MAGNETIC RESONANCE
IN A CORDUBENSIS PERSPECTIVE

SEPTEMBER 5th TO 7th, 2011

Alta Gracia, Córdoba, Argentina

EXTENDED ABSTRACTS BOOK

PROGRAM

MAGNETIC RESONANCE IN A CORDUBENSIS PERSPECTIVE

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Sunday, September 4th

21:00 – Reception for early arrivals

Monday, September 5th

10:00 – Opening session – Organizing Commitee

10:15 – Lecture – Alejandro Vila, Instituto de Biología de Rosario, Argentina

11:00 – Coffee Break

11:30 – Lecture – *NMR dipolar constants of motion in liquid crystals: a test of bed for spin relaxation and decoherence theories in correlated systems.* Ricardo Zamar, Universidad Nacional de Córdoba, Argentina

12:15 – Oral Communication I –

12:35 – Oral Communication II –

13:00 – LUNCH

15:00 – ISMAR Presentation – Hans Wolfgang Spiess, ISMAR President

15:15 – Lecture – *Spin label EPR, a useful tool in biophysical research.* Ana María Gennaro, INTEC, Sante Fe, Argentina.

16:00 – Lecture – Daniel Pusiol, Spinlock SRL ,Argentina

16:45 – Coffee Break

17:20 – Oral Communication III –

17:40 – Oral Communication IV –

18:00 – Oral Communication V –

18:20-19:30 – Poster Session–

Tuesday, September 6th

09:00 – Lecture – Hans Wolfgang Spiess, Max Planck Institute for Polymer Research, Germany

09:45 – Lecture – *Cordubensis developments in field-cycling NMR.* Esteban Anoardo, Universidad Nacional de Córdoba, Argentina

10:30 – Coffee Break

11:00 – Lecture – Claudio Fernandez, Instituto de Biología de Rosario, Argentina

11:45 – Lecture – Patricia Levstein, Universidad Nacional de Córdoba, Argentina

12:30 – LUNCH

15:00 – Lecture – *Applications of fMRI.* Gustavo Foa Torres, Instituto Oulton

15:45 – Oral Communication VI –

16:05 – Coffee Break

17:00 – CITY TOUR / Argentinian NMR Organization Meeting –

21:00 – Conference Dinner –

Wednesday, September 7th

09:00 – Lecture – *New advances in low and zero field NMR.* Tito J. Bonagamba, Instituto de Física de São Carlos, Universidade de São Paulo, Brazil.

09:45 – **Lecture** – Máximo Ramia - MR Technologies SA, Córdoba, Argentina

10:30 – Coffee Break

11:00 – Lecture – *Fast screening and fold elucidation of proteins.* Rodolfo Rasia, Instituto de Biología de Rosario, Argentina

11:45 – Oral Communication VII–

12:05 – Oral Communication VIII–

11:25 – Oral Communication IX–

12:45 – Closing Session

13:00 – LUNCH

LECTURES

MAGNETIC RESONANCE IN A CORDUBENSIS PERSPECTIVE

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Alta Gracia, Córdoba, Argentina

INVISIBLE STATES IN PARAMAGNETIC COPPER PROTEINS

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Keywords: metalloproteins, copper, electronic structure, paramagnetic

NMR of oxidized copper proteins has been largely overlooked, mostly due to the slow electron relaxation times of Cu2+ ion which induce extremely fast relaxation rates in nearby nuclei, rendering them undetectable. It has been shown, however, that these unfavorable electron relaxation features are restricted to T2 copper sites, since T1, T3 and CuA centers display faster electron relaxation rates which make them amenable to NMR studies. In all these cases, the fast electron relaxation stems from to the availability of low-lying excited electronic states which is due to the particular electronic structure of these centers. These features are strongly related to the physiological requirements of these copper centers to perform efficient electron transfer or oxidation chemistry.

The binuclear copper sites CuA and T3 display particularly fast electron relaxation rates which are due to low-lying excited states that can be populated at room temperature and contribute to the reactivity of the metal site. Other magnetic techniques, such as EPR, ENDOR and MCD, normally recorded at cryogenic temperatures, are able to monitor exclusively the ground state. NMR in solution, instead can shed light on the availability of these invisible electronic states. We have studied different mutants of a native CuA site in which small perturbations are able to tune the energy gap between the ground state and the invisible excited state without perturbing the electronic structure of each of them, thus providing a mechanism to regulate the electronic structure of the metal site at room temperature.We have also studied a multicopper oxidase, Fet3 from yeast, in which signals from the T1, T2 and T3 centers could be identified and assigned to each metal site. The temperature dependence of the hyperfine shifts reveals the accesibility of the invisible electronic states in the T3 site of this oxidase, which differ from the description for homologous T3 centers present in other enzymes, again suggesting a role of these excited states in regulating the chemistry of the metal binding site.

ANPCyT and CONICET

NMR DIPOLAR CONSTANTS OF MOTION IN LIQUID CRYSTALS:

A TEST BED FOR RELAXATION AND DECOHERENCE THEORIES IN CORRELATED SYSTEMS.

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Keywords: constants of motion; decoherence; open quantum systems; spin-temperature.

Proton NMR is a very useful tool for studying molecular motions and spin dynamics in liquid crystals (LC), through a variety of relaxation and spin manipulation experiments. In LC's the inter-molecular dipolar spin interaction averages out due to the rapid molecular diffusion, while a large residual intra-molecular dipolar interaction remains. Hence, the molecular proton spins can be treated as small dipole interacting spin clusters, magnetically isolated from spins at other clusters. This characteristic poses a distinction on the way that NMR relaxation and decoherence should be treated and at the same time makes LC an excellent testbed for probing theories on irreversible spin-dynamics of open quantum systems.

The unique cooperative molecular ordering of the mesophases makes the direct application of the theoretical background on the field of solid and liquid NMR inapropriate, both for relaxation and lineshape phenomenae. Early studies of dipolar order relaxation as a function of Larmor frequency and temperature soon showed a singular behavior of T_{1D} relative to other known relaxation parameters, as T_1 and T_{1Q}., since the Redfield-Bloch relaxation theory cannot explain the strong response of T_{1D} to the cooperative motions. Within the same hierarchy of problem is the description of the nature of the initial spin states that can be prepared in the laboratory with the phase shifted two pulse sequence designed to create dipolar order (Jeener-Broekaert). Despite the low dimension of the Hilbert spin space of LC molecules, the phenomenolgy of dipolar signals is quite similar to the solid case. However, since the concept of spin diffusion cannot be evoked to justify quasi-equilibrium in small clusters, the observed build-up of quasi-equilbirium states over a timescale much shorter than thermalization must be understood as being a consequence of decoherence of the spin state due to the coupling of spins with a quantum environment. All these facts urge exploration on the nature of the processes that rule the spin dynamics, along the complete experimentally available timescale.

In this talk, irreversible decoherence as a via to quasi-equilibrium in LC's will be discussed from the perspective of open qantum systems. Refocusing experiments with the magic echo sequence will be used to show the occurrence of a decoherence time scale, intermediate between quantum interference and relaxation, in consistency with theoretical predictions. The possibility of transferring the equilibrium Zeeman order to multi-spin-order states will be illustrated analitically and numerically on spin models of a real LC molecule. In a closed system, constants of motion other than the total energy are expected; their number could rise to about 2^N in an N-spin cluster. However, we show that the quasi-equilibrium matrix can be fully spanned by a few quasi-invariant (e.g. around 10 operators for 8 spins) within the whole scale of experimentally accessible preparation times. Moreover, it is shown that for several preparation conditions, only two of such quasi-invariants are necessary to describe the spin state. This analysis provides the extension of the “spin-temperature” view to spin systems with few degrees of freedom.

SPIN LABEL EPR, A USEFUL TOOL IN BIOPHYSICAL RESEARCH

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Keywords: electron paramagnetic resonance; nitroxide radicals; biomembranes.

Electron paramagnetic resonance spectroscopy (EPR, or ESR) requires the presence of unpaired electron spins in the sample under study. However, it is still possible to study non paramagnetic systems with the aid of spin labels, which are molecules bearing stable nitroxide radicals. Water soluble spin labels allow us to estimate microviscosity in trapped water regions, like the inside of a cell. On the other side, lipid like spin labels can be incorporated to biological membranes or model systems, allowing us to obtain a depth profile of the ordering of the lipid chains from the EPR spectra. This information is relevant in order to characterize the physical state of the lipids, and allows us to estimate changes in their interactions caused, among others, by compositional changes or by specific treatments.

In this talk I will present results obtained in our lab in a wide range of biological systems or their models using spin label EPR. Among them, the measurement of microviscosity in the inside of red blood cells[1]; the characterization of self assembled lipid structures[2]; the detection of phase transitions in simple model systems serving as biomembrane models; and the comparison of lipid ordering between cell membranes and their detergent resistant portions[3]. Other capabilities of this spectroscopy will also be commented.

REFERENCES

1. Gennaro A.M, Luquita A., Rasia M., *Biophys J* 71, 389-393 (1996)
2. Pincelli M.M., Levstein R.R, Fidelio G.D., Gennaro A.M. Chem Phys Lipids 104, 193-206 (2000); Levstein P.R., Gennaro A.M., Pincelli M.M. *Recent Research Developments in Biophysical Chemistry* 2002. Transworld Res. Net.pp- 165-180.
3. Rivas M.G., Gennaro A.M. *Chem. Phys. Lipids* 122, 165-169 (2003); Rodi P.M., Cabeza M.S., Gennaro A.M., *Biophys. Chem.* 122, 114-122 (2006); Rodi P.M., Trucco V.M., Gennaro A.M., *Biophys. Chem.* 135, 14-18 (2008).

WITH SUPPORT FROM CONICET, UNL AND ANPCYT

ON GOING FROM BASIC RESEARCH TO INDUSTRIAL APPLICATIONS: THE SPINLOCK CASE

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In this paper we show an effort to provide discrete and in-line analysis of solids, soft matter and multi-phase products using robust NMR technology. Hardware and Software are tailor-made for industry. Solutions which can be either desktop, transportable and/or in-the-field units and can be adapte for a variety of environments. Succesful track records of scientific achievements and commercial applications in several Industris are actually in use in several innovative leader industries: Food, Oil & Gas, Biodiesel, Pharmaceutical and Security.

We proved that an Young and Multidisciplinary compositions in the SpinLocks Human Resources Matrix is the key to reach an efficient synergia to reach so high scientific and technological goals.

In addition we show how SpinLock can takes advantage of the hughe number (about 150,000) of students and high educated Scientists and Technologists available in the Córdoba City. In particular, the National University of Córdoba posesses Research Groups in Pure and Applied Physics, Mathematics, Chemistry, Biology, Computer Science which can be easily reached by SpinLock for collaborations, giving an extra competitive advantage to the SpinLock Scientific Developments.

MAGNETIC RESONANCE STUDIES OF NANOSTRUCTURED FUNCTIONAL SYSTEMS

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Functional nanostructures are in the focus of current soft matter science. They occur in advanced synthetic as well as in biological systems through self-assembly of carefully chosen building blocks. Secondary interactions such as hydrogen bonding, aromatic pi-interactions, and electrostatic forces are of central importance. Here, magnetic resonance provides unique and highly selective information on structure and dynamics of such systems[1], e.g., on hydrogen bond networks in the solid state,[2] stacking, and cooperative molecular motions of discotics[3] and macrocycles.[4] Solid state NMR is also able to elucidate self-assembly, conformation and dynamics of polypeptides.[5]

The phase separation and dynamic heterogeneities of thermoresponsive dendritic polymers can conveniently be studied with a desktop EPR spectrometer[6]. With more advanced pulsed EPR techniques, such as DEER, distances on the scale of several nm can be measured. This has recently been used to unravel the structure of Human Serum Albumin, a versatile transport protein in the blood. It was found that the functional protein structure contains a more rigid, asymmetric inner part, while the surface of the protein shows much larger structural flexibility[7].

