GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

Under the auspice of:
Gruppo Interdivisionale Risonanze Magnetiche (SCI)

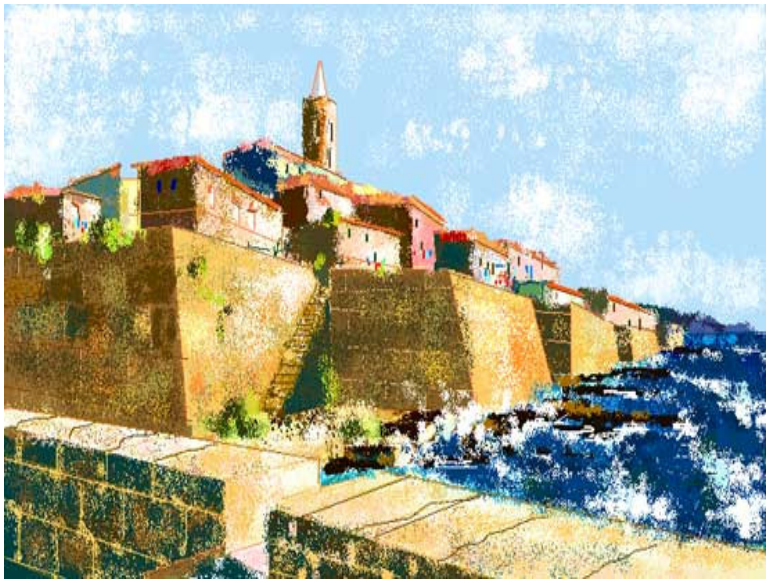
University of Sassari

CNR

University of Cagliari

Porto Conte Ricerche

Porto Conte 2004



XXXIV NATIONAL CONGRESS ON MAGNETIC RESONANCE

Porto Conte Ricerche September 21-24, 2004
Tramariglio (Alghero) - Sardegna (Italy)

www.gidrm.org

GIDRM

Gruppo Italiano Discussione Risonanze Magnetiche

XXXIV NATIONAL CONGRESS ON MAGNETIC RESONANCE

Porto Conte Ricerche - September 21-24, 2004
Tramariglio (Alghero) - Sardegna (Italy)

Scientific Program
Abstracts of the Contributions
Author Index
List of Participants

Under the auspice of:
Gruppo Interdivisionale Risonanze Magnetiche (SCI)

Porto Conte Ricerche

University of Cagliari

University of Sassari

CNR

The Congress has been made possible through the contributions of:

Organizing Committee

Scientific Committee

Lucia Zetta (President of the GIDRM, CNR, Milano)

Carla Marchioro (President of the GIRM, GSK, Verona)

Mauro Andrea Cremonini (UNI, Bologna)

Mauro Fasano (UNI-Insubria, Busto Arsizio (VA))

Stefano Mammi (UNI, Padova)

Mario Piccioli (UNI, Firenze)

Roberto Simonutti (UNI-Bicocca, Milano)

Marco Tatò (Medical Center, Nerviano, Milano)

Local Organizing Committee

Mariano Casu (UNI, Cagliari)

Gianni Cerioni (UNI, Cagliari)

Matilde Chessa (Portoconte Ricerche, Alghero (SS))

Nicola Culeddu (CNR, Sassari)

Costantino Floris (UNI, Cagliari)

Adolfo Lai (UNI, Cagliari)

Maria Antonietta Zoroddu (UNI, Sassari)

SCIENTIFIC PROGRAM

Tuesday, September 21

10:00-18:00 REGISTRATION, Centro Ricerche Porto Conte
18:00-20:00 WELCOME RECEPTION, Centro Ricerche Porto Conte

Wednesday, September 22 – Morning

9:00-9:20 OPENING REMARKS, Conference Room "Auditorium"
9:20-10:50 PLENARY LECTURES, Conference Room "Auditorium" Chair: R. Simonutti

9:20-10:05 **L. Emsley**, *École Normale Superieure de Lyon,France*
Order, disorder and dynamics from high-resolution solid-state NMR

10:05-10:50 **F. Conti**, *Università di Roma "La Sapienza"*
NMR in the post-genomic era: a holistic approach to the study of biological systems

10:50-11:20 COFFEE BREAK

11:20-12:50 PARALLEL SESSIONS

NMR IN BIOMEDICINE

Conference Room "Auditorium"
Chair: M. Gussoni

11:20-11:50 **R. Toffanin**
Istituto di Ricerca PROTOS, Trieste
T_2 quantitation of articular cartilage at 1.5 Tesla: comparison of standard multiecho and grase sequences

11:50-12:10 **C. Scarabino**
Università di Salerno, Baronissi (SA)
Investigation of archaeological bone and teeth by Nuclear Magnetic resonance spectroscopy and X-ray diffraction

12:10-12:30 **M. Sette**
Universià di Roma, Tor Vergata
Role of growth factors in the ontogenesis of pancreatic beta-cells

12:30-12:50 **C. Castro**
Università di Roma "La Sapienza"
NMR based metabonomic studies of transgenic maize

MATERIAL SCIENCE I

Conference Room "Nettuno"
Chair: R. Simonutti

11:20-11:50 **A. Comotti**
Università di Milano – Bicocca
Weak interactions in novel supramolecular frameworks identified by advanced 2D solid state NMR

11:50-12:10 **G. Szalontai**
University of Veszprém, Hungary
Routine ^{2}H NMR in partially oriented phases. Distinction of enantiomers, studies of conformational and anisotropic motions in ternary mixtures

12:10-12:30 **A. Serbescu**
Universitaet Freiburg, Germany
Chain order and dynamics in polymer melts revealed by proton double quantum NMR

12:30-12:50 **F. Presciutti**
Università di Perugia
EPR, SEM-EDS, XRD, MQ-MAS NMR characterization of fired iron bearing clays

12:50-14:30 LUNCH, Centro Ricerche Porto Conte

Wednesday, September 22 – Afternoon

14:30-16:20 PLENARY LECTURES, Conference Room "Auditorium", Chair: M. Fasano

14:30-15:15 **C. Dalvit,** *Nerviano Medical Science, Milano*
Reliable High-Throughput Functional Screening with 3-FABS

15:15-16:00 **L. Mannina,** *Università del Molise, Campobasso*
Characterization of olive oils by High Resolution NMR

16:00-16:20 **S. Hafner,** *Varian Deutschland GmbH, Darmstadt, Germany*
Solid-State NMR at High Magnetic Fields: Technical Challenges, Solutions and Applications

16:20-16:50 COFFEE BREAK

16:50-18:30 PARALLEL SESSIONS

METHODOLOGY I

Conference Room "Auditorium"
Chair: S. Spera

16:50-17:20 **E. Terreno**
Università di Torino
PARACEST agents: a novel class of contrast media for MRI applications

17:20-17:50 **S. Sykora**
Extra Byte,Castano Primo (Mi)
Field-Noise effects in NMR

17:50-18:10 **R. Fogh**
University of Cambridge, UK
Analysis and formatconverter – NMR software from CCPN

18:10-18:30 **M. Gombia**
Università di Bologna
Fracture 3D rendering and pore space properties quantification in reservoir rocks by MRI

FOOD SCIENCE

Conference Room "Nettuno"
Chair: M. Cremonini

16:50-17:20 **H.C. Bertram**
Inst. of Agricultural Sciences, Dk-Tjele, Denmark
NMR as a tool for studying and understanding meat quality

17:20-17:50 **R. Consonni**
ISMAC, CNR, Milano
Ageing indicators in balsamic vinergar: ^{1}H NMR studies

17:50-18:10 **S. Tiziani**
Ohio State University, USA
Separation and identification of extracts of regular and tangerine varieties of tomato juice by ^{1}H NMR

18:10-18:30 **A. Sobolev**
IMC, CNR, Roma
^{1}H High resolution NMR metabolite profiling of truffles (*Tuber Aestivum Vittadini*)

19:30-20:30 DINNER

20:30- GIDRM AND GIRM MEETINGS, Conference Room "Nettuno"
(with after-dinner drinks)

Thursday, September 23 – Morning

9:00-10:30 PLENARY LECTURES, Conference Room "Auditorium" Chair: S. Mammi

9:00-9:45 **G. Barbato,** *IRBM, Roma*
The HIV vaccine and the quest for neutralizing antibody-inducing immunogens: a structure-based rational design approach

9:45-10:30 **B. Maraviglia,** *Università di Roma "La Sapienza"*
i Multiple Quantum Coherences: new NMR tool to study materials and living organisms

10:30-10:50 **R. Kümmerle**, *BRUKER BIOSPIN AG, Fällanden, Switzerland*
Advances in Probehead Technology

10:50-11:20 COFFEE BREAK

11:20-12:50 PARALLEL SESSIONS

PROTEINS I

Conference Room "Auditorium"
Chair: M. Paci

11:20-11:50 **S. Ciurli**
Università di Bologna
Nickel and zinc trafficking at the crossroad: the case of urease

11:50-12:10 **D.O. Cicero**
Università di Roma "Tor Vergata"
Backbone dynamics of MLC1P reveals common features of the calmodulin superfamily

12:10-12:30 **M. Bellanda**
Università di Padova
NMR reveals remarkable properties of 3_{10} helices

12:30-12:50 **D. Picone**
Università di Napoli
Conformational preferences of Aβ-(1-42) and its core fragment (25-35) in polar and apolar media

MR IMAGING

Conference Room "Nettuno"
Chair: B. Maraviglia

11:20-11:50 **M. Gussoni**
Università di Milano
Nitric Oxide: a direct or indirect marker of physiological and pathological processes in biological systems

11:50-12:10 **G. Maddinelli**
Enitecnologie Spa, Milano
Study of flow behaviour and dynamics of large molecules by NMR imaging and relaxation

12:10-12:30 **S. Belfiore**
Università di Torino
MRI-Gd based contrast agents for targeting tumor cells

12:30-12:50 **G. Esposito**
Università di Torino
Gd-Loaded apofferitin as a new vector for delivery MRI contrast agents to specific targets

12:50-14:30 LUNCH, Centro Ricerche Porto Conte

Thursday, September 23 – Afternoon

16:50-18:30 PARALLEL SESSIONS

PROTEINS II

Conference Room "Auditorium"
Chair: N. Niccolai

14:20-14:50 **C. Isernia**
Seconda Università di Napoli
NMR structural study of zinc finger domain containing proteins

14:50-15:20 **T. Beringhelli**
Università di Milano
Ligand-Calycin interactions from the ligand point of view

15:20-15:40 **F. Innocenti**
IRBM, Roma
PRL-3, a phosphatase implied in cancer metastasis: Structure and function

15:40-16:00 **R. Ugolini**
Università di Verona
Liver basic fatty acid binding protein: stability properties as determined by NMR studies

MATERIAL SCIENCE II

Conference Room "Nettuno"
Chair: D. Capitani

14:20-14:50 **M. Geppi**
Università di Pisa
Solid-State NMR of drugs: a study of non steroidal anti-inflammatory drugs and their dispersion with polymeric carriers

14:50-15:20 **G. De Luca**
Università della Calabria
Structural and conformational orientational studies of mesogens and molecules dissolved in Liquid

15:20-15:40 **R. Anedda**
Università di Cagliari
^{29}Si NMR and FT/IR investigation of nanoparticle-silica interactions in a ZnO/SiO_2 Nanocomposite

15:40-16:00 **D. Donghi**
Università di Milano
Hydrogen bonding between BIS(Pentafluoro-phenyl)borinic acid and water: a low temperature NMR study

16:00-18:00 POSTER SESSION, Room "Anghelu Ruju" (with coffee break)

18:00-19:00 PLENARY LECTURE, Conference Room "Auditorium", Chair: L. Zetta

18:00-19:00 GIDRM-GIRM GOLD MEDAL: **L. Banci**, *Università di Firenze*
The NMR role in structural genomics: high throughput determination of solution structures of metalloproteins.

Friday, September 24 – Morning

9:00-9:45 PLENARY LECTURES, Conference Room "Auditorium", Chair: M. Piccioli

9:00-9:45 **M. Delepierre,** *Pasteur Institute, Paris, France*
NMR a tool for biology

9:45-10:45 BEST POSTERS, Conference Room "Auditorium", Chair: A. Spisni

9:45-10:05 1° Prize – **D. Maggioni** *Università di Milano*
LEWIS vs. BRØENSTED acidity in the system $B(C_6F_5)_3/H_2O/NEt_3$: an NMR study

10:05-10:25 2° Prize – **K. Severing** *Universität Freiburg*
^{2}H-NMR investigations on the biaxiality of liquid crystalline side-chain polymers

10:25-10:45 3° Prize – **L. Pinato** *Università di Padova*
Membrane topology of α-synuclein

10:40-11:15 COFFEE BREAK

11:15-12:55 PARALLEL SESSIONS

ORGANIC CHEMISTRY I

Conference Room "Auditorium"
Chair: S. Chimichi

11:15-11:45 **W. von Philipsborn**
University of Zürich, Switzerland
For 45 years in NMR research: some recollections and reflections

11:45-12:15 **L. Gomez-Paloma**
Università di Salerno
Molecular basic for the DNA-Alkylating properties of duocarmycins by NMR spectroscopy and DFT calculations

12:15-12:35 **L. Venturi**
Università di Bologna
Analysis of liquid mixtures by generalized correlation of chromatographic and NMR data

12:35-12:55 **M. Boccalini**
Università di Firenze
Unambiguous structure elucidation of the reaction products of 3-Quinolinonylenaminone with 1,2-dinucleophiles via NMR spectroscopy

METALS IN BIOLOGY

Conference Room "Nettuno"
Chair: T. Beringhelli

11:15-11:45 **G. Natile**
Università di Bari
Reactivity of Cytotoxic trans-Oriented Platinum Complexes towards Nucleotides and Single and Double-Stranded Oligonucleotides Investigated by [^{1}H, ^{15}N] HMQC NMR

11:45-12:15 **B. Jimenez**
Università di Firenze
^{13}C Direct Detection as a Tool for Structural Determination in Metalloproteins

12:15-12:35 **M.A. Zoroddu**
Università di Sassari
Cap43 protein: an attractive motif for Ni(II) in the C-terminal Domain

12:35-12:55 **F. Cesare Marincola**
Università di Cagliari
Multinuclear ^{31}P, ^{2}H, ^{23}Na NMR and polarized light microscopy investigations of divalent metal ion binding to liquid crystalline NaDNA

12:55-14:20 LUNCH, Centro Ricerche Porto Conte

Friday, September 24 – Afternoon

14:20-16:10 PLENARY LECTURES, Conference Room "Auditorium", Chair: H. Molinari

14:20-15:05 **B. Halle,** *Lund University,Sweden*
Biomolecular applications of magnetic relaxation dispersion

15:05-15:50 **B. Meier,** *ETH Hönggerberg, Zürich, Switzerland*
Solid-state MAS NMR: the faster the better?

15:50-16:20 COFFEE BREAK

16:20-17:30 PARALLEL SESSIONS

METHODOLOGY II

Conference Room "Auditorium"
Chair: R. Toffanin

16:20-16:50 **G.M. Contessa**
Università Tor Vergata - Roma
Measurement of different types of Residual Dipolar Coupling on a ^{15}N-^{13}C labeled protein and their contribution to the determination of a solution structure: application to the ApaG protein of unknown structure and function

16:50-17:10 **N. D'Amelio**
Università di Siena
SIMQUADNMR. A tool for getting insight into NMR of quadrupolar nuclei

17:10-17:30 **R. Lamanna**
ENEA CR Trisaia, Rotondella (MT)
Visualization of relaxation properties through the inversion of exponential decays

ORGANIC CHEMISTRY II

Conference Room "Nettuno"
Chair: M. Tatò

16:20-16:50 **D. Potenza**
Università di Milano
NMR in the free and bound state: a tool for the design of glycomimetics

16:50-17:10 **C. Coluccini**
Università di Bologna
Conformational studies by dynamic NMR.94. Cogwheel pathway for the stereomutations of durene derivatives containing the mesityl ring

17:10-17:30 **G. Uccheddu**
Università di Cagliari
Solution ($CDCl_3$) structure of bis(acyloxy) iodoarenes

17:40-18:00 CONCLUDING REMARKS, Conference Room "Auditorium"

20:30 SOCIAL DINNER

XXXIV National Congress on Magnetic Resonance

GIDRM-GIRM GOLD MEDAL

THE NMR ROLE IN STRUCTURAL GENOMICS: HIGH THROUGHPUT DETERMINATION OF SOLUTION STRUCTURES OF METALLOPROTEINS

Lucia Banci

Magnetic Resonance Center CERM and Department of Chemistry, University of Florence, Via Luigi Sacconi 6, 50019, Sesto Fiorentino, Florence, Italy

The knowledge of the genome of a continuously increasing number of organisms, and particularly that of humans, has opened the question of characterizing the products of such genomes. Protein expression and structure determination should exploit high throughput methods in order to address the study of the enormous amount of products of the genome. For this purpose Structural Genomics initiatives are developed worldwide. Our motto for the Structural Genomics Projects is "From Gene to Function through the Structure" (1). We want to reconcile the HTP approach to structure determination with the aim of understanding the protein cellular function. NMR spectroscopy is very well suited, not only for structure determination, but also for characterizing protein/protein interactions and for obtaining functional information.

Our research is focused on metalloproteins, which represent a large share of the proteome. Here our goal is to define an NMR methodology to elucidate metalloprotein structures as well as their interaction networks. The presence of metal ions bound to the protein requires a further step, i.e. the structural characterization of the metal site(s). In most of the cases, it is not possible to obtain direct information on the metal nucleus because it is either NMR silent, or it is quadrupolar or it is paramagnetic. Within our approach, when diamagnetic metal ions are present, EXAFS spectra can be exploited to obtain information on the metal coordination geometry. When metal ions are paramagnetic, i.e. they contain unpaired electrons, they produce dramatic effects on NMR spectra, affecting both chemical shifts and nuclear relaxation. These effects can be exploited to derive structural information. However, in the case of highly paramagnetic metal ions and/or of large molecular weight molecules, the paramagnetic effects on proton resonances are dramatic beyond signal detection. To overcome these problems we are developing specific hardware as well as NMR experiments which exploit direct heteronuclear detection and which avoid any proton detection, as heteronuclei are by far less affected by line broadening caused by paramagnetic effects and/or large molecular size.

The complete strategy for the characterization of metalloproteins has been applied, among other classes, to proteins involved in copper homeostasis in bacteria and eukaryotes, allowing us to determine the structures of both diamagnetic Cu(I)- and paramagnetic Cu(II)-binding proteins. In particular, several members of two large copper trafficking pathways to mitochondria and to the Golgi organelle have been structurally characterized and, in some cases, their interactions have been investigated. The structural characterization contributed to describe, at the molecular level, the mechanisms for copper ion uptake in the cells, copper transport and insertion in the final protein destination. In particular, proteins involved in copper delivery to the redox centers of cytochrome c oxidase have been located through an extensive and comparative search of the gene data banks and several proteins have been expressed, the structure determined, and possible pathways for copper transfer proposed. Similar achievements have been obtained for the proteins involved in copper transport to a multicopper oxidase inside the Golgi organelle. In the presentation methodological as well as functional aspects will be addressed and discussed.

Reference

1) L. Banci and A. Rosato (2003) Acc. Chem. Res. 36, 2

XXXIV National Congress on Magnetic Resonance

PLENARY LECTURES

THE HIV VACCINE AND THE QUEST FOR NEUTRALIZING ANTIBODY-INDUCING IMMUNOGENS: A STRUCTURE-BASED RATIONAL DESIGN APPROACH

G. Barbato[1], E. Bianchi[1], P. Ingallinella[1], J.L. Cole[2], D. Eckert[3], R. Geleziunas[4], M.D. Miller[4], X. Liang[4], W. Hurni[4], J. Joyce[4], D. Hazuda[4], P. Kim[4], J.W. Shiver[4], G. Ciliberto[1], R. Cortese[1], A. Pessi[1]

1 – IRBM, P. Angeletti, Via Pontina km 30.600, Pomezia 00040, Italy
2 – Dep. Mol. Cell Biol., Univ. of Connecticut, CT 06269, USA
3 – Dep. of Biochem. Univ. of Utah, Salt Lake City, UT 84132, USA
4 – Merck Research Laboratories, P.O. Box 4, West Point, PA 19486, USA

The HIV-1 gp41 envelope glycoprotein mediates fusion of the viral and host cellular membranes. Monoclonal antibodies targeting gp41 have been isolated, which display broadly neutralizing activity: these include 4E10, Z13 and 2F5. However, no immunogen so far has been able to generate equivalent antibodies *in vivo*. The core of the gp41 ectodomain undergoes a receptor-triggered conformational transition to form a six helix bundle coiled-coil structure. This trimer-of-hairpins species facilitates fusion of the viral and the host cell membranes. A key intermediate in this transition is the so-called "pre-hairpin intermediate". In this transient state, gp41 is anchored to both the target cell and the viral membranes via the N-terminal fusion peptide and the C-terminal transmembrane domain, respectively, but the six-helix bundle is not yet formed. Our rational, structure-based design seeks to combine structural characterization with traditional SAR, with the goal of producing immunogens which should mimic portions of the gp41 protein intermediates at various stages along the fusion pathway. The designed peptido-mimetics are used both to select for fusion-interfering, neutralizing mAbs *in-vitro,* and as immunogens in the attempt to elicit a neutralizing humoral response *in-vivo*. The progress made so far will be described.

NMR IN THE POST-GENOMIC ERA: A HOLISTIC APPROACH TO THE STUDY OF BIOLOGICAL SYSTEMS.

Filippo Conti

Dip. di Chimica, Università "La Sapienza", P.le Aldo Moro 5, Roma, Italy

The biological systems (cells, tissues, organs, animals and plants) can be assimilated to thermodynamic machines working with a wide range of reactions and transport processes that, by transforming thousands of substances, determine energy and matter fluxes essential for their life and functioning. The metabolism is the ensemble of chemical and physical processes, and from its knowledge we can determine the metabolic potential of a biological system in thermodynamic and kinetic terms.
Metabolites are the end products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to chemical or physical inputs, environmental changes, physio-pathological states, genetic modifications.
The whole metabolic profile and the inherent correlations between different pathways in biological system represent the metabolomics, an emerging and exciting post-genomic holistic approach that span the scope of medicine and biotechnology.
The potentiality of NMR coupled to multivariate data analysis is currently being exploited as a tool to provide metabolic information complementary to genomics and proteomics.
In this lecture some examples will be presented.

RELIABLE HIGH-THROUGHPUT FUNCTIONAL SCREENING WITH 3-FABS

Claudio Dalvit[1], Elena Ardini[2], Gian Paolo Fogliatto[2], Nicola Mongelli[1] and Marina Veronesi[1]

[1]Chemistry and [2]Biology Departments, Nerviano Medical Science, Viale Pasteur 10, 20014 Nerviano (MI), Italy

High Throughput Screening (HTS) is nowadays the mostly used approach within the pharmaceutical industry for identifying potential lead molecules. Unfortunately, the huge number of data generated with HTS since its first applications in the early 1990s did not translate into a significant increase of viable potential drug candidates. A number of important issues concerning the quality of the assays and the quality of the compound collections have emerged. Therefore, the emphasis in HTS today has shifted toward the design of assays that are more reliable and robust, that is, a quality imperative has arisen.
Recently, a method called 3-FABS (three Fluorine Atoms for Biochemical Screening) (1,2) has extended the capabilities of NMR allowing for rapid, high quality and reliable HT functional assay for the identification of inhibitors and for measuring with accuracy their 50% mean inhibition concentration (IC_{50}). The substrate is tagged with a CF_3 moiety and ^{19}F NMR spectroscopy is used for the detection of the substrate and product components. A comprehensive insight into the theory of 3-FABS, a discussion of its strengths and weaknesses when compared to other techniques used in HTS and a presentation of some of its applications to the screening of different enzymes and to multiple screening will be provided.

References

(1) C. Dalvit, E. Ardini, M. Flocco, G.P. Fogliatto, N. Mongelli and M. Veronesi*, J. Am. Chem. Soc.* (2003), 125, 14620-14625.
(2) C. Dalvit, E. Ardini, G.P. Fogliatto, N. Mongelli and M. Veronesi, *Drug Discovery Today* (2004), 9, 595-602.

NMR A TOOL FOR BIOLOGY

Muriel Delepierre

Unité de RMN de Biomolécules Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15 France

NMR and X-ray crystallography are the two of the most powerful tools available for structural determination and have been extensively used to obtain structural information on biomolecules. X-ray crystallography is limited by the necessity to obtain well-ordered crystals and the fact that in most cases structures are determined in a quasi-static state whereas NMR is somehow limited by the size of the molecule and the low sensitivity of the method, making these two techniques complementary. This complementary is particularly evidenced in the biological field. Indeed, NMR, which is particularly useful for determining the structure of small molecules, can also be used to obtain information on the dynamics of macromolecules over a wide time scale range and to study molecular interactions. However, NMR is inherently less sensitive than almost all other analytical methods as a result of the small energy gap between ground and excited states. Indeed, the minimal quantity that can be analysed by NMR is usually in the nanomol range or higher, precluding its routine use in trace analysis. Nevertheless, over the years NMR spectroscopists have learn to do more with less. The sensitivity of NMR has been improved considerably in the last decade by increasing the field strength — 900 MHz is now available — and by increasing the probe performances. The use of cryogenic probes increases the coil quality factor, improving sensitivity by four or five fold compared to conventional 5 mm probes. Sensitivity can be also improved by optimising the sample volume as a function of the specific availability or the solubility of a given sample.

The talk presented here will show how we use NMR to answer some of the biological questions raised by our collaborators. Examples will be given from ongoing projects that illustrate different aspects of NMR such as

(i) **molecular interaction studies** : Conformational studies of oligosaccharide and peptide mimics of the *O*-specific polysaccharide of *Shigella flexneri* 5a in aqueous solution and bound to monoclonal antibodies [1-3]

(ii) **structure determination with scarce amount of compound :** Structure determination of toxins using high resolution NMR at magic angle spinning [4-6]

(iii) **structure determination in relation to function** : Functional and structural studies of HasA, a heme-binding protein involved in a new iron acquisition system.[7-13]

References

1. Mulard L.A, Clément M.J, Segat-Dioury F, Delepierre M, Tetrahedron, 58, 2593 (2002); 2. Mulard L.A, Clément M.J, Imberty A, Delepierre M, Eur. J. Org. Chem., 2486 (2002) ; 3. Clément M.J, Imberty A, Mulard L.A, Phalipon A, Perez S, Simenel C. Delepierre M. J. Biol. Chem. 278, 47928 (2003) ; 4. Delepierre M, Prochnika-Chalufour A, Possani, L. D. Biochemistry 36, 2649 (1997) ; 5. Delepierre M, Prochnicka-Chalufour A, Boisbouvier J, Possani L.D. Biochemistry 38, 16756 (1999) ; 6. Kupce E, Kiefer P, Delepierre M. J. Magn. Reson., 148, 115 (2001) ; 7. Létoffé S, Ghigo, J.M, Wandersman, C. Proc. Natl. Acad. Sci. 91,9876(1994) ; 8. Izadi N, Henry Y, Haladjian J, Goldberg M.E, Delepelaire P, Wandersman C. *et al* Biochemistry 36, 7050 (1997) ; 9. Izadi-Pruneyre N, Wolff N, Redeker V., Wandersman C, Delepierre M, Lecroisey A. Eur. J. Biochem. 261, 562 (1999) ; 10. Izadi-Pruneyre N, Wolff N, Castagné C, Czisch M, *et al* J. Biomol., NMR 14, 193 (1999) ; 11. Arnoux P, Haser R, Izadi N, Lecroisey A, Delepierre M, Wandersman C. *et al*. Nat. Struct. Biol., 6, 516 (1999); 12. Wolff N, Deniau C, Létoffé S, Simenel C, Kumar V, Stojiljkovic I, Wandersman C *et al* Proteins Science 11 757 (2002) ; 13. Deniau C, Gilli R, Létoffé S, Izadi-Pruneyre N, Delepierre M, Wandersman C, *et al*. Biochemistry 42, 10627 (2003)

ORDER, DISORDER AND DYNAMICS FROM HIGH-RESOLUTION SOLID-STATE NMR

Lyndon Emsley

Laboratoire de Chimie, Ecole Normale Supérieure de Lyon, 69364 Lyon, France

In ordered molecular systems resolution is often limited by homogeneous broadening. Proton resolution is determined in rotating powders by the quality of homonuclear dipolar decoupling sequences. We will report the progress we have made in finding improved performance in homonuclear dipolar decoupling. We also show to obtain multi-dimensional proton spectra with very high resolution in all dimensions. The link between experimental ^{1}H spin diffusion curves and the X-ray crystal structure for the model organic compound β-Asp-Ala (12 protons) is investigated through a rate matrix analysis approach. Since dipolar coupling networks extend over relatively large distances, simulations require the molecule in its full crystal environment to obtain good agreement with experiment. The comparison between the experimental data and simulation is shown to depend strongly on the parameters of crystal structure, and quantitative aspects of structure determination by ^{1}H-^{1}H dipolar correlations in solids are discussed in detail. *In disordered chemical systems* resolution is limited by inhomogeneous broadening. We will report progress we have made in measuring structural parameters in disordered systems, including high-resolution chemical shift correlations and scalar *J* couplings. Finally, The determination of molecular dynamics in proteins is one of the key challenges in understanding their structure-function relationships. We will present measurements of nitrogen-15 nuclear longitudinal relaxation rates (R_1) in a micro-crystalline sample of the protein Crh, and provide a qualitative description of the site specific backbone dynamics in the solid-state.

BIOMOLECULAR APPLICATIONS OF MAGNETIC RELAXATION DISPERSION

Bertil Halle

Lund University, Sweden

During the past decade, our group has used multinuclear (^{1}H, ^{2}H, ^{17}O, ^{19}F, ^{23}Na, ^{87}Rb) magnetic relaxation dispersion (MRD) measurements to study the interaction of biomolecules (native and non-native proteins, nucleic acids) with small abundant molecules (water, cosolvents, denaturants, ions). This lecture gives an overview of the MRD method and describes several recent results on low-temperature protein hydration, solvation of non-native proteins, protein self-association, and DNA-ion interactions.

CHARACTERIZATION OF OLIVE OILS BY HIGH RESOLUTION NMR

[a, b]Luisa Mannina, [a] Marco D'Imperio, [b]Annalaura Segre

[a]Università del Molise, Facoltà di Agraria, Dip. S.T.A.A.M, 86100 Campobasso, Italy
[b]Ist.di Metodologie Chimiche, CNR, 00016 Monterotondo Staz., Roma, Italy

A review of our ^{1}H and ^{13}C NMR studies on edible oils is presented.

Authentication of olive oils. ^{1}H NMR can be used to detect olive oil adulteration with hazelnut oils. A detailed ^{1}H NMR protocol developed within the European Project "MEDEO" is presented.

Olive oil quality. ^{1}H NMR data allow a correlation between the olive oil free acidity and the di- and mono- glyceride content to be established. ^{13}C NMR data provide information on the fatty acid composition and the acyl distribution on the glycerol moiety, and allows esterified olive oils to be detected.

Geographical origin classification. ^{1}H NMR spectroscopy allows minor components present in olive oil to be analyzed. These components are useful to determine the geographical origin of olive oils. A proper choice of selected ^{1}H NMR data combined with a suitable multivariate statistical analysis allows the geographical selection.

Varietal classification of olive oils and pedoclimatic effect in olive oil composition. ^{13}C NMR and GC techniques combined with a multivariate statistical analysis allows monovarietal olive oils belonging to particular cultivars to be grouped. In order to find out which cultivar is the best to be grown in extreme climatic conditions, the composition of olive oils obtained from few matched Mediterranean cultivars grown in experimental fields located in Italy and Argentina has been checked. Mediterranean cultivars less affected by extreme pedoclimatic have been selected and selected plants can be proposed as colonizing plants in wild areas.

i MULTIPLE QUANTUM COHERENCES: NEW NMR TOOL TO STUDY MATERIALS AND LIVING ORGANISMS

Bruno Maraviglia

Department of Physics, University of Rome "La Sapienza" and E.Fermi Center Rome

The observable NMR signal , in conventional experiments, is generated by a single quantum coherence.This standard picture is based on some assumptions which in the last decade were thoroughly revised. Today it is well known that Multiple Quantum Coherences (MQc) can produce observable NMR signals also. Several MQc NMR techniques have in fact been developed, in order to gather new information about properties of liquids, not achievable with ordinary NMR procedures.
Multiple Quantum Coherences are due to residual dipolar interactions in liquids. Two main groups of such coherences exist: the intra-molecular multiple quantum coherences and the inter-molecular multiple quantum coherences (iMQc). In ordered systems, in which the molecular motion is anisotropic , dipolar and quadrupolar interactions are not averaged to zero. In this case, intra-molecular multiple quantum coherences can be formed and observed. When fast isotropic motion of the liquid molecules averages out the dipolar interactions, residual long range dipolar couplings between distant nuclei give rise to NMR signal due to inter-molecular multiple quantum coherences. A very interesting feature of the iMQc effect is its dependence on the distance of the coupled spins, which generate the NMR signal. These spins can be even at macroscopic distances.
In this report I will introduce the essential theoretical features of iMQc and show some experimental applications to materials and to biological systems.

References

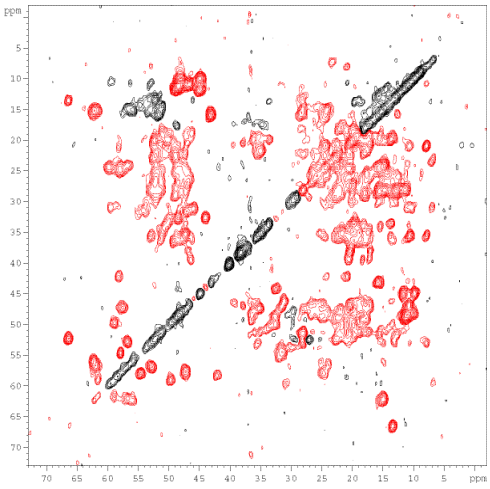
[1] W.Richter,S.Lee and W.S.Warren. Science 267,654 (1995)
[2] R.T.Branca,S.Capuani and B.Maraviglia. Concepts in Magn. Reson. 21A,22, (2004)

SOLID-STATE MAS NMR: THE FASTER THE BETTER?

Beat H. Meier,[‡] Matthias Ernst[‡], Ansgar Siemer[‡], Marcel Meier[‡], Rene Verel[‡], Tiit Tuhem[†] and Ago Samoson[†]

[‡]*Physical Chemistry, ETH Zurich, ETH-Hönggerberg, 8093 Zurich, Switzerland*
[†]*National Institute of Chemical Physics and Biophysics, Akadeemia Tee 23, Tallinn 12618, Estonia*

Recently, MAS Studies with rotation frequencies up to 70 kHz have become possible. While the classical CRAMPS (Combined Rotational and Multiple Pulse Spectroscopy) approach exploits a regime where the averaging by the multiple-pulse sequence appears on a shorter timescale than averaging the by the sample spinning, it becomes now possible to use the opposite approach where the MAS averaging is on the shorter timescale. The implications for pulse-sequence design will be discussed including the question "the faster the better?" and pulse sequences adapted to fast spinning will be presented,



DREAM spectrum at 40 kHz MAS of the catabolic repression HPr-like protein from bacillus subtilis (sample courtesy of Dr. Anja Böckmann)

Applications, in particular to proteins, will also be discussed, e.g. in the context of high-resolution spectroscopy (dipolar and adiabatic J-correlation spectra) in a de novo designed fibril (17-meric peptide) and in the prion protein HET-s (from the fungus Podospora anserina).

XXXIV National Congress on Magnetic Resonance

ORAL COMMUNICATIONS

MRI-GD BASED CONTRAST AGENTS FOR TARGETING TUMOR CELLS

S. Belfiore[1], A. Barge[1], C. Cabella[2], S. Geninatti-Crich[1], L. Tei[1], M. Visigalli[2], S. Aime[1]

[1]*Department of Chimica I.F.M., University of Torino, via P. Giuria 7, I-10125 Torino, Italy.*
[2] *Bracco Imaging,Bioindustry Park,Collaretto Giocosa,Torino,Italy*

The superb anatomical resolution of MR images has made this methodology the technique of choice in modern diagnostic investigations. The endogeneous contrast in a MR image arises mainly from differences in the relaxation times of tissutal water protons. It is now well established that the use of Contrast Agents (CA) can often contribute to strengthen the diagnostic potential of MRI. The CA in the current clinical practice display a non-specific distribution, being unable to recognize any molecular or cellular structure. The next generation of CAs is represented by systems able to recognize specific molecules which act as early reporters of a given pathology(1). In this study, the trasporters of nutrients (amino acids, glucose) or pseudo-nutrients (polyamines) have been considered as targets for the visualization of tumor cells. In fact, it is well established that tumor cells exhibit an overexpression or an up-regulation of such transporting proteins in order to accomplish the needs for the enhanced DNA synthesis and energy metabolism of proliferating cells compared with their non-transformed counterparts.
At the light of the above considerations, two types of CAs have been developed: the first aimed at targeting the Glutamine Transporting system as a representative example of nutrient substrates and the second the Spermidine Transporter as an example of the class of pseudo-nutrients. Therefore the targeting vectors have been bound, either directly or through a spacer, to a Gd(III) chelate. These paramagnetic complexes have been tested *in vitro* on two tumor cell lines, namely HTC (rat hepatoma cells), C6 (rat glioma cells), and on healthy hepatocytes to evidence the different uptake of these CAs.
The cellular internalization of the CA has been assessed either by relaxometry or by MRI. The content of internalized Gd has been determined by treating the cells with concentrated HCl at 120 °C (overnight).Upon this treatment, all Gd^{3+} is transformed into free aquo-ion whose concentration can be easily determined by measuring water proton T_1.
The obtained results show that selective uptake of these CA from tumor cells takes place in “in vitro” experiments and the most promising systems have been selected for further “in vivo” investigations.

Reference:

1) Aime S, Cabella C, Colombatto S, Geninatti Crich S, Gianolio E, Maggioni F.- J Magn Reson Imaging. 2002 Oct;16(4):394-406. Review

NMR REVEALS REMARKABLE PROPERTIES OF 3_{10} HELICES

M. Bellanda*, E. Schievano*, S. Mammi*; M. Rainaldi*, M. Crisma*, F. Formaggio*, Q.B. Broxterman‡, B. Kaptein‡, C. Toniolo*; P. Pengo*, L. Pasquato§ and P. Scrimin*

**Department of Chemical Sciences, University of Padova, Via F. Marzolo 1, 35131, Padova, Italy*
‡ DSM Research, Life Sciences, P.O. Box 18, 6160 MD Geleen, The Netherlands
§Department of Chemical Sciences, University of Trieste, Via L. Giorgieri 1, 34127, Trieste, Italy

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 α-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. The strong propensity of peptides rich in Aib (α-aminoisobutyric acid) and/or (αMe)Val (C^{α}-methylvaline) 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 .

We have synthesized a series of short peptides containing Aib and (αMe)Val, designed to fold in 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. A quantitative measurement of $^{3h}J_{NC'}$ scalar couplings through hydrogen bond [1] clearly revealed only the presence of hydrogen bonds typical of the 3_{10}-helix. All of the measured $^{3h}J_{NC'}$ are in the range 0.11-0.07 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. We showed that direct observation of hydrogen bonds by $^{3h}J_{NC'}$ scalar couplings is not only possible for α-helix and β-sheet peptides and proteins but for 3_{10}-helical peptides as well and that discrimination between α- *versus* 3_{10}-helices is also achievable by this methodology.

Very recently, the outstanding case of a peptide showing peculiar folding properties was published [2]. Not only it is highly folded, but also it exhibits the unique property that the type of helical conformation adopted is dependent on the polarity of the solvent. This peptide, very similar to one of those we have studied, has been characterized by circular dichroism in a series of solvents of different polarity and by molecular dynamics simulations in explicit water. It has been shown to reversibly fold in an α- or 3_{10}-helix in higher or lower polarity solvents, respectively. This result is particularly significant in light of the fact that the 3_{10}-helical conformation is indicated as a thermodynamic intermediate in α-helix folding by a considerable amount of converging evidence. Here, we present a preliminary NMR characterization of this peptide by determining its structure in different solvents.

References

[1]F. Cordier and S. Grzesiek J. Am. Chem. Soc. **121**, 1601-1602 (1999).
[2] P. Pengo, L. Pasquato, S. Moro, A. Brigo, F. Fogolari, Q.B. Broxterman, B. Kaptein and P. Scrimin, Angew Chem Int Ed Engl. **42**, 3388-3392 (2003).

LIGAND-CALYCIN INTERACTIONS FROM The LIGAND POINT OF VIEW

T. Beringhelli,[‡] S. Malaguti,[‡] E. Fontana,[†] A. Sportiello,[§] M. Galliano,[&] S. Capaldi,[$] M. Perduca,[$] H. L. Monaco,[$] L. Galluccio,[#] E. Gianazza,[#] I. Eberini[#]

[‡] Dip. C.I.M.A., Università degli Studi di Milano, via Venezian 21, I-20133 Milano, Italy, [†] PRBN-D Hoffmann-La Roche Grenzacherstrasse 12, CH-4070 Basel, Suisse, [§] Nerviano Medical Science S.r.l., Via Pasteur 10, I-20014 Nerviano (MI) Italy, [&] Dip. Biochimica "A. Castellani", Università degli Studi di Pavia, via Taramelli 3b, I-27100 Pavia, Italy, [$] Lab. di Biocristallografia, Università degli Studi di Verona, Strada Le Grazie, I-37100 Verona, Italy, [#] Dip. Scienze Farmacologiche, Università degli Studi di Milano, via Balzaretti 9, I-20133 Milano, Italy

Calycins are a superfamily of proteins able to bind small hydrophobic molecules. They show a common β-barrel folding [1] and the internal calyx, comprised by the β-strands, is the main binding site of the ligands. Our previous studies on the interaction of long chain fatty acids with two calycins, namely bovine β-lactoglobulin (BLG), a lipocalin, [2] and a fatty acid-binding protein obtained from chicken liver (Lb-FABP) [3], have shown that the binding is modulated by pH and ionic strength. We have now exploited the high sensitivity of ^{19}F to test the binding of the same proteins towards fluorine containing drugs. The tested molecules belong to different therapeutical classes and their structures range from simple aromatic rings to substituted indenic and indolic fragments to steroidal skeletons. The aim of these studies was to check the potential selectivity of each protein for a specific structural moiety.

