TALLER FRONTERAS EN RESONANCIA MAGNÉTICA: DE LOS MATERIALES A LOS SISTEMAS BIOLÓGICOS

NOVEMBER 21-22 2013

ROSARIO, SANTA FE

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ACKNOWLEDGEMENTS

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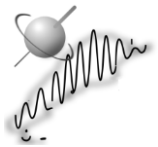
General Index



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Taller Fronteras en Resonancia Magnética, de los Materiales a los Sistemas Biológicos

Sede
Auditorio IRICE, CCT-Rosario
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Programa

Jueves 21 de noviembre

08:00 - 09:00	Recepción e inscripción
09:00 - 09:15	Apertura del Taller
09:15 - 11:15	Elaine Holmes, Imperial College (UK) Mario O. Salazar, Fac. Cs. Bioq. y Farm, Rosario
11:15 - 11:30	Coffee break
11:30 - 12:30	Comunicaciones orales estudiantes
12:30 - 14:00	Almuerzo
14:00 - 15:30	Pierre Florian, Orleans (France) Rodolfo Acosta, FAMAF, Córdoba
15:30 - 15:45	Coffee break
15:45 - 16:45	Comunicaciones orales estudiantes

Viernes 22 de noviembre

09:00 - 10:30	Jean Pierre Simorre, IBS-Grenoble (Francia) Carlos Bertoncini, IBR, Rosario
10:30 - 11:15	Coffee break
11:15 - 12:45	Roberto Gil, Carnegie Mellon (US) Juan Manuel Lázaro Martínez, Córdoba
12:45 - 14:00	Almuerzo
14:00 - 15:30	Leandro Tabares, CEA-Saclay (Francia) Carlos Brondino, UNL, Santa Fe
15:30 - 15:45	Coffee break
15:45 - 16:45	SNRMN

Conferencias

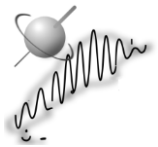
- **Elaine Holmes**, Imperial College, United Kingdom. "The role if spectroscopic profiling in 21st century medicine"
- **Mario O. Salazar**, Fac. Cs. Bioq y Farm, Rosario. "Analysis of differences in chemical profiles of the mixtures from 1H NMR coupled to principal component analysis (PCA)"
- **Pierre Florian**, Orleans, France. "NMR in Material Science: Probing the Atomic-Scale Structures"
- **Rodolfo Acosta, FAMAF**, Córdoba, Argentina. "Relaxation, a new old tool for the study of hyperpolarization and porous media"
- **Jean Pierre Simorre**, IBS, Grenoble, Francia. "Antibiotic resistance and NMR of the bacterial cell wall"
- **Carlos Bertoncini**, IBR, Rosario, Argentina. "Paramagnetic resonance enhancement as a tool to probe low populated species in highly dinamyc protein systems"
- **Roberto Gil**, Carnegie Mellon, USA. "Automated structural analysis of small organic molecules assisted by residual dipolar couplings: from 2d to 3d in one shot"
- **Juan Manuel Lázaro Martínez**, FAMAF Córdoba, Argentina. "SS-NMR applied to the characterization of polymers and gem-diol compounds"
- **Leandro Tabares**, CEA-Saclay, France. "Study of Mn(II) speciation in intact cells by High-Field EPR"
- **Carlos Brondino**, UNL, Santa Fe, Argentina. "CW EPR as a tool to study paramagnetic centers: from simple inorganic systems to complex macromolecules"

Jueves 21/11, 17 hs: Reunión de usuarios de EPR.

Forma propuesta para la actividad: charlas breves (20 min) que permitan tener una perspectiva de las actividades desarrolladas y necesidades en cada centro. Propuestas de colaboraciones que permitan aprovechar al máximo la capacidad instalada en cada centro.

Curso de operadores, 23/11

El día sábado 23/11 se dictará un curso corto de técnicas de adquisición y procesamiento de datos en espectrómetros de RMN. El curso estará orientado a operadores de RMN líquido y estará a cargo de los Dres. Roberto Gil y Eduardo Nascimiento (Bruker BioSpin).



LECTURES

THE ROLE OF METABOLIC PROFILING IN 21ST CENTURY MEDICINE

E. Holmes

Division of Computational and Systems Medicine, Department of Surgery and Cancer. Imperial College London, Exhibition Road, London, SW7 2AZ
e-mail: elaine.holmes@imperial.ac.uk

Keywords: Metabolomics, metabolome wide association, multivariate statistical modelling

Man is a complex ecosystem with thousands of biochemical processes working together through time to maintain health. In order to understand the biology of man and to intervene in an appropriate manner and time to prevent disease, we require knowledge of how the body works at the level of genes, proteins and metabolites. The metabolic phenotype can provide a window onto dynamic biochemical responses to physiological and pathological stimuli. Metabolic profiling strategies for analyzing biosamples, encompassing high-resolution spectroscopic methods in combination with multivariate statistical modelling tools, have been shown to be well-suited to generating metabolic signatures reflecting gene-environment interactions [1]. Spectroscopic analysis has been applied across a wide range of studies with the aim of characterizing classes of disease, different physiological states or response to particular therapies and the natural extension is to derive predictive models for metabolic response from a baseline profile. This approach has also been applied in metabolome wide association studies (MWAS) in order to identify associations between diet and disease prevalence or risk. Using the MWAS approach to characterize the impact of diet on hypertension in a large scale epidemiological, a range of metabolites were identified that differentiated populations with vastly differing blood pressure levels and lifestyles [2].

Several rodent models of diabetes and insulin resistance have been metabolically phenotyped using metabolic profiling strategies. However, substantial effort is now being placed on human population studies using a metabolome-wide association approach. Increasing awareness that the co-evolution has influenced the microbiome of mammals, and that the gut microbiota play a role in the aetiology and/or development of insulin resistance, has been fueled by studies in both animal models and humans showing that obese, lean and insulin resistant individuals carry a different gut microbial composition [3]. Clear differences in microbially-derived metabolites have been shown in profiles from obese individuals with metabolites such as hippurate and phenylacetylglutamine being associated with leaner phenotypes in a range of animal models and in man. Further, it is known that the microbiota are capable of producing many neuroactive chemicals and that the gut-brain axis is important in appetite regulation. This lecture will also explore the metabolic phenotype of type 2 diabetes and discuss the consequences of interventions such as low fat diet, therapeutics and bariatric surgery on the gut-brain axis and its potential role in the resolution of diabetes.

The complexity and interactive nature of biological systems can introduce confounding variation into the metabolic profile data. Methods for characterizing the metabolic consequences of biological processes will be discussed with particular emphasis on accommodating extraneous variation and optimizing biomarker recovery. Additionally a framework for predicting response to interventions at the individual level will be presented and examples drawn from a selection of laboratory and clinical studies.

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ANALYSIS OF DIFERENCES IN CHEMICAL PROFILES OF THE MIXTURES FROM ^{1}HNMR COUPLED TO PRINCIPAL COMPONENT ANALYSIS (PCA)

Mario O. Salazar

Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario-CONICET. Suipacha 531 Rosario (2000), Argentina.
e-mail:msalazar@fbioyf.unr.edu.ar

Keywords: Complex mixtures, ^{1}H NMR, PCA.

Since few decades ago NMR has been developed as a tool to obtain fingerprints of complex mixtures. In order to investigate the differences in such complex chemical profiles, the wide range of data obtained from NMR spectra can be analyzed with the assistance of statistical methods such as PCA. Naturally occurring mixtures like juices present a wealth of metabolites, and therefore chemical information which is characteristic of each mixture. We have used NMR coupled to PCA to analyze compare different orange species of genus citrus observing that discrimination between spices Citrus juice (orange, tangerine, grapefruit, etc.), orange (*C. sinensis*) juice from diferent varieties, orange juice produced from different geographic region (Argentina).

Chemically Engineered Extracts (CEE) are semi-synthetic mixtures of compounds produced by diversification of natural extracts (NE) through chemical transformation of common chemical functionalities in natural products into chemical functionalities rarely found in nature.[1] The analysis of chemical profiles of the mixtures (CEE versus NE) through ^{1}HNMR coupled PCA is a useful methodology to determine the effect of the reactions appied on the total composition of the mixtures.

Through this methodology we have estimated the success of chemical reactions such us sulphonylation and bromination, on the composition of crude extracts.[1,2,3] The score plot showed discrimination between the two groups by principal components (PCs), 1 and 2 (**Figure 1**, sulphonylation of p-toluenesulfonyl chloride). CEEs showed a positive PC2 value mainly due to the positive effect on PC2 of the signals corresponding to the p-toluene moiety introduced to the natural components of the starting mixtures. This effect was confirmed by comparing integrated areas of signals corresponding to the molecular portions from the addition of reagents used.[2]

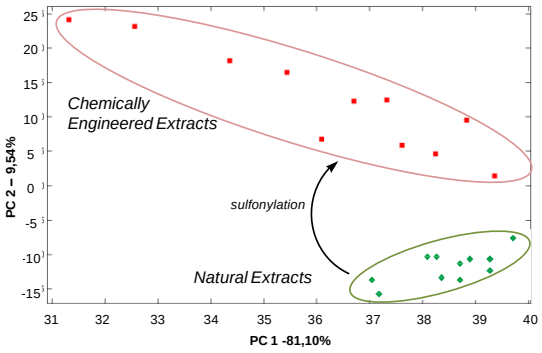

Figure 1: Score plot of PCA of ^{1}H NMR data from 11 NE (green) and 11 CEE (red) produced by reaction with p-toluenesulfonyl chloride.

ACKNOWLEDGEMENTS: Support of this work by grants from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Consejo Nacional de Investigaciones Científicas y Tecnicas (CONICET) is gratefully acknowledged.

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NMR IN MATERIAL SCIENCE: PROBING THE ATOMIC-SCALE STRUCTURES

P. Florian[1], F. Fayon, D. Massiot

CNRS, CEMHTI UPR3079, Univ. Orléans, F-45071 Orléans, France
e-mail: pierre.florian@cnrs-orleans.fr

Keywords: inorganic, materials, disorder, solid-state, high-resolution.

We first introduce briefly the major characteristics of Solid-State Nuclear Magnetic Resonance (SSNMR) applied to material science. We recall the interactions at play, the necessity to use magic-angle spinning and, especially when quadrupolar nuclei are considered, the use of very high magnetic fields.

