ADRIATIC NMR

9–11 July 2026, Rijeka, Croatia

BOOK OF ABSTRACTS

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ADRIATIC NMR CONFERENCE

Rijeka, Croatia, 9–11 July 2026

BOOK OF ABSTRACTS

IMPRESSUM

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Dear Participants,

It is our great pleasure to invite you to attend the Adriatic NMR Conference which will take place in Rijeka, Croatia, from 9 to 11 July 2026.

The wide scope of the Conference includes but is not limited to topics regarding the theoretical basis of NMR, method development, small molecules, Bio-NMR, benchtop NMR, spectral data interpretation and simulation, metabolomics, NMR characterization of supramolecular systems, industrial applications of NMR spectroscopy and solid-state NMR methods and applications.

We strongly believe that Adriatic NMR will foster the exchange of knowledge and experience among students and scientists and will serve as a forum for an extensive networking opportunity. With confidence that it will be a memorable event, we cordially invite you to join us in Rijeka in July 2026.

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ADRIATIC NMR

9–11 July 2026, Rijeka, Croatia

PROGRAMME

THURSDAY, JULY 9	
15:00 – 16:00	**REGISTRATION**
16:00 – 16:05	**OPENING**
CHAIR: Tomislav Jednačak	
16:05 – 16:35	**Leonardo Tenori:** *Deriving Three One Dimensional NMR Spectra From a Single Experiment Through Machine Learning*
16:35 – 16:55	**Alessia Vignoli:** *Development of Low-Field NMR Procedures for Human Urine and Serum Metabolomics*
16:55 – 17:10	**Petra Vuković:** *Characterization of Plavac Mali Wine Variety by GC-MS, HPLC and NMR Spectroscopy*
17:10 – 17:35	**Marina Tišma:** *NMR Spectroscopy as a Key Tool for the Characterization of Lignocellulosic Polymers from Food Industry Side Streams*
17:35 – 17:55	**Mohammad Alwahsh:** *NMR-based Metabolomic Profiling of Treatment Responses in Androgen-Independent Prostate Cancer Using 2D And 3D Culture Models*
17:55 – 18:15	**SPONSOR – Imane Senoussaoui**, JEOL / SCAN

18:30	**WELCOME RECEPTION**

FRIDAY, JULY 10

CHAIR: Janez Plavec	
9:00 – 9:40	**Guido Pintacuda:** *In situ Magic-Angle Spinning NMR of membrane proteins: from vision to practice*
9:40 – 10:10	**Wiktor Koźmiński:** *New Methods for Multidimensional Experiments of Biomolecules.*
10:10 – 10:30	**Peter Podbevšek:** G-quadruplex Remodeling by Oxidative Damage
10:30 – 11:00	**COFFEE BREAK**
CHAIR: Klaus Zangger	
11:00 – 11:40	**Mate Erdelyi:** *Pushing the Limits of Characterising Weak Interactions in Solution*
11:40 – 12:10	**Enrico Ravera:** *NMR for Biomineralization*
12:10 – 12:40	**Patrick Fischer:** Mechanism of NFAT Transactivation Regulation by Phosphorylation
12:40 – 13:10	**Ivan Kodrin:** DFT-supported Conformational Analysis of Ferrocene Peptides
13:10 – 14:30	**LUNCH BREAK**
CHAIR: Dean Marković	
14:30 – 15:10	**István Timári:** *Insights into Carbohydrate-Lectin Molecular Interactions by NMR Spectroscopy*
15:10 – 15:30	**Iva Habinovec:** *LC-SPE/Cryo NMR as a Tool for Macrozone Purity Profiling and Structure Determination*
15:30 – 15:50	**Danijela Šimić:** *The Molecular Handshake: Mapping Macrolide-ribosome epitopes using tr-NOESY & STD-NMR*
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16:10 – 17:10	**COFFEE BREAK + POSTER SECTION**

17:10 – 19:30	**EXCURSION + WINE TASTING**

19:30 – …	**CONFERENCE DINNER**

SATURDAY, JULY 11

CHAIR: Nikola Bregović	
9:30 – 10:00	**Matija Grdičak:** *Reaction Profiling of Visible-light-mediated [2 + 1] Dearomatization of Electron-rich Arenes: Real-time NMR Monitoring*
10:00 – 10:20	**Nikolina Vidović:** *From Structure to Mechanism: The Role of NMR in Peptide Macrocyclization*
10:20 – 10:40	**Antonio Ljulj:** *NMR Study Of Chlorination Of Oxazaphosphorine Anticancer Agents*
10:40 – 11:10	**COFFEE BREAK**
CHAIR: Nikola Bregović	
11:10 – 11:30	**Gyula Batta:** *Antifungal Disulfide Proteins: Structures and Interactions by NMR and in Silico Methods*
11:30 – 11:50	**Ivan Jakovac:** *Insights Into the Magnetic Stucture of Matter*
11:50 – 12:10	**Alma Ramić:** *Synthesis and Characterization of Structurally Related Peptidomimetics*
12:10 – 12:30	**Dajana Barišić:** *From Supramolecular Handshakes to Molecular Tunnels: Controling Calix[4]Arene Self-assemblies*
12:30	**CLOSING**

LEGEND:

PLENARY LECTURES 40'	**INVITED LECTURES 30'**	**SECTION LECTURES 20'**	**SPONSORS 20'**

POSTER SESSION	
Friday, June 2, 16:10 – 17:10	
P01	**Libor Kobera, Valeriia Shabikova:** *Paramagnetic ^{1}H, ^{13}C and ^{19}F VF/MAS Solid-State NMR of SpiroOMeTAD doped with agTFSI*
P02	**Mohammad Alwahsh, Furqan Al-karawi, Aya Hasan, Rahaf Alejel, Lama Hamadneh, Tariq Al-Qirim, Rosemarie Marchan, Roland Hergenröder:** *NMR-Based Metabolomic Profiling of Treatment Responses in Androgen-Independent Prostate Cancer Using 2D and 3D Culture Models*
P03	**Laura Hanić, Jelena Parlov Vuković, Marina Tišma:** *Lignin Isolation From Hemp Pomace with Acid and Deep Eutectic Solvents*
P04	**Réka Pálma Erdei, Gyula Batta:** *NMR Investigation of Entropic Effects in Eremomycin–Ligand Interactions*
P05	**Ana Badenić, Petra Vuković, Ana-Marija Jagatić Korenika, Ivana Tomaz, Tomislav Jednačak, Predrag Novak:** *Characterization of "Graševina" and "Plavac Mali" Wine Varieties by NMR Spectroscopy, HPLC And GC-MS*
P06	**Karla Čargonja, Ana-Marija Jagatić Korenika, Tomislav Jednačak, Ivan Nemet, Nikolina Ilić, Kora Sertić, Franka Sunjka, Tomica Hrenar, Jakov Borovec, Predrag Novak:** *Characterization of Croatian Sparkling Wines by NMR and ICP-MS*
P07	**Gabriela Szczupaj, Andrzej Ejchart, Wiktor Koźmiński, Jacek Wójcik, Igor Zhukov, Michał Nowakowski:** *NMR and Computational Study of Natural Cyclodextrin Inclusion Complexes with Cinchona Alkaloids*
P08	**László Bence Farkas, Zsuzsanna Tardi, Tamás Gáti, István Timári:** *Mapping Ligand Binding Modes with Improved 2D Longitudinal ^{1}H Relaxation-based NMR Experiments*
P09	**Toni Divjak, Alma Ramić, Ines Primožič:** *Variable-temperature NMR Study of Amide Bond Rotation in α-Nicotinoylamino Acetamides*
P10	**Ivona Krznar, Jelena Parlov Vuković, Vanja Jurišić:** *Analysis of Lignocellulose Samples of Wild Millet, Miscanthus and Virginia Mellow by ^{1}H NMR Spectroscopy*
P11	**Franka Sunjka, Andrea Usenik, Karla Kukina Gradečak, Luka Klemen, Katarina Leko, Nikola Cindro, Gordan Horvat, Vladislav Tomišić:** *Conformational Mobility of a Calix[6]arene Derivative and Its Cation Complexes Studied by NMR Spectroscopy*
P12	**Danijela Šimić, Alma Ramić, Iva Habinovec, Tomislav Jednačak, Predrag Novak:** *Interactions of 9a-tetrahydrofurfuryl-macrozone with E. coli Ribosome Studied By NMR Spectroscopy*
P13	**Andrea Usenik, Stella Katovčić, Josip Požar:** *Inclusion of Propofol in β-cyclodextrin Derivatives: Thermodynamic and NMR Insights*

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PLENARY LECTURES

PUSHING THE LIMITS OF CHARACTERISING WEAK INTERACTIONS IN SOLUTION

Manuel A. Herbst,[a] Stefan Peintner,[a] Emma Danelius Rova,[b] Yannik T. Woordes,[a] Hanna Andersson,[b] Ulrika Brath,[b] Mate Erdelyi,[a,c]

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Noncovalent interactions are as important for chemistry and biology as they are cumbersome to characterize in dilute solutions. Standard NMR spectroscopic techniques, such as chemical shift perturbation experiments, are typically incapable of their reliable characterization, and often even of their detection.

I present a strategy[1-4] that expands the current boundaries of the NMR spectroscopic characterization of noncovalent interactions in solution, and demonstrate its scope by the characterization of weak, <2 kcal/mol, halogen bonds in a dilute (< 5 mM) solutions. This new strategy is universally applicable to the geometric and thermodynamic characterization of any type of non-covalent interactions, in any solvent.

This approach makes use of the entropic advantage of studying a weak force intramolecularly, embedded into a cooperatively folding system (Figure 1), and of the combined use of NOE- and RDC-based ensemble analyses.

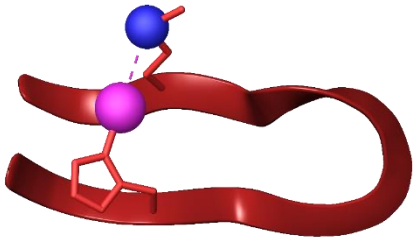

Figure 1. A cooperatively folding β-hairpin model system designed to investigate a single weak noncovalent interaction in solution using NOE and RDC-based ensemble analyses.