Differently from long-chain fatty acids, the solubility of these drugs in phosphate buffer can be significant. Evidence of binding was obtained through the comparison of the relaxation parameters of the ligands (T_1 and heteronuclear n.O.e.) in the presence and in the absence of the proteins. Competition experiments ranked the relative affinities and screening among analogs allowed to point out the relevance of specific substituents for the interaction.

Molecular docking prediction of the interactions between BLG and the same ligands, based on a Monte Carlo/simulated annealing procedure, supported the experimental findings. *In silico* work is in progress for studying also the behavior of Lb-FABP.

References

[1] L. J. Banaszak, N. Winter, Z. Xu *Adv. Protein Chem.* **70**, 89-151 (1994)

[2] T. Beringhelli, I. Eberini, M. Galliano, A. Pedoto, M. Perduca, A. Sportiello, E. Fontana, H. L. Monaco, E. Gianazza *Biochemistry,* **41**, 15415-15422 (2002).

[3] T. Beringhelli, L. Goldoni, S. Capaldi, A. Bossi, M. Perduca, H. L. Monaco *Biochemistry* **40**, 12604-12611 (2001).

NMR AS A TOOL FOR STUDYING AND UNDERSTANDING MEAT QUALITY

H.C. Bertram

Danish Institute of Agricultural Sciences, Department of Food Science, Research Centre Foulum, P.O. Box 50, Dk-8830 Tjele, DENMARK

Consumers' awareness of food quality has never been more pronounced. Meat forms a substantial part of the food consumption, and accordingly techniques to control the quality of meat are needed. In addition, a better understanding of how basic biochemical and biophysical factors influence the final meat quality is also required for optimization of the quality.

Nuclear Magnetic Resonance (NMR) has during recent years gained increasing use within different areas of muscle physiology and meat science. NMR ^{1}H relaxation methodologies enable detection of the mobility of protons in heterogeneous materials and thereby provide possibilities for a characterization of properties of water, which is the main constituent of muscle-based foods and of significance for the over-all texture and structure and for the sensory attributes of these food items. NMR spectroscopy allows detection of different nuclei, which can be used to identify various metabolites in the muscles of significance for the subsequent quality development. Accordingly, NMR spectroscopy is a powerful tool in improving the understanding of the relationship between fundamental biochemical factors and the meat quality.

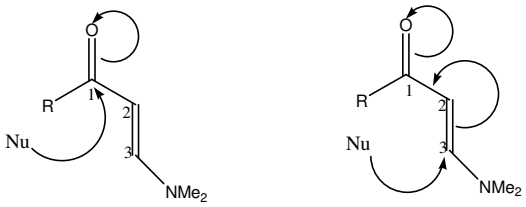
In this presentation an overview of present applications of NMR ^{1}H relaxation and NMR spectroscopy (^{1}H, ^{31}P and ^{13}C) in the determination of various meat quality attributes will be given, and also the use of NMR methodologies to improve the understanding of basic biochemical and biophysical mechanisms substantial for quality development is described. Recently developed magic angle turning techniques, which further enhance the potential of NMR spectroscopy for characterization of meat quality, are also considered.

UNAMBIGUOUS STRUCTURE ELUCIDATION OF THE REACTION PRODUCTS OF 3-QUINOLINONYLENAMINONE WITH 1,2-DINUCLEOPHILES *via* NMR SPECTROSCOPY

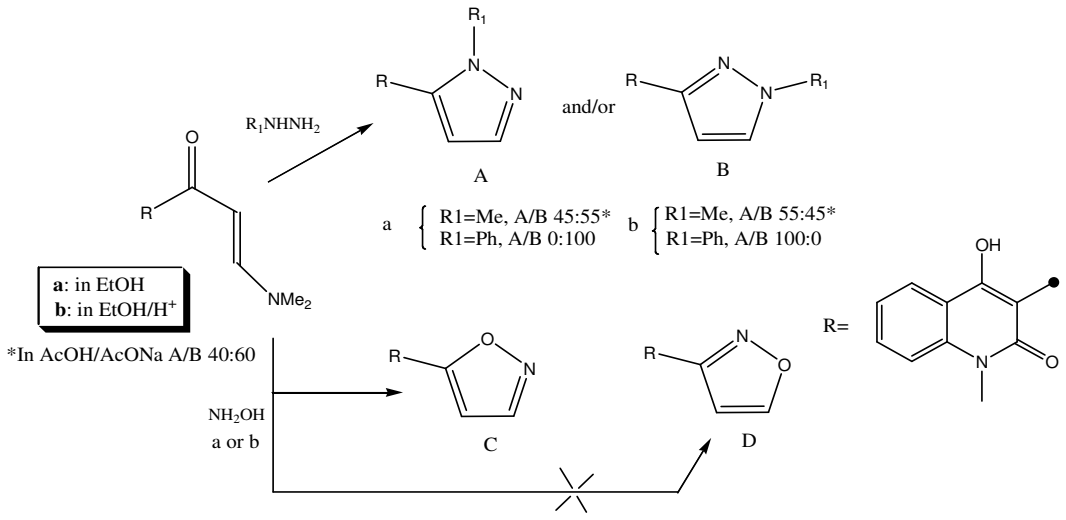
S. Chimichi, M. Boccalini and M. M. Hassan

Organic Chemistry Department, University of Firenze, Via della Lastruccia 13, Sesto F.no, Italy

Enaminones, chemical compounds consisting of an amino group linked to a carbonyl through a C=C, possess both electrophilic and nucleophilic properties and are useful intermediates in the synthesis of heterocycles, such as pyrazoles, isoxazoles ecc. It has been recently reported that in these pushpull ethylenes (*e.g.* 3-dimethylaminopropenoates) C-3 and C-1 are typical electrophilic positions, C-3 being more reactive than C-1. Thus, many papers reporting reactions of 3-dimethylpropenoates with a variety of nucleophiles ends with dimethylamine substitution products [1].



We report here the reaction of a quinolinonyl enaminone with hydrazine, methyl- and phenyl-hydrazine in which the regiochemistry is function of the starting material and of the reaction conditions. No regioisomers were observed employing hydroxylamine. Overall yields are good (60-90%); structure determinations *via* NMR (NOESY1D, gHSQC, gHMBC, ecc.) for all compounds will be illustrated (*e.g.* structure C or D?).



References

[1] B. Stanovnik and J. Svete *Chem. Rev*. **104**, 2433-2480, (2004) and references therein

NMR BASED METABONOMIC STUDIES OF TRANSGENIC MAIZE.

C. Manetti,[‡] C. Bianchetti,[‡] L.Casciani, C. Castro,[‡] G. D'Ascenzo,[‡] M. Delfini,[‡] M.E. Di Cocco,[‡] A. Laganà,[‡] M. Motto,[†] and F.Conti[‡]

[‡]Dipartimento di Chimica Università degli Studi di Roma "La Sapienza", Piazzale Aldo Moro 5 00185 Roma, Italia. [†]Istituto Sperimentale per la Cerealicoltura, Sez. Bergamo, Via Stezzano 24 24126 Bergamo, Italia

Plants are sessile systems unable to escape environmental pressures. As a result, they have evolved to a dazzling array of flexibility in their responses to environmental conditions such as light/dark, drought, temperature, nutritional supply, microbial invasion. Thus, the plant system comprises a genotype by environmental responses, producing a specific gono-phenotype relationship that is heavily dependent on the growth stage and several studies are performed to investigate this kind of "perturbation".

Maize is one of the major crop plant with essential agronomical interest and a model plant for genetic studies. Our study concerns changes in metabolite profiles of maize in response to alterated gene and protein expression.

In the present research, two major points were investigated: 1)characterization of the metabolite profiles induced by genic modification; 2)the possibility of recognising and classifing the different maize inbred lines, by ^{1}H-NMR spectra analysis of their hydro-alcoholic extracts, applying multivariate data analysis, in particular Principal Component Analysis (PCA) and Partial Least Squares (PLS).

MULTINUCLEAR ^{31}P, ^{2}H, ^{23}Na NMR AND POLARIZED LIGHT MICROSCOPY INVESTIGATIONS OF DIVALENT METAL ION BINDING TO LIQUID CRYSTALLINE NaDNA

A. Catte,[†] F. Cesare-Marincola, [†] J. R. C. van der Maarel,[‡] G. Saba,[†] A. Lai[†]

[†]*Dipartimento di Scienze Chimiche, Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, S.S. 554, 09042 Monserrato, Cagliari, Italy,* [‡]*Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands*

Very little is known about the interaction of divalent metal ions and DNA in concentrated polyelectrolyte solutions (hundreds of mg ml^{-1}), where DNA rods spontaneously condense into ordered forms, namely liquid crystalline (lc) phases. Since these molecular rearrangements are very similar to those observed in vivo, investigating the Me^{2+}-lcDNA binding is particularly attractive for understanding the influence of the double helix (de)stabilization by the metal ion interaction on the aggregation of DNA molecules in nature. In this connection, we have analysed the effects of the competitive binding of Mg^{2+}, Cd^{2+}, or Ni^{2+} with the physiological DNA counterion Na^{+} on the liquid crystalline state of cholesteric DNA (molar ratios Me^{2+}/DNA-phosphate ≤ 0.050) by polarised light microscopy and multinuclear ^{31}P, ^{2}H, and ^{23}Na NMR. The overall results indicated unambiguously that the state of cholesteric NaDNA is altered in the presence of $MgCl_2$, $CdCl_2$, or $NiCl_2$ at the same concentration in a different way and to a different extent. In particular, the addition of Mg^{2+} promoted notably the formation of an isotropic phase from the cholesteric one, the presence of Ni^{2+} affected the arrangement of the cholesteric phase mainly by reducing the pitch, while effects strongly reminding those of either Mg^{2+} and Ni^{2+} were observed in the presence of Cd^{2+}. ^{23}Na NMR spectroscopy also showed differences between the binding behaviour of Mg^{2+} and the transition metal ions in the counterion atmosphere around DNA. The results are discussed in terms of different binding modes of the metal ions.

BACKBONE DYNAMICS OF MLC1P REVEALS COMMON FEATURES OF THE CALMODULIN SUPERFAMILY

Daniel O. Cicero[1], Matteo Pennestri[1], Pamela Bielli[2],Alessia Santoprete[1] , Sonia Melino[1]
Antonella Ragnini- Wilson[2] and Maurizio Paci[1]

[1]Department of Chemical Science and Technology, University of Rome "Tor Vergata", via della Ricerca 1, 00133 Rome, Italy. [2.]Dipartimento di Biologia University of Rome "Tor Vergata" via della Ricerca Scientifica, Italy

The EF-hand structural motif was first discovered in the crystal structure of parvalbumin by Kretsinger and co-workers in 1973 (1). It consists of two perpendicularly placed α-helices (for example, helices E and F in parvalbumin) and an interhelical loop, which together form a single Ca^{2+}-binding site. This relatively simple structural motif has been identified in numerous other proteins since, many of which function as Ca^{2+} sensors in the cell. For this reason, these proteins are often named as intracellular Ca^{2+} binding proteins (CaBPs). Ca^{2+}-sensor proteins such as calmodulin (CaM) and troponin C (TnC) enable the cell to detect a stimulatory influx of Ca^{2+} and thereby translate this signal into a variety of cellular processes that often require a rapid response. The mechanism of this molecular switch lies in the conformational change induced by Ca^{2+} binding. Extensive studies have been performed to elucidate the mechanism by which small EF-hand proteins bind in a Ca^{2+}-dependent manner to target proteins (2). Much less is known about the signals that control the Ca^{2+}-independent binding of calmodulin (CaM), ELC or RLC to IQ motifs. This type of interaction is essential for the activity of essential proteins such as myosin motors, IQGAP-like and Ras-GEF signalling proteins.
In the present work we will focus on the backbone dynamics of Mlc1p, a member of the calmodulin (CaM) superfamily involved in vesicle-motor anchoring and movement in yeast cells (3). Although it presents structural homology with CaM, Mlc1p cannot bind Ca^{2+}. There is no structure available for this protein uncomplexed in solution, and we have tackled the structural characterization via NMR of a recombinant Mlc1p sample. ^{15}N relaxation measurements were used to discover regions of the protein showing flexibility that can be linked with its biological activity. These results will be compared with those found for Ca^{2+}-CaM and apo-CaM.

References

[1] R.H. Kretsinger, and C. E.Nockolds, *J Biol Chem* **248**, 3313-26 (1973)
[2] M. S. Cyert *Annu Rev Genet* **35**, 647-72 (2001)
[3] W. Wagner , P. Bielli, S. Wacha and A. Ragnini-Wilson *Embo J,* **21,** 6397-408 (2002)

NICKEL AND ZINC TRAFFICKING AT THE CROSSROAD THE CASE OF UREASE

Stefano Ciurli

Laboratory of Bioinorganic Chemistry, Department of Agro-Environmental Science and Technology, University of Bologna, Italy

The role of metallo-chaperones in the activation of urease, the enzyme responsible for the mineralization of organic nitrogen, consists in the insertion of two essential Ni^{2+} ions in the preformed active site of the apo-protein, without the concurrent loss of this toxic metal ion in the cytoplasm. The structural basis for this functional role has been the focus of the research conducted by our group in the past few years [1-5]. In particular, the structure - function relationships of UreE and UreG have been investigated. UreE appears to act as the soluble simultaneous carrier of both Ni^{2+} ions required for enzyme activation as well as of Zn^{2+} ions. On the other hand, UreG is an intrincally unfolded protein (IUP) acting as a GTPase only in the presence of Zn^{2+} and other chaperones -UreD and UreF - in a complex where the correct fold of UreG is attained (see Fig. 1). The lecture will illustrate the multifaceted approach undertaken to demonstrate such conclusions, an approach which has entailed the application of molecular biology techniques xoupled with NMR and XAS spectroscopies, as well as metal ion titration, fluorescence and mass spectrometry, and molecular modeling. The interplay of Zn^{2+} and Ni^{2+} trafficking will be discussed.

References

[1] H. Remaut, N. Safarov, S. Ciurli and J. J. Van Beeumen *J. Biol. Chem.* **276**, 49365-49370 (2001)
[2] S. Ciurli, N. Safarov, S. Miletti, A. Dikiy, S. K. Christensen, K. Kornetsky, D. A. Bryant, I. Vandenberghe, B. Devreese, B. Samyn, H. Remaut and J. J. Van Beeumen *J. Biol. Inorg. Chem.* **7**, 623-631 (2002)
[3] F. Musiani, B. Zambelli, M. Stola and S. Ciurli *J. Inorg. Biochem.* **98**, 803-813 (2004)
[4] B. Zambelli, M. Stola, K. De Vriendt, B. Samyn, B. Devreese, J. J. Van Beeumen, P. Turano, A. Dikiy, D. A. Bryant and S. Ciurli submitted

CONFORMATIONAL STUDIES BY DYNAMIC NMR. 94. COGWHEEL PATHWAY FOR THE STEREOMUTATIONS OF DURENE DERIVATIVES CONTAINING THE MESITYL RING[1].

Carmine Coluccini, Stefano Grilli, Lodovico Lunazzi, and Andrea Mazzanti*

Department of Organic Chemistry "A. Mangini", University of Bologna, Viale Risorgimento, 4 Bologna 40136, Italy

The low temperature NMR spectra of 1,4-bis-(mesitoyl)durene, **1** (fig.1.) and of 1,4-bis-(mesitylethenyl)durene, **2** (fig.1.) reveal the presence of *syn* and *anti* rotamers at the equilibrium, their relative proportions depending on the dielectric constant of the solvent. In solution the more stable rotamer of **1** is the *anti* whereas, in the case of **2**, the more stable is the *syn*. Depending on the crystallization solvent employed the more (*anti*) and the less stable (*syn*) rotamers were both observed (X-ray diffraction) in the solid state of **1**. On the other hand only the less stable rotamer (*anti*) was found to be present in the solid state of **2**. As shown by MM calculations, the *syn* to *anti* interconversion occurs via a correlated process (cogwheel pathway) involving the mesityl-C and durene-C bond rotations: the dynamic NMR technique yields an experimental barrier of 8.2 kcal mol^{-1} for **1** and 13.1 kcal mol^{-1} for **2**. In the case of derivative **2** a second barrier, due to a second type of correlated rotation process (torsion), was also determined (8.6 kcal mol^{-1}). As a consequence of the restriction of this second torsional motion the *anti* rotamer of **2** displays two distinguishable NMR spectra at –133° C, corresponding to a pair of conformers with different symmetry (*anti* C_i and *anti* C_2)

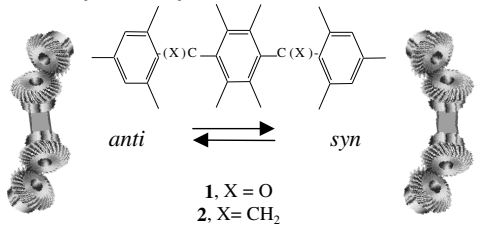


fig.1.

Reference

Coluccini C., Grilli S., Lunazzi L., and Mazzanti A.,*J. Org. Chem.* **200**3, *6*8, 7266-7273

WEAK INTERACTIONS IN NOVEL SUPRAMOLECULAR FRAMEWORKS IDENTIFIED BY ADVANCED 2D SOLID STATE NMR

A. Comotti, R. Simonutti, S. Bracco, P. Sozzani

Department of Materials Science, University of Milano-Bicocca, Italy

2D (^{1}H-^{13}C) HETCOR experiments performed under Fast Magic Angle Spinning (15 kHz) and Phase Modulated Lee-Goldburg homonuclear decoupling allow us to obtain high resolution both in the hydrogen and carbon domains. The integration of the 2D experiment with variable cross-polarization times that rule the communication between the specific spins permit to probe through-space host-guest interactions. In particular, the advanced 2D solid state NMR techniques reveal, through cross-peak intensities, weak intermolecular interactions that sustain nanostructures of polyconjugated molecules (Fig. 1) entrapped as guests in channels formed by an aromatic host (TPP) [1,2]. In addition, the guest molecules aligned parallel to the fully aromatic nanochannel experience intense aromatic ring currents and strong anisotropic diamagnetic susceptibility effects that produce upfield shifts on the guest hydrogen and carbon chemical shifts. Proton resonances shifted 2 ppm upfield indicate the exact topology of the guest hydrogens above the plane of host benzene at a distance of 2.5 Å, as calculated by nucleus-independent chemical shift maps. This data combined with the PMLG 2D HETCOR evidence of the proton proximity to aromatic carbons show that the hydrogens are in a critical position to establish favourable energetic interactions with the π-system and explain the high melting temperature of the adducts. The structures cannot be derived by X-ray diffraction due to the dynamics of the guest molecules in the confined spaces. In conclusion, we have recognized by multinuclear solid state NMR the role of CH•••π interactions cooperating to fabricate novel nanostructured materials that shape exceptionally stable aromatic nanochannels and include polyconjugated molecules of interest for electro-optical applications.

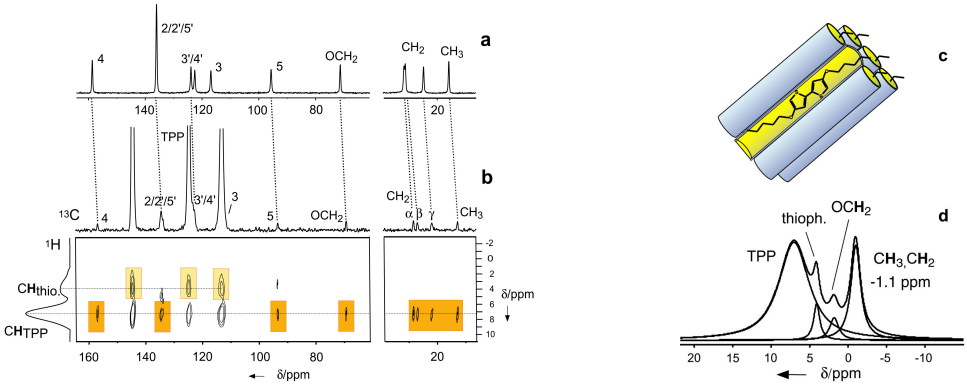


Fig. 1. a) ^{13}C Ramped-CP MAS spectrum of the 4,4'''-dipentoxy-2,2':5',2'':5'',2'''-tetrathiophene. b) Contour plot of 2D PMLG decoupled heteronuclear (^{1}H-^{13}C) dipolar correlation spectrum of the adduct. c) schematic representation of 4,4'-dipentoxy-2,2'-dithiophene confined to the aromatic nanochannels.
d) ^{1}H MAS NMR spectrum of the adduct with a spinning speed of 15 kHz.

References

[1] P. Sozzani, A. Comotti, S. Bracco, and R. Simonutti Angew. Chem. Int. Ed. 43, 2792 (2004).
[2] P. Sozzani, A. Comotti, S. Bracco, and R. Simonutti *Chem. Comm.* 768 (2004).

AGEING INDICATORS IN BALSAMIC VINERGAR: ^{1}H NMR STUDIES

R. Consonni,[‡] A. Gatti[†]

[‡]*Istituto per lo Studio delle Macromolecole, Lab. NMR, CNR, v. Bassini 15, 20133 Milano, Italy.* [†]*Divisione Sensory & Costumer Analysis, NEOTRON S.p.A., Stradello Aggazzotti 104, 41010 S.Maria di Mugnago – Modena, Italy*

Vinegar is a food product obtained by microbiological fermentation of ethanol rich substrates whose origin and composition characterize the final product. Balsamic and traditional balsamic vinegars are obtained from cocked must of selected grapes, following the disciplinary indications. The qualitative characteristics and the peculiarity of these products are essentially dependent from the production procedures and some specific metabolites can be found and used as age markers. Different analytical approaches have been used for ageing discrimination [1-3]: ^{1}H NMR spectroscopy in combination with multivariate statistical methods can give insights into the aging process and asses the quality of the final product [4]. Preliminary studies and comparisons with other approaches are presented.

References

[1] Chiavaro, E.; Caligiani, A.; Palla, G. *It. J. Food Sci*. **10**, 329-337 (1998)
[2] Tesfaye, W.; Morales, M. L.; Garcý`a-Parilla, M. C.; Troncoso, A. M.. *J. Agric. Food Chem.* **50**, 5255-5261 (2002)
[3] Cocchi, M.; Lambertini, P.; Mancini, D.; Marchetti, A.; Ulrici, *J. Agric. Food Chem.* **50**, 7053-7061 (2002)
[4] R. Consonni, A. Gatti *J. Agric. Food Chem.* **52**, 3446-3450 (2004)

MEASUREMENT OF DIFFERENT TYPES OF RESIDUAL DIPOLAR COUPLING ON A ^{15}N-^{13}C LABELED PROTEIN AND THEIR CONTRIBUTION TO THE DETERMINATION OF A SOLUTION STRUCTURE: APPLICATION TO THE ApaG PROTEIN OF UNKNOWN STRUCTURE AND FUNCTION

G.M. Contessa[b,a], T. A. Pertinhez,[¥], F. Marcocci[a], A. M. Katsuyama, [†,¥], M. Paci [a,b], A. Spisni,[¥,§] C.S.Farah[†] and D.O. Cicero[a,c].

[a]Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", via della Ricerca Scientifica, 00133 Rome Italy. [b]INFM, University of Rome "Tor Vergata". [c]SISSA, Settore di Biofisica, Trieste, Italy. [†] Department of Biochemistry, Institute of Chemistry, University of Sao Paulo, Brazil. [¥] BioNMR Laboratory, Center for Structural Molecular Biology, LNLS, campinas, Brazil. [§] Sect. Chemistry and Structural Biochemistry, Department of Experimental Medicine, University of Parma, Italy.

Heteronuclear experiments on ^{15}N-^{13}C labeled samples for the measure of residual dipolar couplings are nowadays an essential step in the determination of the solution structure of proteins and DNA fragments by NMR. ApaG is a protein of unknown structure and function produced by *Xanthomonas axonopodis* pv *citri* (*Xac*) [1], the agent of citrus canker, which causes a severe impact on the economy of Brazil that is one the of the world's leader producer of citrus crop. Since the protein does not present significant sequence homology with any known protein, different types of residual dipolar couplings were collected to assist in the refinement of its solution structure. The protein alignment was obtained using a liquid crystalline solution consisting of filamentous phages and a series of 3D heteronuclear NMR experiments was carried out on a ^{15}N, ^{13}C double labelled protein. In particular: a 3D HNCO spectrum was recorded to measure the ^{1}H dipolar couplings, $^{1}D_{HN}$; a 3D HN(CO)CA was used to measure the $^{1}J_{C\alpha H\alpha}$ and finally, a 2D HSQC without ^{13}C' 180° decoupling pulse during the ^{15}N evolution allowed to calculate the $^{1}J_{C'N}$ and $^{1}J_{C'HN}$. All measurements were performed with the IPAP approach [2]. This procedure implies the acquisition of two subspectra with the antiphase and in-phase doublets, summing and subtracting the two spectra in order to reduce peak superimposition.
All those data were implemented in the simulated annealing protocol. As a reliable initial guess for the axial component of the tensor D_a we used $(1+0.2)D_{max}/2$. A simulated annealing refinement was performed in combination with a grid search to ascertain the value of the rhombicity R [3]. Residual dipolar couplings turn to be the only long range restraints on protein scale, and they were determinant in regions with lack of NOE-type information, like surface exposed loops.

Acknowledgments: The work was partly supplied by MIUR Cofin (Italy) and FAPESP (Brazil)

References

[1] Katsuyama A.M., Cicero D.O., Spisni A., Paci M., Farah C.S., Pertinhez T.A. (2004) J Biomol NMR. 29, 423-4
[2] Bax, A., Kontaxis, G., Tjandra, N. (2001) Methods Enzymol. 339, 127-74.
[3] Clore, G.M., Gronenborn, A.M. & Tjandra, N. (1998) J. Magn. Reson. 131, 159-162.

SIMQUADNMR. A TOOL FOR GETTING INSIGHT INTO NMR OF QUADRUPOLAR NUCLEI

Nicola D'Amelio, Elena Gaggelli, Elena Molteni, Gianni Valensin

Department of Chemistry and the NMR Center, University of Siena, Via A.Moro, 53100 Siena, Italy

Although around two thirds of nuclei usually quoted in NMR tables have spin $I > ½$, the vast majority of studies has been limited to the relatively few $I = ½$ nuclei. However, the interest in some $I > ½$ nuclei (especially form the biological point of view), such as ^{2}H, ^{17}O, ^{23}Na, ^{25}Mg, ^{33}S, ^{35}Cl, ^{39}K, ^{43}Ca, has attracted several investigators who provided many ways of facing the intrinsic complexity of such NMR studies [1-4].
The interpretation of NMR data for the study of the atomic properties of systems containing quadrupolar nuclei, although potentially highly informative due to the large number of parameters involved in both the observed shifts and relaxation rates, is generally hindered by the complexity of the equations describing these phenomena, especially in the case of nuclei with high nuclear spin quantum number, because of the increased number of atomic levels and therefore possible transitions.
A tool for interpreting the experimental spectra in terms of physical parameters (e.g. viscosity of the solution, charge distribution symmetry around the nucleus, molecular weight of the system) and the effects of pulses and delays could therefore be useful for unraveling the chemical processes under investigation.
The presence of slow motions on the NMR time scale (that is when the system is outside the extreme narrowing limit) allows the detection of multiple-quantum coherences which can be originated by the non-monoexponentiality of the relaxation rates or in cases of oriented systems. Such coherences are narrower than the corresponding single-quantum coherence and can be used for a variety of applications. For example, multiple quantum filtered (MQF), and in particular triple quantum filtered (TQF) experiments have been extensively used to reduce the large signal due to extracellular ^{23}Na seen in single quantum (SQ) spectra [5, 6].
SIMQUADNMR is a program which simulates NMR MQF spectra of quadrupolar nuclei based on the physical parameters of the system (motional correlation time, exchange rates, etc.) and on the experimental conditions (magnetic field, pulses and delays) [7]. The program can be used for estimating the possibility to run one kind of experiment (and in case optimize the experimental parameters) or for interpreting from a physical point of view the experimental spectra.

References

[1] R. Kemp-Harper, S.P. Brown, C.E. Hughes, P. Styles, S. Wimperis, *Prog. NMR Spectr.* 30, 157-181 (1997). [2] M.E. Smith, E.R.H. van Eck, *Prog. NMR Spectr.* 34, 159-201 (1999); [3] L.A. Jelicks, R.K. Gupta, *J. Magn. Reson.* 81, 586-592 (1989); [4] A. Lehoux, M. Krzystyniak, E. Baguet, *J. Magn. Reson.* 148, 11-22 (2001); [5] T. Knubovets, H. Shinar, G. Navon, *J. Magn. Reson.* 131, 92-96 (1998); [6] G. Navon, *Magn. Res. Med.* 30, 1-4 (1993); [7] N. D'Amelio, E. Gaggelli, E. Molteni, G. Valensin, *J. Magn. Reson.*, submitted.

HYDROGEN BONDING BETWEEN BIS(PENTAFLUOROPHENYL)BORINIC ACID AND WATER: A LOW TEMPERATURE NMR STUDY

Daniela Donghi[1,2], Daniela Maggioni[1], Peter Tolstoy[2,3], Tiziana Beringhelli[1], Giuseppe D'Alfonso[1], Gleb S. Denisov[3], Nikolai S. Golubev[3], Hans-Heinrich Limbach[2]

[1] Department of Inorganic, Metallorganic and Analytical Chemistry, University of Milan, via Venezian 21, 20133 Milan, Italy. [2] Institute of Chemistry, Free University of Berlin, Takustr. 3, 14195 Berlin, Germany. [3] Institute of Physics, St.Petersburg State University, Ulianovskaja 1, 198504 St. Petersburg, Russia

In previous works [1], it has been shown by variable-temperature ^{1}H NMR spectra that the addition of water to a CD_2Cl_2 solution of bis(pentafluorophenyl)borinic acid $(C_6F_5)_2B(OH)$ leads, at low temperature (T<230 K), to a mixture of compounds. One of this compounds has been recognized as a trimeric species that presents a strong short hydrogen bond between the oxygen of one –BOH group and one proton of a B-bound water molecule (^{1}H signal of bridging proton is at 18.5 ppm). The resulting B-O(H)-H-O(H)-B- moiety shows an hydrogen bond motif similar to that of the protonated water dimer (compound **1**, fig. 1).

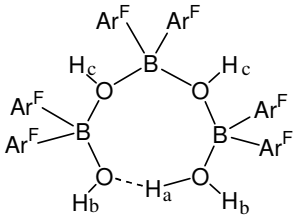


Fig. 1

The possibility to study such a compound in CDF_3/CDF_2Cl mixture as a solvent (in the temperature range 110-170 K) [2] allowed us to prove the asymmetric nature of the hydrogen bond in the moiety, indicated by a big splitting of the signals of H_b (see Fig. 1) at the lowest temperature.

On the other hand, the treatment of solid bis(pentafluorophenyl)borinic acid with water vapor afforded samples whose ^{1}H and ^{19}F spectra in CDF_3/CDF_2Cl revealed the formation of a new single species. This contains a water molecule hydrogen bonded with one of the three OH groups of the trimeric species (compound **2**, fig. 2).

At high temperature, the ^{1}H spectrum shows only one signal, proving the occurrence of a dynamic process in which both the protons of borinic acid and those of water are involved.

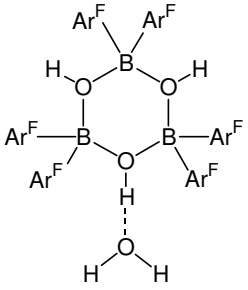


Fig. 2

Further work on H/D substitution in complexes **1** and **2** is in progress in order to gain additional information on the hydrogen bond structure and dynamics.

References

[1] a) T. Beringhelli, G. D'Alfonso, D. Donghi, D. Maggioni, P. Mercandelli, A. Sironi *XXXIII National Congress on Magnetic Resonance* (Brixen, 16-19/09/03). b) T. Beringhelli, G. D'Alfonso, D. Donghi, D. Maggioni, P. Mercandelli, A. Sironi, *manuscript in preparation*.

[2] N. S. Golubev, S. N. Smirnov, V. A. Gindin, G. S. Denisov, H. Benedict, H. H. Limbach, *J. Am. Chem. Soc.* 1994, *116*, 12055.

STRUCTURAL AND CONFORMATIONAL-ORIENTATIONAL STUDIES OF MESOGENES AND MOLECULES DISSOLVED IN LIQUID CRYSTALLINE PHASES BY NMR SPECTROSCOPY

G. De Luca, G. Celebre, M. Longeri, G. Pileio.

Dipartimento di Chimica, Università della Calabria, Via P. Bucci, I-87036 (CS)

Conformational equilibrium of molecules dissolved in liquid phase can be effectively studied by Nuclear Magnetic Resonance and this approach is very useful when the phase is anisotropic. From the analysis of Nuclear Magnetic spectra of small and/or mesogenic molecules dissolved in partially ordering solvents it is possible to obtain many different observables and in particular the partially-averaged dipolar couplings, D_{ij}, directly related to the structure, orientational order and conformational distribution of the molecules[1]. This so-called LXNMR method has a wide range of applicability, and has been used for small, rigid molecules in order to compare at high level of precision the structural parameters obtained in solid, liquid and gas phases[2], and for molecules as large as small proteins to obtain coarse grained structures in their folded states[3]. For "small" flexible molecules i.e. molecules composed by a few "rigid" fragments linked by single bond the situation is more complex[4]:

i) first, obtaining the D_{ij} from the analysis of proton single-quantum spectra can be a very difficulty task since the spectra can be extremely complex even for the simplest molecules. Therefore the analysis of such spectra is impossible if some spectral analysis simplification strategy is not followed[5];

ii) second, the dipolar couplings obtained are averaged over molecular motions, and, since there is a coupling between internal and orientational motions, it is necessary to use theoretical models to related the sets of D_{ij} to the structure, orientational order and conformational state of molecule[6].

In this work we present examples of application of this NMR method to the structure determination of small flexible molecules using the ^{1}H-^{1}H and the ^{1}H-^{13}C dipolar couplings obtained from analysis of ^{13}C selectively enriched samples or detecting the ^{13}C satellites in the proton spectrum. The problem of analysing this very complex spectra is approached in a systematic way which combine experimental strategy[7] and graphical simulation/iteration program[8]. We will also show how the dipolar couplings can be used to test models for conformational distributions using a graphical friendly program to run the calculations and including the effect of small amplitude vibrational motion[9].

References

[1] J. W. Emsley in "*Encyclopedia of NMR*", Eds: D. M. Grant and R. K. Harris, Wiley, New York,1996.
[2] J. Kaski, J. Vaar, and J. Jokisaari, *J. Am. Chem. Soc.,* **118**, 8879 (1996).
[3] E. De Alba and N. Tjandra, *Progr. Nucl. Magn. Reson. Spectrosc.,* **40**, 249 (2002).
[4] G. Celebre and M. Longeri, in " *NMR of ordered liquids*", Eds: E. E. Burnell and C. H. De Lange, Kluwer, Dordrecht, 2003.
[5] G. Celebre and M. Longeri, in *Encyclopedia of NMR*", Eds: D. M. Grant and R. K. Harris, Wiley, New York, 1996.
[6] G. Celebre, G. De Luca, M. Longeri, D. Catalano, M. Lumetti, and J. E. W. Emsley, *Molec. Phys.,* **85**, 221 (1995).
[7] F. Castiglione, G. Celebre, G. De Luca, M. Longeri, *J. Magn. Reson.*, **142**, 216 (2000).
[8] G. Celebre, G. De Luca, M. Longeri, E. Sicilia, *J. Chem. Inf. Comput. Sci.*, **34**, 539 (1994).
[9] a)G. Celebre, G. De Luca, M. Longeri, G. Pileio, J. W. Emsley, *J. Chem. Phys.,* 120, 7075, (2004), b) G. Celebre, G. De Luca, J. W. Emsley, E. K. Foord, M. Longeri, F. Lucchesini, G. Pileio, *J. Chem Phys.,* **118**, 6417 (2003).

GD-LOADED APOFERRITIN AS A NEW VECTOR FOR DELIVERY MRI CONTRAST AGENTS TO SPECIFIC TARGETS

G.Esposito[1], S.G.Crich[1], B.Bussolati[2], C.Grange[2], L. Tei [1], G.Camussi[2] and S.Aime[1]

[1]Department of Chemistry IFM and Center for Molecular Imaging(CIM), University of Torino, Torino Italy. [2] Department of Internal Medicine and Research Center for Experimental Medicine (CeRMS) University of Torino, Torino, Italy.

Magnetic Resonance Imaging (MRI) is a powerful diagnostic technique that allows one to obtain images of tissues and organs which are topological representations of NMR parameters. The contrast arises essentially from differences in T_1 and T_2 of water protons.

The contrast agents which are routinely used in clinical practice are represented by polyaminocarboxylate chelates of Gd(III) ions. These contrast agents enhance tissue contrast by increasing the longitudinal relaxation rate of water protons ($1/T_1$).

To date, all commercially approved gadolinium compounds are extracellular agents with non specific biodistribution. The next generation of contrast agents is represented by systems able to recognize specific molecules on the cellular surface which act as early reporters of a given pathology. Unfortunately, with respect others diagnostic techniques like SPECT, PET, optical imaging. MRI is strongly limited by its low sensitivity. To overcome this limitation it is necessary to develop high contrasting systems able to reach the intended target site at a sufficiently high concentration.

In this study the delivery system represented by Gd-loaded apoferritin has been considered. Following a previously reported procedure, it has been possible to entrap about 10 units of Gd-HPDO3A complexes within the interior of the apoferritin cavity. Interestingly, the relaxivity shown by each Gd(III) complex in the cavity is very high (about 80 mM^{-1} s^{-1}) and makes this "paramagnetic protein" a particularly efficient contrast agent.

Then Gd-loaded apoferritin has been biotinilated accordingly to a standard protein modification protocol using NHS-LC-BIOTIN in order to pursue its selective binding to a specific site of interest, using a streptavidin unit as a linker. The biotinylated apoferritin has been tested "in vitro" on immortalised microendothelial cells derived from a renal carcinoma (Eck-25). These cells overexpress a specific adhesion molecule called N-CAM molecule, able to bind selectively a particular peptide (N-CAM peptide). The N-CAM peptide has been biotinylated without loss of its targeting ability. Using this peptide as a vector for the Gd-loaded apoferritin, the microendothelial cells can be visualized. The MRI visualization of the labelled cells has been carried out using a Bruker Avance 300 equipped with a microimaging probe.

ANALYSIS AND FORMATCONVERTER - NMR SOFTWARE FROM CCPN

R. H. Fogh,[‡] T. J. Stevens,[‡] W. Boucher,[‡] W. Vranken,[†] J. M. C. Ionides,[†] A. Pajon,[†] E. D. Laue[‡]

[‡]*Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, UK.*
[†]*Macromolecular Structure Database Group, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SD, UK*

The talk will be a practical demonstration of the latest release of CcpNmr FormatConverter and CcpNmr Analysis.

The Collaborative Computing Project for NMR (CCPN) is funded by the UK Biotechnology and Biological Sciences Research Council and is dedicated to producing open source software and to advancing collaboration in NMR software. In addition to NMR software, our activities include creating a standard for storing and exchanging data in macromolecular NMR, biochemical laboratory work, and related areas, and writing software for data modeling and automatic code generation.

CcpNmr FormatConverter is a simple menu-driven program to import data from common formats into the CCPN data model, and to write it out again to these formats. FormatConverter can read and write sequences, coordinates, peaks, shiftlists and experiment header information from a total of eighteen different formats. It automatically recognises the naming system used for atoms, and prompts for supplementary information needed for data conversion.

CcpNmr Analysis is an interactive, menu-based program for analysis and assignment of multidimensional NMR spectra. It is based on the CCPN data model, giving it a great flexibility in the data it can store. Analysis includes multiple display windows with multiple spectra per window. Contours are drawn on-the-fly and do not have to be precomputed. A number of commands allow jumping to the plane in a given window that corresponds to e.g. a given peak or frequency. All data, from peaklists and assignments to contour colors and levels can be modified from a series of spreadsheet-type editing windows, and can be accessed from user-written macros. Analysis allows users to group resonances into spin systems and assign peaks to them while assignment is still in progress. There is support for strip plots, display and fitting of T_1 and similar experimental series, ambiguous peak assignment, generation of constraint lists, specification and assignment of protein, DNA, RNA, and organic chemistry molecules, ...

SOLID STATE NMR OF DRUGS: A STUDY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND THEIR DISPERSIONS WITH POLYMERIC CARRIERS

M. Geppi*, S. Guccione**, G. Mollica*, R. Pignatello**, C.A. Veracini*

** Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126 Pisa, Italy.*
*** Dipartimento di Scienze Farmaceutiche, Università di Catania, v.le A. Doria 6, 95125 Catania, Italy*

The formulation of oral controlled-release delivery systems formed by solid dispersions of non-steroidal anti-inflammatory drugs (NSAIDs) and suitable carriers represents a very important task in order to improve the pharmacokinetics and reduce drug side-effects. The NSAID Ibuprofen in two different forms (acid and Na-salt), as well as physical mixtures and coevaporates of each form with the copolymer Eudragit RL100, used as a carrier, have been investigated through several advanced solid state high resolution NMR techniques. A detailed assignment of the different resonances in the ^{13}C spectra of pure drug forms was carried out thanks to spectral editing and 2D-correlation techniques; ^{1}H chemical shift values could be obtained exploiting ^{1}H-^{13}C scalar couplings by using the 2D MAS-J-HMQC technique, while information on conformational properties were revealed by the 2D-HETCOR experiment. The two forms of Ibuprofen showed remarkable differences in their spectral behaviour, also due to their very different dynamics, particularly concerning the internal motions of the isobutyl and phenyl fragments, that were further investigated by means of lineshape analysis in variable temperature ^{13}C CP-MAS spectra. This work put the basis for an estimate of the degree of mixing between each drug form and Eudragit RL100 in their physical mixtures and coevaporates at the hundreds of Å level, obtained by indirectly investigating the ^{1}H spin diffusion process through the measurement of proton T_1's. A comparison between both the relaxation behaviour and ^{1}H spectra of coevaporates and corresponding physical mixtures, suggests the presence of an intimate mixing in the acidic drug form, in agreement with the presence of significant interactions between drug and carrier.

