

**III REUNIÓN BIENAL DEL  
GRUPO ESPECIALIZADO DE RMN  
DE LA RSEQ**



**LIBRO DE RESÚMENES**

**Sant Joan (Alicante)  
15-18 de Octubre de 2006**





## III REUNIÓN BIENAL DEL GRUPO ESPECIALIZADO DE RMN DE LA RSEQ

*Sant Joan (Alicante), 15-18 de Octubre de 2006*

### Comité Organizador

Antonio Donaire  
José Luis Neira  
José Villalaín  
Luis A. Alcaraz  
Olga Ruiz de los Paños  
Miquel Pons  
Marta Bruix

### Organismos Patrocinadores



### Empresas Colaboradoras



OBRAS SOCIALES





## PROGRAMA

### Domingo, 15 de Octubre

- 16:00-20:00 RECEPCIÓN Y ENTREGA DE DOCUMENTACIÓN  
20:30 CÓCTEL DE BIENVENIDA

### Lunes, 16 de Octubre

- 9:00-9:15 PRESENTACIÓN

#### SESIÓN I

*Moderador: José Villalaín*

- 9:15-10:00 CONFERENCIA PLENARIA  
**P1.** “Functional Magnetic Resonance Imaging and Spectroscopy at High and Ultra-High Field”  
**Peter G. Morris** (*University of Nottingham*)
- 10:00-10:30 CONFERENCIA INVITADA  
**I1.** “Biomedical Applications of *In Vivo* and *Ex Vivo* Metabolism by NMR”  
**Bernardo Celda** (*Universitat de València*)
- 10:30-11:00 CONFERENCIA INVITADA  
**I2.** “Use of the *In Vivo* MR Spectroscopy for the Diagnosis and Monitoring of Neurodegenerative Disorders”  
**Ángela Bernabeu** (*Universidad Miguel Hernández de Elche*)
- 11:00-11:30 PAUSA Y CAFÉ

#### SESIÓN II

*Moderador: Mario Piccioli*

- 11:30-12:00 CONFERENCIA INVITADA  
**I3.** “The Use of NMR Spectroscopy to Study Tautomerism”  
**Rosa M. Claramunt** (*Universidad Nacional de Educación a Distancia*)
- 12:00-12:30 CONFERENCIA INVITADA  
**I4.** “Structural Studies on Therapeutically Relevant Proteins: Drug Discovery Implications”  
**Antonio Pineda** (*Centro de Investigación Príncipe Felipe*)
- 12:30-13:00 CONFERENCIA INVITADA  
**I5.** “ESEEM Spectroscopy Applied to the Study of Molecular Systems of Biological Interest”  
**Pablo Javier Alonso** (*Instituto de Ciencia de Materiales de Aragón*)
- 13:00-13:30 CONFERENCIA INVITADA  
**I6.** “*In Vivo* NMR as a Tool in Systems Metabolic Engineering of Dairy Bacteria”  
**Helena Santos** (*Universidade Nova de Lisboa*)

13:30-15:30 ALMUERZO

### SESIÓN III

*Moderador: Jesús Jiménez Barbero*

15:30-16:15 CONFERENCIA PLENARIA  
**P2.** “Peering into Binding Sites of Membrane Drug Targets”  
**Anthony Watts** (University of Oxford)

16:15-16:45 CONFERENCIA INVITADA  
**I7.** “Molecular Recognition of Activated Sugars by Human Blood Group B Galactosyltransferase: Insights from NMR Experiments”  
**Jesús Angulo** (Universidad de Lübeck)

16:45-17:00 COMUNICACIÓN ORAL  
**O1.** “NMR Solution Structure of a Chimeric Protein Mimicking a SH3-Peptide Complex”  
**Adela M. Candel** (Universidad de Granada)

17:00-18:00 PANELES Y CAFÉ

### SESIÓN IV

*Moderador: Carlos González*

18:00-18:15 COMUNICACIÓN ORAL  
**O2.** “Structure of  $\alpha$ -Synuclein Amyloid Fibrils Using H/D Exchange NMR”  
**Marçal Vilar** (The Salk Institute for Biological Sciences)

18.15-18:30 COMUNICACIÓN ORAL  
**O3.** “Simultaneous Alfa/Beta Spin State Selection for  $^{13}\text{C}$  and  $^{15}\text{N}$  from a Time Shared HSQC-IPAP Experiment”  
**Pau Nolis** (Universitat Autònoma de Barcelona)

18:30-18:45 COMUNICACIÓN ORAL  
**O4.** “Millisecond Dynamics in Double Helical DNA”  
**José Gallego** (Centro de Investigación Príncipe Felipe)

18:45-19:00 COMUNICACIÓN ORAL  
**O5.** “Four Stranded DNA Structures Stabilised by Minor Groove Tetrads”  
**Júlia Viladoms** (Universitat de Barcelona)

19.00-19:15 COMUNICACIÓN ORAL  
**O6.** “Edited/Filtered DOSY and STD Experiments with Improved Suppression of Water Signal”  
**Gyula Batta** (University of Debrecen)

19:30 JUNTA GENERAL DEL GERMN

**Martes, 17 de Octubre**

**SESIÓN V**

*Moderador: Carlos F.G.C. Gerales*

- 9:00-9:45            CONFERENCIA PLENARIA  
**P3.** “<sup>19</sup>F NMR-Based Screening for Bona Fide Ligand Identification and Optimization”  
**Claudio Dalvit** (Nerviano Medical Sciences)
- 9:45-10:15        CONFERENCIA INVITADA  
**I8.** “Protein Stability Studied by NMR Spectroscopy”  
**Marta Bruix** (Instituto de Química-Física "Rocasolano")
- 10:15-10:45      CONFERENCIA INVITADA  
**I9.** “Including <sup>13</sup>C Direct Detected Experiments for the Structural and Dynamical Characterization of Cut A1”  
**Beatriz Jiménez** (Universidad de Florencia)
- 10:45-11:15      PAUSA Y CAFÉ

**SESIÓN VI**

*Moderador: Emilio Quiñoá*

- 11:15-11:45      CONFERENCIA INVITADA  
**I10.** “TAR RNA Recognition by a Novel Cyclic Aminoglycoside”  
**Víctor Sánchez** (Universidad de Santiago de Compostela)
- 11:45-12:15      CONFERENCIA INVITADA  
**I11.** “Attempts Applying Ultrafast NMR Spectroscopy”  
**Antonio Herrera** (Universidad Complutense de Madrid)
- 12:15-12:45      CONFERENCIA INVITADA  
**I12.** “PGSE Diffusion NMR: Do We Care about the Role of the Counterions?”  
**Ignacio Fernández** (ETHZ)
- 12:45-13:15      CONFERENCIA INVITADA  
**I13.** “Applications of NMR Techniques to the Conformational Analysis of Small- And Medium-Sized Rings”  
**María Luisa Jimeno** (Centro de Química Orgánica "Manuel Lora Tamayo")
- 13:15-15:30      ALMUERZO

## SESIÓN VII

*Moderador: Miquel Pons*

- 15:30-16:00            CONFERENCIA INVITADA  
**I14.** “Chiral Polyfunctional Compounds: The Disclosure of their Configuration by NMR”  
**Emilio Quiñoá** (Universidad de Santiago de Compostela)
- 16:00-16:30            CONFERENCIA INVITADA  
**I15.** “Medida de Acoplamientos Escalares y Dipolares Residuales en Proteínas”  
**Jorge Santoro** (Instituto de Química-Física "Rocasolano")
- 16:30-17:00            CONFERENCIA INVITADA  
**I16.** “Applications of Diffusion NMR Spectroscopy to Organic Compounds”  
**Nuria Esturau** (Lilly S.A)
- 17:00-18:00            PANELES Y CAFÉ

## SESIÓN VIII

*Moderadora: Teresa Blasco*

- 18:00-18:15            COMUNICACIÓN ORAL  
**O7.** “Tetrahedral Orderin in the NaAlO<sub>2</sub>-NaAlSiO<sub>4</sub> Series: Limits to the Lowenstein’s Rule”  
**Esperanza Pavón** (Instituto de Ciencias de Materiales de Sevilla)
- 18:15-18:30            COMUNICACIÓN ORAL  
**O8.** “Characterization of Morphology in Multiphase Modified Polymers by Solid State NMR”  
**Leoncio Garrido** (Instituto de Ciencia y Tecnología de Polímeros)
- 18:30:18:45            COMUNICACIÓN ORAL  
**O9.** “Estudio de la Litiación de N-bencil-N-metil(1-dinaftil)fosfinamida mediante RMN Multinuclear”  
**Gloria Ruiz** (Universidad de Almería)
- 18:45-19:00            COMUNICACIÓN ORAL  
**O10.** “Studying the Compactness and Aggregation of Dendritic Polymers and Polysaccharides by NMR Relaxation times”  
**Eduardo Fernandez-Megia** (Universidad de Santiago de Compostela)
- 20:00                    ÁGAPE EN EL CASTILLO DE SANTA BÁRBARA

**Miércoles, 18 de Octubre**

**SESIÓN IX**

*Moderador: Manuel Rico*

- 9:00-9:45                    **CONFERENCIA PLENARIA**  
**P4.** “<sup>13</sup>C Direct Detected NMR as a Source of Structural Constraints in Biomolecules”  
**Mario Piccioli** (Universidad de Florencia)
- 9:45-10:15                **CONFERENCIA INVITADA**  
**I17.** “Estructura y Movilidad Superficial en Silicatos Laminares Deducidas con Técnicas RMN de Alta y Baja Resolución”  
**Jesús Sanz** (Instituto de Ciencia de Materiales)
- 10:15-10:45              **CONFERENCIA INVITADA**  
**I18.** “Exploring the Use of Conformationally Locked Aminoglycosides as a New Strategy to Overcome Bacterial Resistance”  
**Juan Luis Asensio** (Instituto de Química Orgánica General)
- 10:45-11:15              **PAUSA Y CAFÉ**

**SESIÓN X**

*Moderador: Pedro Nieto*

- 11:15-12:00              **PRESENTACIÓN BRUKER**  
“New Trends in NMR”  
**Martial Piotto**
- 12:00-12:45              **PRESENTACIÓN VARIAN**  
"New Developments in NMR Technology"  
**Bert Heise**
- 12:45-13:30              **ENTREGA DE PREMIOS**
- 13:30                        **DESPEDIDA**
- 

**SESIÓN “Software en RMN”**

- 15:30-17:00              **Mesa Redonda**  
*Moderador: Antonio Donaire*  
**Participantes:**  
Martial Piotto (Bruker)  
Bert Heise (Varian)  
Blanca López Méndez  
David Pantoja  
Óscar Millet
- 17:00-17:30              **PAUSA Y CAFÉ**
- 17:30-19:00              **MESTRELAB RESEARCH S.L.**



## **CONFERENCIAS PLENARIAS**



# **P1. FUNCTIONAL MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY AT HIGH AND ULTRA-HIGH FIELD**

**Peter G. Morris**

*p.morris@nottingham.ac.uk; Sir Peter Mansfield Magnetic Resonance Centre,  
University of Nottingham, University Park, Nottingham, NG7 2RD, UK*

A 7T MR system has been installed at the Sir Peter Mansfield Magnetic Resonance Centre. The magnet hall includes a 230 ton iron shield to contain the stray field from the unshielded whole body (90 cm bore) magnet. The interpretation and range of applications of functional MRI are limited by the low activation contrast to noise ratio (CNR) of the BOLD effect, even at 3 T. Low CNR is normally addressed by averaging over many trials of a standard block paradigm or a ‘single event’ study. However this assumes that the responses are consistent. Many mental phenomena of crucial importance in understanding brain injury or mental illness are fleeting in nature and must be studied on a single event basis. Working at ultra-high-field will help achieve this<sup>1</sup>.

We have used <sup>13</sup>C MRS to study changes in TCA cycle rate in the human visual cortex on visual stimulation<sup>2</sup>. Analysis of the MRS time-courses reveals increases in  $F_{TCA}$  of about 50%, similar to the increases in CMR<sub>glc</sub> reported in PET studies, but strongly suggest, in contrast to these studies, that cerebral glucose is metabolised oxidatively, even during intense visual stimulation. The same data used to determine  $F_{TCA}$ , can also be analysed to determine the rate of the glutamate/glutamine cycle, via which much of the neurotransmitter glutamate is recycled. Measurements of  $F_{TCA}$  and  $F_{cyc}$  are possible but difficult at current field strengths (3T). We expect them to be substantially improved at higher fields (7T and up).

1. W. van der Zwaag, K.E Head, A. Peters, S. Francis, P. Gowland, R.W. Bowtell and P.G. Morris, *Proc. 14<sup>th</sup> Ann. Meeting Int. Soc. Magn. Reson. Med.*, **2006**, 2776.
2. N. Chhina, E. Kustermann, J. Halliday, I.J. Simpson, I.A. Macdonald, H.S. Bachelard and P.G. Morris, *J. Neuroscience Res.*, **2001**, 66, 737-746.

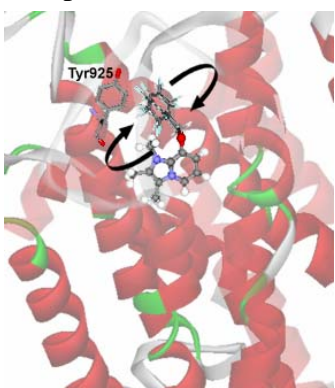
*This work is supported by a JIF Grant from the Wellcome Trust (063654) and a Programme Grant from the Medical Research Council (G9900259).*

## P2. PEERING INTO BINDING SITES OF MEMBRANE DRUG TARGETS

**Anthony Watts**

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

It is now possible to resolve local dynamics within a membrane bound protein at near physiological conditions in natural membrane fragments or in reconstituted complexes, using solid state NMR approaches [1, 2]. This information is obtained by isotopically ( $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{19}\text{F}$ ,  $^{15}\text{N}$ ,  $^{17}\text{O}$ ) labeling selective parts of either a ligand or the protein under study, and observing the nucleus in non-crystalline, macromolecular complexes [3,4].



Ligands with complex structure have differential mobility at their binding sites. Substituted imidazole pyridines, for example, which inhibit the  $\text{H}^+/\text{K}^+$ -ATPase and have therapeutic use, are constrained in the imidazole moiety, but shows significant flexibility at the pyridine group [5] (see figure). It is this group which has a direct interaction with an aromatic (phe198) residue, with the potential for  $\pi$ -electron sharing [6]. Similarly, the steroid moiety of ouabain undergoes motions which are similar to those of the protein, but the rhamnose undergoes a high degree of flexibility at fast rates of motions whilst interacting with Tyr198 [7]. The quaternary ammonium group of acetyl choline, undergoes both kinds of interaction which are driven by thermal fluctuations and may be functionally significant [8].

Membrane protein 2 $^\circ$ -structural elements are often considered as relatively well defined, with connections of (relatively unstructured or mobile) loops, which are not necessarily easily defined structurally. However, these loops may be the most important domains for ligand binding, leading to subsequent activation. In addition, these loops are the regions where protein-protein interactions occur, thereby transferring a signal between an activated receptor and protein transduction in the signal cascade. It has been possible to show that solid state NMR detection (from the  $^{15}\text{N}$  spectral anisotropy of selectively labeled peptides) of loop regions of a receptor embedded in its natural membrane, permits the identification of the available crystal structure which is closest to the structure for the membrane-embedded, physiologically relevant structure [9].

- [1]. Watts, A. (2005) *Nature Drug Discovery*, 4, 555-568
- [2]. Watts, A., Straus, S.K., Grage, S., Kamihira, M., Lam, Y.-H. and Xhao, Z. (2003) Membrane protein structure determination using solid state NMR. In: *Methods in Molecular Biology – Techniques in Protein NMR Vol. 278* (ed. K. Downing), Humana Press, New Jersey, pp. 403-474.
- [3]. Watts, A. (1999). *Curr Op in Biotechn*, 10, 48-53.
- [4]. Watts, A. (2002) *Mol Memb Biol*, 19, 267-275
- [5]. Watts, J.A., Watts, A. & Middleton, D.A. (2001) *J. Biol. Chem.* 276, 43197-43204.
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- [7]. Middleton, D.A., Rankin, S., Esmann, M. and Watts, A. (2000) *PNAS*, 97, 13602-13607
- [8]. Williamson, P.T.F., Watts, J.A., Addona, G.H. Miller, K.W. and Watts, A. (2001). *PNAS*, 98, 2346-2351.
- [9]. Kamihira, M., Vosegaard, T., Mason, A.J., Straus, S.K., Nielsen, N.C. & Watts, A. (2005) *J. Struct. Biol.* 149, 7-16.

See also: [www.bioch.ox.ac.uk/~awatts/](http://www.bioch.ox.ac.uk/~awatts/)

### **P3. <sup>19</sup>F NMR-BASED SCREENING FOR BONA FIDE LIGAND IDENTIFICATION AND OPTIMIZATION**

**Claudio Dalvit**

*Nerviano Medical Sciences, Viale Pasteur, 10, 20014 Nerviano (MI), Italy,*

*claudio.dalvit@nervianoms.com*

NMR-based screening has emerged as a powerful and reliable tool in the identification of potential drug candidates. The technique is now recognized for its impact on the drug discovery process and an increasing number of pharmaceutical companies and universities are investing in this methodology. A plethora of different NMR experiments has been proposed in the literature for performing screening. Two of these approaches utilize the favourable properties of Fluorine NMR spectroscopy. FAXS (Fluorine chemical shift Anisotropy and eXchange for Screening) is a binding assay for the identification of ligands to the target of interest and for the measurement of their dissociation binding constant. 3-FABS (Three Fluorine Atoms for Biochemical Screening) is a functional assay for the detection of inhibitors of an enzyme and for the measurement of the 50% mean inhibition concentration. A WaterLOGSY identified CF or CF<sub>3</sub> ligand that, for his role, is called spy molecule is used in the FAXS and a CF<sub>3</sub> or a magnetically equivalent multiple CF<sub>3</sub> tagged substrate is used in the 3-FABS. The robustness of these methodologies together with the high quality data allow the selection of molecules displaying only a small displacement or a weak inhibitory effect thus capturing the broadest chemical structure diversity for potential lead molecule optimization. The theoretical aspects of these methodologies and some of their applications to different pharmaceutically relevant enzymes and proteins will be presented. These experiments coupled with a NMR-based quality control filter, recently developed (1), generate bona fide ligands or inhibitors that can be considered as starting points for medicinal chemistry efforts directed toward lead optimization.

1. C. Dalvit, D. Caronni, N. Mongelli, M. Veronesi & A. Vulpetti, *Current Drug Discovery Technologies*, (2006), 3, 2 in press.

## P4. <sup>13</sup>C DIRECT DETECTED NMR AS A SOURCE OF STRUCTURAL CONSTRAINTS IN BIOMOLECULES

Mario Piccioli

*Magnetic Resonance Center and Department of Chemistry, University of Florence, Via L. Sacconi, 50019 Sesto Fiorentino, Italy [piccioli@cerm.unifi.it](mailto:piccioli@cerm.unifi.it)*

<sup>13</sup>C direct detected *protonless* NMR spectroscopy<sup>1</sup> is a powerful tool to characterize systems where <sup>1</sup>H signals are difficult to analyze such as paramagnetic systems,<sup>2</sup> unfolded proteins, systems where H<sup>N</sup> signals cannot be observed due to exchange broadening, and possibly molecules with large size. In these cases, structural restraints such as NOEs and dihedral angles may be limited. Favourable relaxation properties and improved instrumental performances allow nowadays to overcome the loss in sensitivity due to the excitation and observation of low  $\gamma$  nuclei vs the established <sup>1</sup>H based approaches. Extensive signal assignment, dihedral angle<sup>3</sup> and residual dipolar couplings restraints<sup>4</sup> can be obtained even when the proton lines are very broad, or even not detected. Furthermore, in paramagnetic proteins structural constraints such as pseudocontact shifts and relaxation rates<sup>5</sup>, as well as a new generation of constraints arising from cross correlation rates<sup>6</sup>, can be obtained at substantially shorter metal-to-nucleus distance.

Among the test cases<sup>7</sup> we will consider, we will characterize the conformational features and the electronic structure of heme iron(III) environment in Hemophore HasA, a 19 kDa iron(III) hemoprotein that participates in the shuttling of heme to a specific membrane receptor. One dimensional and two dimensional <sup>1</sup>H, <sup>15</sup>N and <sup>13</sup>C complementary experiments allowed the identification of the resonances of the metal ligands and of other groups in the second coordination sphere of the iron.<sup>8</sup>

1. Bermel, W.; Bertini, I.; Felli, I. C.; Piccioli, M.; Pierattelli, R. *Progr.NMR Spectrosc.* **2006**, *48*, 25-45.
2. Arnesano, F.; Banci, L.; Piccioli, M. *Q.Rev.Biophys.* **2005**, *38*, 167-219.
3. S. Balayssac, I. Bertini, B. Jiménez and M. Piccioli. *J Magn Reson.* **2006**, in press.
4. S. Balayssac, I. Bertini, C. Luchinat, G. Parigi and M. Piccioli. submitted.
5. I. Bertini, B. Jiménez, M. Piccioli and L. Poggi *J. Am. Chem. Soc.* **2005** *127*, 12226-12227
6. F. Kateb and M. Piccioli. *J Am. Chem. Soc.* **2003**, *125*, 14978-14979.
7. S. Balayssac, B. Jiménez and M. Piccioli. *J Biomol. NMR* **2006**, *34*, 63-73.
8. C. Caillet-Saguy, A. Lecrosisey, M. Delepierre, I. Bertini, M. Piccioli and P. Turano *J Am. Chem. Soc.* **2006**, *128*, 150-158.

## **CONFERENCIAS INVITADAS**



## II. BIOMEDICAL APPLICATIONS OF *IN VIVO* AND *EX VIVO* METABOLISM BY NMR

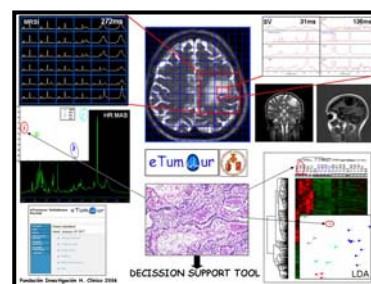
**Bernardo Celda<sup>a,b</sup>, M.Carmen Martínez-Bisbal<sup>a,b</sup>, Daniel Monleón<sup>a,b</sup>, Vicent Esteve<sup>a,b</sup>, M.Beatriz Martínez-Granados<sup>a,b</sup>, Rubén Ferrer-Luna<sup>a,b</sup> and Eva Piñero<sup>a,b</sup>**

<sup>a</sup> *Aplicaciones Biofísicas y Biomédicas de la RMN, Departamento de Química Física, Universitat de Valencia (bernardo.celda@uv.es);* <sup>b</sup> *Laboratorio de Imagen Molecular, UCIM/SCSIE, Universitat de Valencia*

MRS, specially <sup>1</sup>H MRS, was extensively applied to complete and improve the diagnosis and prognosis of Central Nervous System (CNS) pathologies, as Alzheimer [1], epilepsy [2], multiple sclerosis [3-5], schizophrenia [6], paediatric development and in particular to the study of brain tumours [7-9]. A summary of the applications of <sup>1</sup>H MRS to the in vivo diagnosis and prognosis of brain tumours will be presented. In addition, examples of metabolite distribution, infiltration and high cellularity location for neurosurgery applications by MRS molecular images will be shown. Moreover, ex vivo methods for studying the detailed biochemistry of tumour biopsies as metabolomic (HR-MAS) [10, 11], transcriptomic and genetic (DNA microarrays and SNPs) will be discussed as a complementary tool to in vivo MRS and ex vivo HR-MAS (FP6 european project eTUMOUR) [9] (Figure 1) and other tissue techniques like pathology tissue typing. A preliminary comparison between molecular images from PET and <sup>1</sup>H MRS will be also presented. Finally, the application of <sup>1</sup>H MRS to the improvement of prostate diagnosis and prognosis [12], the second leading cause of cancer death, will also be discussed, focusing on the contribution of MRS molecular images to the cancer location.

