4th NMR Young Researchers Meeting

4TH NMR YOUNG RESEARCHERS MEETING

May 7th - 8th 2026
CITIUS II - Celestino Mutis - University of Seville

BOOK OF ABSTRACTS

https://germn.rseq.org/actividades1/germn-junior/

geRMN grupo especializado de resonancia magnética nuclear
NMR Online Limited
BRUKER
GEQB Grupo Especializado de Química Biológica
STAOC RSEQ Sección Territorial de Andalucía Occidental
RSEQ Real Sociedad Española de Química
Palex Improving technologies Improving lives
JEOL
DEUTERO NMR Spectroscopy
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INDEX

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Miguel Mompeán García
Group leader at Blas Cabrera Institute of Physical Chemistry – CSIC (MADRID).

VENUE LOCATION

The scientific sessions will take place at the lecture hall of **CITIUS II - Celestino Mutis** (Av. de la Reina Mercedes, S/N, 41012, Seville). Coffee breaks and sponsor stands will be held on the terrace located right next to the venue. Lunch will be served at the Instituto de Idiomas (Av. de la Reina Mercedes, 4, 41012, Seville), which is situated just in front of the venue.

We are also organizing a cultural visit during the first day of the meeting.

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PROGRAM

Thursday May 7th	
09:00 - 09:30	*Registration*
09:30 - 09:45	Welcome C Opening Remarks
Session I - Chair: Joaquín Tamargo Azpilicueta	
09:45 - 10:30	**IL1 - Irene Marco Rius** “A Nonlinear Path in Science: From Physics to Molecular Imaging (And Everything In Between)”
10:30 - 10:35	**FT1 - José Manuel Rojas-Marcos Hernáez** “CD44 Decoded By NMR: Glycoprofile and Molecular Interactions”
10:35 - 10:40	**FT2 - Cristina Ugedo Pérez** “Non-Canonical Nucleic Acid NMR: Taking Full Advantage Of 1D Spectra”
10:40 - 10:45	**FT3 - Marina Carranza César** “[2.2.1]Heterobicyclic Bromovinyl Sulfones for Thiol-Triggered Strategies in Linker Chemistry”
10:45 - 11:00	**OC1 - Arantxa Forte Castro** “Water-Soluble Lithium and Silver Tris(Pyrazolyl)Methanesulfonates: Multinuclear and Diffusion NMR”
11:00 - 11:15	**OC2: Marta González Lázaro** “Concentration-Independent Molecular Weight Determination of Polymers Via Diffusion NMR: A Universal Approach Across Solvents”
11:15 - 11:45	*Coffee Break*
Session II - Chair: : Inés Lera Lasso	
11:40 - 11:45	**FT4 - Álvaro Fernando García Rodríguez** “Biochar Effects on Dissolved Organic Carbon Assessed By ^{1}H-NMR In Andosol and Alluvial Single-Soil Columns”
11:45 - 11:50	**FT5 - Adelyn M. Betances Mora** “STD NMR Characterization of Glycomimetic Binding to Cancer-Associated Sialyltransferases Reveals Differential Recognition Patterns”
11:50 - 11:55	**FT6 - Francisco J. Moreno Racero** “Solid-State ^{13}C NMR Analysis of Short-Term Organic Carbon Transformations In Hydrochar-Amended Soils”
11:55 - 12:10	**OC3 - Joaquín Tamargo Azpilicueta** “Deciphering Protein Post-Translational Modifications by Experimental and Computational Approaches”
12:10 - 12:25	**OC4 - Mirane Florencio Zabaleta** “Deciphering the Immunoragulatory Axis of Galectin-9 and Tim-3”
12:25 - 12:40	**OC5 - Francisco J. Alonso Martínez** “Exploring Carbohydrate Recognition in Galectins by ^{19}F-NMR Screening, Fluorescence Binding Studies and Structural Analysis”
12:40 - 12:55	**OC6 - Andrea Fernández Veloso** “NMR-Based Metabolomics as a Tool for Monitoring Vernalization in Strawberry Crops”
12:55 - 13:10	**OC7 - Tomás Martín Duque** “Interferogram-Based Pure Shift STD NMR”
13:10 - 13:25	**OC8 - Sara Andrés Campos** “Solid-State NMR Characterization of Amyloid Samples Detected Using CPMAS”
13:25 - 14:55	*Lunch*

Session III - Chair: Mirane Florencio Zabaleta	
14:55 - 15:40	**ST1 - NMR Online** “NMR Online - ELEMENTS: Yesterday's Software for Tomorrow's Solutions!”
15:40 - 15:55	**OCG - Inés Lera Lasso** “Novel NMR Methods for the Study of Fluorinated Glycans”
15:55 - 16:10	**OC10 - Almudena Hornero Romera** “Understanding NHERF1 and ICAM1 Protein Interactions Using Solution NMR”
16:10 - 16:25	**OC11 - M. Paola Oquist Philips** “NMR Characterization of the FGF1-FGFR D2 Interaction: Effects of N-Glycosylation”
16:25 - 16:55	*Coffee Break*
Session IV - Chair: Francisco J. Alonso Martínez	
16:55 - 17:00	**FT7 - Harikrishnan Vijayakumary Krishnakumar** “Investigating RIPK1/RIPK3 Fibril Formation through NMR Spectroscopy and Molecular Dynamics”
17:00 - 17:05	**FT8 - Ignacio Maldonado Lorenzo** “Multinuclear and Diffusion NMR Reveal Structure-Aggregation Relationships in Chiral Amino Acid-Based Anthraquinones”
17:05 - 17:20	**OC12 - María Payá García** “Molecular Insights Into Biologically Relevant CD44-Hyaluronan Recognition by NMR”
17:20 - 17:35	**OC13 - Gabriel Rocha** “Enhancing Ligand Epitope Mapping Analysis by Variable Temperature Saturation Transfer Difference NMR (VT-STD NMR)”
17:35 - 17:50	**OC14 - Cristina Fernández Pérez** “Unravelling Viral Haemagglutinin Preferences to Host Glycans by Chemoenzymatic Synthesis and NMR”
17:50 - 18:05	**OC15 - Antonio Ruiz Albor** “PCNA: One Ring to Bind Them”
18:05 - 18:20	**OC16 - Rafael L. Giner Arroyo** “Tyrosine Phosphorylation Unfolds Nucleophosmin and Disrupts Its Integration Into the Nucleolus”
19:30 - 20:30	*Seville Cultural Visit*
20:30 - ...	*Dinner & After-Hours Networking*

Friday May 8th	
Session V - Chair: Sara Andrés Campos	
9:00 - 9:05	**FTG - José María García de Castro Barragán** “Pushing the Limits of Low-Field NMR: Improving DDIF Method Resolution to Study Biochar Internal Structures”
9:05 - 9:10	**FT10 - Juan de Dios Marín Manzano** “Reconstructing Serum Metabolomic Profiles from Urine NMR Data: Proof-Concept for Indirect Clinical Prediction”
9:10 - 9:15	**FT11 - Laura Gismero Rodríguez** “Historical Land Use and Soil Carbon Dynamics in a Mediterranean Olive Orchard Under Different Agricultural Management Schemes”
9:15 - 9:30	**OC17 - M. Alejandra De Castro Travecedo** “NMR Insights into the Molecular Recognition of Blood Group Antigens by Human Galectin-2”

9:30 - 9:45	**OC18 - Ignacio Nieto Vargas** “Temperature-Dependent Haptotropic Phenomena in Digold(I) Acetylide Complexes”
9:45 - 10:00	**OC1G - Gurudutt Dubey** “NMR Analysis of Small Molecular Inhibitors Binding to Human Proliferating Cell Nuclear Antigen”
10:00 - 10:15	**OC20 - Lourdes Del Álamo Rodríguez** “NMR Study of Self-Assembled Diruthenium Aggregates in Solution”
10:15 - 10:30	**OC21 - Luka Barešić** “An NMR-Based Study of Latanoprost Encapsulation”
10:30 - 10:45	**OC22 - Nuria Andrés Penares** “NMR-Based Measurement of the Guanine Nucleotide Exchange Rate on Gαi3 Protein”
10:45 - 11:15	*Coffee Break*

Session VI - Chair: Tomás Martín Duque	
11:15 - 11:20	**FT12 - Diego Sánchez Pulido** “Allosteric Modulation of TIA-1 Phase Separation by Double Serine Phosphorylation”
11:20 - 11:25	**FT13 - Venetia Psomiadou** “Fluorinated Sulfonate Sialomimetics Reveal Binding Mechanisms of CD33 by STD and ^{19}F-CPMG NMR”
11:25 - 11:30	**FT14 - Beatriz Torres Calvo** “A [2+4] Multicomponent Quantitative Assembly in Polar Media via Hydrogen-Bonds”
11:30 - 11:45	**OC23 - Álvaro Perez Collar** “Metabolic Fingerprinting of Bovine Tuberculosis: Translating Benchtop NMR from Lab to Barn”
11:45 - 12:00	**OC24 - Gonzalo Alonso Reíllo** “Hyperpolarization: What It Is And What Challenges It Poses”
12:00 - 12:15	**OC25 - Pablo Rivero García** “Phosphorylation of the AML-Linked C-Terminal Region of NPM1 Controls Stability, DNA Interaction, and Charge-Driven Phase Separation”
12:15 - 12:30	**OC26 - Lucas García Abuín** “Unraveling the Structure of Wulff-Type Boronate Esters by NMR”
12:30 - 12:45	**OC27 - Diego Martín Arranz** “High and Low-Field Advanced NMR Approaches for Wine Analysis”
12:45 - 13:10	**ST2 - Jeol-Palex** “JEOL ECZ Luminous Series – Overview”
13.10 - 14:40	*Lunch*
Session VII - Chair: Andrea Fernández Veloso	
14:40 - 14:55	**OC28 - María Vila Gonzalo** “Applying NMR for Structural Characterization and Epitope Definition of a Tumor-Associated Carbohydrate Antigen”
14:55 - 15:10	**OC2G - Rocío Rodríguez Marín** “Structural Determination of sp^2-Iminoglycolipids by NMR Spectroscopy”
15:10 - 15:25	**OC30 - Jaime Hiniesta Valero** “From Vineyard to Spectrum: an NMR and Machine Learning Approach for the Authentication of 'Jerez-Xérès-Sherry' Wines”

15:25 - 15:40	**OC31 - Roberto Grosso Bullejos** “Leveraging Liquid-State NMR for Structural Insight and Functional Design of Advanced Biomedical Materials”
15:40 - 15:55	**OC32 - Henry Flatau** “Toward Antivirals against Norovirus: Expanding the NMR Toolkit for Norovirus Drug Discovery”
15:55 - 16:30	*Coffee Break*
Session VIII - Chair: Mirane Florencio Zabaleta	
16:30 - 17:15	**IL2 - David Pantoja Uceda** “  ”
17:15 - 18:00	***Round Table:*** *NMR Career Pathways After the PhD* **Irene Díaz-Moreno** *Full Professor at University of Seville* **Nereida Hidalgo Reinoso** *Marie-Curie Fellow post-doctoral researcher at University of Seville* **David Pantoja-Uceda** *NMR Senior Specialist Technician at Instituto de Química-Física “Blas Cabrera”*
18:00 - 18:10	Award Ceremony and Closing Remarks

INVITED LECTURES

Irene Marco - Rius

Junior Group Leader at Institute for Bioengineering of Catalonia, IBEC (BARCELONA, SPAIN)

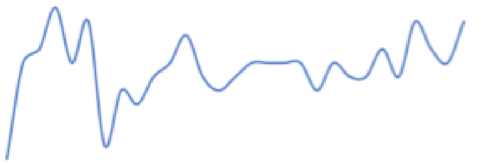
Invited lecture - IL1

“A Nonlinear Path in Science: From Physics to Molecular Imaging (And Everything In Between)”

David Pantoja - Uceda

NMR Facility Manager at the “Manuel Rico” NMR Laboratory, IQF-CSIC (MADRID, SPAIN)

Invited lecture - IL2

“  ”

SPONSORED TALKS

Sponsored talk 1 - ST1 - NMR Online

Simmon P. Skinner

“NMR Online - ELEMENTS: Yesterday's Software for Tomorrow's Solutions!”.

Sponsored talk 2 - ST2 - Jeol-Palex

Carla Merino Ruiz

“JEOL ECZ Luminous Series – Overview”.

JEOL ECZ Luminous Series – Overview

Carla Merino[1], Ahmed Dhifaoui[2]

[1]NMR Product Manager, Analytical Unit, Palex Medical SA
[2]Head of Analytical Instrumentation Division NMR – ESR – MS, European Product Manager, JEOL (Europe) SAS

This presentation provides a comprehensive overview of JEOL's advanced Nuclear Magnetic Resonance (NMR) solutions, with a particular focus on the innovative ECZ Luminous series. The ECZ Luminous console stands out for its hybrid solution-state and solid-state capability, offering both compact and extensible configurations. Its cutting-edge technologies, such as the Multi-Sequencer and Smart Transceiver System (STS), enable multi-resonance experiments using only two channels, significantly enhancing experimental flexibility and efficiency. Additionally, the Multi-Frequency Drive System (MFDS) further expands the instrument's versatility, allowing seamless operation across a wide range of frequencies.

In response to global energy challenges, JEOL introduces the Cryogen Reclamation System (CR80), a sustainable solution for the recovery and recycling of liquid nitrogen and liquid helium, reducing operational costs and environmental impact.

The presentation also highlights JEOL's extensive probe portfolio, including the room-temperature ROYALPROBE series for solution NMR, as well as the SuperCool (nitrogen-cooled) and UltraCool (helium-cooled) cryogenic probes, which deliver superior sensitivity and resolution. Automation in solid-state NMR is exemplified by the ROYALPROBE AutoMAS, streamlining sample handling and data acquisition.

Complementing these hardware innovations, JEOL's DELTA software suite provides robust tools for data acquisition and processing, while the vendor-agnostic JASON software offers enhanced data analysis capabilities, ensuring compatibility and flexibility across diverse NMR platforms.

This overview underscores JEOL's commitment to advancing NMR technology through innovation, sustainability, and user-centric design, addressing the evolving needs of modern research and industry.

A Nonlinear Path in Science: From Physics to Molecular Imaging (and Everything in Between)

Irene Marco-Rius[1]

[1]Institute for Bioengineering of Catalonia, Barcelona

Scientific careers are often presented as linear trajectories. However, reality is frequently far more complex and shaped by uncertainty. And that is not a flaw, but a feature. In this talk, I will share my personal journey from studying Physics in Barcelona, to completing a PhD in Biochemistry at Cambridge, to eventually establishing my own research group at the Institute for Bioengineering of Catalonia (IBEC).

This journey spans over a decade of international mobility, navigating different scientific cultures, expectations and disciplines. I will discuss how moving between physics, chemistry and biology has shaped a multidisciplinary scientific identity, and how exposure to diverse research cultures has influenced my approach to problem-solving, leadership and innovation. Particular emphasis will be placed on the challenges and opportunities associated with navigating disciplinary boundaries, building independence and defining a coherent research vision in emerging fields such as metabolic imaging.

I will also share my experience stepping outside academia to co-found a spin-off company. This was an attempt to translate our research into real-world impact, but also a process that exposed me to uncertainty, responsibility and failure in a very different way than academia does. It challenged many of my assumptions about success, control and risk.

This talk aims to provide an honest account of a non-linear career path and to offer perspectives that may be valuable to early-career researchers navigating their own trajectories.

David Pantoja-Uceda[1],

1. “Manuel Rico” NMR Laboratory, Biomolecular NMR Methodology Group
Instituto de Química Física Blas Cabrera, CSIC, Serrano 119, 28006, Madrid, Spain.
E-mail:dpantoja@iqf.csic.es

Dr. David Pantoja-Uceda was trained as a chemist at the Universidad Autónoma de Madrid (UAM), where he completed both his Bachelor’s and Ph.D. degrees within the protein NMR group at the Institute of Physical Chemistry “Blas Cabrera” (IQF, CSIC, Madrid). He further broadened his scientific perspective through research stays at international research centers such as the Center for Advanced Biotechnology and Medicine (CABM, New Jersey, USA) and the RIKEN Genomic Science Center (Yokohama, Japan), as well as at leading national institutions including CIPF (Valencia) and CNIO (Madrid). His career was subsequently consolidated at the IQF, where it all began, first as a Senior Specialized Officer and later as a Tenured Scientist, with research focused on the structural and dynamical characterization of proteins and their interactions with other biomolecules using NMR spectroscopy. Drawing on his experience across multiple NMR laboratories and his long-term involvement in scientific infrastructure management, he has played a key role in integrating the “Manuel Rico” NMR Laboratory into Spain’s national ICTS roadmap and, at the international level, into the INSTRUCT-ERIC consortium.

In this presentation, he aims to share a personal perspective on his journey through science, offering reflections on how the scientific world has shaped his career, values, and view of research beyond the laboratory bench.