FRACTURE RENDERING AND PORE SPACE PROPERTIES QUANTIFICATION IN RESERVOIR ROCKS BY 3D-MRI

M. Gombia,[‡] V. Bortolotti,[‡] P. Fantazzini[†]

[‡]*Department DICMA, Viale Risorgimento 2, 40136 Bologna, Italy*
[†]*Department of Physics, Viale B. Pichat 6/2, 40127 Bologna, Italy*

The contribution of the fractures to the total porosity and their spatial distribution in fractured oilfield reservoirs are essential parameters for production forecasting and for a correct modeling of stored hydrocarbon volume, fluid dynamics and matrix/fracture exchange. Three dimensional (3D) MRI image analysis has been recently proposed as a particularly useful non-destructive method to visualize and quantify fracture network and flow. ^{1}H-MR imaging is particularly suitable to detect interconnected porosity because water is present only in interconnected fractures and in open matrix after full saturation.

In this work 3D surface images of fractured oilfield rock were obtained and analyzed starting from many different MR images. MRI images were acquired by ARTOSCAN™ tomograph (Esaote S.p.A., Genova) operating at 8 MHz for protons. 3D images were obtained in many different ways: starting 1) from many spin echo (SE) images and from saturation recovery (SR) images; 2) from 3D acquired gradient-echo images; 3) from many two-dimensional T_1, T_2 and spin density (M_0) maps, obtained starting from SE and SR images by the use of the in-house software ARTS [1]. After getting many two-dimensional images, the procedure for the realization of 3D surfaces consists of three steps: first an interactive segmentation is performed, then the interpolation of the curves obtained with the segmentation is realized, and finally these curves are used simultaneously to obtain 3D surfaces. On fractured reservoir rock samples these procedures were followed to get 3D images showing separately the fracture– and the matrix–porosity. These porosities, as well as the shapes and the interconnectivity between fractures and their anisotropic behaviour, were determined.

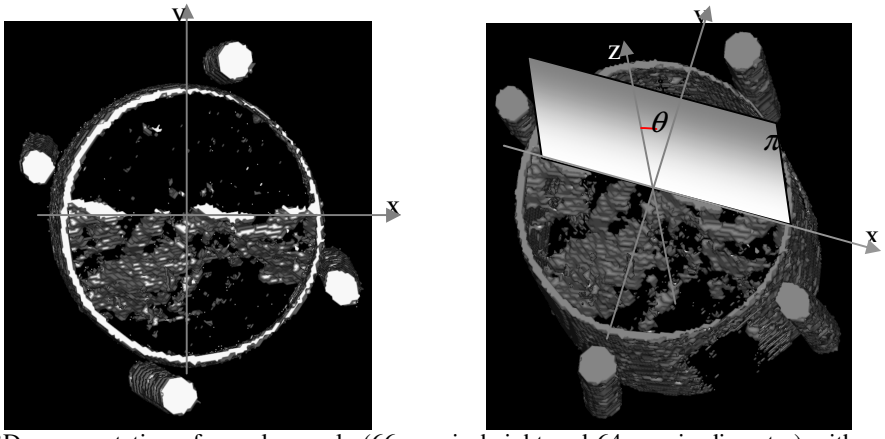


Fig. 1. 3D representation of a rock sample (66 mm in height and 64 mm in diameter) with an about 70 mm long fracture in the direction orthogonal to the x-axis and belonging to the π plane. The two director cosines in x and y directions are respectively 1 and 0. The angle θ is about 15 degrees. The ratio between matrix– and fracture–porosity is about 2, while the total porosity results equal to 7.7%.

References

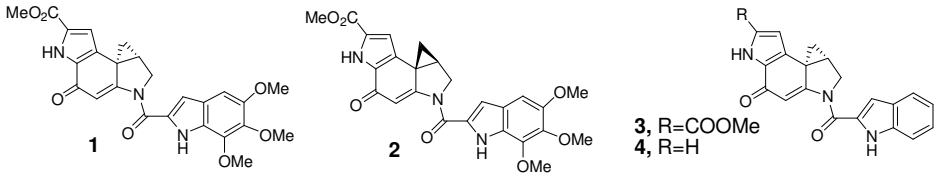
[1] G. C. Borgia, V. Bortolotti, P. Fantazzini, *J. Appl. Phys.,* **90**, 1155 –1163 (2001).

MOLECULAR BASIS FOR THE DNA-ALKYLATING PROPERTIES OF DUOCARMYCINS BY NMR SPECTROSCOPY AND DFT CALCULATIONS

L. Gomez-Paloma,[‡] G. Bifulco,[‡] P. Cimino,[‡] C. Bassarello,[‡] W.J. Chazin,[†] R. Riccio[‡]

[‡] *Dipartimento di Scienze Farmaceutiche, Università di Salerno, via Ponte Don Melillo,84084, Fisciano(SA), Italy.*
.[†]*Department of Biochemistry & Center for Structural Biology, Vanderbilt University, Preston Building 896, Nashville, TN 37232–0146, U.S.A.*

Duocarmycins and CC-1065 are the parent members of a family of extremely cytotoxic natural products endowed with IC_{50}'s in the pM range. They display sequence-specific recognition and covalent binding within the DNA minor groove [1]. Their mechanism of action, involving a so-called *in situ activation*, involves a DNA-catalyzed alkylation at the adenine N3 of AT-rich DNA tracts. The origin of DNA catalysis, the stereoelectronic factors regulating the DNA alkylation process and the molecular basis for their DNA binding properties have all fostered a very intense research activity [2]. Our results on the high-resolution NMR structures of the DNA covalent adducts of (+) and (-) duocarmycin SA (**1**, **2**) [3] and of two simplified derivatives, (+)-DSI (**3**) and (+)-CPI (**4**) [4], proved important for the comprehension of the principles underlying the binding and reactivity of these molecules towards DNA.



Structural and biological data were also complemented, very recently, by results gathered by a fully computational approach. Model systems were analyzed *in-depth* with aim to shed light on the crucial factors influencing the DNA-alkylation properties of these potential new anti-cancer drugs.

References

[1] Reviewed in: D.L. Boger, D.S. Johnson, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1439.
[2] G. Bifulco, J.A. Smith, W.J. Chazin, L. Gomez Paloma, *Advances in DNA Sequence Specific Agents*, **2002**, Vol. 4, G.B. Jones (Editor), Elsevier Science and references cited therein.
[3] J.A. Smith, G. Bifulco, D.A. Case, D.L. Boger, L. Gomez-Paloma, W.J. Chazin *J. Mol. Biol.*, **2000**, 1195.
[4] C. Bassarello; P. Cimino; G. Bifulco; D.L. Boger; J.A.Smith; W.J. Chazin.; L. Gomez-Paloma *ChemBioChem* **2003**, *4*, 1188. P. Cimino.;R. Improta.; G. Bifulco; R. Riccio; L. Gomez-Paloma; V. Barone *J. Org. Chem.* **2004**, *69*, 2816.

NITRIC OXIDE: A DIRECT OR INDIRECT MARKER OF PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES IN BIOLOGICAL SYSTEMS

M. Gussoni,[1] A. Vezzoli,[2] F. Greco,[3] G. Zannoni,[3] M. Fasano[4], M.A. Cremonini[5], L. Zetta.[3]

[1]Dip. di Scienze e Tecnologie Biomediche, Università di Milano, Via F.lli Cervi 93, Segrate (Mi), Italy . [2] Istituto di Bioimmagini e Fisiologia Molecolare, CNR, Via Fratelli Cervi 93, Segrate (Mi), Italy. [3]Istituto per lo Studio delle Macromolecole,CNR, Via Bassini 15, Milano, Italy. [4]Dip. Biologia Strutturale e Funzionale, Univ. dell'Insubria, via da Giussano, Busto Arsizio (Va), Italy. [5] Dip. di Scienze degli Alimenti, Università di Bologna, P.zza Goidanich 60, Cesena, Italy

NO is a gas with one unpaired electron, that reacts with many biological molecules. Originally regarded as an unstable toxic molecule, it was demonstrated to be the responsible of the vascular relaxant properties of endothelium. NO is now known to be an ubiquitous signalling molecule, playing multiple complex roles within many tissues, from the cardiovascular system to the brain. It is being studied currently as a possible therapeutic agent for several disease processes. On the other hand excessive production of reactive oxygen species ROS (NO itself is a ROS) is implicated in the pathogenesis of several chronic disease. In the present study NO was investigated both as an indirect (*a*) and a direct (*b*) marker of physiological and pathological processes by means of MRS, NMRD and MRI techniques.

a) In skeletal and heart muscles, oxymyoglobin (MbO_2) reacts rapidly with NO to yield metMb, otherwise NO might inhibit cytochrome-c-oxidase, a key enzyme of the respiratory chain. Thus myoglobin (Mb) is a NO scavenger, playing a role complementary to that attributed to hemoglobin in the total body NO homeostasis. ^{1}HMRS acquisition of cytosolic Mb resonances is the unique tool for directly measuring the NO-induced conversion [1] and makes possible to quantitate the different binding forms of Mb with O_2 and/or NO simultaneously, where NO is the undirect marker of skeletal and heart muscle fiber oxidative state. The MRS methods adopted in the present study to clearly detect and quantitate Mb resonances are exposed in [2].

b) Nitroxide radicals, showing paramagnetic properties, can easily be conjugated to various (bio)molecules and used as MRI contrast agent, so becoming direct markers of physio-pathological conditions. Poly(amido-amine)s PAAs would be ideal carriers for selective NMR labeling: endowed with ''stealth'' properties, they concentrate by passive targeting at the level of tumors [3]. Two PAA-(AMINO-TEMPO) adducts, where PAA represents the main polymer backbone, characterized by different NO relative concentration were studied. By increasing NO concentration, an increased spin lattice relaxation rate ($1/T_1$) at the same polymer amount could be assessed. The paramagnetic contribution to the solvent water relaxation rate was measured as a function of the applied magnetic field by recording nuclear magnetic relaxation dispersion (NMRD) profiles. Dispersion data were found consistent with a diffusion-controlled dipolar interaction between water protons and unpaired electrons, and are independent of the NO to polymer ratio. The effect of the macromolecular content of solution on polymer relaxivity as well as the polymer uptake, transport and compartimentation within a plant model sample was investigated by MRI technique.

References

[1] U. Flögel, M.W. Merx , A. Gödecke, U.K.M. Decking, J. Schrader *Proc. Natl. Acad. Sci.* **98**, 735-740 (2001).
[2] A. Vezzoli, M. Gussoni, F. Greco,L. Zetta. *XXXIV GIDRM* (2004).
[3] S. Richardson, P. Ferruti, R. Duncan. *J. Drug Targeting* 6, 391-404 (1999)

SOLID-STATE NMR AT HIGH MAGNETIC FIELDS: TECHNICAL CHALLENGES, SOLUTIONS AND APPLICATIONS

Siegfried Hafner

Varian Deutschland GmbH, 64 289 Darmstadt / Germany, Alsfelder Straße 6

Like in NMR of solutions, high magnetic field strengths are advantageous also for many applications in solid-state NMR. Chemical-shift resolution and sensitivity increase with increasing magnetic field strength while the anisotropic line-broadening effects (under MAS spinning) remain constant or, as in case of the second-order quadrupolar interaction, even become smaller. The pre-requisite for exploiting these advantages however is, that the hardware, particularly the probes, perform equally well as in lower fields, which represents a considerable technical challenge.

This presentation provides an overview on problems and technical solutions at high magnetic with emphasis on new approaches to probe design. Unlike classical approaches using variable capacitors for tuning the resonance circuit, the tuning tube technology (T3) provides high-end performance (high-power decoupling) also for magnetic field strength up to 900 MHz, while the Balun design assures B_1 homogeneity that is approaching that of liquids probes. For biological applications, sample heating is also an issue which presently is addressed by new approches to coil design. All these aspects will be demonstrated on the examples of selected applications at field strengths up to 900 MHz.

PRL-3, A PHOSPHATASE IMPLIED IN CANCER METASTASIS: STRUCTURE AND FUNCTION

F. Innocenti[1,2], M. Sette[2], Eleonora Forte[1], P. Lo Surdo[1], Mauro Cerretani[1], Sergio Altamura[1], Licia Tomei[1], G. Barbato[1]

[1] *IRBM, P. Angeletti, Via Pontina km 30.600, Pomezia, Rome, Italy*
[2] *University of Tor Vergata, Rome, Italy*

Protein phosphorylation is one of the major post-translational modification mechanisms that cells utilize to control various regulatory processes. PRL constitute a novel class of phosphatases involved in cell growth modulation. PRL-1 was identified as an intermediate early gene expressed in the early response of regenerating liver tissue to mitogens. The related phosphatases PRL-2 and PRL-3 are involved in growth regulation, proliferation and cell invasion. They are prenylated at their C-terminus, which affects cellular localization and function. In human, PRL-1 and PRL-2 are ubiquitously expressed, while PRL-3 (22-kDa) is expressed in heart and skeletal muscle. Over-expressing PRL-3 is correlated with enhanced growth of human embryonic fibroblasts. PRL-3 is expressed at low levels in normal colorectal and epithelium from benign tumours, at intermediate levels in a subset of malignant stage I or II cancers, and at high level in colorectal cancer metastasis. Only in metastatic cells there is abnormal chromosomal amplification of its gene [1]. The over-expression correlates with increased cell acquired mobility [2]. These characteristics make PRL-3 a potential new cancer therapeutic target.
PRLs contain the PTPase active consensus motif HCXXGXXR (P-loop). Tyrosine specific, dual specificity phosphatases (DSP), and some phosphoinositide (i.e. PTEN) enzymes, share the same signature motif. The key catalytic residues are cys and arg residues in the P-loop and a conserved asp residue, ca. 30 residues upstream of the signature motif.
Here we present the refined 3D atomic structure determined in solution by NMR spectroscopy using ^{15}N and $^{15}N/^{13}C$ double labeled samples. The structure is used as a tool to rationalize the results of the kinetic analysis of de-phosphorylation of a panel of potential substrates. Kinetic and thermodynamic analysis results are presented on a library of peptides (pY, pS, pT) as well on a library of phosphoinositide lipids. The results will be discussed for their relationship with PRL-3 natural substrate.

References

[1] Saha et al. *Science*, 2001, **294**, 1343
[2] Zeng et al. *Cancer res*. 2003, **63**, 2716

NMR STRUCTURAL STUDY OF ZINC FINGER DOMAIN CONTAINING PROTEINS

C. Isernia[*], G. Malgieri[*], M. Leone[#], L. Zaccaro[#], S. Esposito[*], I. Baglivo[*], C. Pedone[#], B. Di Blasio[*], P.V. Pedone[*], R. Fattorusso[*]

[*]*Dipartimento di Scienze Ambientali, Seconda Università di Napoli, via Vivaldi 43, 81100 Caserta.* [#]*Istituto di Biostrutture e Bioimmagini, CNR, Napoli*

Proteins containing classical zinc finger domains (or Cys_2/His_2) represent the largest group of eukaryotic DNA binding proteins known to date, and are present in many different form of lifes. The classical zinc finger domain presents the amino acids sequence $CX_{2-4}CX_3FX_5LX_2HX_{2-5}H$, with the two cysteines and the two histidines tetrahedrally coordinating a zinc ion. The zinc finger domains are present in the proteins in a number varying from 1 to 37 and a minimum of two units is essential for high affinity binding to a specific DNA sequence. Nevertheless, it was recently demonstrated that the single zinc finger domain present in the *Drosophila* GAGA transcription factor is flanked at its N-terminus by basic residues which stabilise the DNA binding [1].

Mutations causing extra stamens to form at the expense of normal carpels development have been mapped in the SUPERMAN (SUP) gene from *Arabidopsis thaliana*, which encodes a protein containing a single classical zinc finger domain presenting the QALGGH sequence, highly conserved in the plant zinc finger proteins [2]. We have demonstrated that the single Cys_2/His_2 zinc finger motif present in the SUP protein is able to specifically bind DNA [3] and the binding is stabilised by two basic regions located on either side of the motif. We have also performed the NMR characterization of a 37 amino acid SUPERMAN region comprising the zinc finger motif [4]. The structure consists of a very well-defined $\beta\beta\alpha$ motif, typical of all the other Cys_2/His_2 zinc fingers, and represent the first high-resolution structure of a classical QALGGH zinc finger domain from a plant protein.

Recently, it has been demonstrated that the *ros* gene in *Agrobacterium tumefaciens,* which encodes a transcriptional repressor, is the first bacterial protein containing a putative single Cys_2/His_2 zinc finger DNA binding domain [5]. Ros is a 15.5 kDa protein which represses the transcription of virulence genes located on the large Ti plasmid present in plant-tumour-inducing Gram-negative bacterium. Ros also regulates expression of the oncogene *ipt*(5). In Ros the peptide loop spacer between the β strands and the α helix of the typical $\beta\beta\alpha$ motif is 9 amino acids as opposed to the invariant 12 amino acids in the classical eukaryotic Cys_2/His_2 motif. This feature is highly conserved in prokaryotic classical zinc fingers. Remarkably, no high-resolution structure of bacterial zinc finger motif is so far available in the literature. In order to study the conformational behaviour of the bacterial zinc finger domains, we have undertaken the structural determination, via NMR, of a Ros C-terminal fragment. The analysis of the threedimensional structure and its comparison with the SUPERMAN zinc finger will help in understanding the structural features involved in the DNA recognition mechanism.

References

[1] Omichinski J.G. et al. (1997). *Nature Struct. Biol.*, **4**, 122-30.
[2] Sakai H. et al (1995) *Nature*, **378**, 199-202.
[3] Dathan, N. et al. (2002). *Nucleic Acids Res.,* **30**, 4945-51.
[4] Isernia, C. et al. (2003) *ChemBiochem*.**2-3**, 171-180.
[5] Chou, A. Y. et al. (1998) *Proc. Natl. Acad. Sci*., **95**, 5293-5298.

^{13}C DIRECT DETECTION AS A TOOL FOR STRUCTURAL DETERMINATION IN METALLOPROTEINS

A. Beatriz Jiménez,[‡] B. Ivano Bertini,[‡] C. Mario Piccioli[‡].

[‡] *Magnetic Resonance Center, University of Florence, Via L. Sacconi, 6, 50019 Sesto Fiorentino, ITALY*

Nuclear Magnetic Resonance spectroscopy (NMR) has developed as a leading technique to study biomolecules because it permits its characterization in terms of structure and dynamics. The major limitation of NMR arise form the increase of transverse relaxation rates which prevents the investigation of molecules with large molecular weight. In the last years cross correlation rates have been shown to be an avenue to investigate them and to increase performance of NMR in structural biology [1]. As relaxation rates depends on the gyromagnetic constant of the nuclei, another way to solve the problem is detecting other nuclei with smaller constant. Our aim is the development of new methodologies based on the direct detection of ^{13}C taking advantage of a prototype probe specially designed by Bruker for our laboratory [2].

When a paramagnetic ion is present in a protein, its behavior is, in terms of relaxation rates, similar to a large molecule because the unpaired electrons increase the nucleus relaxation rates [3]. The Curie relaxation mechanism is due to the presence of a paramagnetic ion in the protein. Cross correlation effects involving Curie relaxation contain not only dynamic information (as in the diamagnetic biomolecules) but also structural information [4]. Extracting structural data from ccr is not so evident in diamagnetic systems. In this sense we are interested on studying the cross correlation effects due to the presence of a paramagnetic ion in the protein. We have focused our efforts in developing new approaches to obtain NMR constraints using a paramagnetic ion as a probe in the system of study.

References

[1] a) Pervushin K., Riek R., Wider G., Wuthrich K. *Proc. Natl. Acad. Sci. USA* **94**, 12366 (1997); b) Pervushin K., Riek R., Wider G., Wuthrich K. *Proc. Natl. Acad. Sci. USA* **96**, 4918 (1999).

[2] a) Arnesano F., Banci L., Bertini I., Felli I.C., Luchinat C., Thompsett A.R. *J. Am. Chem. Soc.* **125**, 7200 (2003); b) Bermel W., Bertini I., Felli I.C., Kummerle R., Pierattelli R. *J. Am. Chem. Soc.* **125**, 16423 (2003).

[3] Bertini, I.; Luchinat, C.; Tarchi, D. *Chem. Phys. Lett.* **203**, 445 (1993).

[4] a) Pintacuda G, Hohenthanner K, Otting G, Muller N., *J Biomol NMR*. **27**, 115 (2003). b) Kateb, F.; Piccioli, M. *J. Am. Chem. Soc.* **125**, 14978 (2003); c) Boisbouvier, J.; Gans, P.; Blackledge, M.; Brutscher, B.; Marion, D. *J. Am. Chem. Soc*. **121**, 7700 (1999).

ADVANCES IN PROBEHEAD TECHNOLOGY

Rainer Kümmerle

NMR Applications Scientist, BRUKER BIOSPIN AG, NMR Division, Industriestrasse 26, CH-8117 Fällanden, Switzerland

A brief overview is given over recent advances in high resolution NMR probehead technology. This covers conventional probeheads as well as CryoProbes. To take most advantage of these improvements, several hints and tips are presented for daily routine NMR and research spectroscopy.

As an example, in case of composite pulse decoupling higher signal-to-noise ratios combined will lead to visible sidebands of decoupled signals. Solutions for reduction and suppression of composite pulse decoupling sidebands are presented.

VISUALIZATION OF RELAXATION PROPERTIES THROUGH THE INVERSION OF EXPONENTIAL DECAYS.

Raffaele Lamanna (1), Donatella Capitani (2), Noemi Proietti (2) and Annalaura Segre (2)

(1) CR ENEA Trisaia SS 106 Jonica Km 419.5 75026 Rotondela (Mt) Italy. (2) Institute of Chemical Methodologies, CNR Area della Ricerca di Roma, M.B.10, 00016 Monterotondo Staz., Roma Italy

Due to instrumental improving recent literature on relaxation properties reports an increasing complexity. It is common to deal with the behavior of multi-component T_1, T_2, T_1rho, FID and other properties defined in the time domain whose representation is somehow obscure and difficult read. A possible tool to improve the global understanding of relaxation behavior is the inversion of exponential decays. This inversion has been previously achieved by many research groups always with different purposes such as the observation of porous systems, the study of water-protein interaction or the study of water in polysaccharidic systems and food stuffs.
Several algorithm for the analysis of complex NMR data deal with the discrete form of the Laplace transform which is calculated assuming a fixed set of relaxation times Tj and then finding the corresponding amplitudes. Several Tj grids, objective and regularization functions, norms etc. are used in the different algorithms.
We discuss the role of discretization algorithms and of the choice of the relaxation time grid on the calculated distribution of relaxation time using both simulated and real relaxation data.

STUDY OF FLOW BEHAVIOUR AND DYNAMICS OF LARGE MOLECULES BY NMR IMAGING AND RELAXATION

G. Maddinelli

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

NMR imaging techniques can be applied in providing data useful in solving complex engineering problems. One the most promising applications are certainly velocity measurements methods based on the combination of pulsed field spin-echo (PGSE) and imaging experiments[1]. This type of technique is quite promising in applications to complex fluid dynamics studies[2], especially required in oil and chemical industry. 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[3]. This feature associated with high spatial resolution makes MRI more attractive than other conventional techniques (e.g. laser Doppler methods). In this application, we have investigated flow dynamics of different polymeric solutions, by assembling a 9 mm (I.D.) flow loop line inside a 2 T, 31 cm horizontal-bore magnet. Flow was assured in a velocity range of 10-100 cm/sec. Studies were realized using different fluids constituted by a mixture of water/glycerine, polymeric solutions and water/oil emulsions, giving information on flow properties and especially on fluid rheology. Viscous fluids with different concentration of added polymers, were adopted and consistent effects on flow regime were observed. Relaxation analysis is very useful in giving information on the dynamic range of large molecules and different chemical species in solution. The results of a study performed at 20 MHz on different solutions of polymers, surfactants and water in oil emulsions are reported giving details on different molecular dynamic regimes. In the study different relaxation times measurements and editing relaxation techniques[4] were applied and the results discussed.

References

1. Callaghan P.T., Eccles C.D. and Xia Y. J. “NMR microscopy of dynamic displacements”. Phys. Sci. Instrum. 21, 820-822, 1988;
2. Xia Y., Callaghan P.T., and Jeffrey K.R., “Imaging velocity profiles: flow through an abrupt contraction and expansion”. AIChE Journal, 38, 9, 1408-1420, 1992;
3. Newling, B., Hall L.D. et al., “Chemical Resolved NMR velocimetry”. Chemical Engineering Science, 52, 13, 2059-2072, 1997.
4. Y.-Q. Song, L. Venkataramanan M.D. Hurlimann , “T_1-T_2 Correlation Spectra Obtained Using a Fast Two dimensional Laplace Inversion”, J. Magn. Reson. 154, 261-268, 2002.

REACTIVITY OF CYTOTOXIC TRANS-ORIENTED PLATINUM COMPLEXES TOWARDS NUCLEOTIDES AND SINGLE AND DOUBLE-STRANDED OLIGONUCLEOTIDES INVESTIGATED BY [^{1}H, ^{15}N] HMQC NMR

Giovanni Natile

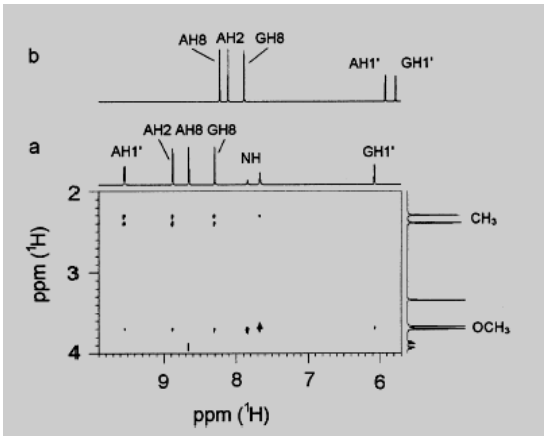
Dept Farmaco-Chimico, University of Bari, via E. Orabona 4, Bari, ITALY

The clinical inactivity of transplatin, *trans*-[$PtCl_2(NH_3)_2$], has been related to the different types of DNA adducts formed by this isomer (which, for steric reasons, cannot form the most prevalent cisplatin adducts, *i.e*. the 1,2-intrastrand cross-links between adjacent purines) and to its greater chemical reactivity (it could undergo deactivation before delivery to the tumour site). However, more recently, several groups have reported on some active *trans*-platinum complexes. The first of these complexes showing *in vivo* antitumour activity in limphoproliferative and solid metastasising murine tumours was the iminoether complex *trans*-[$PtCl_2${*E*-HN=C(OMe)Me}$_2$] (*trans-EE*).[1]

The kinetics and mechanism of binding of the ^{15}N-labeled *trans-EE* to guanosine 5'-monophosphate, nucleotide dimers, single-stranded oligonucleotides, and double-helical duplexes have been investigated by 2D [^{1}H, ^{15}N] HMQC NMR spectroscopy. By this technique different reaction products can easily be identified as cross peaks in the 2D spectra.

The formation of the mono-chloro GMP adduct takes place via a bimolecular process involving the mono-chloro mono-aqua species and the entering nucleotide. Mono-aqua GMP and bifunctional GMP adducts are formed in successive steps.[2]

Reactions of *trans-EE* with r(ApG) and d(ApG) take place through solvolysis of the starting substrate and subsequent formation of *trans* G-N7/monochloro and G-N7/monoaqua adducts. Slowly, the monofunctional adducts evolve to a bifunctional adduct forming an unprecedented and unexpected A-N3/G-N7 platinum cross-link spanning two *trans* positions.[3,4] For the reverse sequence r(GpA), no chelate structure was formed even after a two-week reaction.



^{1}H-NMR 1D and 2D spectra of trans-*EE*/r(ApG) bifunctional adduct M2 (a), and 1D spectrum of free r(ApG) (b).

Reactions of *trans-EE* with the single-stranded oligonucleotides d(CCTCGCTCTC) and d(CCTGGTCC) proceed rapidly through solvolysis of the starting substrate and subsequent formation of G-N7/monochloro *trans-EE* adducts. Quite unexpectedly, the double-helical duplexes, d(TATGGTACCATA)$_2$ and d(TATGGCCATA)$_2$, with no terminal G residues, are practically inert towards *trans-EE*. However, the duplexes d[(CCTGCTCTC)·(GAGAGCAGG)] and d(GATAGGCCTATC)$_2$, which contain both terminal and central G residues, undergo platination only at the terminal, solvent exposed, G residues, thereby confirming that the interior of the duplex is not accessible to *trans-EE* due to steric hindrance. In contrast, cisplatin was found to bind exclusively to the central GG pair in d(GATAGGCCTATC)$_2$.[5]

References

[1] G. Natile and M. Coluccia, *Coord. Chem. Rev.*, **2001**, *216-217*, 383-410.
[2] Y. Liu, F. P. Intini, G. Natile, and E. Sletten, *J. Chem. Soc., Dalton Trans.*, **2002**, 3489-3495.
[3] Y. Liu, C. Pacifico, G. Natile, and E. Sletten, *Angew. Chem. Int. Ed.*, **2001**, *40*, 1226-1228.
[4] Y. Liu, J. Vinje, C. Pacifico, G. Natile, and E. Sletten, *J. Am. Chem. Soc.*, **2002**, *124*, 12854-12862.
[5] J. Vinje, F. P. Intini, G. Natile, and E. Sletten, *Chem. Eur. J.*, **2004**, *10*, 3569-3578.

The University of Bari, the Ministero dell'Istruzione, Università e Ricerca (MIUR), and the EC-COST Chemistry Action D20 are gratefully acknowledged.

CONFORMATIONAL PREFERENCIES OF Aβ-(1-42) AND ITS CORE FRAGMENT (25-35) IN POLAR AND APOLAR MEDIA

D. Picone,[‡] S. Tomaselli,[‡] A.M. D'Ursi[†]

[‡]*Department of Chemistry, University of Naples Federico II, via Cintia 26, I-80126 Naples, Italy.*
[†]*Department of Pharmaceutical Sciences, University of Salerno, via Ponte Don Melillo 11c, I-80084 Fisciano, Italy.*

The fight against the Alzheimer disease is an important challenge of the pharmaceutical research due to the increasing incidence of this pathology correlated to the life elongation.

The design of molecules able to interact with the amyloid peptides as inhibitors of fibril formation or as inhibitors of amyloid membrane pore formation constitutes a basic approach in the development of anti-Alzheimer therapies. Despite the large amount of experimental data, it is not clear whether the fibrils of amyloid peptides are the cause of Alzheimer pathology or if alternatively they have a protective function, while the pathological cause is the amyloid membrane disruption activity. In both the cases the study of the conformational properties of Aβ-peptides in soluble form is the basis for the design of molecules with "anti-amyloid" activity.

In the search for experimental conditions which may favor the monitoring of different conformational states of Aβ-(1-42) and its toxic fragment Aβ-(25-35) under different polarity conditions we resorted to HFIP/water mixtures with different composition. The comparison among the structures obtained might help unveiling the regions that are mainly affected by the environmental conditions.

The CD data recorded in HFIP/water mixtures from 80/20 v:v HFIP/water to 20/80 v:v HFIP/water shows that amount of water higher than 50% produces a drastic modification of the peptide folding. In order to follow the conformational modifications deriving from an increasing of medium polarity we have selected the two extreme conditions resembling the aqueous interface between the membrane surface and the extracellular medium (high water) and the lipid environment (high HFIP) respectively. Interestingly, the structure of Aβ-(1-42) and Aβ-(25-35) in the 80/20 HFIP/water mixture is characterized by a high helical content, suggesting a common neurotoxic mechanism possibly based on the formation of ion channels into the membrane [1].

When the HFIP/water ratio is inverted, the solution structure of Aβ-(1-42) and Aβ-(25-35) is characterized by a decrease of regular folding in the C-terminal region, while a significant regularity in the structure is conserved in the N-terminal region of the full length fragment.

The different structures of Aβ-peptides in HFIP/water mixtures give experimental evidence of the propensity of the peptides to loose the structural regularity in a particular point of the sequence as a function of the environment, behaving as a partially folded intermediate in the aggregation process.

References

[1] O. Crescenzi *et al*., *Eur. J. Biochem* **269**, 5642-5648 (2002).
[1] A.M. D'Ursi *et al.*, J. Med. Chem., in press, (2004).

NMR IN THE FREE AND BOUND STATE: A TOOL FOR THE DESIGN OF GLYCOMOMETICS.

D. Potenza

Università di Milano, Dipartimento di Chimica Organica e Industriale e Centro di Eccellenza CISI, via Venezian 21, 20133 Milano, Italy

Carbohydrate-protein complexes are formed in the initial steps of a large number of physiological and pathological processes. Interference with this recognition events could be used to modulate or alter signal transmission or to prevent the onset of deseaes. We are investigating these problems using as a model one of the best characterised protein-sugar recognition pairs: the cholera-toxin:GM1 complex. New Cholera Toxin (CT) ligands (1) were designed replacing the reducing end of ganglioside head-group with a conformationally restricted cyclohexanediol. Than, we reported a group of second-generation mimics (2) that were conceived by replacing the sialic acid moiety of (1) with different α-hydroxyacids. (Fig. 1)

NMR studies were performed on all the mimics of the GM1 ganglioside oligosaccharide. The conformation of these analogues was investigated first in solution and then upon binding to the B-pentamer of the cholera toxin by transferred nuclear Overhauser effect (TR-NOE) measurements.
STD experiments were also performed, and allowed to define the portions of the ligands which are in closest contact with the protein. The analysis of the NMR data allowed to interpret the experimental observations and to derive workable models of the ligand:toxin complexes. The NMR data were interpreted with the aid of computer models.
The results will be discussed at the meeting.

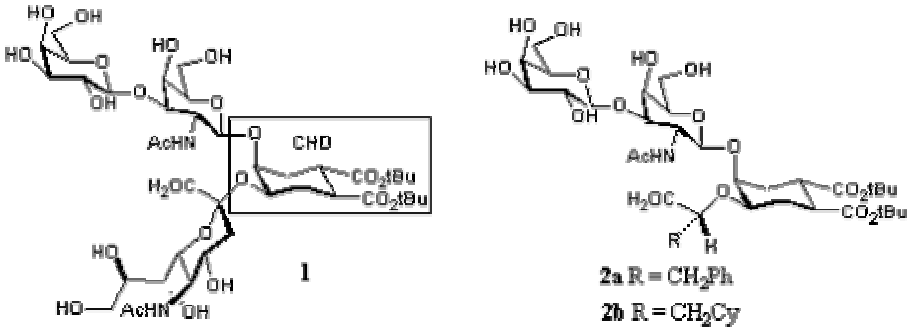


Fig. 1.

References

[1] A. Bernardi, A. Checchia, P. Brocca, S. Sonnino and F. Zuccotto J. Am. Chem. Soc., 1999, **121**, 2032-2036
[2] A. Bernardi, D. Potenza, A. M. Capelli, A. García-Herrero, F. J. Cañada and J. Jiménez-Barbero Chemistry, Eur. J., 2002, **8**, 4597-461

EPR, SEM-EDS, XRD, MQ-MAS NMR characterization of fired iron bearing clays.

[1]Capitani D., [2]Presciutti F., [2]Sgamellotti A., [2]Brunetti G., [1]Viel S., [1]Proietti N., and [1]Segre A.L.

[1]*Inst. of Chemical Methodologies of CNR , MB10 ,Monterotondo Staz. 00016 Rome Italy*
[2]*Dept of Chemistry, Univ. of Perugia*

A natural clay containing 6% of iron was fired at 600,700,800,900,1000,1100 °C in oxidant atmosphere to mime ancient potteries of art - historical interest. Several different techniques were used for the characterization of these fired samples. EPR spectra show an oxidation of iron, $Fe^{++} \rightarrow Fe^{+++}$, with a well defined transition between 800 and 900 °C. After this transition the EPR resonance appears sharpened due to exchange narrowing. Moreover the intensity of EPR signal is too strong and does not correspond to the total iron present, this strong intensity can be interpreted as due to a clustering of the Fe^{+++} into ferromagnetic domains.
The above conclusion is supported by SEM images which, coupled with EDS analyses, show octahedral Fe crystals well defined in the samples fired at the higher temperatures.
A full MAS NMR study was also performed on ^{23}Na, ^{27}Al and ^{29}Si. ^{27}Al MQ-MAS spectra show clearly the presence of two sites, one due to Al in an octahedral environment and the other one due to Al in a tetrahedral environment. The band due to Al in an octahedral environment fully disappears in those samples fired at temperatures higher than 900°C . A similar behavior was observed in the MAS spectra of ^{29}Si and interpreted as due to the loss of a resonance of the Q^4 silicate.
The presence of at least two sites is also supported by ^{23}Na MQMAS spectra.
The clay's phase transitions due to the firing were also studied by XRD analyses. The amount of anorthite, albite, and diopside grows from low to high temperature.
Therefore all data relative to clays fired at higher temperatures can be interpreted as due to $Fe^{++} \rightarrow Fe^{+++}$ oxidation associated with an iron migration and its clustering into octahedral sites.

INVESTIGATION OF ARCHAEOLOGICAL BONE AND TEETH BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND X-RAY DIFFRACTION

Carla Scarabino, Patrizia Oliva, Alexandra R. Albunia,
Davide Alfano and Antonio Proto

Dipartimento di Chimica-Università degli Studi di Salerno, via S. Allende ,84081 Baronissi (SA)

Bones are constituted principally of calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ with additional substances such as carbonate, fluoride, sodium, and magnesium ions with organic portion (collagene).
Many studies[1] have been made on the inorganic component of bone, but less is known about the mineral nature of archaeological bone and the changes that occur after burial (diagenesis)[2].
Herein we report a preliminary investigation of several bone and teeth of ancient people, dated from sixth century b.C. to second century a.C. founded in the archeological site of Paestum.
The scope of this presentation is to compare solid-state NMR and XRD techniques applied to the study of diagenesis of human bone.
X-ray diffraction, that is a powerful technique to determine the crystalline phase composition, is not sensitive to the amorphous phase which is important in archaeological study.
Instead solid state NMR[3],[4], including high power decoupling, magic angle spinning (MAS), cross polarization (CP), allows to observe both organic and mineral parts of bone, in both the crystalline and amorphous phase.
The X-ray patterns and ^{1}H, ^{13}C, ^{31}P NMR spectra have been collected for all the samples and have been repeated either on the samples essiccated at 105 ºC or after treatement with acetic acid, to remove the superficial carbonate.

References

[1] Roufosse, A. H.; Aue, W. P.; Roberts, J. E.; Glimcher, M. J.; Griffin, R. G. *Biochemistry* **23**, 6115 (1984).
[2] Reiche, I. ; Vignaud, C.; Menu, M.; *Archaeometry* **44**, 447 (2002).
[3]Lambert, J. B.; Shawl, C. E.; Stearns, J. A. *Chem. Soc. Rev. 29*, 175 (2000).
[4] Lee, A. P.; Klinowski, J.; Marseglia, E. A.; *J. Arch. Sc*. 22, 257 (1995).

CHAIN ORDER AND DYNAMICS IN POLYMER MELTS REVEALED BY PROTON DOUBLE QUANTUM NMR

A. Serbescu [a], E. Bourgeat-Lami [b], K. Saalwächter [a]

[a] *Institute for Macromolecular Chemistry, University of Freiburg, Stefan-Meier-Str. 31, 79104 Freiburg, Germany.*
[b] *Laboratoire de Chimie et Procédés de Polymérisation (LCPP), CNRS-CPE Lyon, bat. 308 F, 43 Bd. du 11 Novembre 1918, BP 2077, 69616 Villeurbanne Cedex, France*

The project is focused on investigating the changes induced in the dynamic molecular ordering of entangled polymer melts far above T_g by the presence of nanoscopic filler particles with weakly or non-interacting surfaces. We wish to determine the extent and time-scale of the considerable surface-interaction independent ordering effects that were indicated by published results [1, 2].

Newly developed ^{1}H multiple-quantum (MQ) techniques [3], applicable to unlabeled materials, are used. Our method is based on a pulse sequence initially developed by Baum and Pines [4], which relies on the suppression of the perturbing contribution of the uncoupled segments (e.g. chain-ends) from the measured MQ signal, being thus uniquely selective for the determination of residual couplings. The data is analysed in terms of build-up curves of double quantum coherences.

A series of polydimethylsiloxane (PDMS) –based systems have been measured: linear polymers, non-chemically-bonded nanoblends with high-T_g-polymers and silica-filled. The varied parameters include: molecular weights, temperature, phase separation, filler surface and content of the mixtures. The first results indicate a close correlation between the dynamic order parameter, calculated from the measured residual dipole-dipole coupling constants, and the magnitude and distribution of interfaces in the polymer melt.

References:

[1] J. Kraus, P. Müller-Buschbaum, T. Kuhlmann, D.W. Schubert, M. Stamm, (2000) Europhys. Lett. 49, 210
[2] F.W. Starr, T.B. Schroder, S.C. Glotzer, (2001) Phys. Rev. E 64, 1802
[3] K. Saalwächter, O. Spyckerelle, B. Haidar, A. Vidal, J.-U. Sommer, (2003) J. Chem. Phys. 119, 3468
[4] J. Baum, A. Pines, (1986) J. Am. Chem. Soc. 108, 7447

ROLE OF GROWTH FACTORS IN THE ONTOGENESIS OF PANCREATIC BETA-CELLS

Marco Sette[1], Fabiana De Angelis[2], Luca Bova[2] , Davide Lauro[2] and Giulia Donadel[2]

[1]Department of Chemical Sciences and Technology and [2]Laboratory of Molecular Medicine, Department of Internal Medicine, University of Rome, Tor Vergata, Rome, Italy.

The incidence of diabetes mellitus in the population is ~5% and in western society it is becoming an emerging problem (1). Evidences from molecular and epidemiological studies indicate that pancreatic beta-cell dysfunction is crucial in both type I and type II diabetes mellitus (2). In the case of type I diabetes, beta-cells are selectively destroyed after lymphoid infiltration of the islet. This autoimmune destruction results in insulin deficiency and hyperglycemia (3). Type II diabetes is associated with reduced insulin secretion and glucose toxicity that may contribute to beta-cell death (4). In both cases beta-cell death is thought to occur by apoptosis and a number of pathological stimuli involved in type I and type II diabetes have been shown to elicit beta-cell programmed death (5). The regulation process of the human pancreatic beta-cell mass, in terms of beta-cell replication, cell death and regeneration of pancreatic islets, has assumed a prominent role in the last two years since very important goals have been achieved in the field of pancreatic islets transplantation (6). Studies made in many laboratories in the last years have demonstrated that beta-cells can proliferate responding to physiological and pathophysiological stimuli (hyperglycemia, pregnancy, and pancreatectomy) and responding to genetic manipulations which lead to insulin resistance (insulin receptor or its corresponding intracellular signal knock-out) (7). A part from this beta-cells proliferation capacity a source of new beta-cells seems to exist, which would lead to new islets ("islet neogenesis") both during fetal and adult life (8, 9). The cellular precursors, which would originate the new islets, should be located in the pancreatic duct from the main ductal to the small branches (10). The study proposed in this project is based on the observation that pancreatic beta-cell loss, which takes place during diabetes mellitus (type II and I), is not compensed by pancreatic islets massive neogenesis. In this study we focus our attention on the effect of some growth factors which possibly have a biological role mediating the molecular signals in the ontogenetic program of pancreatic beta-cells development from ductal cells. Here we focus our attention on the effect of the placental lactogen hormone (PL) that is known as one of the most potent beta-cells mitogenic agents (11-15, 16). The studies were conducted on intact cells and cellular extracts of human pancreatic ductal cell line in culture (PANC-1) in basal condition and treated with 500ng/ml of recombinant hPL (isoform A) expressed and purified in our laboratory. Using immunofluorescence and NMR spectroscopy we were able to follow morphological and metabolic changes occurring during ontogenetic events of pancreatic beta-cells differentiation from epithelial pancreatic ductal cells.