For full structural and dynamic elucidation, the spectroscopic data have to be combined with other techniques, in particular X-ray scattering, microscopy, dielectric spectroscopy and last, but not least, quantum chemical calculations. The findings will be related to the function of such materials, such as proton- and photo-conductivity.

REFERENCES

1. H. W. Spiess, Macromolecules **2010**, *43*, 5479–5491
2. Y. J. Lee, T. Murakhtina, D. Sebastiani, H. W. Spiess, *J. Am. Chem. Soc.* **2007,** *129***,** 12406.
3. M. R. Hansen, T. Schnitzler, W. Pisula, R. Graf, K. Müllen, H.W. Spiess, *Angew. Chem. Int. Ed*. **2009**, *48*, 4621.
4. M. Fritzsche, A. Bohle, D. Dudenko, U. Baumeister, D. Sebastiani, G. Richardt, H. W. Spiess, M. R. Hansen, S. Hoeger, *Angew. Chem. Int. Ed*. **2011**, *50*, 3030
5. G. Floudas and H.W. Spiess, *Macromol. Rapid Commun.* **2009**, *30*, 278.
6. M. J. N.Junk, W. Li, A. D Schlüter, G. Wegner, H.W. Spiess, A. Zhang, D. Hinderberger, *Angew., Chem. Int. Ed.* **2010**, *49*, 5683.
7. M. J. N.Junk, H.W. Spiess, D. Hinderberger, *Angew., Chem. Int. Ed.* **2010**, *49*, 8755.

CORDUBENSIS DEVELOPMENTS IN FIELD-CYCLING NMR

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The art of cycling the magnetic field was born at the very early days of NMR [1]. With time, it evolved into quite different fields of applications, mainly fast field cycling (FFC) NMR relaxometry [2] and field-cycling MRI [3,4]. The hardware evolution was an important stimulus for the development of new applications, and vice versa, the particular needs of different research fields and industrial applications demanded new hardware engineering. Such development did not only occur at academic environment, but also in connection to industry-related projects. Within this frame, some recent developments and work in progress will be briefly discussed:

1- Acoustically stimulated NMR relaxometry [5,6].
2- FFC NMR elastometry: an application derived from recent studies in liposomes [7,8].
3- Specific hardware development for FFC applications [9,10].
4- Advances on the field-cycling MRI local project.

REFERENCES

1. A. Abragam, *Principles of Nuclear Magnetism*, Clarendon Press, Oxford (1961).
2. R. Kimmich and E. Anoardo, Prog. NMR Spectrosc. **44**, 257 (2004).
3. N. I. Matter, G. C. Scott, T. Grafendorfer, A. Macovski and S. M. Conolly, IEEE Trans. Med. imag. **25**, 84 (2006).
4. D. Lurie, S. Aime, S. Baroni, N. A. Booth, L. M. Broche, C.-H. Choi, G. R. Davies, S. Ismail, D. Ó hÓgáin and K. J. Pine, C. R. Physique **11**, 136 (2010).
5. E. Anoardo and F. Bonetto in *Modern Magnetic Resonance*, G. A. Webb (Ed.), Part III, pp. 1419, Springer (2006).
6. E. Anoardo, C. R. Physique **11**, 160 (2010).
7. C. Meledandri, J. Perlo, E. Farrher, D. Brougham and E. Anoardo, J. Phys. Chem. B **113**, 15532 (2009).
8. J. Perlo, C. Meledandri, E. Anoardo and D. Brougham, J. Phys. Chem. B **115**, 3444 (2011).
9. G. Forte, G. Farrher, L. Canali and E. Anoardo, IEEE Trans. Contrl. Syst. Technol. **18**, 976 (2010).
10. E. Anoardo, H. Segnorile, G. Forte, G. Farrher, P. Novisardi, and L. Revello, Patent application (in progress).

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APPLICATIONS OF FMRI

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Functional magnetic resonance imaging (fMRI) BOLD has provided newknowledge about the brain, providing a noninvasive approach in vivo to assess longitudinal changes in cortical brain activation during task performance and the interesting phenomenon of brain plasticity of the patient the postoperative period. The information can be obtained extends far beyond the cortical maps of sensory, motor, language and attention. Thefunctional maps including the orbitofrontal and medial frontal gyrus,posterior cingulate gyrus, angular gyrus, the amygdala have been described and associated with moral reasoning, emotion and the deepest forms of human thought.

The fMRI has grown into one of the most useful research techniques inmodern cognitive neuroscience, with the participation of a wide variety of researchers in fields as diverse as psychology, neurology, psychiatry, and linguistics. However, the clinical use of MRI is a relatively recent phenomenon, with just over a decade of collective experience.

Functional MRI and other advanced imaging methods continue to mature, the issue of validity becomes increasingly addressed. Therefore, with a growing sense of novelty and breadth of information that neuroimaging can offer about the complexity of human behavior, ethical concerns regarding the potential misuse of data or abuse have reached a first plane.
We must incorporate functional imaging into our research agendas and our clinical practice. These tools should be part of the core competencies ofneuroradiologists, who have the opportunity to work more closely with neurosurgeons, psychologists, mathematicians, physicists and so on.

NEW ADVANCES IN LOW- AND ZERO-FIELD NMR

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Keywords: NMR; magnetic materials, porous media.

During the last years our NMR group decided moving to low- and zero-field NMR areas, not only because they drive us to very rich physical problems, but also because we can transfer to them the principles of the advanced methods applied for high-resolution NMR, getting new and very promising results(1).

In the case of zero-field NMR, used to study magnetic materials, we are going to present one example of zero-field NMR through the study of the polycrystalline intermetallic compound $GdAl_2$. The NMR data were obtained with the sample at 4.2 K in the ordered magnetic state. Using a sequence composed of two rf pulses, we obtained five multiple-quantum echoes for the ^{27}Al nuclei, which were used to construct the zero-field NMR spectra. In order to understand the data observed, we proposed a model based on the density operator theory, in which there are two regions inside the sample with different inhomogeneous spectral line broadenings(2,3). Using this formalism, we proposed a method for selecting each multiple-quantum echo, by using a procedure based on phase-cycling and time-averaging. This method was very successful for constructing spectra from each individual echo, which are formed from the contribution of different multiple-quantum coherences and offer complementary physical information about the nuclear spin interactions.

In the case of low-field NMR, mostly used to study fluid-saturated porous media, we are going to discuss a new procedure to perform two-dimensional T_2-T_2(4) NMR exchange, which allows observing only the migrating fluid molecules and extracting useful permeability(5) data for these important materials for the oil industry. Under this new experimental method, the uppermost signals of the molecules that do not migrate between sites with different T_2's are subtracted from the raw NMR data before processing the 2D Inverse Laplace Transform. This procedure eliminates the intense diagonal ridge, which dominates the 2D T_2-T_2 exchange spectrum and makes it difficult to detect the low off-diagonal peaks, providing a more accurate study of fluid molecules that migrate between distinct sites, where the molecules present different transverse relaxation times.

REFERENCES:

1. Tozoni, J.R.; Teles, J.; Auccaise, R.; Oliveira-Silva, R.; Rivera-Ascona, C.; Vidoto, E.L.G.; Guimarães, A.P.; Oliveira, I.S.; Bonagamba, T.J. *Journal of Magnetic Resonance*, Accepted Manuscript, **2011**.
2. Solomon, I. *Physical Review*, v. 110, n. 1, **1958**, 61.
3. Abe, H.; Yasuoka, H.; Hirai, A. *Journal of the Physical Society of Japan*, v. 21, n. 1, **1966**, 77.
4. Lee, J.H.; Labadie, C.; Springer, C.S.; Harbison, G.S. *Journal of the American Chemical Society*, v. 115, **1993**, 7761.
5. Washburn, K.E.; Callaghan, P.T. *Physical Review Letters*, v.97, **2006**, 175502.

FAPESP, CNPq, and CAPES

NMR PETROPHYSICAL APPLICATIONS

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The use of NMR methods as well as with other techniques have shown to be the choice for evaluating geological formations, not only in drilled wells but also for the study of both crown and lateral plugs in the laboratory. Among the most valuable obtained information the porosity, the pore size distribution, the fraction of movable fluids, and the estimation of the rock permeability integrates the standard set of required results by the petroleum industry. From the pure research field the NMR provides a non destructive tool to study natural porous media. Thus the same NMR principles used to study physical systems or to diagnose anomalies in the human body, or to find macromolecular morphology, etc. can be used to analyze the fluids in the pore spaces of sedimentary rock reservoirs. Complementary techniques to NMR as the determination of resistivity, gravimetric methods, mercury injection, controlled centrifuge are necessary to provide a whole picture of the reservoir properties. Nowadays, the introduction of both multinuclear NMR and Positronium Annihilation Lifetime Spectroscopy has opened the possibility to evaluate tight shale formations with nano-pores. This seminar offers an overview of these techniques and their applications to study porous rocks and the understanding of some of the most relevant information for the petroleum industry.

FAST SCREENING AND FOLD ELUCIDATION OF PROTEINS

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Keywords: Protein NMR, fast acquisition, protein structure calculation

The advent of structural genomics have fostered an increasing interest in the development of NMR methods that provide atom-resolved structural information on proteins in short overall time using sensitive NMR pulse schemes. Previously, several longitudinal relaxation optimized experiments for proteins were introduced that allow for a substantial reduction in acquisition time and for a correlated increase in signal to noise ratio per unit time[1,2]. Reduced NMR data acquisition times become a crucial issue whenever the molecular system under investigation has a short lifetime in the NMR sample tube. It is also important for high-throughput NMR applications in the context of structural genomics initiatives.

We present here the application of these experiments to the screening of protein constructs and a new set of optimized liquid crystal NMR experiments for the obtention of Residual Dipolar Coupling (RDC) restraints. The protein fragments amenable to structural characterization by NMR are first identified from small-scale high-throughput purified samples taking advantage of the enhanced sensitivity. Then RDC datasets are acquired on the selected constructs and used directly to calculate the protein fold using the Rosetta NMR software.

Processing of microRNA in plants involves several multidomain proteins. The procedure outlined above was applied to characterize structurally the RNA binding domains of HYL1[3] and DCL1 from *A. thaliana* and their interaction with RNA substrates.

REFERENCES:

1. Schanda P and Brutscher B, *J. Am. Chem. Soc.* 127:8014-8015 (2006).
2. Lescop E *et al. J. Magn. Reson.* 187:163-169 (2007).
3. Rasia et al., *Biochemistry* 49:8237–8239 (2010)

CONICET, ANCYPT

ORAL COMMUNICATIONS

MAGNETIC RESONANCE IN A CORDUBENSIS PERSPECTIVE

SEPTEMBER 5th TO 7th, 2011

Alta Gracia, Córdoba, Argentina

HMBC key correlation in structural Elucidation of natural compounds

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Keywords: Natural products; dihydro-β-agarofuran sesquiterpenes, HMBC experiments.

Sesquiterpene esters, based on dihydro-β-agarofuran [5,11-epoxy-5 β,10α-eudesm-4(14)-ene] skeleton, are chemotaxonomic indicators of the Celastraceae Family,[1] and have received much attention in medicinal chemistry due to their wide range of biological activity, e.g., insecticidal, cytotoxic, anti-inflammatory, multidrug resistance (MDR) reversal, immunosuppressive, antiviral, anti-HIV, and anti-HSV.[2]

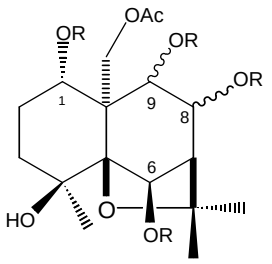
As part of a research program on biologically active metabolites from Celastraceae Family,[3] *Schaefferia argentinensis* species was studied.