Acknowledgements. We thank the European tax payers for financial support via the Swedish Research Council (2024-05496, 2022-06628, 2020-03431) and NSFC–STINT (82211530060). We are grateful to NMR Uppsala and SwedNMR (VR 2021–00167) as well as to NAISS (2025/5-155, VR. 2022-06725) for access to NMR instrumentation and to supercomputers, respectively.

REFERENCES

[1] M.A. Herbst, L. Wu, Y.T. Woordes, S. Peintner, J. Xin, F.M. Boeckler, Z. Xu, A. Navarro-Vázquez, M. Erdelyi, *J. Am. Chem. Soc.* **2026**, 148, 19331-19340

[2] S. Peintner, M. Erdelyi, *Chem. Eur J.* **2022**, 28, e202103559A.

[3] E. Danelius, H. Andersson, P. Jarvoll, K. Lood, J. Gräfenstein, M. Erdelyi, *Biochemistry* **2017**, *56*, 3265-3272.

[4] E. Danelius, U.; Brath, M. Erdelyi, *Synlett*, **2013**, 24, 2407-2410.

INSIGHTS INTO CARBOHYDRATE-LECTIN MOLECULAR INTERACTIONS BY NMR SPECTROSCOPY

László Bence Farkas,[a] Zsuzsanna Tardi,[a] Samar Alnukari,[a] Tünde Zita Illyés,[a] Zoltán Kónya,[b] Anikó Borbás,[c] Zita Képes,[d] Anikó Fekete,[d] István Timári[a]

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Carbohydrates act as critical mediators in cellular signaling and recognition, primarily through interactions with carbohydrate-binding proteins known as lectins. The main target lectins of our recent research work are *human* Galectin-3 (*h*Gal-3), which controls tumor progression and contributes to tumor cell survival, and LecA, produced by a Gram-negative bacterium *Pseudomonas aeruginosa*, which causes chronic pulmonary infections. Since many tumor types express Gal-3, such as melanoma, colon cancer, prostate cancer, and breast cancer, this protein may be considered a suitable diagnostic and therapeutic target for anticancer research. For example, thiodigalactoside (TDG) and its derivatives have been successfully used in the clinical treatment of diseases associated with *h*Gal-3.

As a step towards developing novel *h*Gal-3 inhibitors, we investigated three derivatives of TDG modified with different aralkyl-substituents using NMR spectroscopy and molecular docking calculations. For cancer diagnostic purposes, we designed and synthesized a ^{68}Ga-labeled DOTA chelator-conjugated (2-naphthyl)methylated lactosamine derivative, a promising positron emission tomography (PET) diagnostic agent. The binding of this potential diagnostic agent to *h*Gal-3 protein has been investigated by multiple NMR methods. The binding affinity (K_d) of the compound was determined by competitive Saturation Transfer Difference (STD) ^{1}H NMR experiment using TDG as a reference. Chemical shift perturbation induced by the studied compound in ^{1}H-^{15}N HSQC NMR spectra of ^{15}N-labeled *h*Gal-3 protein has provided residue-specific information allowing to map the binding site. Moreover, *ex vivo* biodistribution and *in vivo* PET studies have indicated that our carbohydrate-based radiopharmaceutical is suitable for the highly selective imaging of *h*Gal-3-expressing tumors by PET.

To inhibit LecA, four thiogalactoside-isochroman derivatives were studied. The selected compounds, exhibiting the highest binding affinities in the series synthesized according to the competitive fluorescence polarization binding assays. STD ^{1}H NMR experiments were performed to identify the atomic groups involved in binding to the LecA protein. The STD NMR results were compared with molecular docking simulations, revealing a good agreement between the experimentally determined binding epitopes and the predicted ligand binding modes.

Acknowledgements. This work has been supported by the KDP-2023 Program (C2305886 to L.B.F. and I.T.) of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund. I.T. is also thankful to the GALECTINVEST 2024-1.2.3-HU-RIZONT-2024-00005 project and the Stipendium Hungaricum Programme.

INVITED LECTURES

DERIVING THREE ONE DIMENSIONAL NMR SPECTRA FROM A SINGLE EXPERIMENT THROUGH MACHINE LEARNING

Leonardo Tenori,[a,b] Alessia Vignoli,[a,b] Stefano Cacciatore[c]

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Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for analyzing complex mixtures due to its ability to manage matrix complexity, provide detailed molecular insights, and preserve sample integrity. Various NMR experiments, such as NOESY, CPMG, diffusion-edited, and J-resolved spectroscopy (JRES), offer complementary insights into biofluids like serum and plasma. For instance, CPMG selectively detects small molecules, diffusion-edited emphasizes signals from macromolecules, and NOESY captures both, and JRES is particularly useful for signal assignment. However, acquiring multiple NMR spectra can be resource-intensive and time-consuming, especially for high-throughput studies. Here, we present a simple and efficient strategy to computationally derive CPMG, diffusion-edited, and projected JRES (pJRES) spectra from a single NOESY acquisition using Partial Least Squares (PLS) regression. Serum samples were used as a case study. We used serum NMR data from a total of 1842 individuals enrolled from 18 recruitment centers. The ^{1}H-NMR spectra for all samples were recorded using a Bruker 600 MHz spectrometer operating at 600.13 MHz. The dataset, comprising serum spectra of samples collected in 17 different recruitment centers, was divided into training (80%) and validation (20%) sets. Furthermore, the spectra of 232 samples from one independent recruitment center were used as the independent test set. Predictive models were created applying PLS regression with 1D NOESY spectra used as independent variables for the prediction of CPMG, diffusion-edited, and pJRES spectra, these latter used as dependent variables. Experimental and predicted spectra were compared in regions with signal intensities at least three times above the noise level. Evaluation metrics included the median relative error (MRE%), root mean square error (RMSE), coefficient of determination (R^2), and ratio of performance to deviation (RPD). In the independent test set, MRE% values of 6%, 4%, and 13% were achieved for predicted CPMG, diffusion-edited, and pJRES spectra, respectively[1]. A future direction is the application of this approach to NMR spectra obtained by low field instruments (i.e. 100 MHz benchtop spectrometer).

Acknowledgements. This work has been supported by Next Generation EU [DM 1557 11.10.2022], in the context of the National Recovery and Resilience Plan, M4C2 Investment 1.3 - Research Program PE8 Project Age-It: "Ageing Well in an Ageing Society" (CUP B83C22004800006). The authors also acknowledge funding from the Italian Ministry of University and Research through the Italian Science Fund under the 2022–2023 Call (FIS 2), project "Benchtop NMR: Translating Metabolomics from High-Tech Laboratories to Point-of-Care Diagnostics (BONSAI)" (Contract Number FIS-2023-00334).

REFERENCES

[1] A. Vignoli, S. Cacciatore, L. Tenori, *Nat Commun* **2025**, *16*, 10159.

NMR FOR BIOMINERALIZATION

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Hen egg-white lysozyme promotes the formation of condensed silica microparticles from silicic acid solutions under milder conditions than the standard sol-gel synthesis [1]. A similar chemistry appears to be applicable to titania and other IV-oxidation state oxides. However, little is known about the mechanism of the reaction, and even less so about the fate of the protein after the reaction is completed.

Over the past years, we applied a wide range of structural biology methodologies - including MAS-NMR and spin-labelling EPR - for understanding the interaction between the protein and theprecursor [2], and for studying the protein-silica interface [3-7]. Our results indicate that the nature of the protein-precursor interaction is mainly electrostatic in nature, and this has been recently confirmed by molecular simulations [8]. After the reaction, the main contribution to the confinement of the protein within the composite is steric, even though electrostatics provide some orientational preference.

We have also investigated the initial phases of mineralization using a variety of biophysical techniques.

Acknowledgements. This work has been supported by Project no. IR0000009 - call MUR 3264/2021 PNRR M4/C2/L3.1.1 and Project no. P2022JSC5Z – call MUR 1409/2022 PNRR M4/C2/L1.1 CUP D53D23016850001 (European Union NextGenerationEU)

REFERENCES

[1] Heather R Luckarift et al., Small, 2006, 2 (5), 640–643
[2] Lucia Gigli et al., Biomolecules, 2021, 11 (1), 43
[3] Enrico Ravera et al., ChemPhysChem, 2015, 16 (13), 2803-2809
[4] Francesco Bruno et al., Langmuir, 2022, 38 (26), 8030-8037
[5] Francesco Bruno et al., Journal of Composites Science, 2023, 7, (5), 188
[6] Francesco Bruno et al., Analytical Chemistry, 2020, 92 (6), 4451-4458
[7] Francesco Bruno et al., Magnetic Resonance in Chemistry, 2023, 61, 373-379
[8] Marina Macchiagodena et al., Chemistry – A European Journal, 2024, in press

MECHANISM OF NFAT TRANSACTIVATION REGULATION BY PHOSPHORYLATION

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The transcription factor NFAT is a central regulator of immune-cell activation. While phosphorylation-dependent nuclear import and export of NFAT are well established, much less is known about how phosphorylation directly regulates NFAT transactivation. This is difficult to address structurally, because key regulatory elements are embedded in intrinsically disordered regions.

Here, we used solution NMR spectroscopy to investigate how single-site phosphorylation regulates the N-terminal transactivation domain of NFAT1. We show that residues 23–41 form a phosphorylation-sensitive transactivation element that binds the KIX domains of CBP/p300 and Med15. Phosphorylation of Ser23 by p38 leads to long-range perturbations throughout the disordered NFAT fragment, indicating that this modification reshapes the conformational ensemble beyond the local phosphorylation site.

Together, our data support a model in which a single phosphorylation event regulates NFAT transactivation by modulating the ensemble, dynamics, and coactivator binding of an intrinsically disordered activation domain.

