemistry based on the magnetic resonance phenomenon. This exploration of brain metabolism, performed without any injection, detects neuronal, glial, and membrane markers. MRS of the brain tends to be a “metabolic "biopsy”, but unique applications for brain MRS are (1) quantitating the oxidative state of the brain and defining neuronal suffering, (2) assessing and mapping neuron damage, (3) evaluating membrane alterations and demyelination, (4) characterizing glial activation or gliosis, (5) identifying macrophagic invasion and/or hypoxia, (6) detecting modification in the metabolism of macromolecules (lipids, proteins), (7) pinpointing anomalies in the metabolism of glial and neuronal aminoacids.

Brain MRS (single voxel spectroscopy and chemical shift imaging) can be performed routinely after conventional MRI, without moving the patient, as a valuable metabolic and functional complement to the anatomical evaluation of the cerebral status of patients. Examples of clinical applications will be presented in the following domains: early diagnosis of HIV-related encephalopathy, differential diagnosis of brain tumors, follow-up of patient response to therapy, characterization of pharmacoresistant epilepsies, multiple sclerosis, stroke, metabolic encephalopathies in children.

AUTOMATED BIOMOLECULAR NMR SPECTROSCOPY FOR LARGE-SCALE SCALE STRUCTURAL GENOMICS

Robert Konrat

Institute of Theoretical Chemistry and Molecular Structural Biology, University of Vienna, Rennweg 95b, A-1030 Vienna

Genomic sequencing efforts have provided the coding DNA sequences of a large number of unknown genes. But knowledge of the sequences of the genes alone is insufficient for an understanding of the function. Structural genomics or structural proteomics attempts to provide three-dimensional structural information of proteins encoded by the sequenced genes and NMR is poised to play a significant role in these activities.

In structural genomics, it is necessary to efficiently screen in a high-throughput manner for the presence of stable structures in proteins which can be subjected to subsequent structure determination by X-Ray or NMR spectroscopy. Here we illustrate that the distribution of the eigenvalues of the ^{1}H chemical shift interaction in a protein as detected by ^{1}H NMR spectroscopy can be used to probe the foldedness (e.g. presence of stable protein structures) of proteins in solution. The method can easily be adjusted for screening purposes, using NMR flow probes and micro manipulator robots, and should consequently prove useful for target selection in high-throughput structural genomics, the identification of experimental conditions to optimize protein stability and crystal formation, and for screening of potential ligands.

For high-throughput structure determination of proteins in solution we have combined bioinformatic tools and NMR spectroscopy. Depending on the quality of a given structure prediction model NMR spectroscopy will be used to validate the model and/or complete the 3D structure in cases of missing date due to imperfect sequence alignment or to identify folds from the structural database if no sequence similarities can be found.

Finally, since the delineation of protein-protein interactions is necessary to fully understand the cellular machinery, a novel NMR approach to monitor protein-protein interactions at the molecular level will be presented. The new approach allows for very sensitive and high-throughput *in vitro* probing and quantification of protein interactions.

The underlying principles and applications to a diverse set of different proteins will be presented.

SELECTIVITY AND FLEXIBILITY IN DNA-PROTEIN INTERACTIONS: THE E2 DNA-BINDING DOMAIN FROM HUMAN PAPILLOMAVIRUS

Daniel O. Cicero

Dept. Chemical Sciences &Technologies, University of Rome `Tor Vergata,' Italy

Papillomaviruses are DNA viruses that infect a wide range of mammals, and represent a serious health threat for humans. Of the over one hundred strains known so far, a few of them are strongly linked to cervical cancer in woman. Among these, HPV-16 is the most frequently found high risk strain. Gene transcription in papillomavirus is regulated by the E2 proteins which can operate either as an activator or repressor of transcription. Integration of the HPV genome into the cellular genome normally takes place through a disruption in the E2 ORF, causing the release of the repression of the transforming protein, E7. In addition, the E2 protein plays an accessory role in viral genome DNA replication. We have tackled the structural and functional study of the DNA-binding domain of the E2 protein (E2C), a homodimer of 18 kDa, and the mechanism behind the recognition of the different DNA binding sites of the viral genome. The use of residual dipolar coupling have been of particular importance in deriving the solution structure, as they provide information regarding the quaternary structure of the dimer. ^{15}N relaxation and quantitative NOE and ROE between HN and water have been measured to derive a dynamic description and solvent accessibility of the free protein in solution. The overall picture has been used to explain mutagenesis results. The complex with one of the DNA binding sites of the viral genome has been also studied. Backbone assignment and dynamics have been obtained, leading to some answers regarding the recognition mechanism, and the possible role of the flexibility in the interaction between the two molecules.

THE *CLOUDS* APPROACH TO BIOMOLECULAR NMR STRUCTURE COMPUTATION

Miguel Llinás

Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.

Standard protocols for the analysis of biomolecules from NMR experiments routinely involve the assignment of resonances as a prerequisite for the derivation of structures from NOESY spectra. In contrast, relatively high numbers of unambiguous NOE *identities* can be consistently obtained and exploited to generate reasonably good protein folds, thus bypassing the assignments. To achieve this aim, we had to develop two approaches, SPI and BACUS, based on Bayesian inference. Starting from homo- and hetero-nuclear multidimensional NMR spectra, SPI (1) quantifies resonance matching probabilities and produces a list of the ^{1}H resonance frequencies grouped into linked spin systems. It takes advantage of redundancies in the number of connectivities revealed by the different types of NMR experiments, systematically tracking the adequacy of each grouping hypothesis. The method estimates the likelihood of nuclear spin resonances appearing at defined frequencies from sets of cross-peaks measured from multi-dimensional experiments. Extending the same concept, BACUS (2) exploits the self-consistency of the NOESY graph by taking advantage of a network of J- as well as NOE-connected "reporter" protons sorted via SPI. BACUS requires neither assignment of resonances nor an initial structural model; thus, it copes with chemical shift overlap without need to cycle through 3D structure calculations.
In the CLOUDS protocol (3), a gas of unassigned, unconnected H atoms is condensed into a structured proton distribution (cloud) via a molecular dynamics simulated annealing scheme in which the NOESY-derived inter-nuclear distances and van der Waals repulsive terms are the only active restraints. A proton density (foc) is generated by combining a large number of such clouds, each computed from a different trajectory. A Bayesian probabilistic approach was developed by which the primary structure is threaded through the unassigned H^{N} and H^{α} foc atoms and the sequence identified. Each foc site, including *cis/trans* proline loci, is thus assigned and side chain H atoms found. The structure is subsequently derived via molecular dynamics embedding into the foc (4). SPI, BACUS and CLOUDS were applied to two protein domains, col 2 and kringle 2, the obtained structures being within 1.0-1.5 Å (backbone heavy atoms) and 1.5-2.0 Å (all heavy atoms) rmsd's from reported X-ray and/or NMR structures.

References:

(1) A. Grishaev & M. Llinás (2002). *J. Biomol. NMR* **24**, 203-213.
(2) A. Grishaev & M. Llinás (2003). *J. Biomol. NMR* **,** in the press.
(3) A. Grishaev & M. Llinás (2002). *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6707-6712.
(4) A. Grishaev & M. Llinás (2002). *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6713-6718.

MORE THAN SEVENTEEN YEARS OF NMR IN POROUS MEDIA AT THE UNIVERSITY OF BOLOGNA

Paola Fantazzini

Dipartimento di Fisica, Università di Bologna
e- mail: fantazzini@df.unibo.it ; web page: http//www.porousmedia.org

In the mid-eighties at the University of Bologna my husband Giulio Cesare Borgia and I initiated a research project for Magnetic Resonance in Porous Media (MRPM). My husband was doing work in petroleum engineering in the Engineering Faculty and I on NMR relaxation in biological systems in the Physics Department. We recognized that both petroleum reservoirs and biological materials can be classified as porous media and that our knowledge could usefully be combined to study porous media in all contexts by Magnetic Resonance. My husband knew of an article by RJS Brown and I Fatt from 1956 (1), in which the fractional wettability of sand was studied by NMR relaxation. We then found material on oilfield applications of NMR by Robert (Bob) J. S. Brown, the leader of an NMR oilwell logging development in the 1950's, and we started to work (2). From then on, MRPM became a central theme in research for both of us. In 1988 the University of Bologna was setting out to celebrate the ninth centennial of its founding, and we wished to organize an international meeting on MRPM, although this meeting could not take place until 1990. In the spring of 1988 we invited Bob Brown to MRPM1, and a collaboration with Bob Brown began and continues to this day, as does the series of international meetings: MRPM7 will take place in Paris in 2004 (3). In these seventeen years the Bologna MRPM Laboratories have produced a steady stream of research results. My husband was able to accumulate a very substantial petrophysics database, with core samples from oil companies such as Agip. My husband's instinct and passion for finding useful patterns among data and his activity in organizing work and establishing connections with people worldwide were essential to the success of the MRPM work at Bologna. New correlations were found among parameters from NMR relaxation measurements and such oilfield parameters as porosity, permeability to fluid flow, irreducible water saturation, residual oil saturation, and pore-system surface-to-volume ratio, and fast algorithms were developed to give these different NMR parameters. Interest in valid interpretation of data led to extensive work also on the inversion of multiexponential relaxation data and the effects of inhomogeneous fields from susceptibility differences on distributions of relaxation times. Extensive developments were made of combined magnetic resonance imaging and relaxation measurements. The porous media studied went far beyond oilfield rocks and included industrial porous materials, elastomers, materials related to cultural heritage preservation, and even biological materials such as bone tissue. A year ago, after years of intense work, we could finally begin to feel satisfied with results accomplished. Then, just a few days after MRPM6, my husband was suddenly and unexpectedly taken from us; nevertheless, our project of MRPM research at the University of Bologna continues.

(1) RJS Brown, I Fatt. Measurements of fractional wettability of oilfield rocks by the nuclear magnetic resonance method. Pet Trans AIME 207:262-264 (1956).
(2) GC Borgia, P Fantazzini, E Mesini. I fondamenti della risonanza magnetica nello studio di alcune proprietà dei mezzi porosi saturati con liquidi idrogenati. Boll Ass Min Sub XXIV:359-375 (1987).
(3) GC Borgia, P Fantazzini, J Gore, M Holz, J Strange Editors. Proceedings of the sixth Intl. Meeting on Magnetic Resonance Applications to Porous Media, Ulm 8-12 Sept 2002, Magn reson Imaging 21, numbers 3/4, April/May (2003).

NEAR THE BORDERLINE OF THE STRUCTURAL AND FUNCTIONAL INFORMATION THAT CAN BE OBTAINED BY NMR SPECTROSCOPY

Alessandra Corazza, Fabio Pettirossi, Giuliana Verdone,
Paolo Viglino and Gennaro Esposito

Dipartimento di Scienze e Tecnologie Biomediche – Università di Udine – P.le Kolbe, 4 – 33100 Udine -ITALY

Over the last few years, theoretical and experimental advancements in high resolution NMR spectroscopy of biopolymers have made possible tackling structural and functional studies of large molecular weight species such as, for instance, heat shock protein aggregates, i.e. assemblies as large as 800 kDa. Prohibitively slow motional regimes can be overcome by clever exploitation of additive and subtractive chemical shift anisotropy contribution to relaxation (Pervushin et al., *Proc. Natl. Acad. Sci. USA* **94**, 12366-71, 1997) to obtain sharp connectivities that solve, at once, the problems expected from limited resolution and low signal-to-noise ratio. In addition, the possibility of measuring residual dipolar couplings that enable the introduction of orientational restraints (Tjandra and Bax, *Science* **278**, 1111-4, 1997) may even abolish the necessity of internuclear distance constraints for restrained structural modeling. As well known, distance constraints are obtained by NOE whose collection becomes harder and harder the more the molecular tumbling rate slows down. Application of these techniques will be presented for the NMR structural determination of the EMILIN-1 C1q-like domain, a 158-residue protein domain that forms a trimer of 51 kDa in solution. This aggregation stoichiometry is functionally relevant and, besides the monomeric structure determination which is still a formidable task, the real challenge remains the elucidation of the interaction interface within the trimer. Similar NMR borderline problems are posed by amyloidogenic proteins that, before precipitating out of solution as fibrillar aggregates, form nucleated oligomers which progressively grow into large fibrils. By classic high resolution NMR techniques we are able to observe just the tip of the iceberg which, nevertheless, provides valuable information about the ongoing nucleation processes. Specific examples will be provided by examination of experimental observations on β_2-microglobulin.

SOLID STATE NMR TO RESOLVE INTIMATE STRUCTURAL AND DYNAMIC DETAILS ABOUT LIGAND-RECEPTOR INTERACTIONS

Anthony Watts

Biomembrane Structure Unit, Biochemistry Department,
Oxford University, Oxford, OX1 3QU, UK

In drug discovery strategies for membrane targets, not only are high resolution structural and dynamic details for a ligand at its site of action required, but also information about the intermolecular binding mechanisms, such as which residues are involved and the mode (electrostatic, steric, π - π sharing, cation-π, etc) of binding. As a result of the lack of membrane crystal structures, we have been developing novel solid state NMR approaches to define such structural and dynamic details of binding for a small, isotopically (non-perturbing, ^{2}H, ^{13}C, ^{19}F, ^{15}N) labelled ligands, prosthetic groups, or solutes when located at their target in a membrane bound protein or ion channel, at near physiological conditions in natural membrane fragments or in reconstituted complexes [1, 2]. In addition, the subtle electrostatic contributions to binding have been resolved and shown to be vital for effective ligand activation or inhibition.

Studies of oriented membranes permit the bond vectors of labelled prosthetic groups to be determined to crystallographic resolution, as shown for retinal-d_3 in bacteriorhodopsin [3, 4, 5] and the 7TMD, GPCR, bovine rhodopsin [6]. The potential for resolving similar details for ligands and drugs bound to their target receptors, has now being realized using the agonist, acetyl choline-d_9 when intercalated into its binding site in the membrane bound, fully functional ligand gated, nicotinic acetyl choline receptor channel [7].

Novel magic angle spinning (MAS) NMR methods on membrane dispersions enable high resolution-like NMR spectra to be obtained for isotopically labelled ligands at their binding site in functional, fully hydrated membrane proteins. To yield structural information, dipolar couplings can be reintroduced into the spectrum of labelled ligands in their binding sites of membrane-bound proteins to give inter-atomic distances to high precision (± 0.5 Å). These approaches have been used to identify sugars, and their structural requirements for binding, in transporters expressed to amplified levels in *E. coli* plasma membranes at high sensitivity (250nmoles of ligand binding sites are detected) [8, 9], and protein folding details [10]. As examples of drug analogues, the structure and binding site details for an imidazole-pyridine and ouabains, which inhibit the gastric-K^+/H^+-ATPase and Na^+/K^+-ATPase respectively, have been defined to high resolution (±0.3Å) whilst at their binding site at their membrane-bound target [11, 12]. In addition, chemical shifts can be measured directly to help provide details of the chemical nature (electrostatic, hydrophobic or aromatic) binding environment for a bound ligand, as shown for acetyl choline in the acetyl choline receptor [13, 14] and the multi drug resistant transport protein, ErmE [15].

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See also (www.bioch.ox.ac.uk/~awatts/)

RELATIVE STEREOCHEMICAL ANALYSIS IN CHIRAL ORGANIC COMPOUNDS WITH CONFORMATIONAL MOBILITY BY QUANTUM MECHANICAL CALCULATION OF ^{13}C NMR PARAMETERS

Raffaele Riccio, Giuseppe Bifulco, and Luigi Gomez-Paloma
Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, via Ponte Don Melillo, 84084 Fisciano (SA), ITALY, Email: riccio@unisa.it

The determination of the relative configuration of chiral organic compounds characterized by multiple conformers equilibria, such as many novel natural or synthetic products, has always been a particularly challenging task, since it requires the simultaneous knowledge of both conformational and configurational features of the molecules under examination. With regard to this context, different approaches based on combined use of NMR spectroscopy and quantum mechanical calculations of ^{13}C NMR parameters are investigated. The application to the determination of the relative configuration of passifloricin A by the approach based on GIAO DFT calculations of ^{13}C chemical shift values of all the relative stereoisomers, performed on the most representative conformers belonging to each stereoisomer and weighted in accordance to their Boltzmann distribution, will be also discussed. In this case, the proposed method appear to be able to provide a correct answer after a careful examination of the conformational problems that are usually associated to flexible carbon chains with multiple stereocenters.

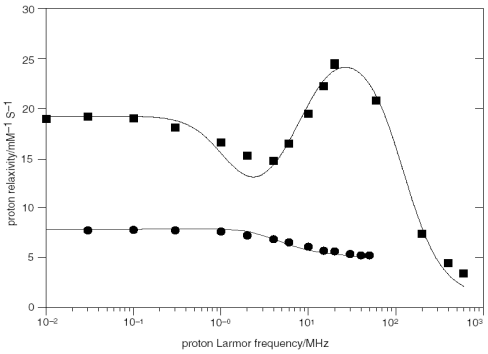
ORAL COMMUNICATIONS

NMR RELAXOMETRY APPLIED TO GADOLINIUM BASED MRI CONTRAST AGENTS

Lothar Helm

Institut de chimie moléculaire et biologique, EPFL-BCH, CH-1015, Lausanne, Switzerlan

The first approval for clinical use of an MRI contrast agent, Magnevist® ($[GdDTPA(H_2O)]^{2-}$), was 15 years ago in 1988. Since then great improvements have been made in the understanding of the molecular factors that determine relaxivity, the relaxation enhancement induced by the contrast agent [1]. A major technique to get information on relaxation enhancement by Gd(III)-complexes is Fast-Field-Cycling NMR relaxometry. Water proton relaxivity is measured over more than three orders of magnitude of magnetic field. Experimental data obtained give access to rotational correlation times, water exchange rates and electron spin relaxation rates, the important factors to be optimized for new higher relaxivity contrast agents.



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PHARMACOLOGICAL MRI: MAPPING DRUG-INDUCED BRAIN ACTIVATION

Angelo Bifone, Alessandra Giarola, Alessandro Gozzi, Christian Heidbreder, Torsten Reese, Adam Schwarz

Biology, Psychiatry CEDD, Glaxo SmithKline Research Centre, Verona, Italy

Functional MRI (fMRI) methods are established in man as a non-invasive means of identifying regions of increased neural activity in response to paradigms such as cognitive or motor tasks. These studies typically employ sequences sensitive to changes in the BOLD component of the haemodynamic response, but cerebral blood flow (CBF) or cerebral blood volume (CBV) changes may also be measured. More recently, these techniques have been employed to investigate the effects of pharmacological compounds on neural activity (phMRI), both in man and animal models.

One class of phMRI experiments involves monitoring the response to acute administration of the compound of interest. We have investigated the response to an acute challenge with cocaine or amphetamine in the rat using a CBV method; both provide a widespread activation of the dopaminergic system and a distributed pattern of phMRI signal changes. We have used this response as a probe for elucidating the effect of selective dopamine antagonists in modulating this response map, and to study the neural mechanism of sensitisation of the dopaminergic system, which is thought to play a crucial role in schizophrenia and other psychiatric diseases.

Since phMRI methods provide an indirect measure of neuronal activity via metabolic coupling to the haemodynamic response, and are subject to peripheral cardiovascular confounds, a sound characterisation of the relationship of the phMRI readout to the underlying mechanisms is crucial. In order to better characterise the MRI response to pharmacological stimuli, and to further validate phMRI methods, we have performed systematic comparisons with more invasive readouts of physiological parameters involved in the response to the drug.

NMR EXPERTS AND MRI QUALITY TEST, AN APPLICATION

Nicola Culeddu*, Matilde Chessa [§] Danilo Aragno[#]

** C.N.R ICB Via La Crucca,3 Baldinca- Li Punti I-07040 Sassari- Italy, nicola@iatcapa.ss.cnr.it*
[§]Porto Conte Ricerche sp. 55 Km 8,4 07040 Alghero – Italy matilde@iatcapa.ss.cnr.it
[#] Azienda Ospedaliera San Giovanni-Addolorata, Via S. Stefano Rotondo, 5a I-00189 Roma - Italy

The physicist/MR scientist must be familiar with the principles of MRI safety for patients, personnel, and the public. The physicist/MR scientist shall be knowledgeable in the field of nuclear MR physics and familiar with MRI technology including function, clinical uses, and performance specifications of MRI equipment, as well as calibration processes and limitations of the performance testing hardware, procedures, and algorithms.
One of the most important steps to obtain a good RM exam is the quality assurance (QA): a group of tests to determine compliance with acceptable or expected standards. In the past years some quality assurance tests has been proposed: AAPM (ref.1), NEMA (ref.2) and other authors (ref 3-8) published some papers on quality assurance. They pointed attention on reliability of technical point of view, showing various method for determine all parameters necessaries to obtain a good QA. Signal to noise ratio (S/N), Uniformity of static magnetic field and RF field, linearity of fields, slice profile/thickness, resolution, precision in T1 and T2, SAR (Specific Absorption Rate), ghosting, gradient noise and presence/absence of artifacts are the main parameters checked in an acceptance and quality test.
In this contribution, we report a view of italian norms concerning QA. A simple theorical treatment on uniformity test (UT) aimed to assessment the influence of the phantom filler composition is also presented. The measure of Signal Uniformity, performed in different equipments with the same static field (1T), using the same phantom filled with various paramagnetic salt solutions ($NiCl_2$ and $CuSO_4$), gave some differences not only related to instrumental setup.

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INTRAVASCULAR CONTRAST AGENTS FOR MR IMAGING: TECHNOLOGIES AND NEW CLINICAL APPLICATIONS IN CARDIAC, ONCOLOGIC, AND NEUROLOGIC IMAGING

Friedrich M. Cavagna

Bracco Imaging SpA, Milano, Italy

Intravascular contrast agents are fostering important innovation in Magnetic Resonance Imaging. This paper will present a review on intravascular contrast agents which are undergoing clinical trials or are still at preclinical stage. All the agents studied today are either based on gadolinium or on superparamagnetic iron oxide particles. Two different technologies are competing in the field of Gd-based agents: macromelucular contrast agents and low molecular weight gadolinium chelates with high affinity for serum proteins.
Two potential, important applications of Gd-based intravascular contrast agents will be shown on the basis of available clinical and preclinical results: 1) expanding the role of MRI in non-invasive cardiac imaging (Magnetic Resonance Coronary Angiography, Myocardial Perfusion Imaging) and 2) Assessing the hyperpermeability of microvasculature, thus acting as a marker of angiogenesis and of success of antiangiogenic treatment
Finally, Intravascular superparamagnetic contrast agents can strongly enhance the signal increase seen in functional MRI (fMRI) upon neuronal activation. Animal imaging experiments will be shown which may open the way to important clinical applications of fMRI for imaging of neurodegenerative and psychiatric diseases.

CHANGES IN ^{1}H MRS SIGNALS FROM MOBILE LIPIDS AND FROM LIPID PRECURSORS DURING CELL GROWTH: A MULTIVARIATE ANALYSIS STUDY.

Sveva Grande, Anna Maria Luciani, Antonella Rosi, Vincenza Viti, Laura Guidoni

Laboratorio di Fisica, Istituto Superiore di Sanità and INFN, Roma, Italia.
Piero Barone
Istituto per le Applicazioni del Calcolo, Consiglio Nazionale delle Ricerche, Viale del Policlinico 137, Roma, Italia.
Vladimir Mlynarik
MR Unit, Department of Radiology, University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria.

Intact tumor cells exhibit intense lipid signals in their ^{1}H MR spectra. The appearance of these signals has been associated either to the presence of lipid droplets in cytoplasm or to membrane microdomains. Different proliferative conditions and the onset of apoptosis were also found to affect the intensity of ML signals.
^{1}H MR spectra were run on tumor cells of different origin, namely MCF-7 from breast carcinoma and HeLa from cervix carcinoma, to reveal differences in lipid and lipid metabolite signals during the growth in culture. Very intense mobile lipids signals were found in the first days in culture while the same signals declined afterwards and started increasing again at confluence and at late confluence. At the same time, signals from the lipid metabolite phosphorylcholine decreased in intensity, while signals from glycerophosphorylcholine in MCF-7 and from choline in HeLa increased as cells approached confluence. We performed a multivariate analysis of a number of ^{1}H MRS experiments of both cell lines. Unsupervised classification on five selected spectral parameters was used to identify the proliferative condition. Cells were correctly classified with respect to the proliferative conditions and the spectra of actively proliferating and non-proliferating cells distinguished, confirming that the selected parameters are valid.

MATERIALS STUDIED WITH A PORTABLE NMR

D.Capitani[†], A.L. Segre[†], N.Proietti[†,‡], S. Viel[†,§]

[†]*Institute of Chemical Methodologies, Cnr, Research Area of Rome, M.B.10 00016, Monterotondo Stazione , Rome, Italy;*
[§]*UMR 6516 Synthèse, Chiralité et Catalyse, Université d'Aix–Marseille III, Av. Escadrille Normandie Niémen, 13397 Marseille, France;*
[‡]*Campec, Via G. Porzio, Centro Direzionale, Isola F4, Naples, Italy.*

NMR spectroscopy is a bulk technique able of measuring several parameters related to the structure of fully different materials such a stones, ceramics, wood and paper. Some of the measurable parameters is strongly sensitive to the state of conservation of the material constituting a manufactured object.
Paper is mostly made by cellulose and water. Using different solid state NMR techniques it was possible to measure the water contained in pores embedded in the cellulose matrix. The dimension of the pores and its distribution was obtained. Moreover we observed that degradation, both chemical and enzymatic, is always accompanied by a reduction of the transversal relaxation time of the water component. This is a very important parameter because it is one of the few which can be measured even in strongly inhomogeneous magnetic field.
Therefore we propose a new instrument based on the concepts of "unidirectional NMR". The new NMR instrumentation is rather inexpensive and transportable. It can be applied near to the surface of most manufactured objects which cannot be moved either because too large or too precious. The depth of penetration is still not very large. However with suitable coils presently we can measure at a maximum depth of about 7-8mm. The methods works well not only on organic solids, wood, textiles, paper, but also on porous stones and ceramics.
In porous stones, porosity, measured with conventional mercury porosimeters, compares well with data obtained using unidirectional NMR.

SOLID STATE NMR INVESTIGATION OF HYDROGEN BOND IN SUPRAMOLECULAR ADDUCTS

Roberto Gobetto

Dipartimento di Chimica I.F.M., Università di Torino, Via P. Giuria 7, 10125 Torino

The aim of crystal engineering is the rational use of intermolecular interactions to design the supramolecular structure of crystalline materials. The strength of hydrogen boning interactions relative to other intermolecular packing forces is well established. Therefore it seem reasonable to expect that the supramolecular arrangements are often strongly dependent on the number and strength of inter- and intramolecular hydrogen bonding interactions. Even though the most common characterization techniques are based on X-ray diffraction (powder and single-crystal) an unambiguous identification of the hydrogen bonding interactions requires spectroscopic tools, because of the intrinsic limitations of X-ray diffraction when dealing with hydrogen atom positions.

It is well known that high-resolution solid-state NMR spectroscopy provides useful information about details of hydrogen bonding and it is a general method for evaluating, for example, the protonation state of carboxylic acids This information can be obtained not only from the small but reproducible change in the isotropic chemical shift upon protonation, but also from the value of the chemical shift tensor obtained from the analysis of low spinning speed spectra. For this reason a comparison between the ^{13}C solid state NMR data and X-ray diffraction data obtained for crystalline or powdered samples can be particularly useful to obtain unambiguous information about the proton transfer.

For instance, mechanical mixing of solid dicarboxylic acids of variable chain length $HOOC(CH_2)_nCOOH$ (n = 1-7) together with solid 1,4-diazabicyclo[2.2.2]octane (dabco) generates the corresponding salts or cocrystals of formula $[N(CH_2CH_2)_3N]$-H-$[OOC(CH_2)_nCOOH]$ (n = 1-7). The samples have been investigated by single crystal, powder diffraction and solid state NMR. The acid-base adducts, whether associated with proton transfer from the -COOH group to the N-acceptor, e.g. forming (-)O---H-N(+) interactions, or with the formation of neutral O-H---N hydrogen bonds, show a melting point alternation phenomenon analogous to that shown by the neutral carboxylic acids.

The carbon chemical shift tensors of the COOH group obtained by the sideband intensity of low speed spinning solid state NMR spectra provide reliable criteria for the investigation of the hydrogen bonding interactions in the adducts: in effect the orientation and values of the principal elements of the nuclear shielding tensor (δ_{11}, δ_{22}, δ_{33}) change significantly with the protonation state of the carboxylic groups. By their values it is possible to confirm the structural parameters (e.g. difference between C-O and C=O distances) obtained by diffraction study and to define the adducts as molecular co-crystal (no proton transfer) or as ionic crystal (proton transfer). Furthermore solid state ^{15}N CPMAS data of the amine groups of the dabco are directly related to the extent of the hydrogen bond and a nice correlation between the N-H distance and the chemical shift of the ^{15}N nucleus is found. Density functional theory applied to explore changes upon hydrogen bonding in the ^{15}N shielding parameters is in nice agreement with the experimental values found by solid state NMR data.

^{2}H-NMR INVESTIGATIONS ON THE BIAXIALITY ON A LIQUID CRYSTALLINE SIDE CHAIN POLYMER

K. Severing*, A. Hasenhindl, E. Stibal-Fischer, H. Finkelmann, K. Saalwächter

Institut für Makromolekulare Chemie, Albert-Ludwigs-Universität Freiburg
Stefan-Meier-Strasse 31, 79104 Freiburg

Biaxial nematic liquid crystals have been theoretically predicted by M. J. Freiser as early as 1970 [1]. Since then, many workers have been trying different approaches towards an experimental proof of this special nematic phase, where the mesogens are not only aligned with their long-molecular axis along the main director, but where the other two molecular axes are macroscopically oriented as well. Yet, the only accepted proof of a biaxial phase was reported for a lyotropic system [2], where the driving force for phase biaxiality is aggregate anisotropy, rather than molecular anisotropy.

We report deuterium NMR experiments on liquid crystalline side chain polymers, in which the rotation about the molecular long axis can be hindered by means of a lateral attachment of the mesogens to the polymer backbone. This rotation about the molecular long axis is believed to be one problem, that opposes the formation of biaxial nematic phases in low molecular weight thermotropic liquid crystals. In comparison to this side-on polymer system, we have studied different end-on polymers, in which the extent of the rotational hindrance presumably depends on the length of the alkyl spacer between the mesogen and the polymer backbone.

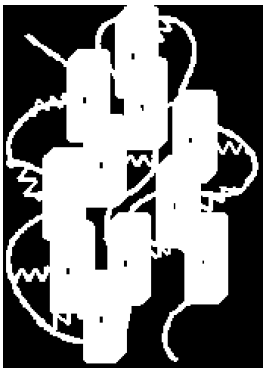

Figure 1: Schematical representation of a side-on liquid crystalline side-chain polymer

Phase biaxiality is investigated by measuring the quadrupole splitting of a spin probe in a macroscopically ordered sample oriented at different angles w/r/t the magnetic field. Director relaxation times have been determined for various temperatures, and were found to be on the order of the time needed for the acquisition of a full spectrum. In order to bypass this relaxation problem we employ a rapid sample flip technique introduced by Frydman [3], in which the sample is rotated to the desired angle only for the acquisition time of a single transient, while it is rotated back to the energetically more favoured 0°-position for the comparatively long recycle delay.
Amongst other problems, the large width of the peaks in polymeric samples represents a serious limitation that has to be overcome in order to provide an experimental proof of the biaxial nematic phase. We demonstrate different one- und two-dimensional echo experiments designed to solve this resolution problem.