CD44 DECODED BY NMR: GLYCOPROFILE AND MOLECULAR INTERACTIONS

José Manuel Rojas-Marcos Hernáez[1], Sandra Delgado[1], M. Azkargorta[2], Felix Elortza,2 Jesús Jiménez-Barbero[1,3], Ana Gimeno[1,3]

[1]Chemical Glycobiology Lab, CIC bioGune, Basque Research & Technology Alliance (BRTA), Bizkaia Technology Park, Building 800, 48160 Derio, Bizkaia, Spain
[2]Proteomics Platform, CIC bioGune, Basque Research & Technology Alliance (BRTA), Bizkaia Technology Park, Building 800, 48160 Derio, Bizkaia, Spain
[3]Ikerbasque, Basque Foundation for Science, Plaza Euskadi 5, 48009 Bilbao, Spain

Aberrant protein glycosylation represents a well-recognized hallmark of cancer, offering strong potential to improve cancer diagnostics, enhance prognostic assessments, and inform the development of personalized therapeutic approaches [1,2]. In this context, elucidating how tumour-associated glycoconjugates mediate the communication between cancer cells and their surrounding microenvironment-and thereby influence cellular behaviour- is essential for advancing our understanding of cancer biology and for developing more effective therapeutic strategies [3,4].

Our research focuses on CD44, a transmembrane glycoprotein which is widely overexpressed in different tumours types and plays a central role in key cancer stem cell functions such as tumour initiation or metastasis [5]. In this study, NMR has been employed as a central tool to characterize the glycosylation profile and molecular interactions of CD44v6, a CD44 isoform associated with increased tumorigenicity across multiple human cancers, including breast, lung, and colon [6].

Additionally, a series of fluorinated monosaccharides has been synthesized and evaluated as chemical probes for the selective tagging of sugar residues within glycoconjugates [7], enabling the use of ^{19}F NMR to monitor molecular interactions in native-like complex systems.

1. Mereinter, S.; Balmaña, M.; Campos, D.; Gomes, J.; Reis, C. *Cancer* Cell **2019**, 36 (1), 6-16
2. Reily, C.; Stewart, T. J.; Ranfrow, M. B.; Novak, J. Nat. Rev. Nephrol. **2019**, 15 (6), 346-366
3. Liao, C.; Wang, Q.; An, J.; Chen, J.; Li, X.; Long, Q.; Xiao, L.; Guan, X.; Liu, J. Front. Oncol. **2022**, 12, 883831.
4. Shan, M.; Yang, D.; Dou, H.; Zhang, L. Progress in Molecular Biology and Translational Science, **2019**, 162, 93-119.
5. Eskhar Sebban, L.; Ronen, D.; Levartovsky, D.; Elkayam, O.; Caspi, D.; Aamar, S.; Amital, H.; Rubinow, A.; Golan, I.; Naor, D.; Zick, Y.; Golan, I. J. Immunol. **2017**, 179 (2), 1225-1235.
6. Heider, K-H.; Kuthan, H.; Stehle, G.; Munzert, G. Cancer. Immunol. Immunother. **2004**, 53 (7), 567-579
7. Dai, Y.; Hartke, R.; Li, C.; Yang, Q.; Liu, J. O.; Wang, L. ACS Chem. Biol. **2020**, 15 (10), 2662-2672

NON-CANONICAL NUCLEIC ACID NMR: TAKING FULL ADVANTAGE OF 1D SPECTRA

Cristina Ugedo Pérez,[1] Irene Gómez-Pinto,[1] Miguel Garavís,[1] Carlos González[1]

[1]Institute of Physical Chemistry Blas Cabrera, IQF-CSIC, Madrid, Spain

Nuclear magnetic resonance (NMR) is a very broad technique that has been refined and expanded during decades [1]. Due to the efforts of many scientists worldwide, it can be used to acquire structural and dynamic information from a wide range of molecules in a diverse set of environments. The tendency has, for a long time, been the design of longer and more complex pulse sequences in order to fully exploit the potential of this technique [2]. In some cases, however, monodimensional spectra are full of structural and dynamic information. Non-canonical nucleic acid structures, particularly, display very specific signals in 1H 1D NMR that make monodimensional spectra very useful for different experiments, such as melting assays, ligand-binding assays or folding assays, which can therefore be performed very quickly with significant and intuitive results. Fluorine atoms within the system provide an NMR probe that enables the extraction of even more information from standard monodimensional spectra.

In this oral communication, I will display monodimensional NMR as a complete experimental methodology by presenting a few experiments that led to publications, performed exclusively with 1D NMR. Additionally, I will comment on the benefits of fluorine modification as an NMR probe that allows for the extraction of even more information through monodimensional NMR.

1. K. Wüthrich, *NMR of Proteins and Nucleic Acids*, First Edition. in Baker Lecture Series. Wiley, **1986**.
2. J.-N. Dumez and P. Giraudeau, *Fast 2D Solution-state NMR: Concepts and Applications*. in New Developments in NMR. *The Royal Society of Chemistry*, **2023**.

[2.2.1]HETEROBICYCLIC BROMOVINYL SULFONES FOR THIOL-TRIGGERED STRATEGIES IN LINKER CHEMISTRY

Marina Carranza César,[1] Antonio J. Moreno-Vargas,[1] Ana T. Carmona Asenjo[1]

[1]Department of Organic Chemistry, Faculty of Chemistry, University of Seville, Spain

[2.2.1]Heterobicyclic bromovinyl sulfones represent versatile platforms for thiol-triggered linker chemistry and controlled drug release. In this work, we investigate the thiol-induced retro-Diels–Alder (rDA) fragmentation of heteronorbornadiene (HND) derivatives through ^{1}H-NMR kinetic studies. We monitored the kinetics of sequential thiol additions and the subsequent rDA, identifying key intermediates, the rate-limiting step and the time required to reach 50% conversion of HNDs into the corresponding furan/pyrrole products. This strategy allowed us to correlate the electronic and steric effects with the fragmentation rates in a family of differently substituted HNDs.[1] Additional experiments under physiologically relevant conditions demonstrated preserved thiol responsiveness in complex media. Overall, this study highlights ^{1}H NMR kinetic analysis as a tool for elucidating thiol-triggered mechanisms and provides a framework for designing HNDs as cleavable linkers for controlled-release strategies.[2]

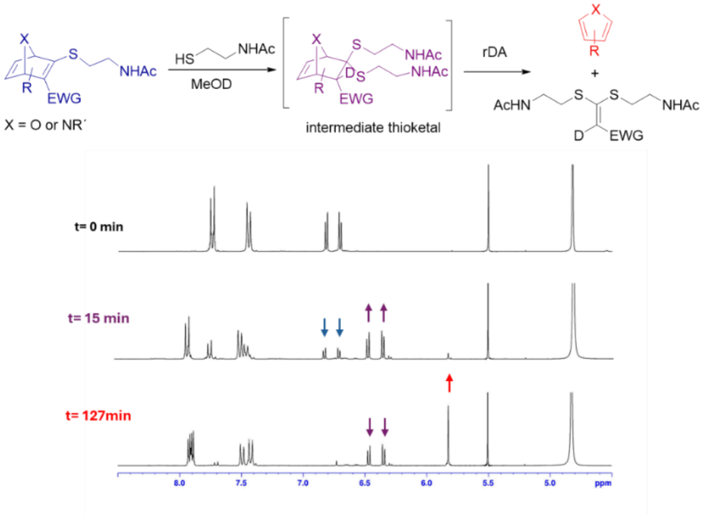


Figure 1. ^{1}H NMR monitoring of thiol-triggered rDA fragmentation.

1. Carranza, M.; Carmona, A. T.; Navo, D. C.; Robina, I.; Fratta, S.; Newburn, C.; Jiménez-Oses, G.; Moreno-Vargas, A. J. Experimental and Theoretical Analysis of the Thiol-Promoted Fragmentation of 2-Halo-3-tosyl-oxanorbornadienes. *Org. Lett*. **2023**, 25, 7481-7485.
2. Carranza, M.; Carmona, A. T.; Maya, C.; Gil de Montes, E.; Vasco, A. V.; Bernardes, G. J. L.; Moreno-Vargas, A. J. [2.2.1]Heterobicyclic Bromovinyl Sulfones for Thiol-Triggered Strategies in Linker Chemistry: Aza- vs Oxa-Norbornadienic Systems. *Bioconjugate Chem*. **2025**, 36, 2079-2089.

WATER-SOLUBLE LITHIUM AND SILVER TRIS(PYRAZOLYL)METHANESULFONATE: MULTINUCLEAR AND DIFFUSION NMR

Arantxa Forte-Castro,[1] Juana M. Pérez,[1] Ignacio Fernández[1]

[1]Department of Chemistry and Physics, Research Centre CIAIMBITAL, University of Almería, Ctra. Sacramento,s/n, 04120, Almería, Spain

Tridentate scorpionate ligands, particularly tris(pyrazolyl)borates (Tp), are among the most versatile and widely employed platforms in coordination chemistry, owing to their robust facial binding mode and tunable steric and electronic properties. The development of the isoelectronic analogue tris(pyrazolyl)methanesulfonate (Tpms) further expands this ligand family by introducing a sulfonate functionality that enhances solubility in polar media, improves hydrolytic stability, and provides an additional anionic donor site. As a result, Tpms ligands offer increased coordination flexibility and enable access to structural motifs and reactivity patterns not readily attainable with classical scorpionates.

We report herein the synthesis and characterization of novel lithium Tpms derivatives and their corresponding silver(I) complexes. X-ray crystallography reveals that these species adopt complex coordination modes in the solid state, often forming organometallic polymers, whereas their behavior in solution differs significantly. To elucidate these differences, a comprehensive nuclear nagnetic resonance (NMR) study was conducted. Routine multinuclear ^{1}H, ^{13}C, and 2D ^{1}H-^{15}N NMR spectroscopy confirmed the tripodal framework, while ^{33}S NMR was employed to probe the coordination of the sulfonate group in the lithiated species. Crucially, ^{1}H and ^{7}Li pulsed-gradient spin- echo (PGSE) diffusion measurements were used to determine the aggregation state in polar media. These diffusion studies reveal the formation of monomeric solvent-separated ion pairs (SSIP), in sharp contrast to the polymeric structures observed in the solid state. Overall, this multimodal NMR approach proves indispensable for elucidating the dynamic solution behavior of these highly flexible systems.

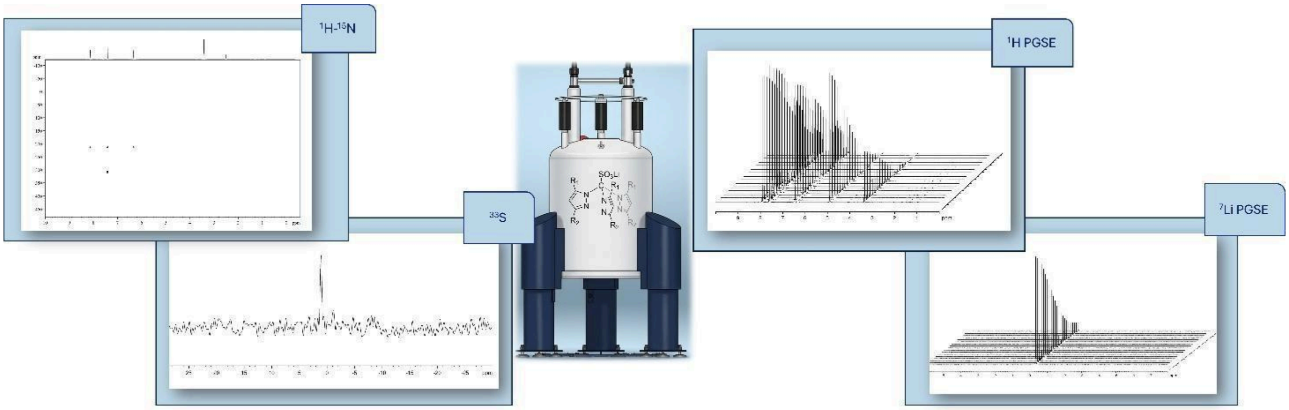


Figure 1. Multinuclear NMR and diffusion analysis of LiTpms: ^{1}H–^{15}N, ^{33}S, and ^{1}H/^{7}Li PGSE measurements.

CONCENTRATION-INDEPENDENT MOLECULAR WEIGHT DETERMINATION OF POLYMERS VIA DIFFUSION NMR: A UNIVERSAL APPROACH ACROSS SOLVENTS[1]

M. González-Lázaro[1], F.M. Arrabal-Campos[2], Juana M. Pérez[1], I. Fernandez[1]

[1]Department of Chemistry and Physics, Research Centre CIAIMBITAL, Universidad de Almería, Carretera Sacramento s/n, 04120 Almería, Spain
[2]Department of Engineering, Escuela Superior de Ingeniería, Universidad de Almería, Carretera Sacramento s/n, 04120 Almería, Spain

Diffusion NMR has established itself as a non-invasive cornerstone for estimating the molecular weight of polymers through scaling relationships based on viscosity-corrected diffusion (Dη).[1] However, the practical application of this technique is often hindered by the "dilution limit"; whereby traditional models typically require measurements at near-infinite dilution to eliminate biases arising from solvent-specific interactions and concentration-dependent viscosity. This necessity not only introduces significant weighing errors at low concentrations but also leads to long acquisition times.

In this work, we present a transformative approach is presented that overcomes these limitations through a self-consistent, iterative scaling framework. Unlike conventional methods, the proposed model explicitly accounts for crowding effects and hydrodynamic interactions by employing a Universal Calibration Curve (UCC) that directly related the diffusion decay coefficient κ to the weight-averaged molecular weight (Mw).[2] By implementing a robust non-linear fitting algorithm that simultaneously optimizes scaling parameters such as a, b, m, n, and ν, together with Mw, the method enables precise determinations across a broad concentration range from 1.5 mg/mL up to 150 mg/mL without the need for physical or mathematical extrapolation to infinite dilution, and independently of the solvent used. The framework was validated using diverse polymer-solvent systems including polystyrene and polypropylene glycol materials in a variety of organic media, demonstrating remarkable accuracy with experimental errors consistently below 3% at a 95% confidence level. By relaxing dilution requirements, the proposed method significantly accelerates data throughput and minimizes solvent-induced bias, making it fully compatible with standard NMR workflows in where experimental time is not a limitation. This approach paves the way for advanced applications in automated quality control, high-throughput screening, and real-time in-line monitoring. Furthermore, this is a flexible method that allows future integration of polydispersity analysis, temperature- dependent dynamics, and the deconvolution of complex polymer mixtures.

1. 1. F. M. Arrabal-Campos, P. Oña-Burgos, I. Fernández, *Polym. Chem*. **2016**, 7, 4326–4329.
2. F. M. Arrabal-Campos, M. González-Lázaro, J. M. Pérez, J. A. Martínez Lao, I. Fernández, *Eur. Polym. J.* **2025**, 226, 113710.

BIOCHAR EFFECTS ON DISSOLVED ORGANIC CARBON ASSESSED BY ^{1}H-NMR IN ANDOSOL AND ALLUVIAL SINGLE-SOIL COLUMNS

Álvaro F. Garcia-Rodriguez[1], Lorena Goretti[2], Heike Knicker[1], Nobuhide Fujitake[2]

[1]Group of Interactions Between Soils, Plants and Microorganisms, Instituto de la Grasa, (IG-CSIC), 41013
Sevilla, Spain
[2]Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe, Hyogo, 657-8501, Japan
*email: alvaro.garcia@csic.es

Abstract: Climate change is increasing the frequency and intensity of extreme rainfall events, enhancing carbon

(C) losses from soils through dissolved organic carbon (DOC) leaching. In Japan, where heavy rainfall events are frequent, understanding how such events alter soil organic carbon (SOC) dynamics and DOC composition is particularly important. Biochar (BC), a carbon-rich byproduct of biomass pyrolysis, is widely applied as a soil amendment due to its potential to improve soil fertility, increase water retention, and promote long-term C sequestration. However, field and laboratory studies indicate that BC particles, especially fine fractions, can migrate downward in soil profiles under intense rainfall or irrigation, potentially affecting BC persistence in topsoil and altering subsurface biogeochemical processes, including nutrient transport and groundwater quality.

To elucidate the effects of BC on SOC mobilization and DOC characteristics, we conducted a controlled single soil column experiment for 4 weeks using Andosol and alluvial soil, subjected to high and moderate irrigation frequency based on their holding capacity (WHC), with and without BC amendment. SOC concentrations were measured in soil leachates, and molecular composition was characterized using ^{1}H-NMR spectroscopy to distinguish changes in aromatic, aliphatic, and oxygenated functional groups associated with BC-derived and native SOC fractions. Preliminary results show higher amounts of TOC in alluvial soils compared to andosol. The ^{1}H-NMR spectra from alluvial-BC soil showed a strong decrease in the O-alkyl region (52.1→34.0%) accompanied by increases in aliphatic (17.9→28.2%), carboxyl-associated (25.8→29.8%), and aromatic (4.2→7.9%) fractions from 0 to 4 weeks.

The results provide mechanistic insights into how soil mineralogy regulates the mobility and transformation of BC- derived and native DOC under simulated rainfall conditions.

This study advances understanding of the fate, stability, and environmental implications of BC in contrasting soil environments and supports the development of sustainable BC management strategies under future climatic scenarios.