DFT-SUPPORTED CONFORMATIONAL ANALYSIS OF FERROCENE PEPTIDES

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Ferrocene-containing peptides represent a versatile class of peptidomimetics in which the organometallic scaffold can restrict conformational freedom, promote intramolecular hydrogen bonding, and induce well-defined turn-like or helical arrangements. Because these systems often exist as ensembles of closely related conformers in solution, their structural characterization requires a combined experimental and computational approach. In this contribution, an overview of recent work is provided, in which conformational analysis based on density functional theory was used to support and refine spectroscopic assignments of ferrocene peptide derivatives. Particular emphasis will be placed on how calculated conformer populations, hydrogen-bonding patterns, and key torsional parameters were correlated with NMR data, including characteristic amide proton chemical shifts, temperature and solvent dependence, and other complementary structural information. The discussed examples cover structurally diverse ferrocene peptidomimetics, ranging from mono- and disubstituted ferrocene conjugates bearing Pro/Ala and other amino acid sequences to systems incorporating hydrophobic residues. Across these families, computational studies helped distinguish between competing intra- and interstrand hydrogen-bonded motifs, including γ-turns, β-turns, and related folded structures. In parallel, TD-DFT calculations of circular dichroism spectra provided a molecular-level interpretation of the ferrocene-based chiroptical response near 470 nm, linking the sign and intensity of the Cotton effect to the preferred geometry of the peptide–ferrocene fragment. Overall, these studies illustrate how computational chemistry can complement NMR spectroscopy in the structural characterization of conformationally flexible bioorganometallic systems.

Acknowledgements. This research was supported by the European Union – NextGenerationEU through the National Recovery and Resilience Plan 2021-2026 via Institutional grants of the University of Zagreb Faculty of Science (*NextGenChem* and *CryoLab*).

REFERENCES

[1] M. Kovačević, M. Čakić Semenčić, M. Bagović, K. Radošević, K. Hanousek Čiča, J. Mrvčić, K. Molčanov, S. Roca, I. Kodrin, L. Barišić, *Organometallics* **2024**, 43, 2608–2625.

[2] M. Nuskol, P. Šutalo, M. Kovačević, I. Kodrin, M. Čakić Semenčić, *Inorganics* **2023**, 11, 225.

REACTION PROFILING OF VISIBLE-LIGHT-MEDIATED [2 + 1] DEAROMATIZATION OF ELECTRON-RICH ARENES: REAL-TIME NMR MONITORING

Matija Grebičak

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The concept of dearomatization has steadily gained significance in organic synthesis, offering a direct route to transform abundant aromatic feedstocks into more complex carbocyclic structures without requiring extensive functional group interconversions. Alongside well-established methodologies, the use of visible light has recently emerged as a dependable strategy, finding broad application across a range of dearomatization reactions.

A detailed mechanistic and kinetic study of the dearomative [2 + 1] reaction between electron-rich arenes and aryldiazoesters is presented, enabling the regio- and diastereoselective synthesis of norcaradienes. Reaction profiling *via* LED-NMR analysis provided valuable kinetic insight into both product formation and competing side processes, while theoretical investigations clarified how the electron-density distribution governs cyclopropanation versus alkylation in the key dearomative step. The synthetic potential of the resulting *meso*-norcaradienes is demonstrated through their desymmetrization into compounds bearing six contiguous stereogenic centers.

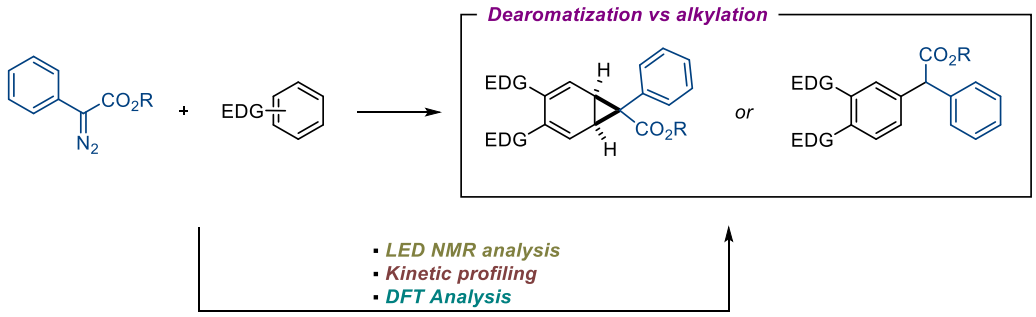

Figure 1. Prediction of the chemoselectivity based on the electron distribution.

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REFERENCES

[1] K. Petrić, A. Čikoš, I. Nikšić-Franjić, G.C. Lloyd-Jones,* M. Grebičak,* N. Topolovčan* *Org. Chem. Front.* **2026**, 13, 2794–2804.

SECTION LECTURES

DEVELOPMENT OF LOW-FIELD NMR PROCEDURES FOR HUMAN URINE AND SERUM METABOLOMICS

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Metabolomics is a promising research field which has been successfully applied in biomedical research for personalized medicine, biomarkers identification, diagnosis, and prognosis. High-field Nuclear Magnetic Resonance spectroscopy (NMR) is one of the main analytical techniques for metabolomics[1]; however, the high experimental costs, particularly those related to analytical instrumentations, prevent its translation in clinical practice. Recently developed Low-Field (LF) NMR spectrometer holds promise for a more accessible format that could break down the cost barrier enabling their application in nearly any settings[2].

We developed specific procedures for the preparation and acquisition of human urine and serum samples using a LF NMR spectrometer. Biospecimens were lyophilized, reconstituted in deuterated water, and supplemented with 10% (v/v) potassium phosphate buffer. Samples were acquired at 100 MHz using a WET (Water Suppression Enhanced through T^1 effects) NMR pulse sequence.

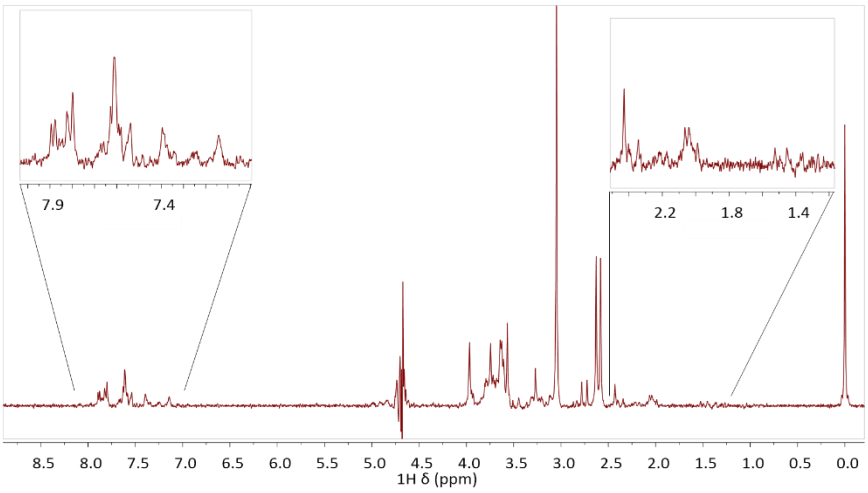

Figure 1. ^{1}H NMR spectrum of a urine sample acquired at 100 MHz.

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REFERENCES

[1] A. Vignoli, V. Ghini, G. Meoni, C. Licari, P. G. Takis, L. Tenori, P. Turano, C. Luchinat, *Angew. Chem. Int. Ed.* **2019**, 58, 968–994.

[2] L. Fantato, M. Salobehaj, J. Patrussi, G. Meoni, A. Vignoli, L. Tenori, *Metabolites* **2026**, 16, 3.

CHARACTERIZATION OF PLAVAC MALI WINE VARIETY BY GC-MS, HPLC AND NMR SPECTROSCOPY

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Plavac mali is the most famous red wine variety in Croatia, planted in Dalmatia region. Chemical and sensory profile of a specific wine vary depending on winemaking techniques, micro-locations and specific terroir.[1] In this study, gas chromatography coupled with mass spectrometry (GC-MS), HPLC and NMR spectroscopy were used to determine characteristic wine components such as volatile organic compounds, organic acids, proanthocyanidins (tannins), phenols, esters, thiols and other metabolites.[2]

Nuclear magnetic resonance spectroscopy (NMR) is an advanced analytical tool that provides information on identity of metabolites present in wine and their quantification.[3,4] The advantages of NMR spectroscopy include rapid and simple sample preparation, fast measurement and high repeatability, non-destructive analysis, and the possibility of obtaining wealth of information from a single spectrum.[3,4] In this presentation a combination of the above mentioned techniques for characterization of Plavac mali will be discussed. This research is a part of the project NMR-WIN which aims to establish a database of authentic Croatian wines providing reliable quality control, authenticity assessment and determination of geographical origin.

Acknowledgements. This work has been supported by the project NMR-WIN (NPOO.C3.2.R3-I1.06.0211)

REFERENCES

[1] D. I. Žeravica, I. Tomaz, D. Preiner, I. Šikuten, D. Stupić, A. Jeromel and E. Maletić, *Beverages* **2025**, 87, 11030087.

[2] I. Tomaz, I. Šikuten, V. Tuščić, N. Rendulić, D. Preiner, N. Buljević, A.-M. Jagatić Korenika, A. Jeromel, OENO One **2024**, 58(2), 1-13.

[3] A.-M. Jagatić Korenika, A. Jeromel, I. Tomaz, T. Jednačak, S. Rončević, I. Nemet, I. Primožič, T. Hrenar, P. Novak, *Food Chem.: X* **2024**, 21, 101162.

[4] J. Parlov Vuković, T. Jednačak, L. Divjak, K. Smetko, M. Tišma, A.-M. Klarić, A.-M. Jagatić Korenika, A. Bucić-Kojić, P. Novak, *Croat. Chem. Acta* **2025**, 98(2), 127–132.

NMR SPECTROSCOPY AS A KEY TOOL FOR THE CHARACTERIZATION OF LIGNOCELLULOSIC POLYMERS FROM FOOD INDUSTRY SIDE STREAMS

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The growing demand for sustainable resources and circular bioeconomy solutions has intensified interest in the valorization of lignocellulosic side streams generated by the food and beverage industry. Large quantities of residues, including oilseed cakes, brewer's spent grain, grape pomace, sugar beet pulp, and fruit and vegetable processing by-products, are generated annually across Europe and represent an abundant and renewable resource for the production of high-value bio-based products. However, both the abundance and structural features of the major lignocellulosic polymers, cellulose, hemicelluloses, and lignin, vary substantially among biomass sources. Since these differences largely determine biomass processing behavior and valorization potential, detailed molecular characterization is essential for selecting appropriate biorefinery strategies and developing targeted bio-based products. Among the available analytical techniques, nuclear magnetic resonance (NMR) spectroscopy has emerged as one of the most powerful and versatile tools for investigating biomass-derived polymers at the molecular level.