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CONTINUOUS FLOW Xe-OPSE NMR APPLIED TO THE CHARACTERIZATION OF MATERIALS

Michele Mauri, Silvia Bracco, Angiolina Comotti, Roberto Simonutti, Piero Sozzani

Università Milano-Bicocca, Dip. Scienza dei Materiali, Via R.Cozzi 53, 20125 Milano

Xenon can be used as a probe gas allowing NMR investigations of porous and lipophilic systems[1]. Interaction of the Xenon atoms with the chemical environment and other Xe atoms generate both isotropic and anisotropic chemical shift, especially in the case of systems with cavities[2] or channels[3,4]. NMR of ^{129}Xe is usually made difficult by a scarce sensitivity, imposing long experimental times, and the use of high Xe pressure. This problem can be addressed by hyperpolarizing the Xe gas using optical pumping spin exchange (OPSE). The first step of this process is the optical pumping of the electronic population of ^{87}Rb using circular polarized laser light. By bringing Xe gas in contact with Rb vapors in proper pressure and temperature conditions, we can stimulate the transfer of polarization from the Rb electronic level to the nuclear spin level of the Xe atoms. This brings the nuclear population of Xenon to a non-equilibrium state. Such state is endowed with a magnetization vector several orders of magnitude greater than what is achieved spontaneously, by Boltzmann distribution of the nuclei among the different energy levels generated by Zeeman splitting. A very diluted Xe mixture can then provide intense signals, without the inconvenience of using sealed tubes, also allowing the fine study of Xe-Xe interaction effects.
A hyperpolarization apparatus has been set up and tested on a number of applications. Our apparatus is based on a 16W optical fiber delivered laser, and can produce a steady flux of 200 cc/h of 3% polarized Xe. In the field of polymers, ^{129}Xe NMR is able to distinguish different kinds of rubbers, provided they are over the glass transition. There are promising indications that the difference of chemical shift associated to different rubbers is not cancelled by mechanical mixing of different samples. We also studied polymeric and copolymeric spheres obtained by polymerization from a Ziegler-Natta catalyst supported by a nanoporous matrix. Work is being made towards relating the porosity of the sphere to the porosity of the catalyst. Another interesting field is that of nanostructured systems. Single crystals with oriented channels have been studied, and different chemical shift values can be obtained by macroscopic reorientation of the crystal. Another nanoporous system is that of the pilastrate hectorite where the xenon can explore the free volume between different silica sheets separated by aminic “pillars”. In this case, a single peak was detected at room temperature (excluding the peak associated to free gas in the detection volume), with a shoulder appearing at lower temperature eventually resulting in a separate peak under 240 K. This can be interpreted by assuming different sites are available to the gas, with an exchange that becomes slow on the NMR time scale only at low T. Filling the space between pillars with C_{16} causes all peaks to disappear, except the one associated to free gas. Finally, preliminary work is being made in the field of analysis and diagnostics of marble surfaces, possibly of historical interest. Extensive trials were performed on a number of untreated surfaces, without degassing, and it was seen that the paramagnetic effects dominate the lineshape. Even without signals associated to accessible porosity, the surface of marbles such as Carrara provides specific modifications to the gas lineshape.

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ACD/COMBI NMR: ^{1}H AND ^{13}C AUTOMATED VERIFICATION

Brent Lefebvre, Tony Williams and Sergey Golotvin

ACD, Toronto, Canada.

With the advent of combinatorial chemistry, the bottleneck has really become the chemist's ability to confirm the identity of a synthesized molecule. With the introduction of ACD/Combi NMR, this "Verification" of identity can be done in a quantitative and automated way. With the help of the NMR predictors, a spectrum-structure match is determined for each sample in a combinatorial plate of data. The results of this comparison can then be displayed on an easy to read plate display, in order to identify the mis-matched structures.
A brand new version of ACD/Combi NMR will be available this fall! With this release, the ability to do ^{13}C verfication as well as ^{1}H is possible in an automated manner. Some other significant improvements will be discussed here as well as a technical review of the entire verification process.

REVERSIBLE OXY-ANION BINDING IN AQUEOUS SOLUTION AT A Gd^{III} CENTER: AN NMR RELAXOMETRIC STUDY

Mauro Botta

Dipartimento di Scienze e Tecnologie Avanzate, Università del Piemonte Orientale "Amedeo Avogadro", Corso Borsalino 54, I-15100, Alessandria, Italy

The ^{1}H and ^{17}O NMR relaxometric properties of two cationic complexes formed by Gd^{III} with a macrocyclic heptadentate tri-amide ligand, L^1, and its N-methylated analogue,L^2, have been investigated in aqueous media as a function of pH, temperature and magnetic field strenght. The di-aqua cationic Gd^{III} chelates interact strongly with a variety of oxy-anions[1] and the formation of ternary complexes is accompanied by large variations of their relaxometric properties. The anions that bind in a bidentate mode (carbonate, lactate, malonate, citrate) displace two water molecules and form adducts with $q = 0$, characterized by relaxivity values about 60% lower than for the free complex. Other anions interact in a monodentate manner (fluoride, phosphate, acetate) and the corresponding ternary complexes maintain one co-ordinated water molecule. In these latter cases the changes of the relaxivity are modulated by the ability of the anion to promote H-bonding interactions with water molecules outside the inner coordination sphere of the Gd^{III} ion. The hydrogen-bonding interactions may involve also the coordinated water molecule and markedly influence its rate of exchange. Furthermore, evidence has been gained of a selective coordination of these anions in the equatorial plane of the complex rather than in the axial position. The N-methylation of the ligand ring nitrogen has little influence on the relaxometric properties of the complex but exerts a profound effect on the affinity constant for the anions. In general, the *K* values for $[GdL^2]^{3+}$ are notably higher than for $[GdL^1]^{3+}$. This result, which may be associated with differential complex hydration, highlights the important fact that minor structural variation of the ligand may modulate in a significant manner the affinity of the complex for the oxy-anions.[2] These data suggest the need of a systematic study to investigate in further detail the nature of the interaction and its dependence from the structural properties of the complexes. The final goal is the preparation of paramagnetic probes for oxy-anions of biological relevance endowed with high selectivity for diagnostic applications.
Finally, in the related complex with an octadentate ligand it has been found that the nature of the counter-anion determines the rate of dissociative water exchange and hence the proton relaxivity of its aqueous solutions. This result represents a strong evidence that the nature of the second hydration sphere is critically important in defining the kinetics of water exchange at metal ion centres.[3]

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^{17}O-WATER MULTIPOLE RELAXATION

N. Ghofraniha[1], W. Nosel[2], S. Capuani[1], M. Paci[3], C. Casieri[4] and F. De Luca[1]

[1]*Department of Physics, University of Rome "La Sapienza", I-00185 Roma*
[2]*Institute of Physics, Jagiellonian University, Pl-30059 Kracow*
[3]*Department of Biochemistry and Molecular Biology, University of Rome "Tor Vergata", I-00173 Roma*
[4]*Department of Physics, University of L'Aquila, I-67010 L'Aquila*

Spin 5/2 oxygen-17 nucleus is of great interest for water dynamics studies, especially when hydration mechanisms are of interest. Apart the absence of cross-relaxation and exchange activity, the transverse and longitudinal magnetization decays of oxygen-17 spins are driven by quadrupolar interaction. Utilizing the formalism of irreducible spherical tensors, the decays may be expressed by a linear combination of dipole, 8-pole and 32-pole terms. Each of these is characterised by three-exponential whose amplitudes and rates are directly connected to the dynamics time scale and sites of the spins residence.
The exact results of the relaxations calculation based on the semi-classical Redfield theory will be presented for 5/2 spins on the basis of multipolar magnetization states. It will also be shown as multiple-quantum filtering is able to select multipole states influencing relaxation and therefore some dependence from dynamics correlation times.
Preliminary results on single-quantum experiments (IR and CPMG) will be presented on aqueous solutions of lysozyme at different concentrations. Utilizing the results of the multipole relaxation theory, the experimental data enabled to estimate the correlation time and the fractions of strongly bound water in a two-state model in fast exchange. The same procedure was used to yield estimates of free, weakly bound and strongly bound water fractions using a three-state model in fast exchange.
It is possible to attain more dynamics parameters using multiple-quantum NMR technique, which allows the dipolar component elimination (triple quantum filter) and makes the linear combination of the other two terms dependent on a sequence parameter. By setting a particular value of this parameter the 32-pole term makes no contribution to the whole signal.

POLYMERIC MATERIALS CHARACTERIZATION BY APPLICATION OF MULTIVARIATE ANALYSIS TO LOW FIELD NUCLEAR MAGNETIC RESONANCE DATA

Lorena Casciani[†], Cecilia Castro[†], Cesare Manetti[†] and Filippo Conti[†]

[†] *Dipartimento di Chimica Università degli studi "La Sapienza" di Roma, 00185, Roma, Italy.*

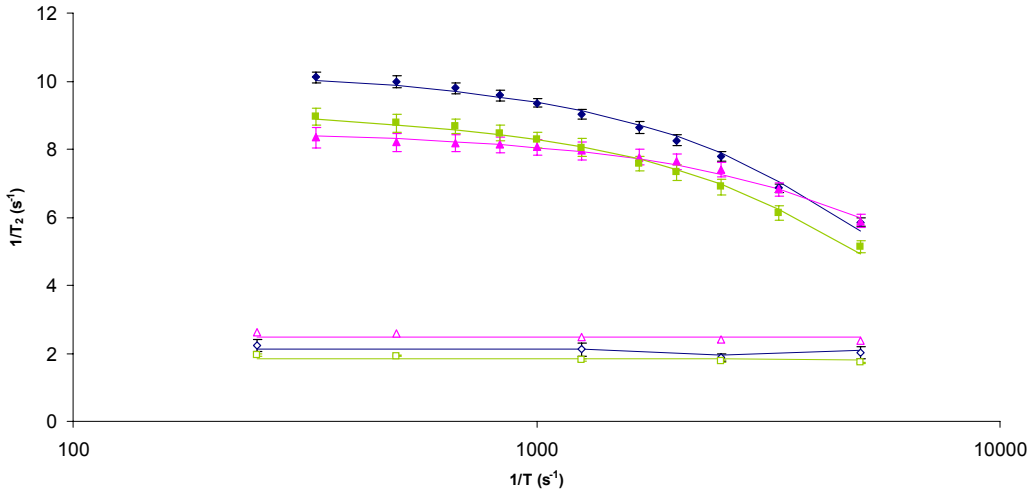
In this work, we are interested in pointing out the structural changes of different kind of contact lenses, once they are brought into contact with artificial tears solutions, that are given to patients affected by dry eye syndrome.
Data are obtained by low field nuclear magnetic resonance (LF-NMR), and they came from samples with completely different characteristics, both for lenses ionicity and water content, and for composition of the artificial tears solutions.
In order to achieve our aim in the best way, we chose to use multivariate techniques to process our data, in addition to multiexponential fitting of each curve: unlike the latter, in fact, in multivariate techniques all the samples are analysed contemporaneously. In addition to be highly conditioned by multiexponential fitting problems, processing each curve alone doesn't allow us to enlighten the aspects the samples have in common, in fact it hasn't to be taken for granted the fact that the components are comparable. Then, we preferred to deal with the problem with a less traditional approach, that, however, allow us to compare the different samples. This technique, not only is speedy and robust, but also it has the peculiarity of overlapping no theoretical model on the data: it simply let the common aspects come out spontaneously among all the samples.
We analysed the different set of contact lenses, put first in physiological solution, then in artificial tears solution, using a MiniSpec (Bruker) instrument, that works at a frequency of 23.2 MHz. The sequence was the Carr-Purcell-Meiboom-Gill (CPMG).
Each resultant curve is the sum of the different situations where water molecules in the sample are: in fact part of these molecules tightly interacts with the lens, given that it enters the network, changing its characteristics a lot; another part, instead, has a lower interaction with the polymeric material. Through a set of plot, that are the result of the data processing by multivariate analysis, we show the changes of the different components among all the samples: in particular in each plot we represent, one as a function of another, the scores of the components in the samples. We are able, in this way, to discuss the structural changes of the lenses, according to the material characteristics and the composition of the artificial tears solutions.

THE EFFECT OF AGEING ON THE EGG WHITE – A WATER PROTON TRANSVERSE RELAXATION STUDY

Mauro A. Cremonini[†], Brian P. Hills[‡], Luca Laghi[†] and Giuseppe Placucci[†]

[†]*Department of Food Science, University of Bologna, Via Ravennate 1020, 47023 Cesena, Italy*
[‡]*Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

The major aspects of the mechanisms causing the weakening of hen egg thick albumen (egg white) during ageing are still unclear, hindering the formulation of new value-added products. It is known that oiling the shell of the eggs immediately after laying dramatically slows down this process, but the mechanism is unknown, though probably related to albumen pH changes. To gain a better understanding of the consequences of ageing on the structure of the thick albumen the interactions between its proteins and water were investigated in eggs of different age, some of which were oiled. Transverse relaxation time dispersions were measured with the Carr-Pourcell-Meiboom-Gill sequence with 90-180 pulse spacing varying between 200 to 3000 µs. The resulting dispersions were fitted with a chemical exchange model describing the fast exchange of water and albumen protons. The albumen dispersions were compared from fresh and old eggs and with old egg albumen whose pH was artificially adjusted to follow the natural modifications occurring after egg laying. In the next figure, as an example, the dispersions for albumen from fresh non oiled eggs (diamonds), 7 days old non oiled eggs (circles) and 7 days old oiled (squares) are compared for two spectrometer frequencies, 300MHz (full symbols) and 23.4MHz (empty symbols). The lines describe the fits of the proton exchange theory and the tables show how the fitting parameters vary with egg ageing. The exchange rate, K_b, shows the biggest change, suggesting that pH changes are indeed dominant.



T_{2a} (s)[1]	δb (ppm)[1]	T_{2b} (s)			K_b (s^{-1})			P_b		
2.000	1.674	4.951E-03	±	1.572E-03	6.762E+03	±	6.073E+02	7.517E-03	±	4.399E-04
2.000	1.674	3.826E-03	±	3.443E-04	1.056E+04	±	5.532E+2	7.645E-03	±	5.814E-04
2.000	1.674	4.965E-03	±	2.774E-04	6.803E+03	±	3.500E+02	6.652E-03	±	6.302E-04

[1] The indicated values for T_{2a} and δ_b were used as a starting point for the calculations

OPTICALLY DETECTED MAGNETIC RESONANCE OF PHOTOSYNTHETIC REACTION CENTERS

D. Carbonera

Department of Physical Chemistry, University of Padova,Via Loredan, 2, 35131 Padova, Italy
e-mail: d.carbonera@chfi.unipd.it

ODMR (Optically Detected Magnetic Resonance) is a double resonance spectroscopy based on the idea to transfer the detection of magnetic resonance to the optical domain The increase on photon energy enhances the sensitivity of the experiment. Zero Field applications involve triplet states and are especially indicated for biologically disordered samples. A triplet state can be seen often as an intrinsic "spin label" for a system, because it carries electronic paramagnetism and is a mild, endogenous, probe of molecular structure and molecular interactions of molecules with their environment.
The principle of ODMR technique is based on the fact that population and decay rates of triplet sublevels are in general different, due to the anisotropy of the molecular mechanisms. Thus under photo-excitation and in the absence of spin-lattice relaxation, condition obtained at cryogenic temperatures, the steady state population of the three sublevels are generally different to each other. Application of resonant microwaves leads to a redistribution of the triplet populations. As a consequence the resonance will produce an increase/decrease of S-S absorption, a decrease/increase of T-T absorption, and an increase/decrease of emission. This technique has been proven to be a powerful tool in the investigation of natural photosynthesis.
ODMR spectra of membranes from the green sulfur bacterium *Chlorobium limicola* and from the green filamentous bacterium *Chloroflexus aurantiacus* have been detected to probe the energy transfer processes and the reaction center structure of these two bacteria in their intact environment. Triplet states localized in the peripheral chlorosomes and, respectively in the FMO and B808-866 core light harvesting complexes, have been characterized. After chemical reduction followed by illumination at 200 K, the recombination triplet state becomes populated under light excitation at low temperature in both systems.
Clear spectroscopic evidence of exciton interaction between the RC and the B808-866 antenna complex in *Chloroflexus* has been found. The analogy of some features of the microwave-induced T-S spectrum with those previously found for *Rb. sphaeroides*, allows to predict a similar coupling among the primary donor and the nearby antenna BChl *a* molecules, assembled as circular aggregate. A topological model for the core-RC complex of *Chloroflexus* based on the ODMR results has been proposed.
Finally a comparison of ODMR spectra detected in membranes of *Chlorobium* with those previously obtained in PSI membranes from higher plants and algae clearly shows the presence of common features in the RC structures of these different species.

APPLICATION OF A NMR-COMPATIBLE MICROGRAVITY-BASED BIOREACTOR FOR ON-LINE MONITORING CELL CULTURES

Silvia Bradamante

CNR – Istituto di Scienze e Tecnologie Molecolari, Milano, Italy.

Microgravity (μg) favours tissue formation providing concurrent lack of structural deformation, displacement of intracellular components and/or reduced mass transfer rates to accommodate molecular scaffolding, and suitable micro-environment. In the case of endothelial cells (EC) the mechanical environment deeply influences their gene expression, structure and function as well as growth, differentiation and apoptosis. Simulated μg affords unique opportunities for novel findings in EC biology.
We developed a NMR-compatible microgravity-based bioreactor (NRG) in which the randomization of the gravitational vector on cells is obtained by combining forces produced in a suitable fluid (medium) by an appropriated cell perfusion system. The reduced gravity conditions in our bioreactor are supported by experimental data.

PEPTIDE-PROTEIN INTERACTIONS STUDIED BY SURFACE PLASMON AND NUCLEAR MAGNETIC RESONANCES

Andrea Bernini, Ottavia Spiga, Maria Scarselli, Arianna Ciutti, Luisa Lozzi, Luisa Bracci, Chiara Falciani, Duccio Calamandrei[$], Daniela Di Maro[$] and Neri Niccolai*

Dipartimento di Biologia Molecolare, Università di Siena, and Biomodem pscrl, Siena.

Peptide and antigen fragment libraries obtained by the phage display technology have been extensively used to study antibody-antigen interaction in order to develop vaccines and diagnostic tools. Moreover, the same technology has been proposed, in general, for investigating any protein-protein or peptide-protein interaction for the design of selective ligands of potential pharmaceutical interest. Understanding the fine details of these intermolecular processes is crucial to design peptide ligands with improved affinity and activity. In this respect, it is now well established that surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) are playing a major role in elucidating dynamics and structural aspects of protein-protein and peptide-protein complex formation. SPR yields accurate estimates of kinetic parameters of the intermolecular interaction, such as k_{on} and k_{off}, while NMR offers precious information on the structural stability of the complex by analysing intramolecular and intermolecular nuclear Overhauser effects (NOEs). A strong correlation between the structure stability and the affinity between protein subdomains has been found from SPR data and a combined use of SPR and NMR measurements has been proposed for investigating peptide-antibody and virus-receptor interactions.
Among the various peptide-protein interactions, the complex formation between acetylcholine receptor (AChR) mimotopes and α-bungarotoxin is here analysed in detail both by SPR and NMR, since many examples of this type have been described.
Thus, a combined analysis of the SPR- and NMR-derived dynamic parameters shows new correlations between complex formation and dissociation and the overall pattern of intramolecular and intermolecular nuclear Overhauser effects. These features could be crucial for a rational design of protein ligands.

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* Prof. Neri Niccolai, Dipartimento di Biologia Molecolare, Università di Siena, via Fiorentina 1, 53100 Siena. Phone 0577-234910; fax 0577-234903; e-mail: niccolai@unisi;it

HIGH-RESOLUTION DIFFUSION-ORDERED SPECTROSCOPY TO PROBE THE MICROENVIRONMENT OF JANDAJEL AND MERRIFIELD RESINS[1]

Celine Gambs, Tobin J. Dickerson, Suresh Mahajan, Laura B. Pasternack and Kim D. Janda

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Solid –phase organic synthesis (SPOS) has become a powerful methodology for the generation of compound libraries. The success SPOS is highly dependent on the accessibility of solvents, catalysts, and reagents to the interior of the resin. In order to explore the applicability of high-resolution ^{1}H DOSY (Diffusion Ordered Spectroscopy) NMR spectroscopy to the analysis of accessibility of reaction components, we undertook a comparative study of the diffusion coefficients of reaction components in two resins. These initial studies were conducted using Merrifield and JandaJel resins since these have different swelling properties and have significantly different kinetic behavior. Results show correlation between chemical reactivity, resin swelling, presence of intermolecular interactions and diffusion of solvents and reagents within the gel as determined by ^{1}H DOSY NMR Spectroscopy.

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FAST MULTI-DIMENSIONAL NMR SPECTROSCOPY

Eriks Kupce

Varian R&D, Oxford, UK

Considerable sensitivity gains provided by cryogenic probes coupled with availability of increasingly high magnetic field strength open new possibilities for development of fast methods for data acquisition in bio-molecular NMR. Such methods can speed up multi-dimensional NMR spectroscopy by up to three orders of magnitude. We shall discuss two new fast NMR methods - multidimensional Hadamard NMR spectroscopy and fast 3D NMR using projection-reconstruction method.

CCPN - NEW NMR ANALYSIS SOFTWARE AND AN NMR DATA STANDARD

Rasmus Fogh[†], Wayne Boucher[†], John Ionides[‡], Anne Pajon[‡], Tim Stevens[†], Wim Vranken[‡], and Ernest Laue[†]

[†] *Department of Biochemistry, University of Cambridge, CB2 1GA Cambridge, UK;*
[‡] *MSD group, European Bioinformatics Institute, CB10 1SD, Hinxton, UK.*

The CCPN project has developed a standard for storage of macromolecular NMR data, to permit easy exchange of data between different programs and deposition into databases, such as BioMagResBank and the PDB. The basic idea is that all programs in the field should eventually be able to exchange data that conform to the CCPN model, so that you can go back and forth between programs without problems with conversion or data loss.
On the basis of the data standard and the extensive subroutine libraries that come with it, we are developing the CcpNmr software suite, a modular, extensible series of programs that will eventually range from processing through analysis and structure determination to validation and deposition of NMR structures. The first functional programs of the suite have been released this summer:
- Analysis, an entirely new analysis and interactive display program, inspired by Ansig and Sparky.
- SpecHandler for setting up new projects, reading data from Bruker and Varian files, and running external processing scripts.
- FormatConverter, that converts data from to and from the formats of ten popular analysis programs, going through the CCPN data standard.

NMR FOLDING INVESTIGATIONS OF RECOMBINANT EF-HAND CALCIUM-BINDING PROTEINS

Elena Babini[#], Ivano Bertini[$], Francesco Capozzi[#], Claudio Luchinat[$], Alessandro Quattrone[‡$] and Maria Turano[§]

[#]*Dipartimento di Scienze degli Alimenti, Università di Bologna, 47023 Cesena (FC), Italy;*
[$]*Centro Risonanze Magnetiche, Università di Firenze, I-50019 Sesto Fiorentino (FI), Italy;*
[‡]*Servizio Genetica Medica, Ospedale Casa Sollievo Sofferenza, I-71010 San Giovanni Rotondo (FG), Italy;*
[§]*Dipartimento di Chimica, Università della Calabria, I-87036 Arcavacata di Rende (CS), Italy.*

The international effort to sequence the 3 billion DNA letters in the human genome is arrived to successful completion on April 14, 2003. These public data represent an enormous information-processing challenge and data-mining opportunity. Human genome exploration allowed us to find almost 200 genes coding for calcium-binding proteins containing the structural EF-hand motif. The 3D structure, either by X-Ray or by NMR, is known for only 15% of the proteins grouped in this super-family, and the sequence analysis, involving the identification of homologous relationships among similar sequences, permitted us to model the EF-hand domain of another 60% of the total. For the remaining proteins, as well as for checking the correctness of modeling in critical cases, it is necessary to investigate their structure by exploiting the heterologous expression in foreign organisms. However, refolding under denaturing conditions is often required, and NMR spectra of recombinant EF-hand proteins provide a quick tool to monitor refolding or to assess the correct folding.

BIOINFORMATIC AND NMR TOOLS FOR THE CHARACTERIZATION OF MOLECULAR FEATURES OF METALLOPROTEINS

Antonio Rosato

Centro Risonanze Magnetiche, Università di Firenze, I-50019 Sesto Fiorentino (FI), Italy

When studying metalloproteins, a special focus is on the metal site, as it is often a key player with respect to the biological function. The presence of the metal results both in additional challenges and additional opportunities with respect to the case of the study of the active site of proteins that do not contain metals. Some details will be provided on how this affects bioinformatic and NMR approaches aimed at the structural characterization of metalloproteins, and on the development of tools specifically targeted for the study of metal ions in proteins.

A DIVIDE & CONQUER APPROACH TO FAST LOOP MODELING

Silvio C. E. Tosatto

CRIBI Biotech Centre and Dept. of Biology, University of Padova, V.le G. Colombo 3, 35121 Padova, Italy

Determination of protein structures that have not been solved experimentally is frequently done by comparative modeling techniques [1]. Copying parts of the target structure, which are assumed to be superimposable, from a known protein structure serves as a framework. Structurally variable regions, referred to as loops, have to be treated separately. Because loops often show the greatest variation in amino acid sequence and are usually less restrained in conformation than the core regions, they cannot easily be taken from the parent structure. Their prediction remains one of the main problems in comparative protein modeling [1]. One problem is the generation of a good set of alternative structures for evaluation with a scoring or energy function.

To be used in typical comparative modeling situations, the loop modeling method should be fast enough to allow the rapid prediction of all loops in the protein.

The newer developments in our fast ab initio method for modeling local segments in protein structures [2] are described. The algorithm is based on a divide and conquer approach and uses a database of precalculated look-up tables, which represent a large set of possible conformations for loop segments of variable length. The target loop is recursively decomposed until the resulting conformations are small enough to be compiled analytically. The algorithm, which is not restricted to any specific loop length, generates a ranked set of loop conformations in 10 to 90 seconds on a desktop PC. The prediction quality is evaluated in terms of global root-mean-square deviation (RMSD). Depending on loop length the top prediction for the original algorithm varies between 1.06 Å RMSD for three residue loops and 3.72 Å RMSD for eight residue loops. Including a local minimization step and re-ranking according to CHARMM [3] energies significantly reduces these values.

Due to its speed the method may be useful to generate alternative starting conformations for complex energy-based simulations or refinement of experimental structures.

The loop modeling tool has been integrated in our comparative modeling package and both will soon be available as web-based prediction servers from the URL:http://protein.cribi.unipd.it/.

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SURFACE DYNAMICS OF LIQUIDS IN POROUS MEDIA: A FIELD CYCLING APPROACH

Jean-Pierre Korb

Laboratoire de Physique de la Matière Condensée, UMR 7643 du CNRS, Ecole Polytechnique, 91128 Palaiseau Cedex, France

We show how nuclear magnetic spin-lattice relaxation dispersion of 1H-water can provide a direct reliable value of the specific surface area of a cement-based material [1]. The remarkable features of the relaxation dispersion support an interpretation in terms of coupled solid/liquid relaxation at pore interfaces, surface diffusion and nuclear paramagnetic relaxation. The measurement is sufficiently fast to be applied continuously during the progressive hydration and setting of the material. We show also how this method is relevant to other chemically reactive porous media in chemical engineering and oil recovery. For instance, we present our recent experiments performed in water/oil saturated petroleum rocks [2, 3]. We believe that these experiments give new information about the surface localization of these two saturating liquids in petroleum rocks.

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NMR DIFFUSION MEASUREMENTS AND MRI IMAGE ANALYSIS IN RESERVOIR ROCKS

Massimo Balzarini

ENI Exploration & Production Division, via Emilia 1, 20097 San Donato Milanese (MI), Italy.

NMR relaxometry and MRI are of great importance for the oil industry to characterise porous media structure and petrophysical properties. From the time-dependence of the apparent diffusion coefficient of the fluid D, interesting information can be derived to characterise geometrical features of the porous media. In particular the surface-to-volume ratio (S/V), estimated from the short time behaviour of D(t), and the connectivity of the pore space, which is probed by the long time behaviour of D(t), could be measured. In addition the permeability could be estimated. As an example of the porous media characterisation, the contribution of fractures to total porosity and their geometrical descriptions have been studied by Image Analysis applied to ^{1}H Magnetic Resonance Imaging (MRI). Images of core plugs of different lithology were acquired with MSME 2D quantitative and 3D sequences. An image analysis procedure, developed ad hoc, was then applied to these acquisitions and the petrophysical parameters computed. These parameters range from fracture porosity to fracture density.

HIGH RESOLUTION MRI FOR THE QUANTITATIVE ANALYSIS OF TRABECULAR BONE ARCHITECTURE

Renato Toffanin,[†,‡] Agostino Accardo,[§] Igor Strolka,[¥] Maria Cova[¥] and Franco Vittur[†]

[†]*Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, I-34127, Trieste, Italy;*
[‡]*PROTOS Research Institute, I-34100 Trieste, Italy;*
[§]*DEEI, University of Trieste, I-34127 Trieste, Italy;*
[¥]*Department of Radiology, University of Trieste, Cattinara Hospital, I-34149 Trieste, Italy*

Recently, high resolution MRI has emerged as the most promising modality to visualise and quantify bone structure directly. Thus far, application of high resolution MRI has targeted the trabecular bone, the site where most osteoporotic fractures occur. However, several technical factors may affect the accurate quantification of the structural parameters characterizing the bone network. For example, as the difference in magnetic susceptibility between bone and bone marrow causes intravoxel phase dispersion, the trabecular thickness may be overestimated in conventional MR images. This artefact can be minimized if the echo-time (TE) is reduced. In this study, we assessed the potential of short-TE projection reconstruction (PR) MR imaging in the in-vivo estimation of the main structural parameters of trabecular bone. The results obtained by the conventional Fourier transform-based method (FT) were utilized for comparison and evaluation.

MODELING BIOLOGICAL AND MATERIAL POROUS SYSTEMS BY ^{1}H NMR T_1 AND T_2 RELAXATION AND IMAGING

Maristella Gussoni [a], Fulvia Greco [b], Alessandra Vezzoli [c] and Lucia Zetta [b]

[a] *Dipartimento di Scienze e Tecnologie Biomediche, Università di Milano, Milan, Italy*
[b] *Istituto per lo Studio delle Macromolecole, CNR, Milan, Italy*
[c] *Istituto di Bioimmagini e Fisiologia Molecolare, CNR, Milan, Italy*

The NMR study of porous materials and of the movement of fluids within them has attracted much interest in recent years. Spin relaxation times give structural and dynamical information on the porous network. By imaging contrast spatially defined processes are described. The non-destructive nature of the technique allows repeated experiments on the same sample, providing a temporal dimension. Biological samples can be considered like porous media as well, owing to the heterogeneous structure of tissues and the intracellular water and lipid compartimentation, even if the great difference between the structure of cells and tissue organization in animals and plants, this latter with air filled intra-intercellular space (1), must be considered. Therefore, once that the appropriate model has been adopted, an unexpected link between biology and materials science is established: biological and non-biological porous systems can be both characterized in terms of fluid-matrix interaction and fluid spatial distribution.

Aim of the present study was the identification of the most suitable model to characterize organic and/or inorganic porous systems. Three model systems have been studied: *a)* germinating seeds of Morning Glory (*Pharbitis nil*) plant; b) caviar eggs fresh and after the addition of salt, which allows long term preservation as high quality food products; c) white Portland cement hydrated with and without organic solvents. On all samples, spectroscopic T_1 and T_2 relaxation, spin-echo and chemical shift selective imaging experiments have been performed (4.7 T Bruker AM WB Instrument, equipped with a Microimaging Unit, 15mm insert, 5-10 mm NMR tube). From the collected data, the conclusions were as follows:

a) the seed germination process could be studied by T_1 magnetization decay. By adopting a multi-exponential model, the state of water in the plant cell compartments and its change in mobility during the growth of the seed, owing to metabolic changes could be assessed. Chemical shift selective images monitored the changes in water-lipid intracellular distribution.

b) Different models were adopted to fit the T_1 and T_2 magnetization decay data, collected on the water and the lipid resonances of the caviar eggs. The models were the same for the fresh and the salted samples: the osmotic effect of the added salt could be described. Selective images showed the cellular organization of the caviar egg, never characterized by NMR.

c) The T_2 decay of fluids could be well described and consistently interpreted in hydrating cement in terms of structural and dynamical parameters, on the basis of a model, largely applied to characterize cross-linked elastomers (2). The model combines an exponential component with a gaussian one, taking into account the residual dipolar interaction. In particular, the analysis of the T_2 values, as a function the hydration time, indicated that a pronounced delay, the 'dormant period', occurs in the cement hardening, when organics are present. Images provided a direct evidence of the 'dormant period', showing large liquid pockets in the cement matrix. Pore network depercolation was imaged as well.