We show that for crystalline minerals such as wollastonite $CaSiO_3$, found in many natural geological compounds, polymorphism can be clearly evidence with ^{29}Si SSNMR.[1] The ability of SSNMR to distinguish crystallographic structures is of outermost importance when very complex compounds are studied, such as Sn- or Ge-based phosphates for which the use of ^{31}P-^{31}P SSNMR correlations has resolved the space groups.[2] This type of "NMR crystallography" strategies have been extended to $AlPO_4$ systems where $^{31}P/^{27}Al$ and $^{27}Al/^{27}Al$ correlations can be implemented.[3,4]

Structural and/or chemical disorder is very often present in real materials, controlling some of their most interesting properties. As opposed to diffraction-type methods, SSNMR is not limited by the lack of sample's crystallinity and hence not only provides information when disorder is present but can also quantify it.[5,6] Chemical disorder in Gehlenite $CaAl_2Si_2O_7$ is the key feature to understand its optical properties and can be finely described using ^{27}Al, ^{29}Si and $^{27}Al/^{29}Si$ SSNMR along with first-principle calculations.[7] In the newly discovered $Ca_3SiB_2O_8$ phase the Neutron diffraction-derived structure has been refined with $^{29}Si/^{11}B$ NMR and DFT-based simulations to fully resolved the local chemical ordering hidden behind the topological disorder.[8]

Finally, SSNMR provide unmatched insights into glassy materials which completely lack long range ordering. It has played a key role in describing the chemical building blocks constituting those industrially and geologically important systems. We illustrate this point with rare-earth aluminosilicate glasses, mimicking the behavior of nuclear waste storage glasses, probing the nature and organization of their building units with ^{27}Al, ^{17}O, ^{45}Sc and $\{^{139}La\}^{27}Al$ NMR.[9]

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RELAXATION, A NEW OLD TOOL FOR THE STUDY OF HYPERPOLARIZATION AND POROUS MEDIA

R. H. Acosta

FaMAF, IFEG -CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.

Keywords: *Porous polymers, hyperpolarization, high resolution*

Dynamics in porous media is a multi-faceted field of great complexity that is found upon studies of a great number of systems such as porous glasses, fine-particle agglomerates, biological tissues, oil reservoirs or catalyzers, among others. A great deal of information, structural or functional, can be obtained by monitoring molecular motions within these complex media either by measuring restricted diffusion properties or by the determination of liquid-solid interactions[1,2]. In this work we address specific porous systems composed by polymer beads. Polymer matrices with well-defined structure and pore sizes are widely used in several areas of chemistry such as catalysis, enzyme immobilization, HPLC, adsorbents or drug controlled release. These polymers have pores in its structure both in the dry and swollen state. Although it is well known that the structures and properties greatly differ between these two states, only a few methods provide information about the swollen state, even though most of the applications involve the matrices in this situation. Nuclear Magnetic Resonance (NMR) is a suitable tool for the study of the molecular dynamics of different liquids spatially confined in macro, meso and nanopores through changes in relaxation times, in particular by determination of T_2 distributions with a Carr-Purcell-Meiboom-Gill (CPMG) sequence. Here we show how measurements of evaporation kinetics can provide information on the swelling of the polymer networks in systems were the percentage of crosslinking is systematically varied.

Two of the major drawbacks in NMR are the low sensitivity and fast decay rates. In porous media it is often important to determine not only the structural properties of the porous network, but also its interconnectivity. This line of work was introduced in the last decade through two dimensional relaxation exchange experiments known as T_2-T_2 maps. Even though this approach brings information on systems with pores in the nanoscale, it is limited by T_1 relaxation in systems with micro or mesopores. Hyperpolarization of noble gases has been successfully applied for instance to the study of the human lung. In this way, low sensitivity and short relaxation times are both accounted for[3]. Recently we have developed a system capable of producing hyperpolarization by parahydrogen (PHIP-ParaHydrogen Induced Polarization). One of the main drawbacks of this technique is the requirement of highly homogeneous fields in order to avoid signal cancelation due to the anti-phase characteristic of the NMR signals[4,5]. We will describe on one hand and approach based on the application of a CPMG train to obtain high resolution *J*-spectra and on the other we will discuss the future application of long-lived singlet states for the study of the porous systems described above.

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ANTIBIOTIC RESISTANCE AND NMR OF THE BACTERIAL CELL WALL

J.P. Simorre

Institut de Biologie Structurale Jean-Pierre Ebel, Grenoble, France.
e-mail: jean-pierre.simorre@ibs.fr

Keywords: Peptidoglycan, beta-lactam, DNP.

The cell wall is essential for the survival of bacteria. It gives the bacterial cell its shape and protects it against osmotic pressure, while allowing cell growth and division. It is made up of peptidoglycan (PG), a biopolymer forming a multi-gigadalton bag-like structure, and additionally in Gram-positive bacteria, of covalently linked anionic polymers called wall teichoic acids (WTA). TAs are thought to play important roles in ion trafficking, host-cell adhesion, inflammation and immune activation.

The machinery involved in the synthesis of this envelop is crucial and is one of the main antibiotic target. Different protein as transpeptidase, transpeptidase activator or hydrolase are recruited to maintain the morphogenesis of the peptidoglycan during the bacterial cell cycle. Based on few examples involved in the machinery of synthesis of the peptidoglycan, we will demonstrates that a combination of liquid and solid-state NMR can be a powerful tool to screen for cell-wall interacting proteins in vitro and on cell..

In particular, structure of the L,D-transpeptidases that results in b-lactam resistance in M. tuberculosis, has been studied in presence of the bacterial cell wall and in presence of antibiotic. The NMR study reveals new insights into the inhibition mechanism.

In parallel, we have investigated the potential of Dynamic Nuclear Polarization (DNP) to investigate cell surface directly in intact cells. Our results show that increase in sensitivity can be obtained together with the possibility of enhancing specifically cell-wall signals. It opens new avenues for the use of DNP-enhanced solid-state NMR as an on-cell investigation tool.**.**

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PARAMAGNETIC RESONANCE ENHANCEMENT AS A TOOL TO PROBE LOW POPULATED SPECIES IN HIGHLY DINAMYC PROTEIN SYSTEMS

Carlos W. Bertoncini, Santiago Estaban-Martín, Jordi Sylvestre-Ryan & Xavier Salvatella

Institute for Research in Biomedicine, Barcelona, Spain & Barcelona Supercomputing Center, Barcelona, Spain. email: carlos.bertoncini@irbbarcelona.org

Keywords: NMR, Structure Calculation, Protein Dynamics, Intrinsically Disordered Proteins, Protein Folding.

In the last decade it has become evident that dynamic states of proteins play important physiological and pathological roles. Disordered states of proteins embody one extreme example of such relevant protein dynamics. It has been shown that transient low populated states are often present in these systems and can play an active role in their biological activity. The structural characterization of such states has so far largely relied on ensemble representations, which in principle account for both their local and global structural features. However, these approaches are inherently of low resolution due to the large number of degrees of freedom of conformational ensembles, and to the sparse nature of the experimental data used to determine them.

We have developed an NMR-based experimental and computational framework to overcome these limitations by the use of extensive sets of nitroxide spin labels on these proteins. Spin labels cause paramagnetic-induced relaxation enhancement (PRE) on nuclei resonances, an effect that is modulated by the distance to the spin label (r^{-6}). Computational studies on synthetic data show that, contrary to ensemble interpretations of PRE data, tertiary interactions in disordered states of proteins can be mapped at high resolution by fitting PRE data to a rather small number of conformations, which can be as low as one. As examples of this application we characterized low populated species present in the acid-unfolded state of apomyoglobin and in the intrinsically disordered state of α-synuclein from experimentally measured PRE data.

These result open up the possibility of determining the topology of cooperatively collapsed and hidden folded states when these are present in the vast conformational landscape accessible to disordered states of proteins.

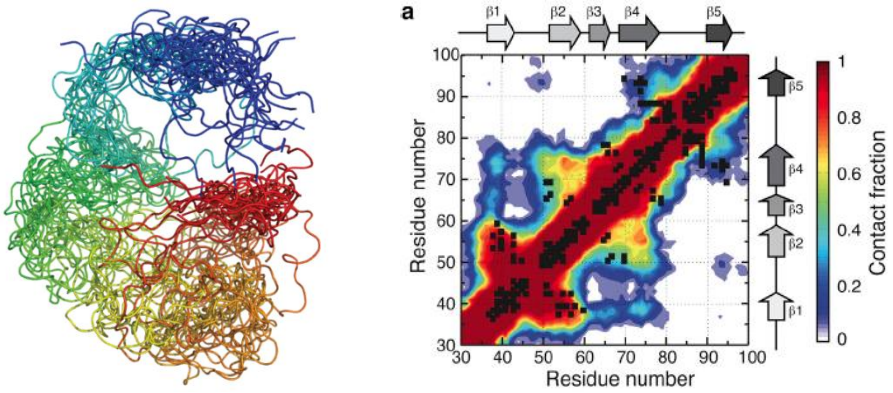

Figure 1: Low populated states of intrinsically disordered α-synuclein probed by PREs show features of pre-amyloid species

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AUTOMATED STRUCTURAL ANALYSIS OF SMALL ORGANIC MOLECULES ASSISTED BY RESIDUAL DIPOLAR COUPLINGS: FROM 2D TO 3D IN ONE SHOT

Roberto R. Gil

Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA.
e-mail:rgil@andrew.cmu.edu

Keywords: Small Molecules, Residual Dipolar Couplings

The 2D structure of most small molecules can be in principle straightforwardly determined by manual or automatic analysis of a set of experimental data that includes the molecular formula, a series of 1D and 2D NMR experiments providing trough-bond connectivity (COSY, TOCSY, HSQC, HMBC and ADEQUATE/INADEQUATE based experiments), and chemical shift predictions. This is the main concept embedded in automatic structure elucidation programs (CASE).*1* Once the 2D structure is available, the determination of the relative spatial arrangement (configuration and preferred conformation) of all atoms in the molecule is a more challenging task that it is commonly addressed in NMR by using NOE and ^{3}J coupling constants analysis, as well as recent developments on the application of DFT calculation of ^{13}C chemical shifts.[2] The development of the application of Residual Dipolar Couplings (RDCs) to the configurational and conformational analysis of small molecules[3] has matured enough in the recent years to perform this task is an almost straightforward way, without even the need of using NOE and ^{3}J coupling analysis; as it will be presented here for the analysis of rigid and semi-rigid small molecules. Selected 2D structures from CASE programs can be fed to molecular modeling packages in order to generate conformational 3D ensembles, which are later supplied to RDC analysis programs. In this way not only the 2D structure but also the correct configuration and conformational space of moderately flexible molecules can be determined. In a last step the absolute configuration can be obtained by computation of population averaged chiroptical properties such as electronic circular dichroism (ECD) or optical rotation using the previously obtained configurational and conformational information. Hence, all necessary steps for complete structural elucidation can be automated with a minimum of human intervention. This procedure has been tested in a series of known alkaloids such as strychnine, yohimbine, and others. Molecules were oriented using the reusable, scalable and reversibly compressible PMMA gels methods developed in our group,[4] and RDCs were collected using *J* Scaled BIRD HSQCs (JSB-HSQC) proton-coupled in F1.