This contribution presents recent research on the isolation and structural characterization of lignin and other lignocellulosic fractions recovered from food industry side streams using both conventional and green fractionation approaches, including deep eutectic solvents (DESs). Advanced NMR techniques were employed to elucidate the molecular architecture of the isolated biopolymers, providing detailed information on monomer composition, interunit linkages, and structural changes induced by biomass fractionation and biopolymer isolation. The results highlight the value of NMR spectroscopy in revealing the structural diversity of biopolymers derived from different food industry residues and in supporting their targeted valorization into high-value bio-based products.

Acknowledgements. This work was carried out within the Project of the Croatian Science Foundation "Valorisation of residues from the vegetable oil industry based on a biorefinery approach—VALREO) (HRZZ-IPS-2023-02- 6614).

NMR-BASED METABOLOMIC PROFILING OF TREATMENT RESPONSES IN ANDROGEN-INDEPENDENT PROSTATE CANCER USING 2D AND 3D CULTURE MODELS.

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Background: Prostate cancer (PC) remains one of the most prevalent solid malignancies worldwide, with a steadily increasing incidence. Despite the availability of multiple therapeutic strategies, there is a pressing need to develop more effective treatment approaches alongside advanced in vitro models that more accurately recapitulate the in vivo tumor microenvironment.

Objectives: In this study, the primary focus was on optimizing in vitro modeling systems by developing and comparing two-dimensional (2D) and three-dimensional (3D) cell culture models to better understand cellular behavior, therapeutic response, and resistance mechanisms. Nuclear magnetic resonance (NMR) spectroscopy was incorporated to investigate treatment-associated metabolic reprogramming in both culture systems.

Methods: Human Prostate cancer cell lines (PC3 and DU145) were treated with bortezomib (Bor), doxorubicin (Dox), and treosulfan (Treo) as monotherapies and in combination to evaluate differential responses across models. Furthermore, the plasma metabolic profiles were analyzed using 1H NMR spectrometer equipped with a cryogenic probe. NMR-based metabolomics profiling was employed to characterize metabolic alterations associated with treatment response in both 2D and 3D systems. This study also investigated the gene expression profiles of key regulatory genes in response to Bor, Dox, and Treo, in 2D and 3D cell culture, administered individually and in combination. Changes in gene expression levels of key regulatory genes using RT-PCR.

Results: In the 3D model, cells exhibited greater resistance to treatment, requiring higher concentrations compared to the 2D model across both cell lines for single and combination therapies. Additionally, PC3 and DU145 cell lines demonstrated higher sensitivity to Bor and Dox, respectively, compared to Treo in both culture systems, as reflected by IC_{50} values. Combination ratios and combination index (CI) values were determined for both 2D and 3D models. NMR-based metabolomic profiling was employed to characterize metabolic alterations across all experimental conditions using both univariate and multivariate data analyses. The results revealed distinct metabolic signatures associated with different treatments and cell lines. PCR analysis showed a significant change in the expression of several genes correlating with the observed metabolic changes.

Conclusions: By identifying a panel of potential metabolic biomarkers, this study identifies key metabolic biomarkers and demonstrates that integrating gene expression with NMR-based metabolomics provides valuable insight into treatment-induced metabolic changes, helping to better understand drug response and resistance in cancer models.[1][2]

Keywords: Prostate cancer, two-dimensional cell culture, three-dimensional (3D) cell culture, NMR-based metabolomics.

REFERENCES

[1] M. Alwahsh, Y. Hamadneh, R. Marchan, L. A. Dahabiyeh, A. A. Alhusban,A. Hasan, ... L. Hamadneh, *Journal of Cancer* **2024**, *15*(*13*), 4047.
[2] M. Alwahsh, O. H. Abusara, A. A. Zaid, A. Hasan, L. Hamadneh, R. Marchan, *Cancer Treatment and Research Communications* **2026**, *47*, 101133.

G-QUADRUPLEX REMODELING BY OXIDATIVE DAMAGE

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Guanine possesses the lowest redox potential among the four DNA nucleobases and is therefore particularly susceptible to oxidative damage. This vulnerability is further amplified in guanine-rich regions that form G-quadruplex structures. Among the oxidation products of guanine, 8-oxo-7,8-dihydroguanine (8-oxoG) is one of the most abundant lesions and a widely used biomarker of oxidative stress. Owing to its altered hydrogen-bonding properties, incorporation of 8-oxoG generally disrupts DNA secondary structure and often destabilizes G-quadruplexes.[1]

8-oxoG can also participate in fully substituted G-quartets, altering the dimensions of the central channel within G-quadruplexes and affecting cation coordination.[2] However, we also identified a biologically relevant G-quadruplex that became markedly stabilized upon incorporation of oxidative lesions, revealing an unexpected level of complexity in the folding landscape of telomeric and promoter G-rich sequences.[3]

Many DNA-processing enzymes translocate along DNA but can be hindered by stable secondary structures such as G-quadruplexes. To investigate how oxidative lesions influence enzyme progression, we employed a polymerase stop assay to examine the effects of structural features in human telomeric and BCL2 promoter G-quadruplexes on the activity of the Klenow fragment.[4] Primer extension profiles revealed that G-quartets, planar assemblies of four guanines, constitute major barriers to enzyme progression, whereas auxiliary base-paired elements are bypassed more readily.

Together, these findings indicate that oxidative lesions can exert diverse and context-dependent effects on G-quadruplex structure and stability, with potential consequences for the recognition, repair, and biological processing of damaged DNA.

Acknowledgements. This work has been supported by the Slovenian Research and Innovation Agency (ARIS, grant P1-0242) and CERIC-ERIC.

REFERENCES

[1] S. Bielskutė, J. Plavec, P. Podbevšek, *J. Am. Chem. Soc.* **2019**, 141, 2594–2603.

[2] S. Aleksič; P. Podbevšek, J. Plavec, *Nucleic Acids Res.* **2025**, 53, gkaf260.

[3] S. Bielskutė, J. Plavec, P. Podbevšek, *Nucleic Acids Res.* **2021**, 49, 2346–2356.

[4] P. Podbevšek, J. Plavec, *Nucleic Acids Res.* **2025**, 53, gkae1157.

LC-SPE/CRYO NMR AS A TOOL FOR MACROZONE PURITY PROFILING AND STRUCTURE DETERMINATION

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Increasing bacterial resistance trends to marketed antibiotics, especially those of Gram-negative bacterial pathogens, pose a growing threat to human health globally. Therefore, there is an urgent need for discovery of new anti-infectives to combat resistance.[1] Recently discovered amide conjugates of azitromycin and thiosemicarbazide derivatives, the macrozones, represent one such class that exhibits promising antibacterial activity against susceptible and resistant bacterial strains. [2] In this research, we employed an approach which combined LC-SPE/cryo NMR, MS/MS and molecular modeling for efficient separation, isolation and characterization of bioactive macrozones and process-related impurities.[3] Identified impurities were classified as macrolide and non-macrolide related by-products and degradation products, as well as macrozone diastereomers. Configurations of diastereomers were proposed on the basis of NOESY NMR spectroscopy data and molecular dynamic simulations.[4] Furthermore, differences in biological activity among diastereomers were detected. LC-SPE/cryo NMR methodology served as a reliable, efficient and sensitive platform for the analysis of complex reaction mixtures, with significantly less sample and solvent consumption compared to standard *off-line* techniques.

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REFERENCES

[1] World Health Organization, WHO: Global antibiotic resistance surveillance report 2025 – summary (https://www.who.int/health-topics/antimicrobial-resistance)

[2] T. Jednačak, V. Stepanić, I. Habinovec, I. Mikulandra, K. Smokrović, H. Čipčić Paljetak, M. Bukvić, J. Parlov Vuković, I. Grgičević, L. Divjak, K. Zangger, P. Novak, ADMET&DMPK **2026**, 3139.

[3] I. Habinovec, I. Mikulandra, L. E. Sekula, J. Gašperov, S. Kazazić, P. Novak, *Molecules* **2021**, 26, 26206316.

[4] I. Habinovec, I. Mikulandra, P. Pranjić, S. Kazazić, H. Čipčić Paljetak, B. Bertoša, A. Barišić, M. Bukvić, P. Novak, *Antibiotics* **2022**, 11, 1738.

INTERACTIONS OF 9A-TETRAHYDROFURFURYL-MACROZONE WITH *E. COLI* RIBOSOME STUDIED BY NMR SPECTROSCOPY

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Macrozones are novel compounds formed by conjugating azithromycin with thiosemicarbazones. Azithromycin, a 15-membered macrolide antibiotic, beside anti-infective activity also posseses favorable pharmacokinetics, including high cellular accumulation and stability. However, the global rise of antibiotic resistance highlights the need for new macrolide compounds with improved antibacterial effect. In this study, macrozone 9a derivatives of azithromycin were purified by semi-preparative HPLC and characterized using 1D and 2D NMR spectroscopy. Furthermore, interactions of 9a-tetrahydrofurfuryl-macrozone with the *E. coli* ribosome were studied by using ^{1}H WATERLOGSY, tr-NOESY and STD NMR, allowing for determination of the binding epitopes.

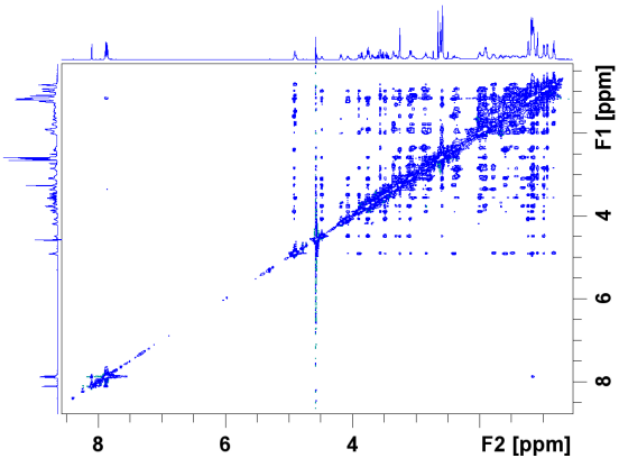

Figure 1. Transferred NOESY spectrum of the ribosome-bound macrozone.