References

[1] Tisch R and McDevitt H 1996 Cell 85: 291-97; [2] Cerasi E, et al. 2001 Diabetes 50 S1: S1-S3
[3] Benoist C and Mathis D 1997 Cell 89 (1): 1-3; [4] Gerish JE 1998 Endocr Rev 19: 491-503
[5] Stassi G, et al. 1997 J Exp Med 186: 1193-200; [6] Garcia-Ocana A, et al. 2001 J Clin Endocr Metab 86 (3): 984-988; [7] Efrat S, et al. 1996 Diabetes Rev 4: 224-234; [8] Fernandes A, et al. 1997 Endocrinology 138: 1750-1762 ; [9] Bonner-Weir S, et al. 1993 Diabetes 42: 1715-1720 ; [10] Krakowski ML, et al. 1999 Am J Pathol 154 (3): 683-691; [11] Finegood DT, et al. 1995 Diabetes 44: 249-256 ; [12] Swenne I, et al. 1992 Diabetologia 35:193-201; [13] Koore M, et al. 1993 J. Clin Invest 92: 113-114; [14] Miyaura C, et al. 1991 Mol Endocrino l 5: 226-234; [15] Scaglia L., et al. 1995 Endocrinology 136: 5461-5468 ; [16] Brelje TC, et al. 1993 Endocrinology 132: 879-887

^{1}H HIGH RESOLUTION NMR METABOLITE PROFILING OF TRUFFLES (*TUBER AESTIVUM VITTADINI)*

Anatoli P. Sobolev,[‡] Michela Cristinzio[†], Luisa Mannina[‡, †] Pietro Ragni[†] and Annalaura Segre[‡]

[‡] *Istituto di Metodologie Chimiche, CNR, c.p.10, 00016 Monterotondo Staz. (Rome), Italy*
[†] *Dipartimento STAAM, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy*

High resolution ^{1}H NMR is a powerful tool to characterize food such as tomatoes, flour, olive oils etc, see references. The method was used to characterize aqueous and organic extracts of truffles (*Tuber aestivum vittadini)* to assess the quality of these mushrooms, widely used in the Italian cuisine. Metabolites of the different classes soluble in water such as amino acids, sugars, organic acids, and other sensory components were detected and assigned by means of 2D experiments (^{1}H-^{1}H COSY and TOCSY, ^{1}H-^{13}C HMQC and HMBC). The ^{1}H and ^{13}C NMR spectral assignments of the cell membranes components such as phospholipides, sterols and fatty acids extracted by mixed organic solvents were also performed.
The method allows to assess that no damage results from a low dose gamma irradiation of tubers.

References

[1] A. P. Sobolev, A. Segre and R. Lamanna, *Magn. Reson. Chem*. **41,** 237-245 (2003).
[2] M. E: Amato, G. Ansanelli, S. Fisichella, R. Lamanna, G. Scarlata, A. P. Sobolev and A. Segre, *J. Agric. Food Chem*, **52**, 823-831 (2004)
[3] L. Mannina, A. P. Sobolev and A. Segre, *Spectroscopy Europe*, **15/3**, 6-14 (2003)

FIELD-NOISE EFFECTS IN NMR

S. Sýkora

Extra Byte, Via R. Sanzio 22C, 20022 Castano Primo (MI)

This presentation reviews the results of the Author's ongoing systematic study [1,2,3] concerned with the effects of magnetic field instabilities on various types of NMR signals and derived NMR data. Despite the pronounced practical impact of this topic, its mathematical aspects have been so far somewhat neglected.

Magnetic field noise arises in practice from many sources, including the magnet system (materials, current generators and stabilizers, field stabilizer, NMR lock system), the NMR instrument (power wiring, transformers) and the enviromental (site-dependent!) magnetic noise (electric wiring, motors, transformers, mains stabilizers, air-conditioning, etc.). Some of the field-noise components are nearly a-periodic (white noise), while others are characterized by frequencies which are multiples of the mains frequency. Quasi-periodic field fluctuations also need to be taken into account (consider, for example, the techniques employing sample rotation).

It is important to understand that a *noiseless field is a theoretical chimera* which, like unicorns, does not exist in Nature!

As a stochastic phenomenon, field noise can be characterized by its auto-correlation function - an approach which unifies the treatement of its stochastic and periodic components. The essence of this study is finding out the correspondence between the field-noise autocorrelation function and the way it affects various NMR signals.

Even in the simplest case of plain FID's, the presence of field noise leads to a multitude of observable effects with well defined features. One can analyse statistics for the effects of field noise on single-scan features (such as spectral-band positions, heights and shapes) as well as those on accumulated data. The latter turn out to be equivalent to multiplying the FID by a well-defined, though definitely non-trivial, weighing function.

Very interesting is the effect of field noise on spin echos. This part of the work is directly pertinent to all multi-pulse NMR techniques which employ refocusing pulse sequence intervals (all 2D and 3D spectroscopy techniques and most MRI methods). Particularly dramatic may be the effects of periodic field fluctuations on data acquired using long, periodic trains of pulses (such as CPMG). In these cases one can occasionally witness disastrous resonance artifacts [4] whose complex nature is now finally well understood.

We shall also discuss the t_1-noise artifacts in multi-dimensional NMR spectroscopies. In practice, t_1-noise is almost totally due to the magnetic field noise and the present theory explains all its characteristic properties.

References

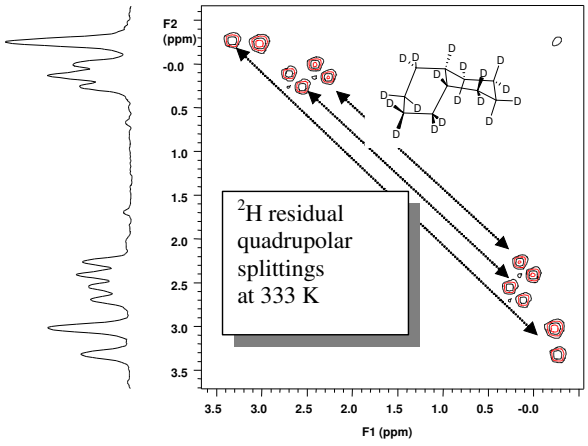
[1] S.Sykora, Field noise effects on NMR signals: FID's and 1D spectra.
[2] S.Sykora, Field noise effects on NMR signals: Hahn echos and CPMG.
[3] S.Sykora, Field noise effects on NMR signals: t1-noise in 2D spectra.
All three papers submitted to *Magnetic Resonance Engineering*.
[4] A.Allerhand, Effect of Magnetic Field Fluctuations in Spin-Echo NMR Experiments, *Rev.Sci.Instr.41*, 269 (1970).

ROUTINE ^{2}H NMR IN PARTIALLY ORIENTED PHASES. DISTINCTION OF ENANTIOMERS, STUDIES OF CONFORMATIONAL AND ANISOTROPIC MOTIONS IN TERNARY MIXTURES

Gábor Szalontai

University of Veszprém, NMR Laboratory, H-8201 Veszprém Pf.158. Hungary.

^{2}H NMR measurements [1] of partially or perdeuterated solute molecules in poly-(γ-benzyl-L-glutamate) (PBLG)/co-solvent (dichloromethane or chloroform or dioxane, … etc.) liquid-crystalline phases are presented. The residual quadrupolar splitting of ^{2}H nuclei of solutes were determined in order to make good use of them in stereochemical and conformational analyses. The results obtained for achiral and chiral model compounds of different size, symmetry, shape and flexibilities are interpreted qualitatively. Emphasis is put on the routine application of the technique. Easy distinction of enantiomers of different origin can be done. Examples taken from organic [2-3] and inorganic chemistry will be presented.



Advantages of working in such lyotropic liquid-crystalline solvents, namely the enhanced signal dispersion, the wide temperature range or the enhanced information content of the ^{2}H spectra compared to those of recorded in isotropic media, will be pointed out.

References

[1] A.Meddour, I.Canet, A.Loewenstein, J.P.Péchiné and J.Courtieu**,** *J. Am. Chem. Soc.* **1994,** 116, 9652-9656.
[2] Gábor Szalontai, *Magn. Res. Chem*. **38**, 872-876 (2000).
[3] Gábor Szalontai, *Eur. J. of Org. Chem*. 3511-18 (2001).

Acknowledgements: the author gratefully acknowledges the financial help of the Hungarian National Scientific Research Foundation (OTKA, grant number T 026503, 34335).

PARACEST AGENTS: A NOVEL CLASS OF CONTRAST MEDIA FOR MRI APPLICATIONS

E. Terreno, C. Carrera, D. Delli Castelli, S. Aime

Department of Chemistry I.F.M., University of Torino, Via P. Giuria 7, 10125 – Torino (Italy)

PARACEST agents (PARACEST = PARAmagnetic Chemical Exchange Saturation Transfer) represent a novel and emerging class of diagnostic media for MRI applications. These chemicals act by reducing the signal intensity of the water protons *via* a saturation transfer mediated by chemical exchange [1]. The two main advantages of these agents over the conventional Gd(III) or Fe(III)-based agents are: i) the ability to generate a contrast only following the irradiation of a frequency characteristic of a given PARACEST agent, and ii) the possibility to design responsive probes whose saturation transfer is not dependent on the absolute concentration of the PARACEST agent [2].

The development of more efficient PARACEST agents requires the optimization of the parameters involved in the saturation transfer process among which the more relevant are the exchange rate of the mobile protons of the agent, k_{ex}, and their number, n.

The optimal k_{ex} value is mainly related to the difference in the resonance frequency ($\Delta\omega$) between the mobile protons of the PARACEST molecule and water protons.

For this reason, a convenient route for designing high-sensitive PARACEST probes is to develop systems endowed with a high number of equivalent mobile protons with optimal k_{ex} values. An increase of n can be obtained by exploiting a molecular recognition between a diamagnetic molecule containing a high number of mobile protons and a paramagnetic shift reagent able to considerably enhance $\Delta\omega$.

In this contribution some example of high-sensitive PARACEST systems as well as some their potential applications will be presented.

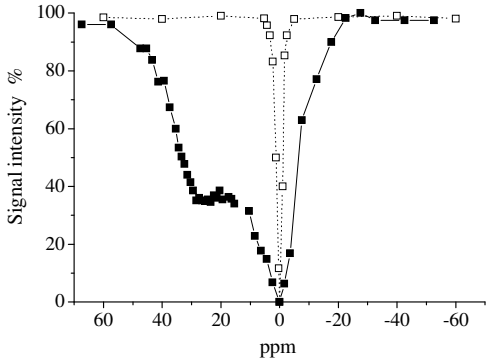


Figure 1: Z-spectra of poly-arginine with (■) and without (□) TmDOTP (pH 7.4, 312 K, 7.05 T).

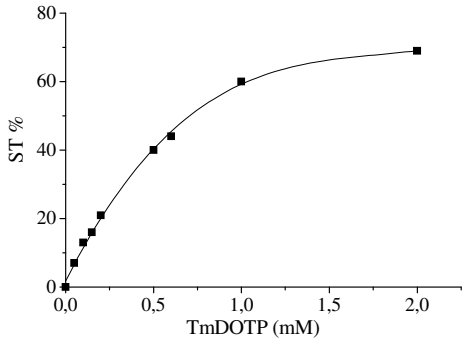


Figure 2: Concentration dependence of ST % for the TmDOTP/poly-arg system (molar ratio 24, pH 7.4, 312 K, 7.05 T).

References

[1] S. Zhang,, M. Merritt, D.E. Woessner, R.E. Lenkinski, A.D. Sherry *Acc. Chem. Res.* **36**, 783-790 (2003)
[2] S. Aime, D. Delli Castelli, E. Terreno *Angew. Chemie Int. Ed.*, **22**, 4334-4336 (2002).

SEPARATION AND IDENTIFICATION OF EXTRACTS OF REGULAR AND TANGERINE VARIETIES OF TOMATO JUICE BY ^{1}H NMR

S. Tiziani, M. Renita, S. Schwartz and Y. Vodovotz

FOOD SCIENCE & TECHNOLOGY DEPARTMENT, THE OHIO STATE UNIVERSITY, COLUMBUS, OH 43210 (USA)

Epidemiological studies have shown the health benefits of tomato consumption for the prevention of chronic diseases, with lycopene and β-carotene being the biologically active components in these tomato based-products [1]. The susceptibility of carotenoids to isomerization may affect and enhance the bioavailability of lycopene [2]. Our objective was to characterize the chemical content of different tomato juice varieties focusing on the different carotenoid profiles. In this study, two different tomato juice varieties, regular (mostly trans-lycopene) and tangerine tomato (containing high levels of cis-isomers), were analyzed by ^{1}H NMR using a Bruker DRX-600 and 800Mhz spectrometers and by reverse-phase HPLC-PDA system. The hydrophilic components of tomato pulp were extracted with D_2O and analyzed by one-dimensional ^{1}H-NMR and by COSY, TOCSY, HSQC and HMBC 2D sequences. The combination of these techniques resulted in the identification of several sugars, organic acids and amino acids that impart the organoleptic properties to the two products. Two different extractions were compared to analyze the hydrophobic compounds in these samples. The first method entailed the extraction of the pulp with 1:1 acetone/hexane followed by drying and further dissolving in CDCl3 prior to NMR. In the second method, hydrophobic compounds were extracted directly by CDCl3 allowing for limited contamination and isomerizations without affecting the identification of carotenoids. COSY, TOCSY, and HSQC 2D spectroscopy were performed and compared with HPLC results. The identification of trans-lycopene and 7-7' 9-9' tetra cis-lycopene were found to be predominant in regular and tangerine tomato juices, respectively, compared to other carotenoids. This work confirms that NMR spectroscopy is a powerful noninvasive technique that allows the characterization not only of sugars, proteins and organic acids [3] but also the identification of minor compounds such as carotenoids.

References

[1] Agarwal, S., & Rao, A.V. (2000). Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association Journal*, **163**, 739-744.

[2] Nguyen, M., Francis, D., & Schwartz, S. (2001). Thermal isomerisation susceptibility of carotenoids in different tomato varieties. *Journal of the Science of Food and Agriculture*, **81**, 910-917.

[3] Sobolev, A.P., Segre, A., & Lamanna, R. (2003). Proton high-field NMR study of tomato juice. *Magnetic Resonance in Chemistry*, **41**, 237-245.

T_2 QUANTITATION OF ARTICULAR CARTILAGE AT 1.5 TESLA: COMPARISON OF STANDARD MULTIECHO AND GRASE SEQUENCES

R. Toffanin,[‡,§] P. Szomolányi,[‡] I. Strolka,[†] F. Vittur,[‡] R. Pozzi-Mucelli,[†] M. Cova[†]

[‡]*Department of Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Via L. Giorgieri 1, I-34127 Trieste, Italy.* [§]*PROTOS Research Institute, P.O. Box 972, I-34100 Trieste, Italy.* [†]*Department of Radiology, University of Trieste, Cattinara Hospital, I-34149 Trieste, Italy*

Evaluation of the T_2 relaxation time of articular cartilage holds great potential for the quantitative assessment of degenerative changes within the cartilage matrix. The purpose of our study was to assess the validity of the GRASE (Gradient and Spin Echo) sequence [1,2] in the fast T_2 mapping of articular cartilage at 1.5 Tesla and compare it with the standard multiecho, single-slice sequence. MR imaging was performed on a 1.5 Tesla Philips Intera scanner equipped with 30 mT/m gradients. A commercially available circular surface coil was used to obtain coronal images of $CuSO_4$-agarose gel phantoms and axial images of patellar cartilage in ten volunteers using GRASE and standard multiecho, single-slice sequences. For all acquisitions, a slice thickness of 3 mm and a matrix of 128×128 with a field of view of 80×80 mm were used. GRASE images (TR/TE in ms 3000/20) were acquired with a TSE factor of 8 and an EPI factor of 3. The number of slices was 10 and the total measurement time was 1 min. 51 s. The measurement time of each individual slice imaged by the standard multiecho, single-slice protocol (TR/TE in ms 3000/20) was 6 min. 30 s. In both cases, the T_2 maps were calculated using an echo train of 8 echoes. As compared with multiecho experiments, the apparent T_2 values calculated from the GRASE images of both gel phantoms and articular cartilage showed a mean overestimation by about +11%. In one volunteer, however, the mean T_2 value of patellar cartilage was 43.64 ±1.04 ms for the GRASE acquisition and 43.37 ± 2.00 ms for the standard multiecho experiment. Our preliminary results demonstrate the applicability of the GRASE sequence in the fast T_2 mapping of articular cartilage at 1.5 Tesla. The T_2 values obtained by the GRASE sequence are, in fact, in good agreement with the corresponding values obtained by the standard multiecho, single-slice sequence. The main advantage of using GRASE is the drastically reduced imaging time. This approach might be of specific interest in the MRI of osteoarthritis.

References

[1] K. Oshio and D.A. Feinberg *Magn. Reson. Med.* **20**, 344-349 (1991)
[2] D.A. Feinberg and K. Oshio *Radiology* **181**, 597-602 (1991)

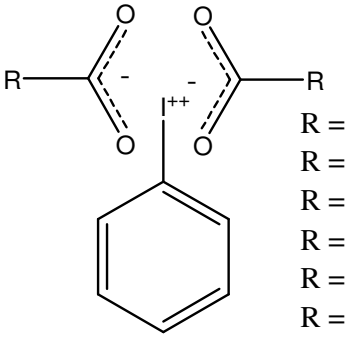
SOLUTION ($CDCl_3$) STRUCTURE OF BIS(ACILOXY)IODOARENES.

Giovanni Cerioni,[‡] Gianluca Uccheddu,[‡]

[‡]*Dipartimento Farmaco Chimico Tecnologico, Via Ospedale, 72 I-09124 Cagliari, Italy.*

Hypervalent iodine chemistry has experienced in the last years a fast and important increase of interest, as shown by the accelerated appearance of reviews [1] devoted to it. Notwithstanding this large body of literature, a full spectroscopic characterization of many I(III) organic derivatives, λ^3 iodanes, is lacking. The solid state,[2] "T-shaped" and I-O covalently bonded structure is usually adopted also in solution, although there is not a definite evidence in favour of it. In recent years, Koser group has shown [3] that [hydroxy(tosyloxy)iodo]benzene is fully ionized in water solution, originating a monomeric positively charged hydroxy(phenyl)iodonium ion. The Ochiai group has been able to stabilize [4] this cation by interaction with 18-Crown-6. In a recent paper [5] we have suggested for bis(acetoxy)iodoarenes an "ion pair" structure, in chloroform solution, even if a "chelate" structure could not be completely ruled out. Main criteria leading to our hypothesis have been detection, at ^{17}O NMR, of only one signal for all the four oxygens of the acetoxy groups and complete invariance of the ^{17}O shifts with *para* substitution.

With the scope to obtain more evidence in favour of this hypothesis, to generalise it to the class of bis(acyloxy)iodoarenes and to explore the influence of acid strength and steric hindrance on the solution structure of this class of λ^3 iodanes, we have analyzed a series of derivates with different acids . ^{17}O NMR data have been compared with those of the free acids. Structures and representative ^{13}C NMR data (C_1) of the studied compounds are shown in Fig. 1.



R = Me	(121,6 ppm)	**1a**	$R = CF_3$	(122.8 ppm)	**1g**
R = Et	(121,6 ppm)	**1b**	$R = C_6H_5$	(122.3 ppm)	**1h**
$R = C(CH_3)_3$	(121.8 ppm)	**1c**	$R = 2,6\text{-}Cl_2\text{-}C_6H_3$	(122.8 ppm)	**1i**
$R = CH_2Cl$	(122.3 ppm)	**1d**	$R = 2,4,6\text{-}Me_3\text{-}C_6H_2$	(122.5 ppm)	**1j**
$R = CHCl_2$	(122.7 ppm)	**1e**			
$R = CCl_3$	(123.3 ppm)	**1f**			

Fig. 1. Structures of compounds 1.

References

[1] a) Stang, P. J. *J. Org. Chem.* **2003**, *68*, 2997-3008; b) Zhdankin, V. V.; Stang, P. J. *Chem. Rev.* **2002**, *102*, 2523-2584; c) Stang, P. J.; Zhdankin, V. V. *Chem. Rev.* **1996**, *96*, 1123-1178.
[2] Alcock, N. W.; Countryman, R. M.; Esperas, S.; Sawyer, J. F. *J. Chem. Soc., Dalton Trans.* **1979**, 854-860.
[3] Richter, H. W.; Cherry, B. R.; Zook, T. D.; Koser, G. F. *J. Am. Chem. Soc.* **1997**, *119*, 9614
[4] Ochiai, M.; Miyamoto, K.; Shiro, M.; Ozawa, T.; Yamaguchi, K. *J. Am. Chem. Soc.* **2003**, *125*, 13006-13007.
[5] Cerioni, G.; Uccheddu, G. *Tetrahedron Lett.*, **2004**, *45*, 505-507.

LIVER BASIC FATTY ACID BINDING PROTEIN: STABILITY PROPERTIES AS DETERMINED BY NMR STUDIES

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

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

In this work we will present the results obtained on the characterization of stability and folding properties of the recombinant ^{15}N-enriched Liver basic Fatty Acid Binding Protein (Lb-FABP), which has been successfully 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. The urea unfolding data could be fitted with a three-state model, with the onset of an intermediate state, which appears to be better defined for the holo protein. For apo Lb-FABP, two regions, exhibiting a different stability pattern, were identified located on opposite beta-sheets. In particular, residues exhibiting a high urea sensitivity were shown to be affected by conformational exchange, on the basis of dynamics studies. Comparison of the thermodynamic figures obtained from urea unfolding data and H/D exchange measurements for the apo and holo protein indicate that complexation with palmitic acid ligand does not significantly stabilize the protein. The data will be discussed in light of the folding results obtained for other FABPs, in order to elucidate common stability determinants among the family.

ANALYSIS OF LIQUID MIXTURES BY GENERALIZED CORRELATION OF CHROMATOGRAPHIC AND NMR DATA

L. Venturi,[‡] M. A. Cremonini,[‡] N. Culeddu,[†] L. Laghi,[‡] G. Placucci[‡]

[‡]*Department of Food Science, University of Bologna, P.zza Goidanich 60, 47023 Cesena, Italy*
[†]*CNR ICB Sezione di Sassari, Via La Crucca 3, Baldinca, 07040 Li Punti Sassari, Italy*

Analysis of natural products extracts aims at identifying the substances contained in complex mixtures. Although HPLC/NMR can be successfully used to achieve this task through the analysis of 1D- and 2D-spectra corresponding to each chromatographic peak, cases may exist where chromatographic separation is difficult and a limited number of compounds may co-elute. In these cases one obtains spectra showing the signals of all the co-eluting species. However, there are chances of obtaining the pure spectrum of each co-eluting compound by exploiting the notion that different extraction conditions would likely change their relative proportions. Accordingly, each extract would yield different chromatographic/NMR responses in which the NMR signals belonging to the co-eluting species increase or decrease altogether and the shape of the chromatographic peak changes, as it is unlikely that all the co-eluting compounds have exactly the same retention time.

When the spectra are crowded, or there are too many lines in the spectrum to allow visual analysis of the NMR dataset, the so called "generalized correlation" (GC) can be of valuable help to finding which lines belong to each co-eluting compound. In its basic form GC is "nothing but a quantitative comparison of spectral intensity variations observed at two different spectral variables" [1], and the so called "synchronous" GC-2D diagram reveals which features of the spectrum vary in synchrony throughout the different extracts.

In this preliminary work the usefulness of GC for the resolution of pure NMR spectra of mixtures components will be tested on a simple dataset obtained by mixing 3 compounds in 9 different proportions. It will be shown that hetero-synchronous GC-2D diagrams (*i.e.* those obtained by GC of cromatographic and NMR data) are particularly powerful in yielding the signals of interest.

References

[1] I. Noda, A. E. Dowrey, C. Marcott, G. M. Story, and Y. Ozaki Appl. Spectrosc. **54**, 236A-248A (2000)

^{129}Xe NUCLEAR SHIELDING AND DIFFUSION IN THE A AND C* PHASES OF A CHIRAL SMECTOGEN

Mario Cifelli [a)], Jani Saunavaara [b)], Jukka Jokisaari [b)*] and Carlo A. Veracini [a)*]

[a)] *Department of Chemistry and Industrial Chemistry, University of Pisa, Italy, Via Risorgimento 35, 56100 Pisa, Italy.* [b)] *NMR Research Group, Department of Physical Sciences, P.O.Box 3000, FIN-90014 University of Oulu*

The ^{129}Xe NMR shielding and diffusion of xenon dissolved in the chiral liquid crystal 1-methylheptyl 4'-(4-*n*-decyloxybenzoyloxy)biphenyl-4-carboxylate (10B1M7) were studied over the temperature range covering the isotropic (I), smectic A (SmA), ferroelectric, ferrielectric and antiferroelectric smectic C* (SmC*) phases. The ^{129}Xe shielding reveals clearly the I – SmA and SmA – SmC*(ferroelectric) phase transitions. The SmC* sub-phase transitions can also be detected by smaller but distinguishable shifts of the shielding accompanied by linewidth changes. A theoretical model developed earlier is applied to the shielding data, confirming a negative anisotropic contribution to the shielding as the SmA phase forms and allowing the evaluation of the tilt angle in the SmC*. Diffusion experiments were mostly performed in the direction parallel to the layer normal, *i.e.* along the external magnetic field, $D_{//}$, in the smectic phases but a few experiments were carried out in the in-plane direction, *i.e*. perpendicular to the external magnetic field, D_{ζ}, as well.
These experiments indicate large anisotropy, $D_{//}/D_{\zeta}$, of the ^{129}Xe diffusion tensor that increases as the temperature decreases. Application of the Arrhenius equation to the temperature dependence of $D_{//}$ reveals different activation energies for each studied phase. This can be correlated to changes in the smectic layer structure that occur between the different smectic phases.

"FOR 45 YEARS IN NMR RESEARCH: SOME RECOLLECTIONS AND REFLECTIONS"

W. von Philipsborn

University of Zürich, Institute of Organic Chemistry, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Unlike any other spectroscopic method nuclear magnetic resonance has shown from the beginning a continuous evolution in theory, methodology, and application to physics and chemistry which even after half a century has not yet slowed down. I have been fortunate to be involved in this development as a witness and active player for more than four decades, which in turn had a profound influence on my chemical research interests.

This reflective lecture will dwell on the interplay between widely different chemical challenges and NMR techniques that evolved from the study of proton spectra and extended to carbon-13, nitrogen-15, oxygen-17, and finally to such demanding nuclei as the spin-1/2 and quadrupolar transition metals. This interplay combined with a global exchange of experimental know-how, experience in spectra interpretation, and mutual teaching is a characteristic feature of our chemical sciences and their success.

In fact, the advent of a physical method eminently suited to study the frame work of chemical structures, chemical bonding, and molecular rearrangements, but also to probe chemical and catalytic reactivity, caused nothing less than a revolution in chemical research, teaching and laboratory training in the nineteen sixties.

The development of the NMR methods will be illustrated by examples from our own studies in organic, organometallic, and biological chemistry ranging from the early beginning to the very recent past, and with an outlook into the future.

References

[1] W. von Philipsborn, *Angew. Chem.*, Intern. Ed. **10**, 472-491 (1971)
[2] W. von Philipsborn, XXI Colloquium Spectrosc. Intern. Cambridge 1979, Heyden & Son Ltd., p. 218-235
[3] V.M.S. Gil and W. von Philipsborn, *Magn. Reson Chem.* **27**, 409-430 (1989)
[4] W. von Philipsborn and R. Müller, *Angew. Chem. Intern. Ed.* **25**, 383-413 (1986)
[5] W. von Philipsborn, *Pure Appl. Chem.* **58**, 513-528 (1986); *Chem. Soc. Rev.* **28**, 95-105 (1999)

CAP43 PROTEIN: AN ATTRACTIVE MOTIF FOR Ni(II) IN THE C-TERMINAL DOMAIN

Maria Antonietta Zoroddu[a]*, Massimiliano Peana[a], Serenella Medici[a], Teresa Kowalik-Jankowska[b], Henryk Kozlowski[b], Max Costa[c]

[a] Department of Chemistry, University of Sassari, Via Vienna 2, 07100, Sassari, Italy. [b] Faculty of Chemistry, University of Wroclaw, Polka. [c] Department of Environmental Medicine, New York University, New York, USA

The carcinogenicity of nickel compounds has been confirmed by numerous epidemiological studies in humans and animals.[1] A possible way to better understand the molecular mechanisms implicated in toxicity and carcinogenicity of nickel compounds is to study the characteristics of the proteins expressed by the genes specifically induced by these carcinogens.

Cap43 is an excellent tumor marker recently discovered. Exposure to either soluble or insoluble nickel compounds strongly activated several hypoxia-inducible genes.[2] Cap43 is one of these genes, and it expressed a 3.0-kb mRNA encoding a Mr 43,000 protein.[3] The primary signal for its induction is an elevation of free intracellular calcium ion caused by nickel ion exposure in cultured human cells, for this reason is named Cap43: Calcium protein 43,000. The peculiarity of protein Cap43 is its new mono-histidinic motif consisting of ten amino acids TRSRSHTSEG repeated three times in the C-terminus.

We have analyzed, for Ni(II) binding, the 30-amino acid C-terminal fragment of the protein, by a combined pH-metric and spectroscopic study. The fragment showed to bind one, two and three metal ions depending on the metal to ligand molar ratio.

The present work supports the existence of an interesting binding site for Ni(II) at the C-terminal domain of Cap43 protein.

References

[1] IARC, Lyon, France Monographs on the evaluation of carcinogenic risks to humans, Vol. 49, Chromium, Nickel and Welding, (1990)
[2] Cangul, H.; Salnikow, K.; Yee, H.; Zagzag, D.; Commes, T.; Costa, M., Met. Toxic., 110, 783 (2002)
[3] Zhou, D.; Salnikow, K.; Costa, M., Cancer Res. 58, 2182 (1998)

XXXIV National Congress on Magnetic Resonance

POSTERS

^{29}Si NMR AND FT/IR INVESTIGATION OF NANOPARTICLE-SILICA INTERACTIONS IN A ZnO/SiO_2 NANOCOMPOSITE

R. Anedda, C. Cannas, M. Casu, A. Musinu, G. Piccaluga

Dipartimento Scienze Chimiche, Università di Cagliari, Monserrato (CA), Italy.

Nanocomposites consisting of metal or metal oxide particles dispersed in glass matrices are very interesting materials because of their potential use in several fields, such as catalysis, optics, magnetism and electronics. In fact, the preparation of nanocomposites represents an effective remedy to the tendency of nanopowders to aggregate. The properties of nanocomposite systems are strongly dependent on their microstructure, not only in terms of dispersion, size distribution of metal oxide nanoparticles, but also as a function of possible interactions that take place among nanoparticles and matrix. In our laboratories the study has been addressed to the characterization of structural and physical properties of several metal oxide-silica nanocomposites obtained through sol-gel and impregnation methods. (1-3)

In this work, ZnO/SiO_2 nanocomposites obtained by a sol-gel process with addition of citric acid are investigated. The procedure allowed to obtain ZnO nanoparticles (zincite phase) homogeneously dispersed into the amorphous silica matrix, as evidenced by X-ray diffraction and transmission electron microscopy. The study is focused on the structural investigation of the precursor formed at the early stage of the synthesis and on the final material after thermal treatment. The influence of the citric acid and silica precursor on the properties of the forming nanoparticles is investigated through ^{29}Si MAS and CPMAS NMR and FT/IR spectroscopy. To this end, the spectra of the nanocomposites have been compared with that of reference samples obtained through the same preparation method but in absence of zinc salts and submitted to the same thermal treatments. The role of citric acid in the zinc oxide nanoparticles formation resulted to be essential to avoid the presence of important interactions among particles and silica matrix in the final nanocomposite.

References

1) C. Cannas, M. Casu, A. Lai, A. Musinu, G. Piccaluga, *J. Mater. Chem*. 9 (1999) 1765.
2) C. Cannas, A. Falqui, A. Musinu, G. Piccaluga, D. Gatteschi, C. Sangregorio, G. Concas, G. Spano, *PCCP* 3 (2001) 832.
3) C. Cannas, M. Bettinelli, M. Casu, A. Musinu, G. Piccaluga, A. Speghini, *J. Non-Cryst. Solids*., 306 (2002) 193.

METABOLIC CHARACTERIZATION OF *OCIMUM BASILICUM* L. BY PROTON NMR SPECTROSCOPY

E. Barrese, I. Piscioneri, N. Sharma, M.L. Miglietta, R. Lamanna

ENEA C.R. Trisaia, S.S. Jonica 106, KM 419.5, 75026 Rotondella (MT), Italy

Ocimum basilicum L, generally known as sweet basil, is an important medicinal plant and culinary herb[1]. It is widely used as kitchen herb in the Mediterranean diet and cultivated for the production of essential oil and oleoresin that possess antifungal, antibacterial and insect-repelling properties [2-3].

The metabolic composition of plant is strongly affected by the type of growing media. In order to study the effect of different substrates on the agronomic and chemical characteristic of basil, a green house pot experiment has been conducted using soil and peat.

Basil leaves extracts have been analysed by proton NMR spectroscopy. The NMR profiles of soil and peat basil samples are compared. The proton NMR spectrum has been assigned through 2D NMR experiments (COSY, TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, DOSY) and various metabolites (organic acid, aminoacids and other minor components.) are identified.

References

[1] Loughrin, J.H.et co. *J. Agric. Food Chem*. **49**, 1331-1335 (2001)
[2] R.Reuveni, A.Fleischer, E.Putievsky, *Phytopath.Z.* **110**, 20-22 (1984)
[3] S.Dube, P.D. Upadhyay, S.C. Tripathi *Can.J.Bot*. **67**, 2085-2087 (1989)

ENANTIODISCRIMINATION OF "COMPOUND B", A DEGRADATION PRODUCT OF SEVOFLUORANE: APPLICATIONS AND NMR INVESTIGATIONS OF CHIRAL RECOGNITION PROCESSES

Gloria Uccello Barretta,[‡] Federica Balzano,[‡] Giuseppe Sicoli,[†] Volker Schurig,[†] Piero Salvadori,[‡]

[‡] *Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, 56126 Pisa, Italy;* [†] *Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany*

In the improvement of the applications of volatile halogenated anaesthetics, the capability to detect and separate their potentially toxic degradation products plays a fundamental role. Sevoflurane is a fluorinated derivative of methyl isopropyl ether which is under development since 1992 for use as an inhalation anaesthetic and 2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane (**1**, compound B) (Fig. 1) was identified as one of its degradation products.
Among the mixed silylated/acetylated cyclodextrins **2**-**4** (Fig. 1), heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (**2**), which is the basis of high efficiency GC methodologies [1] for the enantioresolution of fluorinated chiral anaesthetics and their chiral degradation products, can be proposed as promising chiral solvating agent for their effective and no-time consuming ^{1}H and ^{19}F NMR detection. By using the conditions (in solution) where no interactions between the solvents (C_6D_{12}) and the chiral selector occur, it was possible to obtain a high enantiodiscrimination for compound B, according to the same behaviour observed in the chromatographic separation. On the basis of NMR investigations an interaction mechanism has been proposed, underlining the role of different functional groups on the cyclodextrin rims, besides the action of the inclusion phenomena.

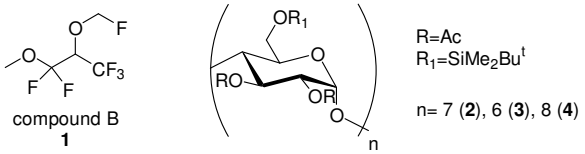


Fig. 1. Compound B and β-cyclodextrin derivatives **2**-**4**

References

[1] Schmidt, R.; Roeder, M.; Oeckler, O.; Simon, A.; Schurig, V. *Chirality* **2000**, *12*, 751-755.

NMR SPECTROSCOPY: A POWERFUL TOOL FOR DETECTING THE CONFORMATIONAL FEATURES OF SYMMETRICAL PERSUBSTITUTED MIXED β-CYCLODEXTRINS

Gloria Uccello Barretta,[‡] Federica Balzano,[‡] Giuseppe Sicoli,[†] Piero Salvadori,[‡]

[‡] *Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35 56126 Pisa, Italy*
[†] *Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18 D-72076 Tübingen, Germany*

During the last ten years persubstituted cyclodextrins have been used as Chiral Solvating Agents (CSAs) [1] able to induce NMR unequivalence of the enantiotopic nuclei of enantiomeric mixtures, making them distinguishable in the NMR spectra. Alkylated or acetylated cyclodextrins, extensively employed in GC [2], have shown great potentialities as CSAs for apolar substrates [3], whereas benzoylated, benzylated and carbamoylated cyclodextrins have been successfully employed as $CDCl_3$ soluble CSAs for enantiodiscriminating polar chiral substrates [4]. These investigations also evidenced the distorsions induced in the α-cyclodextrin structure due to the derivatization [4], which consequently produced dramatic changes of their complexing properties. A recent detailed conformational analysis in solution [5] of the peracetylated β-cyclodextrin revealed the presence of glucopyranose units having strong deviations from the $^4C_1$ conformation. The conformation in solution of exhaustively derivatized mixed carbamoyl/methyl or acetyl cyclodextrins **1**-**4** (Fig. 1) has been defined by NMR spectroscopy. Both tilting of glucopyranose units about the glycosidic linkages and ring deviations from the $^4C_1$ chair conformation are detected, the entities of which are strongly dependent on the nature of the derivatizing groups.

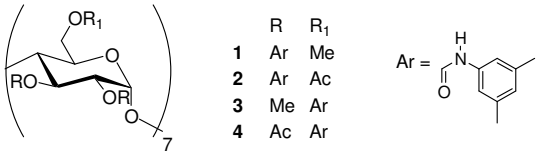


Fig. 1. Persubstituted β-cyclodextrins **1**-**4**

References

[1] (a) Rothchild, R. *Enantiomer* **2000**, *5*, 457-471. (b) Parker, D. *Chem. Rev.* **1991**, 91, 1441-1457.
[2] Schurig, V. *TrAC Trend. Anal. Chem.* **2002**, *21*, 647-661.
[3] (a) Uccello-Barretta, G.; Balzano, F.; Caporusso, A. M.; Salvadori, P. *J. Org. Chem.* **1994**, *59*, 836-839. (b) Uccello-Barretta, G.; Balzano, F.; Caporusso, A. M.; Iodice, A.; Salvadori, P. *J. Org. Chem.* **1995**, *60*, 2227-2231. (c) Uccello-Barretta, G.; Balzano, F.; Menicagli, R.; Salvadori, P. *J. Org. Chem.* **1996**, *61*, 363-365. (d) Uccello-Barretta, G.; Balzano, F.; Salvadori, P.; Lazzaroni, R.; Caporusso, A. M.; Menicagli, R. *Enantiomer* **1996**, *1*, 365-375.
[4] (a) Uccello-Barretta, G.; Cuzzola, A.; Balzano, F.; Menicagli, R.; Iuliano, A.; Salvadori, P. *J. Org. Chem.* **1997**, *62*, 827-835. 8b) Uccello-Barretta, G.; Cuzzola, A.; Balzano, F.; Menicagli, R.; Salvadori, P. *Eur. J. Org. Chem.* **1998**, 2009-2012. (c) Uccello-Barretta, G.; Ferri, L.; Balzano, F.; Salvadori, P. *Eur J. Org. Chem.* **2003**, 1741-1748.
[5] Uccello-Barretta, G.; Balzano, F.; Cuzzola, A.; Menicagli, R.; Salvadori, P. *Eur. J. Org. Chem.* **2000**, 449-453.
[6] Uccello-Barretta, G.; Sicoli, G.; Balzano, F.; Salvadori, P. *Carbohydr. Res.* **2003**, *338*, 1103-1107.

NMR RELAXATION STUDIES OF NEW HEMA HYDROGELS

M. Delfini[‡], M. E. Di Cocco[‡], C. Bianchetti[‡], L. Casciani[‡], C. Manetti[‡] and F. Chiellini[†]

[‡]*Department of Chemistry Roma University "La Sapienza" , P.le Aldo Moro 5, 00185 Roma (Italy)*
[†]*Department of Chemistry and Industrial Chemistry Pisa University , v. Risorgimento 35, 56126 Pisa (Italy)*

Polymer Hydrogels, based on copolymers of 2-Hydroxy-Ethyl-Methacrylate (HEMA) and other methacrylate monomers, exhibit a high degree of chemical stability and mechanical integrity, properties that have been exploited in several applications particularly in the biomedical fields. In many cases, biodegradability is often requested too. To modify the biodegradability of HEMA-based hydrogels, in addition to the monomer, mixture of pentaerityrityl tetrakis(3-mercaptopropionate) (Tetrakis) provided the hydrogels of biodegradability character.

In this study we characterized new poly-2-Hydroxy-Ethyl-Methacrylate (polyHEMA) hydrogels used in the formulation of drug delivery systems and as the basic ingredient for the formulation of scaffolds for tissue engineering applications. The new poly-HEMA hydrogels were obtained by polymerisation with Tetra-(Ethylene-Glycol)-Di-Acrylate (TEGDA) as crosslinking agent and TETRAKIS .