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SS-NMR APPLIED TO THE CHARACTERIZATION OF POLYMERS AND GEM-DIOL COMPOUNDS.

Juan M. Lázaro Martínez[1,2], Graciela Y. Buldain[1], Daniel Vega[3], Gustavo A. Monti[2], Ana K. Chattah[2].

[1]*Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Orgánica – CONICET. Ciudad Autónoma de Buenos Aires, Argentina.*
[2]*FaMAF-Universidad Nacional de Córdoba & IFEG-CONICET, Córdoba, Argentina.*
[3]*Centro Atómico Constituyentes (CNEA), San Martín, Buenos Aires, Argentina.*
e-mail: lazarojm@ffyb.uba.ar

Keywords: Polymers, Coordination chemistry, gem-diol compounds.

Macromolecular chemistry has become of great interest to yield processable materials with unique and valuable properties. Among them, polymer networks offer new possibilities to scientists for the creation of artificial materials. In particular, incorporating coordination complexes into polymeric architectures opens up the possibility of imparting the physicochemical properties of both partners to the resulting material. In particular, metallo-hydrogels containing copper or cobalt ions are particularly useful due to the catalytic activity of these complexes.[1]

In this field, the synthesis of new class of ligands, such as *gem*-diolate molecules, is a growing research area for the obtention of novel polymers. For instance, Perlepes *et al.* have studied the employment of di-2-pyridyl ketone, $(py)_2CO$, as an organic ligand for the synthesis of high nuclearity metal complexes.[2] However, the *gem*-diol form for $(py)_2CO$ exist only in the respective metal complex, since these compounds are rarely stable (and only in aqueous solution). In particular, our group has found that the 2-formylimidazole hydrate is a stable crystalline substance because, in order to revert to the aldehyde-form, a water molecule must be left out, and this is difficult by the electron-withdrawing character of the imidazolium cation (Fig. 1).[3]

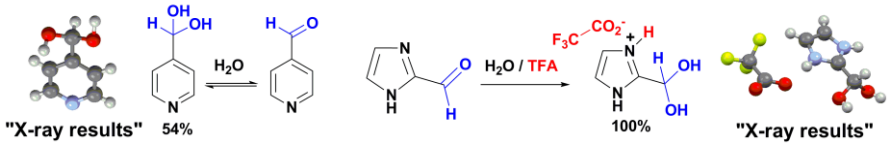

Figure 1: Addition of water to 2-formylpyridine and 2-formylimidazole.

In this work, we describe structural, dynamic homogeneity and the metal ion uptake properties from different hydrogels containing carboxylic acid and heterocyclic azole groups (imidazole, triazole and pyrazole) and in three synthetic variants of *poly*(ethyleneimine) polymers with different molecular weights (22, 87 and 217 kDa). In addition, the existence and stability of the aldehyde-hydrate form of some pyridine and imidazole carboxaldehyde derivatives (Fig. 1) were studied using solution- and solid-state NMR together with single-crystal X-ray crystallography experiments.

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ACKNOWLEDGEMENTS

CONICET, ANCYPT, UBACyT

STUDY OF MN(II) SPECIATION IN INTACT CELLS BY HIGH-FIELD EPR

Leandro C. Tabares, Eduardo M. Bruch and Sun Un

Service de Bioénergétique, Biologie Structurale et Mécanismes, CEA-Saclay, France.
leandro.tabares@cea.fr

Keywords: High-Field EPR, Manganese, D. radiodurans.

High magnetic-field high-frequency electron paramagnetic resonance techniques were used to measure in situ Mn(II) speciation in *Deinococcus radiodurans*, a radiation resistant bacteria capable of accumulating high concentrations of Mn(II) [1]. It was possible to identify and quantify the evolution of Mn(II) species in intact cells at various stages of growth. Aside from water, 95 GHz high-field electron-nuclear double resonance showed that the Mn(II) ions are bound to histidines, glutamates and phosphate groups, mostly from fructose-1,6-bisphosphate but also inorganic phosphates and nucleotides. During stationary growth phase, 285 GHz continuous-wave EPR measurements showed that the majority of the cellular Mn(II) in *D. radiodurans* is bound to a yet unidentified protein and superoxide dismutase. This manganese distribution is affected by the growth conditions but it appears to be not modified by gamma irradiation.

We have extended this studies to other organisms and found that this approach is general and establishes a method for studying Mn(II) speciation and homeostasis in intact cells.

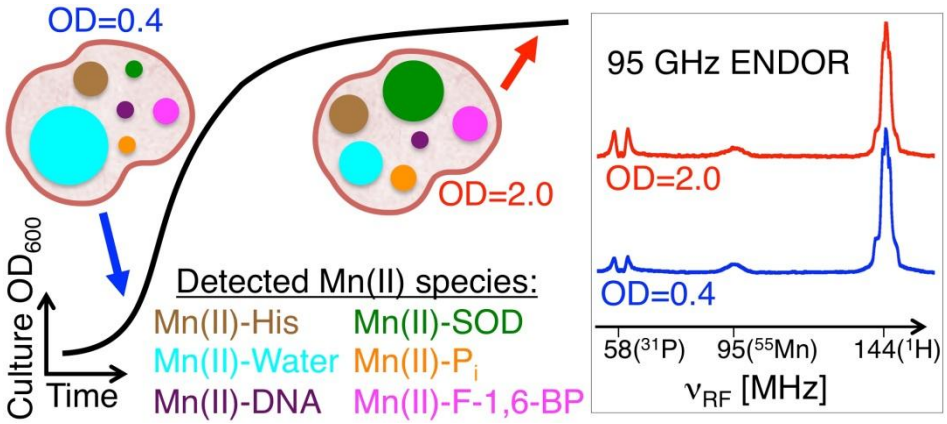

Figure 1: Schematic representation of the different Mn(II) species identified by EPR inside intact cells of *D. Radiodurans* at differents stages of growth.

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CW EPR AS A TOOL TO STUDY PARAMAGNETIC CENTERS: FROM SIMPLE INORGANIC SYSTEMS TO COMPLEX MACROMOLECULES

Carlos D. Brondino

Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.
e-mail: brondino@fbcb.unl.edu.ar

Keywords: CW EPR, metal complexes, single crystal, metalloenzymes

Continuous Wave Electron Paramagnetic Resonance (CW EPR) is a spectroscopic technique extensively employed in the physicochemical characterization of simple inorganic systems and redox enzymes that contain paramagnetic transition metal ions in their structures. Since its beginnings by ~1944, CW EPR was mainly used to evaluate the tensorial magnitudes associated with the anisotropic and isotropic interactions that govern the magnetic resonance phenomenon in simple inorganic/organic systems and to understand how the magnetic interactions between the paramagnetic centers modulate the spectral properties of the isolated ions.[1] In this line, we will analyze some examples of mononuclear and dinuclear inorganic systems studied by single crystal CW EPR to show the potential capability of the technique in the characterization of simple paramagnetic systems.

The use of CW EPR in the characterization of more complex systems, as is the case of redox enzymes that present paramagnetic centers in their structure, is relatively more recent and can be considered as an extension of the experience gained with the above mentioned simpler systems. The redox enzymes we will analyze may include distinct type of redox centers (usually situated ~ 10-20 Å away) connected by long chemical pathways which are involved in electron transfer processes.[2] Despite the long both distances and chemical paths, they can present weak magnetic couplings produced by spin-spin interactions such as dipolar and isotropic exchange.[2,3] We will discuss how CW EPR can be advantageously used to determine intercenter distances, to assign the EPR active centers with those of the structure, to evaluate the integrity of the electron transfer pathways in distinct protein conditions, and how the technique can be used to obtain structural information that cannot be obtained with conventional structural techniques.

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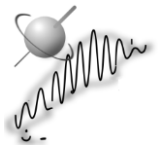


POSTERS

PHOTOINDUCED ELECTRON TRANSFER PROCESSES IN AU/ZNO NANOSTRUCTURES REVEALED BY ELECTRON PARAMGNETIC RESONANCE STUDIES

Matías E. Aguirre, Gonzalo M. Perelstein, Ailén Armanelli, María A. Grela.

Department of Chemistry, UNMdP, Mar del Plata, Argentina.
e-mail:meaguirre@mdp.edu.ar

Keywords: photoinduced processes, gold semiconductor nanostructures, nitroxides.

Hybrid nanostructures composed by noble metals and wide bandgap semi-conductors are interesting materials for molecular optoelectronics, energy conversion and catalysis. Here we present definitive evidence of the plasmon photoinduced electron transfer from Au to ZnO nanoparticles by direct detection of free conduction electrons by EPR spectroscopy. [1] Figure 1 compares the EPR spectra of pure ZnO nanoparticles suspended in ethylene glycol (a), and that of Au/ZnO nanostructures before, (b), and during irradiation at wavelengths longer than 435 nm, (c). The synthesized Au/ZnO nanostructures in the dark, showed a small feature at g = 1.9655, which is assigned to the presence of free electrons in the conduction band. Its origin its attributted to the charge transfer process taking place during the synthesis of the nanostructures to equilibrate their Fermi energy levels. Curve c) shows that visible irradiation ($\lambda > 435$ nm) of the sample leads to a sudden outstanding increase of the singlet at *g* = 1.9623; the shift to higher fields with respect to the dark value indicates that electrons preferentially reside in larger sized particles which favour electron delocalization [1,2].

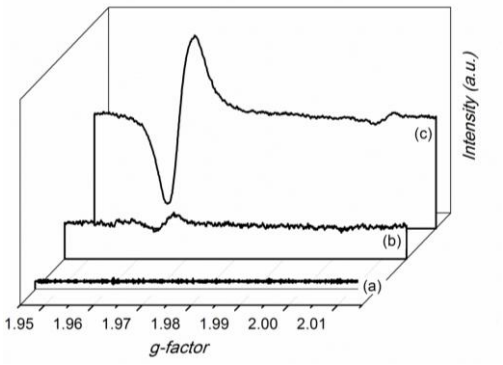
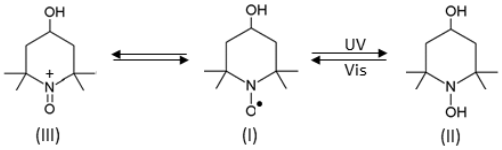


Figure 1: Room temperature EPR spectra of the Au/ZnO nanostructures suspended in ethylene glycol, see text.

Also, by analyzing the redox transformations between the oxidation states of 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl, tempol (**I**), the hydroxylamine (**II**) and its oxoammonium cation (**III**),



we showed that (I) and (II) can be interconverted by selective irradiation of the semiconductor (UV) and the plasmon band (in the visible region). The results indicate that by modulating the excitation wavelength the electron flow direction can be switched, a fact that may be exploited for the development of logical devices.