### References

1. MC. Martínez-Bisbal et al. *Eur. J. Neurol.*, **2004**, *11*, 187-193.
2. B. Martínez-Granados et al.
3. B. Casanova, et al.
4. MC. Martínez-Bisbal et al. *Rev. Neurología*, **2002**, *34*, 807-813
5. M.Gadea et al. *Brain*, **2004**, *127*, 89-98
6. B. Martínez-Granados et al. *MAGMA*, **2004**, *17(S1)*, 219
7. M. C. Martínez-Bisbal et al. *MAGMA*, **2005**, *18(S1)*, 68
8. MC. Martínez-Bisbal et al. *Rev. Neurología*, **2002**, *34*, 309-313.
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10. M.C.Martínez-Bisbal et al. *NMR Biomedicine*, **2004**, *17*, 191-205.
11. B. Martínez-Granados et al. *NMR Biomedicine*, **2006**, *18*, 1-11
12. M.C. Martínez-Bisbal et al. *European Radiology*, **2005**, *15(S1)*, 449.



**Figure 1.- Example of in vivo <sup>1</sup>H MRS biochemical profile FP6 project eTUMOUR (LSH-2004-503094)**

*This work has been carried out with financial aid of the Ministerio de Educación y Ciencia (Project number SAF2004-06297), Instituto Salud Carlos III (ISCIII2003-G03/185, ISCIII2005-PI2695) and the EC FP6 projects eTUMOUR (LSH2004-503094) and HealthAgents (2004-IST27214)*

## 12. USE OF THE *IN VIVO* MR SPECTROSCOPY FOR THE DIAGNOSIS AND MONITORING OF NEURODEGENERATIVE DISORDERS

**Ángela Bernabeu<sup>a,d</sup>, Jaume Morera<sup>b</sup>, C. Serna-Candel<sup>c</sup>, C. Martín-Estefanía<sup>c</sup>, S. Martí<sup>c</sup>, L. Turpín<sup>c</sup> y T. Frutos-Alegría<sup>b,c</sup>.**

<sup>a</sup> *Universidad Miguel Hernández de Elche, angela.bernabeu@umh.es,* <sup>b</sup> *Centro de Diagnóstico Precoz de la Enfermedad de Alzheimer (CDP-ALZ San Vicente). Hospital San Vicente. San Vicente,* <sup>c</sup> *Servicio de Neurología. Hospital General Universitario de Alicante,* <sup>d</sup> *Unidad de Resonancia Magnética. INSCANNER. Alicante.*

Proton magnetic resonance spectroscopy (1H-MRS) has been proposed in conjunction with MR imaging as a method for the evaluation of patients with neurological disorders. There is a substantial body of literature dealing with predictors including the MR spectroscopy in patients with Alzheimer disease (AD) and Amyotrophic Lateral Sclerosis (ALS). These predictors range from a simple delayed recall task on Mini-Mental to sophisticated radiological techniques and CSF biomarkers. Noninvasive markers of disease progression starting from the earliest stages of pathologic involvement are required for determining the evolution and effectiveness of putative disease-modifying therapies that are under development. The <sup>1</sup>H MRS provides a noninvasive way to investigate *in vivo* neurochemical abnormalities providing potentially information about unique *in vivo* pathological processes at the molecular or cellular level. The aim of this work was to analyze the predictive value and the usefulness of the MRS in the study and monitoring of two neurodegenerative diseases: the AD and the ALS. With this purpose two different protocols were performed combining physical, specific psychological test, MRI studies and MRS analyses. For the MR spectroscopy studies the brain regions analyzed were specific for each disease and chosen according to literature. Standard ratios of *N*-acetyl-aspartate/Creatine (NAA/Cr), Glutamate-Glutamine/Creatine (Glx/Cr), Choline/Creatine (Cho/Cr) and Mioinositol/Creatine were calculated by SVS exam in a 1.5T scanner. The spectroscopy data statistical analyses obtained in both cases presented a good correlation with the clinical findings. The results obtained allowed us to generate a more sensitive protocol than the standard MR imaging findings in the detection of both neurological disorders, as well as determine different brain areas implicated in the ALS.

### 13. THE USE OF NMR SPECTROSCOPY TO STUDY TAUTOMERISM

**Rosa M. Claramunt, Concepción López, M. Dolores Santa María, Dionisia Sanz and José Elguero**

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A well-known but clearly established anecdote related the failure of Linus Pauling to reach before Francis Crick and James Watson the structure of DNA, to his use of wrong tautomers for the bases [1]. Although this is certainly not only the difference between both teams, a major role being played by crystallographers Rosalind Franklin and Maurice Wilkins [2], it certainly illustrates the importance of having a clear idea of the structures and general problems involved in the study of tautomerism.

Considering only prototropy, there are six domains to be explored, taking into account the different phases of the matter (gas-phase, solution and solid-state) and the existence of thermodynamic aspects (equilibrium constants  $K$ ) and kinetic aspects (rate constants  $k$ ).

	Gas-phase	Solution	Solid-state
$k$	<1%	5%	2%
$K$	2%	85%	5%

Prototropy is a widespread phenomena that requires only that the proton can migrate between at least two positions. Generally these two positions are linked by a system of double bonds as in  $\mathbf{H-X-Y=Z} \rightleftharpoons \mathbf{X=Y-Z-H}$ . We will examine representative examples that fulfill such condition: carbonyl compounds, heterocyclic derivatives, tropolone and fulvene derivatives to demonstrate that even the solution NMR spectroscopy has in many ways overshadowed the steady growth of gas-phase and solid state, the application of magic-angle-spinning techniques to solid samples has revolutionized NMR spectroscopy of solids [3].

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*This work has been carried out with financial aid of the Ministerio de Educación y Ciencia (Project number BQU2003-00976).*

## **I4. STRUCTURAL STUDIES ON THERAPEUTICALLY RELEVANT PROTEINS: DRUG DISCOVERY IMPLICATIONS**

**Antonio Pineda-Lucena, Rodrigo J. Carbajo, David Pantoja-Uceda, Cheryl Hawkins, Ana M. Fernández-Escamilla, Silvia Mosulén, Leticia Ortí**

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NMR spectroscopy is a versatile biophysical technique with wide applicability in drug discovery research, particularly for the detection and characterization of molecular interactions. In general, biomolecular NMR plays a very important role at the different stages of the drug discovery program: target selection, hit identification, and lead optimization. Furthermore, its role is becoming increasingly important in pharmacokinetic and pharmacodynamic profiling of compounds.

A potential drug target is defined as a protein with a propensity to bind small molecules that modulate its activity (druggable target) and with the potential to be disease modifying. In any case, a prerequisite for initiating structural studies is to obtain protein in enough quantity for NMR characterization. This is probably one of the main "bottlenecks" of the whole process, since it is imperative to produce proteins in a stable and soluble form.

Our laboratory is interested in the structural elucidation of therapeutically relevant proteins, and in the identification of small molecule modulators of their activity. In order to do that, we use NMR in combination with computational chemistry and biochemistry approaches.

In this presentation, I will review some of the projects we are working on at this time, like our efforts to understand the role that solvent-exposed residues play in the stability of proteins using p53 tetramerization domain as a model, the design of peptide models to obtain a deeper insight into protein-protein interactions, the structural characterization of disintegrins, and our initial results on heparanase, an enzyme involved in the degradation of the extracellular matrix, which plays a key role in metastasis.

## **15. ESEEM SPECTROSCOPY APPLIED TO THE STUDY OF MOLECULAR SYSTEMS OF BIOLOGICAL INTEREST**

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The study of the electronic structure of active centres in redox proteins is of great interest in order to determine the structure – function relationship in these systems, which are involved in diverse function of living organisms. As far as they can be found in a paramagnetic state, Electron Paramagnetic Resonance (EPR) spectroscopy can be, in principle, a suitable technique for applying in this kind of studies. So, the analysis of the hyperfine structure provide a detailed map of the unpaired electron distribution on the active molecule. However, the hyperfine structure is scarcely resolved when conventional, cw-EPR spectroscopy is used because of the inhomogeneous broadening of the signals and of the need to use spatial disordered samples. These limitations can be overcome by using advanced EPR techniques based in the Electron Spin Echo Envelop Modulation (ESEEM) phenomena. In particular we have used in our laboratory ESEEM techniques, mainly the 2D-ESEEM HYSCORE spectroscopy, to obtain a detailed unpaired electron map in some radical an heme proteins in their paramagnetic states.

An overview of the ESEEM spectroscopy background together the presentation of some examples recently studied in our laboratory will be given.

*Financial aid of the Ministerio de Educación y Ciencia (Project number BFU2005-07422-C02-02/BMC) is acknowledged.*

## 16. IN VIVO NMR AS A TOOL IN SYSTEMS METABOLIC ENGINEERING OF DAIRY BACTERIA

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Lactic acid bacteria (LAB) are used worldwide in the production of fermented dairy products. *Lactococcus lactis*, a model organism for LAB, is a suitable object for metabolic engineering strategies aiming at the improvement of food quality and human health. However, a deep understanding of the metabolic network as well as of the interdependence relationships at a system level is essential to a rational design of strains. To achieve this ambitious goal, powerful techniques involving global transcriptome and proteome analysis need to be complemented with the analysis of the ensemble of metabolites in the cell, desirably as a function of time.

During the last decade our team directed a considerable effort to the development of *in vivo* NMR techniques with the goal to characterize and quantify metabolite pools directly in living cells [1-5]. It has become possible to monitor the dynamics of those pools in response to well-defined stimuli, such as a pulse of substrate, a switch in the gas atmosphere (anaerobic/aerobic) or pH, as well as the effect of a specific mutation.  $^{13}\text{C}$ -NMR was used for the determination of the dynamic pools of intracellular metabolites with a time resolution of about 30 seconds, since NMR can measure continuously and non-invasively the actual amounts of  $^{13}\text{C}$ -label incorporation into specific carbon atoms of different metabolic intermediates.  $^{31}\text{P}$ -NMR was used to follow phosphorylated intermediates and the energetic status of the cells, giving a detailed picture of the metabolizing cell.

Examples of our work on the application of  $^{13}\text{C}$ -NMR and  $^{31}\text{P}$ -NMR to direct metabolic engineering strategies in *Lactococcus lactis* will be presented.

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## 17. MOLECULAR RECOGNITION OF ACTIVATED SUGARS BY HUMAN BLOOD GROUP B GALACTOSYLTRANSFERASE: INSIGHTS FROM NMR EXPERIMENTS

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Glycosyltransferases are responsible for the synthesis of complex oligosaccharides by transferring a single monosaccharide unit from a nucleotide donor to the hydroxyl group of an acceptor substrate. The histo blood group AB0(H) antigens are defined carbohydrate determinants found on the surface of red blood cells responsible for failure of mismatched blood transfusions, and playing also important roles in cell development, differentiation and oncogenesis. A and B antigens are obtained by the transfer of an  $\alpha$ -D-GalNAc, or  $\alpha$ -D-Gal residue, onto the H-type disaccharide  $\alpha$ -L-Fuc-(1,2)- $\beta$ -D-Gal-OR, employing activated sugars (UDP-GalNAc or UDP-Gal) as glycosyl donors. The transfer reactions are catalyzed by the highly specific enzymes  $\alpha$ 1,3 *N*-acetyl-galactosaminyl-transferase (GTA), or  $\alpha$ 1,3 galactosyl-transferase (GTB). They are highly homologous enzymes, differing at only four amino acids of 354. Recently, the elucidation of the crystal structures of GTA and GTB in complex with UDP and the H-antigen, revealed the structural bases for the discrimination of donor substrates by each enzyme<sup>(1)</sup>. Nevertheless, many questions remain unsolved, since the donor hydrolysis during crystallisation precluded the determination of the bioactive conformation of the residue to be transferred (GalNAc, or Gal), and two loops were disordered and could not be resolved. Furthermore, the catalytic mechanism is still a matter of discussion.

We have carried out a detailed NMR binding analysis with the retaining enzyme GTB and its donor and acceptor substrates utilizing STD NMR and transferred NOESY experiments to deduce relative affinities, binding kinetics, and the bioactive ligand conformations<sup>(2),(3)</sup>. Transfer NOESY experiments reveal that the donor substrate UDP-galactose and the inactive analog UDP-glucose bind to GTB in a folded conformation that is sparsely populated in solution, whereas acceptor ligands bind in a conformation that predominates in solution. STD NMR build up curves show that acceptor substrates dissociate significantly faster ( $k_{off} > 100$  Hz) from the binding pocket than donor substrates ( $k_{off} \approx 10$  Hz), and that proper recognition of the hexopyranose rings of the UDP sugars requires bivalent metal cations. Our experiments lead to a model that suggests a three-step catalytic process in which Asp 302 and Glu 303 act as “molecular tweezers”.

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*This work has been carried out at the research group of Prof. Thomas Peters; I also want to give my thanks to B. Langpap, A. Blume, T. Biet, H.Peters, M.Palcic, B. Meyer and N.R.Krishna. Financial aid by a Marie Curie Intra-European Fellowship (MEIF-CT-2003-500861) is gratefully acknowledged.*

## 18. PROTEIN STABILITY STUDIED BY NMR SPECTROSCOPY

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Refining our comprehension of protein stability is essential for understanding protein structure and function. Stability studies are also important to understand protein folding because many eukaryotic proteins are intrinsically unstructured and only fold upon interaction with another molecules, and since unstructured and partially unfolded conformations are prone to form aggregates that have been implicated in a large number of human diseases. NMR is an excellent technique for stability studies; it gives information that is not obtained by other methods such as the pK<sub>a</sub> of individual charged residues, the tautomeric state of histidine side chains, and the stability of individual amide groups. Moreover 1D or 2D NMR spectra can rapidly reveal whether a protein is folded or not, and are useful for screening different conditions (temperature, pH, denaturing agents, ligands, etc.) that affect protein stability. Different 1D or 2D NMR techniques have been applied including H/D exchange, urea, guanidine hydrochloride or pH titrations monitored by NMR, to study the stability of different systems.

In the last years, we have studied the folding and the conformational and pH dependent stability of several proteins<sup>1-7</sup>. In this lecture we will present some examples covering the folding pathway of the Che Y protein; the specific contribution of  $\alpha$ -sarcin's active site residues to the pH stability; and the per residue contributions to the conformational stability of some Ribonucleases, RNase Sa and RNase A and some related variants.

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## 19. INCLUDING $^{13}\text{C}$ DIRECT DETECTED EXPERIMENTS FOR THE STRUCTURAL & DYNAMICAL CHARACTERIZATION OF Cut A1

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The sequence specific assignment of the NMR signals of a protein is still a mandatory step for solution structure calculations. This often prevents the investigation of molecules of high molar mass as fast transverse relaxation rates quench the efficiency of coherence transfer between different nuclei. Consequently, the experiments commonly used for sequence specific assignment fail.<sup>1</sup>

As  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclei possess smaller magnetic moments, heteronuclear ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) direct detection has been proposed to complement  $^1\text{H}$  detection and so overcome the experimental limitations induced by fast relaxation.<sup>2,3</sup> Technological advances, that have improved the sensitivity on the dedicated  $^{13}\text{C}$  coils, enable the inclusion of a heteronuclear-detected experiment in the usual carnet of experiments available for NMR characterization of a protein in solution.

We demonstrate here that a hybrid approach using proton- and carbon-based experiments permits sequence specific assignment of the complete polypeptide chain of CutA1 from *E. coli*, a trimeric protein of molar mass 36 kDa. CutA1 is a family of proteins distributed widely in bacteria, plants, and animals, including humans. It is expressed as part of the *cop* operon whose specific function is still unknown but may include aspects of copper. The molar mass of this protein represents a limit in the current capacity for NMR signal assignment ability in the absence of fractional deuteration. With the assignment in hand, it was possible to obtain a first structural characterization of this protein in solution to complement the data from X-ray crystallography.<sup>4</sup> It was also possible to study some aspects of its dynamics, metal up-taken and obtain some insights on its unknown function.

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*BJ is a Marie Curie Intra European Fellow (contract MEIF-CT-2005-515039).*

## I10. TAR RNA RECOGNITION BY A NOVEL CYCLIC AMINOGLYCOSIDE

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The TAR RNA hairpin is a key regulatory element of HIV gene expression. To obtain full-length viral transcripts, Tat protein binds TAR and, subsequently, the Tat/TAR complex recruits the positive transcription elongation factor complex (P-EFTb), which interacts with TAR through its cyclin T1 (CycT1) component.<sup>1</sup> The TAR hexanucleotide loop is a crucial region for contacting CycT1.<sup>2</sup> Interfering with the interaction between Tat/CycT1 and the TAR-RNA is an attractive strategy for the design of anti-HIV drugs. Positively charged molecules, like aminoglycosides or peptidomimetics, bind the TAR-RNA, disrupting the Tat/TAR complex.<sup>3</sup>

We have determined the structure of the complex between the HIV-2 TAR RNA and a novel aminoglycoside analogue by NMR spectroscopy. In contrast to other aminoglycosides, this neooligoaminodeoxysaccharide contacts simultaneously the bulge residues required for Tat binding and the A35 residue of the hexanucleotide loop. The novel binding mode of the ligand and the conformational changes experienced by TAR upon binding will be discussed.

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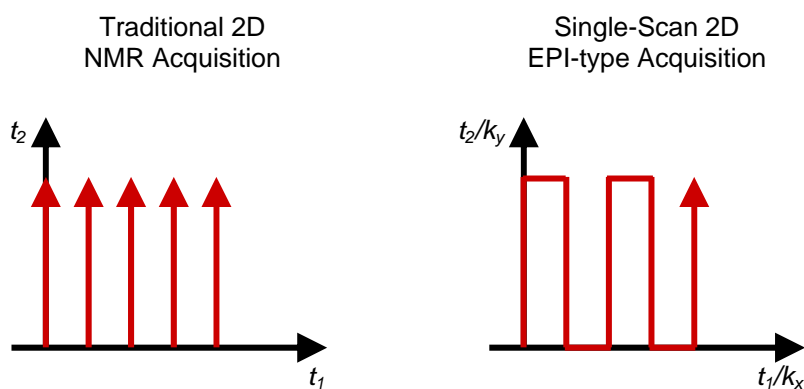
*This work has been carried out with financial aid of the Max Planck Society and the Deutsche Forschungsgemeinschaft (SFB416 to A.K. and T.C.). V.M.S.-P. wants to acknowledge the funding from the European Union (Marie Curie fellowship) and the Ministerio de Educación y Ciencia (Ramón y Cajal contract).*

## I11. ATTEMPTS APPLYING ULTRAFAST NMR SPECTROSCOPY

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NMR offer a well known spectroscopic technique to monitorize dynamic chemical and biochemical processes. However, limitations of 1D NMR appears with complex systems and their associated multiple overlapped lines. A solution to this problem is afforded by multidimensional NMR spectroscopy, which provides a higher spectral dispersion necessary to overcome the overlapping problem. This multiple dimensions are explored via the collection of numerous time-incremented scans. However, the problem associated with the acquisition of this experiments consists of long acquisition times, which are normally required. The possibilities that could be opened by speeding up the multidimensional NMR protocols are very attractive. Lucio Frydman<sup>1</sup> have developed a new methodology called “*Ultrafast NMR Spectroscopy*” based on the application of *echo planar imaging* (EPI) protocols for the data acquisition.



We have starting up to work with this new methodology trying to apply it to the study of dynamic processes. Over our results and problems found will be spoken.

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## I12. PGSE DIFFUSION NMR: DO WE CARE ABOUT THE ROLE OF THE COUNTERIONS?

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Pulsed Gradient Spin Echo (PGSE) NMR diffusion methods are commonly used to assist in determining the relative volume of a compound via the experimental diffusion coefficient,  $D$ , which can be related to the hydrodynamic radius,  $r_H$ . However, additional important applications include the recognition of hydrogen bonding, host/guest encapsulation, ion-pairing, and/or aggregation.<sup>1</sup> The ability to simply inspect the  $D$  values and then make a qualitative estimation of the amount of, for instance ion-pairing, makes the PGSE methodology a very attractive and a valuable method. To properly appreciate the significance of these effects one has to combine PGSE diffusion methods with NOE measurements (*i.e.*  $^1\text{H}$ ,  $^{19}\text{F}$  HOESY in fluorinated ions), since the PGSE results indicate the presence of ion-pairs but do not provide structural insights. HOESY data allow us to obtain a much more detailed understanding of how anions and cations interact in complex salts.

In this lecture, this combined NMR approach will be applied to coordination compounds of ruthenium<sup>2</sup> and some model organolithium compounds,<sup>3</sup> with a view to emphasizing that the solution structure of such salts is often rather specific, *i.e.*, the “innocent” counterion is not taking up a random position.

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## I13. APPLICATIONS OF NMR TECHNIQUES TO THE CONFORMATIONAL ANALYSIS OF SMALL- AND MEDIUM-SIZED RINGS

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In addition to structure elucidation, nuclear magnetic resonance spectroscopy has proved its potential for the study of various kinds of dynamic processes in solution, such as chemical exchange or conformational analysis, whether one deals with stable conformers or molecules where the rapid interconversion occurs at ambient temperature.

We have been interested in the synthesis, conformational analysis, and reactivity of small and medium-sized ring systems with the goal of developing a conformational model to rationalize the physical and chemical properties of these structures.

The rotational processes that allow the interconversion between conformers have been widely investigated by dynamic NMR spectroscopy in solution. These studies are accompanied by theoretical calculations that usually reproduce quite satisfactorily the experimental barriers.

In this communication, several examples of conformational studies on natural products, nucleosides and small peptides will be presented.

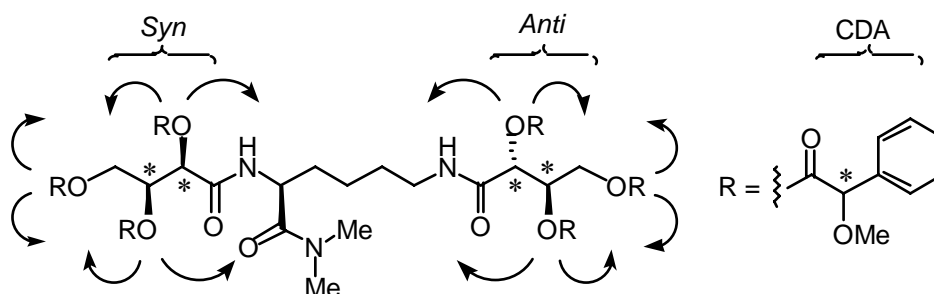
# I14. CHIRAL POLYFUNCTIONAL COMPOUNDS: THE DISCLOSURE OF THEIR CONFIGURATION BY NMR

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In the last decade, NMR has been profusely employed for assignment of the absolute configuration of monofunctional compounds.<sup>1</sup> In most cases, the method is based on the derivatization of the substrate of unknown configuration with the enantiomers of proper chiral derivatizing agents (CDAs) and analysis of the resulting  $\Delta\delta^{RS}$  parameters. The expansion of this approach to compounds bearing two or more chiral functional groups is a recent improvement due to its complexity.<sup>2</sup> In these molecules, the introduction of several CDAs makes the interpretation of the NMR spectra difficult because the chemical shifts depend on the combination of all the different anisotropic effects generated by the different CDA units.