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STD NMR CHARACTERIZATION OF GLYCOMIMETIC BINDING TO CANCER-ASSOCIATED SIALYLTRANSFERASES REVEALS DIFFERENTIAL RECOGNITION PATTERNS

Adelyn M. Betances-Mora[1], Pedro Miguel Ascenso Vieira[2], Giacomo Biagiotti[2] , María Bort[3], Khanh Nguyễn Tấn[3], Ramón Hurtado Guerrero[3], Anne Harduin-Lepers[4], Barbara Richichi[2], Juan C. Muñoz-García[1] and Jesús Angulo[1]

[1]*Instituto de Investigaciones Químicas (CSIC-Universidad de Sevilla), 41092 Seville,* [2]*University of Florence, Department of Chemistry "Ugo Schiff", 50019 Florence,* [3]*University of Zaragoza, BIFI, 50018 Zaragoza,* [4]*Univ. Lille, CNRS, UMR 8576 - Structural & Functional Glycobiology Unit (UGSF) - Sciences & Technologies Faculty, 59655 France.*

E-mail: adelynm.betances@iiq.csic.es

Sialyltransferases catalyze the transfer of sialic acid to cell-surface glycoconjugates, a process often altered in cancer. ST6Gal I is frequently overexpressed and linked to metastasis and immune evasion, while ST3Gal IV has a more context-dependent role in tumor-associated glycosylation [1]. Understanding how these enzymes interact with synthetic inhibitors at the atomic level is crucial for developing new therapeutics.

STD NMR [2] experiments were used to study the interaction of a synthetic glycomimetic ligand with both enzymes. For ST6Gal I, results indicated very weak binding, although epitope mapping identifying the key interacting regions of the ligand could be obtained. In contrast, ST3Gal IV showed relatively higher affinity (still low overall) and stronger STD signals, with similar binding regions but a distinct orientation of the sugar moiety and enhanced interaction.

Computational models were validated against STD NMR data using our software RedMat [3], supporting the proposed binding modes and providing further insights into ligand–enzyme interactions.

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SOLID-STATE ^{13}C NMR ANALYSIS OF SHORT-TERM ORGANIC CARBON TRANSFORMATIONS IN HYDROCHAR-AMENDED SOILS

Francisco J. Moreno-Racero[1], Holger Wissel[2], Otávio dos Anjos Leal[2], Nicolas Brüggemann[2] and Heike Knicker[1]

[1]Group of Interactions Between Soils, Plants and Microorganisms, Department of Food Biotechnology, Instituto de la Grasa (CSIC), Building 46, UPO Campus, Ctra. de Utrera km 1, 41013 Seville, Spain.
[2]Institute of Bio- and Geosciences, Agrosphere (IGB-3), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Straße, 52428 Jülich, Germany.

Email: fjmoreno@ig.csic.es

Keywords: Carbon source partitioning, Soil respiration, Carbon mineralization, solid-state ^{13}C NMR

The application of carbon-rich organic amendments such as hydrochar (HC) has been widely reported to stimulate soil microbial respiration, promoting the mineralization of both HC-derived carbon (C) and native soil organic C (SOC). However, the relative contribution of these C sources to soil CO_2 emissions remains poorly understood, complicating the interpretation of short-term C dynamics in HC-amended soils.

Therefore, we conducted a controlled incubation experiment with Cambisols amended with different doses of chicken manure–derived HC and under contrasting moisture regimes and assessed soil respiration rates and the isotopic composition ($\delta^{13}C$) of evolved CO_2. We used a GasBench interface coupled to an isotope ratio mass spectrometer (IRMS). The HC was applied in doses equivalent to 0 (control), 3.25, 6.5, 13 and 26 t ha^{-1}. The soil and HC proportions were scaled to represent the top 2 cm of soil, and incubated for 45 days in 12 mL glass vials at 24 °C under well-irrigated (60% water holding capacity, WHC) and water-deficit (30% WHC) conditions. This approach enabled the calculation of the partitioning of respired CO_2 into HC- and native SOC-derived C by subjecting the measured isotopic signature to a mixing model. It revealed that HC dose and soil water availability exerted a strong influence on the preferential mineralization of these C sources during incubation.

Building on these findings, solid-state ^{13}C CPMAS nuclear magnetic resonance (NMR) spectroscopy was applied to investigate how short-term incubation and associated C losses are reflected in the chemical composition of soil organic matter. Soils were analyzed immediately after HC application and again after incubation.

Linking the NMR data with the $\delta^{13}C$ signature of evolved CO_2 allowed to identify which organic C groups are preferentially affected during short-term mineralization processes. This combined approach enabled to assess changes in SOC composition at molecular-level and thereby to improve the interpretation of HC–SOC interactions during early stages following soil amendment.

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DECIPHERING PROTEIN POST-TRANSLATIONAL MODIFICATIONS BY EXPERIMENTAL AND COMPUTATIONAL APPROACHES

Joaquín Tamargo-Azpilicueta[1], Rafael L. Giner-Arroyo[1], Alejandra Guerra-Castellano[2], Tobechukwu C. Ezike[2], Dariia Pavlenko[3], José L. Olloqui-Sariego[4], Adrián Velázquez-Campoy[5], Eyal Arbely[3], Miguel A. De la Rosa[1] and Irene Díaz-Moreno[1]

[1]Institute for Chemical Research (IIQ), Scientific Research Centre "Isla de la Cartuja" (cicCartuja), University of Seville and CSIC, Sevilla, Spain
[2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria
[3]Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[4]Department of Physical-Chemistry, University of Seville, Seville, Spain
[5]Institute for Biocomputation and Physic of Complex Systems (BIFI), Joint Unit GBsC-CSIC-BIFI, University of Zaragoza, Zaragoza, Spain

Post-translational modifications (PTMs) fine-tune protein function and drive cell fate by modulating protein folding, localization, and protein-protein interactions. Among these, Tyr phosphorylation and Lys acetylation stand out as the most prevalent PTMs identified *in vivo*. However, their structural and dynamic effects are often overlooked due to the difficulty of obtaining site-specifically modified variants *in vitro*.

In this talk, we present an integrated experimental and computational framework that can overcome these limitations. To do this, we highlight genetic code expansion method as a powerful tool to generate site-specific modified variants, enabling their characterization by NMR and complementary biophysical assays, and we further illustrate how molecular dynamics simulations can complement these experiments.

Using this strategy, we show how Tyr48 phosphorylation of cytochrome *c* (C*c*) reshapes its dynamic behaviour modulating its function across different cellular compartments: as a central player in mitochondrial metabolism, as a trigger of apoptosis in the cytoplasm and as an inhibitor of histone chaperones following its DNA-damage induced translocation to the nucleus [1]. Moreover, we demonstrate that Lys115 acetylation of pyruvate kinase type M2 (PKM2) – a rate-limiting glycolytic enzyme with a central role in tumour metabolism and growth– alters concerted motions within the protein, ultimately impairing substrate catalysis [2].

Altogether, our results on these two different biological systems establish a robust platform that combines experimental and computational approaches to decipher the structural and functional consequences of post-translational modifications in proteins.

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DECIPHERING THE IMMUNORAGULATORY AXIS OF GALECTIN-9 AND TIM-3

Mirane Florencio-Zabaleta,[1] Sandra Delgado,[1] María Jesús Moure,[1] Mikel Azkargorta,[1] Paul J Hensbergen,[2] Marcos Gómez-Redondo,[1] Manfred Wuhrer,[2] Gonzalo Jiménez-Osés,[1,3] Jesús Jiménez-Barbero[1,3,4] and Ana Ardá[1,3]

[1]CIC bioGUNE, Bizkaia Technology Park, Building 800, 48160 Derio, Bizkaia, Spain.
[2]Center for Proteomics and Metabolomics, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands.
[3]Ikerbasque, Basque Foundation for Science, Maria Diaz de Haro 3, 48013 Bilbao, Bizkaia, Spain
[4]Department of Organic Chemistry II Faculty of Science and Technology University of the Basque Country, EHU-UPV Leioa Spain.

Galectin-9 is a tandem-repeat galectin strongly implicated in essential biological processes such as cell growth, differentiation, communication, and death that has emerged as a relevant immunomodulatory biomarker in diverse pathologies.[1,2] Despite its recognized importance, the molecular mechanisms governing its carbohydrate-mediated functions remain unknown. TIM-3 has been identified Galectin-9 ligand and described as a key immune checkpoint, driving tumor immune escape.[3]

We hypothesize that specific glycan motifs on TIM-3, particularly polylactosamine (PLNA) structures, are responsible for high-affinity and selective recognition by Galectin-9, thereby modulating their functional interplay in immune regulation.[4] Our aim is to structurally elucidate the glycan-lectin recognition interface between Galectin-9 and TIM-3.

A multidisciplinary strategy was carried out. For TIM-3, our recently established NMR methodology[5] combined with MS based glycoproteomics, was used to identify the glycan structures present, with particular attention to the presence and of PLNA chains. To understand the selectivity of Galectin-9 towards glycan epitopes, binding experiments towards a with a panel of oligosaccharides, including LacNAc, A- and B-antigen type-2 tetrasaccharides,[6] 3-sialyl-LacNAc, and triLacNAc[7] were performed. The full NMR backbone assignment of both carbohydrate recognition domains (CRDs) of human Galectin-9 was completed, enabling ^{1}H,^{15}N-HSQC-NMR based experiments, which afford site-specific information by defining the protein residues implicated in oligosaccharide binding. Additionally, ligand-based NMR experiments (^{1}H-STD, ^{1}H,^{13}C-STD-HSQC) were employed, together with Isothermal titration calorimetry (ITC) experiments to obtain the thermodynamic fingerprint of these binding events, and molecular dynamic (MD) simulations to generate 3D models and dissect key stabilizing interactions.

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EXPLORING CARBOHYDRATE RECOGNITION IN GALECTINS BY ^{19}F-NMR SCREENING, FLUORESCENCE BINDING STUDIES AND STRUCTURAL ANALYSIS

Alonso Martínez, F.J.[1], Medrano Martín, F.J.[1], Delgado, S.[2], Ardá, A.[2,3], Jiménez Barbero, J.[2,3,4], Cañada Vicinay, F.J.[1,4]

[1]Margarita Salas Biological Research Center (CIB-CSIC), Ramiro de Maeztu, 9, 28040 Madrid, Spain
[2]CICbioGUNE, Bizkaia Technology Park, 48162 Derio, Bizkaia, Spain
[3]Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Spain
[4]CIBER de Enfermedades Respiratorias (CIBERES), 28029 Madrid, Spain.

Galectins are involved in multiple glycan-mediated biological processes. Despite their different structural organizations (prototypical, chimera-type and tandem-repeat) their CRDs are generally considered highly conserved and selective for β-galactoside ligands.[1]

Here we investigate carbohydrate recognition across several galectins using fluorinated monosaccharides as NMR probes. A library of 26 monofluorinated derivatives of glucose, galactose, mannose and fucose was screened against Galectin-3, Galectin-1, Galectin-7, Galectin-8 and GRIFIN using ^{19}F-NMR transverse relaxation (T_2) experiments,[2] enabling direct detection of binding events and comparison of recognition profiles.

A highly conserved pattern was observed, with clear interactions for 2F-β-galactose and 2F-β-fucose. Notably, significant binding was also detected for 3F-β-mannose, revealing an unexpected tolerance of the CRD towards mannose-derived ligands upon OH→F substitution.

Dissociation constants determined by fluorescence spectroscopy for selected systems involving Galectin-3, Galectin-1 and GRIFIN show that fluorinated monosaccharides generally display increased affinity relative to their native counterparts, suggesting that fluorination modulates carbohydrate recognition through subtle electronic and solvation effects. X-ray crystal structures of Galectin-3 complexes further reveal that while 2F-galactose adopts the canonical binding mode, 3F-mannose binds in an alternative orientation that reproduces key hydrogen-bonding interactions typical of galactose recognition.

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NMR-BASED METABOLOMICS AS A TOOL FOR MONITORING VERNALIZATION IN STRAWBERRY CROPS

Andrea Fernández-Veloso[1,2], Jaime Hiniesta-Valero[1], Alejandra Guerra-Castellano[1], Miguel A. De la Rosa[1], Irene Díaz-Moreno[1]

[2]Instituto de Investigaciones Químicas (IIQ), Centro de Investigaciones Científicas Isla de la Cartuja (cicCartuja), Universidad de Sevilla, Consejo Superior de Investigaciones Científicas (CSIC), Spain
[2]ManSciTech S.L., Spain

Strawberry cultivation is an economic activity that holds significant value in Spain, being the world's largest exporter of strawberries, and specifically, in Huelva, that accounts for 87% of the country's total production. Strawberry crops in Spain are characterized for their distinct method for cultivating. Plants are grown first in high altitude nurseries in Castilla y León. Afterwards, strawberry runners are transplanted to production fields in Huelva to flower and bear fruit. This procedure is followed to ensure that strawberry plants undergo vernalization, a physiological process in which dormant plants require a period of cold temperatures—under 10 ºC—to promote flowering and ensure successful fruiting.

Even though vernalization is a process of great interest from an economic point of view, given its close relationship with flowering and fruiting, the mechanisms driving it remain mostly unknown. In this talk, the study of the metabolomic processes that strawberry plants undergo through vernalization will be addressed. To do so, the employed combination of NMR-based metabolomic analysis with computational tools for spectra preprocessing, holistic analysis of metabolomic profile, and metabolite annotation will be presented [1-3].

Our findings suggest that NMR is a useful tool to monitor the main variability sources in the metabolomic profile of strawberry plants throughout vernalization. Besides, molecule annotation revealed that sugar mobility, accessibility, and increased photosynthetic activity in late-vernalization plants may support plant viability post-vernalization. Thus, we propose integrating NMR-based metabolomics with computational tools to aid agricultural workers in monitoring crop progress and improving yields.

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INTERFEROGRAM-BASED PURE SHIFT STD NMR

Tomás Martín-Duque[1], Inés Lera-Lasso[1], Gabriel Rocha[3], Ridvan Nepravishta[2], Noelia de la Cruz[3], Eurico J. Cabrita[4], Javier Ramos-Soriano[3], Javier Rojo[3], Jesús Angulo[3], Ángeles Canales[1], Laura Castañar[1]

[1]Department of Organic Chemistry, Faculty of Chemistry, Complutense University of Madrid, Spain
[2]Cancer Research Horizons and Scotland Institute for Cancer Research, United Kingdom
[3]Institute for Chemical Research, CSIC-University of Seville, Spain
[4]Research Unit on Applied Molecular Biosciences, Chemistry Department, NOVA School of Science and Technology, NOVA University Lisbon, Portugal

^{1}H Saturation Transfer Difference (STD) NMR [1] remains as one of the most widely used techniques for studying ligand-receptor interactions and has numerous applications in biochemistry and drug discovery. Within a single experiment, STD provides a comprehensive view of molecular recognition by enabling the identification of binding events (ligand screening), the mapping of ligand binding epitopes, and the extraction of affinity-related information of the bound state. Despite its usefulness, it is intrinsically limited by the low chemical shift dispersion of ^{1}H signals, which often leads to signals overlap and thereby complicates both chemical assignment and accurate quantification of interactions.

Several strategies have been developed to overcome this limitation, including chemical modification of ligands through isotopic labelling [2,3] or conjugation with lanthanide binding tags [4]. Other strategies involve the use of multidimensional NMR techniques, which spread correlations to additional spectral dimensions [5]. More recently, increasing interest within the NMR community has focused on pure shift NMR methods [6], which suppress the effects of homonuclear scalar couplings in the final spectrum, collapsing multiplets into singlets. This enhances spectral resolution, minimizes signal overlap and facilitates the analysis of complex NMR spectra.

Building on our previous work in real-time pure shift STD NMR [7], we herein introduce an interferogram-based pure shift STD NMR approach with the potential to achieve ultrahigh-resolution STD NMR spectra, even in severely overlapped spectral regions. The new method was validated using a model system (naproxen and bovine serum albumin) and further demonstrated in a biologically relevant case of study involving the interaction between a high-mannose carbohydrate ligand and an HIV-related antibody.

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SOLID-STATE NMR CHARACTERIZATION OF AMYLOID SAMPLES DETECTED USING CPMAS

Andrés-Campos S[1], Mompeán García M[1],

[1]Department of Biological Physical Chemistry (QFB), Biosolids NMR Group, Blas Cabrera Institute of Physical Chemistry (IQF-CSIC), Madrid, Spain.

Amyloids have traditionally been associated with neurodegenerative disorders, but evidence has accumulated demonstrating their participation in physiological processes, including native immune mechanisms such as necroptosis, a form of programmed cell death [1]. Receptor Interacting Protein Kinase 3 (RIPK3), a key regulator of this pathway, forms functional amyloid assemblies through a conserved sequence known as the RIP Homotypic Interaction Motif (RHIM) [2]. Interestingly, viruses have evolved proteins to interfere with these processes and unleash cell death [3]. Understanding the structural basis of these processes is essential to advance our knowledge on immune response activation and viral evasion.