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SOLUTION STRUCTURAL INVESTIGATION OF PIG METMYOGLOBIN CAVITIES AND SURFACE BY ^{129}XE NMR SPECTROSCOPY

Marcella Corda[a], Benedetta Era[a], Antonella Fais[a] and Mariano Casu[b]*

[a]*Dipartimento di Scienze Applicate ai Biosistemi*
[b]*Dipartimento di Scienze Chimiche Università di Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (CA), Italy*

The affinity of xenon for hydrophobic sites in macromolecular interiors has motivated much of the recent work on proteins to identify surface accessibility, hydrophobic cavities and identify protein active sites. In the present work, we report evidence that the use of ^{129}Xe resonance can give information in characterizing the interior structure of the pig myoglobin.
^{129}Xe NMR chemical shift and spin-lattice relaxation times were measured in aqueous solution of pig metmyoglobin (MMb) as a function of xenon and protein concentrations. The experimental data were interpreted using a thermodynamic model which supposes that xenon forms a 1:1 complex with the binding site in proteins, characterized by an equilibrium binding constant $K = [Xe]_{in}/([Xe]_{out}[MMb])$, and exchanges rapidly between a cavity of the MMb (Xe_{in}) and all the other environments (Xe_{out}). By fitting the experimental data, the values of chemical shift of xenon trapped in the protein (δ_{in}=30 ppm), the chemical shift of xenon in all the other possible environments (δ_{out}) and the equilibrium constant K=74 M^{-1} were obtained. The analysis of the spin lattice relaxation times allowed to estimate the Xe-Fe^{3+} distance of 7.4 Å.
By comparing these results with the data published in the literature for horse MMb, the ^{129}Xe NMR data on pig MMb clearly show a lower availability of the internal cavities for the xenon atom (K=74 M^{-1} pig MMb, K=146 M^{-1} horse MMb). Moreover, the observed δ_{in} (30 ppm pig MMb, -42 ppm horse MMb), attributed to the paramagnetic dipolar interactions between the xenon and the high spin paramagnetic Fe^{3+}, and the estimated Fe^{3+}-Xe distance (7.4 Å) in pig MMb longer than that (5.3 Å) in horse MMb, suggest a different orientation and proximity of the xenon binding site to the unpaired electrons of the iron in the heme group. These differences are ascribed to interior structural differences between these proteins.

FOLDING STUDIES ON LIVER BASIC FATTY ACID BINDING PROTEIN

Raffaella Ugolini[1], Marianna Luppi[1], Maddalena Catalano[1,2], Laura Ragona[2] and Henriette Molinari[1]

[1] Dipartimento Scientifico e Tecnologico, Università di Verona, Strada Le Grazie 15, 37134 Verona, Italy; ugolinir@sci.univr.it
[2] Istituto per lo Studio delle Macromolecole, CNR, Via Bassini 15, 20131, Milano, Italy

Lipocalins, fatty acid binding protein (FABP) and Avidins are three families of hydrophobic ligand binding proteins forming the Calycin superfamily. They share functional similarities (hydrophobic ligand binding) and a similar folding pattern (an anti-parallel beta barrel).
In our laboratory we have investigated the structural and folding properties of beta-lactoglobulins from different species, which are important representative of the lipocalin family. We have recently undertaken the structural study of chicken liver basic fatty acid binding protein (Lb-FABP) belonging to the FABP family.
In this work, we present the first results obtained on the characterization of folding and stability of the recombinant ^{15}N-enriched protein, which has been expressed and purified in our laboratory. Hydrogen-exchange and urea-unfolding experiments have been carried out on apo Lb-FABP and on Lb-FABP complexed with palmitic acid. A preliminary analysis indicates a remarkably higher stability of the holo with respect to the apo protein.
The data are discussed in light of the folding results obtained for other FABPs; a comparison with beta-lactoglobulins is also reported in order to elucidate common determinants of stability among the Calycin superfamily.

CONFORMATIONAL BEHAVIOUR OF Aβ(25-35) IN DIFFERENT ENVIRONMENTAL CONDITIONS

Anna Maria D'Ursi,[†] Maria R. Armenante[†], Giuseppe Sorrentino[#] and Delia Picone[‡]

[†] *Dipartimento di Scienze Farmaceutiche, Università di Salerno, Italy*
[#] *Università di Napoli Parthenope*
[‡] *Dipartimento di Chimica Università degli Studi di Napoli Federico II.*

The major components of neuritic plaques found in Alzheimer disease (AD) are peptides known as amyloid β-peptides (A-β-peptides), which range in length between 39 and 42 residues. The A-β-(1-42) is the most prone to aggregation and is produced in larger quantities in familial forms of AD.
A-β-(25-35), sequence GSNKGAIIGLM, is a synthetic derivative of amyloid β-peptide, that is highly toxic and forms fibrillar aggregates typical of β-amyloid.. Since it retains both the physical and biological properties of A-β-peptides, it has been proposed that it represents the biologically active region of full-length fragments.
Like the A-β-(1-42), A-β-(25-35) undergoes a conformational transition from a soluble, alpha-helical form to aggregated fibrillary β-sheet structures which are neurotoxic (1).
A detailed investigation of the influence of different environmental conditions on the conformational state of A-β-(25-35) peptide could help to clarify the mechanism of aggregation and its implications in the formation of fibrillar aggregates.
We have recently studied the solution structure of A-β-(1-42) in a hexafluoroisopropanol (HFIP)/water mixture as determined by homonuclear 2D NMR (2).
Here we report the NMR investigation of A-β-(25-35) in HFIP/water mixtures with different compositions, to evaluate the effect of media with different polarity, and in SDS micelles, which reproduces an anisotropic medium similar to that of cellular membrane interface. 3D structures of A-β-(25-35) were calculated on the basis of NOE data using DYANA software. NOESY spectra in SDS micelles with the presence of the spin labels doxyl- derivatives give interesting information on the orientation of the A-β-(25-35) respect to the micelles. The analysis of the 3D structures in the mentioned solvents and the comparison of the derived structure with the previously solved A-β-(1-42) structure points out an interesting role of the kink region A-β-(25-35).

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STUDY OF AGGREGATION AND FOLDING OF PROTEINS BY NMR AND MOLECULAR DYNAMICS: 1-40 AND 1-28 β-AMYLOIDS

M. Delfini, C. Bianchetti, D. Bonivento, M.E. Di Cocco, M. Valerio and F. Conti

Dipartimento di Chimica, Università "La Sapienza", piazzale Aldo Moro, 5 00185 - Roma

Protein folding and aggregation were investigated by NMR spectroscopy and Molecular Dynamics simulations to elucidate the effects of hydrophobic and electrostatic driving forces, using as model systems the amyloid β peptides.

The Amyoid β-peptide (Aβ) seems to be involved in the Alzheimer's disease and it is constituted by a number of aminoacidic residues variable between 39 and 43. It is the main component of the plaques observed in the central nervous system of patients affected by this pathology.

Recent studies demonstrate that Aβ forms aggregates that interact with cellular membranes and induce irreversible alterations of their properties such as fluidity and permeability to ions.

It was also demonstrated that Aβ, normally present in solution in biological fluids, forms insoluble and toxic aggregates with conformational variations depending on pH, ionic strength and the presence of ions such as Cu^{2+} and Zn^{2+}.

In the present work, effects of pH variations on the conformation of the Aβ 1-40 are studied in the solvent model system constituted by water/hexafluoroisopropanol (HFIP) 30/70 v/v.

The analysis has been performed by TOCSY and NOESY 2D-NMR experiments .

At pH=3,5, Aβ is characterized by two α-helices (residues 4-23 and 27-35) linked by a kink (residues 24-26). Varying pH from 3,5 to 6, isoelectric point of the peptide, a partial destabilization of the first helix has been observed, while no conformational variations are evident in the second helix. Similar experiments were carried out on $A\beta_{28}$.

At pH=3,5 $A\beta_{28}$ is characterized by a single a-helix structure. At pH=6, the peptide forms aggregates, and CD analysis evidence the presence of β-sheet structures.

Finally, Molecular Dynamics simulations of two peptides were performed at the different pH values. The obtained results were in agreement with experimental observations.

The different behaviour between $A\beta_{40}$ and $A\beta_{28}$ are discussed in terms of hydrophobic and electrostatic interactions peptide-peptide and peptide-solvent.

APPLICATION OF NMR TECHNIQUES IN PETROCHEMICAL INDUSTRIAL RESEARCH

G. Maddinelli

EniTecnologie S.p.A., via F. Maritano 26, 20097, S. Donato, Milan, Italy

In the last years, NMR techniques have been extensively applied in several subjects involved in oil industrial research. There is in fact, a great demand of development of new materials and also a substantial need of better knowledge of the principles involved in oil production engineering. A large interest has been devoted to the study and development of new performing polymer materials. NMR relaxation technique, could be particularly suitable to the study of such materials due for instance to great dynamic properties of rubber chains. Some of the knowledge obtained in NMR studies of porous media could be usefully applied to investigation of such materials as demonstrated recently.

The relaxation and imaging techniques have been deeply dedicated to the characterisation of oil rock cores. In fact, a better understanding of phenomena related to flow during oil production operations could be significantly beneficial for economics and efficiency of technology in this field. The great development of scientific knowledge of NMR behaviour in porous media determined impressive technical developments of new tools, such as NMR Logging (NML) equipments which now constitute an important and useful technology applied in oil exploration activity. Also, laboratory characterisation tests of oil cores were greatly improved by the new procedures introduced in the last years..

Another important area which is finding a recent interest is the study of fluid dynamics involved in oil production industrial plants. Improvements in the knowledge of multiphase flow and complex engineering systems were obtained through application of novel NMR imaging techniques. MRI has great potential in discriminating oil/water mixtures even in optically opaque media (e.g. by chemical shift and relaxation differences) and also in measuring velocity profiles over a wide range of values avoiding interference with the flowing system. This feature associated with high spatial resolution makes MRI more attractive than other conventional techniques (e.g. laser Doppler methods).

USE OF MODEL SYSTEMS TO INTERPRET NMR IN-SITU MEASUREMENTS FOR CULTURAL HERITAGE MATERIALS

Cinzia Casieri[1], Francesco De Luca[2], Paola Fantazzini[3], Ilenia Viola[2]

[1]*INFM e Dip. di Fisica, Univ. di L'Aquila, V. Vetoio, I-67010 Coppito L'Aquila, Italy*
[2]*INFM e Dip. di Fisica, Univ. 'La Sapienza', P.le A. Moro 2, I-00185 Roma, Italy*
[3]*Dip. di Fisica, Univ. di Bologna, V.le Berti Pichat 6/2, I-40127 Bologna, Italy*

Determination of penetration depth and distribution of water at surfaces is essential for knowledge of the state of conservation of Cultural Heritage items and materials such as frescos, stone, brick, earthenware and porcelain. The water can penetrate the surface of an artifact, coming from both external and internal sources, and in general the moisture content of the surface region is the cause of different decay phenomena such as surface micro-fractures and disintegration. The approach of Nuclear Magnetic Resonance (NMR) can be very powerful for the evaluation of the state of porous materials by monitoring the T_1 and T_2 distribution functions [1] that are related, to a certain extent, to the pore-size distribution functions, which are due to surface relaxation effects. The drawback is that generally the sample does not fit into standard NMR magnets, so for *in situ* applications single sided NMR instruments have to be used, where strong inhomogeneous magnetic fields are present because of geometrical features of the device. Therefore, the standard methods of getting NMR parameters are not always valid and some alternative procedures have to be performed. For example, when transverse relaxation is measured, molecular diffusion in the field gradient is the major effect, even at the shortest available inter-pulse delay for a CPMG sequence, and surface effects are then largely negligible, so that T_2 distributions no longer represent the pore-size distributions. In this paper we show a method for correction of the de-phasing effect due to the diffusion term on transverse relaxation signal, which correction allows getting the T_2 distribution function due only to the surface effect. The method utilized, just introduced by some of us [2,3] to measure the self-diffusion coefficient of non-confined liquids with a single sided NMR device, is based on the comparison, at the same evolution time, of CPMG echo trains obtained at different inter-pulse delays. The method has been applied to porous reference materials characterized by narrow pore-size distributions, as checked by traditional methods. These materials can then be used as standards for fine-arts materials such as mortar, terracotta and ceramics. In these reference samples, fully saturated with water, the correction method applied to data acquired in the highly inhomogeneous magnetic field of a single sided NMR device provides reliable T_2 distributions, as confirmed by measurements acquired in the homogeneous field of a traditional NMR instrument.

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POROUS STRUCTURE PROPERTIES DETERMINATION BY NMR IMAGING AND RELAXATION

Marcella Alesiani* **,** Silvia Capuani*, Bruno Maraviglia*

Physics Dept. 'la Sapienza' University, and INFM UdR Roma1, Roma, Italy

Porous media represent some of the most important technological materials, whose characterization is thus of considerable interest.
Nuclear magnetic resonance is a very powerful tool for non destructive determination of many materials' features, like porosity and pore size distribution, that are two of the most widely studied properties. The relaxation time measurements and their interpretation in terms of structure related parameters have been confirmed by data obtained via traditional techniques as mercury intrusion porosimetry and gas adsorption methods.
Water presence and transportation within materials' porous matrices is one of the main causes of the structure degradation. This is especially important when the examined material is the stone constituting the façade of an ancient building or a sculpture.
It's therefore evident the need for analysis techniques able to provide maps of water spatial distribution in non invasive way: the use of NMR imaging and relaxation-based methods is thus widely investigated.

A SOLID-STATE NMR STUDY OF β ZEOLITE, CARRIED OUT WITH THE HELP OF PROBE MOLECULES

E. Merlo, G. Spanò and S. Spera

Polimeri Europa SpA – Centro Ricerche Novara "Istituto G. Donegani"

Among the variety of analitycal techniques that can be used in the study of zeolites, solid-state NMR spectroscopy is one of the most powerful, at least for what concerns the local characterization of the cristalline sites.
In order to achieve a complete set of information a "multinuclear" approach is highly useful: it consists in the correlation of the results coming from ^{29}Si, ^{27}Al and ^{1}H NMR spectroscopy for the same kind of sample.
^{29}Si and ^{27}Al NMR spectroscopies are already well-known: with the first it is possible to evaluate the real Si/Al ratio by considering only the Al atoms effectively inserted into the zeolitic framework and, in a few cases, to assign the signals distinguishable in the spectrum to the non equivalent crystallographic sites while with the second it's possible to quantify tetrahedrally coordinated Al nuclei against octahedrally coordinated ones (mainly extraframework sites).
Another important investigation technique is ^{1}H MAS NMR spectroscopy: it allows to characterize and, at least in a very approssimative way, quantify the different types of OH groups on the zeolite surface, OH groups that are usually considered of particular importance in the processes involved in heterogeneus catalytic reactions.
In particular we have worked on zeolite H-β whose importance, for its high activity in the alkylation of aromatic hydrocarbons., is already well-known.
In the course of our studies two aspects have drawn our attention: the oscillating behaviour of the intensities of the aluminum signals in the ^{27}Al NMR spectra and the two families of Brönsted sites in the ^{1}H NMR spectra.
Dealing with ^{27}Al NMR spectroscopy we had to face the problem that, besides tetrahedral and octahedral sites, other aluminium sites turned out to exist, whose lower simmetry make them "invisible" to this kind of spectroscopy. Their presence can be unambiguously stated with an accurate measurement of the intensity of the aluminium signals in samples having different symmetry degree. In particular we found that immediately after the calcination process the quantity of "invisible" aluminium is very high: then, with the process of time (order of hours) part of this aluminium becomes visible. This fact can be due to the rehydratation process that, because of the low accessibility of some channels in the zeolitic structure, requires several hours in order to be complete. Water molecules infact help tetrahedral aluminium sites to assume a less distorted geometry, with a high degree of simmetry and therefore not influenced by quadrupolar interactions.
Also in very high hydratation conditions, zeolitic frameworks having almost the same aluminium content on the basis of elemental analysis, can have very different contents of "invisible" aluminum. The different quantity of "invisible" aluminium can therefore be assumed as a measure of the disorder degree of the zeolitic framework.
In the ^{1}H MAS NMR spectrum, recorded on highly dehydrated samples, zeolite H-β shows, apart from the signal clearly assigned to the silanol groups, two peaks of different broadness, tentatively assigned both to Brønsted acid groups. This assignment is still under debate also in the literature; in order to verify it and to get further information regarding the acidity of this zeolite, we studied the interaction with weakly basic reactants, i.e. acetonitrile-d3. After activation with a termal treatment, the sample was loaded with increasing amounts of various probe molecules, having a basic character; the consequent changes in the ^{1}H-NMR spectrum, due to the interaction with the base could therefore be followed. This technique is already well known in other spectroscopies, i.e. IR spectroscopy, but has been poorly exploited in the solid-state NMR spectroscopy and has allowed us to confirm that both signals can be surely assigned to acidic protons as they have the same low field shift after the interaction with weak bases. Also a semiquantitative estimation of the integrated intensity of the signals before and after the interaction supported this assignment.

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SOLUTION STRUCTURE AND DYNAMIC BEHAVIOUR OF THE K18G/R82E *ALICYCLOBACILLUS ACIDOCALDARIUS* THIOREDOXIN MUTANT: A MOLECULAR ANALYSIS OF ITS REDUCED THERMAL STABILITY

Marilisa Leone[a], Paola Di Lello[b], E. M. Pedone[c], Oliver Ohlenschlaeger[d], Simonetta Bartolucci[c], Mosè Rossi[c], Benedetto Di Blasio[b], Carlo Pedone[a], Michele Saviano[a], Carla Isernia[b] and Roberto Fattorusso[b]

[a] *Istituto di Biostrutture e Bioimmagini, Via Mezzocannone 6/8, 80134 Napoli, Italy.* [b]*Dipartimento di Scienze Ambientali, Seconda Università di Napoli, via Vivaldi 43, 81100 Caserta, Italy.* [c] *Dipartimento di Chimica Biologica, Università di Napoli "Federico II", Via Mezzocannone 16, 80134, Napoli, Italy. Institut für Molekulare Biotechnologie, Beutenbergstraße 11, D-07745, Jena, Germany*

Proteins exhibit modest stabilities, which are equivalent to a small number of weak interactions. In this respect, proteins from thermophiles do not differ strongly from their mesophilic counterpart. No general strategy for thermostability has been yet established, since the extra stability of thermophiles appears to be the sum of different cumulative stabilizing interactions. Among them, the packing efficiency (mainly through van der Waals' interactions), networks of ion pairs and/or hydrogen bonds (including α-helix stabilization), the reduction of conformational strain (loop stabilization) and resistance to chemical modifications have been very often found to be responsible for protein thermal stabilization(1).

Thioredoxins belong to a family of proteins present in all living cells from Archaea to humans. These proteins are involved in several fundamental cellular processes such as regulation of transcription factors, activation of deoxyribonucleotides biosynthesis, regeneration of oxidative damage and regulation of photosynthetic events (2). A novel thioredoxin (105 a.a.) was isolated from the moderate thermophile *Alicyclobacillus acidocaldarius* (first denominated *Bacillus acidocaldarius*) in 1997 and its sequence as well as the physico-chemical properties were described (3). Structural stability studies carried out on *Alicyclobacillus acidocaldarius* thioredoxin (BacTrx) by means of circular dichroism, differential scanning calorimetry and nanogravimetry showed that BacTrx withstands higher temperatures than the thioredoxin from the mesophilic bacterium *Escherichia coli* in spite of their high sequence identity (49%). Molecular dynamics simulation studies performed *in vacuo* and in aqueous solution at different temperatures allowed to derive structural information on the determinants of thermal stability of BacTrx and permitted to design mutants with reduced heat capacity (4). The effects of the point mutations on the protein thermal stability were monitored by CD spectroscopy, spectrofluorimetry, thermodynamic comparative studies and the mutants were shown to be substantially less stable (ΔT_m<12-15°) when compared to the wild-type (5,6). Among all the proteins produced, the K18G/R82E mutant showed the most significant meaningful differences. In this comunication, we report the high resolution NMR structure determination of the K18G/R82E mutant and its comparison with the wild-type protein. In addition, we also present a detailed study, accomplished at different temperatures, of the backbone dynamics of both the K18G/R82E mutant and the wild-type thioredoxins from *Alicyclobacillus acidocaldarius*.

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THE SWAPPING OF TERMINAL ARMS IN RIBONUCLEASES

Delia Picone

Dipartimento di Chimica, Università Federico II di Napoli, Via Cintia, 80126, Napoli, Italy

Bovine seminal ribonuclease (BS-RNase), the only dimeric protein among the pancreatic-like ribonucleases, is endowed with special structural features and with biological functions beyond enzymatic activity. In solution, the protein exists as an equilibrium mixture of two forms, with or without exchange (or swapping) of the N-terminal arms. After selective reduction and alkylation of the two intrachain disulfide bridges, the dimeric protein can be transformed into a monomeric derivative, which has a ribonuclease activity higher than that of the parent dimeric protein, but is devoid of the special biological functions. A detailed investigation of the structural features of this protein in solution in comparison with those of other monomeric ribonucleases may help unveiling the structural details which induce swapping of the N-terminal arms of BS-RNase. The solution structure of the recombinant monomeric form of BS-RNase (mBS), as determined by 3D heteronuclear NMR, shows close similarity with that of bovine pancreatic ribonuclease (RNase A) in all regions characterized by regular elements of secondary structure. However, significant differences are present in the flexible regions. In particular, heteronuclear NOEs of backbone amides for both RNase A and mBS indicate a large difference in the backbone flexibility of the hinge peptide segment 16-22, which could provide the molecular basis to explain the ability of BS-RNase monomers to swap their N-terminal arms. To further investigate the role of the regions which are structurally most affected by the swapping, we have expressed variant proteins by substituting two crucial residues with the corresponding ones of RNase A: Pro 19, within the hinge peptide, and Leu 28, which is located at the interface between the monomeric subunits. We have compared the structural properties of the monomeric forms of P19A-BS-RNase, L28Q-BS-RNase and P19A/L28Q-BS-RNase variants with those of the parent protein, and characterized the exchange behaviour of the corresponding dimers. The results suggest that Pro 19 does not affect the swapping tendency and the stability of BS-RNase, whereas Leu 28 plays a significant role in the dimerization and swapping processes.

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STUDY OF TRANSIENT INTERMEDIATES IN BIOMOLECULAR PROCESSES BY NMR

Roberta Pierattelli

CERM and Department of Chemistry, University of Florence, 50019 Sesto Fiorentino, Italy

The use of NMR to study reactions of proteins in *real time* started some time ago, with the aim of understanding enzymes mechanisms. Since then a variety of *real time* techniques have been developed to investigate the thermodynamics and kinetics of fundamental events in protein.
Procedures involving rapid mixing within the NMR sample tube are now being devised and are beginning to transform NMR into a general and powerful technique for studying a wide range of processes on the 0.1 – 1 s timescale at the level of individual amino acid residues.
However, the requirement for rapid acquisition of NMR data usually compromises the spectral resolution thus decreasing the possibility of monitoring events at the atomic level and analyzing their time-dependence. The task can however still be tackled if nuclei in the region of the protein interested by the phenomenon one wants to follow give rise to signals that are well resolved in the NMR spectrum.
I will show here a few examples on how the *real time* approach can be used to study metal binding and metal exchange processes, as well as protein folding and unfolding.

SURFACE ACCESSIBILITY OF HEW LYSOZYME

Andrea Bernini[†], Ottavia Spiga[†], Maria Scarselli[†], Arianna Ciutti[†], Duccio Calamandrei[‡] Daniela Di Maro[‡], Henriette Molinari[§], Maddalena Catalano[§], Irene Fiaschi[†], and Neri Niccolai[†,‡]

[†]*Dipartimento di Biologia Molecolare, Università di Siena, Siena, Italy*
[‡]*BioModem pscrl, Siena, Italy*
[§]*Dipartimento Scientifico e Tecnologico, Università di Verona, Verona, Italy*

Nowadays structural biology represents an advanced frontier for a detailed understanding of life processes. Several projects of structural genomics are presented world-wide, since predictable or experimentally derived structures, mostly of proteins, represent a solid basis for engineering new therapeutic or diagnostic tools.
However, some missing details still exist as the biological functions result to be not simply correlated to protein shapes and flexibility. The presence of "hot spots" on the molecular surface, always including the protein active sites (1,2), and the anomalous activity of some non-competitive enzyme inhibitors (3) suggest that around the protein surface a non-uniform motion of solvent and other molecules occurs.
NMR seems to be perfectly suited to investigate on this new dimension of structural biology, since two techniques, i.e. water-protein Overhauser (4) effects and paramagnetic perturbation profiles (5), independently or in a combined way can be used to probe the accessibility of the protein surface towards molecules with different polarity. The results obtained from both NMR methods for a series of small proteins such as BPTI, tendamistat and single chain monellin (5,6) suggest that only a limited number of water molecules reside at the protein surface for a time long enough to yield sizeable intermolecular NOEs. The active sites of these proteins never have such tightly bound water molecules nearby. Accordingly, TEMPOL, a stable uncharged nitroxide, efficiently approaches the latter protein moieties due to the reduced hindrance from solvent molecules. Furthermore, there are protein regions where bound water molecules, organised in strong hydration sites, prevent the access of the nitroxide.
Paramagnetic attenuation profiles and water-protein nuclear Overhauser effects observed for HEW lysozyme, obtained in the presence and in the absence of the competitive inhibitor (NAG)3, were analysed in terms of accessibility of water and TEMPOL molecules towards the protein surface.
The combined use of ePHOGSY spectroscopy and TEMPOL induced perturbations resulted to be very powerful also to find, as in the previously reported for BPTI (5), the internal water molecules.

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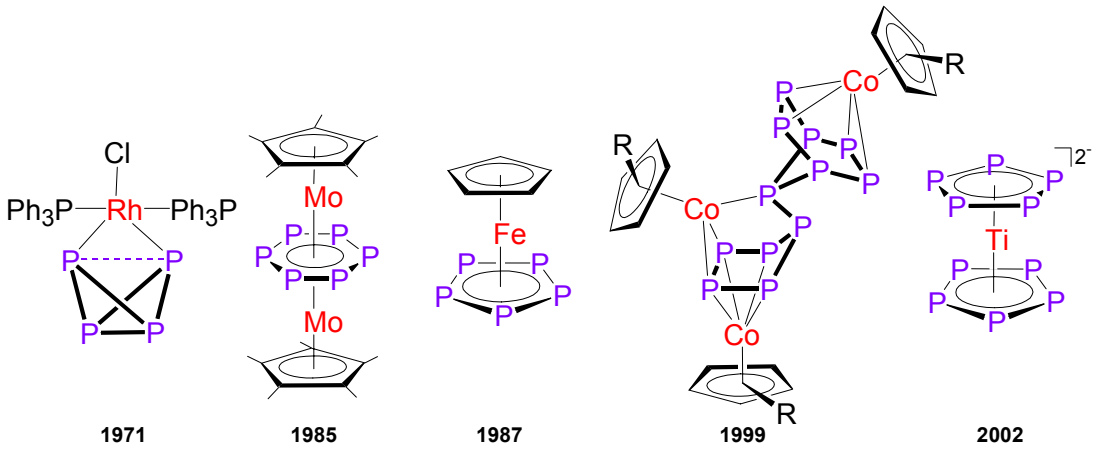
^{31}P NMR SPECTROSCOPY AS AN USEFUL TOOL TO EVALUATE THE SOLUTION STRUCTURE OF TRANSITION METAL COMPLEXES INCORPORATING FRAGMENTS AND UNITS DERIVED FROM WHITE PHOSPHORUS

Maurizio Peruzzini

Istituto di Chimica dei Composti Organometallici
ICCOM CNR, Via Madonna del Piano, snc – 50019 Sesto Fiorentino (FI), Italy

Although virtually unexplored until the 1970s,[1] the coordination chemistry of white phosphorus has developed greatly during the last two decades. These studies have demonstrated that the P_4-tetrahedron can be activated within the coordination sphere of coordinatively unsaturated transition metal-ligand fragments. Once activated, the white phosphorus ligand exhibits a rich chemistry with reactivity patterns different from those of the free molecule.
The sketch below shows some breakthroughs in the area of white phosphorus coordination chemistry.
Most noticeably, the interaction of white phosphorus with transition metal fragments is much more controllable than was originally thought possible and, after reacting with a transition-metal system, the P_4 tetrahedron does not necessarily undergo the often unpredictable and disruptive processes of activation and degradation that characterised the first investigations of P_4-coordination chemistry.[2,3]
^{31}P NMR spectroscopy plays a fundamental and unique role in elucidating the solution structure of these compounds and represents an exceptional tool to study the reaction mechanisms controlling the coordination and the activation steps leading to P_4 degradation and reaggreagation processes.[3]
In this communication, we will illustrate how, a combination of 1D- and 2D-NMR techniques makes possible to gather fundamental information about the solution structure of these complexes and how it helps to shed some light into the processes governing the activation and the reactivity of transition metal complexes incorporating either the P_4 molecule or fragments thereof.



Acknowledgements: MP thanks INTAS/Brussels (project 00-00018) for supporting this activity.

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^{17}O NMR: A QUASI-EXOTIC TOOL FOR THE ORGANIC CHEMIST

Giovanni Cerioni[†]

[†]*Dipartimento Farmaco Chimico Tecnologico, Università di Cagliari, Via Ospedale, 72 I-09124, Cagliari, Italia.*

^{17}O NMR spectroscopy is potentially very useful for an organic chemist, since many functional groups contain oxygen atoms. Its draw back is given by a very low natural abundance (0.037 %), a relatively low resonance frequency (40.662 MHz at 7.05 T), a spin number of 5/2 and a quadrupole moment Q = -2.578 x 10^{-26} electron cm^2. Modern instrumentation relieve these difficulties, at least for compounds with molecular weights minor 800÷1000 Da. The quite short relaxation times ($T_1 \leq 0.02$ s) allow fast accumulation, so that several hundred-thousand transients are obtained in a relatively short time. An advantage is given by ^{17}O large chemical shift range (ca. 1000 ppm for usual organic molecules). The chemical shift trend with hybridization is similar to that observed in ^{13}C NMR spectroscopy, sp^3 oxygens being the most and sp^2 the less shielded, with sp oxygens lying in between. An example is given by ketenes[1] and the importance of cylindrical symmetry for half-height linewidths is discussed. By ^{17}O NMR, evidence of the essential difference in S-O bonds among sulphoxides and sulphones on one side and N-sulfinylanilines and sulfines on the other one is easily reached, and a true π S=O bond has been characterized.[2-4] Conformational information can be gained through ^{17}O NMR and vicinal groups (e. g. α-dicarbonyls[5-7] and *vic*-triketones[8]) have shown to be a suitable subject for such studies. Hydrogen bonding is of course particularly suitable to be studied by ^{17}O NMR and numerous studies can be found in the literature.[9] An application to an important class of compounds, the calix[n]arenes, is discussed.[10]
Several others fields of interest to the organic chemist, e. g. the influence of steric crowding on ^{17}O NMR shifts or the well established relationships among torsional angles and shifts for several funtional groups, are not dealt with in this short communication but some new results on hypervalent iodine derivatives are presented.[11]

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LANTHANIDE-INDUCED SHIFTS (L.I.S.) IN STRUCTURAL AND CONFORMATIONAL ANALYSIS

Raymond J. Abraham[†] and Fernando Sancassan[‡]

[†]*Department of Chemistry, P.O.Box 147, Liverpool L69 3BX, U.K.*
[‡]*Dipartimento di Chimica e Chimica Industriale, Via Dodecaneso 31, I-16146 Genova, Italy, e-mail: fernando@chimica.unige.it*

Twenty five years of researches produced the refinement of this method based on: (i) the use of $Yb(fod)_3$ induced shifts (ΔM_i) to minimize contact contributions on both ^1H and ^{13}C nuclei; (ii) the use of $La(fod)_3$ or, better, of $Lu(fod)_3$ to evaluate diamagnetic (complexation) contributions(ΔD_i) and isolate *experimental* pseudo-contact shifts as (ΔM_i-ΔD_i) values; (iii) the use of a 'starting molecular geometry' and the simulation of chemically reasonable complexation models to obtain *calculated* pseudo-contact shifts by the McConnell-Robertson equation $\Delta M_i^{PC} = k(3\cos^2\varphi - 1)/r^3$ through the scanning of the variables (R, Φ, Ψ, pop) necessary to define lanthanide positions; (iv) the use of Hamilton agreement factor R_{cryst} to compare experimental and calculated pseudocontact shifts.
Given a starting molecular geometry the method can be used to optimize a torsion angle. Alternatively, given the geometries of two conformers, their relative population can be determined. But another stimulating option could be the comparison of the best agreement factors obtained by using different starting geometries, e.g. those obtained by MM (PCModel, Alchemy, Nemesis, etc.), semiempirical (AM-1) or ab-initio (HF, MPn or B3LYP) calculations.
The method can be applied to $CDCl_3$-soluble Lewis bases with a sufficient number of ^1H and ^{13}C nuclei so that the number of experimental data exceeds the number of variables (R, Φ ,Ψ, pop, k and the conformational variable).We have applied the method to aldehydes, ketones, esters, lactones, sulphoxides, sulphones, epoxides. Lactams and amides are currently under investigations as well as the possible use of CD_3CN as solvent.