In this presentation we will show experimental evidence that demonstrates that the absolute configuration of chiral diols, triols and aminoalcohols can be determined in a simple way by use of  $\Delta\delta^{RS}$  and new NMR parameters.

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## **I15. MEDIDA DE ACOPLAMIENTOS ESCALARES Y DIPOLARES RESIDUALES EN PROTEÍNAS**

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La determinación de la estructura tridimensional de macromoléculas biológicas se basa fundamentalmente en la información que proporcionan los efectos Overhauser nucleares, cuya magnitud es función de la distancia entre los dos núcleos entre los que se produce el efecto. Si bien esta información es en general suficiente para la determinación estructural, la obtención de estructuras tridimensionales de alta resolución suele requerir la utilización de información experimental adicional. Entre esta destacan por su utilidad los acoplamiento escalares a tres enlaces y los acoplamiento dipolares residuales. Los primeros están relacionados con el ángulo dihedro alrededor del enlace central y contribuyen por ello a una mejor definición de las estructuras a escala local. Los segundos dependen de la orientación del vector que une a ambos núcleos acoplados, contribuyendo sobre todo a una mayor precisión en la orientación relativa de parejas de núcleos acoplados y con ello de los elementos de estructura secundaria.

En esta conferencia presentaremos la optimización de algunas secuencias de medida de acoplamiento escalares en proteínas para la que se ha tenido en cuenta la introducción de la tecnología PEP, una minimización de la saturación del agua, la optimización de los tiempos de evolución y la minimización de los ciclos de fase. Igualmente se presentarán secuencias desarrolladas en nuestro grupo para la medida de acoplamiento dipolares residuales, ilustrándose su aplicación con algunos ejemplos.

## I16. APLICATIONS OF DIFFUSION NMR SPECTROSCOPY TO ORGANIC COMPOUNDS

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The measurement of the molecular translational self-diffusion coefficients (D) by high-resolution NMR spectroscopy has emerged as an interesting application to solve a great and diverse number of chemical and biochemical issues<sup>1</sup>. Diffusion coefficients are directly related to interesting molecular restraints such as hydrodynamic radius, molecular size or molecular weight<sup>2</sup>. Usually, diffusion coefficients are extracted by analysing the signal decay of specific resonances as a function of the applied gradient strength in 1D pulsed-field gradient spin-echo (PFGSE) related sequences.

Here we report a brief description of the existing sequences and the main sources of possible errors which affect such measurements. We give some tricks to avoid or minimise the undesired convection effects<sup>3</sup>. In addition, we present some applications to analyze mixtures of compounds, to study intermolecular interactions, to estimate molecular weights and to eliminate the signals of the residual nondeuterated solvent and water, from the <sup>1</sup>H spectra of the organic compounds<sup>4</sup>.

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## **I17. ESTRUCTURA Y MOVILIDAD SUPERFICIAL EN SILICATOS LAMINARES DEDUCIDAS CON TÉCNICAS RMN DE ALTA Y BAJA RESOLUCIÓN**

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El estudio RMN de filosilicatos  $R^{+}_{1+x-y}(\text{Si}_{3-x}\text{Al}_x)(\text{Mg}_{3-y}\text{Al}_y)\text{O}_{10}(\text{OH},\text{F})_2$ , ha permitido analizar los factores cristalocquímicos que afectan el desplazamiento químico de las señales RMN. El estudio de las interacciones cuadrupolares en la señal del  $^{27}\text{Al}$  ( $I=5/2$ ) ha mostrado que la posición de la señal RMN no solo depende del entorno químico, sino de las distorsiones geométricas producidas en los poliedros de coordinación. En el caso de la señal de  $^{29}\text{Si}$  ( $I=1/2$ ), el desplazamiento químico depende únicamente del entorno químico de los átomos.

La resolución de varias componentes en la señal de  $^{29}\text{Si}$ , debidas a átomos de Si rodeados por 3Al, 2Al1Si, 1Al2Si y 3Si, ha permitido analizar la distribución Si,Al en las capas tetraédricas de los filosilicatos. En muestras con  $\text{Si}>\text{Al}$ , esta distribución está de acuerdo con la regla de Loewenstein (ausencia de enlaces  $\text{Al}_T\text{-O-Al}_T$ ); sin embargo, en muestras con  $\text{Al}>\text{Si}$ , este criterio no es respetado (técnicas MAS y MQMAS). En la capa octaédrica de filosilicatos con cationes de igual carga,  $\text{Mg}^{2+}$  y  $\text{Fe}^{2+}$ , la substitución de grupos  $\text{OH}^-$  por  $\text{F}^-$  favorece la formación de dominios fluoro-magnésicos. Este estudio ha sido llevado a cabo en las señales de  $^1\text{H}$  y  $^{19}\text{F}$  de muestras monocristalinas.

Finalmente, se ha analizado la disposición y movilidad de las moléculas de agua adsorbidas en filosilicatos. En vermiculitas, las moléculas de agua adoptan dos disposiciones respecto a las láminas del silicato y su movilidad presenta un marcado carácter anisotrópico. En el caso de materiales porosos tipo sepiolita, se ha podido diferenciar el agua zeolítica del agua coordinada a cationes  $\text{Mg}^{2+}$ . En este silicato, las posiciones propuestas para el agua de cristalización han sido confirmadas con la técnica bidimensional HETCOR. La utilización de la técnica PFG permite deducir los coeficientes de difusión de las moléculas de agua y de los cationes compensadores, abriendo así la posibilidad de analizar la dinámica local del agua en estos filosilicatos.

## **I18. EXPLORING THE USE OF CONFORMATIONALLY LOCKED AMINOGLYCOSIDES AS A NEW STRATEGY TO OVERCOME BACTERIAL RESISTANCE**

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The emergence of bacterial resistance to the major classes of antibiotics has become a serious problem over recent years. For aminoglycosides, the major biochemical mechanism for bacterial resistance is the enzymatic modification of the drug. Interestingly, in several cases, the oligosaccharide conformation recognized by the ribosomal RNA and the enzymes responsible for the antibiotic inactivation is remarkably different. This observation suggests a possible structure-based chemical strategy to overcome bacterial resistance; in principle, it should be possible to design a conformationally-locked oligosaccharide that still retains antibiotic activity but, that is not susceptible to enzymatic inactivation. In order to explore the scope and limitations of this strategy, we have synthesized several aminoglycoside derivatives locked in the ribosome-bound ‘bioactive’ conformation. The effect of the structural preorganization on RNA binding, together with its influence on the aminoglycoside inactivation by several enzymes involved in bacterial resistance has been studied. Our results indicate that the conformational constraint has a modest effect on their interaction with ribosomal RNA. In contrast, it may display a large impact on their enzymatic inactivation. Thus, the work presented herein provides a key example of how the conformational differences exhibited by these ligands within the binding pockets of the ribosome and of those enzymes involved in bacterial resistance can, in favorable cases, be exploited for designing new antibiotic derivatives with improved activity in resistant strains.

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## **COMUNICACIONES ORALES**



## O1. NMR SOLUTION STRUCTURE OF A CHIMERIC PROTEIN MIMICKING A SH3-PEPTIDE COMPLEX

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We have designed previously a single-chain chimeric protein<sup>1</sup>, named SPCp41, by connecting a circular permutant of the  $\alpha$ -spectrin SH3 domain<sup>2</sup> to the proline-rich decapeptide p41<sup>3</sup>. SPCp41 mimics in its tertiary fold the interactions found in the SH3-p41 complexes. This property makes of it a very useful tool to analyse the thermodynamic determinants of binding between SH3 domains and proline-rich peptides by stability measurements combined with site-directed mutagenesis. The analysis of the high-resolution structure of SPCp41 and its conformational dynamics is essential to understand the thermodynamic magnitudes observed. We present here the high-resolution structure of SPCp41, together with the analysis of its backbone dynamics using NMR methods. The complete assignment of the NMR spectra has been achieved using a set of triple-resonance NMR experiments with <sup>15</sup>N/<sup>13</sup>C -labelled samples of SPCp41. The three-dimensional structure of the protein was calculated on the basis of both distance and dihedral angle experimental restraints. In addition, the backbone dynamics of SPCp41 have been investigated using <sup>1</sup>H-<sup>15</sup>N relaxation measurements with a <sup>5</sup>N-labelled sample. A detailed analysis of the solution structure of SPCp41 confirms that both the conformations of the SH3 and p41 moieties as well as the interactions at the SH3-p41 interface are extremely similar to those found in complexes between the isolated p41 peptide and SH3 domains.

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## O2. STRUCTURE OF $\alpha$ -SYNUCLEIN AMYLOID FIBRILS USING H/D EXCHANGE NMR

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$\alpha$ -synuclein is a 140 amino acid neuronal protein that has been associated with several neurodegenerative diseases. Mutations in  $\alpha$ -synuclein mediate the intracellular aggregation of the protein into insoluble amyloid fibrils, causing cell-death and eventually Parkinson's disease (PD). Amyloid fibrils are highly organized aggregates formed by peptides and proteins with a wide variety of structures and functions. Because of their inherently non-crystalline and insoluble nature, fibrils are not amenable to characterization with solution-state NMR or X-ray crystallography, and detailed and site-specific structural information has been difficult to obtain. However, different direct and indirect techniques have been successful used to gain structural information of those fibrils <sup>1</sup>. Recently we described the 3-D structures of Het-s prion and A $\beta$ (1-42) peptide amyloid fibrils <sup>2,3</sup>. Here, using quenched H/D exchange NMR, fluorescence spectroscopy, high resolution cryoelectron microscopy and site-directed mutagenesis we determine the secondary structure elements of  $\alpha$ -synuclein full-length (140 residues) and  $\alpha$ -synuclein core (residues 30-110) in the amyloid fibril state. A 3D model of the  $\alpha$ -synuclein core in the amyloid fibril is proposed.

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### **O3. SIMULTANEOUS ALFA/BETA SPIN STATE SELECTION FOR 13C AND 15N FROM A TIME SHARED HSQC-IPAP EXPERIMENT**

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Two novel approaches to achieve alfa/beta spin-state editing simultaneously for  $^{13}\text{C}$  and  $^{15}\text{N}$  in a single experiment are proposed. The pulse schemes are based on a time-shared  $^1\text{H},^{13}\text{C}/^1\text{H},^{15}\text{N}$ -HSQC correlation experiment that combines concatenated echo elements to allow simultaneous  $J(\text{CH})$  and  $J(\text{NH})$  coupling constant evolution, time-shared (TS) evolution of  $^{13}\text{C}$  and  $^{15}\text{N}$  chemical shifts and heteronuclear alfa/beta-spin-state selection by means of the IPAP principle. Heteronuclear alfa/beta-editing for all  $\text{CH}_n$  ( $n=1-3$ ) and  $\text{NH}_n$  ( $n=1-2$ ) multiplicities can be achieved in the detected F2 dimension of a single F2-alfa/beta TS-HSQC-IPAP experiment. On the other hand, an alternative F1-alfa/beta TS-HSQC-IPAP experiment is also proposed to achieve alfa/beta-editing in the indirect F1 dimension. Experimental and simulated data is provided to evaluate these principles simultaneously on backbone and side-chain CH, CH<sub>2</sub>, CH<sub>3</sub>, NH, and NH<sub>2</sub> spin systems in uniformly  $^{13}\text{C}/^{15}\text{N}$ -labeled proteins and small natural-abundance peptides.

## O4. MILLISECOND DYNAMICS IN DOUBLE HELICAL DNA

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Dynamics on the millisecond time range are most important for nucleic acid and protein function and recognition. We describe NMR spectroscopy studies on the complexes of DNA with bisnaphthalimide intercalators, which were unexpectedly found to provide valuable dynamic information on this time scale. The bisnaphthalimides are a family of antitumour agents composed of two planar rings linked by a flexible chain (Fig. 1). When bound to DNA, the two rings of these drugs intercalate between base pairs and at the same time undergo 180° rotating motions that do not affect the linker atoms bound to the DNA groove<sup>1</sup>. These motions have been detected in a broad range of sequence contexts and conditions, and a comparative analysis of frequencies and activation energies indicated that they are a consequence of sequence-dependent nucleotide movements taking place on the millisecond time scale<sup>2</sup>. Intriguingly, nucleotide motions on a similar time range are also observed in DNA duplexes containing the universal base analogue 5-nitroindol<sup>3</sup>. By choosing an appropriate system for NMR analyses, we could determine the solution structure of two 5-nitroindol-containing DNA duplexes, and provide a hypothesis accounting for the observed dynamics. These studies provide insight on the frequencies, activation energies and magnitude of millisecond nucleotide movements.

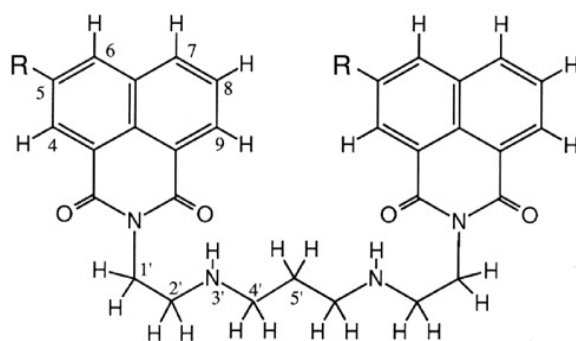


Figure 1: the bisnaphthalimide elinafide

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## O5. FOUR-STRANDED DNA STRUCTURES STABILISED BY MINOR GROOVE TETRADS

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The discovery of a large variety of non-canonical DNA structures has attracted much interest because of their potential biological relevance and therapeutic applications. Particularly, DNA quadruplexes may play important roles in replication, transcription and recombination. In previous studies, we have shown that cyclic octamers can self-associate to form dimeric DNA quadruplexes containing minor groove-aligned tetrads of different types (A:T:A:T, G:C:G:C and G:C:A:T) with four inter-subunit Watson-Crick base pairs. As part of our studies on the requirements for the formation of this unusual DNA motif, we have explored the structure and stability of several linear and cyclic octamers by using NMR and CD spectroscopy.

We have recently observed, on one hand, that Watson-Crick base pairing is not required for the formation of minor groove tetrads and, on the other hand, that short cyclic oligonucleotides can take advantage of the motif to induce hairpinlike structures in linear DNA fragments. This recognition mode between cyclic and linear oligonucleotides may open the door to targeting biologically important DNA hairpins.

Nevertheless, if these structures were to occur naturally in cellular processes, linear oligonucleotides should form this motif in solution. To date, it has only been observed in the X-ray crystal structure of the linear heptamer d(GCATGCT). Attempts to observe it in solution with linear oligonucleotides have failed mainly because of competition with other structures. However, a careful choice of the sequence has now allowed the quadruplex motif to be observed for the first time in solution in the structure of the linear octamer d(TGCTTCGT). For comparison reasons, we also present here the solution structure of the cyclic analogue d<pCGCTCCGT>. In both cases, the overall three dimensional structure is very similar to that of the previously reported dimers, but self-association gives rise to a novel minor-groove G:C:C:G tetrad.

## O6. EDITED/FILTERED DOSY AND STD EXPERIMENTS WITH IMPROVED SUPPRESSION OF WATER SIGNAL

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Saturation Transfer Difference (STD)<sup>1</sup> and Diffusion Ordered Spectroscopy (DOSY)<sup>2</sup> are used to screen small molecule binding to receptors. We have developed new screening protocols and pulse sequences that use labeled receptors or ligands.

We present <sup>13</sup>C-edited and <sup>13</sup>C-filtered PFG DOSY and STD experiments aimed at the individual and accurate measurement of diffusion coefficients and detection of STD-signals, respectively, for a mixture of <sup>13</sup>C-labeled and unlabeled molecules. The proposed experiments utilize an enhanced water suppression scheme based on water flip back, radiation damping suppression and excitation sculpting or Watergate. A gradient pulse scheme featuring two elements is proposed for enhanced editing and filtering. The first editing/filtering element is included in the preparation part of the experiment while the second block is applied before acquisition. This two-step scheme provides perfect separation of signals from labeled and unlabeled molecules. The <sup>13</sup>C-edited experiments detect ligands that compete at the specific sites but not non-specific binding ligands; the <sup>12</sup>C-filtered experiments detect the competing ligands. We will present data for annexin binding to <sup>13</sup>C-labeled and unlabeled nucleotides.

We present initial results on a new <sup>15</sup>N-pumped STD experiment, where amide proton signals of a <sup>15</sup>N-labeled protein can be selectively saturated in the presence of unlabeled peptide ligands. As a result, STD-signals exclusively arise from those peptide ligands which interact with the labeled protein. The <sup>15</sup>N-pumped STD experiment will be tested for studying the binding of S-peptide (1-15) to <sup>15</sup>N-labeled RNase S protein.

The results of a DOSY experiment with improved water suppression will be presented on aqueous 1-10 mg/ml BSA solutions. We can now apply our protocol in D<sub>2</sub>O buffers<sup>3</sup> for the measurement of protein size at 1 mg/ml in H<sub>2</sub>O buffers.

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*Financed by an exchange grant between CSIC and Hungarian Academy of Sciences (2004HU0004).*

## **O7. TETRAHEDRAL ORDERIN IN THE NaAlO<sub>2</sub>-NaAlSiO<sub>4</sub> SERIES: LIMITS TO THE LOWENSTEIN'S RULE**

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Pablo Chain<sup>a</sup>; Miguel Ángel Castro<sup>a</sup>; Alberto Escudero<sup>a</sup>; Moisés Naranjo<sup>a</sup>;  
Carolina Pazos<sup>b</sup>; José M<sup>a</sup> Trillo de Leyva**

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The distribution of aluminium in the tetrahedral sites of the sodium aluminate–carnegieite system (Na<sub>2-x</sub>Al<sub>2-x</sub>Si<sub>x</sub>O<sub>4</sub>, 0≤x≤1) were studied. Samples prepared via a sol-gel route followed by heating between 700° and 1300°C during different times were observed through <sup>23</sup>Na, <sup>27</sup>Al, <sup>29</sup>Si MAS NMR. A range of compositions was studied, including several compositions which did not obeyed Lowenstein's rule as well as others which did. This variety in compositions, along with the changes produced in the local order of the tetrahedral environments as the time and temperature of reaction increase, allows to discuss the application of the classic rule. In those extremes cases in which the rule forces to a highly ordered structure, i.e. x=1, the avoidance of Al-O-Al linkages is not observed, a limitation to the Lowenstein's rule being concluded.

## 08. CHARACTERIZATION OF MORPHOLOGY IN MULTIPHASE MODIFIED POLYMERS BY SOLID STATE NMR

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The modification of polymers is a very active area of research due to its significance to industrial polymers. These materials often include blends of two or more polymers and mixtures with inorganic components leading to multiphase structures. Since the physical and mechanical properties of the polymeric materials depend not only on the intrinsic properties of their components, but on how intimately they are mixed, numerous efforts to improve the compatibility between phases by chemical modification of the polymers of interest are ongoing. Moreover, the characterization of each phase by determining the critical dimensions of domains and interfacial regions, and how the chemical modification alters the polymer microstructure are paramount.

In this presentation, it will be described the characterization by <sup>1</sup>H and <sup>13</sup>C solid-state NMR of a multiphase triblock copolymer *b*-styrene-*b*-(*ran*-ethylene-butylene)-*b*-styrene (SEBS) chemically modified with maleic anhydride (MAH) in the presence of a radical initiator by reactive extrusion\*. This thermoplastic elastomer consists of rigid domains (polystyrene) dispersed in a rubbery matrix (ethylene-butylene random copolymer). In the experiments performed, the concentration of MAH and the temperature profile in the extruder were varied while the concentration of initiator was kept constant. Samples with known degrees of grafting and crosslinking were analyzed with NMR by using techniques based on the spin-diffusion process<sup>1,2</sup> to investigate the microphase structure of the modified copolymers. The results show that the rigid phase having domain sizes of ~17 nm and interdomain distances of ~31 nm is not significantly perturbed by the modification. Alterations in the rubbery phase are illustrated by measured changes in the proton T<sub>1</sub> and T<sub>2</sub> relaxation times and across the interface.

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\*Samples analyzed on this research were obtained under the outsourcing contracted research works signed between Repsol-YPF and ICTP/CSIC (Ministerio de Educación y Ciencia). Kind support from Repsol-YPF to present work is acknowledged.