Repeated chromatography on sephadex LH-20, preparative HPLC, and preparative TLC of the ethanol extract of the aerial parts of *S.argentinensis* yielded, in addition to the two known metabolites, eight new sesquiterpenes. This report aims at showing the application of solution NMR techniques to solve structural determination.

The analysis of NMR spectral data indicate that these compounds contain a skeleton based on 15 carbons, i.e.: three methyl carbons; three methylene carbons; five methine carbons; and four quaternary carbons. These data also suggest that it is a 1,4,6,8,9,15-hexasubstituted-β-dihydroagarofuran skeleton (Fig. 1).[4] All metabolites isolated have the same substituents such as hydroxyl or keto groups as well as acetyl, benzoyl and cinamoyl esters. The full and unambiguous proton and carbon NMR assignments for ten compounds were made using a combination of 1D and 2D NMR experiments.

The only difference found between the compounds was the position and orientation of their substituents.

The locations of the hydroxy and ester functions can be determined on the basis of ^{1}H-^{13}C long-range correlations (HMBC experiments). These positions were established by the key cross-correlation peaks observed for carbinyl protons of the β-dihydroagarofuran skeleton with carbonyl carbons of the substituents. The orientations of H-1, H-6, H-8 and H-9 were determined by analysis of the coupling constants and NOESY experiments.



R=Ac, Bz, Cin, O, H

Figure 1: Metabolites isolated from *Schaefferia argentinensis*

Although the structural diversity in this family of compounds is not particularly wide, a minimal variation in the type of substituent and the relative stereochemistry of each carbon in the molecule results in considerable changes in biological activity.

In the current work, HMBC experiment has proven a key tool which provides valuable information on the structural elucidation of a family of naturally occurring compounds.

REFERENCES:

1. Brüning, R.; Wagner, H. *Phytochemistry*. **1978**, *17*, 1821-1858.
2. González, A. G.;et.al. *Bioorg. Med. Chem*. **2000**, *8*, 1773-1778.
3. Spivey, A. C.; Weston, M.; Woodhead, S. *Chem. Soc. Rev*. **2002**, *31*, 43-59.
4. González, A. G, et.al. *Tetrahedron*. **1993**, 49, 697-702.

DETERMINATION OF COMPONENT FRACTIONS IN HETEROGENEOUS SAMPLES BY TIME-DOMAIN NMR USING DIFFERENCES IN T1

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Keywords: Food, Time Domain Magnetic Resonance, Heterogeneous Molecular Systems.

Many practical applications and quality control in industry make use of a low-resolution NMR pulse spectrometer in order to quantitatively determine the fraction of components in complex samples. A particular case is determining the oil and water content in samples containing water and oil with a relatively high water fraction.

The International Standard ISO 10565 specifies a method for the determination of the oil and water contents of commercial oil seeds using pulsed nuclear magnetic resonance, but it is required for the seeds to have a water content less than 10%. For higher water content, a drying process is necessary before oil content determination[1]. The disadvantages of this method are that only oil content can be determined and the measurement process requires too much time that the method becomes unacceptable when a large number of measurements is needed. Thus, as in many applications it is necessary to determine oil as well as water contents of a sample in very short measurement times, the predrying method turns out to be of scarce practical applicability, complicating their use for chemistry quality control in the industry[2].

The aim of this work was to develop a fast and accurate method for determining the fraction of components in heterogeneous samples with the aid of Time Domain Nuclear Magnetic Resonance (TD-NMR). The method comprises two procedures, the first one is used to calibrate a Low Resolution TD-NMR spectrometer and the second one is used to determine the fraction of components, particulary water and oil, in heterogeneous samples with high relative water content, without pre-drying the samples. The proposed method can be used to determine the fraction of components in systems where at least two of the components of interest have longitudinal-relaxation times (T_1) profiles which do not overlap each other. It is based on the use of inversion-recovery type pulse sequences, choosing proper time intervals between pulses in order to cancel out the signal of the component with larger mean value of T_1[3]. Calibration and measurement processes are performed by a combination of spin-echo and inversion recovery pulse sequences, and require the measurement of T_1 recovery curves. In order to obtain practical measurement times, the proposed method makes use of fast T_1 measurement procedures[4]. The fraction of components is calculated from the NMR signals originated from the different pulse sequences taking into account corrections due to relaxation processes. The method proposed in this work was applied for determination of fractions of oil and water in olive pastes with errors within 2.6 % for water and 0.4 % for oil.

REFERENCES:

1. International Standard ISO 10565, “Oilseeds - Simultaneous determination of oil and water contents - Method using pulsed nuclear magnetic resonance spectroscopy” (1998-08-15)
2. G. Rubel, *Journal of the American Oil Chemists Society*, **1994,** *71*(10), 1057.
3. Bernstein, M. *et al*; “Handbook of MRI pulse sequences”, Elsevier-Academic Press, **2004**, 625.
4. Sigmund, E. E. *et al*; *Sol. State Nuc. Mag. Reson.* **2006**, *29*, 232.

CONICET, FONTAR, SPINLOCK

SOLUTION STRUCTURE AND DYNAMICS OF COX'S SUBUNIT II IN ITS NON-METALLATED FORM

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Keywords: solution structure; protein dynamics; electron transfer copper proteins

Cytochrome *c* oxidase (COX) is a terminal oxidase present in all aerobic organisms, which shuttles electrons from cytochrome *c* to oxygen using the released energy to pump protons across the membrane and thus contribute to its potential[1]. The function of COX depends critically on the correct disposition of two copper centers (Cu_A and Cu_B) and two heme moieties (*b/a* and a_3). We have previously shown that a thiol:disulfide oxidoreductase and a Cu(I) metallochaperone are required for *in vitro* maturation of the Cu_A site in *Thermus thermophilus* ba_3 oxidase (*Tt*Cu_A)[2]. During that work we gathered evidence that the structure and dynamics of the apoprotein are perturbed compared to the copper-bound form.

Here we present the NMR-based solution structure of apo*Tt*Cu_A (Figure 1) and a study of its picoseconds to seconds dynamics, both compared to the reduced holoprotein. Resonance assignment has been completed for most of the 126 residues of the protein and more than 2000 NOE-based restraints have been used for the structure calculation. Dynamics has been studied through ^{15}N-T_1 and T_2, heteronuclear NOE, λ_{NOE} and T_2-relaxation dispersion measurements. Our results show that the loops containing the metal ligands experience mobility in the absence of the metal ions, resulting in significant disorder. This suggests that the copper ions play an important role in the definition of the structure and rigidity of the ligands loops, in line with the requirement of a partially exposed site for metallation.

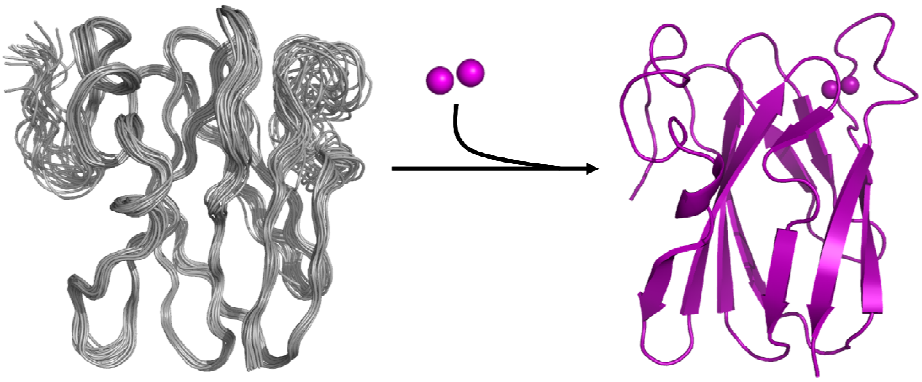

Figure 1. (left) Family of 20 structures calculated in this work for apo*Tt*Cu_A in solution. (right) Structure of the holoprotein formed by binding of 2 copper equivalents to apo*Tt*Cu_A (solved by X-ray diffraction[3]).

REFERENCES:

1. Ostermeier C, Iwata S, Michel H, *Curr Opin Struct Biol* **1996**, *6*, 460-6.

2. Abriata LA, Banci L, Bertini I, Ciofi-Baffoni S, Gkazonis P, Spyroulias GA, Vila AJ and Wang S., *Nat Chem Biol* **2008**, *4*, 599-601.

3. Williams PA, Blackburn NJ, Sanders D, Bellamy H, Stura EA, Fee JA, McRee DE, *Nat Struct Biol.* **1999**, *6*, 509-16.

ANPCyT, CONICET, Ministerio Ciencia e Innovación de España

INTERNAL GRADIENT MAPPING WITH DISTANT DIPOLAR FIELD CONTRAST

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Keywords: porous media, internal gradients, distant dipolar field

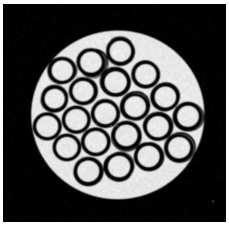
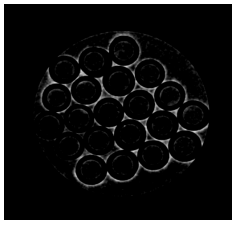
A particle diffusing in a confining medium is a general model for a number of physical, chemical, biological, and industrial processes. It may describe organic molecules in biological cells or brain tissue, oxygen in human lungs, water molecules in cements, etc. When such a particle encounters an interface, they may interact in different ways depending on their physical and chemical properties. NMR technique is of particular interest as being a method to "label" or "encode" Brownian trajectories of spin-bearing particles by using magnetic fields.

The heterogeneous nature of the samples broadens the spectrum of the NMR measurement due to the existence of magnetic susceptibility differences between materials in the samples. For example, between a porous matrix with saturating fluid local magnetic field gradients developed at the interfaces. These local magnetic fields with pronounced spatial variations are commonly referred to as "internal gradients" and the main facts governing their strength depend on the susceptibility difference between the materials, the applied magnetic field and the pore size, shape and the geometry of the pore network.

Decay rates due to susceptibility differences can readily be exploited to obtain characteristics of porous media by using the information provided by the internal gradients [1]. An alternative method to probe the influence of such gradients relies on the use of the Distant Dipolar Field (DDF). In liquids at high magnetic fields the dipolar interaction that is normally averaged out can be reintroduced by the application of sequences like the Cosy Revamped by Asymmetric *Z*-Gradient Echo Detection (CRAZED) [2]. If the symmetry on the sample is broken, as for instance by the application of a magnetic field gradient, intermolecular Multiple Quantum Coherences (iMQC) are converted into observable signal by intermolecular dipolar couplings.

In this work we explore the use of double quantum intermolecular coherences (iDQC) as a contrast agent to probe the internal gradients of a model sample. Here we use a set of capillaries of outer diameter OD=1.4 mm contained in a 10 mm NMR tube which is filled with distilled water [3].

Figure 1a shows a spin-warp spin echo (TE = 3 ms) 2D image with a 1 mm slice selection along the long axis of the sample. In order to generate the iDQC contrast, a CRAZED sequence is applied prior to the imaging sequence, in this way only signals arising from distant dipolar couplings contribute to the image formation. Figure 1b shows the difference between a reference image (Fig. 1a) and an iDQC image. The iDQC signal generation in the presence of the internal gradients can be observed.

a)  b) 

REFERENCES:

1. Y.-Q. Song; S. Tyu; P.N. Sen; *Nature* **2000** 406, 178.
2. W.S. Warren; W. Richter; A.H. Andreotti; B.T. Farmer, II; *Science* **1993**, 262, 2005.
3. H. Cho; S. Ryu; J.L. Ackerman; Y.-Q. Song; *J. Magn. Reson.* **2009** 198, 88.