SOLUTION MOLECULAR STRUCTURE DETERMINATIONS: PARAMAGNETIC NMR OF YTTERBIUM COMPLEXES

Lorenzo Di Bari, Silvia Ripoli, Simona Scarano, Guido Pintacuda and Piero Salvadori

Dipartimento di Chimica e Chimica Industriale - Università di Pisa; CNR, ICCOM-Pisa via Risorgimento 35, 56126 Pisa, Italy

The trivalent lanthanide ions (Ln^{+3}) are employed in several areas that range from chemistry to medicine. Lanthanides and their complexes act, for example, as versatile catalysts in organic reactions, or as contrast agents for magnetic resonance imaging. Particularly, in the case of biological substrates the Ln^{+3} ions constitute useful substitutes of several physiologically important cations which are weakly spectroscopically responsive.[1]
Excluding La^{+3} and Lu^{+3} that do not have unpaired electrons, the other Ln^{+3} are paramagnetic. This characteristic affects the NMR spectra of their complexes, producing a frequency shift and a relaxation rate enhancement (lanthanide induced shift, LIS; lanthanide induced relaxation, LIR).[2] LIS and LIR are connected with the geometry taken by the ligand around the metal. More in detail, these two effects depend on the length and on the orientation of the ion-nucleus vector. On the basis of a well known formalism, structural information can be derived straightforward from the paramagnetic NMR spectra analysis.
In the context of structural determinations, the paramagnetic Yb^{+3} ion has desirable characteristics for NMR studies, especially for relative small ligands. In fact, owing to its electronic properties the use of Yb^{+3} simplify the NMR data treatment to determine the structure of the complex. We set up the program PERSEUS,[3] which allowed us to find the geometry of Yb^{+3}-ligand complexes on the basis of experimental constraints (LIS and LIR) and without imposing symmetry restraints of the complex.
This kind of approach was carried out for calcium binding drugs of the family of the antitumor anthracyclines. Furthermore, a widely used metal carrier (Lasalocid A) was also studied, leading to a solution structure, for the first time based on a large diversity of spectroscopic evidences.

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DOSY – APPLICATIONS AND LIMITATIONS

Rainer Kerssebaum

Bruker BioSpin GmbH, NMR Applications Group, D-76287 Rheinstetten, Germany

The measurement of translational diffusion using magnetic field gradients (1) is already an old application of NMR. Since the development of the DOSY experiment (2) there has been renewed interest in this technique. Instead of evaluating the diffusion coefficients in „normal" diffusion spectra, the signals are spread out in a 2D plane with one axis representing the diffusion behaviour (figure 1).

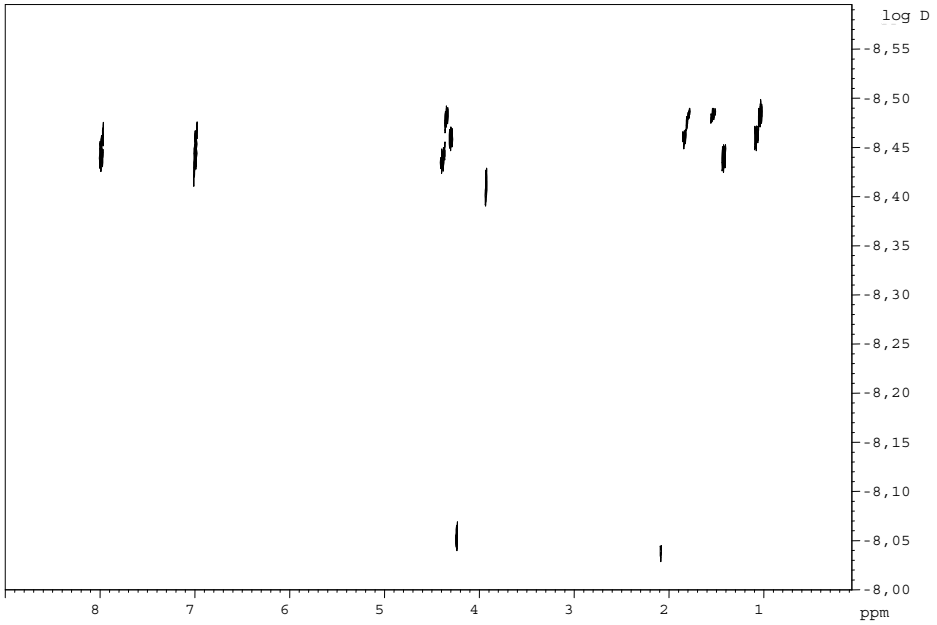

Figure 1: example of a DOSY spectrum; mixture of 4 esters; showing proton chemical shift on the F2 axis (ppm) and diffusion constants on F1 (log D).

Modern NMR equipment with gradient accessory and probes with self-shielded gradient coils make it easy to obtain such spectra. Many different procedures for data acquisition and processing have been reported. The basic technique with different acquisition schemes (STE, LED, LEDbp, ...) and some applications are shown. Another recent application of translational diffusion is the use of diffusion filters to measure diffusion weighted NMR spectra (3). Some examples are shown.

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CYTOPLASMIC DOMAINS OF POTASSIUM ION CHANNELS AND THEIR PROTEIN-PROTEIN INTERACTIONS

Detlef Bentrop[†], Ralph Wissmann[†], Wolfgang Bildl[†], Georgios Spyroulias[‡], Andre Rivard[§], John P. Adelman[§] and Bernd Fakler[†]

[†]*Physiologisches Institut II, Universität Freiburg, D-79104, Freiburg, Germany*
[‡]*Department of Pharmacy, University of Patras, GR-26504, Patras, Greece*
[§] *Vollum Institute, Oregon Health & Science University, OR 97239, Portland, USA*

Small conductance Ca^{2+}-activated potassium (SK) channels underlie the slow afterhyperpolarization that follows the action potential in many types of central neurons. This phenomenon limits the firing frequency of repetitive action potentials and is essential for normal neurotransmission. SK channels are gated solely by intracellular Ca^{2+} in the submicromolar range. This high affinity for Ca^{2+} results from Ca^{2+}-independent association of the pore-forming SK α-subunit with calmodulin (CaM), a property unique among the large family of potassium channels. The talk presents the NMR study of the calmodulin binding domain (CaMBD, residues 396-487 in rat SK2) of SK channels. The CaMBD exhibits an unusually folded helical domain between residues 423-437, whereas the rest of the molecule lacks stable overall folding. Disruption of this core region abolishes constitutive association of CaMBD with Ca^{2+}-free CaM, and results in SK channels that are no longer gated by Ca^{2+}. The results show that the Ca^{2+}-independent CaM-CaMBD interaction that is crucial for channel function is determined by a domain different in sequence and structure from other CaM-interacting proteins. The CaM-CaMBD complex was studied by NMR both in the absence and the presence of Ca^{2+}. Independent of Ca^{2+}, the complex is in intermediate exchange on the NMR chemical shift time scale. Therefore, the structural information about the complex in solution is limited but still complementing the recent crystal structure of the Ca^{2+}-loaded CaMBD/CaM complex (Schumacher M.A. et al, *Nature* **410**, 1120-1124 (2001)).

INSIGHTS INTO PROTEIN-PROTEIN INTERACTIONS WITHIN BIOMEMBRANES BY SOLID-STATE NMR

Eleri Hughes, Jonathan Clayton and David A. Middleton

Department of Biomolecular Sciences, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.

It is emerging that a number of small transmembrane proteins act as regulators of calcium transport in muscle cells by modulating the activity of ATP-driven ion pumps. These proteins activate or inhibit their physiological targets in response to changes in ionic concentrations, expression levels or by the effect of external stimuli such as kinases. We have incorporated the regulatory proteins phospholamban (PLB) and sarcolipin (SLN) together with their physiological target, SERCA2a Ca^{2+}-ATPase, into phospholipid bilayers for structural and functional studies. Activity assays indicated that Ca^{2+}-ATPase retains its function in the lipid bilayers, but maximal activity was partially inhibited by PLB and stimulated by SLN. A range of broad line and high-resolution solid-state NMR methods have been developed to further characterize the interactions between these proteins. Experiments will be outlined in which we have measured the affinity of SLN and PLB for Ca^{2+}-ATPase, examined membrane topology and resolved structural changes in the regulatory proteins when they associate with Ca^{2+}-ATPase. This information is providing a detailed account of the molecular basis for calcium cycling in cardiac and skeletal muscle cells.

NMR STRUCTURAL MODEL OF THE INTERACTION OF ACIFLUORFEN WITH THE PHOTOSYNTHETIC REACTION CENTER FROM RHODOPSEUDOMONAS VIRIDIS

Nicola D'Amelio,[†] Piotr Mlynarz,[‡] Elena Molteni,[†] Elena Gaggelli,[†] Gianni Valensin,[†] and Wolfgang Lubitz[∥]

[†] *Department of Chemistry and NMR Centre, University of Siena, Via Aldo Moro, 2, 53100 Siena, Italy;*
[‡] *Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wroclaw, Poland. Faculty of Chemistry, University of Gdansk, 80-952 Gdansk, Poland*
[∥] *Max-Planck-Institut für Strahlenchemie, D - 45413 Mülheim an der Ruhr/Germany*

The interaction of the herbicide acifluorfen with the photosynthetic reaction center from Rhodopseudomonas viridis has been studied by NMR relaxation measurements. Interaction in aqueous solution has been demonstrated evaluating motional features of the bound form through cross-relaxation terms of protons at fixed distance on the herbicide. Exchange regime was evaluated in the micro-milliseconds time scale. Contributions to longitudinal nonselective relaxation rates different from proton-proton dipolar were inferred, most probably due to paramagnetic effect originating from Fe(II) non-heme iron on the reaction center. Paramagnetic contributions to proton relaxation rates were converted into distance constraints in order to build a model for the interaction. The model places the herbicide between the metal and the QB site, where most herbicides interact, and it could therefore act as a shield between the QA and QB site. The obtained model was validated via docking and *ab initio* calculation of the magnetic susceptibility tensor to qualitatively interpret the experimental shifts induced by the presence of the reaction center.

CALMODULIN MODULATION OF THE OLFACTORY CNG CHANNEL: SOLUTION STRUCTURE BY NMR OF A COMPLEX BETWEEN A FRAGMENT OF THE N-TERMINAL DOMAIN AND CALMODULIN.

Gian Marco Contessa[c], Maria V. Orsale[a], Sonia Melino[a], Vincent Torre[d], Maurizio Paci[a,c], Alessandro Desideri[b,c] and Daniel O. Cicero[ad]

[a]Department of Chemical Sciences and Technologies, [b]Department of Biology and [c]INFM, University of Rome "Tor Vergata", via della Ricerca Scientifica, 00133 Rome Italy, [d]SISSA, Settore di Biofisica, Trieste, Italy

Ion channels sensitive to cyclic nucleotides fall into different subfamilies of distantly related proteins. Members of one subfamily, designated CNG channels, are largely voltage-independent but require cAMP and cGMP to open (Finn et al., 1996). Several studies indicate that the activity of both rod and olfactory CNG channels is modulated by calmodulin (CaM) (Molday, 1996). The binding regions of CaM in the different subunits of the CNG channels have been identified. The olfactory CNG channel presents a binding motif in the N-terminal domain of the α-subunit (Liu et al., 1994). A 26-aminoacid synthetic peptide derived from this binding site was shown to present a high affinity for CaM (Kd 4-12 nM), in the same range of the observed EC50 of CaM (21 nM) (Liu et al., 1994). Varnum and Zagotta (1997) showed that an intramolecular protein-protein interaction between the amino-terminal domain and the carboxyl-terminal ligand-binding domain of the rat olfactory CNG channel exerts an autoexcitatory effect on channel activation. Interestingly, the region of the N-terminal domain involved in the N-C interaction is the same that interacts with CaM (Varnum and Zagotta, 1997). This fact lead to the hypothesis that CaM disrupts the interaction between N- and C-termianl domains and this is the basis for its modulation.

A full understanding at a molecular level of the CaM regulatory mechanism of the olfactory CNG channel will require the structure elucidation of the complexes between CaM and the N-terminal domain, and the complex formed by the intramolecular interaction of N- and C-terminal domains. In the present communication we report the solution structure of the complex formed by CaM and a 26 residue peptide derived from the N-terminal domain of the α-subunit of the bovine olfactory CNG channel. Based on the structure the discussion will focus on the importance that electrostatic and sequence-specific mechanisms ought to have on the target recognition process of calmodulin.

USE OF HPLC-NMR FOR SPEEDING UP THE STRUCTURE ELUCIDATION PROCESS IN PHARMACEUTICAL INDUSTRY

Stefano Provera, Lucilla Turco, Silvia Davalli, Carla Marchioro

GlaxoSmithKline Research Center, Via Fleming 4, 37135, Verona (Italy)

High Performance Liquid Chromatography (HPLC) is a well known and very commonly used technique in the analysis of chemical mixtures but conventional detectors used to monitor the separation (UV, fluorescence, etc.) generally provide poor information on the molecular structure. In the recent past the coupling of HPLC with mass spectrometry (HPLC-MS) has strongly improved the amount of structural data on the mixture components. However, any new chemical entity often requires NMR data in order to prove an unambiguous structural identification and the most common way to proceed involves time-consuming isolation and purification steps.
As a consequence, the HPLC-NMR hyphenation has recently become more and more popular in Pharmaceutical Industry in various fields of application, due to the advantages in terms of speeding up the structure elucidation process. The HPLC-NMR popularity has progressively increased thanks to the development of hardware systems (high field magnets, flow-probes) and of efficient solvent suppression techniques (WET).
Examples of HPLC-NMR applications focussed on the identification of degradation products in drug development phases will be described.

NMR STUDY OF THE STRUCTURE OF SOME IMIDAZOLIUM-BASED ROOM TEMPERATURE IONIC LIQUIDS

Andrea Mele,[†] Matteo Giannone,[†] Walter Panzeri,[†] Chiu D. Tran[‡] and Silvia H. De Paoli Lacerda[‡]

[†]*Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta" del Politecnico di Milano*
[‡]*Department of Chemistry, Marquette University, Milwaukee (USA)*

The importance of room temperature ionic liquids (RTILs) as dissolving media for green chemistry is constanlty growing due to their unique physical and chemical properties. Indeed, they show high dissolving power, chemical stability and virtually no vapor pressure [1]. It is known that RTILs can absorb significant amount of water from air and that some properties (e.g., solubility, polarity, viscosity and conductivity) may consequently vary as a function of absorbed water [2]. This, in turn, affects the rates of chemical reactions and efficiencies of various processes carried out in RTILs. The strict relationship between ionic liquid structure and their behaviour as reaction media stimulated studies with a broad repertoire of techniques (FT-IR, Near-IR, fluorescence, viscosimetry, conductivity and pulsed-gradient spin-echo NMR diffusion coefficient measurements [3]. Unfortunately, these techniques cannot provide direct information on molecular level of structure of RTILs and their interactions with water.
In this communication we report on some results of an NMR study via intermolecular nuclear Overhauser enhancements (NOEs) on model compounds 1-*n*-butyl-3-methylimidazolium tetrafluoborate ($[BMIm]^+$ $[BF_4]^-$, **1**) and 1-*n*-butyl-3-methylimidazolium hexafluorophosphate ($[BMIm]^+$ $[PF_6]^-$, **2**). The comparison of NOEs patterns in the pure liquids and in the samples containing water showed that the presence of water changes the structure of the pure ionic liquid by introducing water-cation interactions. Further details on the type and site of water-imidazolium ion interactions are obtained by the quantitation of water-cation NOEs. Heteronuclear experiments such as $^1H\{^{19}F\}$ NOE difference spectra pointed out the presence of ion-pairs with $C(sp^2)$—H···F typ e hydrogen bonds [4].

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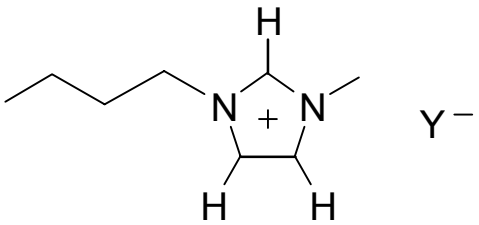

1 $Y^- = BF_4^-$

2 $Y^- = PF_6^-$

ASSOCIATION EQUILIBRIA INVOLVING $(C_6F_5)_2B(OH)$ SPECIES IN TOLUENE-d_8 AND CD_2Cl_2 SOLUTIONS

Tiziana Beringhelli,[†] Giuseppe D'Alfonso,[†] Daniela Donghi,[†] Daniela Maggioni,[†] Pierluigi Mercandelli,[‡] Angelo Sironi[‡]

[†]Dipartimento di Chimica Inorganica, Metallorganica e Analitica; [‡]Dipartimento di Chimica Strutturale e Stereochimica Inorganica, Via Venezian 21; 20133 Milano, Italy
E-mail: daniela.maggioni@unimi.it

The chemistry of bis(pentafluorophenyl)borinic acid $(C_6F_5)_2B(OH)$ (**1**) [1] is at present actively investigated and several applications in catalysis and organic synthesis have been reported [1-3]. This is mainly attributable to some peculiar features of this molecule, where both Lewis and Brønsted acidic sites are simultaneously present, as well as a Lewis basic site (the non bonding electrons on the oxygen substituent).
We have found that in the solid state **1** exists as a cyclic trimer constituted of three $B(C_6F_5)_2OH$ monomers interconnected through B-O(H)-B bridges, resulting in a cyclohexane-like structure with C_2 twist-boat conformation. This is the first example of a trimeric structure based on tetra-coordinated boron atoms only.
The NMR data (particularly ^{11}B) indicate that the dissolution of **1** in toluene-d_8 at room temperature is accompanied by disruption of the trimeric structure to give the $B(C_6F_5)_2OH$ monomer $\mathbf{1_m}$. At low temperature, ^{19}F NMR revealed magnetic unequivalence of the two perfluoroaryl rings of $\mathbf{1_m}$, due to the freezing of the OH rotation around the B-OH bond, while free rotation around the B-C(aryl) bonds is still maintained.
In contrast, the dissolution of **1** in CD_2Cl_2 solution give rise to a mixture (Figure 1) containing both in the monomeric (A) and in the trimeric (B) forms (^{11}B NMR: δ 43.8 for A and 8.4 for B, as typical of tri- and tetra-coordinated Boron atom, respectively). Monomer A and trimer B are in equilibrium, and the relative amount of B increases on increasing the overall concentration and on decreasing the temperature.
Moreover, at the lowest temperatures a new species is observed, with three para (1:1:1) and three protonic (1:2:2) resonances (one of which at δ 18.4). A ^1H-DOSY experiment at 193 K suggested the oligomeric nature of this species.

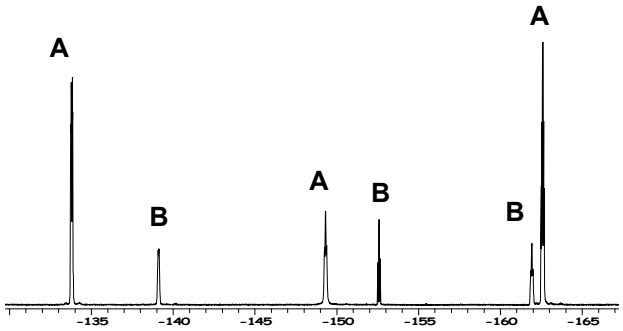

Figure 1. ^{19}F NMR spectrum of a mixture of A and B species (CD_2Cl_2, 193 K, 7.1 T)

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SELECTIVE MONOALKYLATION OF DIHYDROXYCOUMARINS via MITSUNOBU DEHYDROALKYLATION: A REGIOCHEMICAL PROBLEM SOLVED BY NMR

Stefano Chimichi,[†] Marco Boccalini,[†] Giancarlo Cravotto,[‡] and Bruna Robaldo[‡]

[†]*Dipartimento di Chimica Organica "U. Schiff", Università di Firenze, via della Lastruccia 13, I-50019 Sesto F.no (Firenze), Italy*
[‡] *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, via Giuria 9, 10125 Torino, Italy*

The use of high intensity ultrasound to selectively alkylate dihydroxycoumarins using the Mitsunobu reaction and biologically significant alcohols will be presented.
The 400 MHz ^{1}H NMR spectral patterns of all the obtained compounds confirmed that the reaction products were O-alkylated coumarin derivatives and that no Claisen rearrangement occurred. Aesculetin (6,7-dihydroxycoumarin) exclusively afforded in good yields the 7-alkylated derivatives prenyletin, 7-geranyl and 7-farnesylesculetin.
It should be noted that complete regioselectivity is not limited to prenyl alcohols: methanol gave isoscopoletin in good yield. When the same experimental conditions were used with daphnetin (7,8-dihydroxycoumarin), the regioselectivity was low, resulting in a mixture of 7-*O*- and 8-*O*-monoalkylated derivatives together with small quantities of the 7,8-disubstituted compounds, relative yields depending on the molecular size of the alcohol.
The structural assignment for monoalkylated polyphenols (*e.g*. distinction between 6- or 7-, and 7- or 8-substituted coumarins) has always been problematic, as shown by the numerous corrections that from time to time have appeared in the literature.
Solution to the regiochemical problem by NMR experiments (NOEDIF, NOESY) will be discussed.

POSTERS

SEQUENTIAL NMR RESONANCE ASSIGNMENT AND PRELIMINARY STRUCTURE DETERMINATION OF A MUSTARD TRYPSIN INHIBITOR (MTI2)

Ivana Arosio,[†] Teresa Recca, [†] Enrica Alberti, [†] Roberto Consonni, [†] Luigi R. Ceci[‡], Raffaele Gallerani[‡] and Lucia Zetta[†]

[†] *Laboratorio di NMR, ISMAC, CNR Milano, Via Bassini 15, Milano, Italy*
[‡] *Centro Studio sui Mitocondri e Metabolismo Energetico, Bari, Italy*

Plant protease inhibitors have been classified according to the proteolytic enzymes upon which they act (serine, cysteine, aspartic acid and metallo-protease)[1]. In seeds of the Crucifera mustard (*Sinapsis alba*) two different trypsin inhibitors (MTI and MTI2) have been identified[2]. MTI2 was the first protease inhibitor which was sequenced from Crucifera: it consists of 63 amino acids, rich in cysteine and glycine residues, showing no structural homology with other known families of plant serine protease inhibitors. MTI2 is a potent heat-stable inhibitor of trypsin and may have a natural defensive function in plant leaves.
In this work, the recombinant MTI2, expressed in *Pichia pastoris*[3], has been characterised by NMR spectroscopy together with molecular dynamic calculations. Mono-dimensional ^{1}H spectra recorded at temperatures up to 80 °C confirmed the heat-stability of MTI2. Two-dimensional homonuclear experiments enabled us to fully assign of the protein residues and to identify some amino acids possibly located in an hydrophobic clusters. A comparison of the structural homology of MTI2 with a class of toxins have been also presented and discussed. A more refined 3D structure will be pursued in the near future by the analysis of the ^{15}N labelled protein.

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STRUCTURAL ANALYSIS OF THE *E. coli* MONOTHIOL GLUTAREDOXIN GRX4

Massimo Bellanda[†*], Malin Fladvad[‡*], Aristi Potamitou[‡], Stefano Mammi[†] Arne Holmgren[‡], Alexios V. Vlamis[‡] and Maria Sunnerhagen[‡]

†*Department of Organic Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy*
‡*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden*
**These authors have contributed equally to this work*

Glutaredoxins are ubiquitous proteins which have the ability to reduce protein disulfides and mixed disulfides with glutathione. They are therefore central in the response against oxidative stress. The glutaredoxins are divided into two subfamilies, the well characterised dithiols, containing a CPYC active site and the less known monothiols, having a CGFS active site. No structure is available for any monothiol glutaredoxin while that of several proteins of the dithiol subfamily has been solved. Escherichia coli has three dithiol glutaredoxins (Grx1, Grx2 and Grx3) and one recently discovered monothiol, called glutaredoxin 4 (Grx4). Grx4 is a protein of 115 amino acids (13 kDa), with high sequence homology to monothiol yeast Grx5 (37 % identity). In order to gain a better understanding of Grx4 function, we determined the three-dimensional structure of Grx4 using NMR. The solution structure of Grx4 shows a thioredoxin-like fold consisting of a β-sheet in the core, flanked by helices. Comparison of Grx4 with other glutaredoxins reveals significant structural similarities to the pig liver glutaredoxin and to the N-terminal domain of several Glutathione S Tranferase (GST) proteins. The active site in Grx4 (Cys 30-Ser 33), is located on the molecular surface and Pro 72, which is in close proximity to the active site, assumes a conserved cis-peptide configuration found in all dithiol glutaredoxins. However, some regions in the Grx4 structure differ significantly from the dithiol glutaredoxin.

FIRST DIRECT OBSERVATION OF C=O···H─N HYDROGEN BONDS IN PEPTIDES FORMING 3_{10}-HELICES BY INTERRESIDUE $^{3h}J_{NC'}$ SCALAR COUPLING

M. Bellanda[†], M. Rainaldi[†], C. Toniolo[†], M. Crisma[†], F. Formaggio[†], C. Peggion[†], Q.B. Broxterman[‡], B. Kaptein[‡] and S. Mammi[†]

[†]Department of Organic Chemistry, University of Padova, Institute of Biomolecular Chemistry, CNR,Via Marzolo 1, 35131 Padova, Italy
[‡]DSM Research, Life Sciences, P.O. Box 18, 6160 MD Geleen, The Netherlands

Oligopeptides rich in C^{α}-tetrasubstituted α-amino acids present a rather peculiar stereochemistry due to significant constraints imposed on their conformational freedom by these residues. Specifically, most of the C^{α}-tetrasubstituted amino acids have been extensively documented to possess a very high intrinsic helix-forming capacity. The narrow conformational space accessible includes both the classical α-helix and the 3_{10}-helix. It was shown that among the C^{α}-tetrasubstituted chiral amino acids the β-branched C^{α}-methyl-D-valine [(αMe)Val] is the residue with the most pronounced bias toward the left-handed 3_{10}-helix. Furthermore, it was also known that homo-oligopeptides of the non-chiral amino acid α-aminoisobutyric acid (Aib) adopt an incipient 3_{10}-helical structure at the trimer level. The strong propensity of peptides rich in (αMe)Val and/or Aib toward the 3_{10}-helix has been clearly demonstrated in the crystal state by X-ray diffraction and in solution by NOE-based NMR techniques.

The recently developed NMR pulse sequences for the direct measurement of scalar coupling through hydrogen bond has provided a useful tool for the observation of the hydrogen bonding network in doubly labeled proteins, suggesting an independent method to distinguish unambiguously the α-helix from the 3_{10}-helix .

In this work we have synthesized the following series of short peptides containing D-(αMe)Val and Aib:

Z-**D-^{13}C'-(αMe)Val**-Aib$_2$-**D-^{15}N-(αMe)Val**-Aib-OtBu (1)
Z-Aib-**D-^{13}C'-(αMe)Val**-Aib$_2$-**D-^{15}N-(αMe)Val**-Aib-OtBu (2)
Z-**[D-^{13}C'-(αMe)Val]$_2$**-Aib$_2$-**D-^{15}N-(αMe)Val**-Aib-OtBu (3)
Z-**^{13}C'-Gly**-Aib$_2$-**D-^{15}N-(αMe)Val**-Aib-OtBu (4)
Z-Aib-**^{13}C'-Gly**-Aib$_2$-**D-^{15}N-(αMe)Val**-Aib-OtBu (5)

These peptides were designed to fold in left-handed 3_{10}-helices and to incorporate selectively ^{13}C- and ^{15}N-labeled residues at appropriate positions suitable for monitoring C=O···H─N helical hydrogen bonds. The X-ray diffraction structure of peptide (1) was solved and, as expected, a regularly developed 3_{10}-helix was observed. A 2D-version of the experiments proposed by Cordier and Grzesiek (*J. Am. Chem. Soc.* (1999) **121**, 1601-1602) was used to measure quantitatively the $^{3h}J_{NC'}$ scalar coupling through hydrogen bond. With the exception of peptide (4), a signal indicating the presence of a hydrogen bond typical of the 3_{10}-helix was observed. All of the measured $^{3h}J_{NC'}$ are in the range 0.10-0.06 Hz and are much smaller than those measured for α-helical peptides, as expected from the less than optimal hydrogen-bond linearity for 3_{10}-helices. This is the first time that such small constants have been experimentally measured. For peptide (4) containing a Gly at the N-terminus, it was not possible to observe any signal above the detection limit. When an Aib residue was added at the N-terminus [peptide(5)], the peak corresponding to the hydrogen bond between Gly^2 and $(\alpha Me)Val^5$ was clearly observed. Finally, peptide (3) was designed to allow a direct detection of hydrogen bond in case of the 3_{10}- or the α-helical structure. Only the signal indicating the presence of the 3_{10}-helix was seen, unequivocally demonstrating the propensity of these peptides for this type of folding.

CHARACTERIZATION OF THE COMPLEX BETWEEN THE GYRASE-B P24 FRAGMENT AND THE INHIBITOR GR122222 BY NMR AND COMPUTER SIMULATIONS

M. Bellanda[†], L. Monticelli[†], E. Bosa[†], S. Moro[±], E. Peggion[†‡], E. Perdonà[*], E. Domenici[*], C. Marchioro[*] and S. Mammi[†‡]

†Department of Organic Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy
‡Institute of Biomolecular Chemistry, CNR, ,Via Marzolo 1, 35131 Padova, Italy
±Department of Pharmaceutical Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy
**GlaxoSmithKline Medicines Research Center, 37135, Verona, Italy*

DNA topoisomerases are ubiquitous enzymes that control the topological state of DNA in cells. There are several classes of topoisomerases, each with distinct properties. Among them, bacterial DNA-gyrase is able to introduce supercoils into DNA in a reaction coupled to hydrolysis of ATP. Gyrase is essential in all bacteria but it is not found in eukaryotes and is therefore a good target for antibiotics. Gyrase consists of subunits GyrA and GyrB, the active enzyme being an A2B2 complex. The hydrolysis of ATP takes place in the B subunit and binding sites of different inhibitors have been localized in the N-terminal 24kDa fragment of GyrB (P24).The X-ray structures of this fragment complexed with different ligands have been published but structural studies in solution are scarce.
In the present work, the complex between the fragment P24 and the cyclothialidine GR122222 has been investigated in solution by means of different NMR techniques and computer simulations. An initial characterization of the binding site has been performed on the basis of binding-induced changes of backbone amide chemical shift. The conformation of the bound ligand and the contacts between the cyclothialidine and the protein were studied using different isotope-filtered NOESY experiments. Preliminary H-D exchange experiment showed that some amide protons facing the binding site are protected from the solvent although they are not part of defined secondary structure elements. The recognition site was further characterized by means of semirigid docking simulations. Extensive molecular dynamics simulations in explicit solvent allowed the characterization of the hydrogen bonding network highlighting the role of water molecules in mediating some of the interactions. The results were compared with the available X-ray structure of the complex showing that the crystallographic binding site was substantially reproduced.