Acknowledgements. This work has been supported by Croatian Science Foundation, grant number HRZZIP-2018-01-8098, The macrozones

REFERENCES

[1] I. Mikulandra, I. Habinovec, T. Jednačak, P. Novak, *Croat. Chem. Acta* **2026**, 99(2).

[2] T. Jednačak, I. Mikulandra, K. Smoković, A. Hloušek-Kasun, M. Kapustić, K. Delaš, I. Piantanida, M. Jurković, B. Bertoša, K. Zangger, P. Novak, *Bioorg. Chem.* **2024**, 147, 107338.

[3] I. Mikulandra, T. Jednačak, B. Bertoša, J. Parlov Vuković, I. Kušec, P. Novak, *Materials* **2021**, 14, 5561.

[4] I. Grgičević , I. Mikulandra , M. Bukvić, M. Banjanac, V. Radovanović, I. Habinovec, B. Bertoša, P. Novak, *Int. J. Antimicrob. Agents* **2020**, 56, 106147.

FROM STRUCTURE TO MECHANISM: THE ROLE OF NMR IN PEPTIDE MACROCYCLIZATION

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Cyclic peptides represent an important class of bioactive compounds due to their enhanced metabolic stability, bioavailability, and improved affinity toward receptors when compared to their linear analogues.[1–2] However, efficient macrocyclization of short all-*L* peptides remains a major synthetic challenge because successful ring closure requires conformational preorganization of the linear precursor.[3] In this work, NMR spectroscopy was employed as the principal tool to elucidate the mechanism of chloride-assisted peptide macrocyclization.[4]

A series of linear peptide precursors and their cyclic analogues were synthesized and characterized by NMR spectroscopy. While initial ^{1}H NMR titrations showed no significant interaction of the linear peptides with alkali metal cations in DMF, the addition of chloride ions induced pronounced downfield shifts of backbone amide NH signals, demonstrating chloride binding. Stability constants determined by NMR were in excellent agreement with those obtained by isothermal titration calorimetry. Furthermore, 2D NOESY experiments revealed that chloride binding promotes conformational preorganization of the linear precursors by bringing the peptide termini into close spatial proximity, thereby facilitating macrocyclization.

The proposed mechanism was further supported by kinetic studies, which showed that increasing chloride concentration accelerates the macrocyclization reaction and improves product yields, whereas chloride sequestration significantly suppresses the reaction. Molecular dynamics simulations corroborated the experimental findings by demonstrating stable chloride coordination through backbone amide NH groups, resulting in conformations favorable for ring closure.

Together, these results identify chloride, rather than alkali metal cations, as the key templating species in peptide macrocyclization and demonstrate how NMR spectroscopy provides mechanistic insight that enables the rational development of efficient synthetic methodologies for cyclic peptide preparation.

REFERENCES

[1] Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discov.* **2008,** *7*, 608–624. https://doi.org/10.1038/nrd2590.

[2] Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. *Chem. Biol. Drug Des.* **2013,** *81*, 136–147. https://doi.org/10.1111/cbdd.12055.

[3] White, C. J.; Yudin, A. K. *Nat. Chem.* **2011,** *3*, 509–524. https://doi.org/10.1038/nchem.1062

[4] Vidović, N.; Horvat, G.; Riva, D.; Rinkovec, T.; Cindro, N.; Tomišić, V.; Speranza, G. *Org. Lett.* **2020,** *22*, 2129–2134. https://doi.org/10.1021/acs.orglett.0c00036

NMR STUDY OF CHLORINATION OF OXAZAPHOSPHORINE ANTICANCER AGENTS

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Oxazaphorine pharmaceuticals (cyclophosphamide (**CPA**), ifosfamide (**IFO**), and their metabolites) are frequently present in aqueous environment. When hospital and/or wastewaters are treated by free chlorine, oxidation product are formed, some of which are more ecotoxic than the parents. The reaction may be followed by time-resolved ^{1}H and ^{31}P NMR spectroscopy. Upon *N*-chlorination, **CPA** exhibits a uniform downfield shift of its NMR resonances (^{1}H, ^{13}C, and ^{31}P), consistent with net deshielding induced by introduction of an electronegative substituent. In chlorinated ifosfamide, however, the ^{31}P signal shifts markedly upfield relative to the parent **IFO** (Figure). This opposite ^{31}P response cannot be rationalized by a simple inductive argument and instead points to a dominant conformational contribution to the phosphorus shielding. To explain this effect, we performed a full conformational analysis coupled to NMR-GIAO ^{31}P chemical shift calculations for all species involved.

Interestingly, chlorination affects the two isomers differently. For **CPA**, *N*-chlorination preserves the same conformational preference: the axial P=O conformer remains the dominant population in **CPA-Cl**. In contrast, chlorination of **IFO** induces a reversal of conformational preference, rendering the quasi-equatorial P=O conformer most stable for **IFO-Cl** (Figure). This chlorination-driven conformer redistribution provides a natural explanation for the divergent ^{31}P shifts, i.e. the conformational switch governs the sign and magnitude of the ^{31}P chemical shift response.

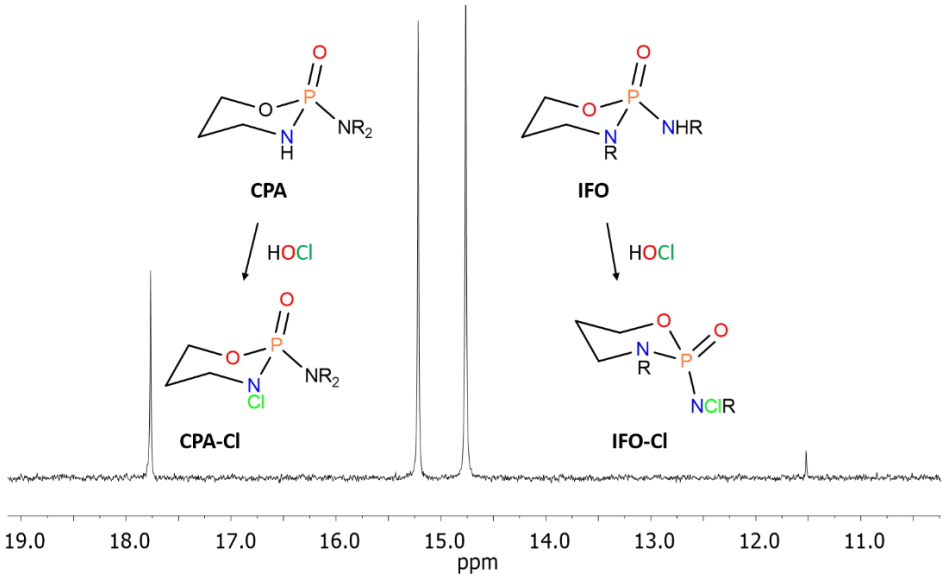

Figure. ^{31}P NMR of reaction mixture of **CPA/IFO** and HOCl in D_2O. R = -CH_2CH_2Cl.

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ANTIFUNGAL DISULFIDE PROTEINS: STRUCTURES AND INTERACTIONS BY NMR AND IN SILICO METHODS

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Understanding the structure of antifungal proteins (AFPs) is crucial for understanding their antifungal mechanisms. We used different AlphaFold (AF) versions to predict the 3D structures of six different AFPs. We found that both AF2 and AF3 models outperform the NMR models in overall structural quality, while AF3 showed the best overall performance. AF3 accurately predicts all disulfide bond patterns, while AF2 is less efficient for pattern identification. We searched possible protein interactions of PAFA, PAFγopt and NFAP2 with candidate targets from *Candida albicans, Aspergillus fumigatus* and *Aspergillus nidulans.* To this end protein–protein docking was applied using HADDOCK and ClusPro. The top-ranked complexes were further refined through molecular dynamics simulations with AMBER and NAMD. In summary, PAFγopt formed stable complexes with the soluble FTase and GGTase I (and also AusH) proteins, whereas PAF and NFAP2 yielded only labile or no complexes with the same targets.

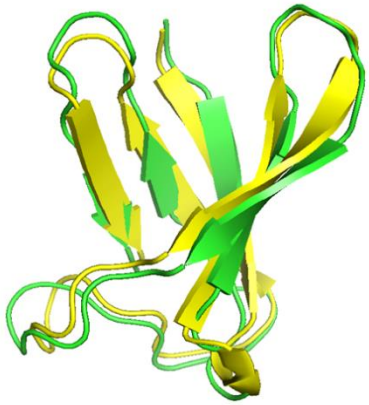

Figure 1. PAF protein (NMR, **2mhv,** green), AlphaFold2 (yellow)[1], RMSD= 1.5 Å

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REFERENCES

[1] J. Gai, M. File, R. Erdei, A. Czajlik, F. Marx, L. Galgóczy, Váradi, G. Batta, *Int. J. Mol. Sci.* **2025,** 26, 1247

[2] M. File, J. Gai, Z. Gáspári, L. Galgóczy, G. Batta, *Submitted,* **2026,**

INSIGHTS INTO THE MAGNETIC STUCTURE OF MATTER

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Magnetic order emerges in many forms, ranging from conventional ferromagnets to antiferromagnets (AFM) and the recently discovered class of altermagnets. Understanding the microscopic structure and dynamics of these states is essential for both fundamental physics and practical applications, including spintronics and magnetic information storage. Magnetism also frequently coexists with and interacts with other collective quantum phenomena, notably unconventional superconductivity.

Experimental techniques commonly used to study magnetic materials, including magnetometry and neutron scattering, face limitations in sensitivity or resolution. Broadband nuclear magnetic resonance (NMR) provides a powerful complementary approach, offering microscopic insight into both magnetic structure and low-energy excitations.