CONICET, FONCYT, SECYT-UNC, MPIP-MPG

INHIBITION OF THE BETA AMYLOID PEPTIDE FIBRILLATION USING SMALL MOLECULES

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Keywords: Alzheimer, misfolding, aggregation

Alzheimer's disease is the most common neurodegenerative disorder and represents one of the leading causes of death in the developed world. The major neuropathological changes in the brains of AD patients are neuronal death, particularly in regions related to memory and cognition, and the presence of intra- and extracellular abnormal protein aggregates, known as neurofibrillary tangles and amyloid plaques, respectively. Extracellular amyloid deposits have revealed to be essentially composed of a hydrophobic 40-43 amino acid peptide called β-amyloid peptide (Aβ). Aβ misfolding and aggregation are probably the first pathological processes in AD. The pathogenic role of Aβ in AD has been supported by the discovery of several mutations in Aβ tightly linked to inherited forms of the disease. Evidence that Aβ plays a causative role in AD pathology has also come from several model systems. Aβ is toxic to neurons in culture, and plaque formation in the brains of transgenic animals overexpressing human Aβ peptide also leads to neuronal toxicity. These evidences and the abundant knowledge accumulated about the molecular mechanism of amyloid formation make the inhibition of Aβ misfolding and oligomerization an attractive therapeutic target for AD. As a consequence, much of the work on Aβ in the last years has focused on the development of amyloid inhibitors. However, the usefulness of most of the studied compounds as amyloid inhibitors was comprised by the unknown structural and mechanistic basis of their inhibitory effects. In this work, we characterized structurally the interaction between Aβ and one of the most potent amyloid inhibitors. Our results emphasized the relevance of aromatic and hydrophobic interactions in the aggregation mechanism of the beta amyloid peptide.

REFERENCES

1. Chiti, F. and C.M. Dobson, Annu Rev Biochem, 2006. 75: p. 333-66.
2. Lamberto, G.R., et al., Proc Natl Acad Sci U S A, 2009. 106(50): p. 21057-62.

1D AND 2D NMR STUDIES OF SYNTHETIC IMIDAZOLES, POLYMERIC MATERIALS AND THEIR METAL COMPLEXES

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Keywords: solid-state NMR; imidazoles; polymers; dynamics.

Synthethic Imidazoles: The few stable crystalline hydrates known are those that have a strongly electronegative group associated with the carbonyl group since, in general, the hydrates can seldom be isolated because they readily revert to the parent aldehyde. So, we focused the study on the imidazole-2-carboxaldehyde (**1**) because it is an important reagent in the synthesis of active compounds. In this field, imidazole derivatives have been synthesized from **1** with relevance in medicinal chemistry. Our results allowed us to conclude that the hydrate form (**1a**) was stable and precipitated at pH = 8.0, and that the aldehyde form was isolated at pH = 6.5 and 9.5 (Fig. 1). Moreover, the presence of the aldehyde-hydrate form was studied through NMR experiments in D_2O at both alkaline and acidic pH.[1] In addition; the tautomeric forms of the 2-substituted imidazole compounds were also analyzed through NMR and X-Ray crystallographic techniques due to its importance in the reactivity of compounds in chemical processes and its effect on biological systems.

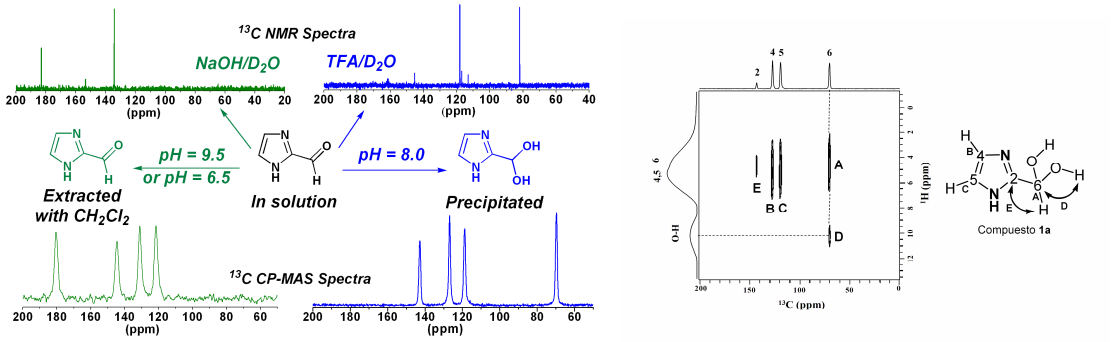

Figure 1: ^{13}C NMR studies and 2D ^{1}H-^{13}C HETCOR spectrum in the solid-state of **1a**.

Polymeric Materials: Two particular interesting groups of functionalized polymeric materials are the polyampholytes and polyelectrolytes.[2] These materials offer the versatility to complex different metal ions due to the presence of azole and/or carboxylate groups in the structure. In particular, in the Cu(II)-Polymer complexes containing imidazole, the heterocyclic ring has been found to be the main group involved in metal ion uptake and the carboxylic group appears to have a coordinating role only at high concentrations of the metal ion from the ^{13}C CP-MAS spectra.[2] The aim of the present work was to determine the effects of copper ion at different concentrations in the molecular dynamics and to study the coordination behavior in synthetic polymers bearing imidazole, triazole or pyrazole and carboxylic acid by 1D- and 2D-solid-state NMR experiments.

REFERENCES:

1. Lázaro Martínez J.M, Romasanta P., Chattah A.K., Buldain, G.Y. *Journal of Organic Chemistry*. 75, **2010**, 3208.
2. Lázaro Martínez J.M, Chattah A.K., Monti G.A., Leal Denis M.F., Buldain, G.Y., Campodall´Orto V. *Polymer*. 49, **2008**, 5482.

CONICET PIP-2010-441, PICT-2010-1096, SECyT-UNC 05/B436, UBACyT-2010-109

NMR-LIKE EXPERIMENTS IN SUPERCONDUCTING QUANTUM SYSTEMS

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Keywords: quantum computation, quantum decoherence, superconducting qubits.

A wide variety of systems, useful for application in quantum computation (or quantum processing of the information), are available by the design of qubits using superconducting Josephson junctions[1]. In those devices the handling of the qubit with microwave pulses is possible. The application of those pulses permits to study decoherence in the signals generated by the qubit, which is produced by the interaction with spurious two level systems (TLS), such as parasitic electric dipoles. In that way, using different NMR-like refocusing techniques[2] we can mitigate the decay of the coherences, and also we can study the properties of the TLS-qubit coupling.

Other interesting system, of recent concern, is a superconducting coplanar waveguide (CPW) resonator coupled with spins embedded in a solid state matrix, for instance a crystal doped with rare-earth's ions[3]. Integrating a qubit with the resonator allows transferring of the state of the qubit to spins coupled to the resonator, thus realizing a quantum memory of the qubit state.

In this work, we present a description of the dynamic of a qubit-TLS system, under the following Hamiltonian (in a z-axis rotating frame, being $\vec{\sigma}_\perp \equiv \sigma_x \hat{x} + \sigma_y \hat{y}$ and $\vec{\sigma}_{\perp(i)} \equiv \sigma_{x(i)} \hat{x} + \sigma_{y(i)} \hat{y}$), $H = \frac{\varepsilon}{2}\, \vec{\sigma}_\perp \cdot \sum_i \zeta_i\, \vec{\sigma}_{\perp(i)} - \frac{\varepsilon}{2} \sum_i \zeta_i^2\, \sigma_{z(i)} + \frac{\varepsilon}{2} \sum_{i<j} \zeta_i \zeta_j\, \vec{\sigma}_{\perp(i)} \cdot \vec{\sigma}_{\perp(j)}$, where σ_α and $\sigma_{\alpha(i)}$ are the Pauli matrix for the qubit and the i-th TLS ($\alpha \equiv x, y, z$), respectively, ε is the energy difference between the qubit's levels and ζ_i is the coupling factor between the i-th TLS and the qubit (dimensionless, $\zeta_i \ll 1$). The results obtained from a simulation of the polarization $\langle \sigma_z \rangle \equiv tr\{\sigma_z \rho(t)\}, \rho(0) = \sigma_z$, show a remarkable enhancement of the coherence time by applying a π_z-pulse sequence (see Figure 1). In addition, we study the coupling between a CPW resonator and the spins in a crystal doped with rare-earth's ions under a semi-classical approach that permits to identify a resistive effect of the spins over the resonator circuit under the resonance condition, which is important for a perfect absorption of the microwave photons.

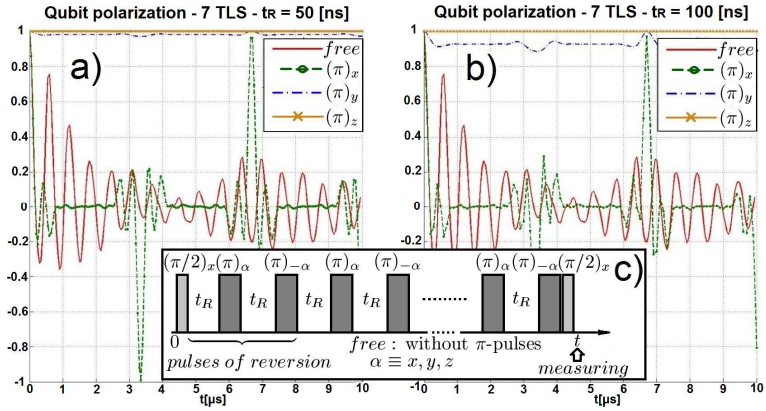

Figure 1: a,b) Simulation of the evolution of the polarization from a qubit interacting with 7 TLS (for a particular ε and set of ζ_i) under different π-pulse sequences. c) Pulses

REFERENCES:

4. G. Wendin; and V.S. Shumeiko, arXiv:cond-mat/0508729v1, **2005**.
5. J. Bylander; S. Gustavsson; F. Yan; F. Yoshihara; K. Harrabi; G. Fitch; D. G. Cory; Y. Nakamura; J.-S. Tsai; and W. D. Oliver, arXiv:1101.4707v1, **2011**.
6. P. Bushev; A. K. Feofanov; H. Rotzinger; I. Protopopov; J. H. Cole; G. Fischer; A. Lukashenko; and A. V. Ustinov, arXiv:1102.3841v1, **2011**.

CONICET, MC2 – CHALMERS

RNA INTERACTIONS AND DYNAMICS OF THE miRNA-PROCESSING ASSOCIATED PROTEIN HYL1

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Keywords: HYL1; miRNA; Protein Dynamics

MicroRNAs (miRNAs), a key member of the family of small non-coding RNA regulators, are 21 nucleotide (nt) molecules processed from endogenous transcripts[1]. The biogenesis of miRNAs is a complex process, which differs in plants and animals. miRNA precursors (pri-miRNAs) are transcribed in the nucleus by RNA polymerase II. The actual miRNAs are located within stem-loop structures in the pri-miRNA and are released through the action of RNase III-type enzymes of the Dicer family. DICER-LIKE1 (DCL1) excises the mature miRNA in a stepwise manner aided by the dsRNA-binding protein HYL1 and the zinc-finger protein SERRATE[2].