^{1}H NMR STUDIES OF ALGINATE INTERACTIONS WITH AMINO ACIDS

Maria Enrica Di Cocco[a*], Cristiano Bianchetti[a], Maurizio Delfini[a], Federica Chiellini[b]

[a] *Department of Chemistry, University "La Sapienza", Piazzale Aldo Moro, 5 – 00185 Roma (Italy)*
[b] *Department of Chemistry & Industrial Chemistry, University of Pisa, Via Risorgimento, 35 – 56126 Pisa (Italy)*

Alginate gels are currently employed in a number of trials to transplant multi-cell types because they meet many requirements for an ideal matrix material. Entrapment of living cells in alginate gel beads can be accomplished under very mild conditions. This procedure is, therefore, widely used for immobilization of micro-organisms as well as of many eukaryotic cells.
Alginate is a polysaccharide obtained from marine algae, with a linear copolymer structure comprising 1,4-linked β-D-mannuronate (M) and α-L-guluronate (G) residues. The gel formation by an alginate solution is due to cooperative binding of divalent cations.
The present work was aimed at investigating the possible interactions between alginate gel and molecules of perfusion media. The ultimate goal being represented by an insight on the influence of the network structure on the real availability of metabolites or on how it may affect concentration gradients in the extra-cellular environment, with the consequent regulation of cells viability and functionality.
A study of the interactions between alginate and hystidine, lysine, phenylalanine and alanine amino acids was carried out by Nuclear Magnetic Resonance dynamic parameters. The characterization of the alginate utilized was performed by 1D and 2D-COSY experiments. Measurements of proton NMR non-selective and selective relaxation rates in the absence and presence of alginate were used to calculate interaction constants between each amino acid and alginate. The trend of the interaction constants was related to the hydrophobicity of the different amino acids side-chains. It was therefore calculated the interaction constant between phenylalanine and Ca^{2+} cross-linked alginate and the behavior of the polymeric network was investigated. The absence of competition between cross-linking processes and amino acid-alginate interaction has been suggested. On the basis of the experimental results, a model of the interaction between amino acids and alginate was proposed.

NMR SPECTROSCOPY METHODS TO STUDY SUBSTRATE–ENZYME INTERACTION: INVESTIGATION ON LIPASE-CATALYSED HYDROLYSIS OF NAPROXEN METHYL ESTER BY RELAXATION RATES

E. Cernia[a], M. Delfini[a], C. Bianchetti[a], M.E. Di Cocco[a], C. Palocci[a], F. Porcelli[b] and S. Soro[a]

[a] *Dipartimento di Chimica, Università "La Sapienza," Piazzale Aldo Moro 5, 00185 Roma, Italia*
[b] *Dipartimento di Scienze Ambientali, INFM sez. B Università della Tuscia, L.go dell'Università, 01100 Viterbo, Italia*

Hydrolases and in particular microbial lipases have found widespread application as biocatalysts for the solution of synthetic organic problems, in particular in the field of regio- and enantioselective reactions (1,2).
In the last decade the crystallographic data for several enzymes have became available providing three-dimensional structures of their active sites. On the other hand, in solution ^{1}H-NMR relaxation rate measurements of substrate–lipase complexes yield the evaluation of the motional and environmental features of bonded substrate.
(±)-2-(6-Methoxy-2-naphthyl)propionic acid methyl ester (methyl ester of Naproxen), the precursor of therapeutically important nonsteroidal anti-inflammatory drugs (NSAIDs) was enantioselectively hydrolysed using as biocatalyst *Candida rugosa* lipase. In research aimed at studing the structure–activity relationship (SAR), NMR spectroscopy methods were employed to identify which Naproxen molecular moiety was essential to the substrate–enzyme interaction (3,4). The substrate exchange from the bound state at a rate fast enough to yield measurable selective relaxation rate enhancements thus provides significant dynamic and structural features. In fact the substrate-enzyme interaction constant measured by NMR relaxation data (not selective, mono-selective and bi-selective relaxation time) (5) showed no tight binding between substrate and enzyme. This is in agreement with reported kinetic data in hydrolysis reaction of Naproxen ester. Moreover, the NMR relaxation data showed that only a portion of aromatic ring protons was the most affected by lipase interaction. This kind of interaction between substrate aromatic rings and enzyme was proposed in previous computer modelling studies (6). The SAR theoretical approach was able to rationalise substrate–enzyme interactions based on enzyme three-dimensional crystal structure and docking studies suggesting some amino acids (particularly Phe345) as playing an important role in determining enzyme–substrate recognition. This finding was confirmed by protein engineering studies (7) and in particular highlights the interaction in solution between the substrate and the enzyme during the catalytic process.

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TOPOLOGICAL CHARACTERISATION OF HUMAN α-SYNUCLEIN IN A MEMBRANE MIMETIC ENVIRONMENT

Marco Bisaglia[†], Isabella Tessari[‡], Elisabetta Bergantino[‡], Luigi Bubacco[‡] and Stefano Mammi[†]

[†]*Dipartimento di Chimica Organica - Università di Padova, Via Marzolo 1 - 35131 Padova*
[‡]*Dipartimento di Biologia - Università di Padova, Via Ugo Bassi 58/B - 35131 Padova*

Human α-synuclein is a 140 amino acid presynaptic protein the physiological role of which is not known. It is the major component of the intracellular aggregates known as Lewy bodies, a central feature of Parkinson's disease.

Although more than 50% of cellular α-synuclein is cytosolic, a portion of the protein is associated with the membrane *via* the first 90 residues that contain an incomplete 11-residue periodicity, found in the A2 class of apolipoproteins and potentially able to fold into an amphipathic α-helix. From the first structural studies, it emerged that α-synuclein has a random structure in water under physiological conditions. Only upon binding to phospholipid vesicles, the N-terminal residues adopt a helical structure. On the contrary, in fibrils found in the Lewy bodies, α-synuclein seems to be in a β-sheet structure.

Aggregation of α-synuclein could be promoted by lipid environments. Moreover, the central region of the protein between the residues 61-95, the so-called NAC region, might be responsible for fibril formation. Interestingly, some predictions indicate that this region could form a transmembrane helix even though there is not general agreement on this.

In order to shed some light on this issue that may be relevant to α-synuclein misfolding in neurodegenerative diseases, we have addressed the problem using an interdisciplinary approach that includes biophysics, functional and structural biology.

In our laboratories, we have cloned the entire coding sequence for either α-synuclein or its 1-99 deletion mutant in fusion with an His-tag in pET28b plasmid (Novagen) to obtain rapid and economic expression of recombinant proteins. The expression system chosen is *E. coli* and we have optimized the bacterial growth conditions to obtain suitable labeling of the proteins to be used for the NMR studies.

To identify the topological orientation of α-synuclein relative to a membrane-mimetic environment constituted by SDS micelles, we have analyzed the effects of water-soluble and membrane-soluble spin probes on the HSQC spectrum of the entire protein. Our data indicate that the 100 N-terminal residues of the protein bind to the membrane whereas the 40 C-terminal ones are exposed to the aqueous solution, in agreement with previous structural studies.

Because of the important overlap of several peaks, to best characterized the NAC region of the protein we have repeated this analysis on the 1-99 deletion mutant of α-synuclein and we have shown that this fragment can be divided in two parts: the first one forms a helix positioned on the micelle surface whereas the second one penetrates into the micelle more deeply. This result seems to support the transmembrane model.

IN VIVO MRI STUDY OF THE DIFFUSION OF LIPOSOMES CONTAINING GADOLINIUM CHELATES IN RAT BRAIN

Giorgio Capuani[†], Luca Denaro[‡], Andrea Masotti[†], Cesare Manetti[†]

[†] *Dip. di Chimica, Università degli Studi "La Sapienza", P.le A. Moro 5 – Roma*
[‡] *Ist. di Neurochirurgia, Università Cattolica del Sacro Cuore, L.go Gemelli 8 – Roma*

Liposomes have been widely used in the diagnostic field as entrapping systems for delivering paramagnetic agents to enhance image contrast in Magnetic Resonance Imaging (MRI).

On the other hand, cationic liposomes have been proposed recently as delivery systems for gene therapy, due to their ability to bind DNA portions and to act as efficient transfecting agents. While the transfection efficiency can be tested *in vitro* on several cell lines, the targeting properties of the whole system (liposome and therapeutic agent) to reach the organ and to transfect it *in vivo*, is still an issue.

On these bases, we have undertaken a study to detect the diffusion and persistence of candidate vehicles for the *in situ* therapy of glioblastoma. The first step has concerned the study of a model delivery system, DOTAP (N-(1-[2,3-dioleoyloxy]propyl)-N,N,N-trimethylammonium): DOPE (dioleyl phosphatidylethanolamine) (1:1 ratio) liposomes including a Gd(III)-DTPA (diethylenetriaminepentaacetic acid) solution inside their cavity. The liposome formulation has been chosen because of its known transfecting efficiency, while the concentration of the Gd(III)-DTPA solution has been selected on the basis of the NMR longitudinal relaxation time of the liposome solution, measured by NMR relaxometry.

The delivery system has been infused in rat brains, either intact or after the induction of a lesion to simulate surgical ablation, and the animals have been examined by MRI imaging on a SMIS IVS NMR spectrometer operating at 4.7T using a fast gradient echo sequence, which contrast is dominated mainly by T_1 values. Images of 600 μm slices of the rat brain, with an in-plane resolution of 40 μm , were acquired in about 5 minutes. The MRI signal enhancement due to the contrast agent has been followed for up to 24 hours and compared with images obtained after the infusion of a Gd(III)-DTPA solution with a T_1 value similar to that of the liposome solution. The results in terms of diffusion and persistence of the contrast agent are discussed.

WATER MOBILITY PROPERTIES INSIDE ALGINATE HYDROGEL BEADS AS A FUNCTION OF THE CROSS-LINKING PROCEDURES BY NMR RELAXOMETRY AND IMAGING

Giorgio Capuani, Cesare Manetti, Lorena Casciani and Filippo Conti

Dipartimento di Chimica , Università "La Sapienza", P.le Aldo Moro 5, 00185 Roma

Biocompatibility, chemical and mechanical stability, permeability properties and the ability to gelify under non-traumatic cell conditions make some classes of hydrogels the elective biomaterials for the immobilization of living cells. In the last few years considerable effort has been addressed to the development of scaffolds for cell entrapment starting from alginates, which are biocompatible polysaccharides of natural origin made by guluronic and mannuronic acid units. Alginates can form physical networks where the three-dimensional structure is kept stable by "physical junction" zones created by portions of chains which are conformationally stiff because of the dimerization of segments which are cross-linked by polyvalent ions. The physical, mechanical and physico-chemical characteristics of the hydrogel matrix play a fundamental role in the definition of the hydrogel biocompatibility, and in turn these characteristics depend on the polymer structure and on the networking process patterns. The most relevant factor influencing the network formation is the nature of the cross-linking cation in terms of ionic radius and ionic charge, this latter determining the cross-linking mechanism. Another important factor is the bead dimension, which can have an effect on the bead homogeneity by limiting the diffusion of the cross-linking agent toward the bead core. This step is influenced by the gelation time as well.

NMR techniques like relaxometry and diffusivity measurements using low-resolution spectroscopy and spatially high-resolved diffusion-weighted imaging have been used to define the structural and physico-chemical properties of hydrogel matrices, that influence the mobility of water and metabolite molecules inside them, and by applying mathematical models to the experimental results starting from the hypothesis that water molecules can exist in different states inside the hydrogels, i.e. "bound" water, strongly associated with the hydrogel hydrophilic segments, and "bulk" water, which motion is reduced by the presence of the hydrogel meshes.

We have applied these NMR techniques to the study of alginate gel beads having different dimensions, obtained by three different cations (Ca^{2+}, Ba^{2+}, Al^{3+}) and three different gelation times (5, 15 and 30 min.). The analysis of the T_2 relaxation times showed that "bulk" water molecules interacts more strongly with the matrix following the order $Ba^{2+} < Ca^{2+} < Al^{3+}$. Shorter relaxation times were observed for the smaller beads, where the cross-linking process occurs more homogenously. This is confirmed by the diffusion maps calculated from the NMR diffusion-weighted images which allow to determine the diffusivity of water molecules in the different regions of the beads. Different compartments were detected in the two sets of bigger beads: an outer region where water molecules are characterized by slower mobility, and a inner region. The extent and the diffusivity properties of these regions are dependent on the cross-linking ion and the gelation time.

^{29}SI CPMAS NMR AND NEAR-IR STUDY OF THE SURFACE STRUCTURE OF MICROPOROUS SILICA

M. Casu, C. Cannas, M. Musinu and G. Piccaluga

Dipartimento di Scienze Chimiche, Università di Cagliari

High-resolution solid-state NMR and Near-IR techniques were used to investigate the surface structure of silica samples with different surface area and similar pore size. ^{29}Si CP-MAS experiments, including CP spin dynamics studies were employed to probe hydrogen bonding and local structural environments of various hydroxyl groups of silica surface. Local information were obtained by the analysis of the characteristic relaxation times $T_{1\rho}$ and T_{SiH}. Two silicon proton polarization times T^{f}_{SiH} and T^{s}_{SiH} were needed for a satisfactory fit of the variable contact time experiments, suggesting the presence of free silanols and hydrogen bonded single and geminal silanols. Near-IR results reveal the existence of a different ratio of hydrogen bonded silanols and isolated silanols on the surface of the silica as a function of surface area. These studies suggest that for high surface area silica samples both isolated and hydrogen bonded silanols are present, while for samples with a lower surface area an higher amount of silanols bonded to water molecules are present.

CHICKEN LIVER FATTY ACID BINDING PROTEIN: NMR INTERACTION STUDIES WITH PALMITIC AND CHENODEOXYCHOLIC ACIDS

Maddalena Catalano[1,2], Raffaella Ugolini[2], Laura Ragona[1], Marianna Luppi[2], Henriette Molinari[2]

[1]Istituto per lo Studio delle Macromolecole, CNR, Via Bassini 15, 20131, Milano, Italy, maddalena.catalano@ismac.cnr.it
[2]Dipartimento Scientifico e Tecnologico, Università di Verona,Ca' Vignal 1, 37134, Verona, Italy

Chicken liver fatty acid binding protein (Lb-FABP) belongs to the basic type fatty acid binding proteins, a novel group of proteins isolated from liver of different non mammalian species, and its structure has been recently obtained, through ^{1}H NMR studies, in our laboratory. We have suggested, on the basis of the high sequence and structural similarity with an orthologous protein, ileal lipid binding protein, that bile acids may be the putative ligands of Lb-FABP. In the present work structural studies have been extended to the recombinant ^{15}N enriched Lb-FABP, complexed with palmitic and deoxycholic acid, a primary bile acid in humans. The ^{1}H and ^{15}N resonance assignments for apo and holo Lb-FABPs have been determined by 2D homonuclear and 3D heteronuclear NMR spectroscopy. The conformational arrangement of the two ligands are analysed and compared in order to elucidate the residues involved in binding. The results are discussed in light of the structural results obtained for other apo and holo FABPs of known structure.

^{1}H NMR DETERMINATION OF PLANT STRESS RESPONSE COMPOUNDS

Roberto Consonni,[†] Teresa Recca[†], Monica Mattana[‡], Candida Vannini[#] Marcella Bracale[#] and Immacolata Coraggio[‡]

[†]*Istituto per lo Studio delle Macromolecole, ISMAC - CNR, Lab. NMR, Milano, Italy*
[‡]*Istituto di Biologia e Biotecnologia Agraria, IBBA-CNR, Milano Italy*
[#]*Dip. Biologia Strutturale e Funzionale - Università dell'Insubria, Varese, Italy*

The expression of several plant genes is regulated by biotic and abiotic stresses through the action of some transcription factors[4]. The *Osmyb4* is a rice gene coding for a transcription factor involved in cold acclimation. We have previously demonstrated[5], that Arabidopsis plants overexpressing *Osmyb4* showed a strong increase in cold and freezing tolerance measured as membrane or PSII stability and as a whole plant tolerance. The process of cold acclimation is acknowledged to be complex, involving a number of biochemical and physiological changes. It has been reported in many papers that in response to low temperatures plants synthesize and accumulate compatible solutes, such as soluble sugars, proline, organic acids, sugar alcohols and glycine-betaine[6,7]. In order to compare the acclimation response of Arabidopsis transformed and wild type plants, we followed the accumulation of proline, sugars and other compatible compounds in a time-course experiment at 4°C for 10 days. Moreover since the response to low temperature is similar to that obtained after a period of drought, as both disturb the intracellular water balance, we performed similar experiments on transformed and wild type Arabidopsis plants subjected to drought.
^{1}H-NMR spectra of wild type and transformed plants water extracts have been recorded. Our data show that *Osmyb4* expression in transgenic plants results in multiple biochemical changes during cold and drought stress. In particular NMR spectra showed changes in the accumulation of several classes of compatible solutes (amino acids, soluble sugars and aromatic compounds). By NMR analysis we observed that the transgenic Arabidopsis plants have a higher concentration of compatible solutes even before the acclimation at 4°C. These results suggest that Myb4 integrates the activation of multiple components of the stress response.

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BALSAMIC VINEGAR AND AGE MARKERS: ^{1}H NMR STUDIES

Roberto Consonni[#], Alberto Gatti[$]

[#]*Laboratorio di NMR, ISMAC, CNR Milano, Via Bassini 15, 20134 Milano, Italy*
[$] *Divisione Sensory & Costumer Analysis, NEOTRON S.p.A. Stradello Aggazzotti 104, 41010 S.Maria di Mugnano – Modena, Italy*

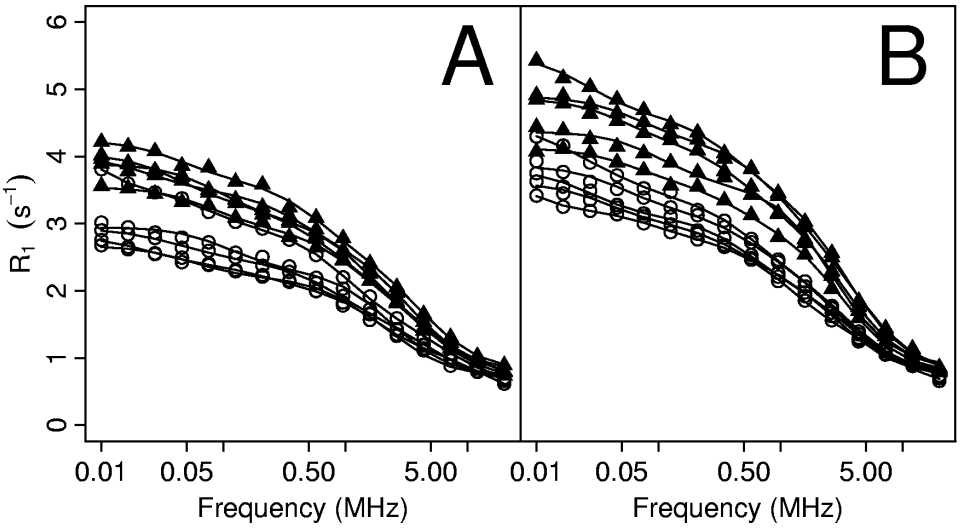
Vinegars are typically produced by microbiological fermentation of ethanol containing substrates, whose origin composition characterise different vinegar types. The Italian traditionally produced balsamic vinegars require specific wine matrixes and well defined production methodologies.
Due to the nature of the production, traditional balsamic, balsamic and commercial vinegars contain substances that can be use as age markers. Here we present the use of ^{1}H NMR studies combined with statistical approaches for the age determination.

FIELD CYCLING RELAXOMETRY OF HEN EGG ALBUMEN

Mauro A. Cremonini,[*] Luca Laghi,[*] Stanislav Sykora,[†] Gianni Ferrante,[‡] Achille Franchini [*] and Giuseppe Placucci [*]

[*]*Department of Food Science, University of Bologna, Italy*
[†]*Extra Byte, Via R.Sanzio 22C, 20022 Castano Primo, Italy*
[‡]*STELAR SRL, I-27035 Mede, Italy*

It is well known that hen egg albumen undergoes a liquefaction process after laying that it is usually referred to as "thinning". Despite the widespread usage of this phenomenon for inferring egg quality
(*i.e.* through the measure of the Haugh index), not many research groups have faced the challenging problem of explaining how is thinning related to the microscopic changes that take place in the albumen structure during ageing.
Some proposals have been put forward along the years. Thinning has been explained as the result of an enhanced interaction between lysozyme and ovomucine caused by the change in the albumen pH from about 7 to about 9 after oviposition. The effect of pH has also been considered as responsible for the chemical cleavage of a O-glicosidically linked trisaccaride to beta-ovomucin. Cleavage of this highly hydrophylic moiety would greatly reduce the interactions between ovomucin and water, thus causing the collapse of the albumen structure. In another explanation albumen thinning was again related to the pH raise through ovomucin depolimerization. Although the above proposals are all supported by experimental data, no general agreement seems to have been reached among the researchers about the very reasons that bring about the albumen liquefaction.
In this work we do not try to give any new explanation of the albumen thinning process. It is our aim to supplement the available literature with new experimental data which were obtained by field cycling relaxometry using albumen samples from oiled and not-oiled eggs. These data may provide a different point of view for the comprehension of this interesting natural phenomenon.



Field cycling relaxometry dispersion curves of albumen from oiled (A) and not oiled (B) eggs. Circles and triangles indicate fresh and aged eggs, respectively.

MOLECULAR CLUSTERING ON LIPID BILAYER

D. D'Emiliano, C. Casieri*, M. Paci** and F. De Luca

INFM and Dipartimento di Fisica, Universita' "La Sapienza", Roma
**INFM and Dipartimento di Fisica, Universdita' dell'Aquila, L'Aquila*
***INFM and Dipartimento di Biochimica e Biologia Molecolare, Universita' "Tor Vergata", Roma*

Nowadays one of the major unsolved topic regarding the study of cellular membranes is the role played by lateral organization of anphiphilic molecules. In the last few years numerous hints have suggested that some of the lipids (in particular glycolipids) that constitute the membrane bilayer experience a high degree of organization that enables them to take part in a great variety of biological process such as selective lipid transport, lateral sorting of membrane proteins and cell signalling. The studies of this phenomenon have led to the hypothesis of the existence in biological membranes of glycolipidis micro domains called lipid rafts. We used high resolution 1H-NMR spectroscopy to investigate the nature of these lipid rafts in liposome composed of mixtures of phospholipid (DOPC) and gangliosside (GM1: a glycolipid abundant in the grey matter of mammal). The choice of proton NMR instead of deuterium NMR, normally preferred for mobility and molecular organization studies, has been decided because the concentrations used for the experiment (GM1 varied from 0,06 to 0,57 mM) the 2H signal was comparable to that naturally present in the phospholipid matrix. All proton spectra were obtained at 400 MHz.
Our analyses showed that the presence of GM1 in the phospholipid bilayer induced a modification in the proton spectra of the DOPC whit the appearance of new resonances not directly imputable to GM1. We were able to establish a relation between these new resonances and the number of DOPC molecules that surrounded ganglioside ones supposing that GM1 affected DOPC locally, changing its physical and chemical environment and, consequently, the chemical shift of some resonances. Over a GM1 concentration of 11% [Mol] our results indicate the formation of gangliosides aggregates, in agreement with results present in literature. Our work provides the first NMR direct prove of the existence of such clusters in model membranes and the means for an estimation of their number and size. It represents a new starting point for further NMR analyses of the physical properties of lipid rafts. More over a broad-line analysis showed that GM1 clusters can effect the DOPC bilayer even in a more extended way, shielding the attractive forces existing between phospholipid polar head groups and making the DOPC bilayer a more mobile environment, in agreement with phase separation studies in model membranes.

A STUDY BY NMR SPECTROSCOPY AT LOW AND HIGH RESOLUTION OF HYDROGELS OF POLY-HEMA

Maurizio Delfini[a], Maria Enrica Di Cocco[a], Cristiano Bianchetti[a], Raffaella Gianferri[a], Cesare Manetti[a], Federica Chiellini[b], Cecilia Castro[a], Giorgio Capuani[a]

[a] *Dipartimento di Chimica, Università "La Sapienza", p.le A. Moro, 5 – 00185 Roma*
[b] *Dipartimento di Chimica & Chimica Industriale, Università di Pisa, Via Risorgimento, 35 – 56126 Pisa*

Among the biomedical materials polymeric hydrogels have the ability to swell in water or in aqueous solutions. At present the most investigated hydrogels are based on 2-hydroxyethyl-methacrylate (HEMA), due to their ascertained lack of toxicity and widespread use.
In the present study we describe by NMR spectroscopy the properties of hydrogels of HEMA with different percentages of ethylene glycol dimethacrylate (EGDMA) and tetraethylene glycol diacrylate (TEGDA) as crosslinking agents.
The high resolution ^{13}C NMR experiments were able to characterize the structure of hydrogels swollen in DMSO solutions and the mobility of the groups of the different copolymers.
The spin-spin relaxation times T_2 of hydrated poly-HEMA and corresponding copolymers were measured to probe the state of imbibed water in these polymers. The decay in the transverse magnetization of water was described by a multiexponential function, where the different components can be assigned to the different water kinds. The translational mobility of water was measured by PFG NMR, a technique offering the opportunity to study the possibly cohexisting different water mobilities in well defined equilibrium conditions.
Consequently, the different hydrogels properties were evaluated on the basis of the mobility of the polymeric chains and of the interacting water.

The present work was performed with the financial support of CNR Progetto Finalizzato "Materiali Speciali per Tecnologie Avanzate II".

HR-MAS CHARACTERIZATION OF ALOE VERA GEL

Anna Dinoi[†], Giuliana Ansanelli[†], Anatoli Sobolev[‡] and Raffaele Lamanna[†]

[†] *ENEA C.R. Trisaia, S.S. Jonica 106, Km 419,5, 75026 Rotondella (MT), Italy;*
[‡] *Institute of Chemical Methodologies of CNR, Rome's Research Area, m.b. 10, 00016 Monterotondo Staz., Rome, Italy.*

In last decades the interest for the therapeutic properties of the inner colourless gel of Aloe Vera leaves increased dramatically. Because of its reported wound healing, antiarthritic, anti-inflammatory, antidiabetic and antibiotic activities [1], Aloe Vera gel is a familiar ingredient in a range of nutritional drinks, healthcare and cosmetic products, widely available and advertised in shops.
Recent publications attribute many of these beneficial effects to acemannan, a storage mucopolysaccharide located in protoplasts, which is composed by β-1,4-linked mannosyl residues, with C2 and C3 acetylated and some side-chains formed by galactose units attached to C6.
The International Aloe Science Council has just adopted Nuclear Magnetic Resonance (NMR) and Size Exclusion Chromatography as official methods to determine the quality and the acemannan content in the Aloe Vera raw material and in commercial products. However, scientific literature about Aloe Vera gel composition is still poor and a little garbled.
In the present work a High Resolution Magic Angle Spinning (HR-MAS) study of Aloe Vera gel is reported. The HR-MAS technique was crucial to obtain well resolved spectra of such viscous samples.
The assignment of the spin systems was accomplished by 2D experiments (J-resolved, COSY, HETCOR, TOCSY) and literature data[2]. Diffusion filtered spectra were performed to confirm the assignment of acemannan signals.
The qualitative comparison between fresh and lyophilised Aloe Vera has been also carried out.

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NMR CHARACTERISATION AND SOLUTION BEHAVIOUR OF HYDRIDO-BRIDGED HETEROMETALLIC RHENIUM-COPPER AND RHENIUM-SILVER COMPLEXES.

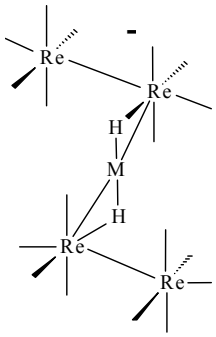
Tiziana Beringhelli, Giuseppe D'Alfonso, Daniela Donghi

Dipartimento di Chimica Inorganica, Metallorganica e Analitica; Via Venezian 21; 20133 Milano, Italy. E-mail: daniela.donghi@unimi.it

Transition metal hydride complexes react with electrophiles E according to two main reaction paths: the σ-donor capability of the metal-hydrogen bond [1] gives rise to L_nM-H-E adducts, that often are (observed or postulated) intermediates in the second reaction path, the hydrido-abstraction process. In the case of metallic Lewis acids, stable hydrido-bridged heterometallic complexes are usually obtained.

In these studies we have investigated the reactivity of "naked" Group 11 M^+ cations (using M^+ salts with poorly coordinating anions, AgOTf, or with labile ligands, $[Cu(NCMe)_4][BF_4]$) towards the rhenium hydride $[HRe_2(CO)_9]^-$ (**1**), used as NEt_4^+ salt [2].

Pentametallic $[(CO)_9Re_2(\mu\text{-}H)M(\mu\text{-}H)Re_2(CO)_9]^-$ complexes, M = Ag (**2**), Cu (**3**) (Scheme 1), were obtained, confirming the tendency of M^+ to be involved in M-H-M' 2e-3c interactions [3]. The two novel complexes were characterised by multinuclear and multidimensional NMR experiments.



Scheme 1

Variable temperature T_1 measurements showed that, while in the case of **2** the relaxation of the hydride is mainly due to the dipolar contribution of Re, in the case of **3** there's a significant dipolar contribution of Cu to the relaxation of the hydride, revealed by smaller T_1 values.

The dynamic behaviour in solution was also investigated. Variable temperature spectra of mixtures of **1** and **3** revealed the occurrence of exchange between the free "ligand" **1** and the "ligand" bound to copper in the adduct **3** (eq. 1). This exchange was confirmed by a [^{13}C-^{13}C] EXSY experiment, performed at 193 K on a ^{13}CO enriched sample of **3** in the presence of a little amount of **1**.

$$[(CO)_9Re_2(\mu\text{-}H)Cu(\mu\text{-}H)Re_2(CO)_9] + [H^*Re_2(CO)_9]^- <=> [(CO)_9Re_2(\mu\text{-}H^*)Cu(\mu\text{-}H)Re_2(CO)_9] + [HRe_2(CO)_9]^- \quad (1)$$

Such exchange occurs through a dissociative mechanism, as revealed by the linear variation of $\Delta\nu_{1/2}$ of the hydridic resonance of **3** on increasing the concentration of **1**. The k_2 constant for the exchange have been obtained at different temperatures, providing the activation parameters for the exchange. The large negative value of $\Delta S^{\#}$ and the small value of the activation energy indicated that the barrier to the process is mainly of entropic nature.

Preliminary investigation of the silver derivative suggested the occurence of the same kind of exchange.

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SOLUTION STRUCTURE OF HPV-16 E2 DNA-BINDING DOMAIN: CONFORMATIONAL AND DYNAMIC ANALYSIS

Tommaso Eliseo[1], Alejandro D. Nadra[2], Gonzalo de Prat-Gay[2], Maurizio Paci[3] and Daniel O. Cicero[3]

[1]*Dipartimento di Chimica,Università di Roma "La Sapienza"*
[2]*Instituto de Ciencias y Investigaciones Bioquimicas-Fundaciòn Leloir, Universidad de Buenos Aires*
[3]*Dipartimento di Scienze e Tecnologie Chimiche,Università di Roma "Tor Vergata"*

A fascinating area of investigation in the genomic era is the conversion of gene sequence information into 3D structural information of specific DNA elements and their cognate binding proteins. NMR techniques and methodologies can play an important role in unraveling the contributes of the structural code of DNA recognition. Papilloma viruses normally infect epithelial tissues in mammals and some strains are cancer-associated. The E2 protein is the unique transcription factor of papilloma virus genome and is an essential regulator of its replication. We have used a multidimensional, heteronuclear strategy to examine the 80 amino-acides DNA-binding domain of E2 from the "human" strain type 16, a dimer consisting of two β-barrel subunits. Three dimensional ^{15}N and ^{1}3C edited NOESY spectra and an HNHA spectrum were performed to extract a set of distance and dihedral angle constraints. Additional orientation restraints were icluded by measuring RDC displayed in a ^{1}H-coupled HSQC specrum by the protein in a poliacrilamide gel matrix. Simulated annealing protocol drove the calculation of monomeric subunits and their assemblies to a final ensemble of low-energy dimeric structures. The dynamic properties of HPV16 E2 were accurately investigated mapping the ^{15}N R_1 and R_2 relaxtion rates and the H-N heteronuclear NOE at two distinct frequencies (400 and 700 MHz). Water-selected NOE and ROE experiments were carried out to identify and quantify the exchange with solvent of individual segments of the protein. In conclusion, HPV16 E2 solution structure shows a common tertiary fold with the other members of the E2 family and a slight but significant differentiation in the relative orientation of the subunits. E2 behaves like a compact but dynamic protein in which local and global fluctuations transiently expose the backbone to exchange with water. Structural and dynamics analysis suggests a correlation between E2 subunit structural arrangements in distinct strains and the affinities toward their intrinsically prebent or flexible DNA targets.