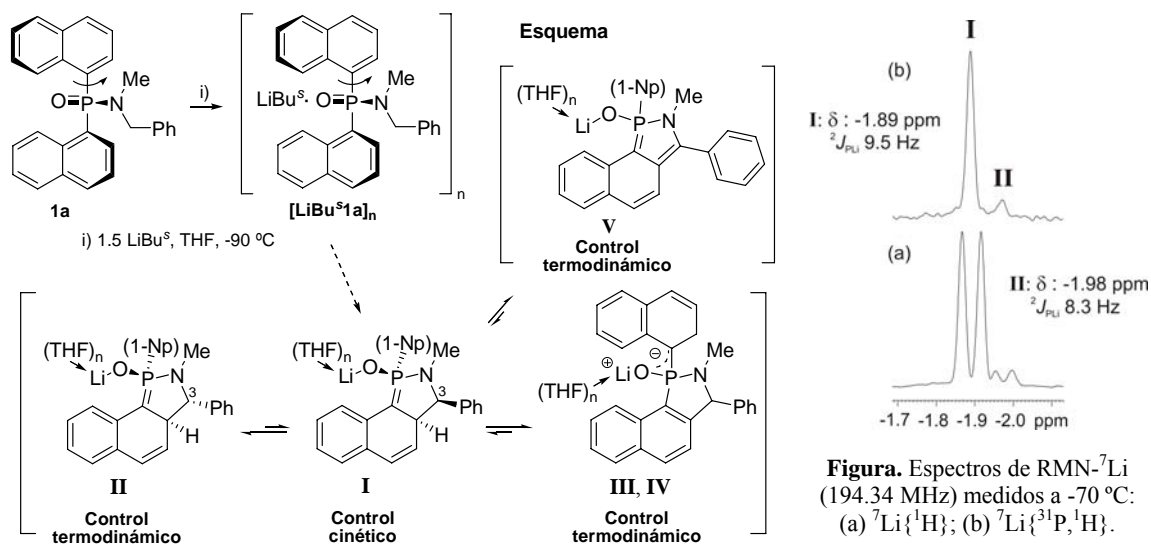
## O9. ESTUDIO DE LA LITIACIÓN DE *N*-BENCIL-*N*-METIL(1-DINAFTIL)FOSFINAMIDA MEDIANTE RM MULTINUCLEAR

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Las *N*-alquil-*N*-bencildinaftilfosfinamidas **1** son precursores adecuados de sistemas dihidronaftalénicos, un tipo de sustancias con elevado potencial biológico. El tratamiento de **1** con LiBu<sup>s</sup> en THF a -90 °C y posterior reacción con electrófilos conduce a productos de desaromatización de uno de los anillos *P*-naftilo mediante ciclación aniónica con excelente rendimiento y diastereoselectividad.<sup>1</sup>

Aquí se describe el estudio de la litiación de **1a** mediante resonancia magnética multinuclear en THF-*d*<sub>8</sub>, en el intervalo de temperatura de -130 °C a +21 °C. Al inicio de la litiación se detectan dos especies aniónicas monoméricas desaromatizadas **I** y **II** epímeras en el carbono metínico C-3 en una proporción 93:7, junto con el complejo [LiBu<sup>s</sup>·**1a**]<sub>n</sub>. A temperaturas inferiores a -115 °C se observan equilibrios entre dos conformeros y al menos un epímero en el centro carbaniónico de **I** y dos conformeros para **II**. Los intermedios **I** y **II** se encuentran asimismo en equilibrio. La concentración relativa de **I** y **II** permanece inalterada entre -90 y -30 °C por un espacio de tiempo cercano a 24 horas. Cuando se mantiene la muestra a -30 °C durante 22 h, aparecen dos nuevos intermedios **III** (monómero) y **IV** epímeros en C-3 asociados a procesos de rearomatización-desaromatización a través de hidruro. Cada una de ellos coexiste como dos epímeros en el carbono C<sub>α</sub>-P, **III<sub>A</sub>/III<sub>B</sub>** y **IV<sub>A</sub>/IV<sub>B</sub>**. Los intermedios **I**, **II** y las especies rearomatizadas **III** y **IV** son estables a temperatura ambiente (Esquema). El intermedio **I** es el producto de control cinético, mientras que **II**, **III**, **IV** y **V** incrementan su proporción en condiciones de control termodinámico.<sup>2</sup>



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## O10. STUDYING THE COMPACTNESS AND AGGREGATION OF DENDRITIC POLYMERS AND POLYSACCHARIDES BY NMR RELAXATION TIMES

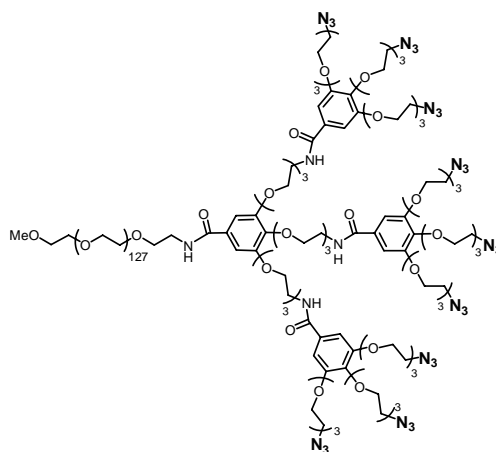
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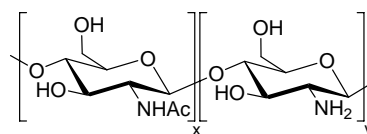
Herein we will present our results on the use of  $^1\text{H}$  NMR relaxation times ( $T_1$  and  $T_2$ ) for the analysis of the compactness and aggregation of dendritic polymers, and the polysaccharide chitosan.

Three generations of dendrimers and PEG-dendritic block copolymers have been studied. From the relaxation results we concluded that  $T_1$  relaxation data alone should not be used for molecular motion studies, because it can induce misinterpretations, and that in order to get reliable molecular motion data,  $T_2$  relaxation times must be better considered.

Accordingly, a radial decrease of density within the dendrimer, leading to more mobile protons at the outermost periphery, and an increasingly higher compactness of the core with generation have been determined by  $T_1$  and  $T_2$  relaxation time studies.



As for chitosan, its aggregation has been studied by pyrene fluorescence, NMR relaxation, and cryotransmission electron microscopy (cryo-TEM). Two limiting aggregation behaviours are proposed as a function of the degrees of acetylation and polymerization ( $\text{DA}$  and  $\text{DP}_w$ ). Looser and less hydrophobic aggregates, characterized by the presence of flexible chains, are produced at high  $\text{DA}$ . On the contrary, more hydrophobic and compact aggregates, constituted by stiffer chitosan chains, are obtained at low  $\text{DA}$ , and on increasing  $\text{DP}_w$  in highly acetylated chitosans.



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**CARTELES**



# C1. COMBINING NMR AND DOCKING TO SOLVE THE SOLUTION STRUCTURE OF MMP-3 COMPLEXES WITH INHIBITORS

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Stromelysin-1 (MMP-3) is a Matrix Metalloproteinase (MMP). MMPs are zinc- and calcium-dependent enzymes and have been associated with invasion, and metastasis of tumors, and other pathologies. MMP-3 is unique in its class as it is able to activate itself and other MMPs. This makes MMP-3 an important medicinal target in rational drug design<sup>1</sup>.

Using a fast approach that combines NMR data and docking software<sup>2</sup>, we have solved the structure of MMP-3 with different inhibitors. As starting point, we take the solution structure of MMP-3 with NNGH, solved by us previously. The comparison between the present solved structures and those of other NNGH-MMP complexes previously studied<sup>3,4</sup> provides clues on the structural features that determine specificity and affinity of MMP-3 inhibitors.

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## C2. NMR study of Nasicon type materials $\text{Li}_{1+x}\text{M}_{2-x}\text{N}_x(\text{PO}_4)_3$ ,

(M, N =Ti, Zr, Al)

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Materials with nominal composition  $\text{Li}_{1+x}\text{M}_{2-x}\text{N}_x(\text{PO}_4)_3$ , M=Ti, Zr and N= Al, Ga, Sc, In, have been prepared by ceramic method and studied with Nuclear Magnetic Resonance (NMR) techniques. Substitution of  $\text{Ti}^{4+}$  by trivalent ( $\text{Al}^{3+}$ ) or tetravalent ( $\text{Zr}^{4+}$ ) cations changes the unit cell dimensions and affects the Li occupancy in the Nasicon framework.

Structural sites occupied by lithium in  $\text{LiTi}_{2-x}\text{Zr}_x(\text{PO}_4)_3$  series have been investigated by  $^7\text{Li}$  NMR spectroscopy. At room temperature, end-members of the series display rhombohedral  $R\bar{3}c$  symmetry in  $\text{LiTi}_2(\text{PO}_4)_3$  and triclinic  $C\bar{1}$  in  $\text{LiZr}_2(\text{PO}_4)_3$ . The first order transition detected at 310K has been followed by NMR ( $^{31}\text{P}$  and  $^7\text{Li}$ ) techniques. In the  $\text{LiTi}_2(\text{PO}_4)_3$  compound, Li ions occupy  $M_1$  sites; however in the  $\text{LiZr}_2(\text{PO}_4)_3$ , Li occupy intermediate  $M_{1/2}$  sites. From the temperature dependence of  $^7\text{Li}$  NMR quadrupole constant ( $C_Q$ ) of the two compounds, the evolution of  $M_1$  and  $M_{1/2}$  sites occupancy in the Nasicon conduction network has been deduced. From relaxation NMR data, a microscopic activation energy of  $E_m = 0.43$  eV was measured in the triclinic phase. Above the phase transition a strong delocalisation of Li over structural sites disposed along conduction paths was detected.

From the analysis of the  $^7\text{Li}$ ,  $^{27}\text{Al}$  and  $^{31}\text{P}$  NMR spectra of  $\text{Li}_{1+x}\text{Ti}_{2-x}\text{Al}_x(\text{PO}_4)_3$  series, the samples composition and the occupancy of  $M_1$  and  $M_{1/2}$  sites by lithium has been deduced. For high  $\text{Al}^{3+}$  content ( $x>0.3$ ), detection of new broad peaks in  $^{31}\text{P}$  and  $^{27}\text{Al}$  MAS-NMR spectra supports the formation of secondary phases ( $\text{AlPO}_4$ ,  $\text{Li}_4\text{P}_2\text{O}_7$ ). In  $\text{Li}_{1.2}\text{Ti}_{1.8}\text{Al}_{0.2}(\text{PO}_4)_3$  compound, the analysis of the quadrupole constant ( $C_Q$ ) and spin-spin relaxation rate ( $T_2^{-1}$ ) deduced from  $^7\text{Li}$  NMR spectra of, two regimes associated with local and long-range motions of lithium have been identified. From the analysis of  $T_1^{-1}$  as a function of reciprocal temperature, the residence times of lithium at structural sites and their dependence on temperature have been estimated.

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### C3. STRUCTURAL INSIGHTS INTO NATIVELY UNFOLDED PROTEINS FROM NMR AND SMALL-ANGLE X\_RAY SCATTERING

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Natively unfolded proteins can perform a large variety of biological functions provided by their inherent conformational plasticity. In addition, some of them are responsible of neurodegenerative pathologies such as Alzheimer's and Parkinson's diseases.

The inherent conformational flexibility of natively unfolded places them beyond the reach of classical structural biology and multidisciplinary approaches are necessary to get insights into their structure and dynamics. Here, we present an approach that allows the characterization of disordered proteins using Residual Dipolar Couplings (RDCs) measured in partially aligned samples, Small Angle X-ray Scattering (SAXS) and computational tools. Ensembles of conformers have been created using an algorithm based on an amino acid specific coil database and a steric term. The resulting ensembles provide quantitative explanations of biophysical data measured with different techniques.

This methodology has been applied to the disordered fragment of the nucleocapsid-binding domain of Sendai virus phosphoprotein and to  $\alpha$ -synuclein, the main component of the aggregates found in Parkinson's disease. The sensitivity of RDCs to the presence of long-range contacts has been shown in this later example.

From this study arises a general view of the structural diversity observed in the unfolded state of proteins, where the conformational sampling observed is essentially dictated by the nature of the amino-acid sequence of those proteins.

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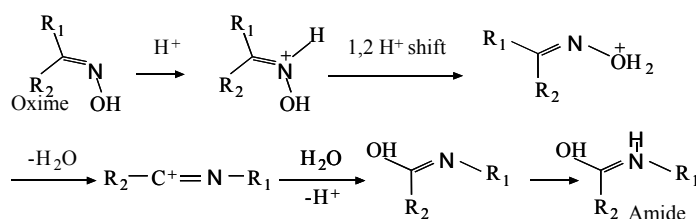
## C4. Beckmann rearrangement of cyclohexanone oxime investigated by ‘in situ’ NMR methods

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The production of  $\epsilon$ -caprolactam, usually obtained by the Beckmann rearrangement of cyclohexanone oxime, is of great industrial interest because it is the intermediate in the fabrication of nylon 6. One of the main problems on the heterogeneous process is the lower lactam selectivity because of the occurrence of parallel and consecutive reactions, which will depend on the pore sizes, the nature of active sites, the reaction temperature and the use of different solvents<sup>1-4</sup>.



We have investigated the Beckmann rearrangement of cyclohexanone oxime into  $\epsilon$ -caprolactam by means of in situ solid state NMR techniques. As catalysts, we have used BEA and MFI type zeolites possessing pores of varying sizes, and containing Brønsted acid or silanol groups as active sites. The results obtained indicate the protonation of cyclohexanone oxime on Al-containing Beta and ZSM-5 zeolites, while only hydrogen bonds are formed on siliceous zeolites. The reaction occurs at lower temperature on acid than on pure siliceous catalysts<sup>5</sup>, although more by-products are formed over acid zeolites.

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## C5. MOLECULAR DETERMINANTS OF LIGAND SPECIFICITY IN FAMILY 11 CARBOHYDRATE BINDING MODULES (CBM11): AN NMR, X-RAY CRYSTALLOGRAPHY AND COMPUTATIONAL CHEMISTRY COMBINED APPROACH

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Nuclear Magnetic Resonance, X-ray Crystallography and Molecular Modelling / Molecular Dynamics and Quantum Mechanical calculations are being used to determine the molecular determinants of ligand specificity of a CBM11 (a sub-unit of the cellulosome from *Clostridium thermocellum*)<sup>1</sup>. The interaction between cellohexaose and CBM11 is being used as a model compound to study the interaction between the soluble enzyme and cellulose.

Saturation transfer NMR experiments were performed to epitope map the interaction in the ligand structure. The results obtained in these studies are in agreement with the average structures obtained with MD simulations. CBM11 also caused line broadening effects for certain NMR resonances of cellohexaose. During the molecular modelling, the geometry of different ligands was created and their parameterization performed. Docking experiments were carried out resorting to software Gold and the program Scramd, that flexibilizes chosen residues in the binding site, was utilized. MD studies were carried out with the Charmm program.

The crystal structure of the native CBM11 was obtained and co-crystallization experiments are under way.

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## C6. NMR BACKBONE ASSIGNMENT OF THE 90 kDa YEAST PCNA HOMOTRIMERIC RING

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PCNA (Proliferating Cell Nuclear Antigen) is an essential factor for DNA replication and repair and the effector through which several cell cycle control and apoptosis signals are realized. We have selected *Saccharomyces cerevisiae* PCNA as a model system to test and implement current TROSY-based NMR methodology in our laboratory. Triply labelled (U-<sup>13</sup>C, U-<sup>15</sup>N, 80%-<sup>2</sup>H) protein was produced in H<sub>2</sub>O-based medium using triply-labelled algal extracts supplemented with <sup>13</sup>C,<sup>2</sup>H-glucose. Almost complete backbone <sup>1</sup>HN, <sup>15</sup>N, <sup>13</sup>C and <sup>13</sup>C<sub>β</sub> NMR assignments is being achieved using a combination of 3D triple resonance out-and-back, TROSY-based experiments (HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB, HN(CO)CACB) and <sup>15</sup>N-edited NOESY experiments (3D NOESY-TROSY, and 4D HSQC-NOESY-HSQC). Use of the HN-HN short distances from the crystal structure of scPCNA was critical for progression of the assignment. In addition, essential to the successful assignment of the 264 amino acid polypeptide has been the production of four residue-specific <sup>15</sup>N-labelled samples in residues of valine, isoleucine, leucine, and phenylalanine. As the 2D TROSY experiment of scPCNA is of high quality even for the non-deuterated protein, the residue-specific samples were not produced in deuterated form. In this presentation, the assignment strategy will be illustrated and the derived NMR parameters will be analyzed to compare the solution structure and dynamics of scPCNA with that observed in the crystal structure. This will set the basis for a detailed analysis of the variety of interactions displayed by PCNA in solution to exert its many functions.

## C7. NMR DETERMINATION OF THE BIOACTIVE CONFORMATION OF PELORUSIDE A BOUND TO MICROTUBULES

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Microtubule stabilizing agents are a chemically diverse set of small molecules which bind to microtubules in different fashions and block microtubule dynamics, leading to mitotic spindle impairment and cell apoptosis; several of them are antitumor drugs. The clinical successes of paclitaxel (taxol) have triggered the search for new agents with similar mechanisms of action but without their disadvantages, i.e., they have relatively low aqueous solubility and they develop of pleiotropic drug resistance. Most of the microtubules stabilizing agents bind at the paclitaxel binding site of  $\beta$ -tubulin. In contrast with these compounds, laulimalide and peloruside<sup>1</sup> apparently share a new binding site, different from the paclitaxel site and still to be mapped on the tubulin molecules, and retain activity in paclitaxel-resistant cells, which holds an important chemotherapeutic potential.

Peloruside A is isolated from a New Zealand marine sponge. The knowledge of the bioactive conformation of these molecules is of paramount interest for the derivation of analogues with improved activity. A variety of conformational studies on paclitaxel, epothilones and other tubulin acting molecules have been performed, both in the free and bound states. The identification of the configuration of the stereogenic centers of Peloruside A, was followed by reports on the synthesis of some fragments and the total synthesis together with the description of its absolute configuration. Moreover, some conformational features of Peloruside A in chloroform solution have been deduced by using NMR.<sup>2,3</sup>

We here report on the determination of the conformation of Peloruside A bound to biochemically stabilized microtubules, by using TR-NOE NMR experiments. As previous step, the conformation of the free molecule in water solution has also been deduced by a combined protocol of NMR data, assisted by modelling procedures. Despite the large size of the ring, Peloruside A mainly adopts two conformations in water solution. A conformational selection process takes place and the microtubules-bound conformer is one those present in water solution, different to that existing in chloroform medium.

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## **C8. HYDROPHOBICITY OF SOLVENT-EXPOSED RESIDUES ON THE BETA-SHEET OF p53 TETRAMERIZATION DOMAIN AFFECTS ITS GLOBAL STABILITY**

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The contribution of individual amino acids to protein stability is largely unknown due to the general interdependency of residue interactions. Several studies have tried to understand the effects of surface amino acids on the stability of globular proteins, but there appears to be no universal mechanism that affords a global understanding of their influence. So far, most efforts have focused on the role played on protein stability by amino acid composition, charge-charge interactions, side-chain hydrophobicity effects, electrostatic mechanisms, etc.. Such role can be analysed employing two complementary approaches: individual site-directed mutagenesis or the design of combinatorial variants of a protein where the effect of all positions of interest can be collectively evaluated.

Here we present an structural study by NMR of the effect on protein stability of solvent-exposed residues, using as a model the tetramerization domain of the tumour suppressor protein p53 (p53TD). A conformationally defined library has been produced with the three solvent-exposed positions in the beta-strand of p53TD randomized. The library has been structurally deconvoluted by CD spectroscopy and as a result a set of peptides containing stabilizing and destabilizing residue combinations has been selected. The influence of the mutations on the tetramer stability has been analysed extensively employing several NMR techniques. The NMR results evidence that while the structure of all the peptides remains globally identical to that of p53TD, small local structural variations in the mutated area, side-chain arrangements and *cation- $\pi$*  interactions are responsible for the differences found in stability.

## C9. $^{23}\text{Na}$ MULTIPLE QUANTUM FILTERED NMR CHARACTERISATION OF $\text{Na}^+$ BINDING AND DYNAMICS IN ANIMAL CELLS

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$^{23}\text{Na}$  NMR is a quite useful technique for biological studies, despite the fact that part of the  $^{23}\text{Na}$  resonance is often not detectable in conventional spectra because of quadrupolar effects. However, when the nucleus bound state is not at the extreme narrowing motional limit and undergoes biexponential relaxation, both double-quantum filtered (DQF) and triple-quantum filtered (TQF) NMR signals can be observed<sup>1</sup>. Multiple-quantum filtered (MQF) NMR spectroscopy has been successfully applied to quadrupolar nuclei (spin  $I = 3/2$ ) in biological systems, both *in vitro* and *in vivo*, in particular to characterize isotropic and anisotropic motions of bound  $\text{Na}^+$ .

In the present work we studied the  $^{23}\text{Na}$  DQF and TQF NMR behaviour of intra- and extracellular  $\text{Na}^+$  in three cellular systems, human erythrocytes in suspension, and agarose gel immobilized bovine chromaffin cells and human neuroblastoma SH-SY5Y cells, in the absence and presence of  $\text{Li}^+$ , an effective drug used in the treatment of bipolar disorder. DQF and TQF  $^{23}\text{Na}$  NMR spectroscopy was used to ensure the separate detection of anisotropic and isotropic motions (given by  $T_{21}$  and  $T_{31}$  signals, respectively) of both intra- and extracellular  $\text{Na}^+$ . Both anisotropic and isotropic contributions were observed for the extracellular  $\text{Na}^+$ , in the three types of cells. Intracellular isotropic  $\text{Na}^+$  motions were detected in all types of cells studied while anisotropic  $\text{Na}^+$  motions were only detected in erythrocytes.  $\text{Li}^+$  was shown to compete with  $\text{Na}^+$  for  $\text{Na}^+$  isotropic binding sites within chromaffin, SH-SY5Y and RBCs, increasing intracellular  $\text{Na}^+$  anisotropic motions in this latter cell type.

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## C10. SEARCH FOR NEW INSULIN MIMETIC DRUGS: STUDY OF THE UPTAKE AND TOXIC EFFECTS OF OXOVANADIUM COMPOUNDS IN HUMAN ERYTHROCYTES

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The uptake of two oxovanadium (IV) compounds, the [N,N'-ethylene bis(pyridoxylaminato)]oxovanadium (IV), V<sup>IV</sup>O(Rpyr<sub>2</sub>en) and bis-[3-hydroxy-1,2-dimethyl-4-pyridonate]oxovanadium (IV), V<sup>IV</sup>O(dmpp)<sub>2</sub>, by human erythrocytes was studied using <sup>51</sup>V and <sup>1</sup>H NMR and EPR Spectroscopy.<sup>1</sup> The V<sup>IV</sup>O(Rpyr<sub>2</sub>en) compound enters the cells in the neutral V<sup>IV</sup> form, not being completely oxidized to V<sup>V</sup> species in aqueous solution under aerobic conditions. In these same conditions the compound V<sup>IV</sup>O(dmpp)<sub>2</sub> originates two V<sup>V</sup> species, one neutral 1:1 species and one negatively charged 1:2 species.<sup>2</sup> The 1:1 species is taken up by erythrocytes through passive diffusion in a temperature dependent process and its depletion from the extracellular medium promotes the dissociation of the 1:2 species as demonstrated by <sup>1</sup>H NMR experiments. The oxidative stress of the oxovanadium compounds in erythrocytes was evaluated based on published methodology<sup>3</sup> by measuring their effect on the pentose phosphate pathway (PP) flux using [2-<sup>13</sup>C]-glucose and <sup>1</sup>H NMR Spectroscopy. The results indicate that both oxovanadium compounds tested do not significantly activate the pentose cycle at the 1 mM concentration studied. The glucose consumption rate by erythrocytes, measured by a biochemical assay, was significantly increased in the presence of 1 mM V<sup>IV</sup>O(dmpp)<sub>2</sub> (0.75 ± 0.13 mM/h) relative to the control value (0.37 ± 0.17 mM/h). No stimulation effect at this concentration was observed for vanadate and for the other vanadium compound studied, V<sup>IV</sup>O(Rpyr<sub>2</sub>en).