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IDENTIFICATION OF A CALMODULIN BINDING SITE AT THE N-TERMINUS OF PARVULIN 17

Noelia Inés Burgardt[1,2], Andreas Schmidt[2], Annika Manns[2], Alexandra Thiele[2], Günther Jahreis[2], Yi-Jan Lin[3], Christian Lücke[2], Matthias Weiwad[2]

[1] Institute of Biochemistry and Biophysics (IQUIFIB), School of Pharmacy and Biochemistry, UBA, Junín 956, C1113AAD, Buenos Aires, Argentina
[2] Max Planck Research Unit for Enzymology of Protein Folding, Weinbergweg 22, 06120 Halle (Saale), Germany
[3] Graduate Institute of Natural Products and Center of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan
e-mail:burgardt@qb.ffyb.uba.ar

Keywords: Protein interaction, Par 17, Calmodulin, Chemical shift index analysis.

Parvulins are peptidyl prolyl *cis*/*trans* isomerases (PPIases), enzymes that accelerate the interconversion between prolyl bond isomers of polypeptide chains[1,2]. Eukaryotic parvulins have been shown to be involved in cell proliferation and cell cycle progression[3,4]. Human Parvulin 17 (Par 17) is encoded by the Par14/Pin4 locus on chromosome Xq13, which also has a second transcription initiation site for the protein Parvulin 14 (Par 14)[1]. As a consequence, they share an identical PPIase domain and the only difference between them is the presence of 25 additional residues at the N-terminus of Par 17. The PPIase domain solution structure was solved by NMR for human Par 14[5,6]. Besides the similarity between Par 14 and Par 17, these proteins have different intracellular localization and show differences in certain biological activities[7-9]. The NMR assignment of Par 17 shows that it features a parvulin-type PPIase domain at the C-terminus, analogous to Par 14, and an unstructured N-terminus encompassing the first 60 residues[10]. In this work we show the identification of a calmodulin binding site located in the N-terminus of Par 17.

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CONICET, ANCYPT

STUDY OF PENDANT CHAIN DYNAMICS IN MODEL PDMS NETWORKS

F. Campise[1], R. Acosta, M. Villar, E. Vallés, D. Vega, G. A. Monti

Facultad de Matemática Astronomía y Física, Universidad Nacional de Córdoba, Córdoba, Argentina.
e-mail:florcampise@gmail.com

Keywords: PDMS, entanglement, defect, Nuclear Overhauser Effect, NOE, activation energy, dynamic, Double Quantum Coherence, Residual dipolar coupling.

Most of the viscoelastic and diffusive properties of polymer melt and concentrated polymer solutions are profoundly influenced by topological interactions[1]. The most succesful model to deal with topological constraints is the tube model [2].

In this work, chain dynamics as well as the contribution of network defects to such dynamics, has been studied by means of proton relaxation NMR experiments in model tri and tetra functional polydimethylsiloxane (PDMS) networks with low concentrations of pendant chains of different molecular weight [3]. Low field NMR experiments were conducted.

We studied the entanglement dynamics of the chains that form the network applying a CPMG pulse sequence at different temperatures. This behaviour characterizes the mechanical response of the polymer. For the analysis of the data we have considered that for the proton NMR time scale, the physical entanglements behave as crosslink points [6]. It could be observed that the contribution of non relaxed pendant material responds to an Arrhenius process enabling the determination of activation energies. The activation energies obtained are in the same range of the obtained in a previous work in which we monitored the magnetization transfer via Nuclear Overhauser Effect (NOE) between pendant and elastic chains.

On the other hand, we present some results of Double Quantum Coherence experiments which might contribute to the analysis of the previous results. This is an ongoing experiment.

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MRI OF FLOW REGIMES INSIDE A TAYLOR-COUETTE CELL WITH APPLICATIONS IN ELECTRHOCHEMISTRY.

Mariela Carpinella[1], Manuel I. Velasco[1], Juan M. Ovejero[2], Sergio A. Dassie[2], Rodolfo H. Acosta[1]

1.Instituto de Física Enrique Gaviola. CONICET – UNC, Córdoba, Argentina.
2. *Instituto de Investigaciones en Físicoquímica, FCQ – UNC, Córdoba, Argentina*
e-mail:mariela.carpinella@gmail.com

Keywords: MRI, Taylor-Couette cell, Taylor Vortex Flow.

Electrochemical cells (EC) and Rotating Disks Electrodes (RDE) are extensively used to describe chemical reactions and investigate transport mechanisms. In order to obtain quantitative data from the cell is necessary to know precisely the flow distribution inside it. More than 50 years ago, Cochran [1] and Levich [2] developed analytical expressions for the approximate speeds on a RDE using several simplifications. In these expressions, the cell dimensions are endless and the electrode thickness negligible. Despite this, the expressions are still widely used to calculate the limiting currents in this type of devices.

A Couette cell is a device composed of two concentric rotating cylinders and represents a simplified model of an EC. In particular, we focused on a cell in which the inner cylinder rotates while the outer one remains stationary. In a simplified description, when the rate of rotation of the inner cylinder exceeds a certain critical value, given by the Critical Taylor number, the laminar azimuthal flow is modified by the appearance of counter-rotating vortices flow (TVF) superimposed on the tangential rotation of the liquid. In the presence of Taylor vortices the device is denominated Taylor-Couette cell (T-C). Despite the big number of works devoted to the study of the flow patterns generated in this kind of devices by means of simulations or Computational Fluid Dynamics [3,4], there are a numerous theoretical inconsistencies that often cannot be clarified because of the difficulties in the experimental measurement of these flows. A precise experimental determination of the velocity field near the electrode through modern techniques such as Particle Imaging Velocimetry becomes difficult due to cell geometry and electrode dimensions, and in many cases requires optically transparent flows [5]. Performing NMR imaging (MRI) has many advantages for this purpose, as it is noninvasive and does not require transparent fluids. MRI has been used for the characterization of the flow inside T-C cells, whose rotating inner cylinder is in contact with the bottom of the cell [6,7]. Although these works are very useful for understanding the flow patterns that are generated, this mechanism is a simplification of the real problem, where the rotating rod length is smaller than the length of the EC.

With the purposed of characterizing the hydrodynamics behavior of the liquid inside a real cell we performed 2D velocity maps using MRI Pulsed Gradients Spin Echo sequence in a T-C cell filled with water, for different heights of the inner cylinder and different rotational speeds. The NMR results are compared with finite element simulations using COMSOL software. Maps were performed in a centered longitudinal plane of the cell of 2 mm thickness with pixel sizes of 0.156 x 0.156 mm.

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13C CPMAS SOLID STATE NMR OF POLYANILINE SALTS WITH INCREASING DOPANT CONCENTRATIONS

C. J. Cattena[1], E. M. Erro[2], R. Iglesias[2], G. A. Monti[1], H. M. Pastawski[1]

[1]Instituto de Física Enrique Gaviola and Facultad de Matemática Astronomía y Física, Universidad Nacional de Córdoba, Córdoba, Argentina.
[2]Departamento de Fisicoquímica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, INFIQC, Córdoba, Argentina
e-mail:cattena@famaf.unc.edu.ar

Keywords: Polyaniline, NMR, Conducting polymers, electronic transport, Decoherence, Disorder

The attention given to conducting polymers have not yet declined since their discovery, more than thirty years ago. In spite of the enormous technological advances and the increasing interest in their use as organic components in nanocomposites and nanodevices, the metal-insulator transition still presents a strong scientific challenge, since it is not well understood from the point of view of basic science [1]. Recent theoretical results give important insights to the conceptual basis of this field, emphasizing the fundamental role of disorder and decoherence processes on the transport properties for these kind of one-dimensional systems [2]. In this work, we report on ^{13}C solid state nuclear magnetic resonance measurements of carefully prepared samples of polyaniline emeraldine base and emeraldine salts, with increasing HCl dopant concentrations, near the critical point of the metal-insulator transition. A simple fitting function, built upon two free parameters, describes surprisingly well the experimental data. We provide a simple and convenient explanation for the observed displacement in the first moment of the spectra with increasing dopant concentrations of the emeraldine salts. Our results shows meticulously that all relevant chemical shifts contribute to a single average and uniform (inhomogeneous) broadening, enforcing the role of disorder as a decisive ingredient in the electronic transport mechanism in polyanilines.

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SSNMR CHARACTERIZATION OF NEW SUPRAMOLECULAR COMPLEXES OF CYCLODEXTRIN AND NORFLOXACIN POLIMORPHS

A. K. Chattah[1], K. H. Mroue[2], A. Ramamoorthy[2], L. Pfund[2] , M.R. Longhi[3], C. Garnero[3]

[1]*FaMAF, Universidad Nacional de Córdoba and IFEG (CONICET),Ciudad Universitaria, Córdoba, Argentina, e-mail: chattah@famaf.unc.edu.ar*
[2]*Biophysics and Department of Chemistry, The University of Michigan, Ann Arbor, Michigan, 48109–1055, USA*
[3]*Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba and UNITEFA (CONICET), Ciudad Universitaria, Córdoba, Argentina*
Keywords: norfloxacin; polymorphism; complexation; characterization; solid state NMR.

Two critical factors resposible for the poor and highly variable human bioavailability of some pharmaceuticals are their low permeability and solubility. On the other hand, selecting a proper polymorph and to control polymorphic transformations has a great impact in pharmaceutical and regulation issues. With these facts in mind, we have focused our work on preparing and characterizing new supramolecular systems of Norfloxacin (NOR) polymorphs B and C with β-cyclodextrin (βCD) (Figure 1). The polymorphs of NOR, their physical mixtures with βCD, and the corresponding NOR–βCD complexes were investigated using a variety of spectroscopic techniques including ssNMR (Figure 2), PXRD, and Fourier transform infrared (FT-IR) spectroscopy.