In this presentation, I will discuss a few case studies that highlight the versatility of NMR spectroscopy and relaxometry: the quasi-one-dimensional system BoNO, the cubic heavy-fermion compound $Ce_3Pd_{20}Si_6$, the frustrated kagome-lattice material $Cs_2Cu_3SnF_{12}$, and the altermagnetic pyrite $MnTe_2$. Together, these examples demonstrate how NMR can reveal the microscopic behavior of magnetic materials across a wide range of temperatures, magnetic fields, and mechanical strain, providing unique insight into emerging quantum magnetic phenomena.

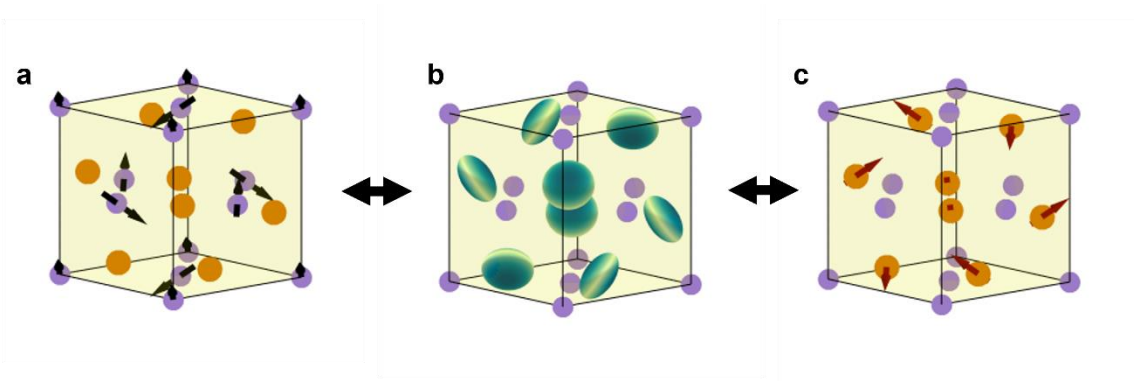

Figure 1. Antiferromagnetic arrangement of magnetic moments on manganese ions (purple) in pyrite $MnTe_2$ (panel **a**). The strong hyperfine coupling (panel **b**) to tellurium sites (yellow, $\gamma(^{125}Te)$ = 13.454 MHz/T) results in a large hyperfine magnetic field exceeding 10 T (panel **c**).

SYNTHESIS AND CHARACTERIZATION OF STRUCTURALLY RELATED PEPTIDOMIMETICS

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Multicomponent reactions, such as Ugi four-component reaction, are efficient and flexible synthetic strategies for the construction of structurally complex organic molecules. These reactions offer significant advantages such as reduced reaction time, lower synthetic costs, and high atom economy through a one-step process. Ugi four-component reaction involves the condensation of an amine, an aldehyde or ketone, an isocyanide, and a carboxylic acid to give divergent peptidomimetic carriers.[1] Structural diversity and potential pharmacological properties make the resulting peptidomimetics attractive intermediates for the development of new therapeutic agents in pharmaceutical research.[2,3]

In this work, Ugi four-component reaction was used for the synthesis of the selected compounds where the carboxyl and isocyanide component were varied whilst paraformaldehyde and benzylamine were selected as other necessary components for Ugi reaction. The products were characterized by FTIR, 1D and 2D NMR and HRMS. The prepared compounds can exist as a mixture of conformers because they contain amide bonds around which rotation is possible that is slow enough so that this effect is observed in the NMR spectra. ^{1}H NMR spectra of prepared compounds show duplicated signals, which indicated the presence of *cis* and *trans* conformers. The ratio of *cis* to *trans* conformers was determined using ^{1}H NMR and compared across different deuterated solvents like a polar aprotic solvent (dimethyl sulfoxide), polar protic solvent (methanol) or non-polar solvent (chloroform).

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REFERENCES

[1] I. Ugi, B. Werner, A. Dömling, *Molecules* **2003**, 8, 56–66.

[2] C. Kalinski, H. Lemoine, J. Schmidt, C. Burdack, J. Kolb, M. Umkehrer, G. Ross, *Synthesis* **2008**, 24, 4007-4011.

[3] L. Lombardi, V. Del Genio, F. Albericio, D. R. Williams, *Chem. Rev.* **2025**, 125, 7099−7166.

FROM SUPRAMOLECULAR HANDSHAKES TO MOLECULAR TUNNELS: CONTROLING CALIX[4]ARENE SELF-ASSEMBLIES

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The rational design of molecular building blocks that undergo controlled self-assembly remains a central goal in supramolecular chemistry, particularly for the development of responsive supramolecular polymers held together by reversible non-covalent interactions.[1] Among the various recognition motifs available, complementary urea–carboxylate hydrogen bonding provides a strong yet reversible interaction that offers numerous possibilities of dynamic supramolecular assemblies.

One of the manifestation of this recognition motif are supramolecular handshakes, heterodimeric capsule-like assemblies formed through hydrogen bonding between two calix[4]arenes, one bearing carboxylate groups and the other ureido functionalities.[2] We have demonstrated that these species can be reversibly disrupted and reassembled in response to external stimuli, namely the addition of acid or base, which is a crucial criterion for their potential use as stimuli-responsive nanoreactors or drug delivery devices. A second application of the same recognition principle is the construction of supramolecular molecular tunnels. For this purpose, we designed and synthesized a new ditopic calix[4]arene monomer incorporating both urea and carboxylate functionalities within a single molecular framework.

The dynamic behavior of both systems was investigated by NMR spectroscopy, with particular emphasis on DOSY experiments. Reversible disruption and reformation of the heterodimeric capsules and the supramolecular polymers were induced by sequential addition of acid and base to solutions containing the complementary urea- and carboxylate-functionalized calix[4]arenes or the ditopic calix[4]arene monomer, respectively. Variable-concentration and -temperature NMR experiments further revealed the dynamic and reversible nature of the aggregation process, providing insight into the stability and responsiveness of these systems.

By demonstrating reversible control over both capsule formation and polymeric self-assembly, this work highlights the potential of complementary urea–carboxylate recognition for engineering dynamic supramolecular materials.

Acknowledgements. This work has been supported by Croatian Science Foundation (CalixCORE, IP-2024-05-3012 and the European Union – NextGenerationEU through the National Recovery and Resilience Plan 2021-2026. via Institutional grants of the University of Zagreb Faculty of Science (NextGenChem and CryoLab).

REFERENCES

[1] M. Martínez-Orts, S. Pujals, *Int. J. Mol. Sci.* **2024**, 25, 4077-4103.

[2] M. Cvetnić, N. Cindro, E. Topić, N. Bregović, V. Tomišić, *ChemPlusChem* **2024**, e202400130.

POSTERS

PARAMAGNETIC ^{1}H, ^{13}C AND ^{19}F VF/MAS SOLID-STATE NMR OF SPIROOMeTAD DOPED WITH AgTFSI

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SpiroOMeTAD (2,2′,7,7′-tetrakis[N,N-di(4-methoxyphenyl)amino]-9,9′-spirobifluorene) is the archetypal hole-transport material (HTM) in perovskite solar cells. Its charge-transport performance is critically governed by p-doping with bis(trifluoromethanesulfonyl)imide (TFSI) salts, which generate a series of stable paramagnetic cation radicals SPIRO^{n+} (n = 0–4). Despite the pivotal role of these species in device operation, the precise localization of the unpaired electron(s) within the molecular skeleton and their influence on local structure remain challenging to establish.

Here we present a systematic multinuclear very-fast MAS paramagnetic NMR (VF/MAS pNMR) investigation of the complete SPIRO^{n+} series (n = 0–4). ^{1}H, ^{13}C and ^{19}F VF/MAS NMR spectra were acquired at two magnetic fields: 11.7 T (500 MHz, 30 kHz MAS) and 16.4 T (700 MHz, 50 kHz MAS). The complementary datasets enable direct assessment of field- and resolution-dependent behavior of the paramagnetically shifted resonances. The paramagnetic character of the SPIROn+ systems is manifested by two distinct NMR signatures: (i) dramatic shortening of T_1 relaxation times via paramagnetic relaxation enhancement (PRE) , and (ii) large paramagnetic shifts and severe broadening of aromatic ^{1}H and ^{13}C resonances. The influence of higher-field/faster-MAS measurement (700 MHz/50 kHz) onto the spectral resolution with fully reproducing the trends observed at 500 MHz/30 kHz was investigated.

Moreover, analysis of the paramagnetic shift patterns across the series reveals that SPIRO^{1+}, SPIRO^{2+} and SPIRO^{3+}exhibit asymmetric spin distribution across the spiro skeleton, while SPIRO^{4+} adopt a symmetric substitution pattern. Relativistic DFT calculations (ZORA/RS:LCY-PBE/TZP, ADF) corroborate the NMR-derived picture: spin-density maps localize the unpaired electron(s) at the nitrogen atoms of the triarylamine arms, and frontier orbital (HOMO/LUMO) visualizations confirm the symmetry assignments.

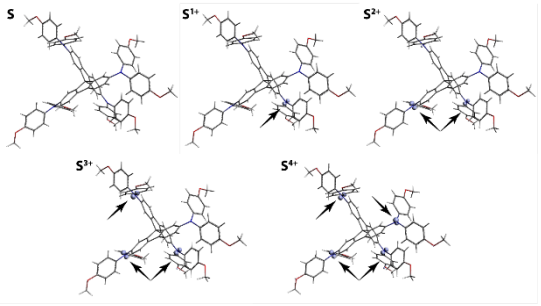

Figure 1. Visualization of spin densities of pristine SPIRO (S), SPIRO^{1+} (S^{+}), SPIRO^{2+} (S^{2+}), SPIRO^{3+} (S^{3+}), SPIRO^{4+} (S^{4+}), respectively. The arrows point toward the calculated most probable allocation of the electron(s).

Acknowledgements. This work has been supported by the Grant Agency of the Czech Republic (GA 24-10199S).