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*This work was carried out with financial support from Fundo Europeu para o Desenvolvimento Regional, Fundação para a Ciência e Tecnologia (FCT), project POCI/QUI/56949/2004, and within the working group "Vanadium Compounds as Insulin-Mimetic Agents" of the COST D21 Action of the EU and and the Portugal-Spain bilateral cooperation Program for 2005-2006. TD, AT and IC were supported by FCT grants SFRH/BD/17010/2004, SFRH/BPD/11536/2002 and SFRH/BPD/13975/2003, respectively.*

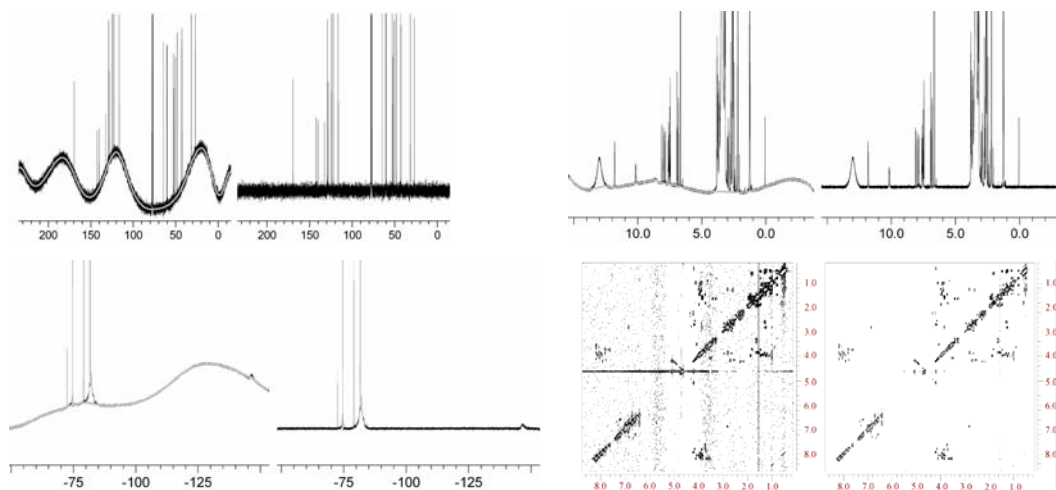
## C11. A New Algorithm for the Automatic Baseline Correction of 1D & nD NMR Spectra

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While spectrometer electronics and probe construction will continue to work towards high-quality, sensitive spectra, baseline distortions still are common in NMR spectra. These are a nuisance at the least, but in many cases the distortions must be eliminated for the data to be useful. Existing approaches have limitations in their effectiveness and universal application.

In this work we present a new algorithm for automatic baseline correction of NMR data sets. It is based on an improved automatic recognition of signal-free regions that uses a Continuous Wavelet Transform derivative calculation,<sup>1</sup> followed by a baseline modelling procedure based on the Whittaker smoother algorithm.<sup>2,3</sup> The method has been proven to automatically flatten 1D and 2D NMR spectra with large baseline distortions arising from different sources, is tolerant to low signal-to-noise ratio spectra, and to signals of varying widths in a single spectrum.



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## C12. Enhanced signal dispersion in the indirect dimension of 2D homonuclear experiments by NMR processing with GEN2D algorithm

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Two-dimensional NMR experiments directly monitor spin-spin interactions and thereby provide unique information about molecular structure and dynamics.

It is well-known that in order to achieve enough resolution in the indirect dimension, an increased number of  $t_1$  increments is necessary, which may result in exceedingly long experimental times. This is especially true for  $^1\text{H}$ - $^1\text{H}$  correlation spectra of dilute samples and/or for correlation spectra with dilute abundant nuclei, e.g.  $^{13}\text{C}$  or  $^{15}\text{N}$ .

Recently, a renewed interest for speeding up multidimensional experiments has emerged and a plethora of new experimental methods have appeared<sup>1</sup>. NMR signal processing schemes such as covariance NMR spectroscopy<sup>2,3</sup> have also proved valuable to reduce NMR acquisition time.

We present a new processing scheme for the generalized 2D correlation NMR (GEN2D) algorithm<sup>4</sup> that provides a fully symmetric spectrum with the same resolution along the indirect dimension  $\omega_1$  as along the detection dimension  $\omega_2$ . The proposed new processing scheme safely removes some potential artefacts in the original method<sup>4</sup>. Using this method only a few acquisition points along the indirect dimension would be necessary to construct a 2D-NMR correlation spectrum.

The implementation of the GEN2D algorithm makes it computationally affordable and amenable to a wide range of routine applications in liquids and solids. The application of GEN-2D algorithm can contribute to reduce the experimental time for two-dimensional correlation experiments and contribute to the rapid resonance assignment, a prerequisite for high-throughput structure determination.

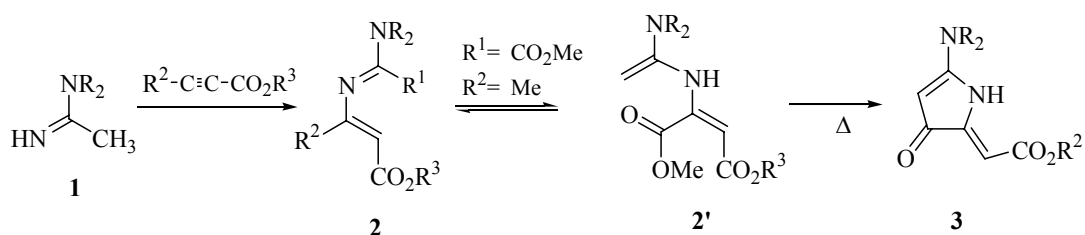
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## C13. NMR AS TOOL FOR THE ELUCIDATION OF Z/E ISOMERY.

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2-Azadienes have proved to be efficient building blocks for the synthesis of a wide range of heterocyclic compounds, alkaloids and natural products.<sup>1</sup> Following with our interest in the study of chemistry of 2-azadienes we prepared 1-amino-2-azadienes **2** derived from  $\beta$ -amino esters readily with very high yields by conjugated addition of *N*-unsubstituted amidines to acetylenic compounds. Thermal treatment of 1-amino-2-azadienes **2** ( $R^1 = R^3 = \text{CH}_3$ ,  $R^2 = \text{CO}_2\text{Me}$ ) in xylene at 140°C in a sealed tube gave directly five-membered cyclic derivatives **3**.



The structure of compounds **3** was assigned on the basis of 1D and 2D-NMR spectroscopic data, including NOESY-1D, HMBC and HMQC experiments. However, with these techniques the *Z/E* configuration of the exocyclic double bond could not be determined. The bidimensional heteronuclear spectroscopic technique HOESY <sup>1</sup>H-<sup>13</sup>C allow us to determine unambiguously the *Z* geometry of the exocyclic double bond in derivatives **3**.

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## C14. STRUCTURAL STUDIES OF PEPTIDE MODELS: INSIGHTS INTO PROTEIN-PROTEIN INTERACTIONS

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Protein interactions play pivotal roles in various aspects of the structural and functional organization of the cell, and their elucidation is critical to understand the molecular mechanisms of biological processes.

The objective of the present study is to characterize independent structural motifs whose interaction could be used as a model for protein-protein interactions. For this work, NMR spectroscopy and biocomputing tools have been used. Initially, two independent and stable domains were chosen: a  $\beta$ -hairpin and an  $\alpha$ -helix, the reason being they are frequently involved in protein-protein interactions. Afterwards, these domains were chemically linked in such a way the  $\beta$ -hairpin and the  $\alpha$ -helix were located at the N- and C-termini of this hybrid molecule, respectively. The linker consisted of glycines because of the “inert character” associated to this residue, and because this string of residues should allow total mobility between both domains. The length of the linker was determined, according to structural studies, to allow potential interactions between residues located at any of the domains.



Finally, if the interaction is observed using NMR spectroscopy, we will try to identify small organic molecules that inhibit such interaction. On the contrary, if the interaction is not observed, we will redesign our model using computational protein design strategies.

## C15. STRUCTURAL STUDIES BY NMR OF SELECTIVELY ELECTROCHEMICAL MODIFICATION OF PROTEINS: TYROSINE NITRATION OF HEN EGG WHITE LYSOZYME

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The electrochemical modification of residues in proteins and other bioactive molecules offers the production of novel proteins, enzymes and other bioactive species, in comparison with traditional methodologies such as protein engineering and the use of chemical reagents<sup>1</sup>. The results have important consequences for the labelling of proteins, specific immobilisation, production of novel modified proteins for pathophysiology in diseases involving oxidative dysfunction, and use in biosensors.

Recently, the relatively robust, stable protein hen egg white lysozyme (HEWL) was exposed in mildly-alkaline aqueous buffer solutions (50 mM disodium tetraborate, pH 9.0) in the presence of sodium nitrite, and electrochemical nitration methods was carried out using a platinum electrode<sup>2</sup>. Much better results in terms of activity retention of HEWL have been achieved when a carbonaceous working electrode like boron doped diamond (BDD) for the different nitrated lysozymes. For both, platinum and BDD electrodes, mass spectrometry showed that electrochemical nitration of HEWL was selective for tyrosine 23 in the initial phase of reaction, followed by bisnitration at tyrosine 20 and 23 at longer times.

The aim of this communication is to investigate the production of electrosynthetically nitrated tyrosine residues in proteins at BDD electrodes, using lysozyme as a model protein, and to correlate enzymatic function and structure by enzymatic assay and NMR studies respectively. Comparison of one-dimensional NMR, TOCSY and NOESY experiments together with DOSY measurements of the native and the different nitrated lysozymes will be shown in this communication. An attempt for determining 3D-structure changes between the native and the nitrated proteins will be presented.

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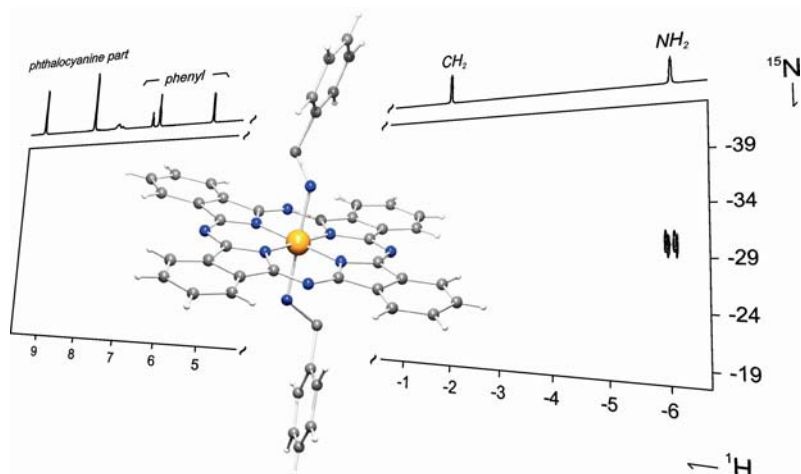
*This work has been carried out with financial aid of Alicante University (Project number GR305-03). J. Iniesta acknowledges the Programme Ramon y Cajal, Spain.*

## C16. SOLUTION NMR AND X-RAY STRUCTURAL STUDIES ON IRON(II) PHTHALOYANINATO-COMPLEXES

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We report here solution NMR and a solid-state study concerned with the structures of the complexes derived from the reactions of decylamine and benzylamine with low spin iron(II) phthalocyanine. In THF solution at room temperature quantitative conversion to the *bis*(decyl) and *bis*(benzylamine) iron complexes, **2** and **3**, is obtained.<sup>1</sup> PGSE <sup>1</sup>H NMR diffusion measurements help on giving a closer view about the aggregation of both species in this solvent. The results show that, at least in **2**, the amine complex which forms, possesses the necessary lability such that a low molecular weight gas molecule, in our case CO, readily complexes the iron center and replaces one amine ligand at 3 bar CO.<sup>1</sup> Photometric studies have been also applied to NO<sub>2</sub><sup>-</sup> and CO-sensing films containing these new complexes in order to establish their use as optical sensors.<sup>2</sup>



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## C17. NMR AND FLUORESCENCE ANISOTROPY STUDIES OF THE INTERACTION BETWEEN THE BACTERIAL NUCLEOID-ASSOCIATED PROTEINS HHA AND H-NS

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The nucleoid associated protein H-NS is a regulator of transcription of a large number of environmentally regulated genes in Gram negative bacteria. The level of expression of a variety of proteins, including virulence factors such as the *Escherichia coli* toxin  $\alpha$ -hemolysin, is modified in response to changes in temperature and osmolarity<sup>1</sup>. H-NS mediated regulation of  $\alpha$ -hemolysin expression requires the participation of Hha. The Hha protein does not bind DNA, but interacts with H-NS forming a complex responsible of the repression of the  $\alpha$ -hemolysin operon under nonpermissive conditions<sup>2</sup>.

The interaction of Hha with the *N*-terminal (dimerization) domain of H-NS has been studied by fluorescence anisotropy and NMR techniques<sup>3</sup>. Complexes of different stoichiometries are formed depending on the salt concentration and a reversible transition between 1:2 and 1:1 complexes is observed when the concentration of NaCl is changed from 150 to 100 mM.

The Hha binding motif has been identified in the *N*-terminal part of the H-NS dimerization domain. Aminoacid residues corresponding to helices 1 and 2 of H-NS are mainly affected upon Hha interaction. In addition, mutation of arginine 12 by histidine in H-NS, which is known to cause a strong loss of function phenotype *in vivo*, abolished Hha binding *in vitro*<sup>4</sup>.

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## C18. Evaluation of Gd(III) chelates of DOTA-type glycoconjugates as liver targeted MRI agents: studies on a Hep-G2 cell line, gamma scintigraphy and MRI of mice

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The development of new and more efficient contrast agents (CA) is an area of intense research. We have developed Ln(III)-thioglycoconjugates of different sugars as potential liver MRI contrast agents for targeting the asialoglycoprotein receptor (ASGP-R) expressed on hepatocyte cells, which recognizes  $\beta$ -galactosyl residues. We will study the effect of valency, topology and sugar type on the liver ASGP-R targeting, using cell and animal models. Internalization of the <sup>153</sup>Sm(III)-labelled thioglycoconjugates in ASGP-R expressing HepG2 cells and their biodistribution and pharmacokinetics in Wistar rats was studied using gamma scintigraphy. Some of the Gd(III)-thioglycoconjugate complexes were studied as MRI CAs by imaging mice at 7.0 T. The radioligand [<sup>153</sup>SmDOTAGal<sub>2</sub>] is taken up by Hep-G2 cells, reduced to 50% after 120 min in the presence of an excess of ASGP-R blocker asialofetuin. Biodistribution, scintigraphic images and time-activity curves at various regions of interest of Wistar rats injected with <sup>153</sup>Sm(III)-DOTA-X (X = Gal, Gal<sub>2</sub>, Gal<sub>4</sub>, Lac<sub>2</sub> and Glc<sub>2</sub>) show strong liver uptake for all cases except Glc<sub>2</sub>, which lasts more than 24 h for Gal<sub>4</sub>. This uptake is strongly blocked (90%) by co-injection of an excess of asialofetuin. Pharmacokinetics of the MRI CAs was analyzed by the time course of signal intensity of several ROIs (liver, kidney medulla, kidney cortex and muscle), during T<sub>1</sub> weighted spin echo MRI experiments in mice with GdDOTALac<sub>2</sub> and GdDOTAGal<sub>2</sub>, and compared with GdDTPA (Magnevist). The liver-to-kidney cortex contrast ratio caused by the glycoconjugates is comparable but not better than that induced by GdDTPA. Despite the specific uptake via the ASGP-R and good scintigraphic imaging performance of the galactose-bearing multivalent <sup>153</sup>SmDOTA compounds, the animal MRI assessment of the Gd<sup>3+</sup> chelates shows liver-to-kidney contrast effects similar to GdDTPA. This probably results from the high hydrophilicity of the complexes, which are quickly washed out from the liver, limiting their use as contrast agents for lectin-mediated molecular imaging.

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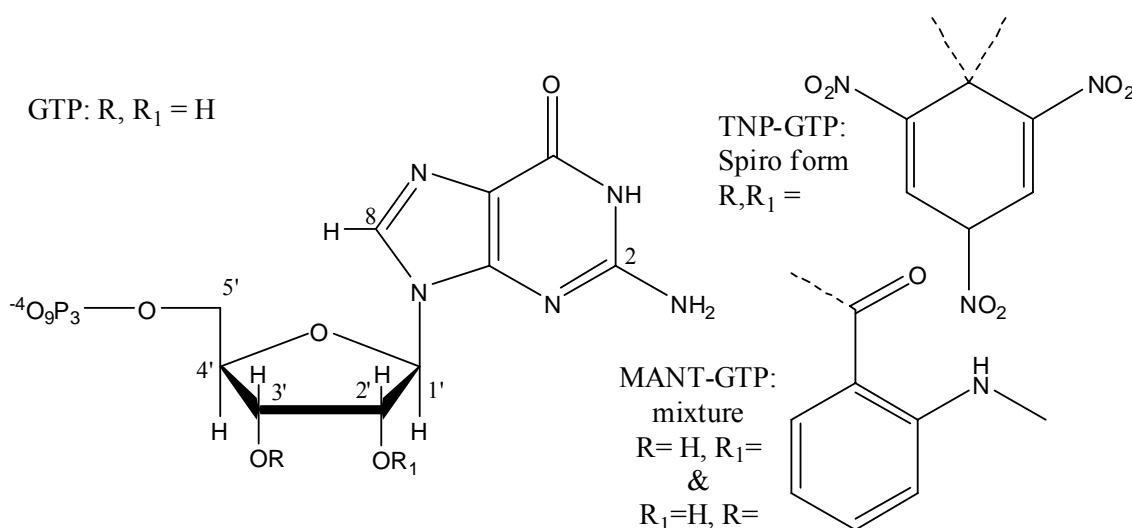
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## C19. ANALYSIS OF ANNEXIN-NUCLEOTIDE COMPLEXES BY NMR

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Annexins bind nucleotides in the micromolar affinity range. There are ~50 structures of annexins in the PDB but none of nucleotide complexes. We obtain information about the annexin A6-bound structure of GTP and analogs by transferred NMR methods.



Transfer NOESY reports the orientation of H8 relative to the ribose sugar. For GTP, ATP and O3-MANT-GTP, H8 eclipses the ribose H2' proton. For O2-MANT, H8 gives similar intensity cross peaks to H1' and H2'. For TNP-GTP, H8 eclipses H1'.

Saturation Transfer Difference (STD) spectroscopy reports which parts of the ligand most strongly contact the receptor. For ATP, relatively weak STD peaks are seen for the ribose resonances, medium intensity for H1' and H8, and strongest for H2 (absent in GTP). GTP and the fluorescent analogs give similar data for the corresponding GTP peaks. The analogs give additional strong STD signals to the fluorescence moieties.

We conclude that annexin A6 has a flexible nucleotide binding site that can bind a range of ligands of different conformation. Potential problems for the use of each of the analogs in fluorescence experiments are revealed.

## C20. THE CONFORMATION OF FREE AND PROTEIN BOUND C-GLYCOSYL COMPOUNDS

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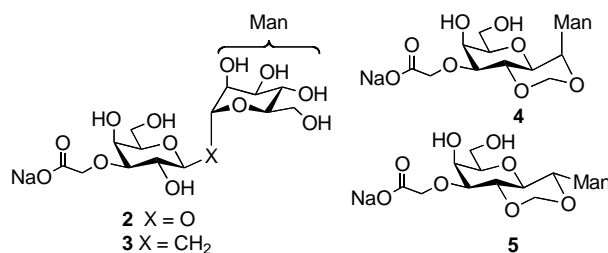
The conformation behaviour of different C-glycosides, synthesized as analogs of a glycomimetic (**2**) of sialyl Lex (**1**), has been studied. Indeed, **1** is a strong antiinflammatory, but it presents lack of stability for glycosidase attack. The first generation of analogs (**3**)<sup>1</sup> showed a moderate biological activity, and thus compounds **4** and **5** (Scheme 1) were designed to restrict their conformational properties and to obtain increased biological activity.<sup>4</sup>

On this basis, the conformation of **2-5** their analogues, **6** and **7**, without the cyclic acetal moiety, has been studied. The conformation has been derived by using an approximation which combines molecular mechanics and dynamics methods with NMR experimental data.<sup>2,3</sup>

It has been demonstrated that the conformation of the compounds depends on the nature of the glycosidic linkages and on the presence or absence of the acetalic bridge.

Moreover, using trNOE and STD NMR methods, the conformation of some of this molecules bound to different lectins has also been derived.

Finally, the inhibition activity of the different compounds has been determined, and this activity has been related with the observed conformation.



Scheme 1

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*This work has been supported by BQU2003 O3550 and a FPI fellowship.*

## C21. STRUCTURE DETERMINATION OF PARTLY UNFOLDED PROTEINS: THE APOFLAVODOXIN THERMAL INTERMEDIATE

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Proteins do not behave always as simple two-state systems, native and denatured states. Under stress conditions (heat, extreme pH values,...) many proteins do not completely unfold but their structures relax into partly unfolded conformations, equilibrium intermediates, deprived from biological function, whose structure and energetic are poorly understood. These equilibrium intermediates have been related to human diseases, such as different types of amyloidosis, and proposed to play physiological roles in some proteins. In spite of its interest, the structural study of intermediates is very difficult. X-ray crystallography is of little use because partly folded proteins do not crystallise. NMR is the technique of choice, but its applicability is complex because the intermediate state may never be dominant in solution, coexisting in equilibrium with the native and the denatured states.

Thermal unfolding of apoflavodoxin from *Anabaena* PCC 7119, an  $\alpha/\beta$  protein, exhibits an intermediate. Its native state has been determined by X-ray<sup>1</sup> and also investigated by NMR<sup>2</sup>. Using this protein as a model, a method based on equilibrium phi analysis has been recently proposed as a tool to obtain low resolution information on the structure of partly unfolded states<sup>3</sup>. The method was validated by partial assignment of the NMR signals in an apoflavodoxin mutant whose thermal intermediate accumulates at high temperature. Based on these results, we have designed an apoflavodoxin mutant whose native structure, the structure that the mutant has at room temperature, is partly unfolded. Here, we describe the NMR structural study of this apoflavodoxin mutant. Its structure is compared with the native structure of wild type apoflavodoxin and with the thermal intermediate. The correspondence between the structure of the mutant and that of the thermal intermediate is excellent.

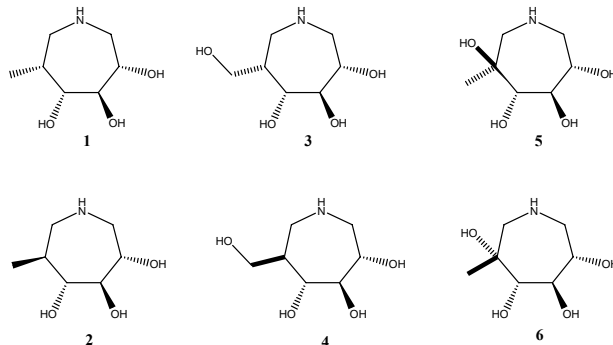
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## C22. NMR BINDING STUDIES OF NEW AZEPAMS AS GLYCOSIDASE INHIBITORS

**Marco Fontanella<sup>a</sup>, Javier Pérez-Castells<sup>a</sup>, Angeles Canales<sup>a</sup>, Yves Blériot<sup>b</sup>, Pierre Sinay<sup>b</sup>, Jesús Jiménez-Barbero<sup>a</sup>**

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Glycosidase inhibitors have been the subject of extensive studies in the past decade because of the biologically widespread interest of the glycoside cleavage. Several compounds with these properties have been observed in the past to have important applications in the treatment of Gaucher's disease, HIV infection, diabetes, viral infection, cancer. A number of natural products acting as efficient glycosidase inhibitors have been found and this led to a considerable effort to realize synthetic analogues<sup>1</sup> of them. Blériot and Sinay<sup>2</sup> have synthesised several azepam derivatives which mimic different monosaccharides and which have been found to be good inhibitors. Starting from this point we have studied, exploiting NMR techniques, the binding properties for  $\beta$ -glucosidase of a new series of azepam seven-member rings (ligands **1-6**).



STD experiments were performed in order to study these complexes and underlined these compounds to be good inhibitors; ligand **1** demonstrated to have a good affinity to almonds beta-glucosidase. Particularly efficient was ligand **2**: some competitive experiments conducted with compound **1** showed the complete disappearance of its STD signals in presence of **2**, and pointed out this latter to be a very tight ligand. Other molecules were also explored, although their spectra showed very low STD peaks in competitive experiments with inhibitor **1**.