HYL1 Is a double-stranded RNA-binding protein harboring two double stranded RNA-binding domains (dsRBDs), involved in miRNA processing in plants. HYL1 enhances the efficiency and the precision of the Rnase III protein DCL1[2]. Here we show the solution structure of both dsRBDs of HYL1 and a dissection of the contributions of the domains of HYL1 to the binding of RNA targets. We found that the first dsRBD is the main contributor to RNA-binding. Mapping of the interaction regions by NMR on the structure of HYL1 RNA-binding domains showed that the difference in binding capabilities can be traced to sequence divergence in loop β2-β3[3]. The characterization by NMR of ps-ns motions of the first dsRBD shows that regions participating in RNA-binding are more flexible than average, suggesting a correlation between flexibility and RNA-binding activity (Figure1).

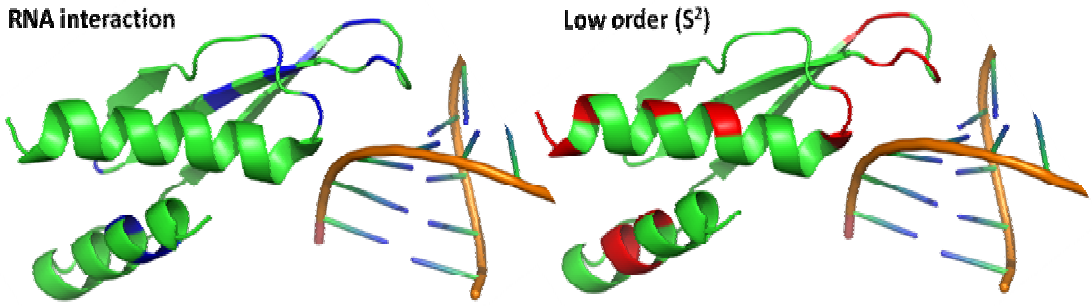

Figure 1: Mapping high mobility residues within the structure shows a good correlation with RNA binding regions

REFERENCES:

1. Filipowicz W.; Bhattacharyya S. N. and Sonenberg N., *Nat. Rev. Genet*, **2008**, 9(2):102-14
2. Dong Z.; Han M. and Fedoroff N., *Proc. Natl. Acad. Sci.,* **2008**, 105: 9970-5
3. Rasia R. M.; Mateos J.; Bologna N. G.; Burdisso P.;Imbert L.; Palatnik J. F. and Boisbouvier J., *Biochemistry*, **2010**, 49 (38): 8237-9

UNDERSTANDING THE COMPLEXATION OF $AlCl_3$ WITH β-CYCLODENXTRIN. A ^{27}Al NMR STUDY

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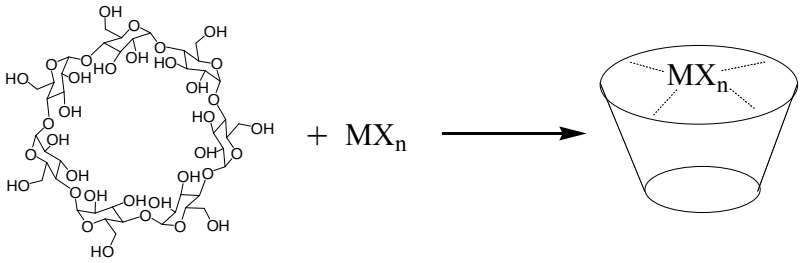
Keywords: ^{27}Al NMR, Carbohydrate, Organometallic complex

Sugars are the building blocks for a large number of biological molecules. Our approach consists of using transition metals with their different assembling properties (depending on the metal and its oxidation state, coordination number, counter ion, etc.) for the creation of novel architectures in coordination chemistry. These molecules may provide (i) chiral metals (ii) chiral cavities, (iii) chiral precursors for aggregates via weak interactions, (iv) novel fragments in organometallic chemistry or (v) oxygen-rich cavities for transition metal salts. These novel complexes have potential application as catalyst in several organic reactions, since the corresponding metallic salts have showed to be very efficient, but their poor stability under atmospheric conditions is a disadvantage for its use. An alternative to improve the physicochemical properties of these salts without loosing their activity is the formation of complexes with cyclodextrins (CD)[1]. Cyclodextrins are cyclic oligomers of glucose with 6, 7 or 8 glucose units for α, β and γ cyclodextrin respectively. We have previously synthesized more than 60 complexes between several transition metals and different CD. The synthetic procedure is simple and leads to reproducible results for most of the studied complexes.



When $AlCl_3$ was used as salt, different complexes were obtained with minimal variations on the procedure. We attribute this to the particular coordination chemistry that Al involves. In this opportunity we will show a ^{27}Al NMR study of these complexes in aqueous or non-aqueous solution and how it helped us to elucidate the structure of these complexes and provided a better understanding of the complexation reaction between β–cyclodextrin and $AlCl_3$.

REFERENCES:

1. Rossi, L.I.; Hoyos de Rossi, R. *J. Supramol. Chem.* **2003**, *2(6)*, 509.

POSTERS

MAGNETIC RESONANCE IN A CORDUBENSIS PERSPECTIVE

SEPTEMBER 5th TO 7th, 2011

Alta Gracia, Córdoba, Argentina

SPHERICAL TENSOR CHARACTERIZATION OF MULTI-SPIN-ORDER QUASI-INVARIANTS IN LIQUID CRYSTALS: AN ANALYTICAL-EXPERIMENTAL STUDY

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Keywords: spherical tensor operators; multi-spin-order; quasi-invariant.

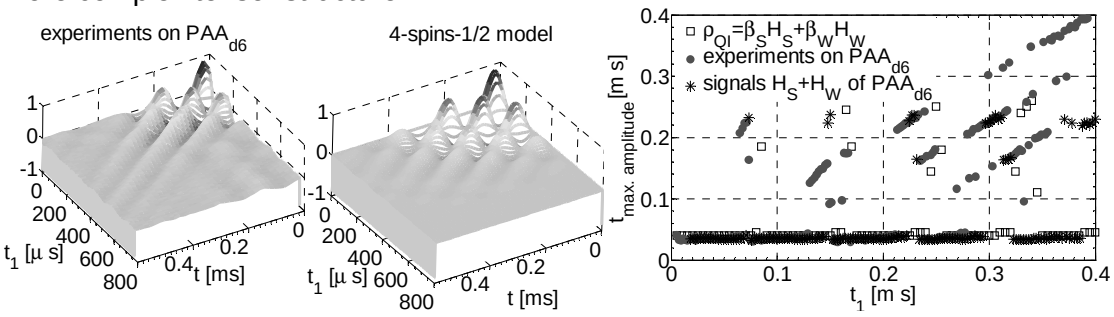
Proton spins of liquid crystal (LC) molecules are small clusters of strongly interacting spins (dipole-dipole), magnetically isolated in the average from spins at other molecules, while mechanically coupled with an orientationally ordered molecular environment. Previous work[1] showed that quasi-equilibrium states (quasi-invariants (QI)) associated with constants of motion of the residual dipolar Hamiltonian Hd, can be prepared in LC through the Jeener-Broekaert (JB) experiment[2]. An efficient application of the QI in many fields, from the basic physics of open quantum systems and quantum information processing, to the study of cooperative molecular dynamics, demands a complete analytical description of the constants of motion.

We report the first complete characterization of a multi-spin-order QI in spherical tensor operator, in a 4-spin-1/2 model, as a first approach for a typical LC (e.g. PAA_{d6}). Describing the spin dynamics over a large time scale generally requires a number of constants of motion compatible with the cluster symmetry, however, we show that for short preparation times t_1 in the JB sequence, the experiment can be described with a similar strategy of truncation of Hd as in hydrated salts[3]. That is, that the initial Zeeman order can alternately be transferred to only two QI, called strong (H_S, dipolar) and weak (H_W, multi-spin-order). We found the following analytical expansion of H_W in spherical tensor operators T_{LM} [4] (numbers in parenthesis indicate the spins)

$$H_W = \sum_{weak-pair}\left(a_{ij}T_{00}(i,j)+b_{ij}T_{20}(i,j)\right)+cT_{00}(1,2,3,4)+\sum_{S=1-5}d_S T_{20}^{(S)}(1,2,3,4)+eT_{40}(1,2,3,4)\,.$$

Consistently with multiple-quantum-coherence encoding experiments in orthogonal basis[5], our expresion for H_W has only zero coherence order in the z-basis and even coherences in the x-basis.

Fig.1 shows the close agreement between the experimental and calculated dipolar signals on PAA_{d6} with H_W expressed as above. In Fig. 1(right) we plot the time at which the maximum of the dipolar signals occurs, vs. t_1. Notice that the linear behavior for t_1>0.3ms cannot be explained by assuming only two QI, which points out that the description of the long time dynamics in PAA_{d6} demands considering additional QI's, of more complex tensor structure.



REFERENCES

1. O. Mensio, C. E. González, and R. C. Zamar, Phys. Rev. E **71**, 011704(2005).
2. J. Jeener and P. Broekaert, Phys. Rev. **157**, 232(1967).
3. H. Eisendrath, W. Stone and J. Jeener, Phys. Rev. B **17**, 47(1978).
4. C. J. Bonin, PhD Thesis (2011) http://www.famaf.unc.edu.ar/publicaciones/documents/serie_d/DFis147.pdf
5. L. Buljubasich, G. Monti, R. H. Acosta, C. J. Bonin, C. E. González, and R. C. Zamar, J. Chem. Phys. **130**, 024501(2009) .

NOESY AND ROESY EXPERIMENTS AS VALUABLE TOOLS TO EXPLAIN THE LACK OF REACTIVITY OF LINEAR-DENDRITIC BLOCK COPOLYMERS

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Keywords: linear-dendritic block copolymers; hydrogen bond; reactivity.

In previous studies, our group reported the synthesis of a family of first-generation linear-dendritic block copolymers[1] (Fig.1) bearing terminal amine groups, and the attempts to use them as substrates in Schiff´s base formation reactions and crosslinking polymerizations with diisocianates. To our surprise, in these reactions the aromatic copolymer **4** exhibited a much higher reactivity than its aliphatic analogues **1**, **2** and **3**.[2] This behavior is unusual for such functional groups and suggests that the molecular structure plays a key role in the reactivity of these compounds in the reaction conditions studied.

The first evidence supporting this hypothesis was obtained from ^{1}H NMR spectra. **1** displayed signals with different peak resolutions in the same spectra. This effect was assumed to be caused by different degree of solvation of the different parts of the copolymers, which directly affected their mobility. The results suggest that the central oligoether segment was well solvated while the terminal arms were much less solvated. Similar results were obtained from compounds **2** and **3**. On the contrary, copolymer **4** showed spectra where all signals were sharp and well resolved, indicating that the entire molecule was well solvated. These differences observed in ^{1}H NMR spectra suggest that the aliphatic copolymers may aggregate, thus reducing their reactivity. Therefore, a series of 2D and 1D selective NOESY and ROESY experiments were performed in different solvents, choosing **1** as aliphatic model copolymer to explain the lack of reactivity. We specially analyzed the trough-space correlation between protons far away form each other in the same molecule. The results demonstrate that the amine-end groups are located spatially close to the central linear segment. A reason for this proximity is formation of hydrogen bonds that terminal –NH_2 groups may form with the oxygen atoms from the ether central block, as well as with the tertiary amine nitrogen atoms. These bonds involve the terminal amine groups into hindered aggregates, thus reducing or limiting their reactivity. Conversely, in the case of **4** the results show no correlation between the dendritic part and the linear segment. Here, the aromatic moieties provide higher stiffness to the dendritic arms and decrease the basicity of the amine groups, rendering unfavorable the formation of hydrogen bonds and leaving the -NH_2 groups accessible to react.