Crosslinker concentrations effects on the mobility polymer chains and on the swelling properties and diffusion behaviour of water were investigated. Data were obtained by High and Low Field Nuclear Magnetic Resonance (NMR) in particular using:

a. the measure of the ^{13}C longitudinal relaxation times (T_1) on samples swelling in DMSO by High Field NMR; *b*. the measure of the dynamical NMR parameters of water, transversal relaxation times (T_2) and self-diffusion coefficients (D) by Low Field NMR. The obtained results pointed out different Hydrogels characteristics and properties owed to the presence of Tetrakis.

STRUCTURAL CHARACTERIZATION OF XENON-BINDING SITES IN PIG AND HORSE CYANOMETMYOGLOBINS BY ^{1}H AND ^{129}Xe NMR SPECTROSCOPY

M. Corda[a], B. Era[a], R. Anedda[b], A. Fais[a], C. Floris[b] and M. Casu[b]

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

The sensitivity of xenon to its local environment has motivated the use of xenon-binding in proteins with the desire to map hydrophobic cavities. Xenon is known to bind specifically to the internal hydrophobic cavities of metmyoglobin (MMb) with very small perturbations in the surrounding environment. Indeed, X-ray crystallographic studies of sperm whale MMb complexed with 7 atm of xenon provided evidence of 4 xenon sites with fractional occupancy ranging from 0.42 to 1 (1). The ^{129}Xe NMR chemical shifts in solution were first studied in 1984 by Tilton and Kuntz (2) in sperm whale MMb. The NMR evidence indicated the presence of xenon binding near the iron in the proximal cavity. This evidence has been recently confirmed in horse MMb (3,4)

In a previous publication (5) by the combined use of the ^{129}Xe chemical shift and spin lattice relaxation rate we were able to reveal structural differences and occupancy of cavities on pig and horse myoglobins. These MMb's differ by 14 aminoacids. One of these, Ile142 in horse MMb, is located in the proximal cavity, which is the major xenon binding site in horse MMb, and is replaced by Met142 in pig MMb. This study supported the presence of more than one type of binding sites in pig MMb. Furthermore, it showed the presence of xenon on the proximal cavity in pig MMb, which also seems to be less populated than in horse MMb

Here, further structural characterization of hydrophobic cavities of pig and horse cyanometmyoglobin (CNMb) are obtained by ^{1}H and ^{129}Xe NMR. Xenon-protein interactions are investigated by ^{129}Xe NMR chemical shifts in aqueous solutions of horse and pig CNMb as a function of the xenon concentration. The results are complemented with ^{1}H NMR spectra to test the dependence of the ^{1}H chemical shift on the addition of xenon. The location of xenon inside the cavity is deduced from 1D ^{1}H NMR and 2D COSY and NOESY experiments. Evidence is given that xenon appears to occupy a cavity in the distal side of CNMb, besides the proximal cavity.

References

1) R. F. Tilton Jr., I.D. Kuntz Jr., and G.A. Petsko, Biochemistry 23, 2849-2857 (1984)
2) R. F. Tilton Jr., I. D. Kuntz, Jr., Biochemistry 21 6850-6857 (1982)
3) S.M. Rubin, M .M. Spence, B. M. Goodson, D. E. Wemmer, A. Pines, Proc. Narl. Acad. Sci. 97, 3472-9475 (2000)
4) E. Locci, Y. Dehouck, M. Casu, G. Saba, A. Lai, M. Luhmer, J. Reisse, K. Bartik, J. Magn. Reson. 150 167-174 (2001)
5) M. Corda, B. Era, A. Fais and M. Casu, BBA, 1674, 182-192 (2004)

DIRECT DELINEATION OF BURIED WATER MOLECULES IN PROTEINS

Arianna Ciutti, Ottavia Spiga, Andrea Bernini, Vincenzo Venditti and Neri Niccolai.

Department of Molecular Biology, University of Siena

Nuclear magnetic resonance dispersion of ^{17}O and ^{2}H relaxation has provided an estimate of bound waters and of their residence times [1]. The use of paramagnetic probes to perturb the intensities of ePHOGSY signals has been also suggested to delineate the distribution of buried water molecules inside proteins [2]. Here we describe a comparison between the hydration network derived from MD calculations with the paramagnetic perturbations of ePHOGSY signals in NMR spectra.of BPTI, hen egg white lysozyme (HEWL) and α-bungarotoxin.

Paramagnetic attenuations of ePHOGSY type signals may depend on two main aspects: i) the rates of the chemical exchange among the water molecules at the different molecular sites and ii) the protons relaxation enhancement of those water molecules which result more exposed to TEMPOL. In this respect, it must be noted that this paramagnetic probe has the unpaired electron spin density entirely distributed on its N-oxyl moiety, a good hydrogen bonding acceptor. This feature may determine very high relaxation enhancement of TEMPOL bound water molecules. The different extent of the paramagnetic relaxation enhancements and the rates of chemical exchange experienced by water molecules interacting with the protein may determine, for the proton relaxation process underlying the observation of the NOEpw's, slow exchange conditions. Accordingly, different approaches of TEMPOL to the protein hydration network can be delineated, resulting in NOEpw's, which are differently perturbed by TEMPOL. This seems to be the case for all the examined proteins, since the presence of TEMPOL induces just small attenuations in the intensities of all the ePHOGSY signals which are predicted by crystallographic and MD data to be close to buried water molecules.

Hence, the presence of structural water molecules or of strong hydration sites may be revealed by the corresponding small Ai values. Other paramagnetic probes might be used, particularly in the case of protein systems with redox properties, which can interfere with the weak oxidant capability of TEMPOL. Thus, neutral complexes of transition metal ions such as Gd(III)DTPA-BMA could be alternatively used, also at lower concentrations, as recently proposed [3].

References

[1] Modig K, Liepinsh E, Otting G, Halle B. J Am Chem Soc. 2004 Jan 14;126(1):102-14.

[2] Niccolai N, Spiga O, Bernini A, Scarselli M, Ciutti A, Fiaschi I, Chiellini S, Molinari H, Temussi PA. J Mol Biol. 2003 332:437-447.

[3] Pintacuda, G., Otting, G. 2002 J. Am. Chem. Soc. 124, 372-373.

^{1}H NMR PROTOCOLE FOR THE DETECTION OF OLIVE OIL ADULTERATIONS WITH HAZELNUT OILS

Marco D'Imperio[1], Enrico Rossi[2], Luisa Mannina[1,2], Annalaura Segre[2], Serge Rezzi[3] and Claude Guillou[3]

[1]*Università degli Studi del Molise, Dipartimento S.T.A.A.M, 86100 Campobasso, Italy*
[2]*Istituto di Metodologie Chimiche, CNR, Monterotondo Staz. Roma, Italy*
[3]*Joint Research Centre of the European Commission, Ispra, Italy*

The NMR technique is a poweful tool to characterize olive oils, see references reported below. A ^{1}H NMR detailed protocol and the whole methodology is proposed for the direct detection of olive oil adulterations with rectified hazelnut oil. The protocole describes: a) the procedure for the acquisition of the ^{1}H NMR spectra of oils, b) the data processing of the ^{1}H spectra, c) the measurement of the ^{1}H selected resonances and finally d) the statistical procedure used to analyse the NMR results. The evaluation of the methodology has been performed by other peer laboratories using different spectrometers operating at 400MHz, 500MHz an 600MHz. The results obtained using different spectrometers operating at different magnetic fields are presented. This work has been funded by the European project "Medeo: Development and Assessment of Methods for the Detection of Adulteration of Olive Oil with Hazelnut Oil".

References

1) C.Fauhl, F.Reniero, C. Guillou, "^{1}H NMR as a tool for the analysis of mixtures of virgin olive oils wity oils of different botanical origin", *Magn.Res. Chem*. (2000), 38, 436-443.

2)L.Mannina, M.Patumi, P.Fiordiponti, M.C.Emanuele, AL.Segre "Olive and hazelnut oils: a study by high-field ^{1}H NMR and gas chromatography", *Italian Food Technolog*y, (2000), 21, 15-24.

3) L.Mannina, M.Patumi, N.Proietti, A.L. Segre, "P.D.O. (Protected Designation of Origin) Geographical Characterization of Tuscan Extra Virgin Olive Oils using High-Field ^{1}H NMR spectroscopy", *Italian Journal of Food Science*, (2001), 13, 53-63

4)L.Mannina, M.Patumi, N.Proietti, D.Bassi, A.L.Segre, "Geographical characterization of Italian extra virgin olive oils using high field ^{1}H-NMR spectroscopy", *Journal of Agriculture and Food Chemistry*, (2001), 49, 2687-2696.

5) L.Mannina, A.L.Segre, "High Resolution Nuclear Magnetic Resonance: From Chemical Structure to Food Authenticity", *Grasas y Aceites*, (2002), 53, 22-33.

6)L.Mannina, G.Dugo,F.Salvo, L.Cicero, G.Ansanelli, C.Calcagni A.L.Segre, "Study of the Cultivar-Compoition Relationship in Sicilian olive Oils by GC, NMR, and Statistical Methods", *Journal of Agriculture and Food Chemistry*, (2003), 51, 126-127.

ON THE CONFORMATIONAL ATTRIBUTES OF BANANA-SHAPED LIQUID CRYSTALS: A PRELIMINARY STUDY

Valentina Domenici[1a], Carlo Alberto Veracini[1b] and Edward T. Samulski[2]

[1] *Dipartimento di Chimica e Chimica Industriale, Universita di Pisa, Via Risorgimento 35, 56126, Italy. (a) e-mail: valentin@dcci.unipi.it; (b) e-mail: verax@dcci.unipi.it*

[2] *Department of Chemistry, University of North Carolina, Chapel Hill, 27514 NC, USA.*

It has often been observed that non-linear (banana-shaped) liquid crystals, consisting of achiral molecules, may form chiral smectic layers and there is a suggestion that this observation derives from a chiral conformation of the banana-shaped molecules[1]. This suggestion, however, may be inaccurate: currently minimal knowledge is available about the detailed molecular structure of these molecules in condensed liquid crystalline phases. To this aim, first we studied the banana mesogen 4-Chloro-1,3-phenylene bis{4-4'-(11-undecenyloxy) benzoyloxy} benzoate, in its nematic phase, by means of ^{2}H NMR. A detailed discussion for either the molecular core or the selectively deuterated fragments, and geometrical features is presented, supported by ^{13}C NMR and Quantum Mechanical considerations[2]. Since the key to understand the conformational properties of these compounds is clearly the central, more rigid, molecular fragment, we have studied two probe molecules with the same molecular structure of the rigid core of several banana molecules (1,3-benzenediol dibenzoate and 4-Chloro 1,3-benzenediol dibenzoate). These probe molecules, selectively deuterated on both lateral rings, have been dissolved in liquid crystal solvents having different symmetries (nematic and cholesteric). The conformation of these probe molecules has been investigated[3] by means of ^{2}H and ^{1}H NMR, *ab initio* calculations and analyzed with a *semi-empirical* approach (*Inertial Frame* model).

References

1. T. Imase, S. Kawauchi, J. Watanabe, J. Mol. Structure, (2001), **560**, 275.
2. R.Y.Dong, K.Fodor-Csorba, J. Xu, V. Domenici, G. Prampolini, C.A. Veracini, J. Phys. Chem. B, accepted.
3. V. Domenici, E. J. Choi, L. A. Madsen, E. T. Samulski, C. A. Veracini, in preparation.

MYRISTIC ACID INTERACTION WITH Mn(III) HEME HUMAN SERUM ALBUMIN – ^{1}H AND ^{17}O NMR CHARACTERIZATION

G. Fanali, [‡] C. Agrati, [‡] P. Ascenzi, [†,§] M. Fasano [‡]

[‡] *Dipartimento di Biologia Strutturale e Funzionale, Università dell'Insubria, via A. da Giussano 12, I-21052 Busto Arsizio (Varese) , Italy*
[†] *Dipartimento di Biologia, Università "Roma Tre", Viale Guglielmo Marconi 446, I-00146, Roma, Italy*
[§] *Laboratorio Interdisciplinare di Microscopia Elettronica, Università "Roma Tre", Via della Vasca Navale 79, I-00146 Roma, Italy*

Human serum albumin (HSA) is the most prominent protein in plasma (its concentration being 45 mg/mL in the serum of human adults) but it is also found in tissues and secretions throughout the body. It is best known for its exceptional ligand binding capacity. It is able to bind a broad variety of ligands as aminoacids, hormones, metal ions and bilirubin. The protein has a high affinity for heme and it is responsible for the transport of medium and long chain fatty acids [1].

The heme-HSA complex is obtained by binding of Mn(III)heme to HSA. Mn(III)heme binding to HSA endows the protein with peculiar spectroscopic properties and it is used as spectroscopic probe to follow a number of events involving the conformation of the protein [2].
The paramagnetic effect of the buried water cluster has been used to follow conformational changes of defatted Mn(III)heme-HSA and ternary Mn(III)heme-HSA-myristate complexes.

Results obtained here show a different contribution to relaxivity depending on the conformational state of the protein and on the occupancy of the myristic acid binding sites. The relaxivity change is more significant between pH 5.5 and 8, where HSA is in its native form (N). This is in part due to the increase of myristic acid molar ratio that makes easier water accessibility to paramagnetic centre, Mn(III)heme. It is clear that without myristic acid water access to paramagnetic centre is either not allowed, or it occurs on a timescale faster than the molecular correlation time of the protein.
By observing the linewidth change of ^{17}O NMR resonance vs. myristate concentration, it appears that the number of water molecules coordinated to the Mn(III) center changes from one to two. This finding is consistent with the change of the functional form of the NMRD profile.
In the range of pH between 8.3 and 11.9, corresponding to the HSA basic form (B), the contribution to relaxivity of Mn(III)heme-HSA fatty acid complexes and ternary Mn(III)heme-HSA-myristate complex does not change significantly from defatted Mn(III)heme-HSA. Therefore, we can assume that myristate binding does not affect the B form of HSA.

References

[1] P. A. Zunszain, J. Ghuman, T. Komatsu, E. Tsuchida, S. Curry. *BMC Struct. Biol.* **3**, 6 (2003)
[2] M. Fasano, S. Baroni, A. Vannini, P. Ascenzi, S. Aime. *J. Biol. Inorg. Chem.* **6**, 650-658 (2001)

^{1}H NMR RELAXOMETRIC CHARACTERIZATION OF THE INTERACTION OF MANGANESE PORPHYRINS WITH HUMAN SERUM ALBUMIN

G. Fanali, [‡] C. Agrati, [‡] E. Caruso, [†] S. Banfi, [†] M. Fasano [‡]

[‡] *Department of Structural and Functional Biology, University of Insubria, via A. da Giussano 12, I-21052 Busto Arsizio (Varese) , Italy.* [†] *Department of Structural and Functional Biology, University of Insubria, via J. H. Dunant 3, I-21100 Varese , Italy*

In recent years there has been a growing interest in the use of porphyrins and related compounds as therapeutic drugs. They are applied in medicine on important areas as cancer detection and as photosensitizer in photodynamic therapy of cancer [1], because these compounds have a tendency to be selectively retained in malignant tumors in comparison with normal tissues [2]. Interactions with macromolecules control the efficacy and biodistribution of porphyrins, which are known to locate preferentially in the cytoplasm and bind poorly to cell membranes. Human serum albumin (HSA), the most prominent protein in plasma, is best known for its exceptional ligand binding capacity. HSA abundance (its concentration being 45 mg/mL in the serum of human adults) makes it an important determinant of binding of a broad variety of ligands as aminoacids, hormones, metal ions and it is responsible for the transport of heme, bilirubin, medium and long chain fatty acids. The interaction of these molecules with proteins is very important to formulate safe drugs and effective dosages. In the present work we investigated the interaction of HSA with the porphyrins shown in Fig. 1.

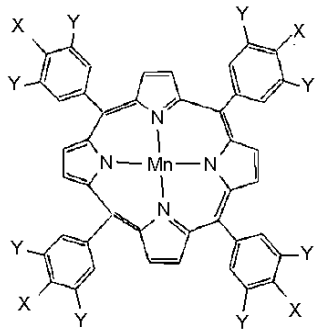


Fig. 1. Structure of the Mn-porphyrins being investigated. X = OH or OMe, Y = H or OMe.

Results obtained here show a remarkable interaction of Mn-porphyrins with HSA, with dissociation constants under the micromolar limit. Analysis of the nuclear magnetic relaxation dispersion profiles, as well as of the pH dependence of the observed relaxivity indicate that the interaction should take place in a binding site other than the canonical heme site. Moreover, NMRD data are consistent with different locations of the binding sites of the three Mn-porphyrins and wih different coordination of the metal ion.

References

[1] R. Bonnet. *Chem. Soc. Rev.* **24**, *19-33* (1995)

[2] S. Chatterjee, T. S. Srivastava. *J. Porphyr. Phthalocyanines* **4**, *147-157* (2000)

STRUCTURE-FUNCTION RELATIONSHIP OF ANALOGUES OF PTH(1-11) FRAGMENTS CONTAINING COMBINATIONS OF AIB AND (αMe)NLE

A. Caporale, N. Fiori, E. Schievano, S. Mammi, and E. Peggion

Department of Chemical Sciences, University of Padova, Institute of Biomolecular Chemistry, CNR,Via Marzolo 1, 35131 Padova, Italy

The N-terminal 1-34 fragment of parathyroid hormone (PTH) is fully active *in vitro* and *in vivo* and it can reproduce all biological responses characteristic of the native intact PTH. Recent studies[1] have demonstrated that analogues of PTH(1-11) and PTH(1-14) fragments with helicity-enhancing substitutions yielded potent analogues of PTH(1-34).
The aim of this work is to develop safer, non-parenteral, non-peptide drugs that stimulate the formation of new bone. To further investigate the role of α-helicity on biological potency, we synthesised and conformationally characterised the following PTH(1-11) analogues containing sterically hindered and helix-promoting C^{α}-tetrasubstituted amino acids, α-amino isobutyric acid (Aib) and α-methyl norleucine (αMeNle).
The following peptides were studied:

I [Aib^1, Ala^3, L(αMe)Nle^8, Gln^{10}, Arg^{11}]-hPTH(1-11)NH_2
II [Aib^1, Ala^3, D(αMe)Nle^8, Gln^{10}, Arg^{11}]-hPTH(1-11)NH_2
III [Ala^1, Aib^3, L(αMe)Nle^8, Gln^{10}, Arg^{11}]-hPTH(1-11)NH_2
IV [Ala^1, Aib^3, D(αMe)Nle^8, Gln^{10}, Arg^{11}]-hPTH(1-11)NH_2
V [$Aib^{1,3}$, L(αMe)Nle^8, Gln^{10}, Arg^{11}]-hPTH(1-11)NH_2
VI [$Aib^{1,3}$, D(αMe)Nle^8, Gln^{10}, Arg^{11}]-hPTH(1-11)NH_2

The peptides were synthesised by SPPS employing Fmoc-protected amino acids. To maximize the total yield, we combined the HBTU/HOBt/DIPEA and the acyl fluoride coupling methods[2]. The latter was used for the incorporation of the C^{α}-tetrasubstituted amino acids.
The correlation between bioactivity and helical structure as found by CD, NMR and MD will be discussed.

References

[1] N. Shimizu., B. D. Petroni, A. Khatri, T. J. Gardella, *Biochemistry*, **42**, 2282-2290 (2003); N. Tsomaia, M. Pellegrini, K. Hyde, T. J. Gardella, D. F. Mierke, *Biochemistry*, **43**, 690-699 (2004)
[2] L. A. Carpino, A. El-Faham, *J. Org. Chem*., **60**, 3541-3564 (1995); L. A. Carpino, M. Beyermann, H. Wenschuh, M. Bienert, *Acc. Chem. Res.*, **29**, 268-274 (1996)

ISOLATION AND NMR STRUCTURAL ELUCIDATION OF SECONDARY METABOLITES FROM MEDICINAL SARDINIAN PLANTS

F. Cottiglia[a], L. Casu[a], L. Bonsignore[a], M. Casu[b] and C. Floris[b]

[a]*Dipartimento Farmaco Chimico Tecnologico, Università di Cagliari, Italy and* [b]*Dipartimento di Scienze Chimiche, Università di Cagliari, Italy*

Plants are known since a remote time as an important source of antitumoral and antiviral medical activity. Sardinian is an island with a large number of endemic plants, most of which are not yet studied from a biological and phytochemical point of view. Some of these plants are still used in some parts of Sardinia in folk medicine.

In our continuous search for pharmacologically active compounds from Sardinia plants, we studied the chemical composition and pharmacological activity of the extracts of *Ephedra nebrodensis (Ephedra)* and of *Bituminaria morisiana* (*Psoralea).*

Some species of the Ephedra genus that produce phenylethylamino alkaloids are commonly employed for diaphoretic, antiasthmatic, and diuretic purposes (1). Two new phenolic glycosides, 4-hydroxy-3-(3-methyl-2-butenyl)phenyl b-D-glucopyranisode and O-coumaric acid b-d-allopyranoside, were isolated from the aerial parts of E. Nebrodensis. In addition, O-coumaric acid gluciside, (-)-epicatechin, and (-)-ephedrine were also isolated.

As much interesting is the isolation and identification of the active extract of plants of Psoralea genus, which is used for skin pigmentation.

A new compound has been isolated from the extract of *Bituminaria morisiana*: 4,5-dihydroxy-6-metoxy-a,4-dimethyl-4,5-dihydro-6H-pirano[7,8,2,3] isoflavone. For the first time, the daizdeina, bidwillon C, pseudoisopsoralene and cumestrolo have been isolated in this plant.

The structure were deduced from extensive 1H and 13C 1D and 2D (DQF-COSY, TOCSY, ROESY, GHSQC and GHMQC) as well as mass spectrometry (EI and HR-MALDI).

References

1) T.H. Huang (1994). Handbook of composition and pharmacological action of commonly-used traditional chinese medicine, Tecnology Press

PROBING PROTEIN-LIGAND INTERACTION BY DYNAMICS STUDY AND LINE SHAPES ANALYSIS: THE CASE OF CRBP

[§]L. Franzoni, [#]T. Mittag, *D. Cavazzini, [§]A. Spisni, *G.L. Rossi and [#]U.L. Günther

*[§]Department of Experimental Medicine, Section of Chemistry and Structural Biochemistry, University of Parma, via Volturno 39, Parma, Italy; [#]Center for Biomolecular Magnetic Resonance, J.W. Goethe University, M. Curie Strasse 9, Frankfurt, Germany; *Department of Biochemistry and Molecular Biology, University of Parma, Parco Area delle Scienze 23/A, Parma, Italy.*

Cellular retinol-binding protein I (CRBP) is an interesting model to study protein-ligand interaction because its β-barrel structure forms a highly specific binding cavity which is fully shielded from the outside medium in both the apo and holo forms [1]. Not even the study of the internal backbone dynamics on the *ps-ns* time scale provided insight into the mechanism of ligand uptake, showing only few residues with higher mobility in apo-CRBP [1]. This is not surprising because many biochemical processes occur on the *μs-ms* time scale. Recent advances in NMR spectroscopy allowed for a reliable and sensitive investigation of slow dynamics and consequently fundamental questions of protein function become accessible.

In this work we have combined ^{15}N relaxation dispersion analysis and line shapes simulation to further investigate the CRBP-ligand interaction and to derive the kinetics parameters at individual residues.

Relaxation compensated CPMG experiments were performed in a time constant manner [2] at two static magnetic fields. Data analysis showed that millisecond dynamics involve extended regions of the apo-form with the highest exchange contributions for a region which has been proposed as a putative portal in other lipid-binding proteins. Interestingly, the high degree of conformational flexibility is almost completely quenched upon retinol binding [3].

High-resolution ^{1}H/^{15}N-HSQC spectra were recorded for different steps of a retinol titration. Line shapes analysis, carried out as recently described by Günther *et al.* [4, 5], showed an unexpectedly complex picture with individual roles of different residues for the ligand interaction.

A possible model of retinol uptake which accounts for the observed kinetics on a per-residue basis, will be presented.

Acknowledgements

Supported by the Large Scale Facility for Biomolecular NMR at the University of Frankfurt (UNIFRANMR) and by grants of the Italian Ministry of Education, University and Research (MIUR).

References

[1] L. Franzoni, C. Lücke, C. Pérez, D. Cavazzini, M. Rademacher, C. Ludwig, A. Spisni, G.L. Rossi and H. Rüterjans. *J. Biol. Chem.* **277**, 21983-21997 (2002); [2] M. Tollinger, N.R. Skrynnikov, F.A.A. Mulder, J.D. Forman-Kay and L.E. Kay. *J. Am. Chem. Soc.* **123**, 11341-11352 (2001); [3] T. Mittag, L. Franzoni, D. Cavazzini, G.L. Rossi and U.L. Günther. In *"Structure, Dynamics and Function of Biological Macromolecules and Assemblies"*, Ed. J.D. Puglisi, IOS Press B.V., Amsterdam, The Netherlands (2004).; [4] U. Günther and B. Schaffhausen. *J. Biomol. NMR* **22**, 201-209 (2002); [5] U. Günther, T. Mittag and B. Schaffhausen. *Biochemistry* **41**, 11658-11669 (2002).

IDENTIFICATION OF COPPER(II) BINDING SITES IN AMINOGLYCOSIDIC ANTIBIOTIC NEOMYCIN B. STRUCTURAL CHARACTERIZATION BY NMR SPECTROSCOPY.

Nicola Gaggelli, Elena Gaggelli, Gianni Valensin

Department of Chemistry and the NMR Center, University of Siena, Via A.Moro, 53100 Siena, Italy

Neomycin B is an aminoglycoside antibiotic produced by Streptomyces fradiae and active against gram-negative aerobic bacteria and Staphylococcus. From the chemical point of view, the evidence for significant toxicity of neomycin B is provided in recent studies [1], where it has been shown that this antibiotic had the highest potency to cleave $tRNA^{Phe}$. Additionally, its cupric complexes disproportionated H_2O_2 to hydroxyl radicals and oxidized plasmid DNA [1].

The coordination of this type of drugs to copper(II) ions was investigated in our recent studies [2,3]. Copper ion is not present at very high levels intracellularly; however its occurrence in the plasma reflects the state of health of the organism. Under conditions when antibiotics are applied, their serum level may increase drastically [4]. Thus chelation of the metal by xenobiotic is not unlikely to occur in vivo.

The coordination abilities of neomycin B (Fig. 1) have not been studied in detail up to date.

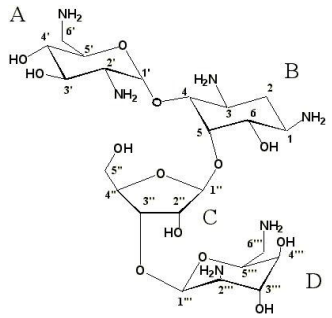


Fig. 1. Molecular formula of Neomycin B.

The aim of the current study is to provide the structural and dynamical characterization of the Cu(II)-neomycin B complex in water solution at pH = 6.8 by ^{1}H- and ^{13}C-NMR. The observed proton and carbon relaxation rate enhancements where converted into H-Cu distances, used to build up molecular models of the resulting complex [5].

References

[1] W. Szczepanik , J. Ciesiołka , J. Wrzesiński , J. Skała, M. Jeżowska-Bojczuk, *Dalton Trans.*, 1488-1494 (2003); [2] E. Gaggelli, N. Gaggelli, D. Valensin, G. Valensin, M. Jeżowska-Bojczuk, H. Kozlowski, *Inorg. Chem.*, **41**, 1518-1522 (2002); [3] N.D'Amelio, E. Gaggelli, N. Gaggelli, E. Molteni, M.C. Baratto, G.Valensin, M. Jeżowska-Bojczuk, W. Szczepanik, *Dalton Trans.*, 363-368 (2004); [4] M.C. Linder, L. Wooten, P. Cerveza, S. Cotton, R. Shulze, N. Lomeli, *Am. J. Clin. Nutr.*, **67**, 965S-971S (1998); [5] M. Jeżowska-Bojczuk, W. Szczepanik, S. Mangani, W. Meyer-Klaucke, E. Gaggelli, N. Gaggelli, G. Valensin, *Dalton Trans.*, submitted.

NMR CHARACTERIZATION OF THE SPECIES FORMED BY REDUCTION OF DICHROMATE BY A BOVINE LIVER HOMOGENATE.

Nicola Gaggelli, Nicola D'Amelio, Elena Gaggelli, Gianni Valensin

Department of Chemistry and the NMR Center, University of Siena, Via A.Moro, 53100 Siena, Italy

Chromium has been the first metal to be recognized as carcinogenic and mutagenic [1]. Beyond the risk associated with inhalation of Cr^0 particles, the adverse biological effects are ascribed to Cr^{VI} compounds, since chromate or dichromate may readily enter cells through the sulfate channel [2]. Within cells, Cr^{VI} is reduced to lower valence states by several potential reductants, including cellular thiols, mainly cysteine, GSH, NADPH and ascorbate [3, 4], with formation of reactive oxygen species, thiyl and carbon-based radicals [5, 6].
Reduction of chromate by GSH has been thoroughly investigated *in vitro* [3, 7, 8]. It has come out that, when reducing chromate, GSH first form a thioester transient species and then a Cr^V complex where two GSH molecules are able to cluster two metal ions [7] with the eventual participation of aspartic acid as clustering agent [8]. The reported investigations have not succeeded so far in ascertaining whether this complex is relevant for detoxification or it is just an intermediate step in the reductive pathway to mutagenic Cr^{III}.
A low-molecular weight chromium-containing fraction of the material resulting from dichromate reduction by bovine liver homogenate was investigated by NMR and ES-MS [9]. The ES-MS spectrum showed a readily detectable peak at m/z = 786.1. The same molecular weight reasonably agreed with the relatively low diffusion coefficient measured by NMR-DOSY experiments on the main species observed in the ^{1}H NMR spectrum. At least two downfield shifted and broad paramagnetic signals were apparent in the ^{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 a tentative proposal of a model for the compound.

References

[1] IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Chromium, Nickel and Welding. IARC, Lyon, 1-214 (1990); [2] H.J. Wiegand, H. Ottenwälder, H.M. Bolt, Sci.Total Environ., **71**, 309-315 (1988); [3] P.H. Connett, K.E. Wetterhahn, J.Am.Chem.Soc., **107**, 4283-4288 (1985); [4] A. Zhitkovich, Y.Song, G. Quievryn and V. Voitkun, *Biochemistry,* **40**, 549-560 (2001); [5] P.C. Kaltreider, C.A. Pesce, M.A. Ihnat, J.P. Lariviere, J.W. Hamilton, *Mol. Carcinogenesis,* **25**, 219-229 (1999); [6] S. Liu, M. Medvedovic, K. Dixon, *Environ.Mol. Mutagenesis,* **33**, 313-319 (1999); [7] E. Gaggelli, F. Berti, N. Gaggelli, A. Maccotta, G. Valensin, *J.Am.Chem.Soc.,* **123**, 8858-8859 (2001); [8] E. Gaggelli, F. Berti, N. D'Amelio, N. Gaggelli, G. Valensin, L. Bovalini, A. Paffetti, L. Trabalzini, *Environ.Health Persp.,* **110, S5**, 733-738 (2002); [9] E. Gaggelli, N. D'Amelio, N. Gaggelli, G. Valensin, L. Bovalini, A. Paffetti, L. Trabalzini, *Bioinorg. Chem. Appl.*, **1**, 285-294 (2003)

SEQUENTIAL NMR RESONANCE ASSIGNMENT AND PRELIMINARY STRUCTURAL ANALYSIS OF A PROTEIN ISOLATED FROM *XANTHOMONAS AXONOPODIS* PV *CITRI*, THROUGH A STRUCTURAL PROTEOMICS STRATEGY

M. Gallo,[‡] M. V. Orsale,[‡] A. M. Katsuyama,[†,¥] T. A. Pertinhez,[¥] A. Spisni,[¥,§] C.S.Farah,[†] M. Paci,[‡] and D. O. Cicero.[‡]

[‡] Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Italy.
[†] Department of Biochemistry, Institute of Chemistry, University of Sao Paulo, Brazil.
[¥] BioNMR Laboratory, Center for Structural Molecular Biology, LNLS, campinas, Brazil.
[§] Sect. Chemistry and Structural Biochemistry, Department of Experimental Medicine, University of Parma, Italy.

Xanthomonas axonopodis pv *citri* (*Xac*) is the agent of citrus canker, which causes many economic losses in the production of citrus fruit. This impact is severe in Brazil, where produces about one third of the world's citrus crop. The complete sequencing of the *Xac* genome revealed several conserved proteins of unknown function and structure [1]. Recognising that the structural characterisation of a protein not only provides information regarding its cellular function but also may disclose new folds, we have been prompted to study the protein encoded by the ORF XACb0070 (79 aa). A protein of unknown function and with low sequence homology with any other protein in the PDB, ORF XACb0070 has been selected for NMR studies by the use of a high-throughput strategy for the screening of targets for structural proteomics [2]. The protein has been over-expressed in *E. Coli* and purified. The CD spectrum showed that the protein is folded and has a high α-helical content. This new protein has also been ^{15}N- and $^{15}N/^{13}C$- isotopically labelled. The "foldedness" of the protein is confirmed by the [^{1}H,^{15}N]-HSQC spectrum, where a narrow range of ^{1}H chemical shift is observed, being this characteristic of proteins with high α-helical content [3]. The sequential NMR resonance assignment of the protein was preformed by three dimensional heteronuclear NMR experiments on the $^{15}N/^{13}C$-labelled sample and the $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ chemical shifts index were calculated. The data suggest the presence, at the N-terminus, of a β-strand, residues N2-S11, and two α-helices, residues V14-A23 and residues L33-E47, respectively. In contrast, the C-terminal region seems to be poorly structured. All these results are supported by the NOEs pattern and by the $^{3}J_{NH}$ values. Furthermore, the T_2 values are, in some cases, too small for a protein of 79 aa. This result seems to indicate that the protein may exist as a dimer. It is expected the characterization of the solution 3D structure as well as of the dynamic properties of this new protein may provide information about its biological function.

Acknowledgments: The work was partly supported by MIUR Cofin (Italy) and FAPESP (Brazil).

References

[1] A. C. da Silva, J. A. Ferro, et al. *Nature* **417**, 459-463 (2002).; [2] F. C. Almeida, G. C. Amorim, V. H. Moreau, V. O. Sousa, A. T. Creazola, T. A. Americo, A. P. Pais, A. Leite, L. E. Netto, R. J. Giordano, A. P. Valente *J. Magn. Reson.* **148**, 142-146 (2001); [3] L. M. Galvao-Botton, A. M. Katsuyama, C. R. Guzzo, F. C. Almeida, C. S. Farah, A. P Valente *FEBS Lett.* **25**, 207-13 (2003).

STRUCTURAL STUDIES BY NMR OF THE PRION α3-HELIX CONFORMATION

M Gallo,[‡] D. Paludi,[†] K. Chiovitti,[†] G. Schettini,[†] S. Melino,[‡] M. Paci,[‡] A. Aceto,[§] T. Florio,[†] and D. O. Cicero.[‡]

[‡] *Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Italy*
[†] *Department of Oncology, Biology and Genetics, Sect. Pharmacology, University of Genova, Italy*
[§] *Department of Biomedical Sciences, Sect. Biochemistry, University "G. D'Annunzio", Chieti, Italy*

Protein nucleation sites are regions of proteins that have an important role on folding. Probably, nucleation sites correspond to peptides that, even isolated of the rest of the molecule, have the tendency to acquire the same conformation adopted in the entire protein [1]. With the aim of identifying possible nucleation sites in the prion protein (PrP), three peptides matching the helices α1, α2, and α3, spanning the entire sequence of the C-terminal region of the human PrP, have been synthesized and their conformations analyzed by far-UV CD spectroscopy. The obtained results showed that α1 and α2 are unstructured in water/2,2,2-trifluoroethanol solutions. On the contrary, the synthesized α3-peptide showed a helical content (47%) significantly higher than that estimated for the two other peptides. These results suggest that α3-helix might represent a nucleation site of the PrP folding [2]. Alternatively, mutations in the α3-helix, D202N and F198S, are associated to GSS disease. A peptide corresponding to α3-helix containing D202N showed a critical diminution of the helical content, according to CD experiments, and has proved to have neurotoxic effects in culture cells experiments [2] Considering these results, and taking into account that the structural link between these specific mutations and the GSS has not has been yet investigated, the solution conformation of the peptide matching the α3 helix has been analyzed by NMR spectroscopy. The NMR results indicate that an N-capping box [3] and a staple [4] motifs are strictly conserved at the beginning of the helix, and that the conformation of this region is strikingly similar to the conformation adopted in the entire protein [5].

References

[1] A. R. Fersht *Curr. Opin. Struct. Biol.* **7**, 3-9, (1997).
[2] D. Paludi, et al., manuscript in preparation.
[3] R. Aurora and G. D. Rose *Protein Sci.* **7**, 21-38, (1998).
[4] A. Aceto *Biochem J.* **322**, 229-334, (1997).
[5] R. Zahn, A. Liu, T. Luhrs, R. Riek, C. von Schroetter, F. Lopez Garcia, M. Billeter, L. Calzolai, G. Wider, K. Wuthrich *Proc. Natl. Acad. Sci. USA* **97**, 145-150, (2000).

DYNAMICS OF LIQUID CRYSTALS: A COMPARISON BETWEEN ^{2}H NUCLEAR MAGNETIC RESONANCE AND DIELECTRIC RELAXATION SPECTROSCOPY

V. Domenici[#], M. Geppi[#], S. Urban[§], C. A. Veracini[#], A. V. Zakharov[†]

 # Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Risorgimento 35, 56126, Italy. § Institute of Physics, Jagellonian University, Reymonta 4, 30-059 Cracow, Poland. † Laboratorium voor Akoestiek en Thermische Fysica, Departement Natuurkunde en Sterrenkunde, Katholieke Universiteit Leuven, Celestijnenlaan 200D, B-3001 Leuven, Belgium

^{2}H NMR relaxation spectroscopy has been established as a very powerful tool for studying structural, orientational and dynamic properties of liquid-crystalline phases in suitably deuterium labelled compounds [1]. In particular the ^{2}H Zeeman (T_{1Z}) and quadrupolar (T_{1Q}) spin-lattice relaxation times are strongly dependent on molecular dynamics in the KHz to hundreds of GHz range. Dielectric spectroscopy is a powerful tool for studying the stochastic reorientation and collective motions of molecules having a permanent dipole moment. A wide frequency range accessible by this method (from sub-hertz to several Gigahertz) enables one to follow very slow as well as very fast rotations of molecules or their polar parts. Through the theoretical treatment of dynamic processes of flexible molecules in an anisotropic medium [2], dielectric relaxation times can be related with $D_{//}$ and $D_{\perp}$, which are the principal components of the diffusion tensor, diagonalized in a molecular frame, and describing the spinning and tumbling motions, respectively. The diffusion coefficients for the overall molecular and internal motions can be also determined by analyzing the ^{2}H NMR relaxation times in terms of specific theoretical models [3].

Here we present two recent works involving two different rod-like liquid crystals, the 4-[4'-(1-methylheptyloxy)] biphenyl'–(10-undecenyloxy) benzoate (11EB1M7) [4] and the 4,4'-di-*n*-heptylazoxybenzene (HAB) [5]. For the first mesogen, the rotational diffusion coefficients and order parameters determined from NMR were used to calculate the dielectric relaxation times τ^1_{00}, τ^1_{10}, τ^1_{01} and τ^2_{00}, as well as the diagonal components of the susceptibility tensor, χ.

For HAB, a complete dynamic study is presented, where the results obtained in both the nematic and SmA phases applying the two experimental techniques (NMR and DR) are treated and compared in terms of the available theoretical models.

References

[1] R. L. Vold, NMR of Liquid Crystals, edited by J. W. Emsley (Reidel), p.231 (**1985**).
[2] A. V. Zakharov, R. Y. Dong, *Phys. Rev. E* **2001**, 63, 011704; A. V. Zakharov, A. Maliniak, *Eur. Phys. J.* **2001**, E4, 435.
[3] See poster: *Dynamics of a ferroelectric liquid crystal by means of ^{2}H NMR spectroscopy: a multifrequency relaxation study*, by V. Domenici *et al.*
[4] V. Domenici, M. Geppi, C.A. Veracini, A.V. Zakharov, in preparation.
[5] V. Domenici, J. Czub, M. Geppi, B. Gestblom, S. Urban, C.A. Veracini, *Liq. Cryst.* **2004**, 31, 91.

DYNAMICS OF A FERROELECTRIC LIQUID CRYSTAL BY MEANS OF ^{2}H NMR SPECTROSCOPY: A MULTIFREQUENCY RELAXATION STUDY

V. Domenici[#], M. Geppi[#], C. A. Veracini[#], R. Blinc[§], A. Lebar[§] and B. Zalar[§]

 # Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Risorgimento 35, 56126, Italy.
§ Department of Solid State Physics, Josef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

In the present work we analysed ^{2}H Zeeman and quadrupolar spin-lattice relaxation times measured for the ferroelectric liquid crystal (FLC) 11EB1M7 at three different Larmor frequencies and on two different isotopomers, one deuterated on the biphenyl moiety (11EB1M7-d_8), the other partially labelled on the phenyl ring (11EB1M7-d_2) [1]. In fact, ^{2}H spin-lattice relaxation is a very powerful tool for studying the molecular dynamics of liquid crystals [2], since it can give very detailed information on individual motional processes occurring in a wide range of characteristic times (10^{-11}-10^{-4} s) [3]. The experimental data have been analyzed in terms of the *Nordio et al*. [4] model to describe overall spinning and tumbling motions, the strong collision [5] and the small step diffusion [6] model to describe the internal reorientations of the aromatic fragments and the theory proposed by *Pincus* [7] and *Blinc et al.* [8] to consider the contribution of order director fluctuations to dynamics. The availability of data for two different isotopomers and three Larmor frequencies allowed both a reliable determination of the diffusion coefficients relative to the tumbling motion ($D_\perp$), usually very critical [9], and an estimate of the contribution of the collective motions. While the small steps diffusion and strong collision models are usually equally suitable to describe the internal reorientations in thermotropic liquid crystals, only the latter gave satisfactory results in the present analysis, confirming a similar observation previously performed on a different FLC [10]. Here we show the results obtained for the SmA phase, and the preliminary analysis extended to the smectic C* phase, where a new data analysis approach recently proposed in the Pisa group was applied [11].