We present recent results on the characterization of RIPK3 RHIM amyloid collected at 14.1 T and 37 C using a triple resonance, 3.2 mm CPMAS CryoProbe [4]. Using two standard 3D experiments (NCACX and NCOCX), we obtained backbone assignments and secondary structure information on ~1.5mg of labelled RIPK3. This information can be complemented with 2D ^{13}C–^{13}C correlation spectra using varied mixing times to collect distance restraints and derive structural models that may help defining side chain orientations that are poorly defined in current EM density maps [5,6].

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NOVEL NMR ADVANCED NMR METHODS FOR THE STUDY OF FLUORINATED GLYCANS

Inés Lera-Lasso[a], Tomás Martín-Duque[a], Adrián Silva-Díaz[b], Javier Rojo[b], Javier Ramos-Soriano[b],
Ángeles Canales[a], Laura Castañar[a]

[1] Department of Organic Chemistry, Faculty of Chemistry, Complutense University of Madrid, Spain
[2] Glycosystems Laboratory, Institute of Chemical Research (IIQ), CSIC – University of Seville, Spain

The development of antiviral vaccines is a major global health priority. Some viruses, particularly enveloped viruses such as HIV, evade the immune response by using host-cell glycosylation to mask surface protein epitopes. However, certain antibodies, such as 2G12 isolated from seropositive individuals, can recognize these glycans[1]. 2G12 has also been shown to recognize other viruses[2], making it a promising target for the design of multivalent vaccines.

The epitope recognise by 2G12 is a high-mannose glycan cluster on the HIV gp120 glycoprotein[1]. However, its preference for the different glycan arms remains under discussion, as distinct binding epitopes have been obsserved in crystallographic studies. NMR spectroscopy is a powerfull tool for studying these interaction in solution, but its application to such complex systems is limited by severe signal overlap in ^{1}H NMR spectra. A common strategy to overcome this limitation is to lablel the glycan with ^{19}F, a sparse and highly sensitive nuclide that enables position-specific characterization of dynamic protein-glycan interactions in solution[3].

Before studying these interactions, detailed characterization and spectral assignment of the fluorinated glycans are required. Due to the complexity of their ^{1}H NMR spectra, advanced NMR methods are needed. Here, we explore the use of two recently developed NMR methods designed to simplify spectral complexity: pure shift NMR and FESTA NMR. Pure shift NMR provides ultrahigh-resolution spectra by removing the effect of homo- and heteronuclear scalar coupling from spectra[4], while FESTA provides simplified ^{1}H NMR subspectra for each fluorine-labeled glycan ring[5]. The utility of both methods is demostrated for the analysis of a fluorinated high-mannose glycan analoge of the Man9GlcNAc2 present in gp120.

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UNDERSTANDING NHERF1 AND ICAM1 PROTEIN INTERACTIONS USING SOLUTION NMR

A. Hornero-Romera,[1] M.Á. Treviño-Avellaneda,[1] D. Pantoja-Uceda,[1] J. Millán,[2] M.Á. Jiménez[1*]

[1]Instituto de Química Física Blas Cabrera (IQF), CSIC, Madrid, Spain
[2]Centro de Biología Molecular Severo Ochoa (CBM), CSIC-UAM, Madrid, Spain

Na^+/H^+ exchanger regulator factor (NHERF) is a multidomain and multivalent protein that recruits different signaling partners. It consists of two PDZ domains, followed by a long mostly disordered region and an ezrin binding domain. [1] It is an adaptor protein, densely distributed in the apical membranes of polarized epithelial cells of several mammalian tissues[2], participates in organizing the trafficking, localization, and membrane targeting of a large number of membrane receptors and channels to which NHERF binds.[2] NHERF is responsible for bridging the interactions between cell membrane and the cytoskeletal actin networks. As a membrane cytoskeleton adapter protein, NHERF enhances the efficiency and specificity of membrane processes by forming multiprotein macromolecular complexes.[3] Specifically, NHERF-1 assembles ezrin-radixin-moesin (ERM) proteins, ICAM-1 (type I transmembrane protein intercellular adhesion molecule) and F-actin into a macromolecule complex in mouse liver. The deletion of NHERF-1 in mice reduces expression of hepatic ERM proteins and ICAM-1 molecules that are up-regulated and are essential for neutrophil migration and infiltration after bile duct ligation (BDL),[4] a surgical procedure used in animal models to study liver diseases. NHERF-1 deficiency also increased synthesis of tetrahydroxylated bile acid, that can suppress bile acid-induced liver damage in the liver. These multiple mechanisms protect NHERF -/- mice against cholestatic liver injury induced by BDL.[4] Pull-down experiments suggested that the PDZ1 and PDZ2 domains of NHERF-1 are involved in the interactions with ICAM-1.[5] To identify the ICAM-1 binding site, constructs of NHERF-1 containing either the PDZ1 or the PDZ2 domain or both were titrated with a peptide corresponding to the cytoplasmic tail of ICAM-1. The absence of changes in the $^{1}H,^{15}N$-HSQC spectra of the PDZ2 construct indicates that this construct on its own shows no interaction with ICAM-1. Whereas chemical shift perturbations (CSP) are observed with PDZ1 construct. And yet more pronounced CSP are observed in the construct containing both domains. These results confirm that ICAM-1 and NHERF-1 interact through PDZ domains, and that both domains are involved in this interaction. Based on the CSP the ICAM-1 binding site was identified. This observation contributes to understand the molecular mechanisms of cholestatic liver injury, which remain largely undefined. It also raises new questions about whether this interaction could be used as a target for new drugs to regulate the inflammatory response that occurs in cholestasis and other hepatic inflammatory diseases.

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NMR CHARACTERIZATION OF THE FGF1–FGFR D2 INTERACTION: EFFECTS OF N-GLYCOSYLATION

Paola Oquist-Phillips[1], Sean A Burnap[2,3], Iker Oyenarte[4], June Ereño Orbea[4],Jesús Jiménez-Barbero[5], Weston B Struwe[2,3], Ángeles Canales[a]

[1]Department of Organic Chemistry, Complutense University of Madrid, Madrid, Spain.
[2]The Kavli Institute for Nanoscience Discovery, Dorothy Crowfoot Hodgkin Building, University of Oxford, Oxford OX1 3QU, United Kingdom.
[3]Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, United Kingdom.
[4]CICbioGUNE, Basque Research and Technology Alliance (BRTA), Derio, Spain.

The interaction between fibroblast growth factor 1 (FGF1) and the D2 domain of its receptor (FGFR) was investigated by solution-state NMR, with particular emphasis on the role of N-glycosylation.[1] Backbone amide assignments of FGF1 were transferred from the reference dataset (BMRB 6875) and mapped onto the crystallographic numbering scheme to enable residue-specific analysis.

^{1}H–^{15}N TROSY titration experiments revealed residue-specific chemical shift perturbations and signal attenuation consistent with binding in the intermediate exchange regime, defining a well-localised interaction surface on FGF1. Comparison of non-glycosylated and glycosylated D2 constructs indicates that both engage the same binding interface. However, the glycosylated form shows more pronounced perturbations and line broadening, suggesting altered dynamic behaviour. Complementary ^{1}H–^{13}C experiments monitoring glycan resonances were performed during titration.

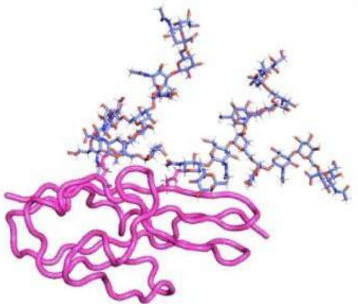

Figure 1. Theoretical structure of glycosylated D2-FGFR

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INVESTIGATING RIPK1/RIPK3 FIBRIL FORMATION THROUGH NMR SPECTROSCOPY AND MOLECULAR DYNAMICS

Harikrishnan Vijayakumary Krishnakumar[1], Miguel Mompean[1]

[1]Instituto de Química Física "Rocasolano", CSIC, Serrano 119, Madrid E-28006, Spain

Receptor-interacting protein kinases RIPK1 and RIPK3 play a crucial role in necroptosis through RHIM-mediated amyloid fibril formation. Understanding the contribution of different regions to fibril assembly remains an important question in elucidating their functional mechanism.

So far, the structures of these homo- and hetero-fibrils have been solved using solid-state NMR along with other biophysical techniques [1,2,3,4,5]. However, the dynamics involved in fibril formation are still not well understood. In this work, we aim to look at the molecular-level mechanisms involved in this process.

In the initial stage of this study, RIPK1 and RIPK3 variants from both mouse and human systems were analyzed to get an overall structural understanding. Secondary structure propensities were estimated from NMR chemical shifts to understand their conformational features in the monomeric state. In addition, NMR relaxation experiments were performed to probe their dynamic behavior [6] .

Currently, molecular dynamics (MD) simulations are being used to study the structural organization and stability of fibrillar assemblies. These simulations aim to explore possible intermolecular interactions in both homomeric and heteromeric systems, complementing the experimental observations.

This combined experimental and computational approach aims to provide a deeper understanding of the molecular determinants governing RIPK1/RIPK3 assembly in necroptotic signaling.

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MULTINUCLEAR AND DIFFUSION NMR REVEAL STRUCTURE–AGGREGATION RELATIONSHIPS IN CHIRAL AMINO ACID-BASED ANTHRAQUINONES

Juana M. Pérez,[1] Ignacio Maldonado-Lorenzo,[1] Jorge Rodriguez-Criado,[2,3] Francisco Quinonero,[2,3,4] Natalia Gutiérrez-Segura,[1]
Mireya E. Lopez-Vargas,[1] Arantxa Forte-Castro,[1] Ana Belén Ruiz-Muelle,[1] Pablo Salcedo-Abraira,[5] Antonio Rodríguez-Diéguez,[5]
Consolación Melguizo,[2,3,4] José Prados,[2,3,4] Ignacio Fernández[1]

[1] Department of Chemistry and Physics, Research Centre CIAIMBITAL, University of Almería, Almería, Spain
[2] Institute of Biopathology and Regenerative Medicine, Center of Biomedical Research, University of Granada, Granada, Spain
[3] Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain
[4] Department of Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada, Spain
[5] Department of Inorganic Chemistry, University of Granada, Granada, Spain

A family of chiral amino acid-based anthraquinones (**3a–f**) and their sodium salts (**4a–f**) was synthesised and characterised by multinuclear $^{1}H/^{13}C/^{15}N$ NMR, with structural assignments supported by two-dimensional heteronuclear experiments. The ^{13}C NMR data revealed a consistent downfield shift of C(9) relative to C(10), indicative of a persistent intramolecular NH···O=C interaction within the anthraquinone framework. Among the series, the phenylalanine derivative **3e** displayed a markedly broadened CO_2H resonance in the ^{1}H NMR spectrum (δ_H 13.84 ppm; $W_{1/2}$ 433.2 Hz), suggesting distinct exchange or association behaviour in solution. This hypothesis was further supported by PGSE diffusion NMR in D_2O (293 K, 60 mM), which showed that **4e** diffuses more slowly than **4f** (D 0.202 vs 0.225 × 10^{-9} m^2 s^{-1}; r_H 10.4 vs 9.3 Å), consistent with enhanced intermolecular association. This behaviour is also in agreement with the π–π stacking interactions observed in the solid state for **3e**. Notably, derivative **4e** exhibited the highest antiproliferative activity (lowest IC_{50}) across the tested tumour cell lines (PANC-1, MCF-7, A549, SF268, HCT116). Overall, these results demonstrate how multinuclear and diffusion NMR provide a coherent framework to correlate structural features with solution behaviour, highlighting aggregation as a key factor potentially underlying the enhanced biological performance of **4e** [1].

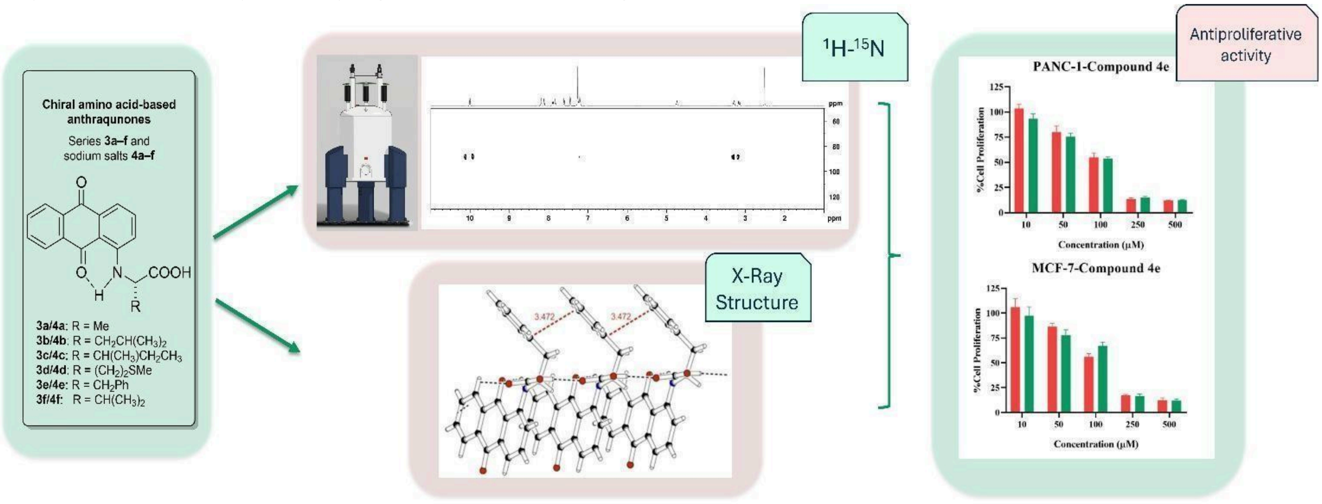

Figure 1. NMR, X-ray and biological features of chiral amino acid-based anthraquinones, highlighting the distinctive behaviour of the phenylalanine derivative.

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MOLECULAR INSIGHTS INTO BIOLOGICALLY RELEVANT CD44–HYALURONAN RECOGNITION BY NMR

María Payá-García[1], Sandra Delgado[1], Mikel Azkargorta[1], Felix Elortza[1], Jesús Jiménez-Barbero[1,2] and Ana Gimeno[1,2]

[1]CIC bioGUNE, Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Derio, Bizkaia, Spain
[2]Ikerbasque, Basque Foundation for Science, Bilbao, Bizkaia, Spain

Glycosylation is one of the most common post-translational modifications of proteins and plays a pivotal role in their folding, stability, and molecular recognition processes. Interestingly, aberrant protein glycosylation, a hallmark of cancer, reshapes receptor-ligand interactions, modulating cell adhesion, growth-factor signaling, immune evasion, and metastatic behavior. These tumor-specific glycoprotein alterations represent unique opportunities for biomarker discovery and development of targeted therapies.

Within this biological framework, the cell-surface receptor CD44 is a transmembrane glycoprotein whose overexpression is widely recognized as a marker of cancer stem cells (CSCs) across multiple tumor types, including breast, colon, lung, prostate, and head and neck squamous cell carcinoma (HNSCC). This glycoprotein acts as a principal binding partner for hyaluronan (HA), as well as a hub for interactions with several extracellular receptors, including osteopontin, selectins, galectins, siglecs, and growth factors. At the molecular level, all variant isoforms of CD44 are highly glycosylated, containing both *N*- and *O*-glycosylation sites within their ectodomain, while only variant 3 (CD44v3) presents a consensus GAG attachment site capable of bearing heparan sulfate (HS) and/or chondroitin sulfate (CS) chains. Through their common hyaluronan binding domain (HABD) all CD44 isoforms recognize HA, thereby regulating cell adhesion, migration, proliferation, and certain signaling pathways that contribute to tumor progression. Notably, this interaction is greatly influenced by CD44 glycosylation profile. Therefore, the CD44-HA crosstalk appears as an interesting target for study to obtain information on how changes in the glycoproteome of cancerous cells contribute to the progression of the disease.

Herein, the glycosylation profile of CD44v3 has been elucidated employing NMR as a central tool. Metabolic ^{13}C-labeling enabled multidimensional NMR analysis of the glycoprotein, revealing a highly heterogeneous and complex glycan composition. These findings were further complemented by mass spectrometry, lectin blotting, and flow cytometry (FACS). Building on this characterization, NMR-based binding studies were then performed to investigate the molecular recognition of hyaluronan by glycosylated CD44, focusing on the role of glycosylation in modulating this interaction.

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ENHANCING LIGAND EPITOPE MAPPING ANALYSIS BY VARIABLE TEMPERATURE SATURATION TRANSFER DIFFERENCE NMR (VT-STD NMR)

Gabriel Rocha[1], Juan C. Muñoz-García[1], Jesús Angulo[1]

[1]Institute for Chemical Research (IIQ), CSIC – University of Seville, Seville, Spain

Saturation Transfer Difference Nuclear Magnetic Resonance (STD NMR) spectroscopy is widely used for the atomic level characterization of receptor-ligand interactions in solution, providing ligand epitope maps that reveal structural details of the main contacts governing molecular recognition [1]. This information is key for understanding the structural basis of biological processes, and drug development. Temperature is a critical factor affecting STD NMR experiments, as it exerts a direct effect on the kinetics and thermodynamic constants of complex formation, thereby modulating the observed STD effects [2]. We have carried out a systematic investigation of the impact of temperature on the parameters affecting and derived from STD NMR data. To do so, we have employed two protein-ligands systems presenting different binding kinetics (fast and intermediate exchange, Figure 1). Our findings evidence that, to obtain epitope maps with well discriminated contact information, temperature is a key parameter to be optimized whenever protein stability permits.