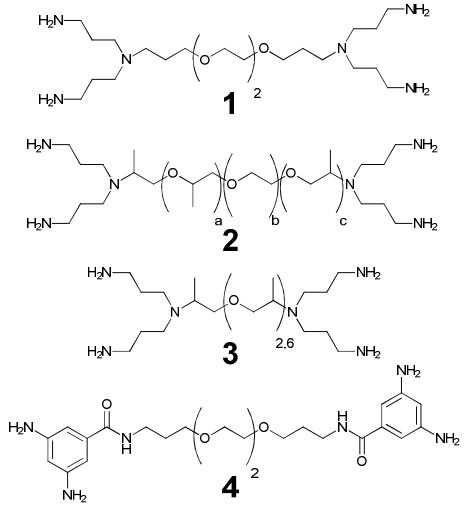

Fig. 1. Family of linear-dendritic copolymers studied.

In summary, we demonstrated the utility of NOESY and ROESY experiments to study the effect of the molecular structure on the reactivity of linear-dendritic block copolymers.

REFERENCES:

1. P. Froimowicz, A. Gandini and M. Strumia, Tetrahedron Lett., 2005, 46, 2653.
2. P. Froimowicz, J. Paez, A. Gandini, N. Belgacem and M. Strumia, Macromol. Symp., 2006, 245-246, 51.

MULTI-BAND AUTOMATICALLY TUNABLE HIGH-SENSITIVE NUCLEAR QUADRUPOLE RESONANCE SPECTROMETER

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Keywords: ^{14}N; ^{35}Cl; nuclear quadrupole resonance; pharmaceuticals; polymorphism; NQR spectrometer

Nuclear quadrupole resonance (NQR) is a nondestructive, highly specific, noninvasive spectroscopic technique. NQR can be used to detect signals from solids and its parameters are highly sensitive to local environment changes. These features make NQR a powerful technique for identifying and study the properties of different polymorphs as well different hydrates forms in pharmaceutical agents, providing effective assistance at the main steps of drug development, manufacturing process and quality control.[1-4]

The aim of this work was the development and implementation of a high sensitive pulse NQR spectrometer, capable of measuring in different frequency bands, allowing the scan of new NQR signals in a fast, automatic and unattended way.

The spectrometer was developed for measuring in two different ranges: 1.9-4.1 MHz (^{14}N) and 34-36 MHz (^{35}Cl). In order to make the spectrometer capable of measuring in both bands, a two capacitively coupled high-Q coils probe-head was built. To cover the whole bandwidth, the high-Q tuning and matching capacitors are mechanically adjustable. The spectrometer also includes a full bandwidth Q-damper system; a switchable transmitter filters; and a sample temperature control.

The tuning and matching capacitors are adjusted by a fast auto-tuning algorithm, feedbacked by reflected power. Q-damper frequency band and power amplifier filters are also fast and automatically adjusted. This allows making a full frequency sweep, performing measurements on the whole range of interest in a complete automatic way.

For operating the spectrometer, a dedicated software was developed. This software allows the selection of parameters as initial, final and step frequency for the NQR signal scan, as well as pulse sequence and other experiment parameters. The software automatically performs every necessary task to scan the defined spectrum. In addition, the software is capable of making spectra reconstruction implementing the spin-echo Fourier transform mapping spectroscopy.[5]

The spectrometer sensibility was tested using the standard samples hexamethylenetetramine for ^{14}N, and p-diclorobenzene for ^{35}Cl. The spectrometer allows to detect 19 mg of hmt in 30 minutes using 16 averages of a 100 pulses SSFP sequence, and 7 mg of pdb in 1 minute using 80 averages of a Spin-Echo sequence.

The spectrometer was tested reconstructing the previously reported spectra of pharmaceutical samples including carbamazepine, furosemide and hydrochloro-thyazide. Nowadays, the spectrometer is been used for finding new NQR signals. Unreported lines were found in diclofenac sodium, aripiprazole and clopidigrel bisulfate. No NQR signal of these last two samples had been reported before.

REFERENCES:

1. Pérez, S.C *et al*, *Int. J. Pharm.* **2005**, *298*, 143-152.
2. Balchin, E. *et al*, *Anal. Chem.* **2005**, *77*, 3925-3918.
3. Latosinsnska, J.N.; *Expert Opin. Drug Discov*. **2007**, *2*(2), 225-248.
4. Limandri, S. e*t al*, *Anal. Chem.* **2011**, *83*(5), 1773–1776.
5. Busandri, A.; Zuriaga, M. J. Magn. Reson. **1998**, *131*, 224-231

CONICET, FONTAR, SPINLOCK

ANALYTICAL STUDIES OF OIL FAT IN FOODSTUFF USING LOW RESOLUTION NMR

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Keywords: NMR; Oil; Food.

During the last years, the studies carried out in the NMR Laboratory in the Physics Department at the UNRC, were oriented to the development of new analytical techniques based on the Hydrogen T2 relaxometry (1) by the pulsed NMR. The aim of these studies is to determine some characteristics of industrial foodstuff, related to the quality of the products. One of such characteristics is fixed from the quantitative determination of diverse fatty acids in oil foods (2) and principally in mixtures of oils foods. Other appears following the gradual fat hydrogenation in typical process by relating it with the quantitative determination of the saturated fatty acids during the process. The quantitative analyses by relaxometry of such fatty acids are interpreted in relation to the analogous models about proton mobilities in the molecular dynamic (3). The analytical results obtained using this technique, are in good agreement compared with others physicochemical methods (4). In the case of hydrogenation process, the relaxometry of the fatty acids is also compared with the measurement of the solid fat content (SFC). From experimental view of the pulsed NMR technique, it can be mentioned that diverse pulse sequences are used (CPMG, Hahn, etc.) (5), the data are analyzed using appropriated T2 profiles with a signal digital filter included in it (6) and by means of a multiexponential algorithm, all of them developed in the research team, which allowed to measure and assign the T2 corresponding to each fatty acid. A commercial equipment of NMR is used –Oxford QP 20+, with external PC control- and the data are transferred to it by a communicational language specially designed for it. Two advantages of this type of analytical technique are worthy of mention, the short analysis time and the fact it does not destroy the sample.

REFERENCES

1. Welser H., Bruker minispec applicat. Notes; Tiwari P,Burj, Journ of AOCS,57,3,**1980**
2. Albert G., Pusiol D and Zuriaga M, Magnetic Res. in Food Science,RS-C,**2003,** 93
3. Price W.S., NMR Studies of translational Motion, Cambridge ed., **2009**, 36.
4. Ortiz F.et al., Congr.Intern. Cs y Tecnol. Alimentos, Córdoba**, 2004**, 13.
5. Farrar T. C., Introduction to Pulse NMR Spectroscopy, Farragut Press, Madison**, 1997**.
6. Diez.,R.,Romero M., Ortiz F., XI RPIC, Rio Cuarto, CD-ISBN 950-665-340-2 pgs. 736-741**-2005**

DYNAMIC OF WATER MOLECULES IN POLYETHYLENE GLYCOL SOLUTIONS. STUDY OF THE LONGITUDINAL RELAXATION TIME (T1) IN 2D AND 1H NMR.

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Keywords: water; polyethylene glycol; ^{1}H-NMR.

Liquid water is a network of molecules microscopically connected by hydrogen bonds, in constant topological reshaping [1]. In solution, ions and hydrophilic residues can immobilize water molecules. This interaction can describe the water in solutions as a set of dynamic subsystems defined by the pattern of water adsorption energies to the different binding sites [2]. Measurements of relaxation times T_1 and T_2 in magnetic resonance, provides information about the time evolution of water distributions, and help to distinguish between the free liquid fractions and structured water in the presence of solutes.

In this work, the dynamics of H_2O in solutions of polyethylene glycol (PEG) of molecular weight of 6000 Da, is studied by analyzing the proton (1H) or deuterium (2D) spin-lattice relaxation times (T_1). The PEG concentrations ([PEG]) ranged between 0 and 70 % W/V, from dilute to highly concentrated systems, simulating the conditions of molecular crowding characteristics of cell cytoplasm. The temperature was set to 37° C in all the experiments, in order to extend the analysis to real physiological systems.

First, we measured 1H in a 20 MHz spectrometer (Minispec, Bruker), observing the free induction decay (FID) and conducting inversion-recovery (IR) experiments to measure T_1. The FID showed no bimodal decay for any of the tested concentrations of PEG, in contrast to the observed decay in heterogeneous systems [3]. On the other hand, a bi-exponential equation could be fitted to data obtained for IR curves. A long (≅3s) and a short (<0.4 ms) time could be distinguished. The long time decreased with [PEG] increment, and the proportion of protons with this T_1 is coincident with the percent of water protons.

Subsequently, we measured 1H T_1 in a Bruker 400 MHz in PEG´s solutions with deuterated water containing residual H_2O. The signals belonging to PEG or H_2O (present in low proportion), showed well resolved resonances. Both peaks were integrated separately and the data were best fitted to a bi-exponential equation in each case. The IR curves showed the presence of two components both in water and in PEG. In water the mean component, showed a long T_1 (≅ 20 s) that decreased with [PEG], and a second component with a short T_1 (0.3 to 0.1 s), in small but measurable proportions. PEG showed a majority component with short T_1 (0.3-0.05 s) and a second component with a longer one (0.8-1.45 s).

Concluding, in solution, the solute (PEG) and solvent (water) showed two populations with different degree of order. Then an equilibrium between two molecular conformations of PEG and the coexistence of two populations of water, is present. The fact that the shorter T_1 of each species had similar values suggests that the immobilization of water stabilizes one of the PEG conformers. Moreover, more flexible PEG conformation would affect the global network of hydrogen bonds of free water.

REFERENCES:

1. Stillinger F.H. *Science.* **1980,** 209(4455): p. 451-457.
2. Lamisovsky D.R.; Schürrer C.A. and Brunetti A.H. *Appl. Mag. Res.* **1996**, 12: p. 61-68.
3. Vackier M.C.; Hills B.P. and Rutledge D.N., *J. of Mag. Res.* **1999,** 138(1): p. 36-42.

CONICET, ANPCyT, SECYT-UNC

THE DSRNA BINDING DOMAINS FROM A. THALIANA DCL1

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Keywords: miRNA; RDC; intrinsically disordered protein

The biogenesis of small RNAs is a complex process involving ribonuclease III like enzymes of the dicer family[1]. In *A. thaliana* four such enzymes, called DCL1-4 participate in different paths leading to the production of the different families of small RNAs (miRNAs, siRNAs, piRNAs)[2]. The processing of miRNA is carried out exclusively by DCL1 which produces the two cuts necessary to precisely excise mature miRNA from its precursors, pri-miRNA[3]. Recognition of the substrate is carried out by the RNA binding domains of the protein. DCL1 protein differs from the canonical Dicer proteins by the presence of a second dsRNA-binding domain at the C-terminus (DCL1-dsRBD2)[2].

In order to understand RNA recognition by DCL1 we studied the two dsRNA binding domains of DCL1. Both domains are located in tandem in the C-terminal region of the protein. We first produced several constructions spanning different parts of DCL1 C-terminus. The construction expressing DCL1-dsRBD2 gives a well folded protein. We assigned the HN, Ha, N, C´, CA and CB protein backbone resonances and acquired Residual Dipolar Coupling (RDC) data on C12E5/hexanol anisotropic phase. The orientational restraints were used to calculate the fold of the protein, which shows some significant differences when compared to canonical dsRBDs: an insertion in loop beta2-beta3 and a change in the orientation of helix 1. This results in a substantial displacement of the relative position of the putative RNA-binding sites, which could give rise to an atypical substrate specificity for this domain. For DCL1-dsRBD1 we produced four constructs including the annotated domain alone and the domain with sourrounding regions. Analysis of the ^{1}H-^{15}N HSQC spectra of the constructs show that the domain is intrinsically disordered in all of them. We explored different solution conditions and additives to test what can lead the domain to acquire an ordered structure, and found evidence that it folds in the presence of RNA.