NMR-BASED METABOLOMIC PROFILING OF TREATMENT RESPONSES IN ANDROGEN-INDEPENDENT PROSTATE CANCER USING 2D AND 3D CULTURE MODELS.

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Background: Prostate cancer (PC) remains one of the most prevalent solid malignancies worldwide, with a steadily increasing incidence. Despite the availability of multiple therapeutic strategies, there is a pressing need to develop more effective treatment approaches alongside advanced in vitro models that more accurately recapitulate the in vivo tumor microenvironment.

Objectives: In this study, the primary focus was on optimizing in vitro modeling systems by developing and comparing two-dimensional (2D) and three-dimensional (3D) cell culture models to better understand cellular behavior, therapeutic response, and resistance mechanisms. Nuclear magnetic resonance (NMR) spectroscopy was incorporated to investigate treatment-associated metabolic reprogramming in both culture systems.

Methods: Human Prostate cancer cell lines (PC3 and DU145) were treated with bortezomib (Bor), doxorubicin (Dox), and treosulfan (Treo) as monotherapies and in combination to evaluate differential responses across models. Furthermore, the plasma metabolic profiles were analyzed using 1H NMR spectrometer equipped with a cryogenic probe. NMR-based metabolomics profiling was employed to characterize metabolic alterations associated with treatment response in both 2D and 3D systems. This study also investigated the gene expression profiles of key regulatory genes in response to Bor, Dox, and Treo, in 2D and 3D cell culture, administered individually and in combination. Changes in gene expression levels of key regulatory genes using RT-PCR.

Results: In the 3D model, cells exhibited greater resistance to treatment, requiring higher concentrations compared to the 2D model across both cell lines for single and combination therapies. Additionally, PC3 and DU145 cell lines demonstrated higher sensitivity to Bor and Dox, respectively, compared to Treo in both culture systems, as reflected by IC_{50} values. Combination ratios and combination index (CI) values were determined for both 2D and 3D models. NMR-based metabolomic profiling was employed to characterize metabolic alterations across all experimental conditions using both univariate and multivariate data analyses. The results revealed distinct metabolic signatures associated with different treatments and cell lines. PCR analysis showed a significant change in the expression of several genes correlating with the observed metabolic changes.

Conclusions: By identifying a panel of potential metabolic biomarkers, this study identifies key metabolic biomarkers and demonstrates that integrating gene expression with NMR-based metabolomics provides valuable insight into treatment-induced metabolic changes, helping to better understand drug response and resistance in cancer models.[1][2]

Keywords: Prostate cancer, two-dimensional cell culture, three-dimensional (3D) cell culture, NMR-based metabolomics.

REFERENCES

[1] M. Alwahsh, Y. Hamadneh, R. Marchan, L. A. Dahabiyeh, A. A. Alhusban,A. Hasan, ... L. Hamadneh, *Journal of Cancer* **2024**, *15*(*13*), 4047.
[2] M. Alwahsh, O. H. Abusara, A. A. Zaid, A. Hasan, L. Hamadneh, R. Marchan, *Cancer Treatment and Research Communications* **2026**, *47*, 101133.

LIGNIN ISOLATION FROM HEMP POMACE WITH ACID AND DEEP EUTECTIC SOLVENTS

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Lignin, cellulose and hemicellulose are biopolymers tightly bound in lignocellulosic biomass (LB). LB includes sources such as forestry residues, agricultural residues and food by-products. Due to the high abundance of LB worldwide, its utilisation has been given a pivotal role. Lignin isolation is one way of using LB to create a diverse set of products, like high-value chemicals. Traditional and novel methods of lignin isolation differ depending on their alignment with the principles of the circular bioeconomy. The former methods usually involve harsh alkali and acid treatments, while the latter entails "green" technologies. Deep eutectic solvents (DES), eutectic mixtures composed of a hydrogen bond donor and a hydrogen bond acceptor, are classified as "green" technologies. DES properties include low toxicity, recyclability and biodegradability. The experimental work consisted of lignin isolation through Klason treatment, which involved the use of concentrated sulphuric acid, and lignin isolation with two different DESs. The aim of this work is to distinguish the difference between Klason lignin and DES-lignin in terms of recovery and lignin properties through NMR analysis. [1,2]

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REFERENCES

[1] M. Tišma, A. Bucić-Kojić, M. Planinić, Chem. Biochem. Eng. Q. 2021, 35, 139–156.

[2] Y. Liu, W. Chen, Q. Xia, B. Guo, Q. Wang, S. Liu, Y. Liu, J. Li, H. Yu, ChemSusChem 2017, 10, 1692–1700.

NMR INVESTIGATION OF ENTROPIC EFFECTS IN EREMOMYCIN–LIGAND INTERACTIONS

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Glycopeptide antibiotics[1] remain essential for the treatment of severe Gram-positive bacterial infections; however, the emergence of resistance increasingly compromises their clinical efficacy, highlighting the need for a deeper understanding of their molecular recognition mechanisms. We investigate the thermodynamic determinants of ligand binding to the glycopeptide antibiotic eremomycin using ^{15}N NMR relaxation[2] measurements, with particular focus on conformational entropy changes accompanying complex formation. Backbone order parameters were quantified for both the free and ligand-bound states, revealing that binding of the cell-wall analogue peptide NAc-D-Ala enhances local internal dynamics at the binding interface, resulting in an entropic gain for the host molecule. In parallel, reduced solvent accessibility was observed in the vicinity of the binding site. These findings provide experimental support for the hypothesis that entropic compensation associated with increased internal mobility contributes to the cooperativity between ligand binding and antibiotic dimerization.[3] In addition, cross-correlated CSA/DD relaxation experiments demonstrate that the cis configuration of the peptide bond at residue 6 is retained upon ligand binding in aqueous solution. Collectively, our results indicate that enhanced conformational entropy may represent an important factor governing glycopeptide antibiotic–ligand interactions.

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REFERENCES

[1] S. Solov'eva, S. Printsevskaia, E. Olsuf'eva, G. Batta, és M. Preobrazhenskaya, „New Derivatives of Eremomycin Containing N-15 or F Atoms for NMR Study", Russian Journal of Bioorganic Chemistry, 34, o. 831–9, 0 2008, doi: 10.1134/S1068162008060162.

[2] N. Tjandra, A. Szabo, és A. Bax, „Protein Backbone Dynamics and 15N Chemical Shift Anisotropy from Quantitative Measurement of Relaxation Interference Effects", J. Am. Chem. Soc.,. 118, sz. 29, o. 6986–6991, jan. 1996, doi: 10.1021/ja960510m.

[3] L. Izsépi et al., „Bacterial Cell Wall Analogue Peptides Control the Oligomeric States and Activity of the Glycopeptide Antibiotic Eremomycin: Solution NMR and Antimicrobial Studies", Pharm. Basel Switz., 14, sz. 2, o. 83, jan. 2021, doi: 10.3390/ph14020083.

CHARACTERIZATION OF "GRAŠEVINA" AND "PLAVAC MALI" WINE VARIETIES BY NMR SPECTROSCOPY, HPLC AND GC-MS

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"Graševina" and "Plavac mali" are among the most important and widely cultivated grapevine varieties in Croatia and possess significant value. "Graševina" is the most widespread white grape variety in Croatia, accounting for approximately 42 % of total wine production, and is predominantly cultivated in the wine-growing regions of Slavonia and the Croatian Danube area. "Plavac mali" is the most significant Croatian red grape variety, representing around 7 % of the total wine production, and is mainly cultivated in central and southern Dalmatia.

Nuclear magnetic resonance (NMR) spectroscopy is currently considered one of the most advanced and reliable methods for the analysis of complex mixtures such as wine. NMR enables the identification and profiling of various metabolites present in wine, including organic acids, alcohols, amino acids, carbohydrates, and phenolic compounds.[1,2] Due to the simple sample preparation and convenient analysis, chromatography coupled with mass spectrometry (MS) was also frequently used technique for analyzing individual wine compounds and metabolites. Hence, the aim of this research is the characterization of Croatian wine varieties "Graševina" and "Plavac mali" using one- and two-dimensional NMR techniques, together with HPLC and GC-MS.

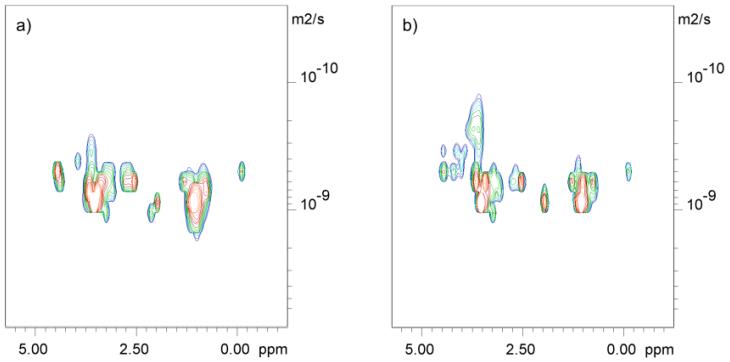

Figure 1. Representative DOSY NMR spectra of a) "Graševina" and b) "Plavac mali" varieties.

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REFERENCES

[1] A.-M. Jagatić Korenika, A. Jeromel, I. Tomaz, T. Jednačak, S. Rončević, I. Nemet, I. Primožič, T. Hrenar, P. Novak, *Food Chem.: X* **2024**, 21, 101162.

[2] J. Parlov Vuković, T. Jednačak, L. Divjak, K. Smetko, M. Tišma, A.-M. Klarić, A.-M. Jagatić Korenika, A. Bucić-Kojić, P. Novak, *Croat. Chem. Acta* **2025**, 98(2), 127–132.

CHARACTERIZATION OF CROATIAN SPARKLING WINES BY NMR AND ICP-MS

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Knowledge of the chemical composition of wine is essential for identifying the compounds that affect its quality, stability and value. Sparkling wines are special wines characterized by the release of CO_2 upon uncorking, resulting in a more or less persistent effervescence. In light of current positive market trends, it may be anticipated that sparkling wine production will become increasingly important in the total wine production and the overall wine market, thereby highlighting the growing importance of further research in this field.