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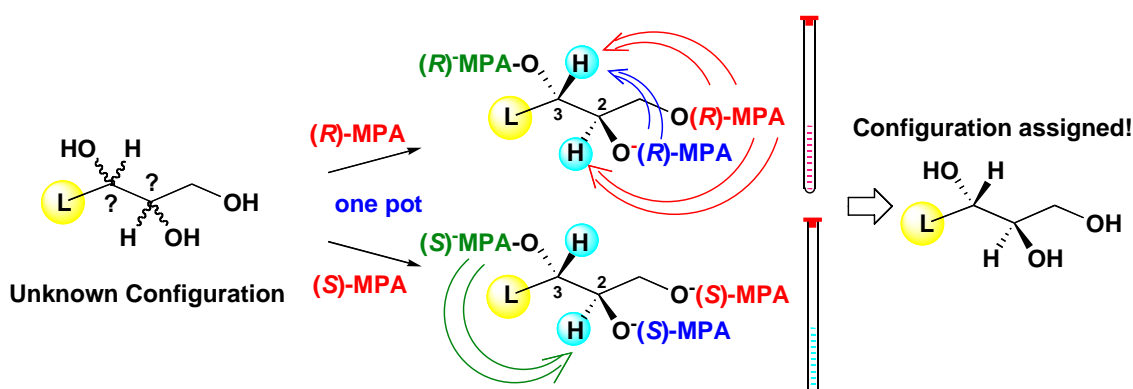
## C23. CHIRAL POLYOLS: APPLICATION OF THE $^1\text{H-NMR}$ APPROACH TO THE CONFIGURATIONAL ASSIGNMENT OF TRIOLS.

**Enrique Lallana<sup>a</sup>, Félix Freire, José Manuel Seco, Emilio Quiñoá and Ricardo Riguera<sup>b</sup>**

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The absolute configuration of 1,2,3-*prim,sec,sec*-triols can be assigned by  $^1\text{H-NMR}$  spectroscopy of the *tris-(R)* and the *tris-(S)*-MPA ester derivatives. This procedure requires the comparison of the signals for H(2) and H(3) that show patterns of  $\Delta\delta^{RS}$  and  $|\Delta\Delta\delta^{RS}|$  signs and values specific for each one of the four possible stereoisomers. Experimental demonstration of this correlation with 24 triols of known absolute configuration and a protocol for its application to the determination of the absolute configuration of other triols are presented.



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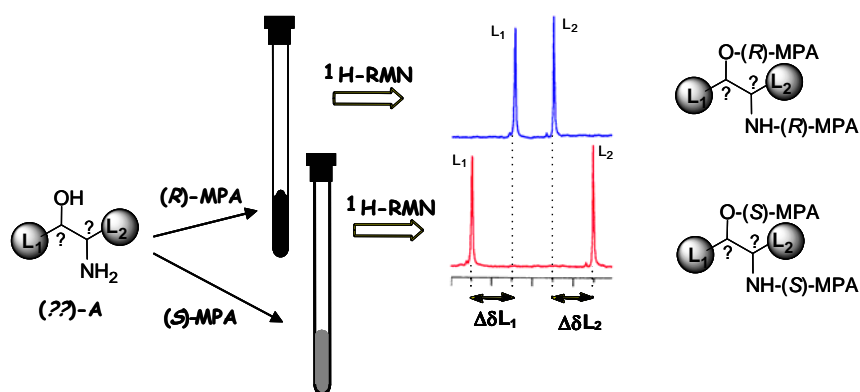
## C24. THE ASSIGNMENT OF ABSOLUTE CONFIGURATION OF POLYFUNCTIONAL COMPOUNDS BY RMN: AMINO ALCOHOLS

**Victoria Leiro<sup>a</sup>, José M. Seco, Emilio Quiñoá and Ricardo Riguera<sup>b</sup>**

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The assignment of absolute configuration by NMR has experienced a remarkable development in recent years.<sup>1</sup> These advances allow a reliable determination of the stereochemistry of a large number of monofunctional chiral compounds of natural and synthetic origin. In the reported cases, these NMR methods were successfully applied to find out the absolute configuration (*R* or *S*) of a single chiral center, usually directly attached to the functional group, acting as a “handle”. The application of this NMR methodology to molecules bearing several chiral centers showed to possess a larger degree of complexity in comparison to monofunctional compounds.

In this communication we will show preliminary results<sup>2</sup> on a general NMR spectroscopy protocol for the determination of the absolute configuration of chiral 1,2-amino alcohols (secondary/secondary), that allows distinguishing among the four possible stereoisomers by analysis of the <sup>1</sup>H NMR spectra of their bis-MPA derivatives.



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*This work has been carried out with financial aid of the Ministerio de Educación y Ciencia (Projects numbers BQU2002-01195 and CTQ2005-05296/BQU).*

## C25. STRUCTURAL BASIS FOR RECOGNITION OF OPERATOR AND CAR S ANTIREPRESSOR BY M. XANTHUS CAR A REPRESSOR

**Esther León<sup>a</sup>, Gloria Navarro-Avilés<sup>b</sup>, M. Cruz Pérez-Marín<sup>b</sup>, Carlos González<sup>a</sup>, Manuel Rico<sup>a</sup>, Francisco J. Murillo<sup>b</sup>, Montserrat Elías-Arnanz<sup>b</sup>, S. Padmanabhan<sup>b</sup> & M. Angeles Jiménez<sup>a</sup>**

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All but one of the enzymes involved in light-induced carotenogenesis in *Myxococcus xanthus* are expressed off the *carB* operon. Its promoter is downregulated in the dark by the CarA repressor binding to its operator. CarA binds operator to effectively impede promoter access to RNA polymerase and repress *carB*. Light induces production of CarS which physically interacts with CarA to dismantle CarA-DNA complexes and relieve repression of *carB*<sup>1-2</sup>.

CarA is organized into two distinct structural and functional domains. One formed by the 209 C-terminal residues is involved in dimerization and binds vitamin B12, a novel ligand for a transcriptional factor. The other domain corresponds to the 78 N-terminal residues of CarA, CarA(Nter). It is a monomeric, highly helical, autonomously folding unit that houses both the operator and CarS-binding specificity determinants of CarA. CarA(Nter) binds operator, and forms a 1:1 complex with either CarS or CarS1 (a gain-of-function CarS mutant). In vitro, CarA(Nter) blocks *M. xanthus* RNA polymerase-promoter binding, and this is relieved by CarS. CarS (111 residues) has no known sequence homologs. CarS1 encompasses the 86 N-terminal residues of CarS<sup>3</sup>.

We have determined the solution structure of CarA(Nter), and have mapped residues crucial for interactions with operator and/or CarS by NMR and by in vivo and in vitro analyses of site-directed mutants. CarA(Nter) adopts the winged-helix topology of MerR-family DNA-binding domains. The DNA recognition helix also mediates CarS-binding, suggesting that CarA-binding site of CarS mimic operator DNA. To confirm this hypothesis, we are currently determining the solution structures of CarS and CarS1.

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## C26. THE FORMATION, STRUCTURE, AND DISSOCIATION OF A TWO CHAIN DOMAIN-SWAPPED DIMER BY RIBONUCLEASE S

**Jorge P. López-Alonso<sup>a</sup>, Marta Bruix<sup>a</sup>, Josep Font<sup>b</sup>, Marc Ribó<sup>b</sup>, María Vilanova<sup>b</sup>, Manuel Rico<sup>a</sup>, Giovanni Gotte<sup>c</sup>, Massimo Libonati<sup>c</sup>, Carlos González<sup>a</sup>, and Douglas V. Laurents<sup>b</sup>**

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Post-translational events, such as proteolysis, are believed to play essential roles in amyloid formation *in vivo*. Ribonuclease A (RNase A) forms oligomers by the three-dimensional domain-swapping mechanism<sup>1</sup>. We demonstrate the ability of Ribonuclease S (RNase S), a proteolytically cleaved form of RNase A, to oligomerize efficiently<sup>2</sup>. This is the first reported case of domain-swapped dimerization by a two chain protein. This unexpected capacity has been investigated to study the effect of proteolysis on oligomerization and amyloid formation. The yield of the RNase S dimer was found to be significantly higher than that of RNase A dimers, which suggest that proteolysis can activate oligomerization via the domain-swapping mechanism. Characterization by chromatography, enzymatic assays, and NMR spectroscopy indicate that the structure of the RNase S dimer is similar to that of the RNase A C-dimer. The RNase S dimer dissociates much more readily than the RNase A C-dimer does. By measuring the dissociation rate as a function of temperature, the activation enthalpy and entropy for RNase S dimer dissociation were found to resemble those for the release of the small fragment (S-peptide) from monomeric RNase S. Excess S-peptide greatly slows RNase S dimer dissociation. These results strongly suggest that S-peptide release is the rate-limiting step of RNase S dimer dissociation.

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*This work supported in part by the McyT (Spain) (CTQ2004-08275-C02-02, BMC2003-08145-CO2-02 (to M. V.)), the DGR, Generalitat de Catalunya (SGR-01-00196) (to M. V.), by the Italian M.U.R.S.T.-PRIN 2004 (to M. L.), and by support from Prof. J. Santoro (McyT BIO2002-00720). J.P.L.A. received a fellowship from the Consejería de Educación de la Comunidad de Madrid y el Fondo Social Europeo.*

## **C27. NMR SOLUTION STRUCTURE OF THE HOMODIMER TRANSCRIPTIONAL REPRESSOR COPG ENCODED BY THE STREPTOCOCCAL PLASMID pMV158**

**Blanca López-Méndez<sup>a</sup>, Javier Pérez-Castells<sup>a</sup>, Teresa Díaz-López<sup>a</sup>, Manuel Espinosa<sup>a</sup>, Gloria del Solar<sup>a</sup>, Peter Güntert<sup>b</sup>, and Jesús Jiménez-Barbero<sup>a</sup>**

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CopG is a 45 amino acid residue transcriptional repressor encoded by the streptococcal plasmid pMV158 involved in the control of plasmid copy number. CopG controls plasmid replication by repressing its own synthesis and that of the initiator of replication, protein RepB. To do so, it binds to a DNA operator that contains a 13 bp pseudosymmetric element. X-ray crystal structure of this protein repressor shows a homodimeric ribbon-helix-helix arrangement with a two-stranded antiparallel  $\beta$ -sheet on the surface of the dimer and responsible for the binding to the major groove of the DNA. CopG oligomerises both in the unbound crystal and in complex with its target DNA to render helical superstructures. Helix B and the turn connecting helices A and B form the dimer-dimer interface in the functional tetramer and residues within these regions should play a main role in protein oligomerization. To gain further understanding of the relevant protein-protein contacts which should be relevant for the highly cooperative binding to target DNA we characterize the CopG7 and CopG8 mutants with point mutations within the helix B (A30E) and the turn connecting helices A and B (G25E), respectively. Here, we will present the solution NMR structure of the wild-type CopG, its comparison with the previously reported X-ray crystal structure and the protocol which will be also used to study the structures of both mutants. The NMR CopG structure was based exclusively on homonuclear NOE (NOESY spectra recorded at 800 MHz) and calculated by means of the combined automated NOE assignment and structure calculation protocol of the program CYANA.

*This work was supported by the Ministerio de Educación y Ciencia of Spain.*

## C28. NMR STUDIES OF BETA-AMYLOID PEPTIDE INTERACTIONS WITH THE GANGLIOSIDE G<sub>M1</sub>

**Jaime López de la Osa<sup>1</sup>, Marta Bruix<sup>1</sup>, Manuel Rico<sup>1</sup>, Carlos González<sup>1</sup>, Avijit Chakrabartty<sup>2</sup>, Douglas V. Laurents<sup>1</sup>**

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**Background:** The beta-amyloid peptide forms small soluble oligomers which can induce synapse dysfunction and neural cell death (1,2). These small oligomers form *in vitro* at micromolar concentrations but at nanomolar concentrations *in vivo*. This strongly suggests the existence of factor(s) that promotes beta-amyloid aggregation *in vivo*. Beta-amyloid peptides associate with gangliosides in Alzheimer's brains (3) and form specific complexes with gangliosides *in vitro* (4). Ganglioside binding promotes beta-amyloid oligomerization (5) and beta-amyloid variants associated with familial Alzheimer's disease show exquisitely selective binding to specific gangliosides (6). These data suggest that gangliosides promote beta-amyloid oligomerization *in vivo*, nevertheless to date the structural details of this process remain incomplete.

**Objective(s):** To determine the molecular structure of the beta-amyloid + ganglioside complex by using high field nuclear magnetic resonance.

**Methods:** The <sup>1</sup>H NMR spectra of beta-amyloid(1-40), ganglioside GM1 pentasaccharide and ganglioside GM1 in small dodecylphosphocholine micelles, alone or in combination, were recorded in a Bruker spectrometer at 800 MHz at 5 °C at neutral pH and assigned.

**Results:** No conclusive evidence from NOEs or saturation transfer experiments for binding between beta-amyloid and the ganglioside GM1 pentasaccharide was observed, which suggests that their union is significantly weaker than that reported for the Tyr10Trp variant of beta-amyloid (5). In contrast, most of the beta-amyloid NMR signals disappear upon mixing the peptide with an excess of ganglioside GM1 in dodecylphosphocholine micelles.

**Conclusions:** The inability of the ganglioside pentasaccharide to bind to beta-amyloid indicates that additional ganglioside moieties are required. The disappearance of the beta-amyloid NMR signals suggests that ganglioside GM1 in dodecylphosphocholine micelles rapidly binds and stimulates the formation of large beta-amyloid aggregates. Experiments are underway to identify near-physiological conditions where beta-amyloid + ganglioside association occurs without further beta-amyloid aggregation.

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## **C29. AUTOMATED PROTEIN STRUCTURE DETERMINATION FROM NMR SPECTRA**

**Blanca López-Méndez<sup>a</sup> & Peter Güntert<sup>b</sup>**

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<sup>b</sup>*Tatsuo Miyazawa Memorial Program, RIKEN Genomic Sciences Center, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan*

Fully automated structure determination of proteins in solution (FLYA) yields, without human intervention, three-dimensional protein structures starting from a set of multidimensional NMR spectra. The three-dimensional structures of three 12–16 kDa proteins computed with the FLYA algorithm coincided closely with the conventionally determined structures, with deviations below 0.95 Å for the backbone atom positions. 96–97% of all backbone and side-chain chemical shifts in the structured regions were assigned to the correct residues. The purely computational FLYA method is suitable to substitute all manual spectra analysis, and thus overcomes a main efficiency limitation of the NMR method for protein structure determination.

*This work was supported by the National Project on Protein Structural and Functional Analyses of the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and by the Tatsuo Miyazawa Memorial Program of RIKEN Genomic Sciences Center.*

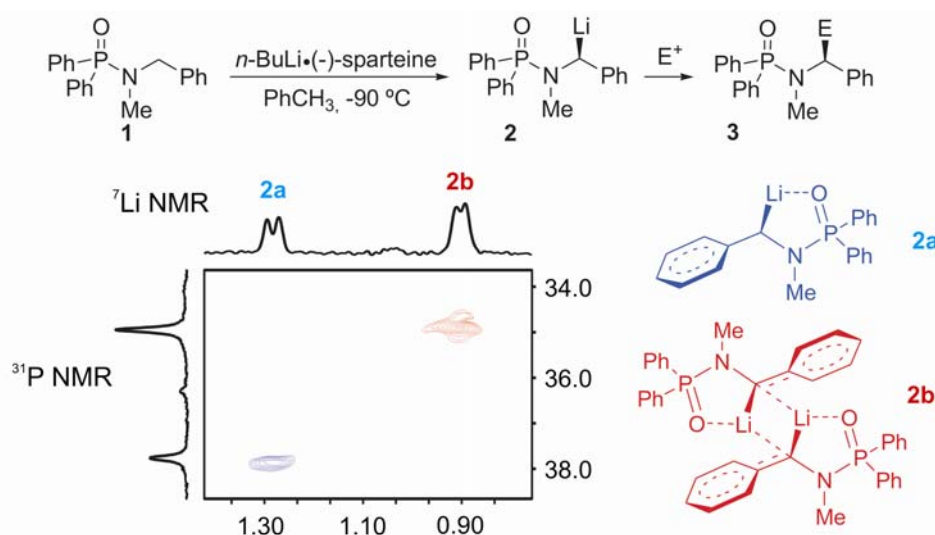
### C30. ENANTIOSELECTIVE LITHIATION OF N-METHYL-N-BENZYL-DIPHENYLPHOSPHINAMIDE: A MULTINUCLEAR MAGNETIC RESONANCE STUDY

Pascual Oña Burgos, M<sup>a</sup> José Iglesias, Ramón Álvarez-Manzaneda, Fernando López Ortiz.

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The deprotonation of N-methyl-N-benzyl(diphenyl)phosphinamide **1** with RLi (R = s-Bu and t-Bu) in diethyl ether and toluene leads to the formation of benzylic anions.<sup>1</sup> We have recently achieved the enantioselective lithiation of phosphinamide **1** upon reaction with the complex [n-BuLi·(-)-sparteine] in toluene.<sup>2</sup> Neutralisation of the resulting lithiated phosphinamide, **2**, with a variety of electrophiles affords adducts functionalised at the benzylic position in yields ranging from good to excellent with high enantioselectivities.

We report herein a study of the solution structure of the organolithium species **2** via multinuclear magnetic resonance (<sup>1</sup>H, <sup>7</sup>Li, <sup>13</sup>C, and <sup>31</sup>P). Temperature and concentration effects have been also evaluated. The results show that intermediate **2** exists as a mixture of two different species, monomer **2a** and dimer **2b**, which are in equilibrium. Based on the <sup>31</sup>P, <sup>7</sup>Li and <sup>13</sup>C, <sup>7</sup>Li coupling constants observed, we conclude that the lithium cation is intramolecularly coordinated to the oxygen of the P=O linkage and that the dimer is constructed by connecting the monomers through carbon-lithium bridges.



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## C31. Mapping the active site of the enzyme uroporphyrinogen III synthase

**Arola Fortian<sup>a,b</sup>, Miquel Pons<sup>a</sup>, Oscar Millet<sup>b</sup>.**

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The term porphyria describes a family of autosomal diseases produced by the existence of one or more mutations in any of the enzymes in the heme group biosynthetic pathway<sup>1</sup>. In particular, sequence alterations in the Uroporphyrinogen III Synthase (U3S) and in the porphobilinogen deaminase (PBGD) result in the erythropoietic and congenital porphyrias respectively. The severity of the disease is usually related to the residual enzyme activity<sup>2</sup>.

Focusing in U3S, we have expressed and purified all the mutant proteins reported in the literature as pathogenic. Experiments of thermal and equilibrium denaturation yield the free energy and thermal stability for each of the recombinant proteins. Some of the mutations like A66V produce a global unfolding of the protein whereas other mutations leave the stability of the protein unperturbed, suggesting a putative role in the binding of the ligand or in the catalysis process. Enzymatic activity tests together with the complete backbone assignment of the protein using high resolution NMR spectroscopy is currently being used to confirm this hypothesis.

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2 Lindberg, R. L. *et al. Nature Gen.*, **1996**, 12, 195-199.

*This work has been carried out with financial aid of the Ministerio de Educación y Ciencia (Project number CTQ2006-09101).*

## C32. SOLID NMR AS FUNDAMENTAL TOOL IN THE STUDY OF ORGANIC MATTER CHANGES DURING THE COMPOSTING PROCESS

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Different spectroscopy methods were used to monitor the composting process, evaluate the degradation rate and thus, determine the maturity of the composts obtained. Methods to assess compost maturity are needed in order to assure optimal benefits with the application of composted materials to lands. The aim of the present paper is to evaluate the maturity degree reached by composts elaborated with residues from the winery and distillery industry using periodic and permanent aeration systems, by means of carbon-13 nuclear magnetic resonance (<sup>13</sup>C-NMR) coupled with cross-polarization magic-angle spinning (CPMAS) and Fourier transform infrared spectroscopy (FTIR). The technique of nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (<sup>13</sup>C CPMAS NMR) is suitable for characterizing directly the main organic composition of complex, insoluble samples as dry powders [1,2].

In this study, changes in the organic matter were produced, as it was shown in an increase in the aliphatic C and carboxyl groups, a decrease in the phenolic groups and total aromaticity, as well as a decline in the polysaccharide levels. From the obtained results, it may be concluded that <sup>13</sup>C CPMAS NMR supported by FTIR could be used to improve the characterization of organic matter changes during the composting process [3].

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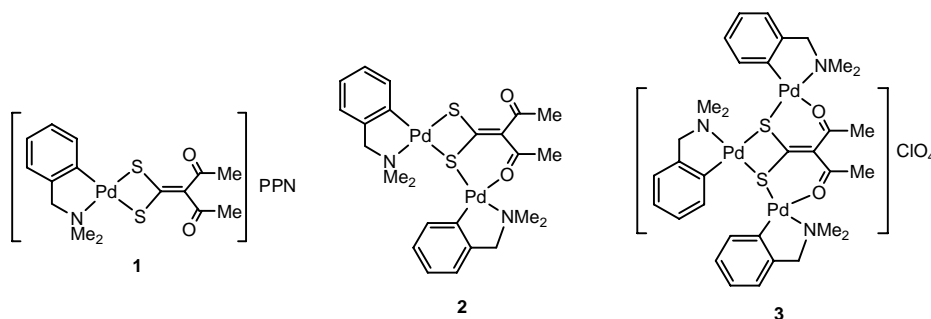
*This work has been carried out with financial aid of the Ministerio de Educación y Ciencia of Spain and has been financed by the CICYT (AGL2002-00296) Project and the University of Alicante (GRJ0508) Project.*

### C33. FLUXIONAL BEHAVIOUR OF MONO- DI- AND TRINUCLEAR Pd(II) COMPLEXES CONTAINING A “JUGGLER” LIGAND.

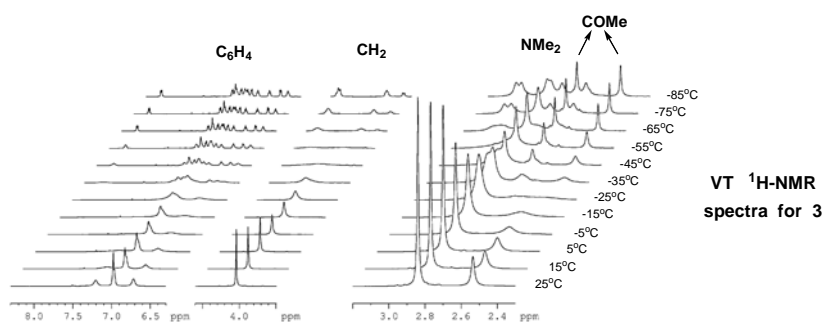
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We have investigated the fluxional behaviour in solution of the Pd(II) complexes **1-3**, containing a 2,2-diacetyl-1,1-ethylenedithiolate ligand bonded to one, two or three [Pd(C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>NMe<sub>2</sub>-2)] moieties. The structures of complexes **2** and **3** have been confirmed in the solid state by X-ray diffraction studies.