^{1}H-NMR RELAXOMETRIC ANALYSIS OF DRUG BINDING EFFECT TO GLOBAL UNFOLDING OF HUMAN SERUM ALBUMIN

Gabriella Fanali[†], Paolo Ascenzi[‡], and Mauro Fasano[†]

[†] *Department of Structural and Functional Biology, University of Insubria, via Alberto da Giussano 12, I-21052 Busto Arsizio (Varese), Italy;*
[‡] *Department of Biology, University 'Roma Tre', Viale Guglielmo Marconi 446, I-00146 Rome, Italy.*

Human serum albumin (HSA), the most prominent protein in plasma, is best known for its exceptional ligand binding capacity. For many compounds, HSA provides a depot so they will be available in quantities well beyond their solubility in plasma. Moreover, HSA abundance (its concentration being 45 mg/mL, in the serum of human adults) makes it an important determinant of the pharmacokinetic behavior of many drugs by binding at two main drug-binding regions. Among hydrophobic molecules, heme binding to HSA is of peculiar relevance for the heme iron reuptake following hemolytic events. Heme binding to HSA endows the protein with peculiar spectroscopic properties [(1)]. Electronic absorption and relaxometric data indicate the occurrence of a high-spin Fe(III) center with no water in the inner coordination sphere, and the occurrence of a strong contribution from a cluster of water molecules buried nearby.
This second-sphere contribution may be employed as a structure-dependent spectroscopic observable to follow a number of events involving the conformation of the protein. Among them, the paramagnetic effect of the buried water cluster has been used to follow chaotropic unfolding of heme-HSA, allowing the determination of the thermodynamic parameters of protein stability in the absence and in the presence of interacting drugs. Chaotropic unfolding of the heme-HSA complex has been obtained by addition of either urea or guanidinium chloride, in the presence or in the absence of stereotypical ligands of the two drug-binding sites, as a function of temperature. Measurements have been performed at three different pH values, i.e., in three different conformational states of the protein [(2)].
Results obtained here show a different stabilization mechanism depending on the conformational state of the protein and on the occupancy of either binding sites. Moreover, NMR relaxometry appears to be a valuable technique to measure thermodynamic parameters for the global unfolding mechanism of paramagnetic proteins.

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RELAXOMETRIC CHARACTERIZATION OF BOVINE LACTOFERRIN

Mauro Fasano[†], Gabriella Fanali[†], Fabio Polticelli[‡], Paolo Ascenzi[‡], and Giovanni Antonini[‡]

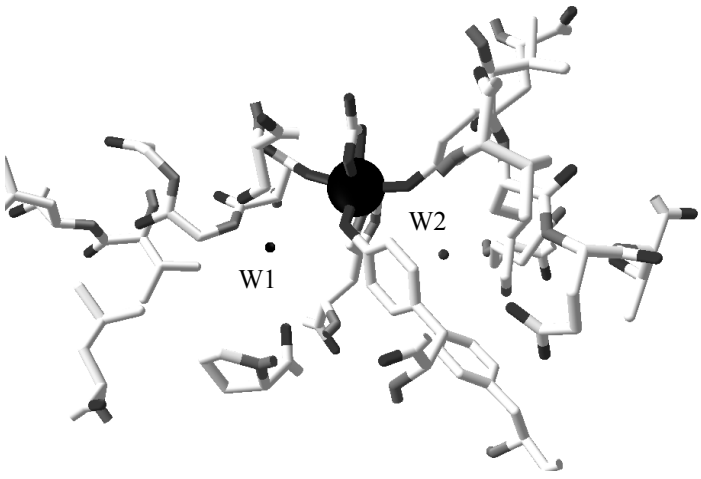
[†] *Department of Structural and Functional Biology, University of Insubria, via Alberto da Giussano 12, I-21052 Busto Arsizio (Varese), Italy*
[‡] *Department of Biology, University 'Roma Tre', Viale Guglielmo Marconi 446, I-00146 Rome, Italy*

Lactoferrin is a mammalian iron binding protein present in external secretions, such as breast milk, tears, saliva, and vaginal secretions, and in polymorphonuclear leukocytes. Its role in host defense mechanisms related to the non-immune defense system against pathogenic bacteria, fungi, and protozoa, both directly and through regulation of the inflammatory response, has been definitively established. Lactoferrin has two identical iron-binding sites, far from each other and magnetically non-interacting. Fe(III) ions are six-coordinated, with four donor atoms provided by protein sidechains (two Tyr, one His, one Asp) and two oxygen atoms from a bridged HCO_3^-. This set of ligands provides an ideal coordination scheme for stable and reversible iron binding.

It is expected that the assessment of the paramagnetic contribution to the NMR relaxation rate of solvent water protons can provide useful insights for a better characterization of the molecular environment of the Fe(III) binding site and of its dynamics.

Like transferrin, NMRD profiles of lactoferrin are characterized by a rather complex functional form. This is in part due to the rather anisotropic spin Hamiltonian of the Fe(III) ions in the coordination scheme of lactoferrin that makes it impossible to obtain a quantitative analysis of the profiles in terms of a feasible physical model. Moreover, temperature dependence data suggest that an important contribution to the overall paramagnetic contribution to the solvent water relaxation rate arises from one or more water molecules in slow exchange with the bulk. An invariant τ_c value of 1.28±0.05 ns is obtained, whereas the temperature dependence of the profiles can be accounted for by a decreasing value of the exchange rate, ranging from 1.2 to 0.7 μs in the observed temperature range. This is clearly indicating that a slow-exchange process is taking place, with an activation enthalpy of 7.3±0.8 kJ mol^{-1}.

Relaxivity values are consistent with a water molecule either with hydrogen atoms at an average distance of 3.3 Å or with a single hydrogen atom at 3.1 Å. By looking at the X-ray structure of Lf (PDB ID code: 1BLF) we can locate a water molecule at 3.95 Å from each Fe(III), labeled W1. This water molecule is bound to several polar groups of the protein backbone and side chains, therefore it is reasonable that its exchange rate suffers of the many hydrogen bonds it is establishing. Moreover, a second water molecule (W2) is present at 4.27 Å from the paramagnetic center.



BIOACTIVE N-TERMINAL UNDECAPEPTIDES DERIVED FROM PARATHYROID HORMONE. THE ROLE OF α-HELICITY

Alessandra Barazza[†], Nereo Fiori[‡], Elisabetta Schievano[‡], Stefano Mammi[‡], Evaristo Peggion[‡], Joseph M. Alexander[†], Michael Rosenblatt[†] and Michael Chorev[†]

†Division of Bone & Mineral Research, Beth Israel Deaconess Medical Center & Harvard Medical School, 330 Brookline Ave., Boston, MA 02215, USA
‡Department of Organic Chemistry, University of Padova, Institute of Biomolecular Chemistry, CNR, ,Via Marzolo 1, 35131 Padova, Italy

The amino-terminal portion of PTH is critical for PTH-1 receptor (P1R) activation. Previously, enhancement of α-helicity in the PTH(1-14) and PTH(1-11) sequences yielded potent analogues of PTH(1-14)NH_2. Here, we present our efforts to stabilize the structure and to increase the helical content of the short *h*PTH(1−11) sequence.

We synthesised and characterized the following *h*PTH(1−11) analogues substituted in positions 1 and 3 by the tetra-substituted amino acid residues Aib, 1-aminocyclopentane-1-carboxylic acid (Ac_5c) and 1-aminocyclohexane-1-carboxylic acid (Ac_6c):

(**I**) [Ac_5c^1,Aib^3, Gln^{10}, Arg^{11}]-PTH(1-11)NH_2,;
(**II**) [Aib^1, Ac_5c^3, Gln^{10}, Arg^{11}]-PTH(1-11)NH_2;
(**III**) [Ac_6c^1, Aib^3,Gln^{10},Arg^{11}]-PTH(1-11)NH_2;
(**IV**) [Aib^1, Ac_6c^3, Gln^{10},Arg^{11}]-PTH(1-11)NH_2;
(**V**) [$Aib^{1,3}$, Gln^{10}, Arg^{11}]-PTH(1-11)NH_2.

The results of biological characterization, including efficacy in stimulating accumulation of cAMP indicated that analogues **I** and **II** are active (10^{-7}-10^{-8} M) while analogues **III-V** are inactive ($\geq 10^{-3}$ M).

The CD spectra of the five analogues in aqueous solution containing 20% TFE (v/v) show a clear correspondence between biological activity and helix content. Only the two bioactive peptides **I** and **II** exhibit the typical CD pattern of the α-helical conformation.

NMR experiments identify the amino acid residues comprising the helical sequence. In analogues **I** and **II,** the chemical shifts differences of αCH protons with respect to the corresponding random coil values identify a helical segment spanning the sequence Val^2-Met^9. In the ROESY spectra, a number of connectivities typical of the α-helix were observed in the segment 2-10 of analogues **I** and **II**. In the inactive analogues **III-V** the tendency of the Ile^5-Met^8 segment to fold into the helical structure is much weaker. Superimposition of the ensembles of the low energy structures resulting from distance geometry and molecular dynamics calculations clearly indicated a much better convergence towards the helical structure of the active analogues compared to the inactive ones.

Taken together, these results stress the importance of the presence of a helical segment at the N-terminus of PTH (1-34) analogues at the effect of biological activity.

DESIGN AND OPTIMISATION OF AN AIR-CORE 1T FIELD CYCLING NMR MAGNET IN THE NOACK–SCHWEIKERT CONFIGURATION

G.M. Ferrante, G. Borissov and L. Dallocchio

Stelar s.r.l. via E.Fermi,4 – Mede (PV)- Italy,
info@stelar.it

The Noack–Schweikert's configuration for Field Cycling NMR magnets makes use of concentric solenoids. Each solenoid is made by cutting metallic tubes in order to obtain the designed number and distribution of windings. The distribution of windings is calculated and made non-uniform on the purpose to maximize the magnetic field and the homogeneity in a defined volume around the centre of the solenoid for a given current. This designing approach offers many well-known advantages compared with other different methods but the practical realization of the real magnet is limited by many technological difficulties and critical manufacturing processes. The present work was done with the objective to develop and manufacture a prototype of 1 Tesla air core FC magnet optimized for the highest Field/Power ratio (the Fabry factor) allowed by the currently available technology.
All electrical and geometrical calculation parameters have been optimized. New materials and new manufacturing process have been introduced. Final results and magnet specifications are presented and discussed.

Work supported by the Commission of the European Communities

MEASURING FAST RELAXING SAMPLES ON CURRENT FFC - NMRD RELAXOMETERS DISCUSSION OF EXPERIMENTAL DIFFICULTIES AND LIMITS

S.Sykora, G.M.Ferrante, A.Galkin

Stelar s.r.l. Via E.Fermi, Mede (PV), Italy
info@stelar.it

Breaking the "barrier" of relaxation rate $R_1 > 1000\ s^{-1}$ and doing so in a reliable and reproducible way is an experimental feat which would have been impossible to dream about even just three years ago. Yet this poster illustrates that the latest FFC instruments make it an affordable reality which lies just slightly beyond the realm of everyday routine. It also shows that with field switching times of 1 ms (corresponding to current state-of-art) one can in certain cases reliably measure relaxation rates R_1 up to 10000 provided that the switching waveforms are linear and perfectly reproducible.

Two samples had been used for this preliminary study of the phenomena occuring when switching times become comparable to, or longer than, relaxation times. Sample I was a 2.1M water solution of dysprosium perchlorate (kindly provided by Dr.L.Helm). Sample II was the commercial Parafilm M foil (American National Can Co.) whose composition is a secret but which appears to consist (NASA/MSFC Materials and Processes Home Page) of a wax (~56%) and a polyolefin (~44%).

As expected, sample I was found to have a flat ^{1}H-NMRD profile over the whole explored field range from 10kHz to 20 MHz (^{1}H Larmor frequency). Its mean relaxation rate at 25 ^{0}C has been found to be $R_1 = 1425\ s^{-1}$ with an expected error of 1.4%. Even using 1ms switching time and slewing rates of 24 MHz/s, its profile has not been easy to measure, despite the fact that the required polarization time is very short, permitting a large number of scans to be taken. This is due to the substantial signal decay during switching period which can not be quite compensated by the number of acquired scans. We feel that the measurement of a reliable NMRD profile of a sample of this kind still requires a cautious and experienced operator and probably is so far beyond the realm of a routine, automated tasks.

Sample II behaves in a quite different way. Its proton R_1 at 25 ^{o}C varies steeply from 39.9 s^{-1} at 18 MHz to over 6400 s-1 at 10 kHz. Despite the fact that its maximum R_1 values are about four times higher than for sample I, it can be easily measured even by a relatively inexperienced operator using the automated profile-acquisition procedure. The only required precaution is setting the switching time value to 1ms, the polarization time to a value adequate for whatever is the chosen polarization field and the field slew rate to the maximum allowed value. The reason for the ease of this measurement is of course the fact that during the switching, the sample is never exposed to fields at which it relaxes really fast for longer than a few tens of microseconds. However, this very fact makes it also evident that those few tens of microseconds must be always the same, implying that the precision and the reproducibility of the field-switching waveforms are both absolutely essential in order to guarantee correct results for this kind of samples.

1H-NMRD PROFILES OF POLYMERS - A PRELIMINARY EXPERIMENTAL REVIEW

S.Sykora*, A.Segre[§] and D.Capitani[§]

**Stelar s.r.l , Via E.Fermi, Mede (PV), Italy- info@stelar.it*
[§]CNR, Area della Ricerca, Monterotondo, Roma-Italy

We have undertaken a systematic exploration of proton dispersion profiles of several families of synthetic polymers. The NMRD profiles of solid samples had been measured at 25 ^{o}C over relaxation fields ranging from 5 kHz to 20 MHz (measured by ^{1}H Larmor frequency). So far, we have explored several classes of elastomers, styrenes, nylons and a polycarbonate.

The study, though still in a preliminary and rather phenomenological phase, allows us already to draw several experimental conclusions of considerable interest in view of the expected rapid growth of NMRD studies of molecular dynamics of bulk polymers.

In many individual profiles, the R_1 values range over more than three orders of magnitude. In virtually all cases, the variation continues down to the lowest measured frequencies, following curves which deviate sharply from the simple BPP model and which rarely exhibit any plateau, be it at high or at low frequencies (only in a few cases there seems to be a plateau between 5 and 10 kHz). This implies a wide spectrum of correlation times and dynamic models much more complex than what might appear from relaxation studies carried out at high (and fixed) frequencies.

Though there are marked differences between individual profiles, the review as a whole exhibits a surprising internal coherence, making it possible to group the polymers into families according to the shapes of the profiles (it is comforting that such classification correlates well with the known chemical composition of the samples).

It is beyond any doubt, however, that the internal coherence of the review would be completely lost if it were not possible to measure reliable R_1 values in the range from 100 to almost 10000 s^{-1}. Indeed, while most of the studied polymers exhibit quite low R_1 values at 20 MHz (in some cases close to 1, often around 10 and always below 100), the situation changes dramatically at fields around 1MHz and lower. Without the R_1 values lying in the two decades above 100 s^{-1}, FFC relaxometry of bulk polymers would be seriously handicapped.

MAGNETIC FIELD COMPENSATION FOR LOW-FIELD RELAXOMETRY*

* E. Anoardo[1], M. Polello[2,3] and G. M. Ferrante[2]

[1]*Facultad de Matemática, Astronomía y Física, Universidad Nacional de Córdoba. Córdoba – Argentina.*
[2]*Stelar s.r.l. Mede – Italia.*
[3]*Bijvoet Center for Biomolecular Research*
Padualaan 8, NL-3584CH Utrecht, The Netherlands

We propose an experimental routine to quantify and compensate external magnetic fields from the environment at the sample position, using the available hardware of the Spinmaster FFC2000 fast field cycling relaxometer, with the inclusion of a special coil-set and its power supply. A compensation of these contributions has successfully been used for the application of field-cycling methods to nuclear magnetic relaxation and double resonance experiments. The feature becomes relevant in samples where local fields are strongly averaged due to motional narrowing, where relaxation experiments can therefore be extended to lower fields. Compensation of external contributions is also crucial for the study of internal processes attributed to the local fields.

The extension of the field-cycling technique to the *ultra low frequency band* (ULF) strictly depends on the quality of the magnetic field compensation. The ULF band is the Larmor frequency range where the effective Larmor frequency sensed by the spin-system has contributions from the magnet, local-internal and external fields. In contrast, we refer to the *low frequency band* (LF) to the kilohertz region but where the Larmor frequency of the spins is well defined by the magnet's field within the limits of its resolution. In this poster we present field-cycling experiments aimed to detect and quantify the contribution from external fields. In addition, we will present details of the calculation of the compensation coil-set.

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OVEREXPRESSION AND PURIFICATION OF THE NS3 PROTEINASE DOMAIN OF DENGUE VIRUS FOR STRUCTURE DETERMINATION BY NMR. DESIGN OF OPTIMIZED SUBSTRATES AND MECHANISM-BASED INHIBITORS

Silvana Fucito[1], Federico Wrubl[2], Andrea Gamarnik[1], Alessandro Padova[2], Maurizio Paci[3,4], Daniel O. Cicero[3] and Sonia Melino[3]

1IIB-Fundación Instituto Leloir, FCEyN, UBA, Argentina
2C4T, Combinatorial Chemistry Center, University di Roma "Tor Vergata" , Italy
3Università di Roma "Tor Vergata", Italy
4Istituto Nazionale di Fisica della Materia, Università di Roma "Tor Vergata", Italy

Dengue virus causes widespread human diseases such as dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. Although there are one million cases of dengue hemorrhagic fever per year, there is currently no effective vaccine or antiviral drug to protect against dengue diseases. The objective of the present study is the detailed biochemical and structural characterization by Nuclear Magnetic Resonance of NS3 protease of dengue virus and its complexes, an enzyme which plays a crucial role in viral maturation. The solution structure of the enzyme will be used to answer yet unresolved questions about the mechanism of action, the role of its cofactor NS2B, and the observed substrate specificity. To this purpose we have overexpressed the NS3 proteinase domain [1-185] in E. coli on minimal medium and are currently optimizing the purification procedure. It is intended to obtain the structure in solution of uniformly 13C,15N-labeled NS3[1-180] by high-field 3D NMR spectroscopy. In parallel, chemically optimized substrates are being designed and tested to afford an efficient in vitro activity assay. Mechanism-based inhibitors are also being designed and will be used to characterize their binding to NS3 through NMR studies.

RADIATION EFFECTS INDUCED BY PROTON BEAMS AND GAMMA RAYS ON BREAST CANCER CELLS EXAMINED BY ^{1}H MRS.

A. M. Luciani[1], L.Guidoni[1], S.Grande[1], V.Viti[1], A.Rosi[1], P.A. Lojacono[2], V.I. Patti[2], L.M. Valastro[2], R. Cherubini[3], S. Gerardi[3]

[1]*Laboratorio di Fisica, Istituto Superiore di Sanità and INFN, Roma, Italia*
[2]*INFN Laboratori nazionali del Sud, Catania, Italia*
[3]*INFN, Laboratori nazionali di Legnaro, Legnaro (Padova), Italia*

Proton beams are believed to be more efficient than gamma rays in inducing cell death and many studies are in progress to exploit proton efficiency to provide new therapeutic modalities for some class of tumors.
The effects of gamma and proton irradiation on lipid and metabolite signals from MCF-7 cells (breast carcinoma) have been examined by means of high resolution ^{1}H MRS after different time intervals. Cells were irradiated with a gamma cell (^{60}Co) and with proton beams of different energy. The experimental setup included measures at different Linear Energy Transfer (LET). Spectral modifications induced by either proton or gamma irradiation were compared. Intensities of relevant peaks were quantified after spectral deconvolution, both in lipid and metabolic cell extracts.
The most relevant effect observed was the increase of the lipid related metabolites Glycerophosphorylcholine (GPC) and Choline (Cho). The radiation quality affects the entity of the effects in a very sensitive way. Also triglycerides concentration, measured in the lipidic samples, was affected by irradiation. Proton beams produced similar effects on the selected molecules at half dose with respect to gamma rays, pointing to a relative biological effect of two.

^{1}H MRS SIGNALS FROM IRRADIATED AND CAFFEINE TREATED TUMOR CELLS CAN MONITOR DIRECT CELL DAMAGE AS WELL AS APOPTOSIS

Alessandra Palma, Sveva Grande*, Laura Guidoni, Anna Maria Luciani, Antonella Rosi, Vincenza Viti

Laboratorio di Fisica, Istituto Superiore di Sanità and INFN, Roma, Italia

MRS study of the metabolic events following irradiation can be of great help in elucidating different mechanisms of tumor cell death. In particular, apoptosis, a strategy of tumor control often induced in tumor cells by chemotherapeutic or hormonal agents, is accompanied by a marked increase of ML signals and by elevated polyunsaturation *in vivo*.
In the present study, we examine the behavior of cultured tumor cells, namely HeLa and MCF-7, at selected time intervals after gamma irradiation. Irradiation of MCF-7 cells induces cell death by apoptosis, while irradiated HeLa cells die predominantly by mitotic death. Otherwise, the treatment with caffeine could induce HeLa cells to undergo apoptosis.
We studied the modifications of mobile lipids (ML) signals 48 hours after irradiation. We observed increase of ML signals in both 1D and 2D COSY 1H MRS spectra of MCF-7 cells, while the same signals decreased in HeLa samples. This decrease is not observed in caffeine treated HeLa cells. Spectral modifications were also observed in metabolites and total lipids extracted from irradiated cells. Modulation in TG concentration and in GPC/PCho ratios were observed paralleling cell behavior.

STRUCTURAL EFFECT OF Cd^{2+}/Zn^{2+} SUBSTITUTION IN A CYS2HIS2 ZINC FINGER DOMAIN

M. Leone[†], G. Malgieri[‡], L. Raiola[‡], L. Zaccaro[†], P.V. Pedone[‡], R. Scandurra[±], C. Pedone[†], B. Di Blasio[‡], R. Fattorusso[‡], C. Isernia[‡]

[†]*Istituto di Biostrutture e Bioimmagini, via Mezzocannone 6, 80134 Napoli, Italy*
[‡]*Dipartimento di Scienze Ambientali, via Vivaldi 43, 81100 Caserta, Italy*
[±]*Dipartimento di Scienze Biochimiche, Ple A. Moro 5, 00185 Roma, Italy*

In the last few years, the role of zinc ions as structural components in proteins grew in appreciation since an increasing number of domains have been found too small to fold by themselves but stably folded when containing a bound zinc. This structural role is well defined in transcription factors that contain zinc finger domains, the largest group of eukaryotic DNA binding proteins know to date. Classical zinc finger domains (also named Cys2-His2) present the amino acid consensus sequence (Phe, Tyr)-X-Cys-X_{2-5}-Cys-X_3-(Phe, Tyr)-X_5-ψ-X_2-His-X_{3-5}-His where X represents any amino acid; the two cysteines and the two histidines coordinate a zinc atom to form a compact structure. NMR studies of single and multiple zinc fingers evidenced that domains fold as isolated units with each finger containing a β-sheet and an α-helix clustered around a compact hydrophobic core. At the center of the fold there is always a single zinc ion chelated in a tetrahedral geometry. Zinc stabilized domain binds to DNA with the α-helix oriented into the major groove, often acting as transcription factor.

Recently, we focused our attention upon the SUPERMAN protein of *Arabidopsis thaliana* that comprises a single QALGGH zinc finger domain and was found to play a role in mantaining the *Arabidopsis* floral whorl boundaries. We reported that a 64 residue fragment of SUPERMAN, containing the single zinc finger domain and two extra N- and C- terminal basic regions, is able to specifically bind a 20 bp DNA sequence and that a 37 amino acids fragment preserves the characteristic folding of these domains.

Metal binding site of these domains was proposed as a possible target for metal substitution with xenobiotic ions that are believed to have etiological roles in carcinogenesis and other disease processes. Little is know about metal-, DNA-binding and folding properties in case of metal replacement. In the present study, the effect of Cd^{2+}/Zn^{2+} exchange on the solution conformation of the 37 amino acids classical zinc finger from SUPERMAN protein is examined in order to clarify the structural effect of metal substitution in this domain.

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DIFFUSION-ORDERED NMR SPECTROSCOPY: A VERSATILE TOOL FOR THE MOLECULAR WEIGHT DETERMINATION OF UNCHARGED POLYSACCHARIDES.

Luisa Mannina[†,‡], Stéphane Viel[†], Donatella Capitani[†]and Annalaura Segre[†]

[†] *Istituto di Metodologie Chimiche, CNR, 00016 Monterotondo Stazione, Italy*
[‡] *Facoltà di Agraria, Università del Molise, Dipartimento S.T.A.A.M, 86100 Campobasso, Italy*

Diffusion-Ordered NMR Spectroscopy (DOSY) experiments have been carried out on dilute aqueous solutions of uncharged saccharidic systems and, in particular, on six well characterized pullulan fractions of different molecular weights. The value of diffusion coefficients and hydrodynamic radii determined for the pullulan fractions is in good agreement with the results obtained with other methodologies such as light scattering. Fitting the diffusion coefficients data as a function of the molecular weight allows the determination of a calibration curve that can be applied to a wide range of mono, oligo and polysaccharides. Therefore DOSY is proposed as a versatile tool for achieving a simple estimation of the molecular weight of uncharged polysaccharides. Mixtures of homopolymers of different molecular weight can be nicely separated. An advantage of the method is that the same sample used for the NMR characterization can be used for the molecular weight determination without any further manipulation. Other water soluble polymers, such as polyethylene oxide and polyvinylpyrrolidone, can be roughly characterized using the same calibration curve.

^{1}H-NMR INVESTIGATION OF CHAIN ORDERING EFFECTS IN POLYMER CRYSTALLIZATION

A. Maus[1]*, K. Saalwächter[1], Jenny Naim[2], M. Gottlieb[2], R. Yerushalmi-Rozen[2]

1*Institute for Macromolecular Chemistry, University of Freiburg, Stefan Meier Str. 31, D-79104 Freiburg, Germany*
[2]*Department of Chemical Engineering, Ben-Gurion-University of the Negev, Beer-Sheva, 84105, Israel*

Polymer crystallization is special in that the chains cannot form a crystal lattice in its classical meaning, because typical features of polymers like (a) polydispersity, (b) the presence of free end-groups, net-points and loops, (c) short side chain branches etc. can be considered as geometric hindrances for the crystallization process. In semicrystalline polymers these geometric barriers accumulate in the amorphous region. Though investigated for over 60 years, the fundamental mechanism of polymer crystallization is not yet well understood and controversly discussed. Classical models suggest that crystallization proceeds via a two step mechanism of nucleation and growth [1]. Recently it was suggested that crystallization from the melt may proceed via the evolution of correlated density and structural fluctuation that develops into a preordered granular crystalline mesophase [2]. In both of these mechanisms as well as in the primary nucleation step, the loss of configurational entropy determines the rate of the process.

In our study, we examine the effect of chemically and physically crosslinked poly(dimethylsiloxane) PDMS. Chemical crosslinking is realized by the formation of networks while physical crosslinks are introduced via entanglements which are present in linear polymer melts with a molecular weight above the entanglement limit M_e. It was shown in DSC measurements that both types of crosslinking enhance the tendency of PDMS samples to crystallize [3]. This is an atypical behavior with respect to the growth rate described in [1] and the geometric considerations mentioned above. We interpret the influence of crosslinking in a sense that they increase the extent of chain order and this, in turn, lowers an entropic barrier which results in an enhancement of the crystallization rate. The lowering of the entropy barrier might be of importance for both primary nucleation and growth.

We present ^{1}H-NMR experiments in order to approve and quantify the results from the DSC measurements. The extent of local chain ordering was quantified by static ^{1}H double quantum experiments [4]. The isothermal crystallization process was observed via analysis of the transverse magnetization relaxation function [5] measured applying a modified CPMG pulse sequence.

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AN INTEGRATED METABOLIC INVESTIGATION OF FATTY ACID OXIDATION AND RELATED GLUCOSE PRODUCTION IN RAT LIVERS USING NMR SPECTROSCOPY.

A. Miccheli, A. Tomassini, F. Pancotti, C. Bianchetti, A. Giuliani* and F. Conti

Department of Chemistry, "La Sapienza" University of Rome, P.le A. Moro 5, Rome, Italy
** Istituto Superiore di Sanità, Viale Regina Elena 299, Rome, Italy*

Free fatty acids (FFAs) have been suggested to play an important role in the regulation of glucose production through the regulation of key-gluconeogenic enzymes and allosteric stimulation of PC by acetylCoA. However, the effects of FFAs on hepatic glucose production, gluconeogenesis, and glycogenolysis in the fasted state are still unclear, particularly for "in vivo" studies, where, high FFA levels may modify the hormonal secretion.
In order to study the role of intrahepatic mechanisms involving the fatty acid oxidation-induced glucose production, we have used isolated livers from fasted rats, perfused with a plasma-like solution containing fixed fatty acid and insuline concentrations, in presence or absence of a competitive inhibitor of Carnitine Palmytoil-Transferase-I (CPT-I).
The metabolite level changes were evaluated in the perfusion fluid by using ^{1}H NMR spectroscopy and applying Principal Component Analysis (PCA) on the variation of the concentrations of metabolites measured at an interval between 2 and 60 minutes (net balance). PCA is a method which allow to evaluate the influence of mitochondrial fatty acid oxidation on hepatic glucose production and gluconeogenesis from lactate in an integrated fashion, i.e.: considering the relations with each involved metabolic pathway. Three Principal Components explained more than 83% of the variability in both control and CPT-I inhibited group. The analysis of factor loadings allowed to describe the system in terms of the relations among the specific metabolic processes, like ketogenesis, aminoacid utilization, gluconeogenesis and acetate release. The quantification of the changes induced by CPT-I inhibition was determined by the factor scores.
^{13}C isotopomeric distribution into liver metabolites was evaluated by ^{13}C NMR spectroscopy supplying [3-^{13}C] lactate to study the effect of mitochondrial fatty acid oxidation on the gluconeogenesis from lactate. The results obtained on ^{13}C enrichments of glucose and glutamate are in agreement with PCA ones, and show a decrease of gluconeogenesis from lactate and of the Pyruvate Carboxylase/Pyruvate Dehydrogenase ratio depending on CPT-I inhibition.

[1-^{13}C]GLUCOSE ENTRY IN NEURONAL AND ASTROCYTIC INTERMEDIARY METABOLISM OF AGED RATS. A STUDY OF THE EFFECTS OF 1,6-DIMETHYL-8β-(5-BROMONICOTINOYLOXYMETHYL)-10α-METHOXYERGOLINE (NICERGOLINE) TREATMENT BY ^{13}C NMR SPECTROSCOPY

Alfredo Miccheli, Caterina Puccetti, Giorgio Capuani, Maria Enrica Di Cocco, and Filippo Conti

Department of Chemistry, "La Sapienza" University of Rome, P.le A. Moro 5, Rome, Italy

Age-related changes in glucose utilization through the TCA cycle were studied using [1-^{13}C]glucose and ^{13}C, ^{1}H NMR spectroscopy on rat brain extracts. Significant increases in lactate levels, as well as in creatine/phosphocreatine ratios (Cr/PCr), and a decrease in N-Acetyl-aspartate (NAA) and aspartate levels were observed in aged rat brains as compared to adult ones following glucose administration. The total amount of ^{13}C from [1-^{13}C]glucose incorporated in glutamate, glutamine, aspartate and GABA was significantly decreased in control aged rat brains as compared to adult brains. The results showed a decrease in oxidative glucose utilization of control aged rat brains. The long-term Nicergoline treatment increased NAA and glutamate levels, and decreased the lactate levels as well as the Cr/PCr ratios in aged rat brains as compared to adult rats. The total amount of ^{13}C incorporated in glutamate, glutamine, aspartate, NAA and GABA was increased by Nicergoline treatment, showing an improvement in oxidative glucose metabolism in aged brains. A significant increase in pyruvate carboxylase/pyruvate dehydrogenase activity (PC/PDH) in the synthesis of glutamate in Nicergoline treated aged rats is consistent with a increase of the transport of glutamine from glia to neurons for conversion into glutamate. In adult rat brains, no effect of Nicergoline on glutamate PC/PDH activity was observed, although an increase in PC/PDH activity in glutamine was, suggesting that Nicergoline affects the glutamate/glutamine cycle between neurons and glia in different ways depending on the age of animals. These results provide new insights into the effects of Nicergoline on CNS.