REFERENCES:

1. Rana TM. *Nat Rev Mol Cell Biol* 8, 23-36 (2007)
2. Margis R, Fusaro AS, Smith NA, Curtin SJ, Watson JM, Finnegan EJ, Waterhouse PM. *FEBS Letters* 580, 2442-2450 (2006)
3. Xie Z, Khanna K, and Ruan S. *Semin Cell Dev Biol* 21, 790-797 (2010)

FUROSEMIDE:MALTODEXTRIN INTERACTIONS STUDIED USING NUCLEAR MAGNETIC RESONANCE

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Keywords: Furosemide complexes; SS-NMR; ^{1}H relaxation times.

Furosemide (FUR) is a loop diuretic used in the oral treatment of edematous states associated with cardiac, renal, and hepatic failure and the treatment of hypertension.[1] However, FUR is practically insoluble in water. An enhanced solubility of FUR is particularly important because its bioavailability is related to in vivo dissolution profile. In addition, seven polymorphic forms are known: four true polymorphs (I, II, III, IV), two solvates (IV—DMS and V—dioxane) and one amorphous form.[2] Maltodextrins (MDs), products obtained from the partial hydrolysis of food grade starch with suitable acids and/or enzymes, have the capability of complexation to various classes of compounds, with the host-guest one being the most common interaction.

Although, a strategy widely used to increase the solubility of drugs is the complex formation with cyclodextrins, the present work is focused to the investigation of the interaction of two polimorphs of FUR (I and II) with MD in solution and solid state, in order to provide an increasing in the solubility of the drug. Moreover, the characterization of pharmeceutical MDs complexes has not been reported in the literature.

The solid samples were obtained by two different methods of preparation: kneading and physical mixture. In order to characterize these new systems, ^{13}C solid-state NMR spectra were obtained to each bynary system and compared to the corresponding components and the physical mixture. In addition, proton spin-lattice relaxation time (T_1) values were measured. Solubility measurements were performed according to the method of Higuchi and Connors.[3]

First, the polymorphs I and II were identified performing the assignments in the ^{13}C solid state NMR spectra. Then, modifications of some signals of the FUR part of the spectrum in the complexes, gave evidence of FUR:MD molecular interaction in solid state. In addition, although the ^{1}H T_1 for the physical mixtures mantain time values similar to those measured in the components, in the complexes a new T_1 value appear together with a value close to the FUR one, indicating that a certain degree of pure drug remains in the complex. Finally, ^{13}C solid state spectra were edited using the T_1 values. In addition, in the ^{1}H-NMR solution spectra of FUR:MD, appreciable shifts were observed in the FUR signals, probably due to conformational changes as a result of interaction in solution.

On the other hand, the solubility diagrams showed an increase in the solubility of each polymorph by the formation of soluble bynary complexes.

This investigation demonstrates the capability of MDs to interact with FUR polymorphs, generating binary complexes both in solution and in solid state.

REFERENCES:

1. Sweetman S, editor. Martindale: The complete drug reference. Electronic version, Pharmaceutical Press, **2009**.
2. Latosinska J. N.; Latosinska M.; Medycki W.; Osuchowicz J. Chemical Physics Letters. **2006**,127.
3. Higuchi T.; Connors K. A. Phase-Solubility Techniques. In Advances in Analytical Chemistry and Instrumentation, Wiley-Interscience: New York, Vol. 4, **1965,** 117.

CONICET, ANPCyT, SECyT-UNC

TOWARDS THE DISCOVERY OF EFFECTIVE POLYCYCLIC AMYLOID INHIBITORS OF ALPHA-SYNUCLEIN PROTEIN LINKED TO PARKINSON´S DISEASE

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Keywords: amyloid; alpha-synuclein; aggregation inhibitor

The misfolding of proteins into a toxic conformation and their deposition as amyloid-like fibrils are proposed to be at the molecular foundation of neurodegenerative disorders including Creutzfeldt-Jakob´s, Alzheimer´s and Parkinson´s diseases (PD).[1-3] A detailed understanding of the mechanism(s) by which proteins of wide structural diversity are transformed into morphologically similar aggregates is therefore of high clinical importance. Neurodegeneration in PD is progressive and characterized by the loss of dopaminergic neurons in the substantia nigra and the presence of fibrillar cytoplasmatic aggregates of alpha-synuclein (AS) in multiple brain regions.[4] Currently, no preventative therapy is available for PD and related synucleinopathies. Identification of therapeutic drugs is not only complicated by a lack of understanding of many of the key aspects of PD pathogenesis but also by the multifactorial etiology of the disease. The aggregation pathway of AS represents then one obvious target for therapeutic intervention in PD. Indeed, one approach to the development of therapeutic agents in neurodegenerative diseases has been the use of small molecules that specifically and efficiently inhibit the aggregation process.[5-10] In this work we report high-resolution structural information of the interaction between AS and some of the most studied fibrillation inhibitors. The elucidation of the structural details of the interactions provided the basis for understanding the role of specific residues in the fibrillation pathway of AS and shed new light into the mechanistic basis that direct the inhibitory process of these anti-amyloidogenic compounds.

REFERENCES

1. Chiti, F., and Dobson, C. M. *Annu Rev Biochem*, **2006**, **75**, 333.
2. Luheshi, L. M., Crowther, D. C., and Dobson, C. M. *Curr Opin Chem Biol*, **2008**, **12**, 25.
3. Sipe, J. D. *Annu Rev Biochem*, **1992**, **61**, 947.
4. Spillantini, M. G., *et al. Nature*, **1997**, **388**, 839.
5. Ehrnhoefer, D. E., *et al. Nat Struct Mol Biol*, **2008**, **15**, 566.
6. Taniguchi, S., *et al. J Biol Chem*, **2005**, **280**, 7623.
7. Chopra, V., *et al. Proc Natl Acad Sci USA*, **2007**, **104**, 16689.
8. Caughey W. S., *et al. Proc Natl Acad Sci USA*, **1998**, **95**, 12122.
9. Zhu, M., *et al. J Biol Chem*, **2004**, **279**, 26857.
10. Lee, E. N., *et al. Biochemistry*, **2004**, **43**, 3715.

ANPCyT, FUNDACIÓN ANTORCHAS, CONICET, MAX PLANCK SOCIETY, ALEXANDER VON HUMBOLDT FOUNDATION, IRB BARCELONA, FP7 MARIE CURIE IEF GRANT, AND DFG HEISENBERG SCHOLARSHIP

STRUCTURAL CHARACTERIZATION OF SYNUCLEINS BY NMR: TOWARDS THE UNDERSTANDING OF THE AGGREGATION MECHANISMS

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Keywords: Synuclein, neurodegeneration, amyloidogenesis.

The synucleins are a family of intrinsically disordered proteins whose physiological functions are poorly understood, although they are brain-enriched and have been implicated in neurodegenerative disorders (Parkinson's disease[1-2] and dementia with Lewy Bodies, among others) and cancer[3]. In humans, the synuclein family consists at least of three members: alpha-synuclein (AS), beta-synuclein (BS) and gamma-synuclein (GS) that range from 127-140 amino acids and are 55-62% identical in sequence, with a similar domain organization. The inability of BS and GS to spontaneously aggregate is a key feature distinguishing AS from these two close relatives. Moreover, evidence that AS aggregation plays a causative role in the development of PD is furnished by a variety of studies. Since AS fibrillization is believed to progress through a soluble oligomeric intermediate rich in β-structure[4], differences in secondary structure propensities in synucleins might provide a possible explanation for their different amyloidogenic potentials[5]. However, our results indicate that other structural factors such as transient tertiary contacts and the nature of the amino acid residue would play also a fundamental role in the structural and toxic mechanisms of AS aggregation. In our laboratory we have performed a detailed analysis to understand the role that different structural and dynamic factors might have on the amyloidogenic capacity of disease-linked proteins, with the aim of establishing a relationship between structure, amyloidogenic potential and toxicity in the synuclein family of proteins.

REFERENCES:

1. Spillantini, M. G.; Schmidt, M. L.; Lee, V. M.; Trojanowski, J. Q.; Jakes, R.; Goedert, M. *Nature*, 388, **1997**, 839.
2. Lee, V. M.; Trojanowski, J. Q. *Neuron*, 52, **2006**, 33.
3. Clayton, D. F.; George, J. M. *Trends Neurosci*, 21, **1998**, 249.
4. Rochet, J. C.; Lansbury, P. T., Jr. *Curr Opin Struct Biol*, 10, **2000**, 60.
5. Marsh, J. A.; Singh, V. K.; Jia, Z.; Forman-Kay, J. D. *Protein Sci*, 15, **2006**, 2795.

QUANTUM DECOHERENCE AND QUASI-EQUILIBRIUM IN 1H-NMR OF LIQUID CRYSTALS

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Keywords: decoherence; quasi-equilibrium; irreversibility

The typical anisotropic molecular orientation together with the rapid liquid-like individual motions, allow considering the proton spins of each liquid crystal (LC) molecule as a dipole-interacting spin cluster, magnetically isolated in the average from other molecules. Previous work[1] showed that despite the low dimension of the Hilbert space of the molecular units, quasi-equilibrium states (QE) associated with the residual dipolar Hamiltonian can be prepared through the Jeener-Broekaert (JB) experiment, which have a similar phenomenology than in solids. It is well known that in closed systems of finite dimension, the evolution under the self Hamiltonian does not produce QE states[2]; approaches based on the spin-diffusion, usually introduced in solids to justify the occurrence of a spin-temperature, are not justified in small systems.

Selective refocusing experiments of multiple quantum coherences conducted in LC's, showed that irreversible decoherence occurs over an intermediate timescale between Liouvillian interference regime (closed system) and thermalization with the lattice. The dissimilar behavior of the different coherence orders observed in the experiment indicates that decoherence is controlled by a full quantum spin-environment interaction that is essentially different from usual coupling models based on thermal fluctuations of the local field, as "pure dephasing" mechanisms[3]. Hence, the quantum openness of the spin system becomes an essential ingredient of any comprehensive theoretical deescription.

The noticeable separation of decoherence and relaxation timescales and the highly correlated nature of the molecular environment, suggest that decoherence in LC is ruled by an energy conserving spin-environment interaction, where the molecular orientational order plays a main role. By analyzing a hypothetical time reversal experiment within a scheme where the angular variables are quantum operators, we identify two sources of coherence loss, of a very different nature, which give rise to distinct timescales of the spin dynamics: reversble or *adiabatic quantum decoherence* and irreversible or *essentially-adiabatic quantum decoherence*, in accordance with the recent experimental results. A fingerprint of quantum decoherence is the process we call "eigen-selectivity"[4], by which the diagonal-in-block structure of the density matrix is preserved over decoherence, allowing the occurrence of a "decoherence-free" subspace, and defines the quasi-invariant structure of the QE states. Therefore, this approach, relying on plausible hypotheses of general character valid for a large class of LC's, also explains the build-up of the QE states in these mesophases.

The occurrence of an irreversible trend towards QE is experimentally demonstrated through spin dynamics refocusing with MREV8 and magic echo pulse sequences starting from the JB initial condition. Numerical calculation of the dipolar signal on a LC molecule supports this conclusion. The effect of eigen-selectivity is experimentally shown through the evolution of the NMR spectrum under refocusing.

REFERENCES:

1. O. Mensio, C. E. González, and R. C. Zamar, Phys. Rev. E 71, 011704 (2005).
2. R. Bruchsweiler and R. Ernst, Chem. Phys. Lett. 264, 393 (1997).
3. C.E.González, H.H.Segnorile and R.C.Zamar, Phys.Rev.E, 83, 011705 (2011).
4. H.H.Segnorile and R.C. Zamar, submitted. H.H. Segnorile, PhD Thesis, Universidad Nacional de Córdoba, Argentina (2009).