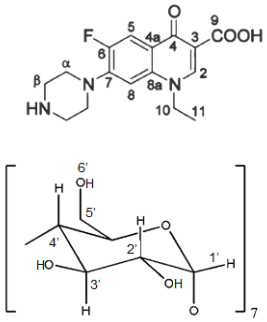

Figure 1: NOR (top), βCD (bottom)

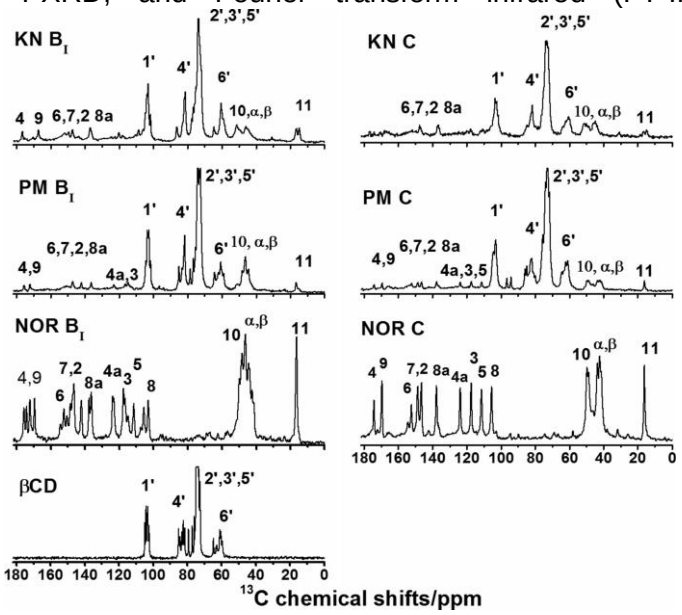

Figure 2: ^{13}C CP-MAS spectra

Additionally, measurement of the proton spin-lattice relaxation times (^{1}H -T_1) using ^{1}H spin-inversion recovery experiments detected in ^{13}C ramped-amplitude (Ramp)-CP-MAS spectra, has enabled us to gain better insights into the complex formation, as well as into the interactions among their individual components at the molecular level. [1]

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CONICET, SECyT-UNC, MINCyT (CBA)

FROM PURPLE TO GREEN CUPREDOXINS: ENGINEERING AZURIN AND AMICYANIN LOOPS INTO A NATIVE CU_A SCAFFOLD

Andrés Espinoza Cara and Alejandro J. Vila

Instituto de Biología Molecular de Rosario (IBR-CONICET), Predio CCT-CONICET, Ocampo y Esmeralda, 2000, Rosario, Santa Fe, Argentina..
e-mail: espinoza@ibr-conicet.gov.ar

Keywords: Cupredoxins, Loop-Directed Mutagenesis, EPR, NMR.

Nature employs metal ions for a wide variety of biological functions. Copper proteins play an important role in electron transfer processes[1]. There are two types of copper electron transfer sites in nature: (a) mononuclear type I centers (T1), also known as blue sites, and (b) the dinuclear CuA center1. Proteins with T1 and Cu_A centers share a conserved fold (cupredoxin fold) despite being from different organisms, revealing a common evolutionary origin[2]. The functional properties of these redox sites cannot be compared directly because of being in different protein matrixes. The T1 and Cu_A centers presents and ideal situation for loop changing as they share the same protein fold and most of the metal ligands are present in loops. There are few examples using this strategy, but all of them suggest that the ligand-containing loops gather most of the structural information that regulates the function of the redox site[3,4]. In this project we employ loop directed mutagenesis in order to introduce different T1 loops in the soluble fragment of the subunit II of the cytochrome *ba3* oxidase from Thermus thermophilus, naturally harboring a Cu_A site ($TtCu_A$). Two T1 variants were constructed, employing the loops from amicyanin (Ami-$TtCu_A$) and azurin (Az-$TtCu_A$). These proteins have distinct characteristics from the native sites. Using distinct biophysical approaches such as optical spectroscopy, EPR and NMR we determined that both proteins have properties that vary along those of rhombic T1 sites. Both proteins can also bind exogenous imidazole in a reversible way giving rise to a copper site similar spectral features as those found in nitrosocyanin[5]. Taken together, these observations discard the “loop defines everything” hypothesis and show how reversible binding of small molecules can be elicited in an otherwise occluded metal site.

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EFFECT OF CHOLESTEROL ON MEMBRANE DYNAMICS IN LIPID BILAYERS FROM FAST FIELD-CYCLING NMR RELAXOMETRY STUDIES OF UNILAMELLAR VESICLES

C. C. Fraenza[1], E. Anoardo, C. Meledandri, D. Brougham

[1]Laboratorio de Relaxometría y Técnicas Especiales (LaRTE), Facultad de Matemática, Astronomía y Física, Universidad Nacional de Córdoba and IFEG (CONICET), Córdoba, Argentina.
e-mail: fraenza@famaf.unc.edu.ar

Keywords: Lipid bilayer, Cholesterol effect, Molecular dynamics, Fast Field-Cycling NMR.

Fast field cycling (FFC) proton NMR spin-lattice relaxation rate dispersions of liposomes have been explained by a physical model that accounts for the molecular and collective dynamics of the lipids, according to a previous work[1,2]. The model was successfully compared with experimental measurements of liposomes prepared with different lipids (DMPC and DOPC), sizes (100-200nm) and temperatures (within the fluid liquid crystalline phase), using values for the different physical constants and parameters available in the literature. The FFC NMR method turned to be a useful tool for the study of the molecular dynamics of lipids and the viscoelastic properties of biological membranes. It was claimed that, up to a limited concentration of cholesterol, the membrane remains in the disordered liquid crystalline phase (l_d)[3,4]. However, other authors define a region around each molecule of cholesterol where lipids become strongly affected (much ordered). In consequence, the lipids population divides in unaffected and affected, depending on the proximity to a cholesterol molecule[5,6,7,8,9]. The unaffected lipids are considered to be in the l_d phase, while the affected lipids are described in an ordered liquid crystalline phase (l_o). In this work we confront both approaches with new experimental data as a further application of our model. We analyze experimental FFC relaxation rate dispersion curves obtained at 298 K for liposomes of radius between 68 and 80 nm composed of DOPC and cholesterol at 10 and 25mol%. The consistence obtained in our analysis suggests that the model previously used to explain the relaxation-rate dispersion in free-cholesterol DOPC liposomes[1,2], can be extended to the study of liposomes containing cholesterol in the membrane.

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MOLECULAR DYNAMICS STUDIES IN POLYMERIC MICELLES USING FAST FIELD-CYCLING NMR RELAXOMETRY

C. Fraenza[1], G. Farrher, E. Anoardo, A. Ordikhani, C. Mattea, S. Stapf, R. Grisoni, A. Sosnik

[1]Laboratorio de Relaxometría y Técnicas Especiales (LaRTE), Facultad de Matemática, Astronomía y Física, Universidad Nacional de Córdoba and IFEG (CONICET), Córdoba, Argentina.
e-mail: fraenza@famaf.unc.edu.ar

Keywords: Polymeric micelles, Molecular dynamics, Fast Field-Cycling NMR.

The administration of drugs presenting low solubility in biological fluids still reptresents a crucial biopharmaceutic limitation for the pharmaceutical industry, being the case for about 50% of the approved drugs and 70% of those in the pipeline. Among the existing strategies to overcome this problem, inclusion of hydrophobic drugs into polymeric micelles is one of the most attractive and versatile alternatives. Amphiphilic poly(ethylene oxide)–poly(propylene oxide) block copolymers are thermoresponsive materials that display unique aggregation properties in aqueous medium. Due to their ability to form stable micellar systems in water, these materials are broadly studied for the solubilization of poorly watersoluble drugs. In this work, molecular dynamics of triblock copolymers (commercial name Pluronic block copolymers) F68 ($EO_{80}PO_{27}EO_{80}$), F108 ($EO_{141}PO_{44}EO_{141}$), and F127 ($EO_{101}PO_{56}EO_{101}$) at different concentrations (10-22.5% w/v) and temperatures (3-25°C) were analyzed using fast field-cycling NMR relaxometry. The frequency range was from 8 KHz to 20 MHz, considering that the measured local field values were lower than 1 KHz for all the samples. This study was complemented with NMR spectroscopy,NMR measurements in the rotating frame, atomic force microscopy (AFM), transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques. Although proton NMR spin-lattice relaxation rate dispersions showed a weak dispersion in the laboratory frame and no dispersion in the rotating frame, they evidenced a bi-exponential behavior that has been attributed to different relaxation of PEO and PPO groups in agreements with other authors[1]. Also, it was observed that the larger the ratio *R,* defined by number of protons of PPO divided number of protons of PEO, the more evident biexponentiality. Efforts will be done in order to explain, using a physical model, this weak dispersion in relaxation times and its bi-exponential behavior.

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TOWARDS A UNIFIED METHODOLOGY TO VALIDATE GLYCOPROTEINS

P.G. Garay[1], O.A. Martin, Y.A. Arnautova, A.A. Icazatt, H.A. Scheraga and J.A. Vila

[1]Instituto de Matemática Aplicada San Luis, CONICET-UNSL, 5700 San Luis, Argentina.
e-mail:garaypablo01@gmail.com

Keywords: Validation, proteins, glycans, glycoproteins, chemical shifts

Over the last several years we have focused our efforts in developing computational tools, such as the *Che*Shift-2 server (Vila et al., 2009, Martin et al., 2012, Martin et al., 2013), for automated validation of X-ray and NMR determined protein structures, provided that the observed $^{13}C^{\alpha}$ and/or $^{13}C^{\beta}$ chemical shifts are available. More than 50% of known protein are glycoproteins (Apweiler et al., 1999), although only 3.5% of the proteins deposited in the the Protein Data Bank (PDB, Bernstein et al., 1977) are covalently bound to a glycan chain and thus can be classified as glycoproteins (Lütteke, 2009). There are multiple reasons why the PDB is heavily biased against glycoproteins, among others the following: glycan chains often hamper crystal growth and, hence, they are removed before-hand, carbohydrates are very flexible molecules and therefore often do not yield sufficient electron density to resolve the three-dimensional structure, etc. (Lütteke, 2009). In addition, around 30% of the PDB-deposited carbohydrates entries contain errors (Lütteke, 2009). Overall, the need for accurate and fast validation methods to detect flaws in protein/glycan structures, at residue/disaccharide level, appears to be crucial. Consequently, here we present a summary of the main features of the *Che*Shift-2 server (Martin et al., 2013) for protein structure validation and also the first steps to expand our methodology to include validation of glycans.

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MAPPING SOLVENT DISTRIBUTION ON PROTEIN SURFACE: CLUES FOR THE PROTEIN-LIGAND STRUCTURE ELUCIDATION

Gauto, Diego, Di Giano Leandro, Modenutti Carlos, Blanco Juan, Martí Marcelo

Departamento de Química Inorgánica, Analítica, y Química Física, INQUIMAE-CONICET, Buenos Aires, Argentina.
e-mail: dgauto@qi.fcen.uba.ar

Keywords: Molecular Dynamics, Molecular Docking, Water, Isopropanol, Carbohydrate

Molecular recognition process is one of the key process related to several cellular functions, and most of them involves the non-covalente protein-ligand asociation. **Therefore, two complementary challenges emerge from this event. On the one hand, the ability to elucidate accurately the binding site from the whole protein, and on the other, the determination of the true protein-ligand complex structure**. At this point, computational methods that combine molecular dynamics simulations (**MD**) complemented with statistical thermodynamics analysis (**TE**) and molecular docking methods, emerge as an excellent choice to tackle these challenges.

In this work, we show how to pursue the first objective (the elucidation of binding site) using MD simulations of proteins inmersed in isopropanol/water solutions and the second challenge, showing how we can combine the results related to the solvent water structure on protein surface and molecular docking method used by AutoDock 4.