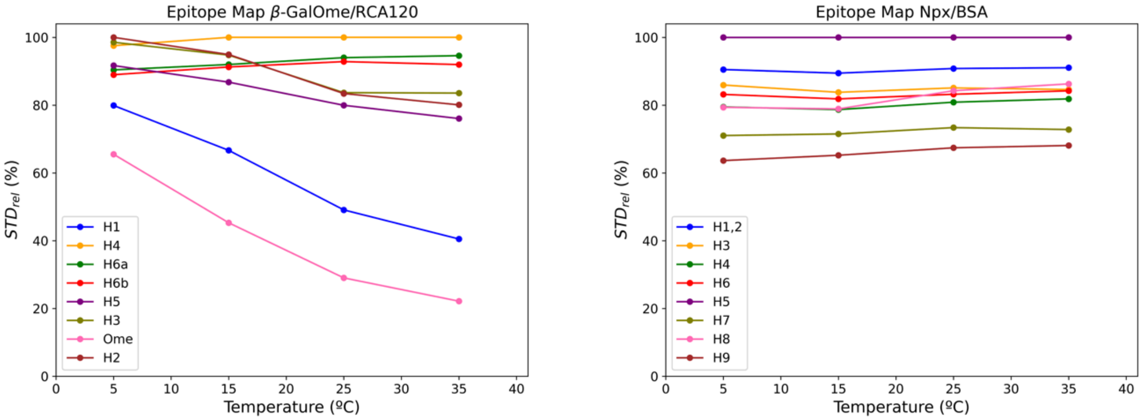

Figure 1. Temperature-dependent evolution of ligand epitope binding mapping for two systems with different kinetics

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UNRAVELLING VIRAL HAEMAGGLUTININ PREFERENCES TO HOST GLYCANS BY CHEMOENZYMATIC SYNTHESIS AND NMR

Cristina Fernández Pérez[1], María Moure Garcia[1], Alberto Marina[1], Iris Bermejo Ruíz[1], Geert-Jan Boons[2], Jesús Jiménez-Barbero[1], Robert P. de Vries[2], Luca Unione[1]
[1]Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), 48160 Derio, Bizkaia, Spain.
[2]Division of Chemical Biology and Drug Discovery Utrecht Institute for Pharmaceutical Sciences Utrecht University, 3584 CG Utrecht, The Netherlands.

The continuous evolution of human influenza A/H3N2 viruses underscores the need to elucidate how the haemagglutinin (HA) protein maintains glycan receptor binding while escaping host immunity [1]. In recent H3N2 variants, such as SG16, HA-mediated entry depends on recognition of α2,6-linked sialylated glycans presented on extended polyLacNAc chains [2]. However, in contemporary strains, including currently circulating variants, substitutions Y159N/T160I and Y195F are highly conserved and have been proposed to modulate HA flexibility and preorganization [3]. Here, we investigate whether and how these mutations also influence HA glycan-binding preferences.

To this end, we combined chemoenzymatic glycan synthesis with ligand-based NMR techniques to obtain atomic-level insights into receptor adaptation in SG16-lineage H3N2 variants, including the circulating A/Darwin/9/21 strain. We synthesized a library of human-type sialylated glycans, including sialyl-LacNAc, sialyl-diLacNAc, sialyl-triLacNAc, and a fluorinated probe (SLNF2), and used these to characterize receptor binding by SG16 HA variants.

Epitope mapping analysis revealed that the Y195F mutant retains the extended binding mode observed in SG16, engaging the full triLacNAc chain for efficient receptor interaction [4]. In contrast, the Y159N/T160I mutant and the Darwin variant preferentially recognize the terminal Neu5Acα2,6Galβ1,4GlcNAc motif within extended glycans. ^{1}H STD and ^{19}F R_2 NMR competition experiments enabled estimation of relative binding affinities.

The Y195F mutant showed efficient binding exclusively to extended sialyl-triLacNAc structures, whereas the Y159N/T160I substitutions resulted in a marked reduction in binding affinity across all tested glycans, irrespective of chain length. Notably, the Darwin strain exhibited strong binding to the short sialyl-monoLacNAc trisaccharide, compensating for the loss of interactions with the underlying polyLacNAc chain.

Overall, our findings demonstrate epistatic interactions between key residues within the HA binding site that synergistically reshape receptor specificity while maintaining immune escape. These NMR-based insights into receptor engagement at atomic resolution are consistent with recent glycan array and molecular dynamics studies by Liang et al. [3], and contribute to understanding the evolutionary trajectory of contemporary A/H3N2 viruses.

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PCNA: ONE RING TO BIND THEM

Antonio Ruiz-Albor[1], Belén Chaves-Arquero[2], Mariola Ferreras-Gutiérrez[2], Miriam Barbera[2], Francisco J. Blanco[1,3]

[1]Instituto de Biomedicina de Valencia (IBV), CSIC, Valencia 46010, Spain
[2]Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, Madrid 28040, Spain
[3]Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to IBV, Valencia 46012, Spain

Murine embryos die early when the Proliferating Cell Nuclear Antigen (PCNA) gene is knocked down [1], consistent with the high importance of the PCNA protein in the replication of DNA [2]. PCNA has a ring-shape structure embracing the DNA duplex, and interacts with many other proteins that modify the DNA molecule or regulate DNA-related processes [3]. These interactions occur frequently through short linear sequence motifs in disordered regions of the PCNA partners [4]. During my period as PhD student, I have studied some of these interactions through various approaches, from NMR to CryoEM, which I will present as a general overview of my work.

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TYROSINE PHOSPHORYLATION UNFOLDS NUCLEOPHOSMIN AND DISRUPTS ITS INTEGRATION INTO THE NUCLEOLUS

Rafael L. Giner-Arroyo[1], Adrián Velázquez-Campoy[2,3,4], Miguel A. De la Rosa[1], Irene Díaz-Moreno[1,*]

[1]Institute for Chemical Research – cicCartuja, University of Seville – CSIC, Seville, Spain
[2]Institute for Biocomputation and Physics of Complex Systems (BIFI), and Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Zaragoza, Spain
[3]Instituto de Investigación Sanitaria Aragón (IIS Aragón), 50009 Zaragoza, Spain
[4]Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas y Digestivas (CIBERehd), 28029 Madrid, Spain
email: rafael.giner@iiq.csic.es

Nucleophosmin (NPM1) is a multifunctional nucleolar protein essential for ribosome biogenesis, genome stability, and stress responses. Its integration into the nucleolus depends on its oligomerization and multivalent interactions that enable liquid–liquid phase separation (LLPS)[1,2]. Here, we investigate how tyrosine phosphorylation at Tyr17, Tyr29, and Tyr67 located within the interface between monomers at the N-terminal oligomerization domain regulates NPM1 structure and function[3]. Replacing tyrosines with *p*-carboxymethyl-L-phenylalanine (*p*CMF) as phosphomimetic substitutions, we show that phosphorylation at Tyr17 and Tyr67 disrupts key inter-monomer interactions and destabilizes the NPM1 pentameric assembly, which drives an order-to-disorder transition as observed by 2D $^{1}H/^{15}N$ transverse relaxation-optimized spectroscopy nuclear magnetic resonance (TROSY-NMR). This impairs binding to nuclear partners and, consequently, dual phosphorylation at Tyr17 and Tyr67 disturbs both homotypic and heterotypic LLPS, thereby impairing incorporation of NPM1 into the nucleolus. This altered localization of NPM1 serves as a hallmark of p53 activation, driven both by nucleoplasmic NPM1 and by the release of ARF from NPM1-dependent sequestration in the nucleolus[4]. Altogether, our results provide a molecular mechanistic explanation on how phosphorylation-induced structural and dynamic changes drive the release of NPM1 from the nucleolus under genotoxic stress.

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PUSHING THE LIMITS OF LOW-FIELD NMR: IMPROVING DDIF METHOD RESOLUTION TO STUDY BIOCHAR INTERNAL STRUCTURES

José María García de Castro Barragán[1], Etelvino Henrique Novotny[2], Heike Knicker[1]

[1]Instituto de la Grasa (IG-CSIC), Carretera de Utrera, km. 1, 41013, Seville, Spain.
[2]EMBRAPA Solos, Rua Jardim Botânico, 1024, Rio de Janeiro, Brazil.

Biochar Pore Size Distribution (PSD) is essential for understanding and optimizing its use as a sustainable horticultural substrate. However, the characterization of its smallest pores remains analytically challenging. The Low-Field Nuclear Magnetic Resonance (NMR) is a promising non-destructive technique to study porous systems. For that, the Decay Due to Diffusion in Internal Field (DDIF) method, which estimates the PSD from the exponential decay of the NMR signal of water molecules is used.

The DDIF acquisition involves two complementary pulse sequences: one that provides signals encoding the diffusion of water molecules through the porous network, and another reference sequence, that reflects signals from only the spin-lattice relaxation contribution. The subtraction of both signals allows the isolation of the DDIF signal from the spin-lattice relaxation background. The encoding time (T_e) was calibrated independently for each sample, and PSD was subsequently obtained via Inverse Laplace Transformation (ILT) of the resulting decay curves.

The resolution of the standard DDIF is limited to approximately 10 µm, since the signals of large macropores often mask those of smaller ones. To improve the resolution, a novel T_1-filter presaturation step was developed and implemented, selectively suppressing the signal from water in large pores and allowing an optimized T_e. This improvement enhanced the detectable pore size range to below 1 µm, an expansion of approximately one order of magnitude.

In the present work, we applied this technique to estimate how washing with distilled water affected PSD of biochars derived from tomato greens, vineyard pruning residues, and Acacia bushes before and after they were applied as planting substrate for tomato and lettuce cultivation for four weeks in the greenhouse. Before the measurement process, the samples were ground, pressed, and saturated with water in vacuum, ensuring a complete porous filling.

Preliminary results, obtained from the combination of the standard and T_1-filtered DDIF experiments reveal a full PSD range (from <1 µm to 1000 µm). We observed that washing of the biochars increased the relative contribution of the small and medium sized pores, most likely due to the loss of ions during the washing process. On the other hand, plant incubation appears to decrease the contribution of larger pores, likely through clogging them by organic matter and root exudates. These observations still need to be confirmed through a more detailed analysis of the full samples set but the preliminary results are promising.

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RECONSTRUCTING SERUM METABOLOMIC PROFILES FROM URINE NMR DATA: PROOF-CONCEPT FOR INDIRECT CLINICAL PREDICTION

Juan de Dios Marín-Manzano[1], Francisco M. Arrabal-Campos[2], Ignacio Fernández[1]

[1]Department of Chemistry and Physics, Research Centre CIAIMBITAL, University of Almería
[2]Department of Engineering, University of Almería

The clinical evaluation of systemic diseases relies on blood-based biomarkers that reflect liver, renal, and metabolic function, as well as infectious status. Although clinically informative, these measurements require invasive sampling and centralized laboratory infrastructure, limiting their suitability for large-scale or repeated monitoring [1]. Urine represents an attractive non-invasive alternative [2]; however, unlike serum— whose metabolic profile reliably reflects systemic host responses—urine composition is highly influenced by diet and lifestyle [3]. In this proof-of-concept study, we propose a machine learning strategy to infer clinically relevant serum metabolic information by reconstructing serum metabolomic profiles from urinary nuclear magnetic resonance (NMR) data. Regression models achieved an R^2 of 0.244 for serum profile reconstruction. The reconstructed serum representations were subsequently used to predict COVID-19 status and clinically relevant biochemical variables, including creatinine, γ-glutamyl transferase (GGT), and HDL cholesterol, and were compared with models trained on real serum data. Classification models trained on real serum achieved macro-F1 scores ranging from approximately 0.57 to 0.61, whereas models based on reconstructed serum profiles showed slightly lower but comparable performance with macro-F1 metrics in the range of 0.49 to 0.56, with broadly similar ROC-AUC values. An information-theoretic analysis confirmed that the reconstruction channel preserves a substantial fraction of clinically relevant signal: the Clinical Signal Preservation Ratio (CSPR) averaged 70.8% (AUC) and 75.5% (macro-F1), with GGT exceeding 0.83, despite the modest global R^2. The effective channel capacity (3.95 bits) further supports that urinary NMR data encodes sufficient metabolic information for indirect clinical prediction.

These results indicate that a remarkable fraction of clinically relevant metabolic information can be inferred from urine with moderate performance attenuation, supporting the potential of this approach as a non-invasive framework for systemic clinical assessment.

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HISTORICAL LAND USE AND SOIL CARBON DYNAMICS IN A MEDITERRANEAN OLIVE ORCHARD UNDER DIFFERENT AGRICULTURAL MANAGEMENT SCHEMES

Gismero-Rodríguez, L. [1]; Valverde, A. [2]; Brüggemann, N. [3]; Leal, O. A. [3] and Knicker, H.[1]

[1] Instituto de la Grasa – Consejo Superior de Investigaciones Científicas (IG-CSIC), Seville, Spain
[2] Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC)
[3] IBG-3: Agrosphere – Forschungszentrum Jülich (FZJ), Jülich, Germany

Soil loss in olive groves is a widespread problem in Mediterranean climates. This is particularly critical in southern Spain, the world's leading olive oil-producing region. To mitigate erosion between olive trees, traditional tillage has increasingly been replaced by cover cropping. While this practice is known to protect the soil structural protection, its effect on soil organic matter (SOM) and carbon dynamics remains insufficiently understood. This study aimed to investigate how long-term management practices influence the degradation capacity of SOM.

Soil samples were collected from an olive orchard under three management regimes established since 2009: conventional tillage (CT), natural cover (MC), and herbicide-based (TL-Herb). Given the sloping nature of the field, soils were sampled from both the upper and lower positions along the slope. A 40-day microcosm incubation experiment was conducted using these soils, both alone and amended with wheat residues (labelled and unlabelled), to investigate carbon turnover and organic matter dynamics.

Preliminary results based on CO_2 flux and $\delta^{13}C$-CO_2 measurements during incubation indicate higher respiration rates in MC soils and in samples from the lower part of the slope. These differences became less pronounced over time, likely reflecting the depletion of more labile, pristine organic matter. Additionally, lower $\delta^{13}C$-CO_2 values in MC treatments suggests a greater contribution of native SOC to microbial respiration.

To further interpret these patterns, mixed-effects model will be applied to quantify fast- and slow-cycling carbon pools and the priming effects across treatments. In parallel, solid-state ^{13}C CP/MAS NMR spectroscopy will be used to evaluate the impact of long-term management on SOM composition. Together, these approaches will provide insights into the role of cover cropping in shaping SOM dynamics and support the development of sustainable soil management strategies and robust soil health indicators.

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NMR INSIGHTS INTO THE MOLECULAR RECOGNITION OF BLOOD GROUP ANTIGENS BY HUMAN GALECTIN-2

Alejandra Travecedo,[1] Montana Manselle,[3] Alejandro Cagnoni,[3] Sandra Delgado,[1] Karina V Mariño,[3] Ana Ardá,[1]
Gabriel Rabinovich,[3] Jesús Jiménez-Barbero,[1,2] Ana Gimeno[1,2]

[1]CIC bioGUNE, BRTA, Bizkaia Technology Park, 48160, Derio, Spain
[2]Ikerbasque, Basque Foundation for Science, Euskadi Plaza 5, 48009 Bilbao, Bizkaia, Spain
[3]Laboratorio de Glicomedicina, Instituto de Biología y Medicina Experimental (IBYME), CONICET, Buenos Aires, Argentina; Laboratorio de Glicómica Funcional y Molecular, (IBYME)
E-mail: mtravecedo@cicbiogune.es

Galectins are a large family of β-galactoside-binding proteins that play critical roles in various physiological and pathological processes.[1] They display striking functional diversity across biological contexts. For instance, Galectin-1 and Galectin-2 exert divergent biological functions, despite sharing the highest homology among this family (amino acid identity 44%).[1] On one hand, Galectin-1 has anti-inflammatory and immune-suppressive properties, and treatments with this protein attenuates atherosclerotic plaque formation.[2,3] On the other hand, Galectin-2 has a pro-inflammatory effect and its inhibition with antibodies has been shown to reduce inflammation and slow the progression of atherosclerosis.[4,5] While Galectin-1 has been extensively studied [6], Galectin-2 remains underexplored, limiting the assessment of its therapeutic potential.

This study aims to address this gap by investigating the molecular basis of Galectin-2 recognition of blood group antigens, which are canonical ligands for galectins. Using a combination of STD-NMR, isothermal titration calorimetry (ITC), and molecular dynamics simulations, we are in the path to characterize Galectin-2's molecular recognition of these ligands at the atomic level. The obtained results strongly suggest that the recognition of blood group antigens by Galectin-2 shares common features with other galectins, where the central β-Gal moiety serves as key epitope for ligand binding. Moreover, additional decorations in the common β-Gal scaffold of the blood group antigens, such as α-Gal, α-GalNAc and α-L-Fuc, enhance the binding affinity. Eventually, these investigations will provide critical insights for the rational design of novel therapeutic strategies targeting Galectin-2.