FLOW REGIME ANALYZER BASED ON LOW-FIELD NUCLEAR MAGNETIC RESONANCE AND HALBACH TYPE MAGNETS

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Keywords: Flow-rate, Low-Field NMR, Halbach magnet, 2D image, velocity image.

In oil industry, to assess the efficiency of extraction methods it is necessary to be able of evaluating, under field conditions, the flow regime and the composition of the complex fluid being extracted. In this work we present a system, based on a Halbach magnet type, designed for determining in real-time, mean velocity and fraction of components of complex fluids directly in the production vein by means of low-field Nuclear Magnetic Resonance[1].

The apparatus includes a main Halbach magnet of 60 cm in length, with a cylindrical region of interest (ROI) of 10 cm in diameter and 10 cm in length, and two pre-polarization stages, one at each side of the main magnet, in order to have the capability of measuring bi-directional flows (Figure 1). By means of a method based on the analysis of the early behavior of the echo amplitudes of a CPMG sequence and without applying any static or pulsed gradients, the cuts and mean velocity of oil and water mixtures were measured for flow-rates between 5 and 60 m^3/h. The experimental results are in agreement with the theoretical framework[2]. On the other hand, density contrast between phases with different longitudinal relaxation times T_1 was accomplished by changing the effective length of the pre-polarizing magnetic field. The pre-polarization field of variable effective length was achieved by means of Halbach stacks with rotation capabilities. Furthermore, in order to test imaging proficiencies of the system, 2D images of the cross section of the ROI were obtained for different configurations of static solutions of $CuSO_4$ in water (Figure 2). Experimental results showed a spatial resolution better than 1 cm. In addition, a CPMG-based method for velocities determination was used in combination with imaging techniques to obtain 2D images of velocities profiles on the cross section of the ROI.

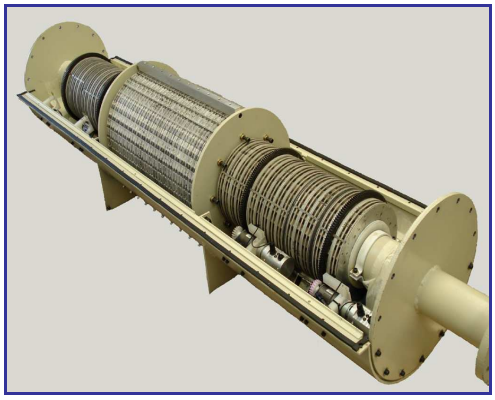

Figure 1: Flow regime analyzer including main magnet and pre-polarization stages

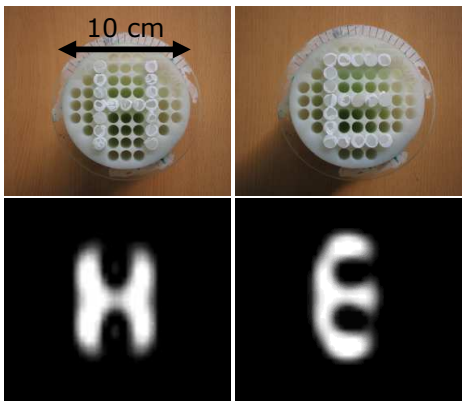

Figure 2: $CuSO_4$-doped water phantoms and their respective NMR images

REFERENCES:

1. Pusiol D. J. et al., US Patent US 7,872,747 Date: Jan. 18, **2011**
2. Osán T.M. et al., *Journal of Magnetic Resonance* **2011**, 209, 116-122

CONICET, SPINLOCK

NUCLEAR MAGNETIC RESONANCE IMAGING SENSITIVE TO THE MOLECULAR ORDER

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Keywords: Magnetic Resonance Imaging, Contrast, Molecular Order

Nuclear magnetic resonance (NMR) is a noninvasive technique capable to provide information about chemical, structural, and dynamical properties of molecules [1]. The Larmor frequency of nuclear magnetic moments in a magnetic field is proportional to the intensity of that field and to the nuclear gyromagnetic ratio. This behavior allows us to encode the space in a given volume using magnetic field gradients, and therefore to obtain images based on the principle of NMR [2]. NMR tomography is a technique capable of producing cross-sectional images oriented in any direction or three-dimensional images of an object. This technique is used in medicine to get high-resolution images inside the human body. However, the application of NMR imaging in materials has a wide range of applications both in basic research and industry [3]. This technique can be applied to biological tissues, plants, food and a wide variety of synthetic materials.

In nuclear magnetic resonance imaging (MRI) experiments, it is possible to identify different regions along an object, whereby, between these regions, there must be differences in contrast due to the physical properties that may differ from one region to another. These contrasts can be obtained depending on various parameters such as spin density (ρ), the longitudinal relaxation time (T_1) and transverse relaxation time (T_2), which are the most common examples. In the medical field, the values of T_1, T_2, and ρ is specific to each tissue or pathology. This allows, for example, identify healthy tissues from diseased tissues.

The aim of this work is to develop an experimental technique to obtain information from the spatial dependence of the state of order in material systems of different nature. With this technique would be possible to visualize, with spatial localization, natural or induced dynamic effects in a variety of materials, reflected in the state of order.

We are interest in the effect produced by an acoustic field at the molecular order in partially disordered systems. We are particularly interested in the spatial dependence of the magnitude of the effect achieved by the distance to the sonotrode, and understand whether there are regions in which the molecular system has isotropization. This could occur as a result of high pressure and temperature gradients to which the sample is subjected during sonication in the vicinity of the sonotrode. Existing studies suggest that the acoustic field can influence the dynamics and molecular order in nematic and smectic liquid crystals [4]. In particular, some experiments suggest that the molecular order can be averaged by the acustic field, without the existence of isotropization [5]. However, a suitable method to study this issue must necessarily be based on a positional determination of physical parameters representative, ie with MRI techniques. The experiments were performed at low intensity of the magnetic fields (0.3-0.7T), using as the main parameter for contrast the attenuation of the magnetization in the rotating frame due to the presence of local fields.

REFERENCES:

1. A. Abragam, *Principles of Nuclear Magnetism,* Clarendon Press, Oxford, **1961**.
2. P. T. Callagham, *Principles of Nuclear Magnetic Resonance Micrsocopy,* Clarendon Press, Oxford **1991**.
3. B. Blümich, *NMR Imaging of Materials,* Clarendon Press, Oxford, **2000**.
4. E. Anoardo and F. Bonetto, en *Modern Magnetic Resonance*, Graham A. Webb Ed., Springer, Amsterdam, **2006**.
5. E. Anoardo, F. Bonetto and R. Kimmich, Phys. Rev. E68, **2003,** 022701.

EXPLORING DYNAMICS IN METALLO-BETA-LACTAMASES BY NMR

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Keywords: protein dynamics, protein evolution

Metallo-Beta-Lactamases (MBLs) are a very important type of enzymes that inactivate most clinically useful beta-lactam antibiotics,[1,2] representing one of the most relevant resistance mechanisms to them. Knowledge of the structural determinants of their catalytic mechanism and the substrate binding mode is of great importance for the development of MBL inhibitors, yet unavailable.

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, L10 and L12). The crystal structure of BcII, the MBL from *B. cereus*, discloses the absence of electron density for residues located in loop L3, suggesting local flexibility.[3] Related to this, we have shown that mutations which alter the hydrogen-bond network connecting loops L3 and L12 gives rise to evolved enzymes with an extended substrate spectrum.[4] In order to explore the role of this mutations in the loop flexibility, and (within a broader perspective), the role of flexibility in protein evolution, we decided to study the backbone dynamics of wild-type BcII and *in vitro* optimized mutants. We have measured backbone R_1 and R_2 relaxation rates, and heteronuclear NOE for wild-type BcII, which were analyzed based on the model-free approach. The enzyme exhibits a relatively rigid backbone in the pico-nanosecond time scale, with an average order parameter of 0.9, with a slight evidence of loop L3 flexibility within this timescale. We also performed CPMG relaxation dispersion experiments with the purpose of detecting conformational exchange processes that occur on micro-millisecond time scales, important for binding and catalityc events. We observed that several residues along the entire sequence show relaxation dispersion profiles in this time scale. On the other hand, we carried on relaxation dispersion experiments on an optimized variant which presents two mutations: G262S and N70S. An important issue to determine is if these mutations could be responsible of an increase in protein dynamics or flexibility that can be important in conferring a broader substrate spectrum. Interestingly, we observed an increase in the relaxation profile of several residues that are located around the active site, particularly in loops L3 and L10. These findings prompt new experiments on other optimized variants in order to correlate protein dynamics with the ability to acquire new functions.

REFERENCES:

1. Fisher, J. F., Meroueh, S. O., and Mobashery, S. *Chem. Rev.* **2005**, 105.
2. Crowder, M. W., Spencer, J., and Vila, A. J. *Acc. Chem. Res.* **2006**, 39.
3. Fabiane, S. M. And Sutton, B. J. *Biochemistry* **1998**, 37.
4. Tomatis, P. E., Fabiane, S. M., Simona, F., Carloni, P., Sutton, B. J., and Vila, A. J. *Proc. Natl. Acad. Sci. U. S. A.,* **2008**, 105.

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NMR STUDIES OF THE ELECTRONIC FACTORS OPTIMIZING BIDIRECTIONAL ELECTRON TRANSFER THROUGH CuA CENTERS

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Keywords: paramagnetic centers; NMR; copper proteins

Cytochrome c oxidase (COX) is a terminal oxidase present in all aerobic organisms, which shuttles electrons from cytochrome c to oxygen using the released energy to pump protons across the membrane and thus contribute to its potential[1]. These inter- and intramolecular electron transfer (ET) reactions take place through a binuclear copper center termed the CuA site, located on the exterior part of the oxidase. In this center, the copper ions are bridged by two Cys thiolates, and each coordination sphere is completed by one His and one weak axial ligand.

Electron transfer through CuA is highly efficient despite the low driving force and long distances involved. This has been attributed to its unique electronic structure (maximizing the superexchange coupling)[2,3] and its intrinsic rigidity (lowering the reorganization energy)[4]. We have used functional measurements and spectroscopic techniques, in particular NMR[5], to study the structural basis of these phenomena, using the CuA-containing fragment of *T. thermophilus* ba_3 oxidase as a model system.

We found that the low reorganization energy is mostly due to the rigidity of the Cu_2S_2 core. This core is highly covalent, with a large amount of unpaired spin density on the cysteins, while the rest of the ligands play an important role in fine-tuning the electronic structure of the center. In particular, NMR reveals a low-lying excited state whose energy can be easily tunable by substitutions of a weak axial Met ligand. Functional measurements show that this low-lying excited state is competent for electron transfer. Given the different electron density distribution in this excited state we propose that it is involved, together with the ground state, in bidirectional ET.

REFERENCES:

1. S. Ferguson-Miller, G. T. Babcock *Chem. Rev.* **1996**, 96
2. E. I. Solomon, X. Xie, A. Dey *Chem. Soc. Rev.* **2008**, 37
3. S. I. Gorelsky, X. Xie, Y. Chen, J. A. Fee, E. I. Solomon *J. Am. Chem. Soc.* **2006**, 128
4. O. Farver, Y. Lu, M. C. Ang, I. Pecht *Proc. Natl. Acad. Sci. U. S. A.* **1999**, 96
5. L. A. Abriata, G. N. Ledesma, R. Pierattelli, A. J. Vila *J. Am. Chem. Soc.* **2009**, 131

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