In solution, the VT <sup>1</sup>H-NMR spectra show that a fluxional process interconverts the two acetyl groups of each complex as well as, for **2** and **3**, all the [Pd(C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>NMe<sub>2</sub>-2)] fragments. We show the spectra for **3** in the Figure. Thus, the dithiolate group in **3** acts as a “juggler” ligand, with the metallic fragments moving around it. Additionally, a “flip-flop” process makes the two faces of **2** and **3** equivalent in solution.



We have used the **simulation program** *gNMR* to determine the rate constants for these processes at a wide range of temperatures. With these data, we have determined the **activation parameters** and proposed a **mechanism**.

*This work has been carried out with financial aid of the MEC and FEDER (Project number CTQ2004-05396), as well as the Fundación Seneca. E. M. V. is grateful to the MEC and the University of Murcia for her Ramón y Cajal contract.*

## C34. Enhanced signal dispersion in Saturation Transfer Difference experiments by conversion to a 1D-STD-homodecoupled spectrum

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The Saturation Transfer Difference (STD) experiment<sup>1</sup> is a rich source of information on topological aspects of ligand binding to a receptor. The epitope mapping is based on a magnetization transfer after signal saturation from the receptor to the ligand, where interproton distances permit this process.

Signal overlap in the STD spectrum can cause difficulties to correctly assign and/or quantitate the measured enhancements. Therefore, modifications of the basic protocol of STD experiments such as 2D-STD-TOCSY<sup>2,3</sup> or the selective version 1D-STD-selective TOCSY<sup>4</sup> have already been proposed. To address this issue we propose a modified pulse sequence of the routine experiment and a processing scheme that provides a 1D-STD Homodecoupled spectrum (i.e. an experiment in which all STD signals appear as singlets) with line widths similar to those in original STD spectrum. These refinements and the possibility to incorporate signal selectivity in the basic scheme contribute to alleviate problems of signal overlap. The experiment is based on 2D-J-resolved spectroscopy, one of the fastest 2D experiments under conventional data sampling in the indirect dimension, and provides excellent sensitivity per time unit, a key factor for difference spectroscopy experiments such as STD.

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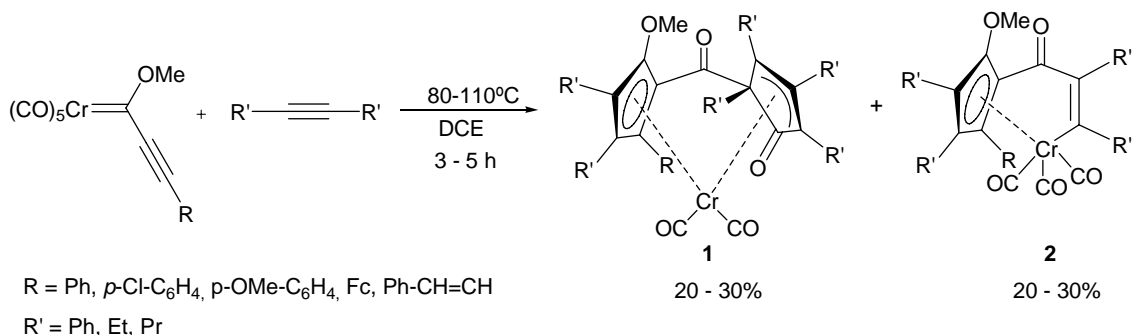
## C36. REACTIVITY OF ALKYNYL FISCHER CARBENE COMPLEXES TOWARD INTERNAL ALKYNES

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Fischer Carbene Complexes (FCC) are valuable reagents in organic synthesis, recognized as chemical multitailents<sup>1</sup> due to the versatility of their reactivity towards a large variety of substrates to produce highly functionalized structures in multi-component reactions<sup>2</sup>. While the reaction between alkenyl and aryl FCC with alkynes is well documented, the reactivity of alkynyl FCC with alkynes has been less studied. Novel research in our group has revealed that acetylenes undergo insertion reactions with alkynyl FCC and that the evolution of the initial insertion adduct, as well as the final products, depends on the type of acetylene. Whereas alkynyl FCC react with terminal acetylenes to give six and seven membered rings<sup>3</sup> which do not contain metal, their reaction with internal alkynes produce a mixture of two elaborated products both containing the metallic fragment in their structures.



The two compounds **1** and **2** are stable products that were separated by column chromatography and identified by <sup>1</sup>H and <sup>13</sup>C NMR experiments. The structure of **1** was confirmed by x-ray analysis. This molecule incorporates three alkyne units and two CO ligands through a process in which 7 new C-C bonds and two cycles are created. In contrast, compound **2** incorporates only two alkyne units and one CO ligand to give a molecule which contains a σ Cr-Csp<sup>2</sup> bond, as has been established by the analysis of its 2D HSQC and HMBC spectra.

Mechanistic considerations suggest that **2** is an intermediate in the formation of **1**, and therefore will be a reactive specie that may undergo insertion of a third alkyne molecule. So, the reactivity of this compound toward other alkynes has been tested, as well as its behavior in acidic media. Their reaction products have been characterized through 1D and 2D NMR spectroscopic techniques.

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## C37. THE INFLUENCE OF THE HOFMEISTER ANIONS IN PROTEIN STABILITY AS STUDIED BY THERMAL DENATURATION AND CHEMICAL SHIFT PERTURBATION.

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### ABSTRACT

Thermal stability of the B1 domain of protein L (ProtL) is effectively modulated by the addition of a suite of anions and follows the Hofmeister series. The maximum increase in thermostability (corresponding to 14 deg) was observed in the presence of 1M sodium sulphate. A detailed mechanistic analysis of the effect of cosolutes in the alteration of wild type ProtL stability was employed to estimate the relative contributions from excluded volume and preferential anion solvation for each anion. As expected, the excluded volume terms stabilizes the native conformation of ProtL for all the cosolutes but opposite effects in protein stability arise from anion's solvation depending on their tendency to interact with or to become excluded from the protein surface. This dichotomic behaviour has been corroborated by independent NMR experiments: only the anions predicted to preferentially interact with the protein surface produce significant perturbations in the amide protein chemical shift ( $\Delta\delta^{\text{HN}}_{23}$ ). The correlation obtained between  $\Delta\delta^{\text{HN}}_{23}$  and the temperature coefficients for the different amide protons provide qualitative information about the structural determinants for the interaction between the protein surface and the cosolute.

*This work was financially supported by grants from the Ministerio de Educación y Ciencia (MP) and the Programa Ramón y Cajal (OM).*

## C38. NMR STRUCTURAL INVESTIGATION OF VEGF AND VAMMIN DERIVED PEPTIDES

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Angiogenesis is crucial in tumour development and metastasis. Hence, anti-angiogenic therapy constitutes an alternative strategy in cancer treatment. Vascular endothelial growth factor, VEGF, is a very important pro-angiogenic agent whose mode of action is by binding to specific membrane receptors, like KDR. One approach in novel anti-angiogenic factors research is the modulation of protein-protein interactions playing a key role in angiogenesis, such as VEGF-KDR. Loop 3 in VEGF has a  $\beta$ -hairpin conformation and is involved in KDR binding. This  $\beta$ -hairpin structure represents an excellent template for the design of peptides which may inhibit binding of VEGF to receptor KDR.

This communication describes NMR structural studies of peptides encompassing the native sequences of loop 3 in two members of VEGF family proteins, VEGF-A and Vammin, and also of VEGF- and Vammin-derived peptides. These peptides were designed to increase  $\beta$ -hairpin stability by cyclizing with disulfide bonds or/and by incorporating Trp residues. Several NMR parameters, NOE,  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts, have been analysed to investigate  $\beta$ -hairpin formation. In the future, the ability of these peptides to adopt the native VEGF or Vammin  $\beta$ -hairpin structures will be compared with results on their binding to KDR and on their anti-angiogenic activities. This will improve our knowledge of the VEGF-KDR interactions and open the way towards the rational design of new KDR antagonists.

## C39. STRUCTURAL STUDIES OF JERDOSTATIN. A NOVEL SNAKE VENOM DISINTEGRIN

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Disintegrins represent a family of polypeptides found in the venoms of various vipers that selectively block the function of the integrin receptors. The common characteristic feature to all disintegrins is the similar pattern of cysteines and the presence of the so-called "integrin-binding" loop. The integrin-inhibitory activity of disintegrins depends on the appropriate pairing of cysteine residues, which determines the conformation of the inhibitory loop.

Our work is centred on r-jerdostatin, a novel disintegrin from *Trimeresurus jerdonii* recombinantly produced in *E. coli*. r-jerdostatin is a 43 amino acid polypeptide that functions as a specific antagonist of the  $\alpha 1\beta 1$  integrin. The distinct features of r-jerdostatin are located in the sequence of the integrin-binding loop and the C-terminal tail. r-jerdostatin presents a novel RTS motif in the binding loop, in contrast to the KTS sequence found in all other reported disintegrins that selectively target the  $\alpha 1\beta 1$  integrin. Additionally, it contains two extra C-terminal residues (Asn<sup>42</sup>Gly<sup>43</sup>) which in all venom-isolated members of the  $\alpha 1\beta 1$ -specific short disintegrins are post-translationally removed. Although synthetic peptides bearing the RTS motif appears to be more potent than those possessing KTS inhibiting the  $\alpha 1\beta 1$  integrin, r-jerdostatin is less active than the KTS-disintegrins obtustatin (from *Vipera lebetina obtusa*), viperistatin (*Vipera palestinae*), and lebestatin (*Macrovipera lebetina transmediterranea*), strongly suggesting that substitutions outside the integrin-binding motif and C-terminal proteolytic processing are responsible for the decreased inhibitory activity<sup>1</sup>.

Here we present structural studies by NMR of wild type r-jerdostatin and mutants in the integrin-binding loop and C-terminal sequences of the protein, both regions considered responsible for the biological activity of the disintegrins.

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## C40. A MODULAR SOFTWARE APPROACH FOR THE PREDICTION OF SCALAR AND DIPOLAR COUPLING CONSTANTS FROM 3D STRUCTURE

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Established relationships between scalar coupling constants and geometrical parameters, mostly dihedral angles are a well known tool in the determination of stereochemistry of organic compounds. Most examples involve the relationship between the vicinal  $^3J_{\text{HH}}$  coupling constant and the dihedral angle between the coupled nuclei. Starting from the simple Karplus equation, several modifications account for the influence of substituents. or computation of  $^nJ$  constants in biomolecules such as saccharides, peptides, etc.<sup>1</sup> The use of residual dipolar coupling constants (RDCs) obtained in oriented media provides also stereochemical information. In general, computation of the alignment tensor, either by *brute-force* or *a priori* methods is required to be able to extract the required information.<sup>2</sup>

We present here a modular software approach implemented inside the NMRDev<sup>3</sup> platform which allows for very easy implementation of different equations, both for scalar coupling  $J$  automatic calculation or computation of RDCs through the prediction of the alignment tensor by different methodologies. We have applied the developed software to a preliminary study on the performance of several RDC computing methodologies for the prediction of RDCs in small sized molecules.

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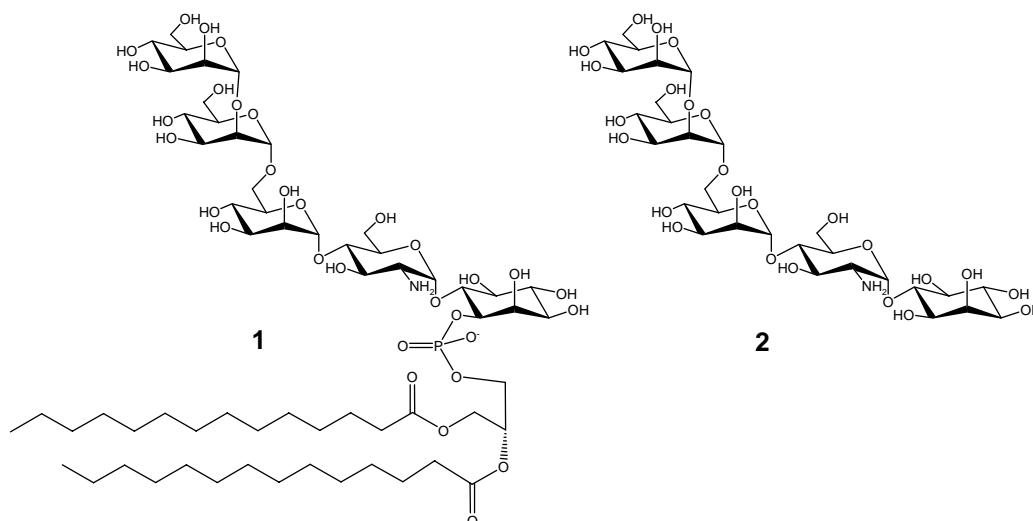
*R. Santamaría thanks MestReLab Reseach for granting of a student fellowship.*

## C41. CONFORMATIONAL STUDIES OF A SYNTHETIC GPI ANCHOR INSERTED INTO MICELLES.

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The synthetic glycolipid **1** represents the consensus structure of the known GPI anchors which are often used as system for the attachment of peripheral proteins to the cell plasmatic membrane. We have studied the conformational properties of the GPI anchor **1** inserted into DPC micelles by combined NMR and MD techniques in order to determine the influence of the micellar media on the structure and dynamics of the carbohydrate moiety. The NMR structural study by NOESY and off-resonance ROESY of **1** in DPC-d<sup>38</sup> at several temperatures and fields suggests a similar three-dimensional structure than that determined for the pseudopentasaccharide **2** in solution. The qualitative analysis of the flexibility by H relaxation and the hydrodynamic properties denote the influence of the larger macromolecular size of the micelles in the internal flexibility of the glycan.



## C42. STRUCTURAL STUDIES OF ISOFORMS OF THE PRO-METASTATIC ENZYME HEPARANASE

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Cancer cells require the ability to degrade the extracellular matrix (ECM) in order to turn into invasive and metastatic cancer cells. Many proteases and glycosidases are essential in the process of dissolving the components of the ECM. An endo- $\beta$ -D-glucuronidase, heparanase, is capable of specifically degrading one of the ECM components, heparan sulfate. It has been found that heparanase expression is often dramatically upregulated in invading cells, notably in metastatic tumor cells. Consequently, heparanase represents an excellent molecular target for the development of novel drugs with the potential for being antiangiogenic, antimetastatic, and antiinflammatory agents.

In this work we present initial studies directed toward the expression, purification and structural analysis of different isoforms of human heparanase in order to understand the molecular basis of its biological function. We have expressed several constructs of heparanase: the full length enzyme (53 kDa), the catalytic domain (38 kDa) and the renal-fetal isoform (26 kDa). The main problems we have encountered so far are the low solubility and the high tendency to aggregate of the enzyme. These issues have been partially overcome with the aid of detergents. Preliminary NMR experiments on different isoforms are shown.

## C43. IDENTIFICATION OF A FLEXIBLE SEGMENT IN REV PROTEIN FIBERS BY TRIPLE RESONANCE HR-MAS NMR SPECTROSCOPY

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The connexion between the formation of fibers by certain proteins in vivo and several human pathologies has given a boost to the development of new methods to determine the structure of these supramolecular assemblies. Advances in sample preparation, electron microscopy and specially the new developments in solid state NMR have been fundamental to describe with increased resolution the structure of some of these fibers<sup>1</sup>. However, structural data on the more flexible zones of the filaments are scarce. HR-MAS NMR spectroscopy provides the possibility of studying these kind of fibers by reducing the line broadening due to residual dipolar or chemical shift anisotropy interactions, resulting in quasi- liquid NMR spectra<sup>2</sup>. We have recorded standard triple resonance spectra under MAS on fibers of uniformly <sup>13</sup>C and <sup>15</sup>N enriched HIV Rev protein as well as on the fibers formed by Rev bound to an unlabelled 45 bases long RNA fragment of the Rev Response Element (RRE). The results indicate a similar behaviour at the molecular level of the protein in the context of the fiber either free or bound to RRE: the existence of two different segments, a flexible disordered one consisting of the last 25 residues at the C-terminus, and a rigid, invisible region that presumably forms part of the core of the fiber, spanning 90 residues from the N-terminus.

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## **C44. NMR CHARACTERIZATION OF THE SPECIFIC RECOGNITION OF METHYLATED HISTONE 3 TAILS BY THE PLANT HOMEODOMAIN FINGER OF THE TUMOUR SUPRESOR ING4**

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Plant homeodomain (PHD) fingers are frequently present in proteins involved in chromatin remodelling and bind to nucleosomes [1, 2]. The family of tumour suppressors Inhibitors of Growth (ING) contains a PHD at the C-terminus. PHD finger has a basic region at the C-terminus (RKKK) that it could be involved in binding to phosphoinositides, the same way that occurs with PHD of ING1 and ING2. We show that the PHD of ING4 does not bind to phosphoinositides, but specifically recognises ( $K_D = 4\mu\text{M}$ ) histone 3 trimethylated at lysine 4 (H3K4me3), that is a hallmark of active genes. H3 and H3K9me3 bind to PHD with a two orders of magnitude larger  $K_D$  while H4K20me3 does not bind to PHD, all of them are associated with gene silencing.

Recently, the binding and functional implications of the PHD finger of ING2 to H3K4me3 have been characterised, and shown to be essential for transcriptional repression of the corresponding gene [3] while the interaction of PHD NURF with H3K4me3 module transcription initiation [4]. Consequently, although the precise functional implications of H3K4me3 recognition by ING4 PHD domain are still to be determined, the results presented here show that it behaves differently from the ING2 PHD finger, which performs as a dual specificity module for both H3K4me3 and phosphatidylinositol-5-phosphate.

The structure of ING4 PHD and its affinity and binding site for histone 3 derived peptides have been measured by NMR for the wild type and for a mutant detected in lung cancer, which shows differences in binding affinities.

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## C45. APLICACIÓN DE MÉTODOS DE ASIGNACIÓN AUTOMÁTICA A LA PROTEÍNA UBC13

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Una de las aplicaciones más importantes de la RMN en el campo de la biología es la determinación de la estructura en disolución de proteínas de pequeño tamaño. Esta aplicación es de particular importancia tras la secuenciación completa del genoma de numerosas especies, lo que está dando lugar a la identificación de una gran cantidad de nuevos genes que codifican proteínas de estructura y función desconocida. En los próximos años, se espera que el número de proteínas „problema“ cuya estructura sea interesante determinar crezca enormemente. Para que la RMN sea una herramienta más eficaz en esta tarea es necesario acelerar el proceso de determinación estructural.

En este trabajo se ha utilizado el método de asignación automática MARS<sup>1</sup> para realizar la asignación de proteínas a partir de espectros 2D y 3D de la proteína doblemente etiquetada con <sup>15</sup>N y <sup>13</sup>C. Además, se han desarrollado nuevos módulos que combinados con programas de asignación automática pueden facilitar la resolución de estructuras de proteínas. Estos nuevos módulos han sido aplicados en proteínas cuyas estructuras han sido resueltas mediante RMN, y en un futuro serán aplicados a la proteína UBC13<sup>2</sup>. Esta proteína está formada por 152 aminoácidos y está implicada en el proceso celular de ubiquitinación.

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## C46. SOLUTION NMR STRUCTURE OF A NEW SODIUM CHANNEL INACTIVATOR TOXIN FROM THE SEA ANEMONE *CONDYLACTIS GIGANTEA*

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The three-dimensional structure of a new peptide toxin from the sea anemone *Condilactys Gigantea* exhibiting a MW of 5043 Da and comprising 47 amino acid residues and three disulfide bonds has been determined by NMR spectroscopy. The peptide was isolated from the sea anemone *Condylactis gigantea* and is a member of type I sea anemone sodium channel toxins containing the typical pattern of the six cysteine residues.<sup>1</sup> A restraint set of 1618 NOE interproton distances were used for the structure calculations which were carried out using the CYANA<sup>2</sup> program and refined with the AMBER<sup>3</sup> package. CgNa adopts in solution a compact structure of 4 strands of  $\beta$ -sheets connected by loops. The final ensemble of 20 structures has RMSDs values of 1.20 Å for the whole protein backbone and 0.28 Å for the well-defined region excluding the flexible loop comprising residues 8-17. This loop is conserved in other anemone toxins with sodium channel binding ability, and is thought to be important for toxin affinity.

Studies with type 3 sea anemone toxins have shown that there are well conserved amino acid residues that seem to be involved in the binding affinity to the Na<sup>+</sup> channel (Gly10, Gly15 and Gly20 in the flexible loop and Trp33 and Lys37 outside the loop).<sup>4</sup> These residues are also present in CgNa except the replacement of Lys37 by a Glu37 that introduces an additional negative charge in the molecule.

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## C47. STRUCTURAL BASIS OF mRNA RECOGNITION BY HRP1

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Processing of the 3'-end of messenger RNA is a crucial process during the biogenesis of this molecule. Nearly all mRNA in eukaryotes are cleaved and polyadenylated at the 3'-UTR of the transcript by a large and complex machinery exclusively made of protein factors<sup>1,2</sup>. The presence of this modification is crucial for mRNA stability and other downstream processes like transport and translation. There are several proteins that contact directly the RNA during the assembly of the polyadenylation machinery. Among them, the *S. cerevisiae* Hrp1 recognises the so-called Polyadenylation Enhancement Element (PEE) and is crucial for the efficient usage of the polyadenylation site. The molecular bases of this interaction are revealed here by the solution structure of a complex between Hrp1 and an oligonucleotide mimicking the PEE<sup>3</sup>. Six consecutive bases (AUAUAAU) are specifically recognised by two RNA binding domains (RBDs) arranged in tandem. Both protein and RNA undergo significant conformational changes upon complex formation with a concomitant large surface burial of RNA bases. Key aspects of RNA specificity can be explained by the presence of intermolecular aromatic-aromatic contacts and hydrogen bonds. In particular the role of a key tryptophan, that has been further investigated by NMR competition experiments with two mutants. All together, the Hrp1-PEE structure represents one of the first steps towards understanding of the assembly of the cleavage and polyadenylation machinery at the atomic level.

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*This work has been carried out with financial aid of the European Union and Ministerio de Educación y Ciencia (Ramón y Cajal program).*

## C48. MULTI-PURPOSE THE “MIX AND SHAKE” METHOD FOR CONFIGURATIONAL ASSIGNMENT BY NMR

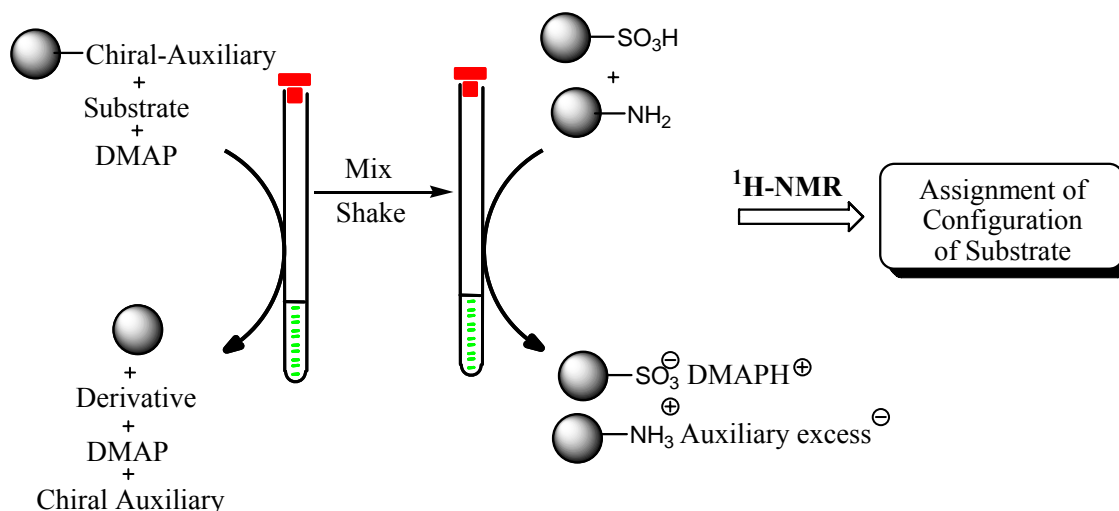
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A methodology for determining absolute configurations by NMR in just a few time and one pot is presented.<sup>1,2</sup> The required derivatives are obtained by mixing a solid matrix-bound auxiliary reagent (MPA, MTPA, BPG or 9-AMA) with the chiral substrates (secondary and primary alcohols, cyanohydrins, secondary-secondary diols, secondary-primary diols, secondary-secondary aminoalcohols and secondary-primary aminoalcohols) directly in the NMR tube. The transformation into derivatives requires the presence of DMAP and for its elimination were added scavengers resins. Finally the NMR spectra of the derivatives are obtained without any type of workup, or manipulation.