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TEMPERATURE-DEPENDENT HAPTOTROPIC PHENOMENA IN DIGOLD(I) ACETYLIDE COMPLEXES

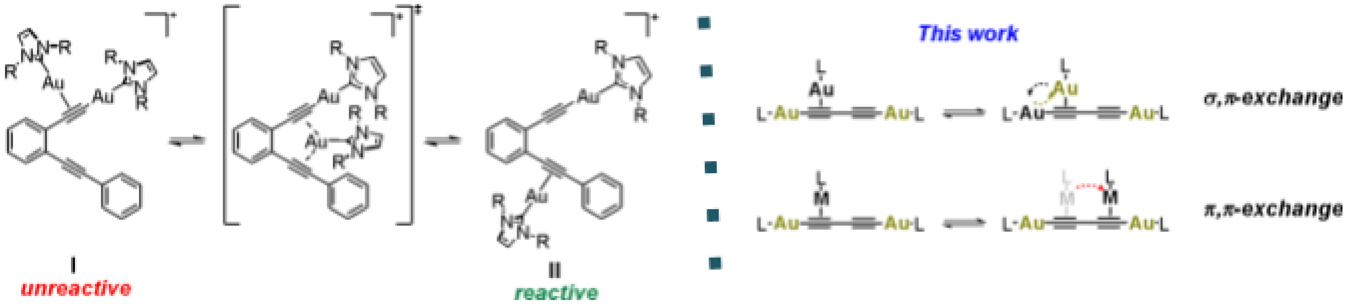
Ignacio Nieto-Vargas[1], Juan Cayuela-Castillo[1], Francisco J. Fernández-de-Córdova[1], Israel Fernández*[2], Pablo Ríos*[1].

[1]Instituto de Investigaciones Químicas (IIQ), Departamento de Química Inorgánica, Centro de Innovación en Química Avanzada (ORFEO-CINQA), CSIC and Universidad de Sevilla, 41092 Sevilla, Spain.
[2]Departamento de Química Orgánica I and Centro de Innovación en Química Avanzada (ORFEO-CINQA), Facultad de Químicas, Universidad Complutense de Madrid, Madrid 28040, Spain
e-mail: inieto@us.es

Keywords: gold acetylides, VT-NMR, dynamic exchange, EDA analysis, trimetallic complexes.

Over the last decades, the chemistry of gold(I) acetylide complexes has experienced a paradigm shift, transitioning from a focus on C–C activation (typically centered on a single Au atom) to new reactivity pathways, including dinuclear gold cooperativity.[1] In the context of these latter species, some *σ,π*-digold acetylides have been proposed as key reactive intermediates in catalysis, including the cycloisomerization of 1,5-allenynes.[2] Furthermore, there are examples in the literature where the fluxional behavior of the gold fragments seems to be crucial to understand some of these transformations.[1,3] Nonetheless, very limited experimental evidence has been published so far on this matter, and hence justifies a deeper study of these thermodynamic equilibria, with the goal of increasing our understanding of the factors governing Au(I)-mediated reactions.

In this regard, NMR spectroscopy plays a key role in exploring such dynamic phenomena. Using variable-temperature experiments (VT-NMR) on a series of gold-based multimetallic platforms, we were able to not only detect these fluxional exchanges but also to obtain crucial thermodynamic information.[4] On this account, we report a, to the best of our knowledge, new *π,π* metallotropic shifts in the cationic gold and copper units of the presented complexes. These findings are supported by additional low-temperature NOESY experiments, DFT calculations and Energy Decomposition Analysis (EDA), showcasing the potential of NMR spectroscopy techniques in this area.



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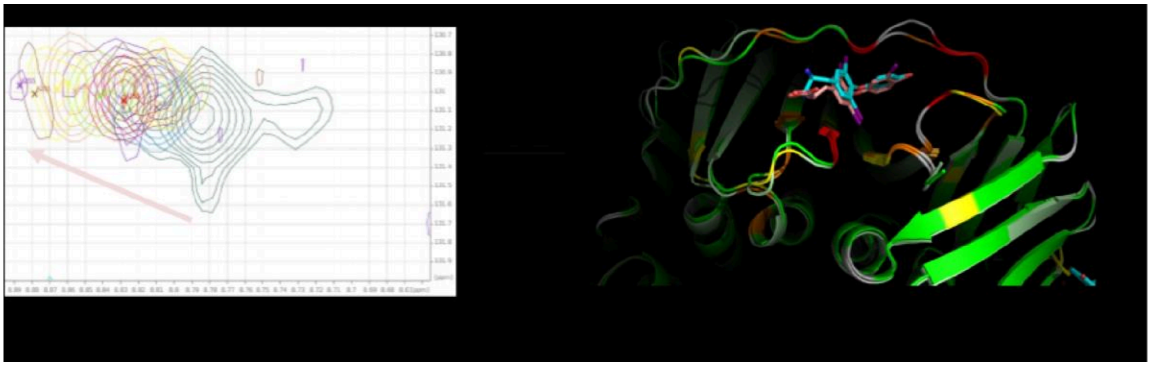
NMR ANALYSIS OF SMALL MOLECULAR INHIBITORS BINDING TO HUMAN PROLIFERATING CELL NUCLEAR ANTIGEN

Gurudutt Dubey[1,2], Alain Ibáñez de Opakua,[3] Francisco J. Blanco[1,2]

[1]Instituto de Biomedicina de Valencia (IBV), CSIC, Valencia 46010, Spain
[2]Centro de Investigación Príncipe Felipe, Unidad Asociada a IBV, Valencia 46012, Spain
[3]CIC bioGUNE, Derio 48160, Spain

Proliferating cell nuclear antigen (PCNA) is a central regulator of DNA replication, repair, and cellular proliferation, and plays a significant role in cancer development and progression, making it an important biomarker and therapeutic target [1]. Several small-molecule inhibitors of PCNA have been identified, among which T3, T2AA, I1, AOH1996 and AOH1160 have advanced to clinical trials, highlighting its potential in cancer therapy [2]. Although the cocrystallized structures of PCNA with some of these small molecules are already reported, exploring the binding mode and effect of these molecules on the structural integrity of PCNA can help advancing the drug discovery process of PCNA inhibitors. To provide more insights to the structural changes and residue level details on the binding site, TROSY experiments were conducted on the small molecule inhibitors of PCNA, namely, T3 and T2AA. Chemical shift perturbation (CSP) analysis of PCNA interacting with T3, revealed that T3 binds to the PIP-box, evident by large shifts in the signals of PIP-box residues especially A252, T250, L235 and K125. CSP analysis and titrating PCNA with T2AA, an analogue of T3, revealed that it also binds to PIP-box. T2AA interacts with weak H-bonding with E109 and S183 shows C–H•••π interaction with T2AA, supporting the binding of second molecule of T2AA at the interprotomer junction of PCNA, as reported in the crystal structure. Additionally, TROSY experiments performed to analyze the effect of DMSO solvent on PCNA by titrating DMSO from 2% to 14% suggested that DMSO binds to several sites on PCNA with very low affinity. Small structural changes start occurring at 2% . Partial denaturation of PCNA occurs at 6% DMSO, which is evident from the intensity reductions of the dispersed backbone amido signals.



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NMR STUDY OF SELF-ASSEMBLED DIRUTHENIUM AGGREGATES IN SOLUTION

Lourdes del Álamo[1], Rodrigo González-Prieto[1], José Luis Priego[1], Laura Castañar[2], Ivo Krummenacher[3], José Manuel Pérez-Cañadillas[4], Santiago Herrero[1,5] and María J. Mayoral[1]

[1]Inorganic Chemistry Department, Faculty of Chemical Sciences, UCM, 28040 Madrid, Spain
[2]Organic Chemistry Department, Faculty of Chemical Sciences, UCM, 28040 Madrid, Spain
[3]Institute for Inorganic Chemistry, Julius-Maximilians-Universität Würzburg, 97074 Würzburg, Germany
[4]Institute of Physical-Chemistry "Blas Cabrera", CSIC, 28006 Madrid, Spain
[5]Instituto de Tecnología del Conocimiento, UCM, 28223 Pozuelo de Alarcón, Madrid, Spain

The design of functional magnetic materials through the self-assembly of paramagnetic units remains a challenge. Metal-metal bonded **diruthenium** complexes are excellent candidates for this approach due to their unique electronic structure. In this work, **Nuclear Magnetic Resonance** (NMR) is presented as a dual probe for monitoring both the self-assembly process and the magnetic behavior in solution.

Characterizing this system is intrinsically challenging due to the **paramagnetic nature** of the diruthenium core (Figure 1a).[1] By employing a combination of concentration-dependent ^{1}H-NMR experiments (Figure 1b) and 2D Diffusion Ordered Spectroscopy (DOSY) in $CDCl_3$, it is possible to monitor the chloride-driven self-assembly of the diruthenium monomers.[2]

Complementary, NMR was further used to probe the magnetic properties of the system. Through the **Evans Method**, the paramagnetic susceptibility in solution was quantified (Figure 1c),[3] tracking the magnetic changes upon assembly. These results, complemented by **Electron Paramagnetic Resonance** (EPR), demonstrate that the monomer's paramagnetic character is successfully transferred to the supramolecular polymer, giving rise to magnetically active nanomaterials.

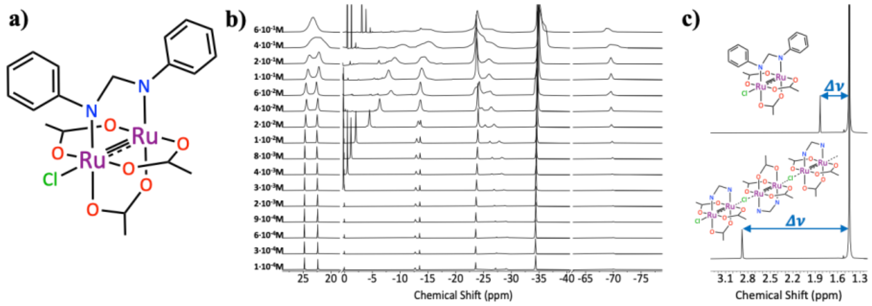

Figure 1. a) Structure of the diruthenium monomer. b) Concentration-dependent ^{1}H-NMR experiment in $CDCl_3$. c) Evans Method ^{1}H-NMR using cyclohexane as a reference for the monomer (top) and supramolecular polymer (bottom).

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AN NMR-BASED STUDY OF LATANOPROST ENCAPSULATION

Luka Barešić[1], Ana Čikoš[1], Leo Štefan[2], Maša Safundžić Kučuk[2]

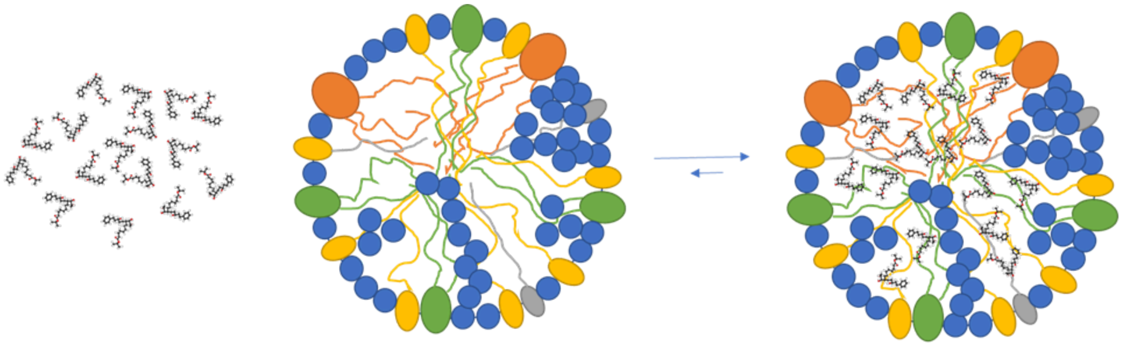
[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Jadran-Galenski Laboratorij d.d., Rijeka, Croatia
lbaresic@irb.hr

Latanoprost, a prostaglandin F2α analogue, is a cornerstone in glaucoma treatment due to its ability to reduce intraocular pressure.[1] However, its clinical efficacy is compromised by adsorption to container surfaces and hydrolysis, both of which lower its active concentration.[2] To address these limitations, various surfactants can be used as drug delivery systems.

This study investigates the encapsulation of latanoprost in benzalkonium chloride (a cationic surfactant) micelles compared to three non-ionic surfactant micelles (macrogolglycerol hydroxystearate, macrogolglycerol ricinoleate, and macrogol 15 hydroxystearate), and assesses the impact of sodium hyaluronate on such systems.

NMR analysis revealed strong interactions between latanoprost and the surfactants: chemical shift perturbations indicated binding, NOE interactions confirmed micellar encapsulation, and reduced diffusion coefficients supported incorporation into the micellar system. Notably, sodium hyaluronate did not perturb NMR signals, suggesting no interaction with latanoprost or the surfactants.

These findings demonstrate that the tested surfactants effectively encapsulate latanoprost, even in the presence of sodium hyaluronate, demonstrates their potential to stabilize ophthalmic formulations.



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NMR-BASED MEASUREMENT OF THE GUANINE NUCLEOTIDE EXCHANGE RATE ON GAI3 PROTEIN

Nuria Andres-Penares[1], Mariola Ferreras-Gutiérrez[2], Francisco J. Medrano[2], Mikel García-Marcos[3,4], Francisco J. Blanco[1,5]

[1] Instituto de Biomedicina de Valencia (IBV), CSIC, Valencia 46010, Spain
[2] Centro de Investigaciones Biológicas Margarita Salas (CIB), CSIC, Madrid 28040, Spain
[3]Department of Biochemistry & Cell Biology, Chobanian & Avedisian School of Medicine, Boston University, Boston, MA 02118, USA
[4]Department of Biology, College of Arts & Sciences, Boston University, Boston, MA 02115, USA
[5]Centro de Investigación Príncipe Felipe, Unidad Asociada a IBV, Valencia 46012, Spain

The rate of guanine nucleotide exchange (GDP for GTP) on the α-subunit of heterotrimeric G-proteins (Gα) regulates their downstream cellular signaling [1]. Traditional methods for measuring this rate require the use of radioactive GTP and meticulous sample manipulation. Alternative approaches employ GTP analogs with bulky fluorophores, which bind with reduced affinity. We aim to assess the efficacy of NMR spectroscopy as a direct method for measuring the nucleotide exchange rate of Gα subunits, specifically the 41 kDa protein Gαi3. An NMR sample of uniformly ^{2}H-^{15}N labeled GDP-bound Gαi3 protein was prepared, and a 10-fold concentration of GTPγS (a non-hydrolyzable analog of GTP) was added before recording a series of 30-minute- long ^{1}H-^{15}N TROSY NMR spectra, at 800 MHz over 15 hours. The intensities of NMR signals corresponding to the indole side chains of Gαi3's tryptophan signals, distinctive for GDP-bound and GTPγS-bound states, were quantified and fitted to an exponential decay or association to obtain the exchange rate constant. Our results indicate an average nucleotide exchange rate of $5.5 \pm 0.6 \cdot 10^{-5}$ s^{-1} at 20 ºC, about twice the value measured by intrinsic fluorescence at 30 ºC ($2.32 \pm 0.01 \cdot 10^{-4}$ s^{-1}) or by radioligand-based assays at 30 ºC (2.0 10^{-4} s^{-1}) [2]. These results demonstrate the feasibility of using NMR spectroscopy to directly measure nucleotide exchange rates in Ga subunits, with the potential to measure them in complex media like cellular extracts.

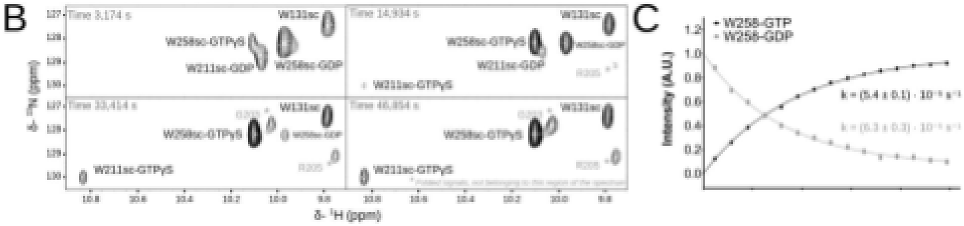

Figure: **(B)** ^{1}H-^{15}N TROSY spectra of GDP-Gαi3 at increasing times after the addition of GTPγS at a 10-fold molar excess (only the region of the Trp indole signals is shown). Asterisks (*) indicate folded backbone amido signals. **(C)** Time- dependent change of the intensity of W258 indole signal (W258sc) and fitting to a single exponential. The rate constant is indicated.