The results show that **MD simulation of proteins in Isopropanol/water solutions are able to sample the ligand binding site and the inclusion of thermodynamics waters molecules information into the conventional docking schemes used by Autodock, increase the accuracy of the protein-carbohidrate complex dilucidation**.

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INVERSE SPIN HALL EFFECT IN METALLIC BILAYERS

J. Gómez[1], N. Alvarez[1] and A. Butera[1].

Bariloche Atomic Center - CONICET, CNEA, San Carlos de bariloche, Argentine.
e-mail:gomezj@cab.cnea.gov.ar

Keywords: Ferromagnetic resonance, Spin Hall effect, spin pumping.

The inverse spin Hall effect, present in some metals, produces a charge accumulation at the end of a metallic when a spin current passes through it [1,2]. Particularly, when a ferromagnetic resonance experiment is performed on a ferromagnetic/non-magnetic bilayer (FM/NM), a pure spin current is injected on the NM layer when the FM layer fulfills the resonance condition. In this case, if the NM metal presents the inverse spin Hall effect, it is possible to detect this spin injection by measuring a voltage at the end of the sample [3]. We present in this work some recent results obtained in this kind of systems and, based on the experimental results, we discuse the origin and properties of this effect.

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UNVEILING CONFORMATIONAL SUBSTATES IN METALLO-β-LACTAMASES DURING EVOLUTION

M. M. Gonzalez, L. A. Abriata, P. E. Tomatis and A. J. Vila

Instituto de Biologia Molecular y Celular de Rosario (IBR), Rosario, Argentina

Metallo-Beta-Lactamases (MBLs) represent one of the most relevant bacterial resistance mechanisms.[1] The broad substrate spectrum of MBLs is attributed to the particular topology of the active site, a shallow groove formed and flanked by several loops (L3, L7, L10 and L12). We have shown that mutations N70S and G262S, which alter the hydrogen-bond network connecting loops L3 and L12, give rise to evolved enzymes with an extended substrate spectrum.[2] In order to explore the role of these mutations in the loops flexibility and (within a broader perspective) the role of flexibility in protein evolution, we decided to study the backbone dynamics of wild type BcII (BcII wt) and *in vitro* optimized mutants.

BcII wt, N70S, G262S and N70S/G262S exhibit a relatively rigid backbone in the pico-to-nanosecond time scale[3], with a similar S^2 profile and a slight increase in flexibility in loop L3.

Conformational exchange processes that occur on micro-to-millisecond time scales, were probed by using CPMG relaxation dispersion methods.[4] We observed that in the wt enzyme there is only eight residues in conformational exchange. During the analysis of mutation N70S, which is deleterious in the wild type background, there is no residues with conformational dynamics. Interestingly, the G262S mutation displays an enhanced relaxation profile for several residues, most of which are located in and near loops L7 and L10. In addition, the N70S/G262S double mutant showed residues located in and near loops L3, L7, L10 and L12 with an enhanced relaxation dispersion profile. This conformational dynamics in the active site suggests that flexibility is a key trait for MBLs evolution.

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LOCAL STABILITY ON A DISORDERED DOMAIN FROM DCL1 OF A. THALIANA AND ITS MODULATION BY POINT MUTATIONS

G. Hails, I. Suarez, P. Burdisso, R. Rasia.

Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina
e-mail:guillermohails@gmail.com

Keywords: Intrinsically disordered proteins, miRNA, Dicer proteins

MicroRNAs are small RNA molecules of about 22 nucleotides which regulate gene expression at a post-transcriptional level in multicellular organisms. In plants, miRNA are processed in the nucleus from an endogenous transcript (pri-miRNA). The processing complex is comformed by DICER-LIKE1 (DCL1), HYL1 and SERRATE. DCL1 has a central role is the recognition and processing of heterogeneous plant precursors. A. thaliana´s DCL1 has two double strand RNA binding domains (dsRBD) displayed in tandem in the C-terminal end of the protein. In a previous work, we have seen that both dsRBDs bind with either dsRNA or dsDNA with similar affinities but are more specific for dsRNA together as a complex. We have also observed that the first dsRBD is intrinsically disordered. However, it adopts a structured conformation when binding to its ligand dsRNA and shows evidence of defined structure in presence of SDS micelles.

We demonstrated that the unstructured nature of the first dsRBD in DCL1 is characteristic of *A. thaliana* by evaluating the folded conformation of the homologue domain in mouse Dicer. Using in silico modelling of the first dsRBD, we designed four mutants in order to induce a structured conformation of the domain even in absence of ligand. We performed urea titration and evaluated the interaction with SDS micelles over the wild type domain and its four mutants. Both experiments were followed by NMR spectroscopy. We were able to divide the domain in regions with different folding level. The differences in these regions could have biological implications.

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SPECIFIC ROLES OF THE SCO PROTEINS IN THE ASSEMBLY OF THE MITHOCONDRIAL Cu_A SITE

Morgada MN[a], Abriata LA[b], Vila AJ[a]
[a]*Instituto de Biología Molecular y Celular de Rosario (IBR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario-Conicet, Rosario, Argentina*
[b]*Swiss Federal Institute of Technology, École polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland*
e-mail: morgada@ibr-conicet.gov.ar

Keywords: Metallochaperones, Cu_A sitefont, Protein NMR

The dinuclear copper center Cu_A is the electron entry point of the cytochrome *c* oxidase (COX). It funnels the electrons from reduced cytochrome *c* to the Cu_B center at subunit I of COX where O_2 is reduced to water molecules. The correct assembly of this metal center is essential for the function of the complex and thus for the survival of the cell.

Metallochaperones are proteins capable of binding copper ions and transfer them to their target proteins that use them as a cofactor, and at the same time reduce the toxic effect. In the case of the assembly of the Cu_A site in humans, two proteins of the Sco family have been found as essential for its formation: Sco1 and Sco2.[1]

Both proteins binds copper ions through a $CxxxCx_nH$ motif, being able not only to transfer copper but also able to transfer the electrons to maintain the Cys from the Cu_A protein in its reduced state for the binding of the copper ions.[2]

Using a model of the eukaryotic oxidase, we followed by NMR the interactions between these two putative metallochaperones finding that Sco1 is able to transfer to copper while Sco2 is important to maintain the Cys ligands in the reduced state.

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CONICET, ANCYPT

MAGNETIC INTERACTIONS IN CO(II)FUMARATE AND CO(II)-DOPED ZN(II)FUMARATE

N.I. Neuman[1], E. Winkler, O. Peña, M.C.G. Passeggi, A.C. Rizzi, C.D. Brondino

[1]*Departamento de Física, Facultad de Bioquímica y Cs. Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.*
e-mail:niconeuman@gmail.com

Keywords: Cobalt, EPR, Exchange, Zero-field splitting

High spin Co(II) (S = 3/2) compounds possess highly anisotropic magnetic properties which are very sensitive to the coordination environment; hence they are used as spectroscopic probes replacing ions such as Zn(II) in metalloproteins[1] and also in the synthesis of molecular magnets.[2] In a ligand field, the Co(II) ion is subjected to a zero-field splitting (ZFS) of its S = 3/2 quartet into two doublets, separated by an energy 2*D*, each of which possess an effective **g**'-tensor.

We aim to study an octahedral Co(II) complex (Co(II)Fumarate) by single crystal Electron Paramagnetic Resonance and thermodynamic magnetic measurements, in order to determine the magnetic parameters of the Co(II) site (**g**-, **D**- and **A**-tensor) as well as magnetic interactions between sites.

Single crystal spectra were taken at 9.4 GHz and 5 K of Co(II)Fumarato and Co(II)-doped Zn(II)Fumarato for several orientations of the magnetic field **B** in three orthogonal crystal planes. The spectra of the diluted sample showed two octets of resonance lines from which centers the molecular g'-factor was calculated, whereas those of the pure compound showed for all orientations a single broad line.

Least-squares fitting of the resonance lines allowed us to determine the molecular **g**'-tensor of the Co(II) site (g'_1=5.14, g'_2=4.89, g'_3=2.61, Fig. 1). These effective g'-factors, associated with the ground doublet, are related to the real **g**-tensor and to the rhombicity E/D factor of the ZFS.[3] Combination of these results with magnetization and magnetic susceptibility measurements allowed us to obtain the *D* factor of the ZFS (*D* = 65 cm^{-1}), typical of an octahedral Co(II) site, and to determine weak antiferromagnetic interactions between Co(II) ions (*zJ* ~ -0.38 cm^{-1}). The analysis of single crystal EPR spectra allowed us to discriminate interactions transmitted through H-bonds and the fumarate ligand.

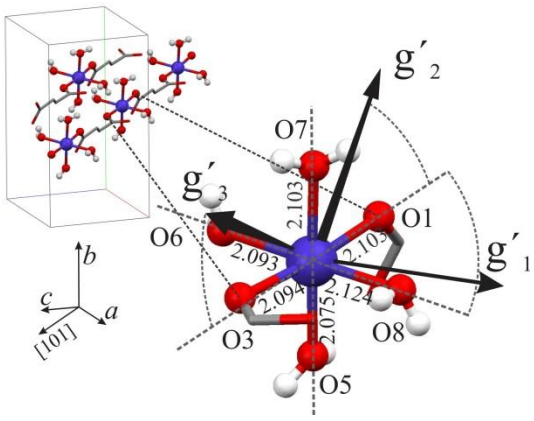

Figure 1: Orientation of the effective **g**'-tensor in the Co(II) site.

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CONICET, ANPCYT, UNL

ESR SPECTROSCOPY: AN USEFUL TOOL TO STUDY INTERACTIONS IN TWO DIFFERENT BIOLOGICAL SYSTEMS

G. Facorro, A. Cimato, M. Martínez Sarrasague, L. Piehl.

Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Argentina. gfacorro@ffyb.uba.ar

Keywords: pulmonary surfactant, sperm cells, ESR, lipids.

1) Studies of interaction between an exogenous pulmonary surfactant (EPS) and serum components, in order to elucidate the mechanisms by which pulmonary surfactant is inactivated by serum. Some serum components generate changes in the surfactant properties and activity. Using spin labels and ESR we found that these components interact with EPS phospholipids and increase rigidity of the bilayer in both, the hydrophobic core and the proximity to the polar region. Statistical analysis showed that surfactant activity correlated with the fluidity in the polar area but not with that in the hydrophobic region. We obtained strong evidence that among all the serum components tested, HDL is the one that causes the structural changes that compromise surfactant performance.