INFERENCES ON CR(V) OR CR(IV) SPECIES FORMED BY REDUCTION OF DICHROMATE BY BOVINE LIVER EXTRACT: UV-VIS, EPR, NMR AND MASS-SPECTROMETRIC STUDIES

Nicola D'Amelio, Elena Gaggelli, Nicola Gaggelli, Elena Molteni, Gianni Valensin,
Department of Chemistry, University of Siena, Via A.Moro, Siena 53100, Italy

Lucia Bovalini, Alessandro Paffetti, Lorenza Trabalzini
Department of Molecular Biology, University of Siena, Via Fiorentina, Siena 53100, Italy

The material resulting from dichromate reduction within the bovine liver homogenate was investigated by NMR and ES-MS. ES-MS spectrum of the chromium-containing material was showing a readily detectable peak at m/z = 1572.1 revealing the presence of some high molecular weight compound. DOSY experiment confirmed that the main species observed in ^{1}H NMR spectrum displays a low diffusion coefficient in agreement with the molecular weight measured in the ES-MS experiment. At least two downfield shifted and broad paramagnetic signals were apparent in ^{1}H NMR spectrum. Temperature dependence of chemical shift was exploited in order to estimate the diamagnetic shift of the signals in the diamagnetic region of the spectrum. 2D TOCSY, NOESY, COSY and ^{1}H-^{13}C HMBC spectra revealed the presence of aromatic protons (which were assigned as His residues), Gly and some other short chain amino-acids. Combinations of the molecular masses of such components together with acetate (which is present in the solution) and chromium atoms allowed to propose some models for the compound.

ON THE MECHANISMS OF PEPTIDE-PROTEIN INTERACTION

Andrea Bernini, Ottavia Spiga, Maria Scarselli, Arianna Ciutti, Luisa Lozzi, Luisa Bracci, Chiara Falciani, Duccio Calamandrei[$], Daniela Di Maro[$] and Neri Niccolai*

Dipartimento di Biologia Molecolare, Università di Siena, and Biomodem pscrl, Siena

Peptide and antigen fragment libraries obtained by the phage display technology have been extensively used to study antibody-antigen interaction in order to develop vaccines and diagnostic tools. Moreover, the same technology has been proposed, in general, for investigating any protein-protein or peptide-protein interaction for the design of selective ligands of potential pharmaceutical interest. Understanding the fine details of these intermolecular processes is crucial to design peptide ligands with improved affinity and activity. In this respect, it is now well established that surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) are playing a major role in elucidating dynamics and structural aspects of protein-protein and peptide-protein complex formation. SPR yields accurate estimates of kinetic parameters of the intermolecular interaction, such as k_{on} and k_{off}, while NMR offers precious information on the structural stability of the complex by analysing intramolecular and intermolecular nuclear Overhauser effects (NOEs). A strong correlation between the structure stability and the affinity between protein subdomains has been found from SPR data and a combined use of SPR and NMR measurements has been proposed for investigating peptide-antibody and virus-receptor interactions.
Among the various peptide-protein interactions, the complex formation between acetylcholine receptor (AChR) mimotopes and α-bungarotoxin is here analysed in detail both by SPR and NMR, since many examples of this type have been described.
Thus, a combined analysis of the SPR- and NMR-derived dynamic parameters shows new correlations between complex formation and dissociation and the overall pattern of intramolecular and intermolecular nuclear Overhauser effects. These features could be crucial for a rational design of protein ligands.

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* Prof. Neri Niccolai, Dipartimento di Biologia Molecolare, Università di Siena, via Fiorentina 1, 53100 Siena. Phone 0577-234910; fax 0577-234903; e-mail: niccolai@unisi;it

TWO DISTINCT CALCIUM-CALMODULIN INTERACTIONS WITH N-TERMINAL REGIONS OF THE OLFACTORY AND ROD CYCLIC NUCLEOTIDE GATED-CHANNELS CHARACTERIZED BY NMR SPECTROSCOPY

Maria Orsale[a], Sonia Melino[a], Gian Marco Contessa[a,c], Vincent Torre[d], Giuseppina Andreotti[e], Andrea Motta[e,f], Maurizio Paci[a,c], Alessandro Desideri[b,c,*] and Daniel O. Cicero[a,d]

[a]Department of Chemical Sciences and Technologies, [b]Department of Biology and [c]INFM, University of Rome "Tor Vergata", via della Ricerca Scientifica, 00133 Rome Italy, [d]INFM and SISSA, Settore di Biofisica, Trieste, Italy, [e] Istituto di Chimica Biomolecolare del CNR, Pozzuoli, and [f] INFM, University of Naples, Italy

The interactions of calcium-calmodulin with two fragments of the N-terminal domains of the olfactory α-subunit and rod β-subunit CNG channels have been investigated using NMR spectroscopy. The results indicate that in the two cases both the N-terminal and the C-terminal calmodulin lobes are involved in the interaction. The olfactory CNG channel segment forms a 1:1 complex with calmodulin, whereas the rod fragment forms a 2:1 complex. The correlation times of the two complexes, as estimated by ^{15}N relaxation studies, are compatible with the observed stoichiometries These results indicate differences in the mode of action by which calmodulin modulates the activity of both channels, and suggest that either the rod channel is modulated through a simultaneous interaction of two β-subunits with calmodulin or that other regions of the N-terminus are necessarily implicated in the binding.

A ^{13}C- AND ^{1}H-NMR STUDY OF GLUCONEOGENESIS AND MITOCHONDRIAL FATTY ACID METABOLISM USING [3-^{13}C] LACTATE AND [U-^{13}C] OLEATE IN BIOREACTORS CONTAINING RAT HEPATOCYTES ENTRAPPED IN ALGINATE GEL BEADS

Alfredo Miccheli, Alberta Tomassini, Caterina Puccetti, Elisabetta Sartori, Federica Pancotti, Roberta Di Clemente, Maria Enrica Di Cocco and Filippo Conti

Dept. Of Chemistry, University "La Sapienza", P.le A.Moro 5, 00185, Rome, Italy

Due to its physico-chemical properties (chemical and mechanical stability, porosity, permeability), calcium alginate is becoming a promising biomaterial for cell immobilization. We have previously shown, by elettronic and confocal microscopy, that the entrapment at high cell density and culture in a fixed bed bioreactor under continuous flow favour the development of aggregates displaying a three-dimensional tissue-like structure after only 6 hours of culture (1). Gluconeogenesis and mitochondrial fatty acid oxidation are some of the specific hepatic processes which are known to decrease in the traditional models of hepatocyte culture as a consequence of a reduction of phenotype expression

The present work concerns the study of gluconeogenesis from lactate and of the fatty acid oxidative metabolism in hepatocytes entrapped in alginate gel beads in a fixed bed bioreactor perfused with a medium containing glucose, aminoacids and oleate under strictly controlled conditions (temperature, pH, oxygen). The studies have been performed, by using ^{1}H and ^{13}C NMR spectroscopy, supplying [3-^{13}C]lactate and [U-^{13}C]oleate, in presence of unlabeled oleate and lactate repectively.

The comparison of ^{13}C isotopomeric distribution in liver metabolites detected from medium and cell extracts, supplying [3-^{13}C] lactate or [U-^{13}C] oleate, allowed us to describe the metabolic rates and the relations between gluconeogenesis and fatty acid metabolism, including cholesterol and tryglyceride synthesis.

The NMR spectroscopic data on the medium have allowed to study cellular metabolic processes in working bioreactors, representing a powerful tool to evaluate the effects of biological active substrates and to develop new drugs for metabolic diseases.

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CONFORMATIONAL STUDIES OF FRAGMENTS FROM THE PROTEIN STICHOLYSIN II BY NMR SPECTROSCOPY

Aline L. Oliveira[1], Eduardo M. Cilli[2], Yohanka Martinez [3], Carlos Alvarez[3], Fábio Casallanovo[4], Shirley Schreier[4], Alberto Spisni[1,5] and Thelma A. Pertinhez[1]

[1]Centro de Biologia Molecular e Estrutural, LNLS, Brasil; [2]Instituto de Química, UNESP, Brasil; [3]Facultad de Biología, Universidad de La Habana, Cuba; [4]Instituto de Química, USP, Brasil; [5]Dipartimento di Medicina Sperimentale, Università di Parma, Italia

Sticholysin II (St II) is a cytolytic protein produced by the Caribbean Sea anemone *Sthichodactyla helianthus*. The toxin interacts with biological and model membranes destroying their permeability barrier with a mechanism that is believed to be related to pore formation. As for the lytic activity of St II, an important region that could be involved in toxin-membrane interaction is the amphipathic α-helix located at the N-terminus. Such interaction has been demonstrated for Equinotoxin II, that reveals a 64-66% sequence identity with St II. In this work we have studied the structure of three synthetic fragments of St II with the aim of understanding the molecular mechanism underlying St II-membrane interaction. These protein fragments were selected since they encompass regions predicted to adopt an amphipathic α-helix conformation, and therefore are expected to be involved in toxin-membrane interaction. The structural analysis has been carried out by circular dichroism (CD) and ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR). The CD results showed that among the three synthetic peptides studied, VLDKVLEELGKVSRKIAVGI-NH_2, St II (16-35), turns out to be the peptide exhibiting the highest helicity in the presence of SDS micelles. Interestingly, this fragment presents a certain hemolytic activity and when incubated with erythrocytes, inhibits the hemolytic activity of St II. Based on these structural and functional features, the 3D structure of St II (16-35) was determined in SDS micelles by ^{1}H-NMR spectroscopy. The results show that indeed the peptide is predominantly helical, with a regular amphipathic α-helix, spanning the region from Leu^{17} to Ala^{32}. From the H/D exchange experiments it has been possible to identify the location of the peptide St II (16-35) in the SDS micelle. The peptide lays on the surface of the micelle with the hydrophobic residues buried inside the micelle hydrophobic core and the hydrophilic ones exposed to the water external medium. This findings support the hypothesis that, upon interacting with the erythrocyte membrane, the peptide might form a sort of a screen that would hamper the interaction of St II with the membrane, thus leading to the inhibition of the toxin's hemolytic activity.

NMR AND CALORIMETRIC INVESTIGATION OF WATER IN A SUPERABSORBING CROSSLINKED NETWORK BASED ON CELLULOSE DERIVATIVES.

N.Proietti[†,‡], D.Capitani[†],G.Mensitieri[§] and A.L. Segre[†]

[†]*Institute of Chemical Methodologies, Cnr, Research Area of Rome, M.B.10 00016, Monterotondo Stazione , Rome, Italy*
[‡]*Campec, Via G. Porzio, Centro Direzionale, Isola F4, Naples, Italy*
[§]*Department of Materials and Production Engineering, University of Naples "Federico II", P.Le Tecchio 80, 80125 Naples, Italy*

In this study we have investigated the state of water in a superabsorbing network based on hydroxyethylcellulose (HEC) and carboxymethylcellulose sodic salt (CMC-Na) crosslinked with divynilsulfone (DVS). This network is able of adsorbing an amount of water as high as 1000 times its own weight .
Dehydrated-rehydrated networks containing different amounts of absorbed water have been studied using differential scanning calorimetry (DSC) and NMR relaxometric methods. DSC analysis allowed the evaluation of freezable and non-freezable fractions of absorbed water showing also the presence of two types of freezable water. On the other hand, NMR relaxometry evidenced the presence of two hydration shells, characterized by a different mobility, which in both cases is lower than that of bulk water.
An excellent quantitative agreement was found between the two techniques for the determination of the amount of freezable water.
A comparison of the state of water in the crosslinked network and in the corresponding uncrosslinked mechanical mixture shows that in the last case micro-heterogeneity arises.

THREE-DIMENSIONAL SOLUTION STRUCTURE OF SSO7D INACTIVE MUTANT

Teresa Recca,[†] Enrica Alberti,[†] Ivana Arosio, [†] Roberto Consonni, [†] Paola Fusi [‡] and Lucia Zetta[†]

[†]*Istituto per lo Studio delle Macromolecole, Lab.NMR, CNR, Via Bassini,15 20134 Milano, Italy*
[‡]*Dipartimento di BioTecnologie e Bioscienze, Università di Milano-Bicocca, Piazza delle Scienze, 2 Milano, Italy*

Sso7d is a thermostable RNAse[8] and DNA binding protein isolated from the thermoacidophilic archeobacterium *Sulfolobus solfataricus*. We have determined the solution structure of the recombinant form[9] (1JIC PDB entry), which consists of a compact globular unit that includes a double-stranded antiparallel ,-sheet onto which an orthogonal triple-stranded antiparallel ,-sheet is packed, and a small helical stretch at the C-terminus. The protein displays a very compact hydrophobic core[10] consisting of side chains at the interface of the two β-sheets, in particular, the aromatic residues Phe5, Phe31, and Tyr33. We have investigated the piezo and thermostability of some single point mutants[11],[12] and determined the possible residues involved in the catalytic activity[13]. Particularly intriguing is the lack in Sso7d of the two histidine residues usually involved in the catalytic mechanisms of most ribonucleases. In the present study we report the structural characterisation of K12L mutant, which in contrast to the wild type protein, does not show RNase activity and possesses a higher thermal stability. Comparison between K12L and Sso7d three-dimensional structures both obtained from NMR data and molecular dynamic calculations, allowed to explain these different properties.

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A STUDY OF THE INTERACTION BETWEEN NEW CARBAMATES AND ACETHYLCHOLINESTERASE BY NMR MOTIONAL PARAMETERS

Anna Borioni[a], Maria Rosaria Del Giudice[a], Andrea Rodomonte[a], Cristiano Bianchetti[b], Maurizio Delfini[b], Maria Enrica Di Cocco[b], Fernando Porcelli[c]

[a] *Istituto Superiore di Sanità*
[b] *Dipartimento di Chimica, Università "La Sapienza", Roma*
[c] *Dipartimento di Scienze Ambientali, INFM sez. B Università della Tuscia*

In the present work, the interaction between Acethylcholinesterase (AchE) and new carbamates (2-[2-(2-Dimethylcarbamoyloxyphenyl)ethyl]-1-methylpiperidine and 2-[2-(3-dimethylcarbamoyloxyphenyl)ethyl]-1-methylpiperidine) (1) has been studied. In fact, carbamates by inhibiting the AChE in the brain may improve the cholinergic transmission and enhance the cognitive activity and memory.
Costitutional characterization of the carbamates was carried out by mono- and bidimansional NMR experiments.
The study of the motional parameters, non-selective, mono-selective and bi-selective spin-lattice relaxation times at two different magnetic fields, allows the calculation of correlation times of the different protons in the molecules, in the absence and presence of AchE. The variation of relaxation times in the absence and presence of AchE, made it possible the identification of the binding sites of the carbamates with AchE.
Measurements of the enhancement of selective relaxation rates, as carbamates concentrations vary in the presence of a fixed AChE concentration, allowed the evaluation of the interaction constants, according to IC_{50} values determined pharmacologically.

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Eur. J. Med. Chem. 37 (2002) 91-109

pH-DEPENDENT INTERACTIONS OF A TRANSLOCATING PEPTIDE DERIVED FROM HSV1 DNA POLYMERASE WITH MICELLES AND VESICLES: A CD AND NMR APPROACH

E. Schievano, I. Menegazzo, T.Calisti, G. Palù[#], S. Mammi and A. Loregian[#]

University of Padova, Department of Organic Chemistry, Istituto di Chimica Biomolecolare, CNR, I-35131, Padova, Italy
[#]University of Padova, Department of Histology, Microbiology and Medical Biotecnology, 35121, Padova, Italy

Pol peptide, an oligopeptide corresponding to the 27 C-terminal amino acids of DNA polymerase from herpes simplex virus type 1, has recently been demonstrated to be able to translocate from endosomal compartments into the cytosol after being intracellularly delivered via a protein carrier. While an acidic environment was speculated to be important for Pol peptide proteolytic cleavage from the carrier and/or for membrane translocation, the mechanism of Pol peptide translocation remained undefined.
To investigate the influence of an acidic environment on the conformational properties of the peptide and on its propensity to interact with lipid bilayers, we studied the conformational behaviour of Pol peptide at different pH values. The study was conducted in the presence of DPC micelles and also of DMPC:DMPG 3:1 vesicles, both by circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy. Our CD results indicate that the peptide presents a random conformation in aqueous solution at both acidic and basic pH, whereas in the presence of micelles it assumes a C-terminal α-helical structure which is significantly pH-dependent. Different orientations of Pol peptide relative to the DPC micelle at pH 4.0 and pH 6.5 were found using paramagnetic probes. The NMR diffusion experiments showed strong interaction of Pol peptide with vesicles at pH 4.0, while low affinity was revealed at the higher pH value. Based on results both in micelles and in vesicles, a first model which might explain the mechanism of Pol peptide translocation from acidic endosomes to the cytosol is discussed.

BACKBONE DYNAMICS OF HUMAN PARATHYROID HORMONE (1-34): MOBILITY OF THE CENTRAL REGION UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Michele Scian[‡], Massimiliano Marin[‡], Massimo Bellanda[‡], Tou Liqiang[§], Joseph M. Alexander[§], Michael Rosenblatt[§], Michael Chorev[§], Evaristo Peggion[‡], and Stefano Mammi[‡]

[‡]*Institute of Biomolecular Chemistry, CNR, University of Padova, Department of Organic Chemistry, via Marzolo 1, 35131 Padova, Italy*
[§]*Bone and Mineral Metabolism Unit, Charles A. Dana and Thorndike Laboratories, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, 02215 USA*

The presence of a stable tertiary structure in the bioactive N-terminal portion of parathyroid hormone (PTH), a major player in the maintenance of extracellular calcium homeostasis, is still debated. In this work, ^{15}N relaxation parameters of the 33 backbone amides of human PTH (1-34) were determined in phosphate buffered saline solution (PBS), and in the presence of dodecylphosphocholine (DPC) micelles. The relaxation parameters were analyzed using both the model-free formalism [Lipari, G., and Szabo, A. (1982) *J. Am. Chem. Soc.* **104**, 4546-4549] and the reduced spectral density function approach [Lefevre, J.-F., Dayie, K. T., Peng, J. W., and Wagner, G. (1996) *Biochemistry* **35**, 2674–2686]. In PBS, the region centered at Gly12 possesses a high degree of mobility and the C-terminal helix is less flexible than the N-terminal one. In the presence of DPC micelles, the mobility of the entire molecule is reduced, but it is still relatively higher at residue 12. The presence of micelles increases the stability of the N-terminal helix relative to the C-terminal one and generates a new site of local mobility at residues 16-17. These results support the hypothesis that, in the absence of the receptor, the relative spatial orientation of the two N- and C-terminal helices is undefined. The mobility in the mid-region of hPTH(1-34) may enable the correct relative disposition of the two helices, favoring a productive interaction with the receptor.

NMR AND MOLECULAR DYNAMICS IN BINARY MIXTURES

R. Sinibaldi*, C. Casieri**, S. Melchionna*, G. Onori^, A. L. Segre+, S. Viel+, L. Mannina++ and F. De Luca*

**INFM and Dipartimento di Fisica, Universita' "La Sapienza", Roma*
***INFM and Dipartimento di Fisica, Universita dell'Aquila, L'Aquila*
^INFM and Dipartimento di Fisica, Universita' di Perugia, Perugia
+Istituto di Metodologie Chimiche, CNR Area della Ricerca di Roma, Montelibretti
++Dipartimento di Agraria, Universita' del Molise, Campobasso

We investigated by molecular dynamics simulation, chemical shift and DOSY measurements, two binary mixtures composed by small amphiphylic molecules, TMAO and TBA, in rich water conditions. TMAO is an osmolyte while TBA is a monohydrical alcohol, both characterised by the same hydrophobic group made by three methyl groups and unlike "heads" possessing rather different electrostatic dipoles. The different dipole moments are potentially responsible for effect that these solutes induce on water and therefore over protein or phospolipids. It is known in fact that TBA induces some proteins damage while TMAO favors their conformational stability. The mechanisms of water coordination and of "hydrophobic hydration" involved in TBA-water and TMAO-water mixtures are quite general and responsible of basic biological processes. The comparison between the effects induced by two very similar solutes on water can help to understand the role that molecular branches can play on water-solute dynamics.
The results achieved by molecular dynamics, especially on hydrogen bond networking, solute-solute spatial correlations and self-diffusivity, found full support in the NMR experimental data and agree with previous numerical studies on similar mixtures. On the basis of this many-sided study, we reached some conclusions about the microscopic characteristics of the mixtures. For TBA solutions, the results support a description in which the competition between hydrophobic and dipolar interactions is responsible of a structural change of mixtures over a certain concentration in which TBA aggregates. This does not take place in TMAO mixtures, where the mechanism of hydration seems independent by concentration and water shows a more strong coordination around solute.
These structural changes of the hydration water could have correlation with the effect that TBA and TMAO induced over other hydrophobic groups present in solution like phospholipids or proteins apolar residues.

NMR 3D STRUCTURE DETERMINATION OF ApaG/CorD PROTEIN OF THE PHYTOPATHOGEN *Xanthomonas axonopodis pv. citri*

Katsuyama, A.M.[1,2], Cicero, D.O.[3], Farah, C.S.[1], Spisni, A.[2,4] & Pertinhez, T.A.[2]

[1] *Center for Structural Molecular Biology, LNLS, Campinas, Brazil*
[2] *Dept. Biochemistry, Institute of Chemistry, University of São Paulo, Brazil*
[3] *Dept. Chemical Sciences &Technologies, University of Rome `Tor Vergata,' Italy*
[4] *Dept. Experimental Medicine, University of Parma, Italy*

Xanthomonas axonopodis pv. *citri* (*Xac*) is the agent of citrus canker, which causes many economic losses in production of citrus fruit. This impact is severe in Brazil, which produces about one third of the world's citrus fruit crop. The complete sequencing of the *Xac* genome revealed several conserved proteins of unknown function and structure. In this context, we looked for ORFs located in clusters of genes associated with pathogenecity, secretion systems or yet of unknown function. Recognizing that structural characterization of a protein not only provides information regarding its cellular function but also may disclose new folds, the protein encoded by ORF 2684 has been selected, over-expressed in *E. coli* and purified. ORF 2684 codes for the protein ApaG which is located in a multifunctional operon and which has homology with CorD, a protein of *Salmonella typhimurium* associated with Co^{2+} and Mg^{2+} control. Circular dichroism spectroscopy has been used to initially characterize the protein fold. Backbone assignments derived from 3D triple resonance NMR experiments allowed to derive a preliminary description of the protein secondary structure. Diffusion measurements enabled to estimate the protein hydrodynamic radius and hydrogen-deuterium exchange permitted the identification of regions with diverse degrees of exposure to the solvent. The description of the internal backbone dynamics was derived from ^{15}N relaxation data (T_1, T_2, and heteronuclear nuclear Overhauser effect). We envisage that the knowledge of the protein 3D solution structure will provide clues on its molecular function and will allow to model ApaG/CorD analogs, including mammalian F-box proteins involved in ubiquitin-linked protein degradation. FAPESP

MOLECULAR PENDULUMS. VARIABLE TEMPERATURE MULTINUCLEAR MAGNETIC RESONANCE STUDY OF DIMERIC PALLADIUM(I) BISPHOSPHINE COMPLEXES

Gábor Szalontai[1], Gábor Besenyei[2] and László Párkányi[2]

[1]*University of Veszprém, NMR Laboratory, H-8201 Veszprém Pf.158., Hungary*
[2]*Institute of Chemistry, Chemical Research Centre, MTA, H-1525 Budapest Pf.17., Hungary*

Identification and study of molecular machines such as "propellers", "turnstiles", "rotors", "pendulums", *etc*. became intense in the last few years. We have conducted solution and solid state ^{31}P NMR studies on a series of $[Pd_2X_2(dppm)_2]$ complexes (X = Cl, Br, I) and methyl substituted derivatives such as $[Pd_2Cl_2(dppm)(dppmMe)]$, *syn*-$[Pd_2Cl_2(dppmMe)_2]$ and *anti*-$[Pd_2Cl_2(dppmMe)_2]$ (dppmMe = 1,1-bis(diphenylphosphino)ethane) in order to study the conformational behaviour of the eight-membered ring. These mimic a pendulum-like motion (twisting) of the frame around the Pd–Pd axis and, perhaps, a separate flipping of the bridging methylene groups.

According to crystallographic studies, the coordination planes of the two Pd-centres are twisted about the Pd–Pd bond by 37°-50° allowing to form enantiomers. While the interconversion of the clockwise and anticlockwise forms is rapid in CD_2Cl_2 at RT, below 200 K the solution spectra became similar to the MAS spectra of polycrystalline samples observed in solid state at room temperatures. A semi-quantitative approach was used to account for these observations. Replacement of one hydrogen atom by a methyl group on the dppm methylene carbon atom, increases the conformational barriers significantly, exchange rate constants (swinging rates) could be estimated from the coalescence temperatures. Steric interactions between the phenyl groups are thought to be the driving force behind the pendulum-like motions.

MAS spectra support the presence of a single crystalline phase in the polycrystalline samples.

APPLICATION OF HPLC-NMR IN CHEMICAL AND PHARMACEUTICAL DEVELOPMENT

Lucilla Turco, Stefano Provera, Silvia Davalli, Carla Marchioro

GlaxoSmithKline Research Centre, Via Fleming 4, 37135 Verona

HPLC-NMR is a powerful technology, widely used in Pharmaceutical Research process, which allows separation and structural characterization of complex mixture of unknown compounds. Its main application in drug development regards the identification of degradation compounds or byproducts in final drug batches or in formulation. Other interesting applications are monitoring chemical reactions, studying metabolites from biological fluids and identifying isomers from a synthesis or a process.
We will focus here on the application of HPLC-NMR in structure elucidation of drug impurities and related degradation products.

CHANGES IN ENERGY COST OF ANAEROBIC METABOLISM IN ISOLATED RESTING FROG MUSCLE BY ^{31}P AND ^{1}H MRS

Alessandra Vezzoli [a], Maristella Gussoni [b], Fulvia Greco [c] and Lucia Zetta [c]

[a] *Istituto di Bioimmagini e Fisiologia Molecolare, CNR, Milan, Italy*
[b] *Dipartimento di Scienze e Tecnologie Biomediche, Università di Milano, Milan, Italy*
[c] *Istituto per lo Studio delle Macromolecole, CNR, Milan, Italy*

Ischemic and anoxic conditions occur routinely under normal physiological an pathophysiological conditions. In this simplified model (anaerobic metabolism), owing to oxygen lack, the only active energy yielding mechanisms are the hydrolysis of high energy phosphates, mainly phosphocreatine (PCr), and the glycogenolysis, the end product of which is lactate. Nuclear Magnetic Resonance Spectroscopy (MRS) is nowadays the technique of choice for investigating muscle energetics in O_2 deprived preparations. ^{31}P and ^{1}HMRS high resolution measurements at 4.7 T were carried out in isolated frog (Rana esculenta) gastrocnemius muscle during anoxia to assess, using reference compounds (1), the concentration of all phosphate metabolites and lactate. Intra and extracellular pH (pHi, pHe) were determined too. The rate of the anaerobic exergonic processes occurring in isolated resting muscles was determined at 15, 20 and 25°C and at pHe values higher (7.9), similar (7.3) and lower (7.0) than the physiological pHi. The rate of PCr hydrolysis and La accumulation at the investigated T and pHe conditions, as well as their energetic equivalents (rate of ATP resynthesis calculated as the sum of PCr hydrolysis and lactate accumulation) during the first 5 hours are shown in Table 1. The high-energy phosphates metabolism was confirmed to be the initial and preferential energetic source. The anaerobic glycolysis was found proportional to the metabolic request which is closely coupled to temperature much more than of PCr depletion The rate of PCr hydrolysis did not result to be affected by pHe changes. On the contrary, a lowering of the pHe produced a depression in lactate accumulation, particularly at the highest T. This mechanism resulted to be determined by the transmembrane protons concentration gradient that regulates the glycolysis, probably throughout a reduced lactate efflux depending on the activity of the lactate-H^+ co-transporter (2). The overall energy turnover tends to drop significantly with decreasing pHe at 20 and 25°C. An energy saving mechanism can reduce the ATP cost of pHi regulation by a shift from less to more ATP-efficient ion transporters as reported previously in aerobic conditions by Reipschläger and Pörtner (3).

Table 1

PCr splitting (μmol·g^{-1}·h^{-1})

pHe	T = 15°C	T = 20°C	T =25°C
7.9	2.26	3.86	5.18
7.3	2.59	3.90	5.36
7.0	2.33	3.56	5.22

La accumulation (μmol·g^{-1}·h^{-1})

pHe	T = 15°C	T = 20°C	T = 25°C
7.9	0.15	1.05	2.16
7.3	0.13	0.64	1.20
7.0	0.15	0.72	0.80

Total ATP resynthesis (μmol·g^{-1}·h^{-1})

pHe	T = 15°C	T = 20°C	T = 25°C
7.9	2.49	5.44	8.42
7.3	2.79	4.86	7.16
7.0	2.56	4.64	6.42

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NMR IN BIOTECHNOLOGY. MODULATION OF PROCESS VARIABLES IN LOVASTATIN AND MEVASTATIN PRODUCTION FROM *ASPERGILLUS TERREUS*

Silvia Bradamante,[†] Alessandro Villa,[†] Livia Barenghi,[†] Fabio Nicoli,[†] Marinella Bonfà[‡]

[†]*CNR – Istituto di Scienze e Tecnologie Molecolari, Milano, Italy*
[‡]*Dipt. Scienza dei Materiali, Universita' di Milano Bicocca, Milan, Italy*

The combined approach of statistic and DOSY NMR, applied to fermentation processes, indicated that lovastatin best production is induced with glycerol, glucose, defatted soybean flour and `reciprocal agitation` at 25°. Lovastatin is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl CoA reductase, the major regulatory enzyme for cholesterol synthesis. In general, lovastatin is produced in association with variable quantities of mevastatin, an useful intermediate for pravastatin synthesis. Pravastatin, another inhibitor of cholesterol synthesis, is characterized by better adsorption. Here we consider to modulate process variables as carbon and nitrogen sources in order to maximize the lovastatin or alternatively the mevastatin production. Addition of D,L-methionine to the medium has been also considered, as this compound is known to modulate methylation.. NMR analysis of aqueous and lipid extracts from flours (soybean and peanut) have been performed. DOSY NMR has been used to investigate the culture broths and to identify cometabolites and impurities.

^{1}H, ^{2}H AND ^{17}O NMR WATER MOBILITY IN HETEROGENEOUS FOOD MODEL SYSTEMS AS RELATED TO MICROBIAL RESPONSE

Elena Vittadini,[†,‡] and Pavinee Chinachoti[†*]

[†]*Departmant of Food Science, University of Massachusetts, Amherst, MA 01003, USA*
[‡]*Current address: Dipartimento di Chimica Organica e Industriale, Universita' degli Studi di Parma, Parco Area delle Scienze 17/A, 43100 Parma, Italy*
[*]*Current address: Hill's Pet Nutrition, Inc., Science and Technology Center, P.O. Box 1658, Topeka, KS 66601-1658, USA*

The role played by water activity (a_w), "mobility" (molecular and structural) and physico-chemical properties of the media in modulating microbial response has been the object of large debate in the food science community as tools to assess safety and stability of food products. Multi-component model food systems at variable a_w (of different physical state, ranging from solid to liquid) were characterized for both NMR water molecular (^{1}H ^{2}H and ^{17}O NMR) and structural mobility. The correlation of such parameters with microbial activity was evaluated.