References

[1] V. Domenici, M. Geppi, C. A.Veracini, R. Blinc, A. Lebar, B. Zalar, *ChemPhysChem* **2004**, *5*, 559-563.
[2] L. Calucci, M. Geppi, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1006-1014.; [3]R.Y. Dong, *Prog. Nucl. Magn. Reson.* **2002**, *41*, 115-151.; [4] P.L. Nordio, P. Busolin, *J. Chem. Phys.* **1971**, *55*, 5485-5490.; [5] P.A. Beckmann, J.W. Emsley, G.R. Luckurst, D.L. Turner, *Mol. Phys.* **1986**, *59*, 97-125.; [6] D.E. Woessner, *J. Chem. Phys.* **1962**, *36*, 1-4.; [7] P. Pincus, *Solid St. Commun.* **1969**, *7*, 415-418.; [8] R. Blinc, D. Hogenboom, D. O'Reilly, E. Peterson, *Phys. Rev. Lett.* **1969**, *23*, 969-972.; [9] D. Catalano, L. Chiezzi, V. Domenici, M. Geppi, C. A. Veracini, *J. Phys. Chem. B* **2003**, *107*, 10104-10113.; [10] L. Chiezzi, V. Domenici, M. Geppi, C.A. Veracini, R.Y. Dong, *Chem. Phys. Lett.* **2002**, *358*, 257-262; D. Catalano, M. Cifelli, M. Geppi, C.A. Veracini, *J. Phys. Chem. A.* **2001**, *105*, 34-40. ; [11] V. Domenici, M. Geppi, C.A. Veracini, *Chem. Phys. Lett.* **2003**, 382, 518-522.

USE OF ^{13}C AND ^{1}H SOLID STATE NMR TECHNIQUES TO PROBE THE CHANGES INDUCED IN FLOUR BY ACCELERATED AGING OF BREAD WHEAT SEEDS

L. Calucci[1], M. Geppi[2], G. Mollica[2]

[1] *Istituto per i Processi Chimico-Fisici, CNR, Area della Ricerca, via G. Moruzzi 1, I-56124 Pisa*
[2] *Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento 35, I-56126 Pisa*

Different ^{13}C and ^{1}H Solid State Nuclear Magnetic Resonance techniques were employed to study the changes induced in structural and dynamic properties of flour by accelerated aging (storage at 40 °C and 100 % relative humidity) of bread wheat (*Triticum aestivum* cv. Centauro) seeds for periods ranging from 0 to 10 days. Structural information was obtained through ^{13}C Single Pulse Excitation, Cross Polarization, Non Quaternary Suppression and other selective experiments performed under Magic Angle Spinning (MAS) and Dipolar Decoupling conditions, as well as ^{1}H-fast MAS spectra and 2D-WISE (WIdeline SEparation) experiments [1]. Proton FID analysis and wideline measurements of proton spin-lattice relaxation times in both the laboratory (T_1) and rotating ($T_{1\rho}$) frames were performed over a broad range of temperatures in order to get insight into the changes induced by the aging process of seeds in the molecular dynamics of flour components. To this aim, we used two recently developed procedures: (a) the use of the Power Wighted Rate Average [2] which allows the proton relaxation to be completely interpreted in terms of dynamics, eliminating the spin diffusion effects; (b) the $T_2/T_{1\rho}$ correlation experiment [3] which yields the degree of correlation between the different exponential components of the $T_{1\rho}$ decay and the different FID components. Static and MAS ^{1}H spectra at different spinning rates were also recorded in order to investigate the nature of the magnetic interactions responsible for spectral line broadening.
The experimental results highlighted a clear correlation between the dynamic properties of flour at a molecular level and the effects of seed aging.

References

[1] N. Zumbulyadis, *Phys. Rev. B*, **33**, 6495 (1986); K. Schmidt-Rohr, J. Clauss, H. W. Spiess, *Macromolecules*, **25**, 3273 (1992).
[2] M. Geppi, R. K. Harris, A. M. Kenwright, B. J. Say, *Solid State NMR*, **12**, 15 (1998).
[3] M. Geppi, A. M. Kenwright, B. J. Say, *Solid State NMR*, **15**, 195 (2000).

METHODS FOR THE EXTRACTION OF DYNAMIC INFORMATION FROM ^{1}H SPIN-SPIN AND SPIN-LATTICE RELAXATION TIMES IN A SOLID BLOCK COPOLYMER

V. Ferrini*, C. Forte**, M. Geppi*, S. Pizzanelli** and C.A. Veracini*

** Dipartimento di Chimica e Chimica Industriale, Università di Pisa, V. Risorgimento 35, 56126 Pisa, Italy*
*** IPCF-CNR, Area della Ricerca di Pisa, V. G. Moruzzi 1, 56124 Pisa, Italy*

The analysis of T_1 and $T_{1\rho}$ spin-lattice proton relaxation times in terms of dynamic parameters in solid polymers is usually a difficult task because of the scarce spectral resolution and the averaging effect of spin diffusion. In particular, the multi-exponential relaxation behaviour commonly observed for the spin-lattice relaxation times in the rotating frame has been sometimes mistakenly interpreted in terms of different components of a heterogeneous system.

We have performed an extensive study of a styrene-butadiene-styrene (SBS) block copolymer, in a range of temperatures below and above the styrene and butadiene glass-transition temperatures, respectively.

Proton NMR experiments were performed in a wide temperature range by means of low-resolution techniques. In particular, analyses of ^{1}H FIDs, recorded on-resonance by solid-echo, were performed using a large variety of analytical functions (Exponential, Weibullian, Pake, Brereton, Gaussian, etc.) by means of a specifically developed Mathematica software package; this allowed spin diffusion-free parameters to be obtained, getting insight into the dynamic heterogeneity of the system and its changes with the temperature.

^{1}H spin-lattice relaxation times in both the rotating and the laboratory frame were measured in order to obtain information on dynamic processes in the mid-kHz and MHz ranges, respectively. While T_1 was always found to exhibit a mono-exponential behaviour, a bi-exponential decay was observed at all temperatures for $T_{1\rho}$. A two-dimensional T_2-$T_{1\rho}$ correlation experiment [1] was applied in order to associate the two $T_{1\rho}$ components to protons of either the styrenic or butadienic blocks, also highlighting the contribution to the relaxation arising from the two blocks. Very different behaviours were observed at the low- and high-temperature limits of the temperature range investigated; the results were further supported by ^{13}C CP-MAS and 2D-WISE high-resolution experiments.

References

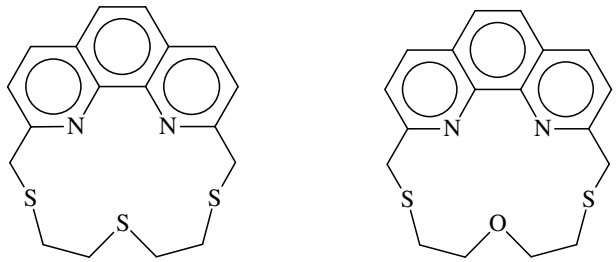
[1] M. Geppi, A.M. Kenwright and B.J.Say, Solid State NMR **15**, 195 (2000)

A ^{13}C-NMR STUDY OF Pd^{II}, Pt^{II}, Rh^{III} AND Ru^{II} COMPLEXES WITH TWO PENTADENTATE MACROCYCLES CONTAINING THE 1,10-PHENANTHROLINE SUB-UNIT

Francesco Isaia, Alessandra Garau, Vito Lippolis

Dipartimento di Chimica Inorganica ed Analitica, Università degli Studi di Cagliari, S.S. 554 Bivio per Sestu, 09042 Monserrato- Cagliari

We have recently reported the synthesis of mixed aza-thioether crowns contaning a 1,10-phenanthroline sub-unit (Fig. 1) and their complexes with Pd^{II}, Pt^{II}, Rh^{III} and Ru^{II} [1, 2].



L1 **L2**

The crystal structures of [Pd(**L1**)]$(PF_6)_2$, [Pt(**L1**)]$(PF_6)_2$, [Pd(**L2**)]$(BF_4)_2$ show the ligands in a N2S2 coordination; in the case of complexes [Pd(**L1**)]$(PF_6)_2$ and [Pt(**L1**)]$(PF_6)_2$ a long interaction between the metal ion and the central sulphur is observed. In the complexes [Ru(**L1**)Cl]BF_4 and [Ru(**L2**)PPh_3]$(BF_4)_2$ the ligands are acting as N2S3- and N2S2O-donors, respectively. An octahedral coordination sphere around Ru^{II} is completed by a Cl^- ion in [Ru(**L1**)Cl]BF_4, and by a triphenylphosphine ligand in [Ru(**L2**)PPh_3]$(BF_4)_2$.
A ^{13}C-NMR measurements have been carried out on all the complexes. An octahedral coordination is inferred for the Rh^{III} complexes from NMR data.

References

[1] F. Contu, F. Demartin, F.A. Devillanova, A. Garau, F. Isaia, V. Lippolis, A. Salis, G. Verani, *J. Chem. Soc., Dalton Trans*., 4401 (1997).
[2] M. Arca, A. J. Blake, J. Casabò, F. Demartin, F. A. Devillanova, A. Garau, F. Isaia, V. Lippolis, R. Kivekas, V. Muns, M. Schröder, G. Verani, *J. Chem. Soc., Dalton Trans*., 1180 (2001).

^{13}C CPMAS AND ^{1}H NMR STUDY OF THE INCLUSION COMPLEXES OF β-CYCLODEXTRIN WITH CARVACROL, THYMOL AND EUGENOL PREPARED BY SUPERCRITICAL CARBON DIOXIDE

E. Locci, S. Lai, A. Piras, B. Marongiu, A. Lai

Dipartimento di Scienze Chimiche, Università di Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (CA), Italy.

β-cyclodextrin inclusion complexes with carvacrol, thymol and eugenol (components of essential oils of vegetable origin) were prepared by the supercritical carbon dioxide technique and their structural characterisation was made by means of 1H NMR in aqueous solution and 13C CPMAS NMR in the solid state. Evidence of the formation of the inclusion complexes for all the examined systems was obtained by 1H NMR in solution, while 2D-ROESY NMR experiments were used to investigate the geometry of inclusion. In addition, the dynamics of these inclusion complexes in the kilohertz timescale was investigated by analysis of the proton and carbon spin-lattice relaxation times in the rotating frame.

LEWIS vs. BRØENSTED ACIDITY IN THE SYSTEM $B(C_6F_5)_3/H_2O/NEt_3$: AN NMR STUDY

D. Maggioni,[‡] D. Donghi,[‡] T. Beringhelli, [‡] G. D'Alfonso[‡], A. Di Saverio,[†]
I. Camurati,[†] L. Resconi[†]

[‡] *Dip. C.I.M.A., Università degli Studi di Milano, via Venezian 21, I-20133 Milano, Italy*
[†] *Basell Poliolefine Italia S.p.A , P.le Privato G. Donegani 12, I-44100 Ferrara, Italy*

The addition of one equivalent of $B(C_6F_5)_3$ to a CD_2Cl_2 solution of the salt $[HNEt_3]^+[B(C_6F_5)_3OH]^-$ provides a rational synthesis of $[HNEt_3]^+[(C_6F_5)_3B(\mu\text{-}OH)B(C_6F_5)_3]^-$ (**1**). At low temperature the anion moiety shows structural rigidity. The fifteen ^{19}F resonances observed at 173 K, in agreement with the C_2 symmetry found in the solid state [1], have been assigned and the solution structure established (Figure 1).

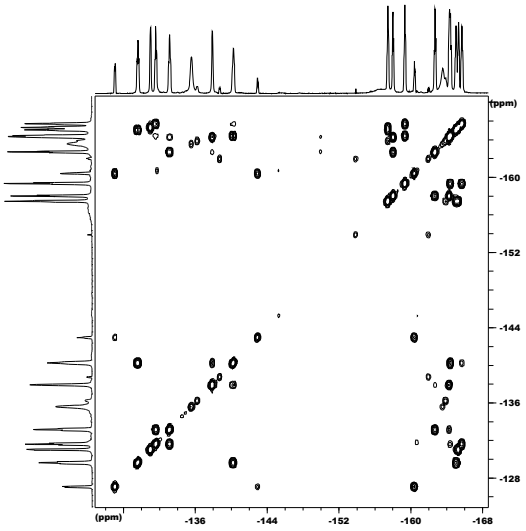


Fig. 1. ^{19}F COSYGS (175 K, CD_2Cl_2, 7.1 T) of a solution containing **1**, **2** and some $B(C_6F_5)_3$.

^1H-^{19}F COSY and HOESY showed that the found conformation is stabilized by *intra*molecular hydrogen bonds between the hydroxylic proton and two fluorines in the *ortho* positions of the phenyl rings. On rising the temperature this unique conformation is destroyed by the onset of three different dynamic processes, revealed both by ^1H and ^{19}F spectra.

The reactivity of **1** with NEt_3 and with water has been studied. The amine does not react as a Brøensted base towards the hydroxylic hydrogen, but rather it undergoes hydrogen abstraction with formation of $[HNEt_3]^+[(C_6F_5)_3BH]^-$ and $Et_2N^+{=}CHCH_2{-}B(C_6F_5)_3$.

The reaction of **1** with water affords the ion pair $[HNEt_3]^+[(C_6F_5)_3BO(H)H\cdots O(H)B(C_6F_5)_3]^-$ (**2**): water inserts into a B-O interaction, acting as a Lewis base towards one of the acidic boron centers and as a Lewis acid towards the oxygen of the remaining borate moiety, giving rise to a very strong hydrogen bond. Indeed, while at room temperature a unique resonance at 8.55 ppm is observed for the three protons of anion **2** (indicating the presence of a fast exchange on the NMR time scale), on lowering the temperature they give rise to two signals at 17.9 and 4.5 ppm (intensity ratio 1:2). However, even at low temperature, ^{19}F spectra show just three signals indicating that in **2** no peculiar interaction slow down the fast rotation of the perfluorinated phenyl rings around the B-C and the B-O bonds.

References

[1] a) A. A. Danopoulos, J. R. Galsworthy, M. L. H. Green, S. Cafferkey, L. H. Doerrer, M. B. Hursthouse *Chem. Commun.*, 1998, 2529-2530.

THE INTERACTION BETWEEN LB-FABP AND BILE ACIDS: A ^{19}F NMR STUDY

S. Malaguti,[‡] T. Beringhelli,[‡] S. Capaldi,[†] M. Perduca,[†] H. L. Monaco[†]

[‡]*Dip. C.I.M.A.,Università degli Studi di Milano, via Venezian 21, I-20133 Milano*
[†]*Laboratorio di Biocristallografia, Università di Verona ,strada le Grazie 15, Cà Vignal, Verona*

The basic fatty acid-binding protein obtained from chicken liver (Lb-FABP) belongs to a family of intracellular, low molecular weight, nonenzymatic proteins, that exhibit high affinity binding constant for small lipophilic ligands [1]. It shows a typical β barrel structure [2] , the interior of which is the binding site of the ligands [3]. Its specific function has to be ascertained still, however the high sequence and structural similitary with ileal lipid binding protein suggested that bile acid may be the putative ligands [4]. Indeed the crystal structure of a complex containing two cholate molecules has been recently refined [5]. We report here the results of a solution study on the interaction between Lb-FABP and bile acids through ^{19}F-NMR titration experiments.

We have previously proved, through ^{19}F relaxation parameters (T_1 and NOE), that Lb-FABP binds small ligands containing fluorine atoms, such as drugs with steroidal structure or long-chain fatty acids analogs [6]. On adding progressive amounts of bile acid to preformed complexes of the above mentioned ligands, changes of the chemical shifts, of the line width and of the relaxation parameters indicated the occurrence of the interaction with the bile acid.

In the case of steroidal derivatives complete replacement occurred.

The titration of the complex with 2-F-palmitic acid showed the high affinity of the protein toward both the ligands. In fact 2-F-palmitic acid (that is totally insoluble in water) is bound jet after the addition of 2 equivalents of bile acid as confirmed by T_1 and NOE measurements. The change of chemical shift and shape of 2-F-palmitic acid shows that bile acid is bound as well (Fig 1) in the cavity.

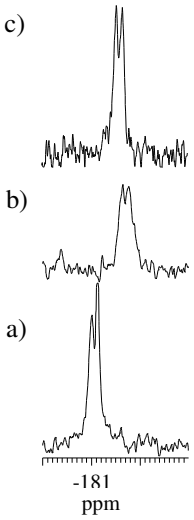


Fig1: a) Lb-FABP complex with 1 eq of 2-F-palmitic acid (Lb-FABP/2FP); b) Lb-FABP/2FP complex after the addition of 2 eq of sodium cholate; c) Lb-FABP/2FP complex after the addition of 2 eq of sodium chenodeoxycholate.

References

[1] L. J. Banaszak, N. Winter, Z. Xu *Adv. Protein Chem.* **70**, 89-151 (1994)
[2] M. Perduca, A. Bossi, L. Goldoni, H. L. Monaco, P. G. Righetti *Elecrophoresis* **21**, 2316-2320 (2000)
[3] T. Beringhelli, L. Goldoni, S. Capaldi, A. Bossi, M. Perduca, H. L. Monaco *Biochemstry* **40**, 12604-12611 (2001)
[4] F. Vasile, M. Perduca, H. L. Monaco, H. Molinari *J. Biomolecular NMR* **25**, 157-160 (2003)
[5] S. Capaldi, M. Perduca, H. L. Monaco *in press*
[6] T. Beringhelli, S. Malaguti, S. Capaldi, M. Perduca, H. L. Monaco *in preparation*

PRODUCTION AND STRUCTURAL ANALYSIS OF THE PATHOLOGICAL FRAGMENT OF HUMAN α–SYNUCLEIN

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

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

Human α-synuclein plays a principal role in many neurodegenerative disorders, referred as α-synucleinopathies, that include Parkinson's disease and Alzheimer's disease. The central region of the protein, known as non-Aβ component of amyloid plaques (NAC), seems to be responsible for the pathogenic role of the wild-type protein.

At present, the structure of α-synuclein has not been yet determined; several conformational models exist that are often contradictory. To shed some light on this important question that can be relevant to understand the pathological process of fibrils formation, we have undertaken a structural analysis on the NAC region of α-synuclein.

Since the NAC fragment is very sparingly soluble in aqueous buffer, we have cloned a slightly larger portion of α-synuclein, αsyn57-102, which contains some charged residues at both extremities of the NAC region. The conformational preferences of purified αsyn57-102, in solution and when bound to SDS micelles, were studied by CD spectroscopy and three dimensional heteronuclear NMR experiments. Our results indicate that the protein is largely unfolded in solution, but exhibits an extended helical conformation in the lipid-associated state. To identify the topological orientation of αsyn57-102 relative to the micellar environment, we also analyzed the effects of water-soluble and membrane-soluble spin probes on the HSQC spectrum of the protein.

SOLUTION STRUCTURE AND BACKBONE DYNAMICS OF THE HUMAN TRANSCRIPTION FACTOR P63 SAM DOMAIN

S.Mele,[‡] D.O.Cicero[†], B.Cadot[§], E.Candi[§], G.Melino[§], S.Rufini[‡] and A.Desideri[‡]

[‡]*Department of Biology, University of Rome "Tor Vergata", Italy*
[†]*Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Italy*
[§]*Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Italy*

For over 20 years, p53 has been recognized as a guardian against cellular stressors, particularly those that inflict DNA damage. Surprisingly, in view of the central importance of p53 in cellular stress responses, it is only within the past six years that two p53 homologues, p73 and p63 [1] , which are also transcription factors, have been identified. Unlike p53-deficient mice, p73 and p63 knockout animals do not develop spontaneous tumours, but show defects in neuronal and epidermal development, respectively. This striking differences in function between p53, p73 and p63 are unexpected because all three proteins have a similar basic domain structure and there is very high amino acid identity in the DNA-binding domain. However, p63 and p73 are markedly more complexes than p53, largely owing to their extended C-terminal sequence. They also exhibit alternatively spliced C-terminal isomeric forms.The longest (α) isoforms of p63 and p73 contain a sterile α motif (SAM), which, in other molecules, has been implicated in protein-protein interactions. Two structures, one solved by NMR [2] and one by X-ray crystallography [3], are available for the p73SAM domain, but no structure was available for the p63 SAM domain . We have determined the three-dimensional structure of the SAM domain of p63 (505-579) using multinuclear and multidimensional NMR. The resulting structure shows the characteristic five-helix bundle topology observed in SAM domains. Comparison with the recently determined STE50 SAM domain [4] allowed us to propose possible key residues involved in the interaction with molecular partners. ^{15}N relaxation times and ^{1}H-^{15}N steady state NOEs measured for backbone amides were fitted using the model free formalism developed by Lipari and Szabo. The global correlation time of the molecule clearly demonstrate that p63 SAM domain is a monomer in solution. This behaviour is in clear contrast to that observed for other SAM domains. Two regions presenting large motions were observed at the N- and C-terminus. The observed flexibility can be used to explain, partially, the low tendency towards the homodimerization observed for p63 SAM domain.

OVEREXPRESSION AND STRUCTURAL CHARACTERIZATION OF THE ACTIVE FORM OF THE NS3 PROTEINASE DOMAIN OF DENGUE VIRUS. DESIGN OF OPTIMIZED SUBSTRATES AND INHIBITORS.

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

[1]Dep. of Science and Tenc. Chemistry of University of Rome "Tor Vergata", Italy; [2]IIB-Fundación Instituto Leloir, FCEyN, UBA, Argentina; [3]C4T, Combinatorial Chemistry Center, University of Rome "Tor Vergata" Italy

Dengue virus causes widespread human diseases such as dengue fever, dengue hemorrhagic fever and dengue shock syndrome. The viral genome is a positive RNA strand that encodes for a single polypeptide precursor. Processing of the polyprotein precursor into mature proteins is carried out by the host signal peptidase and by NS3 serine protease. The three dimensional structure of NS3 protease domain [1-185] NS3pro has been elucidate (1). Recently a new construct of the recombinant form of the NS3pro, was engineered (2). We have expressed in *E. coli* the His-tag-CF40.gly.NS3pro protein a new construct of the recombinant form of the NS3pro linked to a 40 - residue co-factor, corresponding to a part of NS2B, via a noncleavable, flexible nonapeptide ($Gly_4SerGly_4$), and have currently optimized the purification procedure. Chemically optimized substrates, peptides and depsipeptides, were designed and tested to afford an efficient *in vitro* activity assay, using FRET spectroscopy and HPLC. The data suggest that the amino-terminal region of the 40-amino acid co-factor domain may be involved in additional charged interactions with NS3 that are essential for activity as previously described. This form showed a catalytic activity and spectroscopic studies (CD and NMR) were performed to evaluate the folding of the protein. Moreover, experiments of limited proteolysis have been performed to identify the essential enzymatic domain of the protein and to stabilize the role of the cofactor in the activity and in folding stabilization of the enzyme. After 2h of the limited proteolysis with endoproteinase Asp-N the product was analyzed by SDS-PAGE and activity assay, showing a high reduction of the molecular mass and only a loss of the activity of the 20%. CD and ^{15}N-^{1}H-HSQC spectra of this protein fragment were performed and other functional and structural characterizations are going on in our laboratory. It is intended to obtain the structure in solution of the essential active domain of the uniformly ^{13}C, ^{15}N-labeled CF40.gly.NS3pro by high-field 3D NMR spectroscopy. 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.

References

1. Krishna Murthy H.M. et al (1999) J- Biol. Chem. 274, 5573-5580
2. Leung D. et al (2001) J.Biol. Chem. 276,45762-45771

CHARACTERIZATION OF CAROTENOIDS IN RAW VEGETABLES BY HR-MAS ^{1}H NMR

M.L. Miglietta,[‡] E. Barrese,[‡] R. Lamanna[‡]

[‡]*ENEA C.R. Trisaia, S.S. 106 Jonica Km 419,500, 75026 Rotondella (Mt), Italy*

Carotenoids are linear C_{40} tetraterpenoid hydrocarbons and represent a wide categories of natural pigments. Many important physiological properties of carotenoids are related to their structures. In fact, they act as light energy carriers and quenchers of singlet oxygen, protecting cells and organisms against lipid peroxidation. For this reason carotenoids, such as lycopene and β-carotene, play an important role in the human diet. Lycopene and other carotenoids also play an important role as anti-cancer agents [1,4].

Carotenoids have a strong lipophilic character and are usually analyzed in organic solvents. However, due to their biological activity, the characterization of these compounds in an aqueous environment or in the natural matrix is very important.

Because of their low concentration and strong interaction with the biological matrix, β-carotene and lycopene have never been observed by NMR [5].

In the present work, both β-carotene and lycopene are identified in raw carrots and tomatoes by HR-MAS ^{1}H NMR. The identification of the NMR resonances has been done comparing the raw material spectra with those of β-carotene and lycopene adsorbed on a polysaccharidic matrix and on lyophilized matrices of carrots and tomatoes saturated with D_2O.

The comparison of β-carotene and lycopene spectra in raw vegetables and model systems in aqueous environment shows significant differences with respect to the spectrum obtained in $CDCl_3$.

References

[1] Moden, B., Chuckle, H., H. & Lubin, F. *Int. J. Cancer*, **28**, 421-432 (1981)
[2] Colditz, G., Branch, L., Lipnick, R., Willet, W., Rosner, B. & Hennekens, D. *Am. J. Clin. Nutr.*, **41**, 31-37 (1985)
[3] Olson, J., *J. Nutr.*, **116**, 1127-1145 (1986)
[4] Micozzi, M. S., Beecher, G. R., Taylor, P. R. & Khachik, F., *J. Nat. Cancer Inst.*, **82**, 282-288 (1990)
[5] Mele, A., Mendichi, R., Selva, A., *Carbohydrate Research*, **310**, 216-267 (1998)

DYNAMICS OF 1,2 DIHALOGENATED MOLECULES CLATHRATED IN SYNDIOTACTIC POLYSTYRENE POWDERS AND ORIENTED FILMS: A ^{2}H NMR INVESTIGATION

Patrizia Oliva, Alexandra R. Albunia, Gaetano Guerra, Alfonso Grassi

Dipartimento di Chimica-Università degli Studi di Salerno, via S. Allende , 84081 Baronissi (SA)

Dynamics of discrete molecules hosted in inclusion compounds can be very complex but of interest to gain insight into host-guest interactions. Molecular motions with time scale of 10^5-10^8 Hz are appropriately investigated by ^{2}H NMR spectroscopy using properly deuterated guest molecules.

Investigation of the structure and mobility of organic molecules trapped in crystalline polymers is less common and in this case the guest molecules are typically sorbed in the amorphic part of the polymer[1] Recently the δ crystalline form of syndiotactic polystyrene has been described in the literature as a nanoporose material containing pores with well defined size (120-180 A^3) and location where solvents can be readily hosted.[2] This behavior assimilates syndiotactic polystyrene to the zeolites or other classical inorganic porous materials suggesting for this material the definition of thermoplastic molecular sieves.

A preliminary investigation of the mobility of aromatic molecules, namely benzene and toluene, clathrated in syndiotactic polystyrene powders was previously undertook by means of ^{2}H NMR spectroscopy.[3]

Herein we report an extension of this study to clathrates of 1,2-dihalogenated molecules that show a complex motional reorientation of the guest in the polymer powders.

A comparison of the properties of these clathrates with the same obtained by including the guest in uniaxially oriented films is also reported to gain insight into the complex dynamics of these guest molecules in the syndiotactic polystyrene cavities.

References

[1] Blochowicz, Th.; Karle, C.; Kudlik, A.; Medick, P.; Roggatz, I.; Vogel, M.; Tschirwitz, Ch.; Wolber, J.; Senker, J.; Rössler, E. *J. Phys. Chem. B* **103**, 4032 (1999).

[2] (a) De Rosa, C.; Guerra, G.; Petraccone; V.; Pirozzi, B. *Macromolecules* **30**, 4147 (1997).
(b) Milano, G.; Venditto, V.; Guerra, G.; Cavallo, L.; Ciambelli, P.; Sannino, D. *Chem. Mat.* **13**, 1506 (2001).

[3] (a) Trezza, E.; Grassi, A. *Macromol. Rapid. Commun.* **23**, 260 (2002).
(b) Albunia, A. R.; Graf, R.; Guerra, G.; Spiess, H. W.; in preparation.

^{13}C-NMR ISOTOPOMERIC DISTRIBUTION ANALYSIS: A METHOD FOR MEASURING METABOLIC FLUXES IN FATTY ACID AND CHOLESTEROL SYNTHESIS IN HEPATOCYTE BIOREACTOR

Puccetti C., Miccheli A., Pancotti F., Valerio M., Di Clemente R.,
Tomassini A. and Conti F.

Department of Chemistry, University "La Sapienza", Rome

^{13}C NMR spectroscopy associated with the use of ^{13}C-enriched substrates is a powerful tool to investigate intracellular metabolism because of the wealth of information contained in the distribution of isotopes in key metabolites. A new method of using 13C label distribution measurements in carbon skeletons of metabolites to estimate metabolic fluxes through biochemical reaction networks has been previously reported (1). NMR isotopomer distribution analysis (NMR-IDA) involves the introduction of a ^{13}C-enriched precursor, and measurements of the ^{13}C positional enrichment at just one carbon atom position of the product via ^{13}C NMR spectroscopy. Information on isotopomer distribution is obtained, and data are analyzed according to a mathematical model based on multinomial probability expression to obtained the best fit between theoretical and experimental ^{13}C label distribution.
In this study, we have applied NMR-IDA to the ^{13}C NMR results of the experiments obtained on fixed bed bioreactor containing hepatocytes entrapped in alginate beads by using [U-^{13}C]oleate and [1,2-^{13}C]acetate. In particular, the metabolic fluxes involved in synthesis of β-hydroxybutirate, cholesterol and fatty acids have been estimated starting from substrates, like oleate and acetate, which have a different metabolic utilization in terms of mitochondrial or cytosolic Acetyl-CoA production.

References

1) Puccetti C. *et al* NMR Biomed 2002; 15:404-415.

NICKEL INTERACTION WITH METAL BINDING SEQUENCES OF HISTONE H4

Maria Antonietta Zoroddu[a]*, Massimiliano Peana[a], Serenella Medici[a], Max Costa[b]

[a] *Department of Chemistry, University of Sassari, Via Vienna 2, 07100, Sassari, Italy.* [b] *Department of Environmental Medicine, New York University, New York, USA*

Nickel compounds are well known as human carcinogens.[1] The leading concepts in nickel carcinogenesis involves oxidative promutagenic DNA damage and epigenetic effects in chromatin resulting from nickel binding inside the cell nucleus.[2-5] The nuclear proteins, and in particular the most abundant among them, the histones, are able to compete for metal ions with even higher affinity metal binding sites in other less abundant nuclear proteins or smaller molecules. Phagocytosis of insoluble particles of Ni_3S_2 by either macrophages or epithelial cells causes buildup of very high levels of nickel inside the cells after its intracellular dissolution catalyzed by the acidic pH of endocytic vacuoles, thus providing a continuous source of Ni(II) ions.[6]

We investigated the issue of Ni(II) binding within the histone octamer. Using histone sequences in conjuction with the structural data we identified a binding site for Ni(II) ions located in the N-terminal tail of the histone H4.

References

[1] IARC, Lyon, France Monographs on the evaluation of carcinogenic risks to humans. Chromium, Nickel and Welding, 1990, Vol. 49
[2] K. Salnikow, S. Cosentino, C. Klein and M. Costa, Mol. Cell. Biol., 1994, 14, 851
[3] W.Bal, V.Karantza, E.N.Mondriakis, K.S. Kaprzak, Arch.Biochem. Biophys. 1999, 364, 161
[4] L. Broday, W. Peng, K. Salnikow, M.A. Zoroddu, M.Costa, Cancer Res. 2000, 60, 238
[5] W.Bal, R.Liang, J.Lukszo, S. Lee, M.Dizdaroglu, K.S. Kaprzak, ChemRes. Toxicol. 2000, 13,616
[6] R.M.Evans, P.J.Davies, M.Costa, Cancer Res. 1982, 42, 2729

STRUCTURAL STUDY OF THE INTERACTION OF CALCIUM-INDEPENDENT BINDING OF MYOSIN LIGHT CHAIN 1 TO IQ MOTIFS OF CLASS V MYOSIN MOTOR IN YEAST CELLS

Matteo Pennestri[1], Pamela Bielli[2],Alessia Santoprete[1], Maurizio Paci[1], Sonia Melino[1] Antonella Ragnini- Wilson[2] and Daniel O. Cicero[1]

[1] Dipartimento di Scienze e Tecnologie Chimiche, University of Rome "Tor Vergata",[2] Dipartimento di Biologia University of Rome "Tor Vergata" via della Ricerca Scientifica, Italy

INTRODUCTION: The mechanism of organelle-vesicle anchoring is conserved in eukaryotes and requires the interaction between class V myosins and Rab/Ypt family members. Vesicle-motor anchoring and movement in yeast cells occurs via the formation of a complex that contain a Rab/Ypt protein, a class V myosin member and a member the calmodulin superfamily, the Mlc1p (1). A similar machinery was found to be required for melanosome transport in melanocytes dendrides in mammals. Defects in this machinery are at the basis of genetic human dysfunction having as common features defects in organelle transport (2). Mlc1p regulates vesicle/organelle delivery at a specific location and at a determined stage of the cell cycle and differently to other calmodulin-like EF hand proteins does not respond to Ca2+- signals (3), is conceivable to think that Mlc1p-regulated interaction with IQ motifs of the components of class V myosin is controlled either by signals that come from the cell cycle or from Rab/Ypt activation.

We have started a multidisciplinary approach in which genetic in vivo interactions and NMR in vitro studies will be used to clarify the determinants of Mlc1p interaction with Myo2p IQ motifs.

The recombinant His-tagged Mlc1p protein has been expressed and labelled for NMR studies. The ^{1}H-^{15}N HSQC spectrum shows a remarkable dispersion of chemical shift and line-shape characteristics, indicating a stable fold of the protein. Using standard multinuclear and multidimensional NMR experiments on the $^{15}N/^{13}C$ labelled Mlc1p we have obtained the almost complete assignment of backbone resonances. Chemical shift indexes and $^{3}JHNH\alpha$ were used to establish the secondary structure of the protein, for which no structure in the uncomplexed form exists. Finally, the interaction with two IQ motifs from class V myosin, IQ2 and IQ4, were followed by a series of ^{1}H-^{15}N HSQC spectra. The chemical shift perturbation analysis reveals differences in the two interactions. This type of studies are the starting point for other interaction studies involving Mlc1p mutants presenting complex phenotypic effects. These results will be used to clarify the link between the observed physiological effect of the mutations and the deviations in the protein-protein interaction pattern.

References

1. Wagner et al., *EMBO. J* **21**,6397-6408 (2002)
2. Wu et al., *Nature Cell Biol.* **4**, 271-278 (2002).
3. Terrak et al. *EMBO J.* **22**, 362-371 (2003).
4. Marley J. et al (2001) *J. Biomol. NMR* 20, 71-77.

SOLUTION STRUCTURE DETERMINATION BY NMR AND STRUCTURE-BASED INVESTIGATION OF FUNCTION OF THE UNKNOWN PROTEIN ApaG OF THE PHYTOPATHOGEN *Xanthomonas axonopodis* pv. *Citri*

G.M. Contessa[b,a], A. M. Katsuyama, [†,¥] C.S.Farah,[†] M. Paci [a,b], D.O. Cicero[a,c], A. Spisni[¥,§] and T. A. Pertinhez,[¥]

[a]*Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", via della Ricerca Scientifica, 00133 Rome Italy.*
[b]*INFM, University of Rome "Tor Vergata".*
[c]*SISSA, Settore di Biofisica, Trieste, Italy.*
[†] *Department of Biochemistry, Institute of Chemistry, University of Sao Paulo, Brazil.*
[¥] *BioNMR Laboratory, Center for Structural Molecular Biology, LNLS, campinas, Brazil.*
[§] *Sect. Chemistry and Structural Biochemistry, Department of Experimental Medicine, University of Parma, Italy.*

Xanthomonas axonopodis pv. *citri* is the agent of citrus canker, a severe disease of the citrus fruit. ApaG protein belongs to the group of proteins of unknown structure and function encoded by this pathogen [1]. Recognising that the structural characterisation of a protein may provide information regarding its cellular function we have been prompted to determine the 3D solution structure of that protein. To reach this goal we used standard multinuclear and multidimensional NMR experiments, including various types of residual dipolar coupling. The preliminary results indicate that the protein has a global β-immunoglobulin-like fold. The main feature is a seven-stranded cup-shaped anti-parallel β-pleated sheet, with an accessible central hydrophobic cavity. The use of different types of residual dipolar coupling was essential to characterize the loops connecting the β-sheets.
A structural homology study was carried out with the aid of the server Dali, and we selected the structures with the highest z-values. A striking similarity was obtained with the GM2-Activator protein, that acts as cofactor in the sequential degradation of gangliosides [2].
The final refined structure will be discussed in the light of understanding the possible biochemical function of the ApaG protein.

Acknowledgments: The work was partly supplied by MIUR Cofin (Italy) and FAPESP (Brazil)

References

[1] Katsuyama A.M., Cicero D.O., Spisni A., Paci M., Farah C.S., Pertinhez T.A. (2004) J Biomol NMR. 29, 423-4
[2] WRIGHT CS, LI SC, RASTINEJAD F. (2000) J MOL BIOL. 304, 411-22.

MEMBRANE TOPOLOGY OF α-SYNUCLEIN

Luca Pinato[#], Marco Bisaglia[#], Massimo Bellanda[#], Isabella Tessari[¶], Sabrina Giraudo[§], Mauro Fasano[§], Elisabetta Bergantino[¶], Luigi Bubacco[¶], Stefano Mammi[#].

[¶]Department of Biology, University of Padova, Viale G. Colombo 3, 35121, Padova, Italy
[#]Department of Chemical Sciences, University of Padova, Via F. Marzolo 1, 35131, Padova, Italy
[§]Department of Structural and Functional Biology, University of Insubria, Via Jean H. Dunant 3, 21100 Varese, Italy

Human α-synuclein is a small presynaptic protein of unknown physiological function abundantly expressed in the brain and found in Lewy bodies, a characteristic feature of Parkinson's disease. α-Synuclein is random in water under physiological conditions but, in the presence of vesicles, the N-terminal 100 residues interact with phospholipids and adopt an ordered conformation. The rest of the molecule remains disordered in the bulk of the solution. The conformation of the N-terminal portion of the molecule in SDS was described as an extended helix [1], as two distinct α-helices interrupted by a two-residue break [2] or as a non-canonical conformation, the α11/3 helix [3]. In the present study, we characterized the topology of the different regions of α-synuclein relative to the surface of SDS micelles. We made use of independent experimental approaches, such as spin-probe-induced broadening of NMR signals, ^{15}N relaxation measurements, fluorescence spectroscopy and CD. Our results support the presence of two N-terminal α-helices, separated by a flexible stretch and positioned on the membrane surface. The region 61-95 of the protein also adopts a helical conformation, but it is partially embedded in the membrane. To our knowledge, this is the first report that provides sufficient experimental evidence to suggest a topological model of the structuring effect of lipids on α-synuclein. On these premises, an attempt was made to define the physiological structure and consequentially the potential functions of α-synuclein.

References

[1] Eliezer D, Kutluay E, Bussell R Jr and Browne G *J Mol Biol*, **307**, 1061-1073 (2001).
[2] Chandra S, Chen X, Rizo J, Jahn R and Sudhof TC *J Biol Chem*, **278**, 15313-15318 (2003).
[3] Bussell R Jr and Eliezer D *J Mol Biol*, **329**, 763-778 (2003).

THREE DIMENSIONAL SOLUTION STRUCTURE OF A SINGLE POINT MUTANT OF SSO7D AND ANALISYS OF THERMOSTABILITY DETERMINANTS

I. Arosio, T. Recca*, E. Alberti, L. Zetta and R. Consonni

Istituto per lo Studio delle Macromolecole, Lab.NMR, C.N.R., v. Bassini, 15, 20133 Milano, Italy
**present address: Dipartimento di Chimica Organica e Industriale, Università degli Studi, v. Venezian, 21, 20133 Milano, Italy*

Sso7d is a protein isolated from the archeobacterium Sulfolobus solfataricus, a thermophilic bacterium that lives at 87° in volcanic hot springs. It is composed of 62 amino acid residues and shows DNA binding property and RNase activity [1]. We have structurally characterized the wild type protein (WT) and different single point mutants and the structures have been discussed in terms of determinants for activity and thermostability [2,3]. The overall folding of these proteins is that of compact and globular small beta barrels.
In this paper, we present the 3D solution structure of the most thermostable mutant of Sso7d found up to now, in which a lysine residue has been substituted with a leucine (PDB code 1R83). We have compared this structure with those of the WT (PDB code 1JIC) and of the mutant in which a phenylalanine residue has been mutated with an alanine (PDB code 1B4O), analysing the principal factors affecting the thermostability of this family of proteins.

References

[1] E. Shehi, S. Serina, G. Fumagalli, M. Vanoni, R. Consonni, L. Zetta, G. Dehò, P. Tortora, P. Fusi "The Sso7d DNA-binding protein from Solfolobus solfataricus has ribonuclease activity" (2001) FEBS Letters 497, 131-6
[2] R. Consonni, L. Santomo, P. Fusi, P. Tortora and L. Zetta "A single point mutation in the extreme heat- and pressure-resistant Sso7d protein from Sulfolobus solfataricus leads to a major rearrangement of the hydrophobic core" (1999) Biochemistry 38, 12709-17
[3] R. Consonni, I. Arosio, B. Belloni, F. Fogolari, P. Fusi, E. Shehi and L. Zetta "Investigations of Sso7d catalytic residues by NMR titration shifts and electrostatic calculations" (2003) Biochemistry 42, 1421-1429

STRUCTURAL STUDIES OF THE BINDING OF H-NS TO ITS TARGET DNA

Stefano Stella[1], Gabriele Risi[2], Gaetano Barbato[3], Claudio Gualerzi[1],
Lorenzo Stella[2] and Marco Sette[2]

.
[1]*Laboratory of Genetics, Department of Biology MCA, University of Camerino, 62032 Camerino, Italy,* [2]*IRBM-Merck, via Pontina Km 30,600 Rome, Italy,* [3]*Department of Chemical Sciences and Technology, University of Tor Vergata, via della Ricerca Scientifica, Rome Italy*

INTRODUCTION: H-NS (Histone-like Nucleoid Structuring protein) is an abundant protein associated with the bacterial chromosome acting as a transcriptional repressor of many genes [1]. H-NS consists of two structurally and functionally independent domains [2,3] connected by a flexible linker. The N-domain (residues 1-64) and C-domain (residues 90-136) are held responsible for protein oligomerization and DNA binding, respectively. The structure and function of the linker (residues 65-89) is not yet well defined. Native H-NS is ill-suited for spectroscopy studies since it has a limited solubility, is very sensitive to pH and ionic strength and forms aggregates at high concentrations. On the other hand, the C-domain was reported to be soluble and monomeric even at millimolar concentrations so that its structure was determined by homonuclear NMR spectroscopy [4]. Likewise, the regions corresponding to the C-domain (residues 90-136) and linker region (residues 60-89) were used to map the H-NS residues that interact with DNA. However, a protein fragment which is likely a dimer in solution and a synthetic DNA, whose structure is presumably different from that of a natural target of H-NS [5], were used in these studies. The results obtained have therefore suggested that the linker region may contribute to H-NS oligomerization and, directly or indirectly, to DNA binding. To identify the DNA and protein regions involved in a specific H-NS-DNA interaction, in this work we are examining, by several spectroscopic approaches, the interaction of the C-domain of H-NS with a specific fragment of DNA representing an in vivo target of H-NS binding.