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ALLOSTERIC MODULATION OF TIA-1 PHASE SEPARATION BY DOUBLE SERINE PHOSPHORYLATION

Diego Sánchez-Pulido[1], Alejandro Velázquez-Cruz[1], Laura Corrales-Guerrero[1], Ana B. Uceda-Mayo[1], Emanuela Tumini[2], Sofía M. García-Mauriño[1], Saboora Waris[3], Rafael L. Giner-Arroyo[1], Fionna E. Loughlin[3], Rafael Fernández-Chacón[2], Jacqueline A. Wilce[3], Miguel A. De la Rosa[1] and Irene Díaz-Moreno[1]

[1]Institute for Chemical Research — Centro de Investigaciones Científicas Isla de la Cartuja (cicCartuja), Universidad de Sevilla – Consejo Superior de Investigaciones Científicas (CSIC), Seville, Spain.
[2]Institute of Biomedicine of Seville (IBiS, University Hospital Virgen del Rocío – CSIC – University of Seville, Department of Medical Physiology and Biophysics, School of Medicine, and CIBERNED ISCIII, Seville,Spain
[3]Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia.

In response to diverse harmful stimuli, eukaryotic cells generate cytoplasmic stress granules (SGs), mainly composed of mRNAs and RNA-binding proteins (RBPs). RBPs are fine-tuned by a diverse array of post-translational modifications (PTMs), with important consequences for the assembly, dynamics and clearance of SGs. One of the best characterized SG nucleators is the RBP T-cell intracellular antigen 1 (TIA-1), although knowledge about the structural and functional impact of its identified PTMs is very limited. TIA-1 is organized into three RNA-recognition motifs (RRMs) and a C-terminal prion-related domain (PRD) that drives its phase separation from the cytosol. Microscopic observations revealed an increased propensity of the phosphomimetic TIA-1 S198/199E to undergo liquid-liquid phase separation (LLPS) and self-assemble into SGs independently of stress stimuli.

We analyzed the effect of TIA-1 double phosphorylation at serines 198 and 199 on TIA-1 function and structure. Firstly, computational simulations suggested that such phosphorylations promote the formation of a β-hairpin motif at the beginning of the PRD. NMR experiments (i.e., 15N-HSQC, HNCA, and HN(CA)CO) were performed for constructs of 13C or 15N-labelled TIA-1 WT and TIA-1 S198/199 phosphomimetic mutant (S198/199E). Our results suggested that the glutamate mutations induce the folding of part of the N-terminal quarter of the PRD, generating a more rigid PRD region which results in the formation of a β-hairpin motif, thus supporting the computational prediction. Moreover, the ALS-associated mutation V283M in TIA-1 was predicted to lead to the formation of an aberrant structure in the β-hairpin region, highlighting the fine balance between physiological and pathogenic TIA-1 phase transition, and the importance of a better understanding of the molecular mechanisms underlying the liquid demixing of this RBP.

FLUORINATED SULFONATE SIALOMIMETICS REVEAL BINDING MECHANISMS OF CD33 BY STD AND 19F- CPMG NMR

Venetia Psomiadou[1], Frank de Kleijne[1], Maurice Peene[1], Kumarage Nuwanthika[1], Willem Titulaer[1], Pablo Valverde[2], Iker Oyenarte[2], Jesús Jiménez-Barbero[2], June Ereño-Orbea[2] and Thomas J. Boltje[1]

[1]The Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands
[2]Chemical Glycobiology Lab, CIC bioGUNE, Derio, Spain

Siglec-3 (CD33) is a sialic acid-binding immune receptor involved in the regulation of innate immune responses and represents an important therapeutic target [1,2]. While canonical recognition is mediated by a conserved interaction between the sialic acid carboxylate and a key arginine residue, sulfated sialoglycans are known to enhance binding affinity [3,4]. However, the relative contributions of canonical and additional electrostatic interactions remain insufficiently understood. Here, we designed fluorinated sulfonate sialomimetics to dissect canonical versus electrostatic contributions to CD33 binding.

Ligand–protein interactions were characterized using saturation transfer difference STD NMR and CPMG experiments. STD NMR enabled epitope mapping, revealing dominant interactions at the sialic acid moiety, consistent with a canonical binding mode. In parallel, dissociation constants (K_D) were determined independently by both techniques [5], yielding comparable values. Notably, CPMG provided improved precision, reduced experimental time, and enhanced sensitivity, further optimized through implementation of a SHARPER-CPMG sequence. Binding studies revealed that para-sulfonylbenzyl sialosides display the highest affinity, followed by benzyl analogues, while 6′-sialyllactose binds weakly. These findings are consistent with previous observations on Siglec ligand specificity [3] and demonstrate that electrostatic interactions from sulfonate groups enhance binding but are insufficient in the absence of the canonical sialic acid-arginine interaction. Molecular dynamics simulations further support secondary electrostatic contributions stabilizing ligand binding.

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A [2+4] MULTICOMPONENT QUANTITATIVE ASSEMBLY IN POLAR MEDIA VIA HYDROGEN-BONDS

Beatriz Torres-Calvo[1], Alberto de Juan[1,2], Isabel López-Martín[1], and David González-Rodríguez[1,2]

[1]Nanostructures Molecular Systmens and Materials Group, Departamento de Química Orgánica, Universidad Autónoma de Madrid, 28049 Madrid, Spain.
[2]Institute for Advanced Research in Chemical Sciences, Universidad Autónoma de Madrid, Spain.

Porphyrins play crucial roles in biological processes such as electron transfer (cytochromes), oxygen transport (hemoglobin), etc. Inspired by *Nature*, numerous researchers have focused on developing **organized structures** based on porphyrin units, assembled through covalent bonds, metal-ligand interactions or supramolecular interactions.[1]

In our research group, **nanostructured systems with well-defined inner cavities** are prepared by combining a tetratopic porphyrin functionalized at the *meta* position of the *meso* arene groups and a ditopic linker bearing complementary *meta* substituted groups, leading to the corresponding cofacial **porphyrin cage**.[2] This work focuses on the synthesis of **self-assembled cages using non-covalent amidinium-carboxylate interactions**. So far, multicomponent porphyrinic systems based on this interaction have only been assembled in non-polar media.[3] Therefore, a key objective is to develop an **assembly process** that can robustly occur **in polar media**.

The synthetic development of these cages requires mixing two components under stoichiometric conditions: **porphyrins**, which incorporate **carboxylic acid** moieties in their structure, and **linkers** bearing complementary **amidine groups** (***Figure 1***). The resulting self-assembled supramolecular cage will feature an inner cavity with two cofacially arranged (metallo)porphyrins, capable of encapsulating guest molecules of suitable size. The self-assembly process will be studied through NMR techniques, including 1D, DOSY, temperature, solvent, and concentration-dependent experiments, as well as titrations studies.

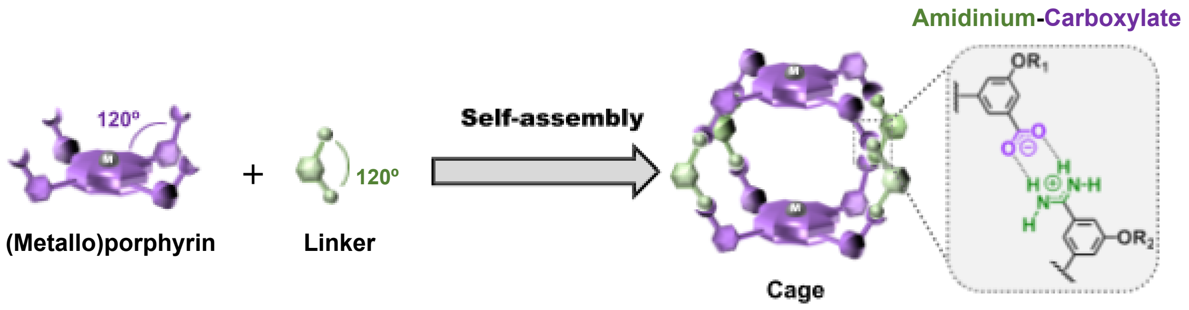

Figure 1. *Self-assembly of cages by means of amidinium-carboxylate bonding.*

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METABOLIC FINGERPRINTING OF BOVINE TUBERCULOSIS: TRANSLATING BENCHTOP NMR FROM LAB TO BARN

Álvaro Pérez-Collar,[1] Luis A. de Benito-Rodríguez,[1,2] Pilar Alonso-Moreno[1,2], Carlos Velasco-Reinaldos,[3,4] J. Ortega-Martin,[3] Javier Bezos-Garrido,[3,4] José L. Izquierdo-García[1,2,5,6]

[1]NMR and Imaging in Biomedicine Group, Pluridisciplinary Institute, Complutense University of Madrid, Madrid, Spain
[2]Department of Chemistry in Pharmaceutical Sciences, Pharmacy School, Complutense University of Madrid, Madrid, Spain
[3]Centro de Vigilancia Sanitaria Veterinaria, VISAVET, Complutense University, Madrid, Spain
[4]Department of Animal Health, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain
[5]ICTS Bioimagen Complutense, BIOIMAC, Complutense University of Madrid, Madrid, Spain
[6]Centre for Biomedical Networked Research in Respiratory Diseases (CIBERES), Carlos III Health Institute

Bovine tuberculosis diagnosis is based on the detection of the host immune response, primarily through tests such the interferon-gamma release assay (IGRA). This immune response is complex and can be influenced by multiple factors, compromising the accurate identification of infected animals. Additionally, exposure to environmental mycobacteria or prior sensitization may lead to false-positive results, reducing diagnostic specificity.

NMR-based metabolomics represents a promising approach for identifying metabolic profiles that could improve bovine tuberculosis diagnosis. Its application has traditionally been limited by high costs and infrastructure demands. However, the emergence of benchtop NMR spectrometers offers a novel and accessible alternative.

The aim this study was to identify metabolomic biomarkers associated with bovine tuberculosis infection and to evaluate the feasibility of implementing benchtop NMR in routine veterinary diagnostics.

Two groups were analysed: the first comprised calves from herds with confirmed bovine tuberculosis (N = 20), classified as true positives; the second included calves from tuberculosis-free herds that tested IGRA-positive (N = 20), classified as false positives. Whole blood samples were incubated with PBS (A), avian PPD (B), and bovine PPD (C), followed by plasma separation and NMR-based metabolomic analysis. Both high-field (Bruker AVANCE III 500 MHz) and low-field (Magritek Spinsolve Ultra 80 MHz) spectrometers were employed.

Partial Least Squares Discriminant Analysis (PLS-DA) enabled discrimination between true-positive and false-positive animals, achieving an accuracy of 92.5% using high-resolution NMR and 90% with benchtop NMR spectrometers. The high-field approach fulfilled an AUC-ROC of 88,1%, with 89% sensitivity and 81% specificity, while benchtop NMR achieved an AUC-ROC of 92%, with 94% sensitivity and 86% specificity.

These findings highlight the potential of NMR-based metabolomics as a more specific diagnostic approach for bovine tuberculosis, supporting its application in to clinical veterinary practice.

HYPERPOLARIZATION: WHAT IT IS AND WHAT CHALLENGES IT POSES

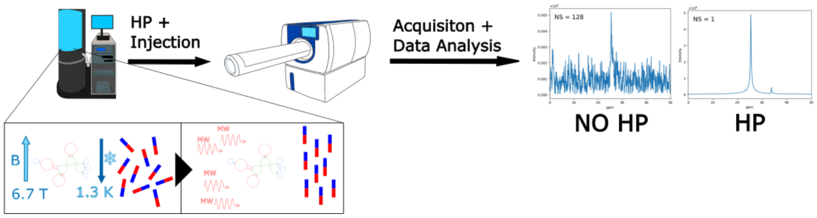
Gonzalo Alonso Reíllo[1]

[1]Institute of Bioengineering of Catalonia, IBEC

Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI) are well-established non-invasive techniques, widely used in both clinical and preclinical settings for metabolic studies. Among their extensions, carbon 13 Magnetic Resonance (^{13}C MR) allows to detect chemically specific information on metabolic pathways, though its application is limited by intrinsically low sensitivity. Dissolution dynamic nuclear polarization (dDNP) addresses this limitations by providing over 10^5-fold signal enhancement over conventional ^{13}C MRI [1]. This enhancement enables non-invasive, real-time tracking of cellular metabolic activity, making ^{13}C hyperpolarized (HP) MR a powerful tool for detecting cancer-induced metabolic reprogramming in vivo and in vitro [2]. ^{13}C-pyruvate is one of the most widely used HP tracers, allowing direct monitoring of glycolytic flux, a pathway consistently altered in liver malignancies such as hepatoblastoma [3].

However, the lifetime of hyperpolarized signals is, in the best cases, limited to tens of seconds: each radiofrequency pulse consumes a fraction of the non-recoverable magnetization, and T_1 relaxation further constrains the acquisition time window. These limitations impose strict conditions on pulse sequence design, forcing to develop fast acquisition procedures to exploit the maximum information [4].

In this contribution, we present our work on the development and optimization of HP-MR pulse sequences targeting hepatic metabolic fluxes in hepatoblastoma. The work is carried out using two complementary platforms, a 1.4T benchtop NMR spectrometer (Oxford Instruments) [5] for the study of 3D cell models, and a 3T preclinical MRI scanner (Bruker BioSpec Maxwell) for in vivo acquisitions. Dedicated reconstruction and post-processing approaches are being developed alongside the pulse sequences to maximize the signal- to-noise-ratio and metabolic kinetic rates readout within the constrained acquisition time window.



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PHOSPHORYLATION OF THE AML-LINKED C-TERMINAL REGION OF NPM1 CONTROLS STABILITY, DNA INTERACTION, AND CHARGE-DRIVEN PHASE SEPARATION

Pablo Rivero-García[1], Rafael L. Giner-Arroyo[1], Joaquín Tamargo-Azpilicueta[1], Abbey Telfer[2], Elisa Frezza[3], Adrián Velázquez-Campoy[4,5,6], Sofía Díaz-Moreno[2], Miguel A. De la Rosa[1], Irene Díaz-Moreno[1]

[1]Institute for Chemical Research – cicCartuja, University of Seville – CSIC, Seville, Spain
[2]Diamond Light Source – Harwell Science & Innovation Campus, Didcot, UK
[3]Université Paris Cité, CiTCoM, CNRS, F-75006 Paris, France
[4]Institute for Biocomputation and Physics of Complex Systems (BIFI), and Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Zaragoza, Spain
[5]Instituto de Investigación Sanitaria Aragón (IIS Aragón), 50009 Zaragoza, Spain
[6]Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas y Digestivas (CIBERehd), 28029 Madrid, Spain

Nucleophosmin (NPM1) is a nucleolar protein commonly mutated in ~30% of newly diagnosed acute myeloid leukemia (AML) cases. These mutations occur in the terminal exon of the NPM1 gene, affecting the C-terminal DNA-binding domain of the protein and causing its delocalization to the cytoplasm—a hallmark of NPM1-mutated AML. NPM1 shuttling to the nucleoplasm is tightly regulated by post-translational modifications (PTMs), such as phosphorylation of Ser254, Ser260 and Tyr271 of the DNA-binding domain. However, the structural mechanisms underlying this process remain unclear. In this work, we show that Ser-to-Asp (S254D-S260D) and Tyr-to-pCMF (para-carboxymethyl phenylalanine) (Y271pCMF) phosphomimetic mutations induce significant structural and dynamical rearrangements, as well as drastic modifications in electrostatic surface potential (ESPs). These changes compromise recognition of a G-quadruplex sequence from the c-MYC promoter by reducing DNA-binding affinity, reshape histone capturing dynamics, and fade charge segregation in the histone-binding domain. Combination of such substitutions in a triple phosphomimetic variant (S254D-S260D- Y271pCMF) further destabilizes the domain's structure and triggers protein aggregation. Altogether, these findings suggest that phosphorylation of Ser254, Ser260 and Tyr271 of the C-end DNA-binding domain weakens both DNA affinity and charge block–driven liquid-liquid phase separation, offering a molecular explanation for the delocalization of NPM1 outside of the nucleolus.

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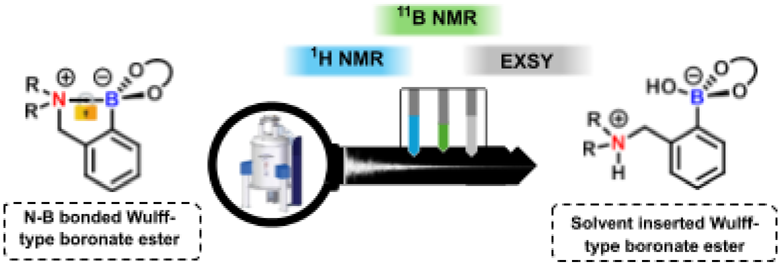
UNRAVELING THE STRUCTURE OF WULFF-TYPE BORONATE ESTERS BY NMR

Lucas Garcia-Abuin,[1] Samuel Parcero-Bouzas,[1] and Eduardo Fernandez-Megia[1]

[1]Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, Jenaro de la Fuente s/n, Santiago de Compostela 15782, Spain.