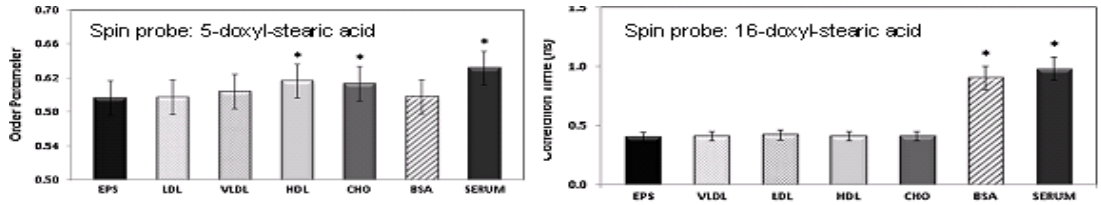

Figure 1: Order parameter (S) and the Rotational correlation time (τ) of EPS and EPS added with VLDL, LDL, HDL, cholesterol, albumin (BSA) or serum.

2) Studies of interaction between sperm cells (SC) and additives used in cryopreservation, a process that severely reduces sperm viability, in order to improve SC quality after thawing. Cryocapacitation is a deleterious pathway which occurs during SC cryopreservation. Cholesterol is a key molecule in this process. Boar SC present the lowest membrane cholesterol/phospholipids ratio (0,26) and this cells are the most susceptible to cryocapacitation. Egg yolk (EY) is used as cholesterol source in cryopreservation protocols. We studied boar SC interaction with EY and with liposomes made of EY or commercial lipids labeled with 3β-doxyl-5α-cholestane, a cholesterol analogue. Results showed SC-cholesterol interaction when lipids were provided as liposomes but not when EY was added directly.

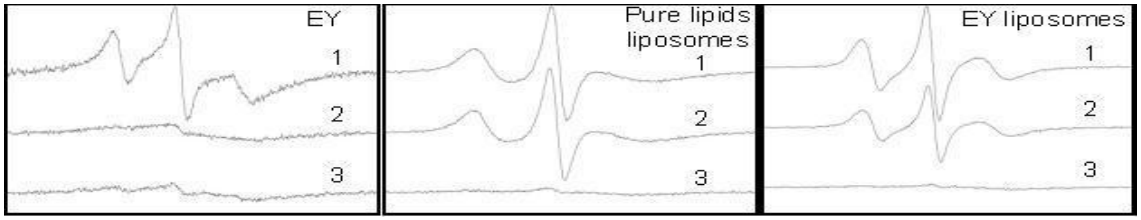

Figure 2: ESR spectra of 3-β-doxyl-5α-cholestane: 1: additive alone, 2: boar SC after incubation with the additive, 3: additive control processed as SC but without SC.

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HIGH RESOLUTION HYPERPOLARIZED J-SPECTRA WITH PARAHYDROGEN DISCRIMINATION.

I. Prina, L. Buljubasich, and R.H. Acosta

FAMAF-Universidad Nacional de Córdoba, IFEG CONICET, X5016LAE Córdoba, Argentina.
racosta@famaf.unc.edu.ar

Keywords: PHIP, parahydrogen, NMR Spectroscopy, Pulse sequence, Spin Echoes, CPMG.

Hyperpolarization by parahydrogen (PHIP)[1] has become a powerful tool not only to overcome the low intrinsic sensitivity of Nuclear Magnetic Resonance (NMR) but also as a probe for catalytic reactions, contrast agent in magnetic resonance imaging (MRI) or in analytic chemistry. In complex systems the enhanced antiphase signals steaming from parahydrogen in a PASADENA (Parahydrogen and Synthesis Allow Dramatically enhanced Nuclear Alignment)[2] experiment can be partially cancelled by the presence of large thermally polarized signals or due to the magnetic fields inhomogeneities. Recently we have presented a method to obtain high resolution spectra even in the presence of magnetic field inhomogeneities by application of a modified CMPG sequence[3,4]. In this work we show a simple method to separate the thermal and hyperpolarized contributions by taking advantage of their very different evolution during this pulse sequence. The separation is obtained in combination with a property of the Fast Fourier transform algorithm (FFT). The technique is experimentally demonstrated for a mixture of hyperpolarized 1-Hexene and a large amount of CH_2Cl_2.

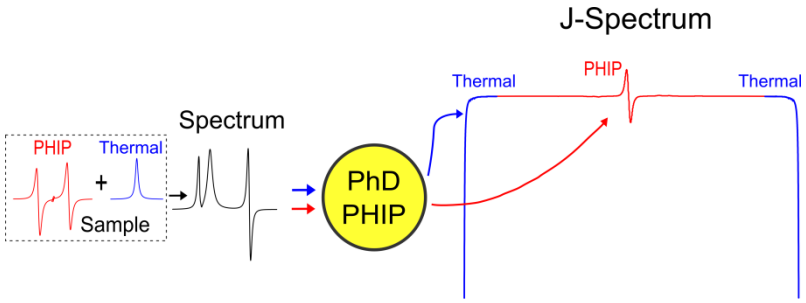

Figure: A simple NMR experimental protocol discriminates hyperpolarized PHIP signals from thermal ones. Destructive interference from both types of signals is removed and additionally highly resolved *J*-spectra are obtained.

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ELECTRON PARAMAGNETIC RESONANCE: CHARGE TRANSFER AND SPIN DELOCALIZATION ANALYSIS.

Cristina Ramirez, Alejandro Parise, Mariano Vera[1]

Dpto. Qca, FCEN, UNMdP, Funes 3350, Mar del Plata, BsAs, Argentina.

e-mail:farmramirez@yahoo.com.ar

Keywords: EPR, Charge Transfer, Arylamines, calculated spin density

The effect of the temperature on mixed-valence compounds EPR spectra has been shown to be an useful tool to determine or corroborate the capability of these compounds for charge transfer. Figure 1 shows two intervalence systems, formed by two triarylamine fragments connected by different bridges. In $B^{\cdot+}$, only little coupling has been assigned since the arylamine nitrogens have very different coupling constants (a_{N1}=6,48 G y a_{N2}=1.98 G). Interestingly, for the case of $A^{\cdot+}$, when a rigid aliphatic bridge, bearing two nitrogens is used as a bridge, unexpected charge transfer properties were found. This fact was supported by the EPR analysis, which in turn suggest that the splitting pattern assigned to N atoms indicates that the spin is symmetrically delocalized between the two arylamine fragments at room temperature, and that bridge's nitrogens contribute significantly to the electronic coupling[1]. These results were supported by both the simulated spectra and the spin density distributions obtained at the ab initio CAM-B3LYP/EPRII//6-31+G* level of theory.

The coalescence phenomenon[2,3,4] has been experimentally characterized as the localization of the electron/hole as the temperature decreases in mixed valence systems; its extent depending on the following four variables: a) the value of *kT,* b) the reorganization energy (dependent on both compound and solvent), c) the electronic coupling between the redox sites and, d) the characteristic time scale of the physical phenomenon involved in the spectroscopic experiment. The relationship between them determines whether specific spectroscopic marks will appear in a particular spectrum, either related to a single fragment (if the electron exchange between sites is slow) or

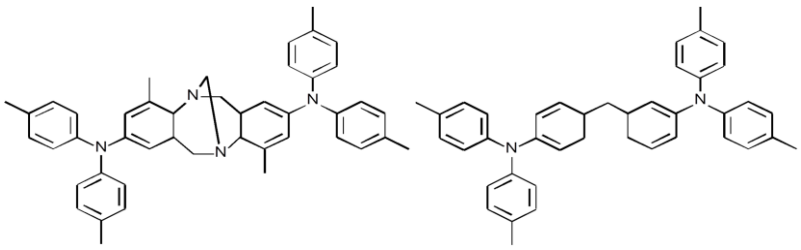

Figure 1: Trôger Based bridge and methylene bridge. Insight: spin distribution calculated. Isosurface: 0.025 a.u.

both sites (if the exchange is fast).

The EPR spectrum of $A^{\cdot+}$ showed an expected increase in intensity and a mild change in shape when temperature decreases. However, even at -100° C, the four-nitrogen splitting pattern is still identifiable. The absence of a clear electron-localized signature in this system contrasts with other bis-arylamines, for which complete EPR-coalescence phenomena in the range -100º C to 0º C, with full localization at low temperature, have been reported. This findings emphasizes the unusual charge transfer properties for this mixed-valence system bridged by an aliphatic moiety.

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FOLLOWING THE FORMATION OF CHAPS MICELLES WITH SPIN LABEL EPR AND FACTOR ANALYSIS

P.M. Rodi[1], M.D. Bocco Gianello[1], A. M. Gennaro[1,2]

[1]*Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, UNL, Santa Fe*

[2]*IFIS Litoral (CONICET-UNL), Santa Fe*.

e-mail: prodiar@yahoo.com.ar

Keywords: detergents, self- aggregation, EPR spectroscopy.

CHAPS is a zwitterionic synthetic detergent derivative from bile salts. It is a facial detergent, and due to its peculiar characteristics, CHAPS self assembly has been subject of several works in the last years, and there are open discussions regarding the characteristic of the resulting micelles. In this work we obtain EPR spectra of the spin label 12-SASL in CHAPS solutions of a wide range of concentrations. The spectra show strong changes with concentration. Using Principal Factor Analysis we demonstrate that all the spectra can be reproduced as linear combinations of only three fundamental spectra, and obtain their relative weights at each detergent concentration. One of the spectra corresponds to 12-SASL free in water, and the other two correspond to the label in motionally restricted environments. One of the motionally restricted components appears at a concentration coincident with the experimentally reported cmc of CHAPS, and it was assigned to 12-SASL in a “Type 1” micelle. The relative weight of this component gradually decreases at increasing concentrations, with the rise of the component assigned to a “Type 2” micelle. Rotational correlation times of the spin labels for each of the micelles were obtained by fitting the spectra with “slow motion” conditions. From these times, lower bounds for the micelle radii were estimated. Molecular Dynamics simulations of CHAPS micelles are in progress.

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CONICET, UNIVERSIDAD NACIONAL DEL LITORAL

INFLUENCE OF THE INCORPORATION OF FIBERS IN BISCUIT DOUGH. CHARACTERIZATION BY TIME DOMAIN NMR.

M.R Serial[1], M.S. Blanco Canalis[2], M. Carpinella[1], A.E León[2], P.D. Ribotta[2], R.H. Acosta[1]

[1]FAMAF-Universidad Nacional de Córdoba, IFEG-CONICET, Córdoba, Argentina.
[2]Instituto de Ciencia y Tecnología de Alimentos Córdoba, CONICET-UNC, Argentina
e-mail:raquelserial@gmail.com.

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Several epidemiological and experimental studies show that consumption of determined food products can act either as protective or as risk factors on non-transmissible diseases. Recommendations have emerged that aim to reducing the consumption of sugars and fats, or to increase the fiber content (particularly soluble) in the diet. Biscuit dough is a complex system containing abundant components in different states, such as starch, gluten, lipids (flour constituents), sugars, fats and water. In biscuits, both, the incorporation of fibers or reduction of sugar and fat, create number of technological problems in processing and, in some cases, a loss of acceptability [1]. For this reason, it is of great interest to study the effects on the physico-chemical and structural characteristics of biscuit dough upon the incorporation or reduction of these ingredients, as well as their partial replacement with fiber [2].