In solid and semisolid systems (gums with and without mannitol, cellulose with and without sorbose), water molecular mobility was studied by applying liquid (T_1, inversion recovery and T_2, CPMG) and solid (wide-line) state ^{1}H and ^{2}H NMR. The polymers used in this study showed no observable glass transition as analyzed by DSC and/or DMA suggesting no relation between structural mobility and microbial response. However, at the molecular level, NMR mobility was consistently observed (although retarded). In these slow exchange conditions, *Aspergillus nidulans* germination and growth, as well as survival of *Rizobium japonicum* under extreme osmotic conditions, were found to be highly dependent on the molecular mobility of water. In such cases, a_W alone was a poor indicator for food stability.

In liquid media, ^{17}O NMR water spectra of protein broths (fast exchange) were collected (WALTZ pulse sequence) and evaluated with the "anisotropic, two correlation time model" (Halle and Wennestrom, 1981; Belton et al., 1991). ^{17}O NMR water mobility (R_2, P_{bw} and τ^{s}_{bw}) and physical (solid content, a_w, kinematic viscosity) parameters of the media were obtained and correlated with *Staphylococcus aureus* growth parameters. In these high moisture, liquid and homogeneous media *S. aureus* growth related to all the physico-chemical and molecular mobility parameters analyzed in a similar manner and it was found to be influenced more significantly by added NaCl than by the physico-chemical and molecular mobility of the media. *S. aureus* growth correlated better with a_w than with any other parameter considered.

The results of this study indicate that NMR water mobility is a possible tool to describe water availability in solid and semi-solid systems (dry and intermediate moisture foods) while in high moisture, liquid systems the use of water activity is still a preferred indicator for food safety and stability.

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AUTHOR INDEX

LIST OF PARTICIPANTS

(as of September 8th)

Massimo Adobati
Sapio Industrie
Via Silvio Pellico 48
20052 Monza (MI) Italy
Tel.: 39 039 8398711
Fax: 39 02 95743 571
m.adobati@sapio.it

Enrica Alberti
Istituto per lo Studio delle
Macromolecole, CNR
Via Bassini 15
20133 Milano Italy
Tel.:
Fax:
enrica.alberti@ismac.cnr.it

Marcella Alesiani
Dip. di Fisica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3928
Fax: 39 06 4991 3484
marcella.alesiani@roma1.infn.it

Giuliana Ansanelli
ENEA CR TRISAIA
SS Jonica 106 Km 419,5
75026 Rotondella (MT) Italy
Tel.: 39 0835 974 716
Fax: 39 0835 974 724
giuliana.ansanelli@trisaia.enea.it

Massimo Balzarini
ENI Divisione E&P
Via Emilia 1
20097 San Donato Milanese (MI) Italy
Tel.: 39 02 520 62309
Fax:
massimo.balzarini@agip.it

Livia Barenghi
Istituto di Scienze e Tecnologie
Molecolari, CNR
Via Golgi 19
20133 Milano Italy
Tel.: 39 02 5031 4146
Fax:
nmrone@istm.cnr.it

Massimo Bellanda
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5324
Fax: 39 049 827 5239
massimo.bellanda@unipd.it

Detlef Bentrop
Physiologisches Institut II,
Universität Freiburg
Hermann-Herder-Str. 7
D-79104 Freiburg Germany
Tel.: 49 761 2035149
Fax: 49 761 203 5191
detlef.bentrop@physiologie.uni-
freiburg.de

Andrea Bernini
Dip. di Biologia Molecolare,
Università di Siena
Via Fiorentina 1
53100 Siena Italy
Tel.: 39 0577 234911
Fax: 39 0577 234903
andrea.bernini@unisi.it

Angelo Bifone
GlaxoSmithKline Research Center
Via Fleming 4
37135 Verona Italy
Tel.: 39 045 921 9707
Fax: 39 045 921 8047
angelo.2.bifone@gsk.com

Marco Bisaglia
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5743
Fax: 39 049 827 5239
marco.bisaglia@unipd.it

Giovanni Bizzaro
Bruker Biospin S.r.l.
Via Giovanni Pascoli 70/3
20133 Milano Italy
Tel.: 39 02 70 63 63 70
Fax: 39 02 23 61 294
gb@bruker.it

Massimo Boerci
Rivoira
Via Stephenson, 43/A
20157 Milano Italy
Tel.: 39 02 3579 31
Fax: 39 02 3579 3388
Massimo_Boerci@praxair.com

Maria Bonfà
Dip. di Farmacologia -
Laboratorio NMR
Via Vanvitelli 32
20129 Milano Italy
Tel.: 39 02 5031 7090
Fax: 39 02 5031 4139
nmrone@mater.unimib.it

Anna Borioni
Dip. Farmaco,
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma Italy
Tel.: 39 06 4990 2518
Fax: 39 06 4990 2516
annab@iss.it

Mauro Botta
DiSTA, Università del Piemonte
Orientale "Amedeo Avogadro"
Corso Borsalino 54
15100 Alessandria Italy
Tel.: 39 0131 287431
39 011 6707602
Fax: 39 0131 287416
39 011 6707855
mauro.botta@unito.it

Silvia Bradamante
Istituto di Scienze
e Tecnologie Molecolari, CNR
Via Golgi 19
20133 Milano Italy
Tel.: 39 02 503 14146
Fax: 39 02 503 14139
silvia.bradamante@istm.cnr.it

Lorenza Broccardo
S.IN - Soluzioni Informatiche S.a.s.
Via Salvemini 9
36100 Vicenza Italy
Tel.: 39 0444 920 920
Fax: 39 0444 533 954
consulting@s-in.it

Paul T. Callaghan
School of Chemical
and Physical Sciences
Victoria University of Wellington
Wellington New Zealand
Tel.: 64 4 4635945
Fax: 64 4 4635237
paul.callaghan@vuw.ac.nz

Donatella Capitani
Istituto di Metodologie Chimiche,
CNR
Via Salaria Km. 29,300
00016 Monterotondo Stazione
(Roma) Italy
Tel.: 39 06 9067 2476
Fax: 39 06 9067 2477
donatella.capitani@imc.cnr.it

Francesco Capozzi
Dip. di Scienze degli Alimenti,
Università di Bologna
Via Ravennate 1020
47023 Cesena (FO) Italy
Tel.: 39 0547 636105
Fax: 39 0547 382348
fcapozzi@foodsci.unibo.it

Giorgio Capuani
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
giorgio.capuani@uniroma1.it

Donatella Carbonera
Dip. di Chimica Fisica,
Università di Padova
Via Loredan 2
35131 Padova Italy
Tel.: 39 049 827 5144
Fax: 39 049 827 5135
d.carbonera@chfi.unipd.it

Lorena Casciani
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
lorena.casciani@uniroma1.it

Cinzia Casieri
Dip. di Fisica, Università dell'Aquila
Via Vetoio – Coppito
67100 L'Aquila Italy
Tel.: 39 0862 43 3052
Fax:
cinzia.casieri@roma1.infn.it

Mariano Casu
Dip. di Scienze Chimiche,
Università di Cagliari
Cittadella Universitaria di Monserrato
09042 Monserrato (CA) Italy
Tel.: 39 070 6754416
Fax: 30 070 675 4388
mariano@mvcch3.unica.it

Maddalena Catalano
Dip. Scientifico e Tecnologico,
Università di Verona
Ca' Vignal 1, Strada Le Grazie 15
37134 Verona Italy
Tel.: 39 045 8027906
Fax:
maddalena.catalano@ismac.cnr.it

Friedrich M. Cavagna
Bracco Imaging S.p.A.
Via E. Folli 50
20134 Milano Italy
Tel.: 39 02 2540 660
Fax: 39 02 2641 0678
Friedrich.Cavagna@bracco.com

Giovanni Cerioni
Dip. Farmaco Chimico Tecnologico,
Università di Cagliari
Via Ospedale 72
09124 Cagliari Italy
Tel.: 39 070 675 8553
Fax: 39 070 675 8550
cerioni@unica.it

Alberto Cerri
NiKem Research S.r.l.
Via Zambeletti 25
20021 Baranzate di Bollate (MI) Italy
Tel.: 39 02 35694 1
Fax: 39 02 35694 7606
alberto.cerri@nikemresearch.com

Matilde Chessa
PortoConte Ricerche
loc.Tramariglio sp.55 km 8.4
07041 Alghero (SS) Italy
Tel.: 39 079 998646
Fax: 079 998648
m.chessa@iatcapa.ss.cnr.it

Stefano Chimichi
Dip. di Chimica Organica,
Università di Firenze
Via della Lastruccia 13
50019 Sesto F.no (FI) Italy
Tel.: 39 055 457 3537
Fax: 39 055 457 3568
stefano.chimichi@unifi.it

Daniel Cicero
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.: 39 06 7259 4449
Fax: 39 06 7259 4328
cicero@scienze.uniroma2.it

Roberto Consonni
Istituto per lo Studio delle
Macromolecole, CNR
via Bassini 15
20133 Milano Italy
Tel.: 39 02 2369 9620
Fax: 39 02 2369 9578
roberto.consonni@ismac.cnr.it

Gian Marco Contessa
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.: 39 06 7259 4410
Fax: 39 06 7259 4328
contessa@uniroma2.it

Filippo Conti
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4455 278
Fax: 39 06 4455 278
filippo.conti@uniroma1.it

Patrick J. Cozzone
CRMBM, UMR CNRS n 6612,
Faculté de Médecine de Marseille
27 Boulevard Jean Moulin
13005 Marseille France
Tel.: 33 (0)4 91 25 65 29
Fax: 33 (0)4 91 25 65 39
patrick.cozzone@medecine.univ-mrs.fr

Mauro Andrea Cremonini
Dip. di Scienze degli Alimenti,
Università di Bologna
Via Ravennate 1020
47023 Cesena (FO) Italy
Tel.: 39 0547 636106
Fax: 39 0547 382348
mac@foodsci.unibo.it

Nicola Culeddu
Istituto di Chimica Biomolecolare,
CNR
Via La Crucca 3 Baldinca-LiPunti
07100 Sassari Italy
Tel.: 39 079 3961033
Fax: 39 079 3961036
nicola@ss.cnr.it

Nicola D'Amelio
Dip. di Chimica, Università di Siena
Via A. Moro
53100 Siena italy
Tel.: 39 0577 234511
Fax: 39 0577 234254
damelio@unisi.it

Antonia De Capua
Dip. di Scienze Ambientali,
Seconda Università di Napoli
Via Vivaldi 43
81100 Caserta Italy
Tel.: 39 0823 274 637
Fax: 39 0823 274 605
decapua@chemistry.unina.it

Maria Rosaria Del Giudice
Dip. Farmaco,
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma Italy
Tel.: 39 06 4990 2516
Fax: 39 06 4990 2516
mr.delgiudice@iss.it

Maurizio Delfini
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3124
Fax: 39 06 490631
maurizio.delfini@uniroma1.it

Roberta Di Clemente
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
Roberta_DiClemente@hotmail.com

Maria Enrica Di Cocco
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3728
Fax: 39 06 490631
mariaenrica.dicocco@uniroma1.it

Silvia Di Stefano
Bruker Biospin S.r.l.
Via Giovanni Pascoli 70/3
20133 Milano Italy
Tel.: 39 02 70 63 63 70
Fax: 39 02 23 61 294
sidis@bruker.it

Anna Dinoi
ENEA CR TRISAIA
SS Jonica 106 Km 419,5
75026 Rotondella (MT) Italy
Tel.: 39 0835 974 717
Fax: 39 0835 974 724
anna.dinoi@trisaia.enea.it

Anna Maria D'Ursi
Dip. di Scienze Farmaceutiche,
Università di Salerno
Via Ponte Don Melillo 11c
84084 Fisciano (SA) Italy
Tel.: 39 089 964355
Fax: 39 089 962828
dursi@unisa.it

Gennaro Esposito
Dip. di Scienze e Tecnologie
Biomediche, Università di Udine
P.le Kolbe 4
33100 Udine Italy
Tel.: 39 0432 494321
Fax: 39 0432 494301
gesposito@mail.dstb.uniud.it

Paola Fantazzini
Dip. di Fisica, Università di Bologna
Viale Berti Pichat 6/2
40127 Bologna Italy
Tel.: 39 051 209 5119
Fax: 39 051 209 0457
fantazzini@df.unibo.it

Mauro Fasano
Dip. di Biologia Strutturale e
Funzionale, Università dell'Insubria
Via Alberto da Giussano 12
21052 Busto Arsizio Italy
Tel.: 39 0331 339450
Fax: 39 0331 339459
mauro.fasano@uninsubria.it

Roberto Fattorusso
Dip. di Scienze Ambientali,
Seconda Università di Napoli
Via Vivaldi 43
81100 Caserta Italy
Tel.: 39 0823 274 637
Fax: 39 0823 274 605
roberto.fattorusso@unina2.it

Gianni Ferrante
Stelar S.r.l.
27035 Mede (PV) Italy
Tel.: 39 0384 820096
Fax: 39 0384 805056
ferrante@stelar.it

Nereo Fiori
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5743
Fax:
nereofiori@yahoo.it

Rasmus Fogh
Dept. of Biochemistry,
University of Cambridge
80 Tennis Court Road
CB2 1GA Cambridge UK
Tel.:
Fax: 44 (0)1223 766002
r.h.fogh@bioc.cam.ac.uk

Paolo Formaglio
Dip. di Chimica, University of Ferrara
Via L. Borsari 46
44100 Ferrara Italy
Tel.: 39 0532 291123
Fax: 39 0532 240907
frp@unife.it

Mariana Gallo
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.: 39 06 7259 4410
Fax: 39 06 7259 4328
mari_gallo@yahoo.com.ar

Neda Ghofraniha
Dip. di Fisica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.:
Fax: 39 06 4463 158
neda.ghofraniha@roma1.infn.it

John Gilbert
JEOL (U.K.) Ltd.
JEOL House
Silver Court - Watchmead
AL7 1LT Welwyn Garden City - Herts
England
Tel.: 44 (0)1707 377117
Fax: 44 (0)1707 373254
johng@jeoleuro.com

Roberto Gobetto
Dip. di Chimica I.F.M.,
Università di Torino
Via P. Giuria 7
10125 Torino Italy
Tel.: 39 011 670 7855
Fax: 39 011 670 7523
roberto.gobetto@unito.it

Sveva Grande
Dip. Tecnologie e Salute,
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma Italy
Tel.: 39 06 4990 2125
Fax: 39 06 4938 7075
sveva.grande@iss.it

Fulvia Greco
Istituto per lo Studio delle
Macromolecole, CNR
VIA BASSINI 15
20133 Milano Italy
Tel.:
Fax:
f.greco@ismac.cnr.it

Laura Guidoni
Dip. Tecnologie e Salute,
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma Italy
Tel.: 39 06 4990 2804
Fax: 39 06 4938 7075
guidoni@iss.it

Maristella Gussoni
Dip. di Scienze e Tecnologie
Biomediche, Università di Milano
Via Fratelli Cervi 93
20090 Segrate (MI) Italy
Tel.: 39 02 5033 0417
Fax:
maristella.gussoni@unimi.it

Lothar Helm
Institut de Chimie Moléculaire
et Biologique
EPFL-BCH
CH-1015 Lausanne Switzerland
Tel.: 41 (0)21 693 98 76
Fax: 41 (0)21 693 98 75
lothar.helm@epfl.ch

Carla Isernia
Dip. di Scienze Ambientali,
Seconda Università di Napoli
Via Vivaldi 43
81100 Caserta Italy
Tel.: 39 0823 274 637
Fax: 39 0823 274 605
isernia@chemistry.unina.it

Rainer Kerssebaum
Bruker BioSpin GmbH
Silberstreifen
D-76287 Rheinstetten Germany
Tel.: 49 721 5161 139
Fax: 49 721 5161 297
Rainer.Kerssebaum@bruker-biospin.de

Robert Konrat
Institute of Theoretical Chemistry
and Structural Biology,
University of Vienna
Vienna Austria
Tel.:
Fax:
robert.konrat@univie.ac.at

Jean-Pierre Korb
Laboratoire de Physique
de la Matière Condensée
Ecole polytechnique
91128 Palaiseau France
Tel.: 33 1 69 33 47 39
Fax: 33 1 69 33 30 04
jean-pierre.korb@polytechnique.fr

Eriks Kupce
Varian Ltd
28 Manor Road
KT12 2QF Walton-on-Thames
Surrey England
Tel.: 44 1932 898000
Fax: 44 1865 435402
eriks.kupce@nmr.varian.com

Luca Laghi
Dip. di Scienze degli Alimenti,
Università di Bologna
Via Ravennate 1020
47023 Cesena (FO) Italy
Tel.: 39 0547 636106
Fax: 39 0547 382348
llaghi@foodsci.unibo.it

Raffaele Lamanna
ENEA CR TRISAIA
SS Jonica 106 Km 419,5
75026 Rotondella (MT) Italy
Tel.: 39 0835 974 262
Fax: 39 0835 974 724
raffaele.lamanna@trisaia.enea.it

Brent Lefebvre
ACD/Labs
Toronto Canada
Tel.:
Fax:
brent.lefebvre@acdlabs.com

Miguel Llinas
Dept. of Chemistry,
Carnegie Mellon University
4400 Fifth Avenue
15213 Pittsburgh PA U.S.A.
Tel.: 1 412 268 3140
Fax: 1 412 268 1061
llinas@andrew.cmu.edu

Anna Maria Luciani
Dip. Tecnologie e Salute,
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma Italy
Tel.: 39 06 4990 2804
Fax: 39 06 4938 7075
luciani@iss.it

Giuseppe Maddinelli
Enitecnologie S.p.A
Via F. Maritano 26
20097 S. Donato (MI) Italy
Tel.: 39 02 5205 6559
Fax: 39 02 5203 6347
gmaddinelli@enitecnologie.eni.it

Daniela Maggioni
Dip. C.I.M.A., Università di Milano
Via Venezian 21
20133 Milano Italy
Tel.: 39 02 5031 4352
Fax: 39 02 5031 4405
daniela.maggioni@unimi.it

Gaetano Malgieri
Dip. di Scienze Ambientali,
Seconda Università di Napoli
Via Vivaldi 43
81100 Caserta Italy
Tel.: 39 0823 274 637
Fax: 39 0823 274 605
gaetano.malgieri@unina2.it

Stefano Mammi
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5293
Fax: 39 049 827 5239
stefano.mammi@unipd.it

Cesare Manetti
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3058
Fax: 39 06 4455 278
manetti@caspur.it

Andrea Manni
Spectra2000
Via A. Domenico Gabbiani, 137
00133 Roma Italy
Tel.: 39 06 2063 0997
Fax: 39 06 2063 0997
info@spectra2000.it

Luisa Mannina
Facoltà di Agraria,
Università del Molise
Via De Sanctis
86100 Campobasso Italy
Tel.: 39 06 9067 2700
Fax: 39 06 9067 2477
mannina@imc.cnr.it

Carla Marchioro
GlaxoSmithKline Research Center
Via Fleming 4
37135 Verona Italy
Tel.: 39 045 921 8362
Fax: 39 045 921 8072
carla.2.marchioro@gsk.com

Michele Mauri
Dip. di Scienza dei Materiali,
Università di Milano - Bicocca
Via R. Cozzi 53
20100 Milano Italy
Tel.: 39 02 6448 5132
Fax: 39 0335 671443
michele.mauri@mater.unimib.it

Andreas Maus
Institut für Makromolekulare Chemie,
Universität Freiburg
Stefan-Meier-Str. 31
79104 Freiburg Germany
Tel.: 49 761 203 6312
Fax: 49 761 203 6306
maus@makro.uni-freiburg.de

Andrea Mele
Dip. di Chimica, Materiali
e Ingegneria Chimica "Giulio Natta",
Politecnico di Milano
Via L. Mancinelli 7
20131 Milano Italy
Tel.: 39 02 2399 3006
Fax: 39 02 2399 3080
andrea.mele@polimi.it

Sonia Melino
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.:
Fax: 39 06 7259 4449
melinos@uniroma2.it

Ileana Menegazzo
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5260
Fax: 39 049 827 5239
ileana.menegazzo@unipd.it

Alfredo Miccheli
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
alfredo.miccheli@uniroma1.it

David Middleton
Dept. of Biomolecular Sciences,
UMIST
Sackville Street
M60 1QD Manchester UK
Tel.: 44 161 204217
Fax: 44 161 2360409
david.a.middleton@umist.ac.uk

Maria Chiara Mimmi
Dip. C.I.M.A., Università di Milano
Via Venezian 21
20133 Milano Italy
Tel.: 39 02 5031 4416
Fax: 02 5031 4405
chiara.mimmi@unimi.it

Anna Minoja
Bruker Biospin S.r.l.
Via Giovanni Pascoli 70/3
20133 Milano Italy
Tel.: 39 02 70 63 63 70
Fax: 39 02 23 61 294
apm@bruker.it

Giorgio Modesti
Institut für Makromolekulare Chemie,
Universität Freiburg
Stefan-Meier-str. 31
79104 Freiburg Germany
Tel.: 49 761 203 6312
Fax: 49 761 203 6306
giorgio.modesti@makro.uni-freiburg.de

Henriette Molinari
Dip. Scientifico e Tecnologico,
Università di Verona
Ca' Vignal 1, Strada Le Grazie 15
37134 Verona Italy
Tel.: 39 329 212 6125
Fax: 39 045 8027 929
molinari@sci.univr.it

Elena Molteni
Dip. di Chimica, Università di Siena
Via A. Moro
53100 Siena Italy
Tel.:
Fax:
molteni@unisi.it

Francesco Moneta
Bruker Biospin S.r.l.
Via Giovanni Pascoli 70/3
20133 Milano Italy
Tel.: 39 02 70 63 63 70
Fax: 39 02 23 61 294
lia@bruker.it

Alejandro D. Nadra
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.: 39 06 7259 4410
Fax: 39 06 7259 4328
anadra@leloir.org.ar

Neri Niccolai
Dip. di Biologia Molecolare,
Università di Siena
Via Fiorentina 1
53100 Siena Italy
Tel.: 39 0577 234910
Fax: 39 0577 234903
niccolai@unisi.it

Maria Orsale
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.: 39 06 7259 4410
Fax: 39 06 7259 4328
orsale@uniroma2.it

Maurizio Paci
Dip. di Scienze e Tecnologie
Chimiche,
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma Italy
Tel.: 39 06 7259 4446
Fax: 39 06 7259 4328
paci@uniroma2.it

Federica Pancotti
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
federica_pancotti@hotmail.com

Laura Pasternack
The Scripps Research Institute
10550 N. Torrey Pines Rd.
92037 La Jolla, CA U.S.A.
Tel.: 1 858 784 2338
Fax: 1 858 784 2332
lpastern@scripps.edu

Evaristo Peggion
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5262
Fax: 39 049 827 5239
evaristo.peggion@unipd.it

Thelma A. Pertinhez
Dept. of Biochemistry,
Institute of Chemistry,
University of São Paulo,
Brazil
Tel.:
Fax:
thelma@lnls.br

Maurizio Peruzzini
Istituto di Chimica dei Composti
Organometallici, CNR
Via Madonna del Piano, snc
50019 Sesto Fiorentino (FI) Italy
Tel.: 39 055 5225 289
Fax: 39 055 5225 203
mperuzzini@iccom.cnr.it

Mario Piccioli
CERM & Dip. di Chimica,
Università di Firenze
Via L Sacconi 4
50019 Sesto Fiorentino Italy
Tel.: 39 055 457 4265
Fax: 39 055 457 4253
piccioli@cerm.unifi.it

Delia Picone
Dip. di Chimica, Università di Napoli
Via Cinthia
80126 Napoli Italy
Tel.: 39 081 674406
Fax: 39 081 674409
picone@chemistry.unina.it

Roberta Pierattelli
CERM & Dip. di Chimica,
Università di Firenze
Via L. Sacconi 6
50019 Sesto Fiorentino Italy
Tel.: 39 055 4574265
Fax: 39 055 4574253
pierattelli@cerm.unifi.it

Noemi Proietti
Istituto di Metodologie Chimiche, CNR
Via Salaria Km. 29,300
00016 Monterotondo Stazione (Roma) Italy
Tel.: 39 06 9067 2476
Fax: 39 06 9067 2477
noemi.proietti@imc.cnr.it

Stefano Provera
GlaxoSmithKline Research Center
Via Fleming 4
37135 Verona Italy
Tel.: 39 045 921 9860
Fax: 39 045 9218072
stefano.2.provera@gsk.com

Caterina Puccetti
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
forredeltreja@tin.it

Shaun Quill
JEOL (ITALIA) S.p.A.
Via dei Tulipani 1
20090 Pieve Emanuele (MI) Italy
Tel.: 39 02 9041 431
Fax: 39 02 9041 4343
quill@jeol.it

Luca Raiola
Dip. di Scienze Ambientali,
Seconda Università di Napoli
Via Vivaldi 43
81100 Caserta Italy
Tel.: 39 0823 274 637
Fax: 39 0823 274 605
luca.raiola@unina2.it

Simona Ravetta
Sapio Industrie
Via Senatore Simonetta 27
20040 Caponago (MI) Italy
Tel.: 39 02 957 05 610
Fax: 39 02 95743 571
s.ravetta@sapio.it

Teresa Recca
Istituto per lo Studio delle Macromolecole, CNR
Via Bassini 15
20133 Milano Italy
Tel.:
Fax:
teresa.recca@ismac.cnr.it

Raffaele Riccio
Dip. di Scienze Farmaceutiche,
Università di Salerno
Via Ponte Don Melillo 11c
84084 Fisciano (SA) Italy
Tel.: 39 089 962818
Fax: 39 089 962828
riccio@unisa.it

Silvia Ripoli
Dip. di Chimica e Chimica Industriale,
Università di Pisa
Via Risorgimento 35
56126 Pisa Italy
Tel.: 39 050 918298
Fax: 39 050 918260
silviar@dcci.unipi.it

Andrea Rodomonte
Dip. Farmaco,
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma Italy
Tel.: 39 06 4990 2345
Fax: 39 06 4990 2627
andrea.rodomonte@iss.it

Antonio Rosato
CERM & Dip. di Chimica,
Università di Firenze
Via L. Sacconi 6
50019 Sesto Fiorentino Italy
Tel.: 39 055 4574267
Fax: 39 055 4574253
rosato@cerm.unifi.it

Fernando Sancassan
Dip. di Chimica e Chimica Industriale,
Università di Genova
Via Dodecaneso 31
16146 Genova Italy
Tel.: 39 010 353 6120
Fax: 39 010 353 6118
fernando@chimica.unige.it

Paolo Santino
VARIAN S.p.A.
Viale Assunta 101
20063 Cernusco s/N (MI) Italy
Tel.: 39 02 9273 402
Fax: 39 02 9273 4025
paolo.santino@varianinc.com

Elisabetta Schievano
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5742
Fax: 39 049 827 5239
elisabetta.schievano@unipd.it

Michele Scian
Dip. di Chimica Organica,
Università di Padova
Via Marzolo 1
35131 Padova Italy
Tel.: 39 049 827 5324
Fax: 39 049 827 5239
michelescian@yahoo.it

Anca Serbescu
Institut für Makromolekulare Chemie,
Universität Freiburg
Stefan-Meier-Str. 31
79104 Freiburg Germany
Tel.: 49 761 203 6313
Fax: 49 761 203 6306
ancas@makro.uni-freiburg.de

Kirsten Severing
Institut für Makromolekulare Chemie,
Universität Freiburg
Stefan-Meier-Str. 31
79104 Freiburg Germany
Tel.: 49 761 203 6313
Fax: 49 761 203 6306
kirsten@makro.uni-freiburg.de

Roberto Simonutti
Dip. di Scienza dei Materiali,
Università di Milano - Bicocca
Via R. Cozzi 53
20125 Milano Italy
Tel.: 39 02 6448 5132
Fax: 39 02 6448 5400
Roberto.Simonutti@mater.unimib.it

Silvia Spera
Polimeri Europa
Via Fauser 4
28100 Novara Italy
Tel.: 39 0321 447425
Fax: 39 0321 447219
silvia.spera@polimerieuropa.com

Ottavia Spiga
Dip. di Biologia Molecolare,
Università di Siena
Via Fiorentina 1
53100 Siena Italy
Tel.: 39 0577 234911
Fax: 39 0577 234903
spiga@unisi.it

Alberto Spisni
Dip. di Medicina Sperimentale,
Università di Parma
Via Volturno, 39
43100 Parma Italy
Tel.: 39 0521 903807
Fax: 39 0521 903802
aspin@ipruniv.cce.unipr.it

Gábor Szalontai
NMR Laboratory,
University of Veszprém
Boglárka utca 11.
H-8200 Veszprém Hungary
Tel.: 36 88 422 022/ 4356
Fax: 36 55 421 869
Gabor.Szalontai@sparc4.mars.vein.hu

Marco Tatò
Dip. di Chimica, Pharmacia Italia
S.p.A.
Viale Pasteur 10
20014 Nerviano (MI) Italy
Tel.: 39 02 4838 3147
Fax: 39 02 4838 3965
marco.tato@pharmacia.com

Pierandrea Temussi
Dip. di Chimica, Università di Napoli
Via Cinthia
80126 Napoli Italy
Tel.: 39 081 674416
Fax: 39 081 674409
pat@chemistry.unina.it

Renato Toffanin
Istituto di Ricerca PROTOS
P.O. Box 972
34100 Trieste Italy
Tel.: 39 040 3994965
Fax: 39 02 70040 0633
toffanin@protos-institute.org

Alberta Tomassini
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
tomassinialb@hotmail.com

Silvio C. E. Tosatto
CRIBI e Dip. di Biologia,
Università di Padova
V.le G. Colombo 3
35121 Padova Italy
Tel.: 39 049 827 6165
Fax: 39 049 827 6159
silvio@cribi.unipd.it

Lucilla Turco
GlaxoSmithKline Research Center
Via Fleming 4
37135 Verona Italy
Tel.: 39 045 921 9432
Fax: 39 045 9218072
lucilla.2.turco@gsk.com

Raffaella Ugolini
Dip. Scientifico e Tecnologico,
Università di Verona
Ca' Vignal 1, Strada Le Grazie 15
37134 Verona Italy
Tel.: 39 045 802 7906
Fax: 39 045 8027 929
ugolinir@sci.univr.it

Maria Cristina Valerio
Dip. di Chimica,
Università di Roma "La Sapienza"
P.le Aldo Moro 5
00185 Roma Italy
Tel.: 39 06 4991 3576
Fax: 39 06 4455 278
mcrivalerio@yahoo.it

Aldrik Velders
Molteni Farmaceutici
S.S. 67
50018 Loc. Granatieri, Scandicci (Fi)
Italy
Tel.: 39 055 736 1330
Fax: 39 055 736 1288
a.velders@moltenifarma.it

Alessandra Vezzoli
Istituto di Bioimmagini
e Fisiologia Molecolare, CNR
Via Fratelli Cervi 93
20090 Segrate (MI) Italy
Tel.: 39 02 5033 0417
Fax: 39 02 5033 0414
Alessandra.Vezzoli@ibfm.cnr.it

Alessandro Villa
Istituto di Scienze e Tecnologie
Molecolari, CNR
Via Golgi 19
20133 Milano Italy
Tel.: 39 02 503 17090
Fax: 39 02 503 14139
a.villa@fastwebnet.it

Elena Vittadini
Dip. di Chimica Organica
e Industriale,
Università degli Studi di Parma
Parco Area delle Scienze 17/A
43100 Parma Italy
Tel.: 39 0521 905472
Fax: 39 0521 905413
evittadi@nemo.unipr.it

Anthony Watts
Dept. of Biochemistry,
University of Oxford
South Parks Road
OX1 3QU Oxford UK
Tel.: 44 1865 275268
Fax: 44 1856 275234
awatts@bioch.ox.ac.uk

Lucia Zetta
Istituto per lo Studio delle
Macromolecole, CNR
Via Bassini 15
20133 Milano Italy
Tel.: 39 339 3330871
Fax:
lucia.zetta@ismac.cnr.it