Boronic acids (BA) bind diols in a reversible pH-dependent manner, recognized as an archetypal example of dynamic covalent chemistry,[1] making them attractive for the design of stimuli-responsive systems. However, since boronate ester formation only occurs at pH values higher than the p*K*a of the BA, the relatively high p*K*a values of traditional BA (8.8 for phenylboronic acid) limits their applicability under physiological conditions. To address this limitation Wulff-type BA have been developed, drastically reducing the p*K*a and thus enabling boronate ester formation at physiologycal pH.[2] In this context, our group has recently reported a strategy based on a Wulff-type BA functionalized dendrimer for gene therapy and the formation of coacervate-based synthetic cells.[3,4] Despite these advances, the structure of Wulff-type boronate esters in solution remains poorly understood, particularly regarding the equilibrium between intramolecular N–B bonded and solvent-inserted species. Similar electronic environments in both structures make this distinction difficult to establish experimentally. Moreover, structural assignments remain unclear due to limited experimental evidence which has led to multiple models proposed over the years.[5] In this context, our work aims to provide a more detailed and systematic investigation to unravel the complex equilibria of Wulff-type boronate esters in solution.



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HIGH AND LOW-FIELD ADVANCED NMR APPROACHES FOR WINE ANALYSIS

Diego Martín-Arranz[1], Tomás Martín Duque[2], Ángeles Canales[2], Laura Castañar[2]

[1]Department of Compounding Pharmacy and Food Technology, Faculty of Veterinary, Complutense University of Madrid, Madrid, 28040, Spain
[2]Department of Organic Chemistry, Faculty of Chemical Sciences, Complutense University of Madrid, Madrid, 28040, Spain

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for the analysis of complex matrices such as wine [1], providing simultaneous structural and quantitative information on multiple compounds. In this work, the applicability of NMR spectroscopy, both at high and low field, is evaluated for the chemical analysis of wines, addressing both qualitative and quantitative approaches.

The main objective of this study is to validate the use of NMR as an analytical technique in oenology through the optimization of experimental protocols, the determination of alcohol content, and the structural identification of major wine components. For this purpose, 15 representative wine samples were analysed, selected according to grape variety, aging time, and geographical origin. 1D ^{1}H NMR experiments with selective suppression of dominant signals (water and ethanol) were employed [2], together with two-dimensional techniques (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) for metabolite structural elucidation. Advanced NMR techniques such as pure shift NMR [3] and ultra selective methods [4] were employed for structural assignment of the signals.

The results show that high-field NMR enables the identification and characterization of a large number of compounds, including alcohols, organic acids, and other relevant metabolites, as well as the accurate determination of alcohol content. Furthermore, comparative analysis of the spectra reveals significant differences associated with aging, grape variety, and geographical origin, in agreement with previous NMR-based metabolomics studies. In contrast, low-field NMR presents limitations in terms of resolution and sensitivity, which reduce the number of identifiable compounds; however, it allows the detection of major components and reproduces comparative trends observed at high field.

Overall, this work highlights NMR as a versatile and robust tool for wine analysis. High-field NMR remains the method of choice for detailed structural characterization, whereas low-field NMR offers a practical alternative for quality control and routine applications, despite its inherent limitations

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APPLYING NMR FOR STRUCTURAL CHARACTERIZATION AND EPITOPE DEFINITION OF A TUMOR-ASSOCIATED CARBOHYDRATE ANTIGEN

Vila-Gonzalo, María[1,2], Real-Arévalo, I[1]., Viñuela, M.[1], Cañada, F. J.[2,3]

[1]INMUNOTEK SL., Alcalá de Henares, Spain.
[2]Center of Biological Research Margarita Salas (CIB-CSIC), Madrid, Spain.
[3]CIBER de Enfermedades Respiratorias (CIBERES), 28029, Madrid, Spain.

Cell surface glycosaminoglycans (GAGs) are essential mediators of intercellular signalling and tumor progression. Due to their high heterogeneity and complex sulfation patterns, characterizing their interaction with the immune system requires high-resolution biophysical tools. In this study, we focus on Ca10, a high molecular weight GAG fraction derived from murine Ehrlich tumor (ET) cells, which serves as a target for the monoclonal antibody (mAb) A10, which inhibits ET growth *in vivo* and *in vitro* [1] and shows cross-reactivity with human tumor antigens, making it a potential candidate for cancer immunotherapy. [2]

To elucidate the molecular basis of this recognition, we employed an NMR-based multidisciplinary approach. The primary structure of the Ca10 polysaccharide and its structural analogues were determined through a combination of 1D/2D homonuclear and heteronuclear experiments, while Diffusion-Ordered Spectroscopy (DOSY) was utilized to monitor the hydrodynamic behaviour and purity of the glycan fractions and to determine the MW distribution and followed-up of chemical and enzymatic reactions to obtain the structure.

To define the minimal recognition epitope, we performed systematic enzymatic depolymerization using specific GAG-lyases. The resulting library of well-defined oligosaccharides was analyzed to identify the key structural motifs, specifically un-substituted glucosamine units (GlcN), required for antibody binding. The interaction dynamics were characterized at the atomic level using Saturation Transfer Difference (STD-NMR). [3] These experiments allowed us to map the precise epitope contact surface and identify the specific carbohydrate residues that mediate the binding within the A10. Our results demonstrate the power of advanced NMR techniques in decoding the "glyco-code" of tumor antigens.

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STRUCTURAL DETERMINATION OF sp^2-IMINOGLYCOLIPIDS BY NMR SPECTROSCOPY

Rocío Rodríguez-Marín[1], Elena M. Sánchez-Fernández[1], José M. García Fernández[2], Carmen Ortiz Mellet[1]

[1]Department of Organic Chemistry, University of Sevilla, Spain
[2]Instituto de Investigaciones Químicas (IIQ), CSIC - University of Sevilla, Spain

sp^2-Iminoglycolipids (sp^2-IGLs) constitute a prominent family of α-glycomimetics that stand out for its structural versatility and remarkable beneficial properties in different fields, including a variety of cancers, infections caused by Leishamnia parasites as well as different pathologies caused by diabetes disease.[1] Extensive structure-activity relationship (SAR) studies through a wide variety of structural modifications in both the glycone and aglycone space of this family of glycoconjugates, have revealed the importance of certain structural features in their therapeutic performance.[2, 3] From the large library of sp^2-IGLs reported over the years, herein we have selected some examples in which a combination of different 1D and 2D Nuclear Magnetic Resonance (NMR) techniques has displayed a central role in their structural elucidation (^{77}Se, ^{19}F, TOCSY or HMBC spectra, among others). These examples include the structural assignation of selenium and fluorine-containing sp^2-IGLs (**1** and **2**), epimeric azido-derivatives at C2 position (**3**), and the *C*-glycosylterpenoid (**4**) (Figure 1).

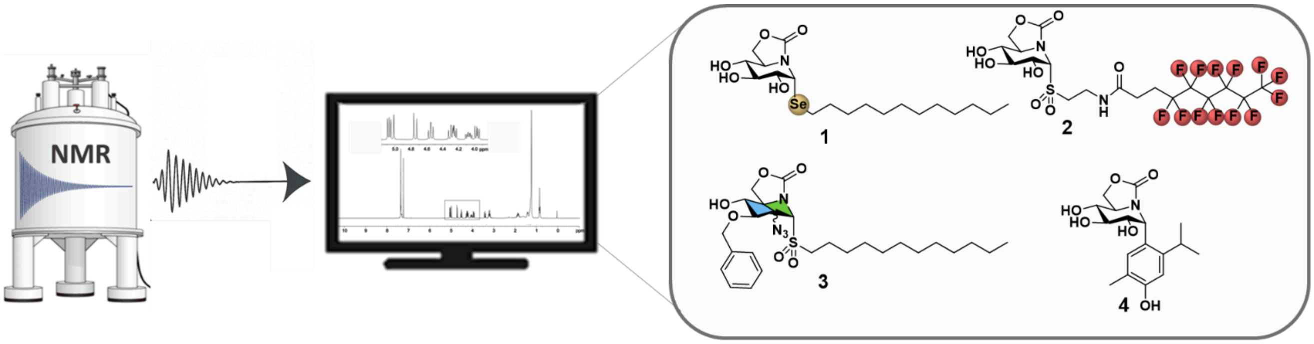

Figure 1. Structural elucidation of selected sp²-IGLs by NMR spectroscopy.

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FROM VINEYARD TO SPECTRUM: AN NMR AND MACHINE LEARNING APPROACH FOR THE AUTHENTICATION OF 'JEREZ-XÉRÈS-SHERRY' WINES

Jaime Hiniesta-Valero, Alejandra Guerra-Castellano, Andrea Fernández-Veloso[2], Miguel A. De la Rosa, Irene Díaz-Moreno

[1]Instituto de Investigaciones Químicas (IIQ), Centro de Investigaciones Científicas Isla de la Cartuja (cicCartuja), Universidad de Sevilla, Consejo Superior de Investigaciones Científicas (CSIC), Spain
[2]ManSciTech S.L., Spain

The authentication and quality control of products under Protected Designation of Origin (PDO), such as "Jerez-Xérès-Sherry" wines, are vital for protecting both producers and consumers against fraud. While traditional sensory analysis remains the standard, there is a growing need for objective, high-throughput analytical methods to certify the identity and "*terroir*" of these wines. In this study, we present an authentication model based on Nuclear Magnetic Resonance (NMR) combined with Machine Learning (ML) algorithms. These models not only support the classification of Sherry wines under the Jerez-Xérès-Sherry D.O. but also facilitate the discovery of complex patterns that might otherwise go unnoticed. This enables a more reliable certification process, reinforcing the application of machine learning as a powerful tool for ensuring the authenticity and typicity of wines.

The robustness and versatility of this NMR-based metabolomic workflow [2] have been previously demonstrated in other complex agri-food systems, such as the investigation of molecular mechanisms in strawberries (*Fragaria ananassa*) during vernalization [3]. In that context, the methodology successfully captured critical metabolic shifts in sugar mobility and photosynthetic activity necessary for crop development. By applying this same analytical rigor to the viticultural sector, we established a unique "*Terroir* fingerprint" for Sherry wines. Our model achieved high accuracy in differentiating between various wine categories, including Fino, Amontillado, and Oloroso, based on their specific metabolic profiles. By offering an objective approach to identifying the complex variables that influence wine production, this framework provides a valuable foundation for advancing quality control and origin certification tools within the wine industry, paving the way for more accurate, data-driven methods in the future.

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LEVERAGING LIQUID-STATE NMR FOR STRUCTURAL INSIGHT AND FUNCTIONAL DESIGN OF ADVANCED BIOMEDICAL MATERIALS

R. Grosso,[1] N. Iglesias,[1] F. Díaz-Carrasco,[1] E. Galbis,[1] E. Benito,[1] M.-V. de-Paz[1]

[1]Department of Organic and Pharmaceutical Chemistry. Faculty of Pharmacy,
Universidad de Sevilla. 41012, Sevilla. Spain

Liquid-state NMR spectroscopy is a key analytical tool in the design and development of advanced biomedical materials, providing detailed molecular-level insights throughout synthesis, purification and functional evaluation. Firstly, it enables the precise determination of copolymer composition in molar terms, and helps confirming the absence of residual monomers, ensuring polymer purity after dialysis. It allows accurate quantification of repeating units, even in complex amphiphilic block copolymers comprising multiple segments. This structural control is essential for tuning self-assembly in aqueous media, leading to the formation of micellar systems that can be core-crosslinked and functionalized for the encapsulation of lipophilic compounds. [1, 2] Such nanostructures have been successfully tested as drug delivery systems (DDS) *in vitro*, both as colloidal suspensions and as nanocomposites embedded in chitosan-based hydrogels for colon-targeted therapies. [3, 4]

Beyond structural characterization, NMR can also provide valuable insight into polymer network formation and crosslinking kinetics in aqueous environments, enabling identification of optimal reaction conditions and real-time monitoring of gelation. [5] These molecular-level observations can be directly correlated with rheological behavior, confirming hydrogel formation and mechanical stability. NMR also plays an important role in evaluating the degradation of DDS. For example, in combination with UV–Vis spectroscopy, it allows quantitative assessment of polymer degradation and drug release kinetics of gastroretentive DDS in simulated gastric conditions across both physiological and pathological pH ranges, which is particularly relevant for antibiotic delivery against *Helicobacter pylori*. [6, 7] In addition, NMR can be instrumental in the study of thermoresponsive, self-healing copolymer networks that rely on reversible bond exchange processes, such as retro-Diels–Alder reactions. These analyses confirm the dynamic equilibrium between bond dissociation and reformation, underpinning key properties such as injectability, mucoadhesion and self-repair. [8] Altogether, these findings highlight the versatility of liquid-state NMR in enabling the rational design of functional materials with strong potential in tissue engineering and stimuli-responsive drug delivery.

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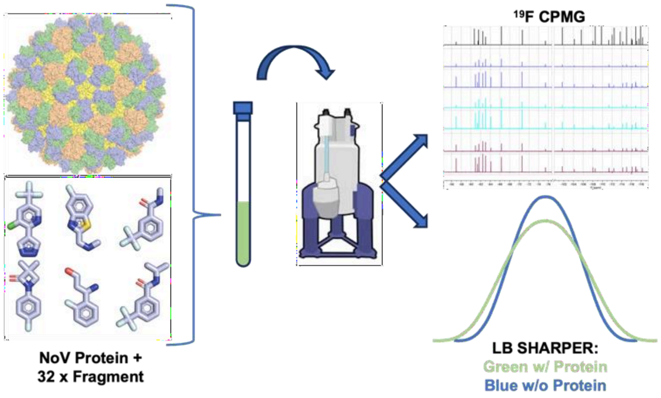
TOWARD ANTIVIRALS AGAINST NOROVIRUS: EXPANDING THE NMR TOOLKIT FOR NOROVIRUS DRUG DISCOVERY

Henry N. Flatau[1], Siyu Lin[2], Luca Pisapia[3], Francisco José Morais Esteves[4], Marco Fragai[2], Annabelle Varrot[3], Anne Imberty[3], Mirandea De Graaf[4], Juan Carlos Muñoz-García[1], Jesús Angulo[1]

[1]Instituto de Investigaciones Químicas, CSIC – Universidad de Sevilla, Américo Vespucio 49, E-41092, Seville
[2]Department of Chemistry – DICUS, University of Florence, via della Lastruccia 3-13, 50019 Sesto (FI), Italy
[3]CERMAV-CNRS, UPR 5301 CS40700 38041 Grenoble cedex 9, France
[4]Department of Viroscience, Erasmus Medical Centre, Wytemaweg 80, 3015 CN Rotterdam, Netherlands

Norovirus (NoV) is one of the most contagious human pathogens in our world. Statistically speaking, every person is infected approximately every eight years.[1] Despite causing at least 700 million infections annually and posing serious death risks to newborns and elderly individuals, no approved drugs or vaccines are currently on the market. The key interaction driving infection is the binding of the NoV Protruding Domain (P-domain) to Human Blood Group Antigens (HBGAs) on intestinal cells.[2] Due to the shallow and hydrophilic nature of the carbohydrate binding site Drug Discovery campaigns against this binding site of the P-Domain are challenging.



Graphical abstract: NoV Protein (VLP or P-Dimer) were screened against 530 fragments (32 fragments per cocktail) by ^{19}F CPMG NMR. The hits against the carbohydrate binding site were ranked with LB SHARPER.

Here we present the groundwork for new drug discovery campaigns by screening 530 fluorinated fragments with a focus on the P-domain carbohydrate binding site. Using competition ^{19}F CPMG NMR experiments we observed specific binding of 6 molecules to the carbohydrate binding site. Further, we have ranked the hits based on their affinity by the state-of-the-art LB-SHARPER NMR methodology[3], obtaining dissociation constants within the µM range for all of them - an increase of two to three orders of magnitude compared to the natural HBGA ligands. Additionally, we introduce in this study for the first time the combined use of Virus-Like Particles (VLPs) in ^{19}F NMR-based FBDD and their combination with LB-SHARPER NMR, thereby expanding the NMR FBDD toolkit for viruses. Our results establish a foundation for targeting NoV-carbohydrate interactions and expand the methodological landscape of NMR-based fragment screening against viruses.

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LIST OF PARTICIPANTS

Adelyn Maria Betances Mora - IIQ-CSIC
Ainhoa MartínMuñoz - Universidad de Alcalá
Alejandra De Castro Travecedo - CIC bioGUNE
Alejandra Guerra Castellano - IIQ (US-CSIC)
Almudena Hornero Romera - Instituto Física Química Blas Cabrera
Alvaro Perez Collar - Instituto Pluridisciplinar - UCM
Alvaro Fernando Garcia Rodriguez - Instituto de la Grasa (IG-CSIC)
Ana Molina Teba - Universidad de Sevilla
Andrea Fernández Veloso - Universidad de Sevilla
Antonio Ruiz Albor - IBV
Arantxa Forte Castro - Universidad de Almería
Beatriz Torres Calvo - Universidad Autónoma de Madrid
Belén Fernández Alfaro - Servicio de RMN - CITIUS
Carla Merino Ruiz - Palex Medical
Carmen Hidalgo Montero - University of Seville
Christoph Schnieders - Deutero GmbH
Cristina Fernández Pérez - CIC
bioGUNE Cristina Ugedo Pérez -
IQF-CSIC Cristina Mora Cordero -
IIQ-CSIC
David Pantoja Uceda - IQF-CSIC
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Catalonia Gurudutt Dubey - IBV-CSIC
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