MATERIALS AND METHODS: The DNA fragment (15 bp) having the desired, specific sequence was obtained from MWG. Fluorescence spectroscopy was performed on either the C-domain (residues 89-136) or the C-domain containing part of the linker (residues 64-136). NMR spectra were recorded at 400 and 600 MHz. 2D-TOCSY and NOESY experiments were recorded on the isolated DNA fragment. 3D-TOCSY-HSQC and NOESY-HSQC experiments were performed on the ^{15}N-labelled C-domain fragment.

RESULTS: One-dimensional NMR spectra titrations clearly showed that the selected DNA fragment binds specifically to the C-domain of H-NS. Furthermore Trp108, a residue which has been reported to be involved in DNA binding [6], was found not to be affected by the interaction, a result which was also confirmed by fluorescence spectroscopy. Since the binding affinity is not very high (K_a ranging from 10^3 to 10^5 M^{-1} depending on the experimental conditions) a protein fragment comprising the C-domain and part of the linker region (residues 64-136) was prepared. This fragment binds the DNA with higher affinity (K_a ranging from 10^5 to 10^7 M^{-1} depending on the conditions) thus allowing us to establish the conditions to obtain a stable complex in solution. It is noteworthy that the DNA resonance shifts observed upon addition of the protein are the same with the C-domain, the C-domain plus the linker or with the whole protein suggesting that even when using a small protein fragment we are observing an interaction similar to that occurring *in vivo*. Fluorescence anisotropy experiments suggest that both the C-domain and the fragment with the linker are monomeric in solution. The NMR spectrum of the C-domain has been reassigned using an ^{15}N-labelled sample and the NMR spectrum of the DNA is currently under study to have the full assignment of the DNA resonances. Ultimately, this will provide insight into the regions of the DNA and of the protein involved in the interaction and clarify the structural changes occurring in the two ligands upon complex formation.

References

1. Pon et al., (2004) In DNA Conformation and Transcription, edited by T. Ohyama, Landes Biosciences.
2. Smyth et al., (2000) Mol. Microbiol. 36, 962-972.; 3. Schroder et al., (2001) Biochem. Biophys. Res. Comm., 282, 219-227.; 4. Shindo et al., (1995) FEBS Letters 360, 125-131; 5. Shindo et al., (1999) FEBS Letters, 455, 63-69.; 6. Tippner et al., (1995) J.Biol.Chem. 270, 38, 22243-22247.

TWO PARAMAGNETIC PROBES ARE BETTER THAN ONE

Ottavia Spiga, Arianna Ciutti, Andrea Bernini, Vincenzo Venditti and Neri Niccolai.

Department of Molecular Biology, University of Siena

The protein surface accessibility to neutral small organic molecules has been investigated in the crystal [1] and in solution [2] suggesting the presence of hot spots driving the intermolecular recognition process. In this respect, NMR spectroscopy offers big opportunities to explore, through independent measurements and suitable experimental strategies, the mechanisms of interaction of a protein with its molecular environment. Thus, accessibility probes, such as the soluble spin-label TEMPOL [3,4] and, more recently, Gd(III)/DTPA-BMA complex [5], have been proposed on the critical assertion, so far always met, that these species are not involved in any specific interaction with particular amino acid side chains or structural moieties.
Here, we present direct evidence that, at least in the limits of the used experimental conditions, TEMPOL and Gd(III)DTPA-BMA cause, at different extent but in a very similar way, enhancements of spin-lattice relaxation rates of bovine pancreatic trypsin inhibitor (BPTI) protons, which strongly reflect their surface exposure.
Conventional paramagnetic perturbation analysis of HSQC signals of α-bungarotoxin has been also performed by using both probes. The obtained data indicate that the toxin active site is the most exposed, and in a similar extent, to TEMPOL and Gd(III)DTPA-BMA in spite of their different polarity and shape.
In the case of BPTI, dilution studies of the paramagnetic solutions suggest the presence of anomalous surface accessibility dynamics, most likely due to nascent BPTI aggregation phenomena, which cannot be monitored by other conventional NMR parameters.

References

[1] Mattos C, Ringe D. Nat Biotechnol.**14**, 595-599 (1996).
[2] Liepinsh E, Otting G. Nat Biotechnol. **15**, 264-268 (1997).
[3] Niccolai N, Ciutti A, Spiga O, Scarselli M, Bernini A, Bracci L, Di Maro D, Dalvit C, Molinari H, Esposito G, Temussi PA. J Biol Chem. **276**, 42455-42461 (2001).
[4] Niccolai N, Spiga O, Bernini A, Scarselli M, Ciutti A, Fiaschi I, Chiellini S, Molinari H, Temussi PA. J Mol Biol. **332**, 437-447 (2003).
[5] Pintacuda G, Otting G. J Am Chem Soc.**124**, 372-373 (2002).

NULL-BIASED FAST-FIELD-CYCLING SEQUENCES

S.Sýkora and G.M.Ferrante

Stelar Srl, Via E.Fermi 4, 27035 Mede (PV)

The standard pre-polarized and non-polarized measurement sequencies used in fast-field-cycling (FFC) NMR relaxometry [1] suffer from two major drawbacks:

(a) Due to the magnetization evolution during switching intervals, the longitudinal magnetization evolves with increasing τ towards non-zero values. Consequently, FFC-NMR decay curves must be fitted with an adjustable 'offset' parameter, in addition to the decay rates and weights of individual sample components (for example, three parameters in the case of a mono-exponential fit).

(b) Measurements are carried out using different sequences at high relaxation fields (NP) and at low relaxation fields (the crossover field being approximately half of the polarization field). For reasons which are not yet quite clear, this sometimes leads to a statistically significant discrepancy between the two 'sections' of the resulting NMRD profile.

We propose new measurement sequences consisting in a combination of RF pulse-phase cycling, receiver cycling and magnetic field cycling, such that all switching-time interval effects get cancelled, while the sample pre-polarization effects and the subsequent decay are enhanced.

In such sequences, the acquired signal $S(\tau)$ decays rigorously to zero for $\tau \rightarrow \infty$ (which is why we call them ***null-biased***). Essentially all classical FFC-NMR sequences can be cast in this way (the basic NP/PP, IR, IR_CPMG, ...), some of them in a way which does not at all reduce the efficiency of the measurement (i.e., the total signal range which can be achieved in a given time).

The null-biased sequences offer several important advantages:

(1) Since $S(\infty)$ is strictly null and does not have to be estimated experimentally, there is no need to acquire portions of the decay curve where the signal is already almost stable (this leads to a time saving).

(2) For the same reason, the data can be fitted without the adjustable offset parameter (for example, a two-parameter fit is sufficient in the case of a mono-exponential decay). Considering the effect of number of adjustable parameters on their confidance intervals, this fact alone improves the precision of the estimated T_1 by a large factor (~10).

(3) In most cases, the same sequence can be used to acquire the NMRD profile over the complete range of field values (for example, from 5 kHz to 40 MHz). As a result, the measured NMRD profiles are internally more coherent and no discrepancy between high-field data and low-field data can occur.

We discuss also an apparent drawback of the new sequences consisting in the fact that they enhance the visual impact of random field variations between consecutive scans. Analysis shows, however, that traditional measurements are burdened by the same instabilities, even though they are to a large extent masked by the switching-interval signal components.

References

[1] G.Ferrante, S.Sykora, Technical Aspects of Fast Field Cycling NMR Relaxometry, in Advances in Inorganic Chemistry, R.van Eldick, Editor, Vol.57 (2004), in press.

[2] C.Radhakrishna Rao, Linear Statistical Inference and its Applications, John Wiley & Sons, 1973

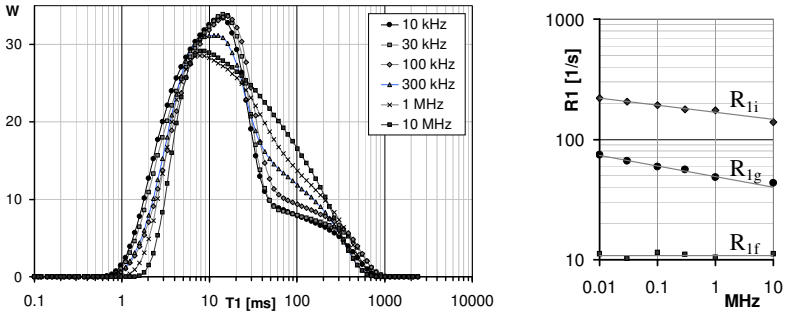
FIELD-DEPENDENCE OF RELAXATION TIME DISTRIBUTIONS IN ROCK SAMPLES

V.Bortolotti[a], R.J.S.Brown[b], P.Fantazzini[c], G.Ferrante[d], S.Sýkora[e]

[a]*Univ. of Bologna, Dept. DICMA, V.le Risorgimento 2, 40136 Bologna, villiam.bortolotti @unibo.it*
[b]*953 W. Bonita Ave., Claremont CA 91711-4193, USA; rjsbmeb@mailaps.org*
[c]*Univ. of Bologna, Dept. of Physics, Viale Berti Pichat 6/2, 40127 Bologna, paola.fantazzini@unibo.it*
[d]*Stelar Srl, Via Enrico Fermi 4, 27035 Mede (PV), ferrante@stelar.it*
[e] *Extra Byte, Via R. Sanzio 22C, 20022 Castano Primo (MI), sykora@ebyte.it*

^{1}H-NMR relaxation times of water-saturated rock samples are widely employed to characterize the architecture of pore space and other petrophysical properties. So far, such NMR studies were always carried out at fixed frequencies and thus could be affected by the fact that relaxation rates are field dependent [1]. Mono-exponential analysis of T_1 decay curves so far published [2] indicate that the field dependence, though modest, does exist. However, it has been amply shown [3] that the mono-exponential hypothesis is not applicable to rocks, where one observes wide distributions of relaxation rates, due to the wide distributions of pore sizes and properties. For this reason, we have investigated the relaxation rate distributions in several carbonates and sandstones at a number of relaxation field values, ranging from 10 kHz to 30 MHz.

The T_1 decay curves of water-saturated rock samples were measured by a Stelar Srl FFC-NMR Relaxometer and then subject to T_1-distribution analysis by UPEN [3].



The results, exemplified above by the Pietra Serena sandstone data, indicate that:

(a) There are significant and systematic field-dependent variations in the continuous distribution curves obtained by UPEN.

(b) Geometric (R_{1g}) and arithmetic means (R_{1i}) of the T_1's do depend on field intensity, while no field-dependence can be detected for the longest relaxation rates R_{1f}. It thus appears that different components of the sample exhibit different field dependencies.

Analysis of a set of T_1 distribution curves obtained at a number of relaxation field values can allow one to separate different sample components and associate a distinct field-dependence profile with each of them. Theoretical model aimed at achieving this goal have already been established [4] and further work is presently in progress.

References

[1] N.Bloembergen, E.M.Purcell, R.V.Pound, *Phys. Rev.* **73**, 679 (1948); [2] S.Godefroy, J.-P.Korb, M.Fleury, R.G.Bryant, *Phys. Rev. E*, **64**, 21605 (2001); [3] G.C.Borgia, R.J.S Brown, P.Fantazzini, *J. Magn. Reson.* **132**, 65 (1998); **147**, 273 (2000); [4] V.Bortolotti, R.J.S. Brown, P.Fantazzini, G.Ferrante, S.Sýkora, in Press.

^{1}H NMR RELAXATION DISPERSION PROFILES OF SOLID BSA

S.Sýkora[a], M.A. Cremonini[b], L.Laghi[b], P.Fantazzini[c]

[a] *Stelar Srl, Via E.Fermi 4, 27035 Mede (Pv); sykora@ebyte.it*
[b] *Department of Food Science, University of Bologna, P.zza Goidanich 60, 47023 Cesena, Italy; mauro.cremonini@unibo.it and luca.laghi2@unibo.it.*
[c] *Department of Physics, University of BolognaViale Berti Pichat 6/2, 40127 Bologna, paola.fantazzini@unibo.it*

Introduction: Bovine Serum Albumin is a representative of nearly globular proteins of intermediate molecular weight. During the last two decades, a considerable amount of research [1-5] has been dedicated to collecting and interpreting its NMR relaxation data, in particular T_1 dispersion profiles of both solid BSA and of its solutions. Although there is so far no convincing consensus on the nature of the relaxation processes in BSA (and in proteins in general), one feels that an in-depth understanding might be in reach.

Here we present high quality proton NMRD profiles of solid BSA, measured over more than five decades of relaxation fields (Larmor frequencies ranging from 5 kHz to 600 MHz) which, hopefully, might contribute to reaching such an understanding.

Experimental: Lyophilized, ultra-pure (>99 %) and globulin-free BSA purchased from Sigma (product number A7638) was kept for 3 hours in a 10 mm NMR sample tube under high vacuum in order to remove all free/adsorbed oxygen and any traces of humidity before sealing. FID's, NMRD profiles, CPMG decays and other NMR signals of the sample were measured using the following instruments:

(a) Frequency range of 5 kHz - 30 MHz: fast-field-cycling (FFC) relaxometer (1T FFC SpinMaster produced by Stelar Srl, Mede, Italy).

(b) Frequency range 15 - 50 MHz: Stelar SpinMaster relaxometer of traditional type used with a recycled Jeol electromagnet.

(c) Fixed frequencies of 200, 400 and 600 MHz: respectively, Bruker AC200, Varian Mercury VX 400 and Varian INOVA 600.

Sample temperature has been carefully calibrated and rigorously controlled during all measurements. Since the FID's of the dry, solid BSA are very short and decay below the noise level in about 25 μs, we have used low Q-factor probes (~10) with very short dead-times (~7 μs) at frequencies below 100 MHz. Standard HR probes were used for experiments at higher resonance frequencies fields.

Experimental results: The NMRD profiles show a steep dispersion extending from less than 10 kHz to over 300 MHz. Data acquired with completely different instruments are perfectly coherent with each other. The dispersion curves, plotted as log(R) against log(ω), with R being the relaxation rate (inverse of T_1) and ω being the ^{1}H Larmor frequency at relaxation field, exhibit two nearly linear regions, a low- and a high-frequency plateau and four superposed ^{14}N glitches. These observations were combined into an empirical reference formula which describes the data with a precision better than the measurement errors.

References

[1] Kimmich R.,Gneiting T., Kotitschke K.,Schnur G., Biophysical Journal 58,1184 (1990); [2] Koenig S.H.,Brown R.D., Magn.Resonance in Medicine 30, 685 (1993); [3] Halle B.,Johannesson H.,Venu K., J.Magn.Reson. 135,1 (1998); [4] Halle B.,Denisov V.P.,Venu K., in Biological Magnetic Resonance, Ed.s Krishna, Berliner, Vol.17, p.10, Kluwer Academic/Plenum Publishers 1999; [5] Bertini I.,Fragai M.,Luchinat C.,Parigi G., Magn.Reson.Chem. 38,543 (2000).

ANALYSIS OF PAESTUM'S CERAMICS AND MODERN POTTERY BY NMR RELAXOMETRY AND MRI

P. Tedesco,[‡] R. De Amicis, [‡] G. Patimo[‡], S.Pace[‡], I.Rabuffo[‡], A. Pontrandolfo[†]

[‡]*Dipartimento di Fisica "E.R:Caianiello", Università degli Studi di Salerno, via S.Allende, Baronissi (SA), Italia.*
[†]*Dipartimento dei Beni Culturali, Università degli Studi di Salerno, via S.Allende, Baronissi (SA), Italia*

The nuclear magnetic resonance imaging can be used as a non-destructive method to study the evolution of the properties of porous media and the spatial distribution of liquid water confined in a porous material[1-7].
In this work we analyze some ceramics, whose provenience is Paestum's archeological site, and two different kind of modern pottery, obtained by several sintering processes of fireclay and kaolin.
The measurements are performed by a benchtop imaging system (MARAN Ultra/DRX Resonance Instrument console) with a permanent magnet at 0.5 T. The water penetrating rate via capillary rise for the different samples has been observed by proton density weighed images. The distribution of spin-lattice (T_1) and spin-spin (T_2) relaxation times in water saturated samples was derived. The values of relaxation times of bulk water are on the order of seconds, while for the water confined in porous materials these can be on the order of milliseconds. Thus a study of T_1 and T_2 gives an information about the porosity of materials. [4,5]
In some clay the NMR measurements on the confined water is complicated by magnetic impurities [such as FE(III)] [1,3]. The effective spin-spin relaxation time of the bulk pore fluid decreased linearly with increasing Fe(III) concentration. The iron impurities have been estimated by a Scanning Electron Microscopy with an Energy Dispersion Spectrometer probe. The NMR relaxometry data are analyzed considering the different amount of iron impurity in different samples.

References

[1] T.R.Bryar, C.J.Daughney and R.J.Knight, J. Magn. Reson. **142**, 74-85 (2000)
[2] P.Coussot, Magn. Reson. Imaging **16**, 621-623 (1998)
[3] L.Pel, K.Kopinga, G.Bertram and G. Lang, J. Phys. D: Appl. Phys. **28**, 675-680 (1995)
[4] G.C.Borgia, M.Camaiti, F.Cerri, P.Fantazzini, F.Piacenti, J. Cult. Heritage **1**, 127-132 (2000)
[5] L.Appolonia, G.C.Borgia, V.Bortolotti, P.Fantazzini, G.Rezzaro, Magn. Reson. Imaging **19**, 509-512 (2001)
[6] P.T. Callaghan, S. Godefroy, and B.N.Ryland, J. Magn. Reson. **162**, 320-327 (2003)
[7] S. Sharma, F. Casanova, W.Wache, A. Segre, B. Blümich, Magn. Reson. Imaging **21**, 249-255 (2003)

CHARACTERIZATION OF WHEAT AND SOY BREADS BY ^{1}H AND ^{13}C NMR DURING STORAGE

Stefano Tiziani, Yu Chu Zhang, Alessia Lodi, and Yael Vodovotz

Food Science & Technology Department, The Ohio State University, Columbus, OH 43210 (USA)

Addition of soy to wheat bread has been shown to affect the physico-chemical properties of the final product. In this study, the molecular changes in wheat (used as reference) bread [1] and soy containing (60% of wheat flour replaced by soy ingredients) bread during storage at 4°C for 7 days were investigated using ^{1}H and ^{13}C NMR. Bread samples were placed in 5mm tubes and ^{1}H Cross Relaxation measurements [2] were performed on a Bruker DMX 300MHz, spin-lattice relaxation time (T_1) of ^{1}H and ^{13}C were measured on a DRX 600MHz NMR. The T_1 curves for both fresh soy and wheat breads were single exponential. Wheat bread had a greater ^{1}H T_1 (0.56s) than soy bread (0.44s). During storage, the T_1 decreased for wheat bread but did not change for soy bread. ^{13}C T_1 was best fitted with a bi-exponential curve for fresh wheat bread (3.2 and 0.09s) and a single-exponential curve for fresh soy bread (0.53s). Over time, the ^{13}C T_1 of both fresh soy and wheat bread changed to be more heterogeneous. Cross-relaxation results showed a higher mobility of solid like protons in fresh soy bread than in fresh wheat bread [3]. During storage, the mobility of solid like protons increased for soy bread but did not change for wheat bread (Fig. 1). These results indicate that the addition of soy to bread significantly influence ^{1}H and ^{13}C mobility and therefore will impact storage stability.

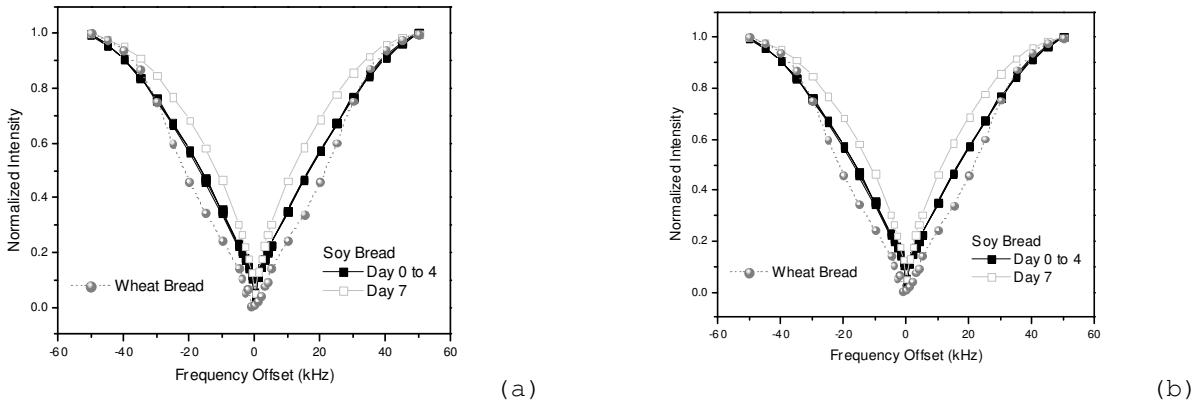


Fig. 1. Cross-relaxation spectra during storage of wheat bread (a) and comparison with soy bread (b).

References

[1] Baik, M.Y., Dickinson, L.C., and Chinachoti, P. 2003. Solid-state 13C CP/MAS NMR studies on aging of starch in white bread. *Journal of Agricultural and Food Chemistry*. **51**: 1242-8.

[2] Wu, J.Y., Bryant, R.G., Eads, T.M. 1992. Detection of solid-like components in starch using cross-relaxation and fourier transform wide-line 1H NMR methods. *Journal of Agricultural and Food Chemistry*. **40**:449-455.

[3]Vodovotz, Y., Vittadini, E., and Sachleben, J. R. 2002. Use of 1H cross-relaxation nuclear magnetic resonance spectroscopy to probe the changes in bread and its components during aging. *Carbohydrate Research.* **337**: 147-53.

METABONOMIC ANALYSIS ON PLASMA OF DIALYSED PATIENTS BY ^{1}H-NMR: EFFECT OF CARNITINE TREATMENT

G. Capuani,[‡] A. Tomassini[‡], M. Valerio[‡], A. Giuliani[†], M. Colafranceschi[¶], F. Pancotti[‡], A. Miccheli[‡] and F. Conti[‡]

[‡] *Department of Chemistry, "La Sapienza" University, P.le Aldo Moro 5, 00185 Roma (Italy)*
[†] *Environmental and Health Department, Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Roma (Italy)*
[¶] *Department of Human Physiology and Pharmacology, "La Sapienza" University, P.le Aldo Moro 5, 00185 Roma (Italy)*

Metabonomics based on NMR data analysed by statistical methods such as multivariate data analysis is going to reveal as an powerful method for the study of physio-pathological states, as well as of the effect of therapeutic treatments, by taking into account the metabolic profile of biological fluids.
In the present study, the NMR profile of 33 patients, 6 of which underwent to chronic treatment with carnitine, before and after renal dialysis was studied. The aim of the study was to detect modification of the metabolic system as a function of the dialysis and of the carnitine treatment. The analysis was conducted both by "unsupervised" pattern recognition method and by "supervised" determination of 18 metabolites, which signals could be unambiguously assigned and integrated.
Metabonomic analysis was performed by PCA as applied to the entire set (before and after dialysis) and to the subset given by data collected before dialysis. Unsupervised analysis was able to recognise the metabolic processes involved with dialysis and with carnitine treatment, and the supervised analysis clarified their biological meaning.

MOLECULAR ORDER AND STRUCTURE OF PYRIDINE–D_5, ALANINE-^{15}N AND PHENYLALANINE IN THE NEMATIC OR LAMELLAR PHASE OF THE LYOTROPIC CSPFO/WATER MIXTURE.

Silvia Borsacchi, Donata Catalano, C. A. Veracini

Dipartimento di Chimica e Chimica Industriale, via Risorgimento 35, 56126 Pisa, Italia.

The system CsPFO/H_2O (or D_20) is an extensively studied mixture [1], forming two discotic micellar phases, nematic and lamellar respectively. The former has been used as an orienting solvent for obtaining residual dipolar couplings of a solute protein [2], usefull as constraints in the determination of the protein structure [3]. On the other hand, in the last few years, conformational analysis of some oligosaccharides have been published, entirely based on ^{1}H-^{1}H and/ or ^{1}H-^{13}C residual dipolar couplings in another lyotropic solvent [4,5]. The CsPFO/H_2O lamellar phase has also been used as orienting solvent for the conformational analysis of alanine dipeptide from ^{1}H-^{1}H and ^{1}H-^{13}C dipolar couplings [6a] and the orientation of some simple amides at the bicelle-water interface has been discussed in detail [6b].
The aim of our work is the study of molecular order and structure of water soluble, suitably deuterated molecules, dissolved in the liquid crystalline phases of CsPFO/H_2O, also exploiting ^{2}H NMR spectroscopy [7]. As a starting point, we have investigated pyridine–d_5, a small, rigid molecule of well-known structure, and alanine-^{15}N, which presents a quite simple ^{1}H NMR spectrum even in the oriented phases. Then, the ^{2}H and ^{1}H NMR spectra of phenylalanine-d_8 and phenylalanine, respectively, have been analyzed. From the experimental quadrupolar and/or dipolar couplings, detailed orientational, geometrical and conformational indications have been obtained.

References

[1] N. Boden, S.A. Corne, K. W. Jolley J. Phys. Chem. **1987**, 91, 4092
[2] A. Kimura, N. Kuni, H. Fujiwara J. Am. Chem. Soc. **1997**, 119, 4719.
[3] J. H. Prestegard, H. L. Al-Hashimi, J. R. Tolman Quart. Rev. of Biophys., **2000**, 33, 371.
[4] M. Martin-Pastor, C. A. Bush Biochemistry **2000**, 39, 4674.
[5] F. Tian, H.L. Al-Hashimi, J.L. Craighead, J.H. Prestegard J. Am. Chem. Soc. **2001**, 123, 485.
[6] C. F. Weise, J. C. Weisshaar J. Phys. Chem. B **2003**, 107, p.3265 (a); p.6552 (b)
[7] C. A. Veracini in "Nuclear Magnetic Resonance of Liquid Crystals" edited by J. W. Emsley, NATO ASI Series C, Vol. 141, Reidel Publishing Company, Dordrecht 1983, p. 99.

MUSCLE OXYGENATION QUANTITATIVELY ASSESSED BY ^{1}H-MRS

A. Vezzoli,[1] M. Gussoni,[2] F. Greco,[3] L. Zetta.[3]

[1]*Istituto di Bioimmagini e Fisiologia Molecolare, CNR, Via Fratelli Cervi 93, Segrate (Mi), Italy*
[2]*Dip. di Scienze e Tecnologie Biomediche, Università di Milano, Via F.lli Cervi 93, Segrate (Mi), Italy*
[3]*Istituto per lo Studio delle Macromolecole,CNR, Via Bassini 15, Milano, Italy*

Oxygen has multiple roles in cells, which usually are highly dependent on its amount. Generally the measurements of oxygenation are made in the circulatory system but unfortunately these can have considerable limitations due in some cases to invasivity (blood gases, polarography, fluorescence quenching), in other cases by indirect determination (NIRS). However the real limitation, for all these methods, is that they do not report on the oxygen levels in the sites where most of the oxygen-dependent interactions take place, that is in tissues. By contrast, NMR methods are not invasive and can localize metabolite signals from tissues and reveal their changes under different physiological and pathological conditions. This technique is useful in tissues that contain myoglobin (Mb) like skeletal muscle and heart for investigating conditions with low oxygen levels such as exercise and/or ischaemia. Mb is a relatively small protein acting as an oxygen store during periods of reduced blood oxygen supply and as a mobile carrier of oxygen from the sarcolemma to the mitochondria within the muscle fiber. Up to now ^{1}HMRS acquisition of cytosolic Mb resonance is the unique tool for detecting intracellular oxygenation, following the electronic structure alteration that accompanies oxygen binding. The unpaired electrons of heme Fe(II) in deoxyMb interact directly with the histidine F8 by means of its proximal histidyl NδH proton that generates a signal appearing at ~80 ppm. On the contrary, when Mb is bound to O_2, the Val E11 γCH_3, positioned on the distal side of heme experiences a ring current shift to about –2.8 ppm (DSS spectrum reference) [1]. Since the total Mb concentration is not known a priori, only a deoxyMb/oxyMb ratio would lead directly to a quantitative value of oxygen partial pressure. In light of Mb low concentration and of the interfering endogenous signals in the diamagnetic spectral region, a 1331-pulse sequence was used in the literature to suppress the water signal [1,2]. Unfortunately, the sequence suffers of a intrinsic phase modulation showing up in a baseline distortion so that it is difficult to phase the resonances making a reliable absolute quantitative determination impossible[1]. In the present study, different pulse sequences were tested aiming to reach the best compromise between an efficient water suppression and the detection of the oxy-Mb resonance. All NMR experiments were carried out on a 400 MHz Bruker Avance (^{1}H/BB BBI 5mm probe, with gradient unit on Z axis (50 Gauss/cm)). Water suppression was obtained by means of z-gradient pulses. Different pulse sequences were tested: by using the excitation sculpting method for selective excitation and water suppression, water resonance was almost completely suppressed and baseline and phase errors removed. Nevertheless oxyMb resonance was mostly undetectable even at high biological concentrations (1mM). The best compromise was reached by using the WATERGATE sequence to suppress the solvent signal, using a very short binomial inter-pulse delay, to maximize the excited spectral band. A quantitative calibration of the oxyMb concentration was assessed in the range 1- 0.05 mM.

References

[1] U. Kreutzer, D.S. Wang, T. Jue *Proc. Natl. Acad. Sci.* **89**, 4731-4733 (1992).
[2] U. Flögel, M.W. Merx , A. Gödecke, U.K.M. Decking, J. Schrader *Proc. Natl. Acad. Sci.* **98**, 735-740 (2001).

NMR INVESTIGATION OF A NEW CLASS OF π–π COMPLEXES FORMED BY SMALL ORGANIC MOLECULES IN DILUTE AQUEOUS SOLUTIONS

S. Viel,[‡,†] L. Mannina,[‡,†] C. Sanna,[†,*] P. Stano,[&] A. L. Segre[†]

[‡] *S. T. A. A. M. Department, University of Molise, Via De Sanctis, 86100 Campobasso, Italy*
[†] *Institute of Chemical Methodologies, CNR, Via Salaria km 29.3, 00016 Monterotondo Stazione, Italy*
[*] *Chemical Sciences Department, University of Rome 'Tor Vegata', Via Orazio Raimondo 18, 00173 Rome, Italy*
[&] *Biology department, University of Rome III, Viale Marconi 446, 00416 Rome, Italy*

The formation of π–π complexes through π–π staking interactions is of the utmost importance in the study of biological systems and in organic chemistry. In the present contribution, we describe a new class of π–π stacked complexes formed in dilute aqueous solutions by low molecular weight organic molecules (200 < MW < 400 Da). These molecules contain an aromatic ring and have a rather low water solubility. When they are dissolved in aqueous solutions at a concentration higher than their reported solubility, they form rather large molecular sized π–π complexes through stacking interactions of their aromatic rings. These π–π complexes were investigated by NOESY, ROESY, Diffusion-ordered NMR (DOSY) as well as Dynamic Light Scattering (DLS) experiments. In particular, DOSY and DLS experiments allowed us to estimate the effective size of the π–π complexes in solution.

NMR STUDY OF CURVATURVE OF E2 BINDING SITES

Tommaso Eliseo [‡f], Alejandro D. Nadra [†], Edoardo Trotta[‡], Valentina Morino[‡], Gonzalo de Prat-Gay [†], Maurizio Paci [‡] and Daniel O. Cicero [‡]

[‡f] *Dipartimento di Scienze e Tecnologie Chimiche, Universita' di Roma 'Tor Vergata', via della Ricerca Scientifica 1 (00133), Rome, Italy*
[†a] *Instituto de Investigaciones Bioquimicas Fundaciòn Leloir, Facultad de Ciencias Exactas y Naturales and CONICET, Universidad de Buenos Aires, Patricias Argentinas 435 (1405) Buenos Aires, Argentina;*

The mechanism of DNA recognition often requires important structural changes in the protein and/or the nucleic acid. DNA intrinsic curvature and flexibility play an important role in the thermodynamics and kinetics of binding, predisposing the molecule to favourable interactions with the protein. The E2 protein is the only transcription factor of the Papillomavirus genome and recognizes the consensus sequence ACCGNNNNCGGT [1]. The E2 protein presents a differential affinity toward the E2 binding sites on the viral genome. Although the four central bases don't make direct contacts with the protein, they affect the curvature and flexibility of this DNA tract and so have important effects on the hierarchy of occupancy of the viral promoters, leading to a fine regulation of the virus life-cycle.
Traditional solution NMR methodologies make use of NOEs and torsion angle restraints to determine the structure of DNA oligomers. Due to the elongated shape of these molecules and to the short-range nature of these restraints in nucleic acids, only the local parameters are well defined, while an high degree of uncertainty usually characterizes the global conformation. Long-range structural data can be obtained by measuring Residual Dipolar Couplings, extremely useful to fix the relative orientations of distant structural elements. One-bond RDCs are readily measurable even at natural abundance in concentrated solutions of DNA oligonucleotides. With this regard, the philamentosus phage is an ideal tool to align nucleic acids [2]
We studied structural features of the ACCGACGTCGGT and ACCGAATTCGGT E2 promoter sequences by NMR techniques. The analysis of Chemical Shifts and of the NOE's profile doesn't reveal any significant difference, being substantially affected by local factors. ^{1}H-^{13}C RDCs were accurately measured in phagus philamentosus solutions. A direct comparison of RDCs in the two DNAs was carried out to assess structural analogy or diversity between them, with particular regard to the degree of bending. The conformational differences between the two oligos can help to get insight into the role of sequence on the DNA curvature.

References

[1] R. S. Hegde. *Ann. Rev. Biophys. Biomol. Struct.* **31**, 343-360 (2002)
[2] H. Zhou, A. Vermeulen, F. M. Jucker and A. Pardi. *Biopolymers.* **52**, 168-180 (2000)

MULTILAMELLAR VESICLES FROM $C_{10}E_3/D_2O$: NMR LINE SHAPE AND PULSED GRADIENT DIFFUSION MEASUREMENTS

Felix Kleinschmidt[‡] and Claudia Schmidt[†]

[‡] Albert-Ludwigs-Universität Freiburg, Institut für Makromolekulare Chemie Stefan-Meier-Straße 31, 79104 Freiburg, Germany; [†] Universität Paderborn, Fakultät für Naturwissenschaften Warburger Straße 100, 33098 Paderbon, Germany

Lyotropic lamellar phases under shear flow can form assemblies of close packed multilamellar vesicles (MLVs) [1]. The size of these onion-like structures depends on the shear-rate and can be varied from a few microns to a tenth of a micron. In situ 2H NMR spectroscopy of lamellar phases under shear has shown that, while the normal lamellar phase shows a quadrupole doublet, the vesicles give rise to broad single peaks whose width depends on the size of the vesicles [2]. In this contribution further NMR studies aiming at a better understanding of the relationship between NMR line shapes and phase structure are reported. The system investigated is a binary mixture of $C_{10}E_3/D_2O$ containing 40 wt. % surfactant [3], from which vesicles have been prepared at shear rates between 2 and 20 s^{-1}.

The type of motion responsible for the different line shapes is the diffusion of the D_2O molecules. As long as the phase consists of extended domains of flat lamellae the average orientation of the water molecules is not influenced by their diffusion and the residual quadrupole splitting due to the motional averaging by the fast anisotropic tumbling of the molecules is not affected. However, when the layers get curved, as in the vesicles, lateral diffusion is connected with a change in orientation, leading to additional motional averaging. The line shapes of the vesicles are a superposition of the spectra from the onion shells with largely varying radii. This inhomogeneity becomes evident by the strong dependence of the solid line shapes on the pulse separation time τ. While at short τ a broad single peak is observed, the spectra et long t values (up to 30 ms) have largely reduced intensities and consist of both a central peak and a doublet. This can be explained by the slow-motion effect on NMR line shape which predicts a strong signal reduction with increasing τ only for motions on the time scale of the echo experiment. In our case, the signal from shells of intermediate size falls into the slow-motion regime of the reorientation by diffusion and therefore has decayed for long pulse separation times, leaving only the signals from the large outer shells (almost rigid limit) and the small inner shells (fast-motion limit).

In addition to an analysis of the solid line shape direct measurements of D_2O self diffusion by the pulsed gradient stimulated echo technique have been performed [4]. Experiments on magnetically aligned lamellar samples show that the diffusion coefficients parallel and perpendicular to the layers differ by almost two orders of magnitude. Measurements of the stimulated echo attenuation due to diffusion for vesicles of different sizes (prepared at different shear rates) show that the echo attenuation depends strongly on the diffusion time Δ. MLVs are thus an int.eresting but complex model system for restricted diffusion.

References

[1] O. Diat, D. Roux, and F. Nallet. Effect of shear on a lyotropic lamellar phase. J. Phys. II France, 3:1427-1452 (1993).

[2] S. Müller, C. Börschig, W. Gronski, and C. Schmidt. Shear-induced states of orientational of the lamellar phase of $C_{12}E_4$/water. Langmuir, 15:7558-7564 (1999).

[3] T. D. Le, U. Olsson, K. Mortensen, J. Zipfel, and W. Richtering. Nonionic amphiphilic bilayer structures under shear. Langmuir, 17: 999-1008 (2001).

[4] E. Stejskal and J. Tanner. Spin diffusion measurements: Spin echoes in the presence of a time-dependent field gradient. J. Chem. Phys., 42:288-292, (1965)

ORDER PHENOMENA IN POLYMER CRYSTALLIZATION

A. Maus and K. Saalwaechter[‡], R. Yerushalmi-Rozen[†] and M. Gottlieb[†]

[‡]*Institut fuer Makromolekulare Chemie, Albert-Ludwigs-Universitaet Freiburg, Stefan Meier Str. 31, D-79104 Freiburg, Germany*
[†]*Dept. of Chemical Engineering, Ben Gurion University of the Negev, Ber-Sheva 84105, Israel*

Polymer crystallization is special since not all the chains can form a crystal lattice in its classical meaning, because typical features of polymers like polydispersity, the presence of free end-groups, net-points and loops, short side chain branches etc. hinder the crystallization process. In semicrystalline polymers these geometric barriers accumulate in the amorphous region. Although investigated for over 40 years, the fundamental mechanism of polymer crystallization is not yet well understood. Classic 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 [2,3]. In DSC measurements it was found that chemical and physical crosslinks (net points and entanglements) in polydimethylsiloxane (PDMS) affect the crystallization behavior in an unexpected manner [4]. Both types of crosslinks enhance the tendency of PDMS samples to crystallize which is not explainable in terms of the classic concepts. We present 1H-NMR experiments in order to approve and quantify the results from the DSC measurements. The isothermal crystallization process was observed via analysis of the transverse magnetization relaxation function [5] measured applying a modified CPMG pulse sequence and the extend of local chain ordering was quantified by static ^{1}H multiple quantum (MQ) experiments [6]. The same methods have been applied to investigate the memory effect (self seeding) which occurs in the crystallization of many polymers. Here we investigated syndiotactic polypropylene, where the kinetic is influenced by the temperature of the melt prior to cooling to the crystallization temperature, as was found by dilatometry [7]. In our measurements we reproduced this effect and could resolve subtler differences in the crystallization kinetic. Possible causes are investigated using MQ and relaxation experiments.

References

[1] J. D. Hoffman et al., in Treatise on Solid State Chemistry, Vol. 3 (1976)
[2] G. Strobl, Eur. Phys. J. E., 3, 165-183 (2000)
[3] P. D. Olmsted et al., Phys. Rev. Let., 81, 373-376 (1998)
[4] T. Dolase et al., Eur. Phys. Let., 60 (3), 390-396 (2002)
[5] R. H : Ebengou, J. P. Cohen-Adad, Polymer, 35, 2962-2969 (1994)
[6] K. Saalwaechter et al., J. Chem. Phys., 119, 3468-3482 (2003)
[7] B. Heck, G. Strobl, Colloid Polym. Sci, 282, 511-513 (2004)

SOLUTION STRUCTURE DETERMINATION OF THE FIRST PHD FINGER OF AIRE1, THE PROTEIN INVOLVED IN AUTOIMMUNE POLYENDOCRINOPATHY ECTODERMAL DYSTROPHY.

Matthew Bottomley (1) , Bernd Simon (2), Gunter Stier (2), Gaelle Legube(2), Julya Krasotkina (3) , Asifa Akthar (2) , Michael Sattler (2), Giovanna Musco (3)

1) IRBM/Merck, Roma, 2) EMBL-Heidelberg, 3) Dulbecco Telethon Institute c/o Dibit San Raffaele Scientific Insitute

Background: Mutations in the autoimmune regulator gene AIRE1 give rise to a rare monogenic autosomal recessively inherited disease: autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, OMIM 240300, Finnish and German Consortium 1997), characterized by destructive autoimmune disorders of the endocrine organs and chronic mucocutaneous candidiasis. AIRE1 encodes a multidomain protein (545 amino acids) of unknown function expressed in immunological relevant tissues (thymus, spleen). It has transcriptional activation properties and harbors a homogenously staining region HSR, 4 LXXLL motifs, a SAND domain and two plant homology (PHD)-type zinc fingers. The PHD fingers constitute a mutational hot spot for APECED, as several pathological point mutations (V301M, C311Y, P326Q, P326L) and truncations have been reported (Pitkanen 2003). The PHD finger is a ~60 amino acid long domain found in more than 600 eukaryotic proteins, many of which are involved in the regulation of gene expression, and in the maintenance of chromatin structure (Aasland 1995). The real function of PHD domains is still elusive and controversial, as a variety of functions have been suggested, ranging from E3 ligase activity in the ubiquitination pathway to histone binding (Coscoy 2003).