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## C49. ORIGIN OF THE NMR DETECTED “MOBILE LIPID” INTENSITY CHANGES IN C6 GLIOMA CELLS

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NMR-visible mobile lipids (ML) resonances at 1.28 and 0.9ppm have been described in the spectral pattern of aggressive tumours and in cultured cell types<sup>i</sup>. These ML mostly originate from triacylglycerol (TAG) in droplets (1-10 micrometers of diameter)<sup>ii iii</sup> and have been related to necrosis and hypoxia in tumours<sup>iv</sup> and proliferation rate in cultured cells<sup>ii</sup>. Proper understanding of the biochemical and biophysical origin of these ML could help MRS of human brain tumours to provide useful information for diagnosis, prognosis and therapy planning<sup>v</sup>. C6 cells display variable ML content (increase by 5-27 times) with proliferation arrest under defined culture conditions<sup>ii,vi</sup>. Our objective has been to ascertain whether ML intensity changes with proliferation rate in normally growing C6 cells are caused by biochemical changes (variation in TAG content per cell) or a different origin needs to be considered.

C6 total lipid cell extracts<sup>vii</sup> of cultured cells (day 4 cells, log phase, n=3, and day 7 cells, postconfluent, n=3) and {1}-13C-glucose 99% enriched grown cells (day 4 cells, n=3, 24h incubation with <sup>13</sup>C enriched glucose, day 3-day 4, and day 7 cells, n=3, 48h incubation with <sup>13</sup>C enriched glucose, day 5-day 7) were studied by <sup>1</sup>H-<sup>13</sup>C HMQC in an ARX Bruker 9.4T (SeRMN, UAB) and data analysis was performed using SPSS 11.5 for Windows (SPSS Inc., USA), with significance at p<0.05.

Quantitation of C6 lipid extracts by <sup>1</sup>H-<sup>13</sup>C HMQC showed no statistically significant changes in TAG content between day 4 and day 7 cultured cells. When comparing <sup>1</sup>H-<sup>13</sup>C HMQC experiments of extracts of normal growing C6 cells and C6 cells incubated with {1}-13C-glucose, the increase in the ratio TAG  $\alpha'$ ,  $\gamma'$ /choline (normalized to phosphatidylcholine content) is 4.3 times higher comparing day 4 cells and day 4 (3+1) labeled cells, and 11.3 times higher between day 7 cells and day 7 (5+2) labeled cells. On the other hand, we found an increase of 50 times in the glycerol  $\alpha$  carbon of phospholipids (PL $\alpha$ )/choline ratio between day 4 and day 4-labeled cells, while this ratio increased 26 fold between day 7 and day 7- labeled cells. Synthesis of PL and TAG is necessary for cell duplication if their content is to remain constant. Then, PL content per cell does not change between log and post-confluent cells (result not shown), while TAG content per cell does not change either. Also PL labelling, during log phase incubation with {1}-13C-glucose, is about 10 times higher than TAG labeling for the same time interval. This suggests a differential cellular compartmentalization of the synthesis pathway for PL and TAG, additionally requiring an origin different from glucose for TAG synthesis under the experimental conditions here assayed.

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<sup>ii</sup> Barba, I et al. *Cancer Res* 1999; 59:1861-1868.

<sup>iii</sup> Pérez Y et al. *Cancer Res.* 2002 Oct 15;62(20):5672-7.

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<sup>v</sup> Murphy PS et al. *Br J Radiol.* 2003 Jul;76(907):459-63.

<sup>vi</sup> Barba, I et al. *NMR in Biomed* 2001;14, 1: 33-40

<sup>vii</sup> Folch J et al. *J Biol Chem.* 1957; May; 226(1):497-509.

## **C50. *Geobacter sulfurreducens* TRIHEME CYTOCHROMES: SMALL DEVICES DRIVING ATP SYNTHESIS**

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The bacteria *Geobacter sulfurreducens* (Gs) can naturally oxidize organic compounds using Fe(III) or other metal oxides as terminal electron acceptors. Several of these metals are toxic and/or radioactive. Additionally, Gs can couple sediment organic matter oxidation with electron transfer to electrode surfaces with concomitant electricity production<sup>1</sup>. During growth in presence of Fe(III) oxides, Gs expresses several cytochromes. One of these proteins is a small periplasmatic triheme cytochrome with 71 residues, designated by PpcA, which was shown to have important role in the bioenergetic of Gs. In fact, this protein is known to be involved in the reduction of metal ion species such as Fe(III) and U(VI)<sup>2</sup> and recently we have shown that it participates in the e<sup>-</sup>/H<sup>+</sup> energy transduction in these bacteria<sup>3</sup>. The analysis of the Gs complete genome revealed the existence of four PpcA homologues, which function is presently unknown<sup>4</sup>. NMR has been shown to be an excellent technique to study the redox properties of multiheme electron transfer cytochromes, since it allows probing individually each redox centre providing information essential to elucidate the protein's physiological functions. The thermodynamic properties of the four PpcA homologues were studied using NMR and visible spectroscopy techniques and the preliminary results appear to indicate that, together with PpcA, these proteins may form a periplasmic cytochrome pool, which contributes to the H<sup>+</sup> electrochemical potential gradient across the periplasmic membrane that drives ATP synthesis.

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This work was supported by Project Grant POCI/QUI/60060/2004, awarded to Carlos A. Salgueiro.

## C51. APLICACIÓN DE LA RMN A LA DETERMINACIÓN ESTRUCTURAL DE FOTOPRODUCTOS DE PLAGUICIDAS

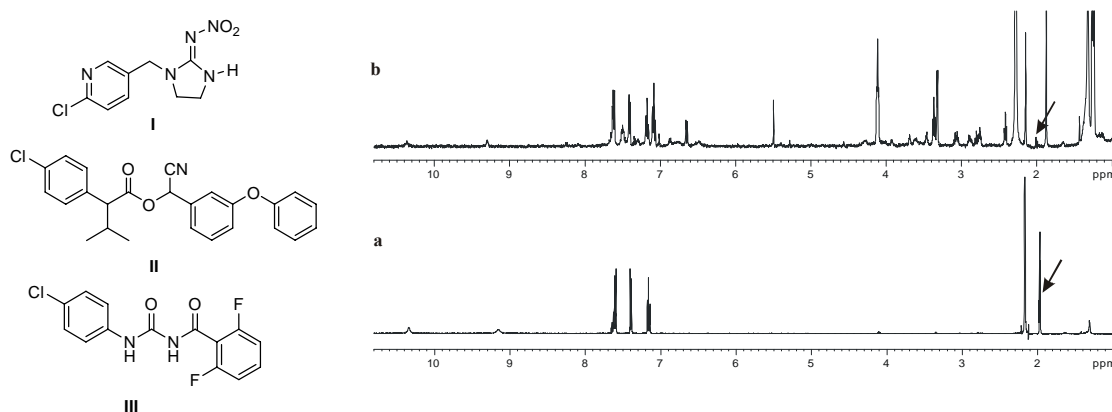
**Estela M. Sánchez<sup>a</sup>, Isidro Sánchez<sup>b</sup>, María Martínez\*<sup>b</sup>, Fernando López-Ortiz\*\*<sup>a</sup>**

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Mediante irradiación con una lámpara UV de Xe de 150 W se ha llevado a cabo el proceso de derivatización fotoquímica para tres insecticidas de uso generalizado en los cultivos en invernaderos de Almería: *imidacloprid* (**I**), *fenvalerato* (**II**) y *diflubenzurón* (**III**) (Figura 1). Dado que dichos plaguicidas no poseen fluorescencia nativa, pero por irradiación con luz UV se transforman en fotoproductos altamente fluorescentes,<sup>4</sup> se ha estudiado la cinética de las reacciones fotoquímicas a través de medidas de la intensidad de fluorescencia de los productos de fotólisis. De este modo, se ha optimizado la naturaleza del disolvente empleado para llevar a cabo cada reacción, así como el tiempo de irradiación óptimo de los compuestos, a la vez que se han realizado estudios de estabilidad de los fotoproductos almacenados a temperatura ambiente y a -4 °C.

La identificación estructural de los productos de fotodegradación de los insecticidas se ha abordado mediante técnicas de RMN mono y bidimensional. Para minimizar las posibles pérdidas de productos de vida media corta, las medidas de RMN se han realizado sobre alícuotas de las muestras irradiadas, lo que supone una concentración de trabajo de 20 ppm en disolventes no deuterados.<sup>5</sup> La utilización de la secuencia WATERGATE permite una eliminación excelente de la señal del disolvente (Figura 1).



**Figura 1:** Insecticidas objeto de estudio y espectros de RMN-<sup>1</sup>H (500.13MHz) adquiridos sobre una sonda 5mm TBI. a) Diflubenzurón (ns 128, S/N 5563, disolución saturada, aprox. 86x10<sup>3</sup> ppm) en CD<sub>3</sub>CN. b) Fotoproductos de diflubenzurón (ns 1024, S/N 3766, 20 ppm de soluto) en CH<sub>3</sub>CN. Eliminación de la señal del disolvente mediante WATERGATE.

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<sup>2</sup> G. A. Martin *Ann. Rep. NMR Spectrosc.* **2005**, 56, 1.

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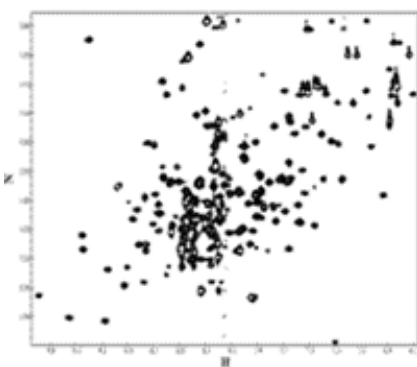
## C52. NMR ASSIGNMENT OF THE GROWTH ARREST AND DNA DAMAGE PROTEIN, GADD45 $\alpha$

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Gadd45 $\alpha$  is one of the three human members of the GADD45 family, originally identified as growth arrest and DNA damage inducible genes. Gadd45 $\alpha$  is an 18 kDa acidic protein that is induced by genotoxic and certain other cellular stresses. The exact function of this protein is unknown, but there is evidence that is involved in growth control, maintenance of genomic stability, DNA repair, cell cycle control and apoptosis. Oligomerization of Gadd45 $\alpha$  has been demonstrated<sup>1</sup>: several assays showed that recombinant Gadd45 $\alpha$  forms mainly a dimeric species in vitro.

We have overexpressed and purified Gadd45 $\alpha$  from a pET21 plasmid. Uniformly <sup>15</sup>N labelled and doubly labelled (<sup>15</sup>N and <sup>13</sup>C) samples were obtained. We have also produced selective <sup>15</sup>N-amino acid samples with Leucine and Phenylalanine/Tyrosine with high yields. NMR samples up to 1.4 mM have been obtained, but we acquired all spectra using a 0.45 mM sample at which concentration it was confirmed to be in monomeric form by analytical ultracentrifugation.



The HSQC <sup>1</sup>H-<sup>15</sup>N presented on the left displays two set of signals: a first set of peaks, presenting dispersion which is characteristic of a high content of defined secondary structure and a second set of peaks with low dispersion, probably arising from residues located in loops or flexible regions of the protein. We have also acquired a complete battery of triple resonance experiments to assign the resonances of the protein. At the moment, we have assigned 40% of the backbone of the protein. In the poster, we expect to show the complete assignment of the backbone. Those results will help us to determine the secondary structure of Gadd45 $\alpha$  in solution.

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*The pET21-Gadd45 $\alpha$  plasmid was provided by Dr. A.J. Fornace, Jr., Massachusetts School of Public Health, Harvard, USA.*

## C53. SPECIFIC BINDING OF PWWP DOMAIN TO HISTONE METHYLATED PEPTIDES

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The “histone code” hypothesis postulates that specific patterns of covalent modifications of histones can be recognized by different protein modules and correlate with a specific biological role<sup>1</sup>. Chromo domains bind to methylated lysine residues of histones while Tudor domains have been reported to recognize both symmetrical dimethylated arginine and methylated lysine residues. The PWWP domain is a protein module with a conserved Pro-Trp-Trp-Pro motif present in nuclear proteins of eukaryotes<sup>2</sup>. Although specific function of the PWWP domain is still unknown, the remarkable structural and sequence similarities with the Tudor, chromo and MBT domains suggest that are all members of the same protein superfamily<sup>3</sup>.

Methylated lysine residues on histones seem to be likely binding partners for the PWWP domain. Using chemical shift mapping experiments, we have shown that the PWWP domain from the *S. pombe* protein SPBC215.07c<sup>4</sup> binds specifically to trimethylated H3-K36. It does not show binding to the corresponding mono-, di- or unmethylated peptides. Fluorescent anisotropy measurements with fluorescein-labelled peptides have shown that trimethylated H3-K36 recognition, although highly specific, is of modest affinity (100  $\mu$ M range). PWWP domains of the human proteins LEDGF and HDGF show the same binding specificity than the yeast one, suggesting a role of this protein module in transcription activation. The binding site involves highly conserved aromatic residues analogously to the cluster of aromatic residues responsible for the recognition of methylated histone residues by chromo and Tudor domains.

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L.M. Slater, M.D. Allen, and M. Bycroft *J. Mol. Biol.*, **2003**, 330, 571-576.

## **C54. NMR SOLUTION STRUCTURE AND INTERNAL DYNAMICS OF THE C-TERMINAL DOMAIN OF OLE E 9, A MAJOR ALLERGEN FROM OLIVE TREE POLLEN**

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Olive tree pollen is one of the main causes of seasonal respiratory allergies in Mediterranean countries. Ten allergens from olive pollen, Ole e 1 to Ole e 10, have been described. Ole e 9 is a 46 kDa protein composed by an N-terminal domain (36 kDa) and a C-terminal domain (10.5 kDa). The N-terminal domain exhibits 1,3- $\beta$  glucanase activity. To gain further insights into the structural bases of its biological function and allergenic properties, the solution structure and the internal dynamics of the C-terminal domain has been undertaken by NMR methods. NMR spectra were acquired on 600 and 800 MHz spectrometers, and assigned following the standard methodology based on 3D <sup>15</sup>N-<sup>13</sup>C strategies. The three-dimensional structure was calculated on the basis of distance and angular experimental restraints. Ct-Ole e 9 is composed by two  $\alpha$ -helices spanning residues 22-32 and 56-70, a short  $\beta$ -sheet (residues 13-65 and 82-85) and a long and unstructured N-tail. The internal dynamics was determined from the analysis of <sup>1</sup>H-<sup>15</sup>N relaxation data and shows a high degree of mobility in the C- (residues 98-101) and N-terminus (1-12) of the protein. Subsequent analysis of this data will be essential to infer information about the accessibility, shape and mobility of the different regions of the molecule, which could lead to the design of new vaccines specific for olive tree pollen. In addition, the structure refinement will provide a basis for understanding the physiological function of this novel plant protein.

## **C55. THE HIGH-RESOLUTION STRUCTURE OF THE R21A SPC-SH3:P41 COMPLEX: UNDERSTANDING BINDING AFFINITY BY COMPARISON WITH ABL-SH3**

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<sup>2</sup>*Bijvoet Center, Department of NMR Spectroscopy, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands.*

SH3 domains are small protein modules of 60-85 amino acids that bind to short *proline-rich* sequences with moderate-to-low affinity and specificity. Interactions with SH3 domains play a crucial role in regulation of many cellular processes (some are related to cancer and AIDS) and have thus been interesting targets in drug design. The decapeptide APSYSPPPPP (p41) binds with high affinity to Abl-SH3, while it has low affinity for Spc-SH3. The binding affinity, however, is increased 2-fold upon mutation of the arginine at position 21 to an alanine residue (R21A Spc-SH3). Here we present the high-resolution structure of the complex between R21A Spc-SH3 and p41 derived from NMR data using a combination of automatic assignment protocols (CANDID and ARIA) in conjunction with modern docking methods (HADDOCK). The mutation has no major consequences, as the overall structures of the wild type and R21A mutant are almost identical. The 2-fold higher binding affinity upon mutation of arginine 21 to alanine in Spc SH3, can be rationalized by elimination of the steric clash induced by the longer arginine side chain. The structure of the R21A Spc-SH3:p41 complex resembles that of the Abl-SH3:p41 complex that was solved previously by X-ray crystallography. The difference in affinity for p41 between Spc-SH3 and Abl-SH3 can be rationalized by the different interactions that are involved in the binding to the ligand and can be partially ascribed to the absence of at least three out of five buried water molecules that were shown to contribute largely to the highly negative binding enthalpy in the Abl-SH3:p41 complex.

## C56. $^1\text{H}$ and $^{29}\text{Si}$ Solid State NMR studies of the Si-OH groups in pure silica zeolites, CHA and LTA

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Zeolite materials are synthesized hydrothermally from a mixture of water, a source of silica, alumina and/or the heteroatoms to be incorporated into the framework, and the organic molecules acting as structure directing agent. Moreover the synthesis can be carried out using fluoride or  $\text{OH}^-$  as mineralizing agent. The preparation of zeolites in fluoride medium usually leads to crystalline materials with low number of structural or connectivity defects (Si-OH groups). Here, we have investigated the nature of the residual Si-OH groups existing in pure silica CHA and LTA type zeolites obtained in fluoride medium by using  $^1\text{H}$ ,  $^{29}\text{Si}$  MAS and  $^1\text{H}$  to  $^{29}\text{Si}$  CP/MAS NMR.

The  $^1\text{H}$  MAS NMR spectra of zeolites CHA and LTA show narrow signals in the range 1.7-2.2 ppm assigned to isolated Si-OH terminal groups,<sup>1</sup> and a weak signal centred at 5.5 ppm. The  $^{29}\text{Si}$  single pulse spectra of these materials show a main signal at -110.9 for LTA and at -111.5 for CHA, attributed to  $\text{Si}(\text{OSi})_4$  of their unique Si crystallographic position.<sup>2</sup> Beside this, it is possible to distinguish a group of very weak signals at about -100 ppm, whose intensity is enhanced in the  $^1\text{H}$  to  $^{29}\text{Si}$  CP MAS spectra, indicating that it must be due to Si-OH groups. The  $^{29}\text{Si}$  signals attributed to the Si-OH groups, observed in the spectra recorded under cross polarization conditions, can be tentatively associated with the different oxygen crystallographic positions in the zeolites structures.

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## **C57. EVALUATION OF THE BINDING OF NOVEL UNNATURAL ANTIANGIOGENIC AND ANTIMETASTATIC INHIBITORS TO THE I-DOMAIN OF THE INTEGRIN LFA-1.**

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Lymphocyte function-associated antigen-1 (LFA-1), a member of the integrin family of adhesion receptors that is expressed on the surface of leukocytes and is involved in leukocyte trafficking and extravasation. Intercellular adhesion molecule-1 (ICAM-1), a cell surface ligand for LFA-1 is a member of the immunoglobulin protein super-family. The interaction of LFA-1 and ICAM-1 is critical to many immunological reactions, including T lymphocyte antigen-specific responses and leukocyte accumulation in inflamed tissues. LFA-1 also plays an integral role in the mechanisms of cancer cell adhesion, growth, invasion, and metastasis and has been shown to influence the immune response to malignant cells.

The extracellular domains of LFA-1 are composed of the large and complex  $\alpha_L$  and  $\beta_2$  subunits, however, the ligand binding site is located exclusively in the inserted (I) domain of  $\alpha_L$ . Within the I-domain, there is a single metal ion dependent adhesion site (MIDAS) that preferentially binds divalent cations. Cation binding is required for ligand interaction and is believed to induce the conformational changes in LFA-1 necessary for binding. Using NMR, we have analyzed a library of organic compounds designed to compete with ICAM-1 for binding to LFA-1. Some of the compounds are known to be biologically active: in vitro they block the adhesion of lymphoma cells to liver sinusoidal endothelial cells, and in vivo they reduce the number of liver metastatic tumors in treated mice. We have utilized 2D heteronuclear techniques to map the interaction of the compounds with the I domain, and after assigning the protein backbone using 3D heteronuclear techniques, we have identified key residues involved in compound binding. This data provides clues for the design of a second generation of compounds.

## C58. $^1\text{H}$ MAS NMR studies of structure-directing agents in a paramagnetic-metal-doped $\text{AlPO}_4$ microporous material

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The presence of paramagnetic species in samples drastically alters their NMR signals. In general, it has been considered as a serious limitation as it uncontrollably accelerates both longitudinal ( $T_1$ ) and transversal ( $T_2$ ) relaxation times of the neighbouring NMR-active nuclei. As a consequence, the resultant NMR spectra consist on very broad signals, which in addition could be shifted in a hard-predictable way. However, liquid/solution NMR techniques has sometimes took advantage of the presence of paramagnetic species for proposes as diverse as to quickly acquire some spectra otherwise very longer or to separate signals otherwise overlapped.<sup>1</sup> On the contrary, paramagnetic species has been scarcely (but ingeniously) applied in solid-state NMR.<sup>2,3</sup>

We have prepared two high-doped  $\text{AlPO}_4$ -based microporous materials of the same topology (chabazite), with the same metal ion content, with the same structure-directing agent (cyclohexylamine) but doped with different transition-metal ions:  $\text{Zn}^{2+}$ , diamagnetic ( $d^{10}$ ), and  $\text{Co}^{2+}$ , paramagnetic ( $d^7$ ). Dramatic differences were found in the  $^1\text{H}$  MAS NMR spectrum of the CoAPO sample compared to that of the diamagnetic ZnAPO sample, which were explained by the presence of paramagnetic species. Thus, several orders of intense spinning side bands were found in the spectrum of CoAPO, even at high spinning rates (16 KHz). More importantly, a new signal at an unusual  $^1\text{H}$  chemical shift of 18 ppm appeared, which could be due to the protonated group  $\text{NH}_3^+$  directly interacting with the paramagnetic  $\text{Co}^{2+}$  ions of the framework, since it is absent in the spectrum of ZnAPO.

This work opens the possibility of using  $^1\text{H}$ -contained probe molecules to study the paramagnetic character of the accessible metal ions in solids.

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