The study of a multicomponent system is a complex subjet, scarce research can be found on this topic and just a few focuses on the microscopic properties related with the interactions between principal ingredients (flour, water, type of fat and sucrose). Low resolution NMR is an important tool as it allows the study of water mobility by means of relaxation time measurements (T_2) in the sample, in a non-invasively, fast and accessible way. Bread dough has been analyzed in several articles by low field NMR [3,4], however systems containing sugar such as cake and biscuits, are less found in literature [5,6]. In such complex systems, many components that reduce the mobility of the water present in the dough coexist, giving rise to a distribution of mobilties. In this way it is possible to identify flour constituents, sugars and fats, by measuring the different proton populations associated with a given relaxation time [6].

In this work we study the proton water mobility in standard biscuit dough through relaxation profiles obtained from CPMG sequence at 0.5 Tesla using a Bruker minispec mq20 spectrometer. Different populations are assigned: the first two correspond to intra and inter-granular water respectively, and can be attributed to protons in interaction with flour constituents, while the third population is associetted with the fat components of the dough. We also corroborate that the second population is the more sensitive to water content and therefore the most affected in the cooking process [6]. Finally, the dependence of mobility as a function of the dough temperature in a cooking process is correlated with the quality of the final product upon the incorporation of soluble and insoluble fiber (inulin and oat fiber respectively) with flour reduction.

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INTRA-ANEURYSMAL FLOW DETERMINATION BY 3D VELOCITY MAPS USING LOW FIELD NMR TOMOGRAPHY

J. Perlo[1], E.V. Silletta[2], E. Danieli[1], G. Cattaneo[3], R.H. Acosta[2], B. Blümich[1], F. Casanova[1]

[1]ITMC, RWTH Aachen University, Aachen, Germany
[2]FaMAF-Universidad Nacional de Córdoba & IFEG-CONICET, Córdoba, Argentina
[3]AcandisGmbH u. Co. KG, Theodor-Fahrner-Straße 6, 75177 Pforzheim, Germany.

e-mail: silletta@famaf.unc.edu.ar.

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Dynamic NMR imaging is the only method capable of measuring velocity patterns within flowing systems in a complete non-invasive way [1]. This makes MRI velocimetry an attractive technique to study flow phenomena in many areas of research, in particular medical technology, where stents are use to treat aneurysms. An aneurysms is caused by a local weakness of a vessel wall, which enlarges the size of an artery or vein. The two events that dominate the evolution of an intracranial aneurysm are growth and rupture, being both dependent on intra-aneurysmal flow [2]. Decreasing the intra-aneurysmal flow is one way of treating intracranial aneurysms. This can be achieved by inserting a stent. Knowledge of the velocity patterns in aneurysms is essential to understanding and treating the disease.

In this work we present low-field NMR imaging to study intra-aneurysmal velocity patterns under steady flow conditions before and after insertion of a stent. 3D velocity maps of fluids with rheological properties similar to those of blood were measured with spin-echo imaging using a portable low-field tomograph (0.22T) [3] to study the flow behavior in a phantom of an aneurysm. It was possible to determine the influence of curvature of the artery on the flow pattern in the aneurysm. In absence of a stent, a rotational vortex is observed when the artery is curved. In the presence of a stent the flow in the aneurysm is greatly attenuated.

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CONICET, MINCYT

DCL1-DSRBD1: AN INTRISICALLY DISORDERED DOMAIN WHICH FOLDS UPON BINDIN TO THE SUBSTRATE RNA

I. P. Suarez[1], P. Burdisso, G. Hails, R. M. Rasia

[1]*Institute for Molecular and Cellular Biology of Rosario*, Rosario, Argentina.
e-mail: isuarez@ibr-conicet.gov.ar

Keywords: DCL1, miRNA, intrisically disordered protein.

Biogenesis of small RNAs is a complex process involving ribonuclease III like enzymes of the Dicer family[1]. In A. thaliana the processing of miRNA is carried out exclusively by DCL1, which performs two sequential cleavage steps necessary to precisely excise mature miRNA from its longer precursors, pri-miRNA[2]. Structural features which allow DCL1 to process this heterogeneous group of precursors still remain to be elucidated.

In order to understand RNA recognition by DCL1 we studied its first double-stranded RNA binding domain (dsRBD1) from a structural perspective. This domain is the first one out of tow located in tandem in the C-terminal region; and it is conserved among all Dicer like proteins. A mutant harboring an insertion that truncates the domain is embryonic lethal[3]. We produced four different constructs of the protein, spanning the annotated domain alone and including surrounding regions. The domain is unstructured in every case. We explored different solution conditions and additives to test what could lead the domain to acquire an ordered structure, and found evidence that it folds in the presence of dsRNA. Acquiring of standard set of NMR experiments for resonance assignment (HN-HSQC, HNCo, HN(Ca)Co, HNCa, HN(Co)Ca, HNCaCb, HN(Co)CaCb) we were able to assigne most backbone resonances corresponding to the free unfolded and bound folded protein. Analysis of NMR data of the free protein shows it has some tendency to acquire secondary structure on the C-term end. We have calculated the structure of the folded protein in complex with dsRNA employing CS-Rosetta. The structure corresponds to a canonical dsRBD, bearing some differences. One of the three typical regions for RNA binding is missing, but affinity for the substrate is not affected. Finally we found that in the presence of excess dsRNA the unfolded form is still present, but it is not the same as the free unfolded form. On ZZ exchange experiments, we found this unfolded form is in slow exchange with the folded form. Based on these results, we can propose a model for binding event, which involves a complex equilibrium between free unfolded, bound unfolded and bound folded species.

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A MULTI-PROBE APPROACH TO STUDY A PDZ DOMAIN FOLDING

Torchio, Gabriela[1,2]; Arán, Martín[2,3]; Gallo, Mariana[2,3]; Burgos, Inés[2,4]; Fidelio, Gerardo[2,4]; Ermácora[1,2], Mario; Sica, Mauricio[2,5]

1. Laboratorio de Plegado y Expresión de Proteínas, Depto de Ciencia y Tecnología, Universidad Nacional de Quilmes-IMBICE (La Plata)-CIC-CONICET, Argentina.
2. Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
3. Fundación Instituto Leloir-CONICET, Argentina.
4. Centro de Investigaciones de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional d|e Córdoba, Argentina.
5. Centro Atómico Bariloche

gabrielatorchio@gmail.com

Keywords: protein folding, PDZ domains

Here we present a preliminar study of the unfolding of the PDZ domain of the β2-syntrophin protein (β2S-PDZ), which is directly involved in the regulation of insulin secretion. PDZ domains are modules of protein-protein interactions, and for the role they play in the cell, they are expected to have some degree of conformational plasticity.

Protein folding is usually analyzed using the equilibrium thermodynamics approach. The experimental techniques most frequently used for this purpose, as circular dichroism spectroscopy (CD), differential scanning calorimetry (DSC) and flourescence spectroscopy, provide information about the thermodynamic states and parameters, but they probe only global reaction coordinates, without providing details of what happens at residue level. In recent years, protocols that introduce Nuclear Magnetic Resonance (NMR) as a high resolution probe for protein folding have been developed. The idea is to analyze unfolding curves of individual residues, using traditional (un)folding models. This approach has been already applied to the study of the folding of BBL domain from the 2-oxo-glutarato deshidrogenasa multienzymatic complex from *E. coli*, and the gpW protein from λ bacteriophague (1-3). □

Our previous results, obtained from thermal unfolding experiments followed by CD and DSC, show that β2S-PDZ does not behave as expected for a two-states or three-states protein unfolding process. Instead, we believe that β2S-PDZ follows a multistate mechanism (4)□. Our goal is to determine if the NMR approach is suitable to study β2S-PDZ folding at residue level.

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CHARACTERIZATIÓN OF POROUS ORGANIC MATRICES IN THE SWOLLEN STATE BY NMR

M. I. Velasco[1], E. .V. Silletta[1], C. G. Gomez[2], M. C. Strumia[2], G. A. Monti[1] y R. H. Acosta[1]

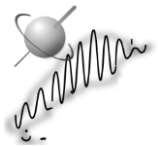
1)FaMAF, IFEG -CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.
2)Depto. de Química Orgánica, Facultad de Ciencias Químicas, IMVIB- CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.
e-mail:mvelasco@fcq.unc.edu.ar

Keywords: *Porous polymers, CPMG,*

Polymer matrices with well defined structure and pore sizes are widely used in several areas of chemistry such as catalysis, enzyme immobilization, HPLC, adsorbents or droug controled release. These polymers have pores in its structure both in the dry and swollen state. Although it is well known that the structures and properties greatly differ between these two states, only a few methods provide information about the swollen, even though most of the applications involve the matrices in this situation. Nuclear Magnetic Resonance (NMR) is a suitable tool for the study of the molecular dynamics of different liquids spatially confined in macro, meso and nanopores through changes in relaxation times. In transverse relaxation experiments, either diffusion inside the pore, or relaxation induced by mobility restriction of the liquid near the wall, are additional sources of relaxation, which are extremely useful in the determination of structural and functional properties. In particular the use of ^{1}H-CPMG allows the determination of the transverse relaxation time (T_2) of the molecules of liquid, and this can be related to the pore size at which it has bound. Hence through relaxometry data it is not only possible to perform the characterization of the material in the swollen state, but also to monitor the behavior of the matrix during evaporation.

For this work we synthesized polymers of 2-hydroxyethyl methacrylate (HEMA) cross-linked with ethylene glycol dimethacrylate (EGDMA), varying the proportion of crosslinking between 6 and 33%. The amount of crosslinking has a marked effect on the final properties of the material. Given the polar characteristics of the matrices, heptane was used to characterize the dry state, since the molecules enter in the pores but do not solvate the polymer chains, so the network morphology is not altered. On the other hand, water was used to study the properties of the matrix in the swollen state. Analizing the distribution of T_2 valuesis possible to determine parameters that are useful in the characterization of the material, such as pore size distribution, porosity and the contribution of each pore population to the total porosity. Furthermore, this technique allows the observation of the variation in the distribution of pore populations as the swelling liquid evaporates. This enables to infer specific properties of each organic matrices under studied.

Sumarizing, NMR relaxometry provides information of the pore structure of different polymeric matrices. These parameters are useful for the characterization of porous materials and, unlike most conventional techniques, this information can be obtained in both the dry and the swollen state. The swollen state study also provides information on the structure of the mesh network. This technique represents a direct, simple, quick and non-destructive approach to determine the porperties and architecture of organic porous materials.



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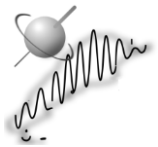

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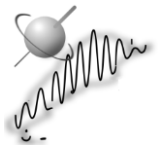

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