Aim of the work: In order to gain more insights into the structure-function relationship of the PHD finger(s) of AIRE1 and the related role in the pathogenesis of APECED, the project had the following goals:

- determination of the solution structure of the first PHD finger of AIRE1 (AIRE1-PHD1 residues 293-354)
- structural rationale of the effects of pathological AIRE1-PHD1 missense mutations performing conformational analysis of AIRE1-PHD1 mutants.
- analysis of the possible role of AIRE1-PHD1 as E3 ligase in the ubiquitination pathway.

Results: The domain (AIRE1-PHD1) folds into a compact monomeric $\beta\beta\alpha$ motif stabilized by two Zn ions coordinated in an interleaved scheme (Figure 4a). It is characterized by the presence of a negative surface potential, thus excluding its involvement in nucleic acid binding (Figure 4b). Conversely, in agreement with the transcriptional activation properties associated to the second PHD domain of AIRE1 (Uchida et al 2004), an homology model of AIRE1-PHD2 reveals the presence of a pronounced positive electrostatic surface, indicative of possible interactions with nucleic acids (Figure 4c). In order to elucidate the structural impact of disease causing mutations we have expressed, purified and analyzed the corresponding AIRE1-PHD1 mutants by NMR spectroscopy. NMR analysis shows that the C311Y mutation, affecting the second Zn binding site, impairs metal coordination, thus destroying completely the fold.

Both mutations involving proline 326 (P326L, P336Q), located on a flexible loop of the structure, compromise stability leading to a partial unfolding of the domain. Conversely the V301M mutation, which involves a surface residue, does not affect the fold of the domain, thus suggesting a functional role for this residue (Figure 5a-d).

Based on the fold similarity with RING fingers, PHD domains have been proposed to work as E3 ligases in the ubiquitination pathway (Coscoy et al. 2003). This hypothesis is debated in the literature (Scheel et al. 2003); we have therefore investigated the E3 ligase activity of AIRE1-PHD1, and at variance to what has been previously published (Uchida et al. 2004) we could not observe any evidence of activity.

References

Aasland R, Gibson TJ, Stewart AF. The PHD finger: implications for chromatin mediated transcriptional regulation. *Trends Biochem Sci*.20, 56-9 (1995)

Coscoy L, Ganem D. PHD domains and E3 ubiquitin ligases viruse make the connection T*rends Cell Biol. 13*, 285-7 (2003)

Pitkanen J, Peterson P. Autoimmune regulator: from loss of function to autoimmunity. *Genes Immun.4*,12-21 (2003)

Scheel H, Hofmann K. No evidence for PHD fingers as ubiquitin ligases. *Trends Cell Biol. 13,*7-12 (2003)

LOCAL AND GLOBAL CONFORMATIONAL PLASTICITY OF THE HPV-16 E2 DNA BINDING DOMAIN

Alejandro D. Nadra[‡], Tommaso Eliseo[†], Diego U. Ferreiro[‡], Maurizio Paci[†], Gonzalo de Prat-Gay[‡] and Daniel O. Cicero[†]

[‡] *Instituto Leloir - Inst. de Investigaciones Bioquímicas, FCEyN-UBA and CONICET, Argentina.*
[†]*Dipartimento di Chimica, Università di Roma "La Sapienza", Rome, Italy.*

Gene transcription in papillomavirus is controlled by the E2 protein. We analyzed the structure and dynamics of the high risk strain HPV16 DNA E2 binding domain as a prototypic case. Using NOE constrains and residual dipolar couplings (RDC), we have obtained a high resolution structure and were able to address several issues related to folding and functional particularities of this unique fold. Despite being a highly stable and cooperatively folded dimer (DG ~12.0 kcal/mol), exchange measurements show that over 90% of the protons are exchanged after 12 hrs. Careful backbone dynamics measurements indicated that the flexibility of the DNA recognition helix is particularly high for its N-cap residues. The few residues that remain unexchanged after 24 hrs are I286 and V287 of b-strand 1, I330 and L333 of b-strand 3 and residues N343 and F344 of a-helix 2, and these long range interactions are critical for the overall stability of the fold. Thus, the recognition helix, far from being disordered as found in several transcriptional regulatory domains, acts as a structural hinge, providing an overall plasticity with a stable b-barrel architecture. This plasticity provides potential regulatory conformational changes in regions far from the DNA recognition interface, an essential necessary role in these and other transcriptional regulators. In addition, we have completely assigned the backbone of the HPV16 E2C domain bound to a 18mer DNA duplex containing the specific E2 recognition site. The perturbation of backbone chemical shifts is small, except for the residues in the major DNA binding helix and adjacent residues, and a in the b2-b3 loop. Backbone amide exchange rate shows a larger overall protection but most notably in the DNA binding helix. The C-terminus located oppostite to the DNA binding helix and locking the central b-barrel interface with crytical H-bonds, becomes more protected, supporting a role of these interactions in DNA bending. These results suggest that the overall plasticity of the fold is not restricted to the DNA binding interface.

EXPLOITING THE INTERMOLECULAR NUCLEAR OVERHAUSER EFFECT IN THE INVESTIGATION OF PREFERENTIAL SOLVATION PHENOMENA: TRANSIENT 1D NOE SPECTROSCOPY OF BINARY MIXTURES

Alessandro Bagno[†], Federico Rastrelli[†] and Gianfranco Scorrano[†]

[†]*Dipartimento di Scienze Chimiche, Università degli Studi di Padova via Marzolo 1, 35131 Padova (Italy)*

Many recent studies have employed two-dimensional NOESY to probe preferential solvation,[1-3] but the use of such a technique is often limited by the low sensitivity which stems from the intrinsic weakness of the observed intermolecular dipolar interactions. One-dimensional NOESY experiments based on DPFGSE (Double Pulsed Field Gradient Spin-Echo) sequences [4] can partially overcome the aforementioned limitations by delivering high sensitivity at low time costs. In typical applications of these 1D techniques, the solvent resonance is selected and a solvent-to-solute NOE enhancement is observed. Yet, this straightforward approach may suffer from subtle drawbacks when applied to the case of binary mixtures of non-deuterated solvents. In order to detect small *inter*molecular NOEs, it is desirable that both solvents provide high proton concentrations, so as to increase the rate of *inter*molecular dipole-dipole relaxation between solute and solvents (the opposite requirement holds for a typical *intra*molecular NOE experiment). In order to attain a higher efficiency, we have sought to implement a NOE experiment relying on *solute-to-solvent* magnetization transfer which, in principle, allows for simultaneous observation of NOE buildup on multiple solvent signals provided that the whole solute spectrum is effectively filtered by a uniform, tunable and band-selective excitation scheme. A pulse sequence tailored to investigate the preferential solvation of carbohydrates in binary aqueous mixtures is presented in Fig.1 [5]. This same experiment has been subsequently adapted to investigate the preferential solvation of fatty acids derivatives in binary mixtures of organic solvents. Advantages and limitations of this technique are discussed in the present communication.

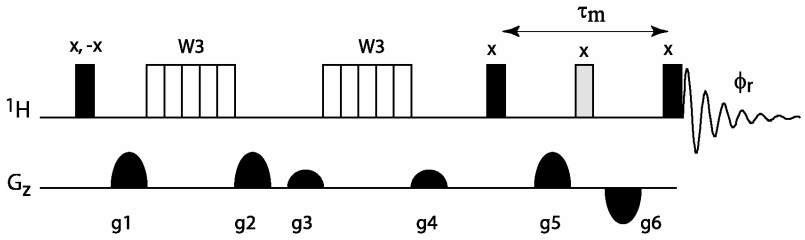


Fig. 1. W3-filtered DPFGSE NOE pulse scheme.

References

[1] A. Bagno, M. Campulla, M. Pirana, G. Scorrano, S. Stiz,, *Chem. Eur. J*. 5 (**1999**) 1291-1300.

[2] A. Bagno, G. Scorrano, S. Stiz, *J. Am. Chem. Soc*. 119 (**1997**) 2299-2300.

[3] M. Fioroni, M. D. Diaz, K. Burger, S. Berger, , *J. Am. Chem. Soc.* 124 (**2002**) 7737-7744.

[4] K. Stott, J. Stonehouse, J. Keeler, T-L Hwang, A. J. Shaka, *J. Am. Chem. Soc.* 117 (**1995**), 4199-4200.

[5] A. Bagno, F. Rastrelli and G. Scorrano, *J. Magn. Reson.* (**2004**) *167*, 31-35

^{2}H-NMR INVESTIGATIONS ON THE BIAXIALITY OF LIQUID CRYSTALLINE SIDE-CHAIN POLYMERS

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; Germany

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. Until recently 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. Samulski and coworkers have shown phase biaxiality in low molecular weight liquid crystals made up of banana shaped mesogens with a transverse electric dipole moment [3].
We report on deuterium NMR experiments conducted on liquid crystalline side chain polymers, in which the rotation about the molecular long axis is hindered by means of a lateral attachment of the mesogens to the polymer backbone [4]. 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. In these systems we have observed phase biaxiality for end-on polymers with short spacers. Phase biaxiality is investigated by measuring the quadrupole splitting of a spin probe in a macroscopically ordered sample oriented at different angles with respect to the magnetic field applying, a rapid sample flip technique indroduced by Frydman [5]. Different one- and two-dimensional echo experiments as well as computer simulations were performed in order to verify the accuracy of the measured biaxiality parameter, and investigate slow dynamics and distribution effects of the director.

References

[1] M. J. Freiser (1970) Phys. Rev. Lett. 24, 1041
[2] L. J. Yu, A. Saupe (1980) Phys. Rev. Lett. 45, 1000
[3] L. A. Madsen et al. (2004) Phys. Rev. Lett. 92, 145505
[4] K. Severing and K. Saalwächter (2004) Phys. Rev. Lett. 92, 125501
[5] Grinshtein et al. (2001) J. Chem. Phys 114, 5415

XXXIV National Congress on Magnetic Resonance

AUTHOR INDEX

XXXIV National Congress on Magnetic Resonance

LIST OF PARTICIPANTS

Massimo Adobati
Sapio Industrie S.r.l.
Via Silvio Pellico 48
20052 Monza
Italy
Tel: 0039 039 83981
Fax: 0039 02 95743571
E-mail: m.adobati@sapio.it

Roberto Anedda
Dipartimento di Scienze Chimiche-
Università di Cagliari
09042 Monserrato (CA)
Italy
Tel: 0706754354
Fax: 0706754388
E-mail: aneddar@unica.it

Federica Balzano
Dipartimento di Chimica e Chimica
Industriale - Università di Pisa
via Risorgimento 35
56126 Pisa
Italy
Tel: 0502219232
Fax: 0502219260
E-mail: fb@dcci.unipi.it

Lucia Banci
Centro Risonanze Magnetiche
Università di Firenze
Via Luigi Sacconi 6
50019 Sesto Fiorentino (Fi)
Italy
Tel : +39 055 4574 263
Fax: +39 055 4574 253
E-mail banci@cerm.unifi.it

Gaetano Barbato
IRBM spa
Via Pontina km 30.600
00040 Roma
Italy
Tel: +39.06.91093403
Fax: +39.06.91093225
E-mail: gaetano_barbato@merck.com

Elena Barrese
ENEA
SS 106 Jonica KM 419,500
75026 Rotondella (Mt)
Italy
Tel: 0835.974262
Fax: 0835.974724
E-mail: elena.barrese@trisaia.enea.it

Simona Belfiore
Università di Torino
Via P. Giuria, 7
10127 Torino
Italy
Tel: +390116707946
Fax:
E-mail: simona.belfiore@unito.it

Massimo Bellanda
Department of Chemical Sciences,
Università di Padova
Via Marzolo 1
35131 Padova
Italy
Tel: +39 049 827 5742
Fax: +39 049 827 5239
E-mail: massimo.bellanda@unipd.it

Francesca Benevelli
Bruker Biospin S.r.l.
Via Giovanni Pascoli 70/3
20133 Milano
Italy
Tel: 39 02-70636370
Fax: 39 02 23 61 294
E-mail:
Francesca.benevelli@bruker.it

Tiziana Beringhelli
Dip. Chimica Inorganica
Metallorganica e Analitica, Univ.
degli Studi Milano
Via Venezian 21
20133 Milano
Italy
Tel: ++39 02 503 14350
Fax: ++39 02 503 14405
E-mail: tiziana.beringhelli@unimi.it

Hanne Christine Bertram
Danish Institute of Agricultural
Sciences, Dept. Food Science
P.O. Box 50 8830
Dk-Tjele
Denmark
Tel: +45 89 99 15 06
Fax:+45 89 99 15 64
E-mail: hannec.bertram@agrsci.dk

Roberto Biancardi
Nerviano Medical Science Srl
Viale Pasteur, 10
20014 Nerviano
Italy
Tel: 0331581440
Fax: 0331581057
E-mail:
roberto.biancardi@nervianoms.com

Cristiano Bianchetti
Dipartimento di Chimica Università
degli Studi di Roma “La Sapienza"
Piazzale Aldo Moro 5
00185 Roma
Italy
Tel: 06 49913728
Fax:
E-mail:
cristiano.bianchetti@uniroma1.it

Giovanni Bizzarro
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
E-mail: gb@bruker.it

Marco Boccalini
Organic Chemistry Dept.
Università di Firenze
Via della Lastruccia, 13
50019 Sesto F.no
Italy
Tel: +39 055 457 3478
Fax: +39 055 457 3568
E-mail: m.boccalini@moda.unifi.it

Andrea Boninsegni
RIVOIRA S.p.A.
Via Tiburtina 271
00162 Roma
Italy
Phone: 06-44702113
Fax: 06-4940985
E-mail:
andrea_boninsegni@praxair.com

Donatella Capitani
Istituto di Metodologie Chimiche, CNR
Via Salaria Km 29,300
00016 Montelibretti
Italy
Tel: 0690672700
Fax: 0690672477
E-mail: capitani@imc.cnr.it

Lorena Casciani
Dip. Chimica, Università "La Sapienza" di Roma
Piazz.le Aldo Moro, 5
00185 - Roma
Italy
Tel: 0649913058
Fax:
E-mail: lorena.casciani@uniroma1.it

Cecilia Castro
Dip. Chimica Università "La Sapienza" di Roma
Piazz.le Aldo Moro, 5
00185 Roma
Italy
Tel: 0649913058
Fax:
E-mail: cecilia.castro@uniroma1.it

Mariano Casu
Dipartimento di Scienze Chimiche
Università di Cagliari
Cittadella Universitaria di Monserrato
09042 - Monserrato (Ca)
Italy
Tel: 070 675 4416
Fax: 070 675 4388
E-mail: mcasu@unica.it
mariano@mvcch3.unica.it

Giovanni Cerioni
Dept. Farmaco Chimico Tecnologico
Via Ospedale, 72
09124 Cagliari
Italy
Tel: +390706758550
Fax: +390706758553
E-mail: cerioni@unica.it

Alberto Cerri
NiKem Research srl
Via Zambeletti, 25
20021 Baranzate di Bollate Milano
Italy
Tel: 02 35 694 7471
Fax: 02 35 694 7606
E-mail:
alberto.cerri@nikemresearch.com

Flaminia Cesare Marincola
Dipartimento di Scienze Chimiche-
Università di Cagliari
Cittadella Universitaria di Monserrato
09042 Monserrato (Ca)
Italy
Tel: + 39 070 675 4356
Fax: + 39 070 675 4388
E-mail: flaminia@mvcch3.unica.it

Matilde Chessa
Porto Conte Ricerche
Loc.Tramariglio sp.55 km 8.4
07041 Alghero (SS)
Italy
Tel: 39 079 998646
Fax: 079 998648
E-mail: m.chessa@iatcapa.ss.cnr.it

Stefano Chimichi
Organic Chemistry Dept.
Università di Firenze
Via della Lastruccia, 13
50019 Sesto Fiorentino
Italy
Tel: +39 055 457 3537
Fax: +39 055 457 3568
E-mail: s.chimichi@moda.unifi.it

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

Stefano Ciurli
Dept. Agro-Environmental Science and Technology,
Università di Bologna
Viale Giuseppe Fanin 40
40127 Bologna
Italy
Tel: +39-051-209-6204
Fax: +39-051-209-6203
E-mail: stefano.ciurli@unibo.it

Arianna Ciutti
Dip. Biologia Molecolare Università di Siena
Via Fiorentina 1
53100 Siena
Italy
Tel: 0577/234911
Fax: 577234903
E-mail: ciutti@unisi.it

Carmine Coluccini
Dipartimento di Chimica Organica
Università di Bologna
Viale del Risorgimento 4
40136 Bologna
Italy
Tel. 0512093633
Fax 0512093654
E-mail: carmine@ms.fci.unibo.it

Angiolina Comotti
Università di Milano-Bicocca
Via Cicognara 7
Milano
Italy
Tel: 02-64485140
Fax:
E-mail: angiolina.comotti@unimib.it

Roberto Consonni
ISMAC, CNR
Via Bassini 15
20133 Milano
Italy
Tel: 02-23699578
Tel: 02-23699620
E-mail:
roberto.consonni@ismac.cnr.it

Gian Marco Contessa
Università di Tor Vergata a Roma
Via della Ricerca Scientifica, 1
00133 Rome
Italy
Tel: +390672594410
Fax: +390672594328
E-mail: contessa@uniroma2.it

Marcella Corda
Scienze Applicate ai Biosistemi
Università di Cagliari
09046 Monserrato
Italy
Tel: +390706754548
Fax: +390706754523
E-mail: corda@unica.it

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

Mauro Andrea Cremonini
Dept. of Food Science
P.zza Goidanich 60
47023 Cesena
Italy
Tel: +39 0547 338106
Fax: +39 0547 338106
E-mail: mauro.cremonini@unibo.it

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

Cinzia Cuomo
Dipartimento di chimica Università di Salerno
via S. Allende
84081 Baronissi
Italy
Tel: 089/965254
Fax: 089695296
E-mail: c.cuomo@unisa.it

Claudio Dalvit
Nerviano Medical Science
Viale Pasteur, 10
20014 Nerviano (MI)
Italy
Tel:
Fax:
E-mail:
claudio.dalvit@nervianoms.com

Nicola D'Amelio
Department of Chemistry
Università di Siena
Via Aldo Moro 2
53100 Siena
Italy
Tel: 0577234511
Fax: 0577234233
E-mail: damelio@unisi.it

Muriel Delepierre
Unité de RMN de Biomolécules
Institut Pasteur
28 rue du Dr Roux,
75724 Paris Cedex 15
France
Tel: 33(0)1 45 68 88 71
Fax: 33 (0)1 45 68 89 29
murield@pasteur.fr

Maurizio Delfini
Dipartimento di Chimica
Università degli Studi di Roma "La Sapienza"
Piazzale Aldo Moro 5
00185 - Roma
Italy
Tel: 0649913124
E-mail: maurizio.delfini@uniroma1.it

Giuseppina De Luca
Dipartimento di Chimica, Università della Calabria
Via Pietro Bucci, Arcavacata di Rende
87036 Cosenza
Italy
Tel: 0984-493323
Fax: 0984-492044
E-mail: g.deluca@unical.it

Roberta Di Clemente
D.pt of Chemistry, University "La Sapienza"
P.le Aldo Moro, 5
00185 Rome
Italy
Tel: +39064455278
Fax: +39064455278
E-mail:
Roberta_DiClemente1@hotmail.com

Maria Enrica Di Cocco
Dipartimento di Chimica Università degli Studi di Roma "La Sapienza"
Piazzale Aldo Moro 5
00185 Roma
Italy
Tel: 0649913728
Fax:
E-mail:
mariaenrica.dicocco@uniroma1.it

Marco D'Imperio
Università degli studi del Molise
Montagano (CB)
Vico Nottole 7
86023 Montagano (CB)
Italy
Tel:
Fax:
E-mail: marcodimpe@hotmail.com

Nicola Di Pasquale
RIVOIRA S.p.A.
Via Tiburtina 271
00162 Roma
Italy
Tel: 06-44702113
Fax: 06-4940985
E-mail:
nicola_dipasquale@praxair.com

Anna Di Rosa
Sigma Aldrich
Via Gallarate 154
20151 Milano
Italy
Tel: 39 02 33417286
Fax: 0238010737
E-mail: adirosa@europe.sial.com

Daniela Donghi
Dip. CIMA
Università degli Studi di Milano
Via Venezian 21
20133 Milano
Italy
Tel: 00390250314352
Fax: 00390250314405
E-mail: daniela.donghi@unimi.it

Tommaso Eliseo
Dipartimento di Scienze e
tecnologie Chimiche
Via della Ricerca Scientifica 1
00133 Roma
Italy
Tel: 0672594410
Fax:
E-mail:
tommaso.eliseo@uniroma1.it

Lyndon Emsley
Laboratoire de Chimie,
Ecole Normale Superieure de Lyon,
46 Allée d'Italie
69364 Lyon cedex 07
France
Tel: +33 4 72 72 84 86
Fax: +33 4 72 72 84 83
E-mail: Lyndon.Emsley@ens-lyon.fr

Monica Epis
Sapio Industrie S.r.l.
Via Senatore Simonetta 27
20040 Caponago (MI)
Italy
Tel: 0039 02 95705611
Fax: 0039 02 95743571
E-mail: m.epis@sapio.it

Benedetta Era
Dip.Scienze Applicate ai Biosistemi,
Università di Cagliari
Cittadella Universitaria
09042 Monserrato
Italy
Tel: 070/6754506
Fax: 070/6754523
E-mail:

Giovanna Esposito
Università di Torino
Via P.Giuria 7
10127 Torino
Italy
Tel: 011/6707946
Fax:
E-mail: giovanna.esposito@unito.it

Antonella Fais
Dip.Scienze Applicate ai Biosistemi,
Università di Cagliari
09042 Monserrato
Italy
Tel: 070/6754506
Fax: 070/6754523
E-mail: fais@unica.it

Mauro Fasano
Dip. Di Biologia Strutturale e
Funzionale, Università dell'Insubria
Via Alberto da Giussano 12
21052 Busto Arsizio (Va)
Italy
Tel: +39 0331 339450
Fax: +39 0331 339459
E-mail: mauro.fasano@uninsubria.it

Roberto Fattorusso
Department of Environmental Sciences
Via Vivaldi 43
81100 Caserta
Italy
Tel: +39 0823 274637
Fax: +39 0823 274637
E-mail: roberto.fattorusso@unina2.it

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

Nereo Fiori
Dept Chemical Sciences
Via Marzolo 1
35131 Padova
Italy
Tel: 0498275324
Fax:
E-mail: nereofiori@yahoo.it

Costantino Floris
Dipartimento di Scienze Chimiche -
Università di Cagliari
09042 Monserrato (Ca)
Italy
Tel: 070/6754392
Fax: 070/6754388
E-mail: cfloris@unica.it

Rasmus Fogh
University of Cambridge
80 Tennis Court Road
CB2 1GA Cambridge
UK
Tel: 0044 (0)1223 766022
E-mail: r.h.fogh@bioc.cam.ac.uk

Paolo Formaglio
University of Ferrara
Via L.Borsari 46
44100 Ferrara
Italy
Tel: 0532291123
Fax: 0532240907
E-mail: frp@unife.it

Lorella Franzoni
Dept. Experimental Chemistry - Sect. of Chem. and Struct. Biochem.
Università di Parma
Via Volturno 39
43100 Parma
Italy
Tel: 0521-903823
Fax: 0521-903802
E-mail: lfranz@unipr.it

Nicola Gaggelli
Department of Chemistry
Università di Siena
Via Aldo Moro 2
53100 Siena
Italy
Tel: 0577234242
Fax: 0577234233
E-mail: gaggellin@unisi.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
E-mail: mari_gallo@yahoo.com.ar

Marco Geppi
Dipartimento di Chimica e Chimica Industriale
Via Risorgimento 35
56126 Pisa
Italy
Tel: +39-0502219289
Fax: +39-0502219260
E-mail: mg@dcci.unipi.it

Clelia Giannini
Dipartimento di Chimica Organica e Industriale
Via Venezian 21
20133 Milano
Italy
Tel: 0250314148
Fax: 0250314139
E-mail: clelia.giannini@unimi.it

Mirko Gombia
Dept. DICMA - University of Bologna
Viale Risorgimento 2
40136 Bologna
Italy
Tel: +390512090459
Fax: +390512090457
E-mail:
mirko.gombia@mail.ing.unibo.it

Luigi Gomez Paloma
Dipartimento di Scienze Farmaceutiche
Università di Salerno
Via Ponte Don Melillo
84084 Fisciano (SA)
Italy
Tel: +39-089-962811
Fax: +39-089-962828
E-mail: gomez@unisa.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

Siegfried Hafner
Varian Deutschland GmbH
Alsfelderstrasse 6
64289 Darmstadt
Germany
Tel: +49 6151 70 31 47
Fax: + 49 6151 70 32 00
E-mail:
siegfried.hafner@varianinc.com

Bertil Halle
Department of Biophysical Chemistry
Lund University
Box 124 SE-22100 LUND
Sweden
Tel: +46 - 46 222 9516
Fax: +46 - 46 222 4543
E-mail: bertil.halle@bpc.lu.se

Federica Innocenti
IRBM P. Angeletti
Via Pontina Km 30,600
00040 Pomezia (Rm)
Italy
Tel: 0691093457
Fax:
E-mail:
federica_innocenti@merck.com

Francesco Isaia
Dip. Chimica Inorganica ed Analitica
Cittadella Universitaria di Monserrato
09042 Monserrato (Ca)
Italy
Tel: 070 6754496
Fax: 070 6754456
E-mail: isaia@unica.it

Carla Isernia
Seconda Università degli Studi di Napoli
Via Vivaldi 43
81100 Caserta
Italy
Tel: 0823/274636
Fax: 0823/274605
E-mail: carla.isernia@unina2.it

Raffaele Lamanna
ENEA CR Trisaia
SS 106 Jonica Km 419.5
75026 Rotondella (Mt)
Italy
Tel: 0835974262
Fax: 0835974724
E-mail:
raffaele.lamanna@trisaia.enea.it

Emanuela Locci
Dipartimento di Scienze Chimiche,
Università di Cagliari
Cittadella Universitaria di
Monserrato, S.S. 554 Bivio per
Sestu
09042 Monserrato (CA)
Italy
Tel: +39 070 675 4356
Fax: +39 070 675 4388
E-mail: manu@mvcch3.unica.it

Beatriz Jiménez
Center of Magnetic Resonance
(CERM) Università di Firenze
Via Luigi Sacconi 6
50019 Sesto Fiorentino (Fi)
Italy
Tel: +39 055 457 4237
Fax: 00 39 055 457 4271
E-mail: jimenez@cerm.unifi.it

Felix Kleinschmidt
Institute of Macromolecular
Chemistry, University of Freiburg
Germany
Stefan-Meier-Strasse 31
79104 Freiburg
Germany
Tel: +761-203-6314
E-mail:
Felix.Kleinschmidt@makro.uni-
freiburg.de

Rainer Kümmerle
NMR Applications Scientis,
BRUKER BIOSPIN AG, NMR
Division,
Industriestrasse 26,
CH-8117 Fällanden
Switzerland
Tel:
Fax:
E-mail

Maria Antonietta Macrì
Dipartimento di Medicina Sperimentale
e Patologia
Università “La Sapienza”
P.le Aldo Moro 5
00185 Roma
Italy
Tel: 06 49913484
Fax:
E-mail:

Giuseppe Maddinelli
Physical Chemistry Enitecnologie
Spa
Via Maritano 26
20097 S. Donato M.se
Italy
Tel: +39.02.520.56559
Fax: +39.520.3647
E-mail:
gmaddinelli@enitecnologie.eni.it

Daniela Maggioni
Dip. di Chimica Inorganica,
Metallorganica e Analitica
Università degli Studi di Milano
Via Venezian, 21
20133 Milano
Italy
Tel: 02 503 14352
Fax: 02 503 14405
E-mail: daniela.maggioni@unimi.it

Marco Maioli
Dipartimento di Chimica
Università "La Sapienza"
Piazzale Aldo Moro
00194 Rome
Italy
Tel: 0649913124
Fax:
E-mail: m.maioli@inwind.it

Silvia Malaguti
Dip C.I.M.A.
Università di Milano
Via Venezian 21
20133 Milano
Italy
Tel: 02 50314352
Fax: 02 50314405
E-mail: Silvia.Malaguti@unimi.it

Gaetano Malgieri
Seconda Università degli Studi di
Napoli
Via Vivaldi 43
81100 Caserta
Italy
Tel: 0823/274636
Fax: 0823/274605
E-mail: gaetano.malgieri@unina2.it

Stefano Mammi
Department of Chemical Sciences
Università di Padova
Via Marzolo 1
35131 Padova
Italy
Tel: 39 049 827 5293
Fax: 39 049 827 5239
E-mail: stefano.mammi@unipd.it

Cesare Manetti
Dip. di Chimica, Università "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
E-mail: info@spectra2000.it

Luisa Mannina
Università del Molise
Via De Sanctis
86100 Campobasso
Italy
Tel: 00390690672700
Fax: 00390690672476
E-mail: mannina@unimol.it

Suraj Manrao
Spectra Stable Isotope Group
8109 A, Guilford Road
21046 Columbia, MD
USA
Tel: 301-776-9029
Fax: 410-740-1328
E-mail: surajmanra@aol.com

Bruno Maraviglia
Department of Physics - Università di Roma "La Sapienza"
P.le Aldo Moro, 5
00185 Rome
Italy
Tel: +39 06 4454859
Fax: +39 06 49913484
E-mail:
bruno.maraviglia@roma1.infn.it

Andreas Maus
Institut fuer Makromolekulare Chemie, University Freiburg
Stefan Meier Strasse 31
79104 Freiburg
Germany
Tel: +49-761-203-6314
Fax: +49-761-203-6306
maus@makro.uni-freiburg.de

Beat H. Meier
Physical Chemistry
ETH Hönggerberg
CH-8093 Zürich
Switzerland
Tel:
Fax:
E-mail: beme@ethz.ch

Sonia Mele
Department of Biology
Via della Ricerca Scientifica
00133 Roma
Italy
Tel:
Fax: +39 062023500
E-mail: soniamele@hotmail.com

Sonia Melino
Università di Roma "Tor Vergata"
Via della Ricerca 1
00133 Rome
Italy
Tel: +390672594449
Fax: 00390672594328
E-mail: melinos@uniroma2.it

Ileana Menegazzo
Università degli studi di Padova
Via Marzolo, 1
35131 - Padova
Italy
Tel: 0498275260
Fax: 0498275239
E-mail: ileana.menegazzo@unipd.it

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

Maria Lucia Miglietta
ENEA C.R. Trisaia
SS 106 Jonica Km 419.500
75026 Rotondella (MT)
Italy
Tel:
Fax:
E-mail: miglietta@trisaia.enea.it

Anna Minoia
Bruker Biospin S.r.l.
Via Giovanni Pascoli 70/3
20133 Milano
Italy
Tel: 39 02 70636370
Fax:39 022361294
E-mail: anna.minoja@bruker.it

Giorgio Modesti
Institut für Makromol Chemie
Stefan-Meier-str. 31
79104 Freiburg i. B.
Germany
Tel: +49(761)203 6312
Fax: +49(761)203 6306
E-mail:
giorgio.modesti@makro.uni-freiburg.de

Henriette Molinari
Università di Verona
Strada Le Grazie 15
37014 Verona
Italy
Phone: 329 2126125
Fax: 02 23699 620
E-mail: molinari@sci.univr.it

Alejandro Nadra
Instituto de Investigaciones Bioquimicas - Instituto Leloir
Patricias Argentinas 435
C1405BWE Ciudad de Buenos Aires
Argentina
Tel: 00 54 1 48634011
Fax:
E-mail: anadra@leloir.org.ar

Giovanni Natile
Dipartimento Farmaco-Chimico,
Università di Bari,
Via E. Orabona 4
70125 Bari
Italy
Tel: 39 080 544 27 74
Fax: 39 080 544 22 30
E-mail
natile@farmchim.uniba.it

Neri Niccolai
Dipartimento di Biologia Molecolare
Via Fiorentina 1
53100 Siena
Italy
Tel:
Fax:
E-mail: niccolai@unisi.it

Patrizia Oliva
Dipartimento di Chimica
Università di Salerno
Via S. Allende
84081 Baronissi (Salerno)
Italy
Tel: 089/965254
Fax: 089/965296
E-mail: poliva@unisa.it

Maurizio Paci
Dept.ChemSci&Techn.University Rome Tor Vergata
Via Ricerca Scientifica 1
00133 Roma
Italy
Tel: 06 7259 4446
Fax: 06 7259 4328
E-mail: paci@uniroma2.it

Federica Pancotti
Dept. of Chemistry, University "La Sapienza"
P.le A. Moro, 5
00185 Rome
Italy
Tel: +3964455278
Fax: +3964455278
E-mail: federica_pancotti@hotmail.com

Massimiliano Peana
Dip. Chimica Università di Sassari
Via Vienna 2
07100 Sassari
Italy
Tel:
Fax:
E-mail: peana@cerm.unifi.it

Matteo Pennestri
Scienze e Tecnologie Chimiche
Universita di Roma 'Tor Vergata'
Via della Ricerca Scientifica
00133 Roma
Italy
Tel: 0672594410
Fax: 0672594328
E-mail: matteo.pennestri@tiscali.it

Ornella Perini
GlaxoSmithKline
Via Fleming 4
37135 Verona
Italy
Tel: 0459219965
E-mail: ornella.2.perini@gsk.com

Vanni Piccinotti
NMR Service
Via delle Gore, 39A
50141 Firenze
Italy
Tel: +39 055 434841
Fax: +39 055 434841
email vpnmr@facile.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
Dept. of Chemistry
Via Cintia (Monte S. Angelo)
I-80126 Naples
Italy
Tel: +39-081-674406
Fax: +39-081-674409
E-mail: delia.picone@unina.it

Luca Pinato
Dept. of Chemical Science
University of Padova
Via F. Marzolo 1
35131 Padova
Italy
Tel: 049/8275324
Fax:
E-mail: luca.pinato@unipd.it

Donatella Potenza
Dip. Chimica Organica e Industriale-Univ. degli Studi di Milano
Via Venezian, 21
20133 Milano
Italy
Tel: 02-50314085
Fax: 02-50314072
E-mail: donatella.potenza@unimi.it

Federica Presciutti
Dept. di Chimica Università di Perugia
Via Elce di sotto n.8
06123 Perugia
Italy
Tel: +390755855569
Fax: +390755855606
E-mail: federica@thch.unipg.it

Luca Raiola
Seconda Università degli Studi di Napoli Facoltà di Scienze Ambientali
Via Vivaldi
81100 Caserta
Italy
Tel:
Fax:
E-mail: luca.raiola@unina2.it

Federico Rastrelli
Università degli Studi di Padova
Dip. Scienze Chimiche
Via Marzolo 1
35131 Padova
Italy
Tel: +39 049 827 5273
Fax: +39 049 827 5239
E-mail: federico.rastrelli@unipd.it

Teresa Recca
Università degli Studi - Dip. Chimica Organica e Industriale
Via Venezian, 21
20133 Milano
Italy
Tel: 02-5031.4069
Fax:
E-mail: teresa.recca@unimi.it

Enrico Rossi
C.N.R. Istituto di Metodologie Chimiche
Via Salaria Km. 29,300
00016 Monterotondo Stazione (RM)
Italy
Tel: 0690672700
Fax: 0690672477
E-mail: emrico.rossi@imc.cnr.it

Fernando Sancassan
Dipartimento di Chimica e Chimica Industriale, Università di Genova
Via Dodecaneso 31
16146 Genova
Italy
Tel: +39 010 353 6120
Fax: +39 010 353 6118
E-mail: 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
E-mail: paolo.santino@varianinc.com

Alessia Santoprete
Dip. di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata"
Via della Ricerca Scientifica 1
00133 Roma
Italy
Tel:
E-mail:

Carla Scarabino
Dip. Chimica Università di Salerno
Via Allende
84081 Baronissi (SA)
Italy
Tel: 089/965254
Fax: 89965296
E-mail: scarab.carla@libero.it

Michele Scian
Università degli Studi di Padova
Dipartimento di Scienze Chimiche
Via Marzolo1
35131 Padova
Italy
Tel: 0039 049 8275324
Fax: 0039 049 8275239
E-mail: michele.scian@unipd.it

Anca Serbescu
Institut fuer Makromolekulare Chemie / Universitaet Freiburg
79104 Freiburg
Germany
Tel: +49-761-203 6254
Fax: +49-761-203 6306
E-mail: anca.serbescu@makro.uni-freiburg.de

Marco Sette
Dept. of Chemical Sciences and Technology, Università Tor Vergata
Via della Ricerca Scientifica 1
00133 Rome
Italy
Tel: +39-0672594424
Fax: +39-0672594328
E-mail: sette@uniroma2.it

Kirsten Severing
Institut fuer Makromolekulare Chemie, University Freiburg
Stefan Meier Strasse 31
79104
Freiburg
Germany
Tel: 0049-761-203 6312
Fax: 0049-761-203 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
E-mail :
Roberto.Simonutti@mater.unimib.it

Stanislav Sykora
Extra Byte
Via Raffaello Sanzio 22C
20022 Castano Primo (Mi)
Italy
Tel: 0331-880281
Fax:
E-mail: sykora@aliceposta.it

Anatoli Sobolev
IMC, CNR
c.p. 10, Monterotondo Stazione
00016 Roma
Italy
Tel: +39 6090672385
Fax: +39 6090672477
E-mail: anatoli.sobolev@imc.cnr.it

Piero Sozzani
Università di Milano Bicocca
Via Cicognara 7
Milano
Italy
Tel: 02-64485124
Fax: -
E-mail: piero.sozzani@unimib.it

Silvia Spera
Ist. G. Donegani
Via Fauser 4
28100 Novara
Italy
Tel: 0321-447219
Fax: 0321-447862
E-mail:
silvia.spera@polimerieuropa.com

Ottavia Spiga
Dip. Biologia Molecolare Università di Siena
Via Fiorentina 1
53100 Siena
Italy
Tel: 0577/234911
Fax: 0577234903
E-mail: spiga@unisi.it

Alberto Spisni
Università di Parma
Via Volturno, 39
43100 Parma
Italy
Tel: +39 0521 033807
Fax: +39 0521 033802
E-mail: aspin@unipr.it

Gábor Szalontai
University of Veszprém, NMR laboratory
H-8200 Veszprém Pf.158.
Veszprém
Hungary
Tel: 36 88 422 022 / 4356
Fax:
E-mail:
Gabor.Szalontai@sparc4.mars.vein.hu

Marco Tatò
Nerviano Medical Science Srl
Viale Pasteur 10
20014 Nerviano (Milano)
Italy
Tel: +39 0331 581088
Fax: +39 0331 581360
E-mail: marco.tato@nervianoms.com

Patrizia Tedesco
Dept. of Physics "E.R.Caianiello", University of Salerno
Via S. Allende
84081 Baronissi
Italy
Tel: 089 965255
Fax: 089 965275
E-mail: patted@sa.infn.it

Enzo Terreno
Dipartimento di Chimica
I.F.M. Università di Torino
Via P. Giuria 7
10125 Torino
Italy
Tel:
Fax:
E-mail: enzo.terreno@unito.it

Stefano Tiziani
Department of Food Science & Technology
The Ohio State University
110 Parker Food Science & Technology Bldg.
2015 Fyffe Court
Columbus, Ohio 43210 (USA)
Tel: 614-247-7686
Fax: 614-292-0218
email: tiziani.1@osu.edu

Renato Toffanin
Istituto di Ricerca PROTOS
C.P. 972
34100 Trieste
Italy
Tel: +39 040 3994965
Fax: +39 02 700400633
E-mail: toffanin@protos-institute.org

Alberta Tomassini
Dep.t of Chemistry,
Università "La Sapienza"
P.le Aldo Moro, 5
00185 Rome
Italy
Tel: +39064455278
Fax: +39064455278
E-mail: tomassinialb@hotmail.com

Gianluca Uccheddu
Università di Cagliari
Via Ospedale, 72
I-09128 Cagliari
Italy
Tel: 0706758581
Fax:
E-mail:
gianlucauccheddu@hotmail.com

Raffaella Ugolini
Dipartimento Scientifico e Tecnologico, Università di Verona
Strada Le Grazie 15
37134 Verona
Italy
Tel: 00390458027906
Fax: 00390458027929
E-mail: ugolinir@sci.univr.it

Maria Cristina Valerio
Dip. di Chimica Università
“La Sapienza di Roma"
P.zzale Aldo Moro
00185 Roma
Italy
Tel: 0039-06-4455278
Fax: 0039-06-4455278
E-mail: mvalerio@caspur.it

Luca Venturi
Università di Bologna,
Department of Food Science
Via Ravennate 1020
47023 Cesena
Italy
Tel:
Fax:
E-mail: lucave78@hotmail.com

Aldrik H. Velders
Supramolecular Chemistry & Technology
Faculty of Science and Technology
University of Twente (UT)
P.O.Box 217
7500 AE Enschede, The Nederlands
Tel: +31 (0)53-4892988 (0)53-4892980 (secr)
Fax: +31 (0)53-4894645
E-mail: a.h.velders@utwente.nl

Carlo Alberto Veracini
Dipartimento di Chimica e Chimica Industriale
Via Risorgimento 35
56126 Pisa
Italy
Tel: +502219227
Fax: +502219221
E-mail; verax@dcci.unipi.it

Alessandra Vezzoli
Istituto di Bioimmagini e Fisiologia Molecolare - C.N.R.
Via Fratelli Cervi 93
20090 Segrate (MI)
Italy
Tel: 02 50330417
Fax: 02 50330414
E-mail:
Alessandra.Vezzoli@ibfm.cnr.it

Stéphane Viel
National Research Council (CNR),
Institute of Chemical Methodologies
Via Salaria km 29.300
00016 Monterotondo Staz.(ROME)
Italy
Tel: 06-90672385
Fax: 06-90672477
E-mail: viel@imc.cnr.it

Wolfgang von Philipsborn
Institute of Organic Chemistry
Winterthurerstrasse 190
University of Zurich
CH-8057 Zurich,
Switzerland
Tel: +1-635 4260
Fax: +1-635 6836
e-mail: egysi@oci.unizh.ch

Lucia Zetta
CNR
Via Bassini 15
20133 Milano
Italy
Tel: 3393330871
Fax: 02 23699620
E-mail: lucia.zetta@ismac.cnr.it

Maria Antonietta Zoroddu
Dip. Chimica Università di Sassari
Via Vienna 2
07100 Sassari
Italy
Tel:
Fax:
E-mail: zoroddu@uniss.it