132 Croatian sparkling wine samples were analyzed using NMR spectroscopy and inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS analysis can provide information on trace elements, while the interpretation of NMR spectra provides insight into various metabolites serving as a wine fingerprint. Different NMR techniques were used, including ^{1}H NMR with solvent suppression for water and ethanol signals, quantitative ^{13}C NMR and diffusion-ordered NMR spectroscopy (DOSY NMR). Experimental data were further processed and analyzed by PCA and deep reinforcement learning methods. The obtained model has great potential for wine classification.

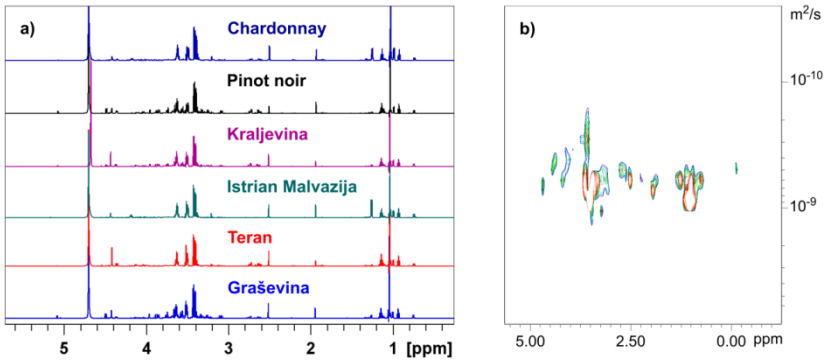

Figure 1. a) Representative ^{1}H NMR spectra of different wine varieties; b) DOSY NMR spectrum of the Chardonnay sample.

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REFERENCES

[1] A.-M. Jagatić Korenika, A. Jeromel, I. Tomaz, T. Jednačak, S. Rončević, I. Nemet, I. Primožič, T. Hrenar, P. Novak, *Food Chem.: X* **2024**, 21, 101162.

[2] J. Parlov Vuković, T. Jednačak, L. Divjak, K. Smetko, M. Tišma, A.-M. Klarić, A.-M. Jagatić Korenika, A. Bucić-Kojić, P. Novak, *Croat. Chem. Acta* **2025**, 98(2), 127–132.

NMR AND COMPUTATIONAL STUDY OF NATURAL CYCLODEXTRIN INCLUSION COMPLEXES WITH CINCHONA ALKALOIDS

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NMR spectroscopy is a well-established method for studying inclusion complexes at an atomic level, [1] providing quantitative data from changes in NMR parameters with NMR titration, and revealing enantioselective binding parameters through differences in guest enantiomer behavior in the presence of chiral hosts. [2]

Understanding physicochemical properties of natural cyclodextrins inclusion complexes is crucial for ensuring their safe and effective application and for exploring potential new therapeutic uses. This study investigates the influence of pH on the chemical shifts of the Cinchona bark alkaloids and examines their interactions with natural cyclodextrins (αCD, βCD, and γCD) using NMR spectroscopy. The stoichiometry and association constants of the inclusion complexes were determined, providing insights into the interactions of alkaloids with different cyclodextrins. Additionally, temperature-dependent NMR studies (10–35°C) were conducted to assess the thermodynamic parameters of complexation. Computational modeling, including structural optimization and molecular docking, was performed to complement the experimental data.

The results enhance the understanding of alkaloid-cyclodextrin complexation, with implications for improving drug solubility, stability, and bioavailability. Moreover, the findings could aid in the stereoselective separation of Cinchona alkaloid enantiomers using cyclodextrins. This research contributes to the broader study of alkaloid-cyclodextrin interactions, with potential applications in pharmaceuticals, food additives, and cosmetics.

REFERENCES

[1] G. Szczupaj, J. Wójcik, A. Ejchart, M. Nowakowski, *J Incl Phenom Macrocycl Chem* **2024**, 104:129–136.

[2] M. Nowakowski, A. Ejchart, *J Incl Phenom Macrocycl Chem* **2014**, 79:337–342.

MAPPING LIGAND BINDING MODES WITH IMPROVED 2D LONGITUDINAL ^{1}H RELAXATION-BASED NMR EXPERIMENTS

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Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most valuable analytical techniques in modern drug discovery. Structural information — particularly the epitope mapping of protein-bound ligands — plays a key role in revealing structure–activity relationships and in identifying molecular regions that can be modified to achieve favorable pharmacological properties of lead compounds.

The ligand-detected 1D ^{1}H DIRECTION NMR method developed by Mizukoshi et al. utilizes differences in the longitudinal relaxation rates of ligand proton signals in the presence and absence of selective protein irradiation to identify ligand moieties involved in binding interactions.[1] However, the applicability of the method is significantly limited by the relatively narrow chemical shift range of ^{1}H NMR spectra, resulting in incomplete signal separation. Consequently, in the case of larger or structurally similar molecules, proton resonances frequently overlap, complicating the reliable assignment and quantitative evaluation of individual signals. To overcome these limitations and improve spectral resolution, our research group has developed two alternative approaches for obtaining such information. We modified the original 1D DIRECTION pulse sequence by incorporating either a 2D CLIP-COSY relay module or a homonuclear broadband proton-decoupled ("pure shift") ^{1}H–^{13}C HSQC experiment.[2,3] These modified methods enable the determination of ^{1}H longitudinal relaxation times (T_1) through correlation peaks with substantially improved spectral resolution.

The applicability of the novel experiments is demonstrated using two well-established biological model systems from the literature: the *human* serum albumin (HSA) – (*S*)-ibuprofen and the *human* galectin-1 (*h*Gal-1) – methyl-β-lactoside protein-ligand complexes, on which ligand epitope mapping studies were performed.

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REFERENCES

[1] Y. Mizukoshi, et al., *Angew Chem Int Ed Engl.* **2012**, 51(6), 1362-1365.

[2] T. Gyöngyösi, et al., *ChemPlusChem* **2018**, *83* (1), 53–60.

[3] I. Timári, et al., *Anal. Chem.* **2019**, 91(3), 2304-2311.

VARIABLE-TEMPERATURE NMR STUDY OF AMIDE BOND ROTATION IN α-NICOTINOYLAMINO ACETAMIDES

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α-Acylamino amides have been developed as a new class of cholinesterase inhibitors.[1] ^{1}H and ^{13}C NMR spectra of various *α*-acylamino amides show double signals, indicating the presence of *cis* and *trans* conformers with respect to amide bonds.[2] The rate of interconversion between *cis* and *trans* conformers is slow enough to be detected by NMR spectroscopy. Upon increasing the temperature, the distinct NMR signals corresponding to individual conformers gradually coalesce into a single signal at a specific temperature.[3] The energy barrier for restricted bond rotation can be estimated using the modified Eyring equation.[4]

In this study, novel α-nicotinoylamino acetamides were synthesized via the Ugi[5] multicomponent reaction using nicotinic acid, paraformaldehyde, isobutylamine, and either *tert*-butyl isocyanide or benzyl isocyanide. The Ugi reaction was performed using microwave-assisted synthesis, the classical method of organic synthesis, solvent-free ball milling and/or liquid-assisted grinding (LAG). Subsequently, quaternization of the nicotinamide pyridine nitrogen atom with benzyl bromide led to the formation of novel α-nicotinoylamino acetamide derivatives, prepared under both microwave-assisted and conventional conditions. The compounds were characterized by standard analytical methods (FTIR, 1D and 2D NMR, HRMS). Variable-temperature ^{1}H NMR spectra (−20 °C to 80 °C) were recorded in various deuterated organic solvents (including mixtures of dimethyl sulfoxide and water, acetonitrile, ethanol and chloroform). The energy barrier for amide bond rotation was determined (using the modified Eyring equation) to be approximately 16 kcal mol^{-1}, which is consistent with the previously reported literature range of 14–18 kcal mol^{-1}.[4]

Acknowledgements. This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-2022-10-9525 and European Union – NextGenerationEU through the National Recovery and Resilience Plan 2021-2026. via Institutional grant NextGenChem of the University of Zagreb Faculty of Science.

REFERENCES

[1] T. Sun, T. Zhen, C. H. Harakandi, L. Wang, H. Guo, Y. Chen, H. Sun, *Eur. J. Med. Chem.* **2024**, 275, 116569.

[2] M. C. de Koning, M. J. A. Joosen, F. Worek, F. Nachon, M. van Grol, S. D. Klaassen, D. P. W. Alkema, T. Wille, H. M. de Bruijn, *J. Med. Chem.* **2017**, 60, 9376–9392.

[3] M. Sadvakassova, A. Khlebnikov, A. Bakibaev, O. Kotelnikov, R. Erkassov, M. Yelubay, M. Issabayeva, *Molecules,* **2023**, 28, 535.

[4] M. Huggins, T. Kesharwani, J. Buttrick, C. Nicholson, *J. Chem. Educ.* **2020**, 97, 1425–1429.

[5] I. Ugi, B. Werner, A. Dömling, *Molecules* **2003**, 8, 56–66.

ANALYSIS OF LIGNOCELLULOSE SAMPLES OF WILD MILLET, MISCANTHUS AND VIRGINIA MELLOW BY ^{1}H NMR SPECTROSCOPY

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Lignocellulosic biomass (LB) is an abundant and renewable resource originating from agriculture, forestry, and the food industry. [1-2] As it can provide a sustainable alternative for producing various value-added products, LB plays a key role in the global shift away from fossil resources. It is a very complex mixture, mainly composed of polysaccharides cellulose and hemicelluloses and an aromatic polymer lignin. A detailed understanding of composition and properties of LB is crucial for its correct application which is the reason why the field of lignocellulose chemistry has attracted much attention in recent years.

NMR spectroscopy has been used for many years as a powerful tool for qualitative and quantitative analysis of lignocellulosic biomass samples. It can provide data on functional groups, molecular architecture, and linkage profiles.

We report here ^{1}H NMR characterization of three different lignocellulosic samples. The samples of wild millet, miscanthus and Virginia Mellow were disolved in two different solvents (deuterated DMSO and $CDCl_3$ and NMR spectra showed different portion of lignocellulosic components.

REFERENCES

[1] J. Parlov Vuković, M. Tišma, *Food Chem.: Mol. Sci.*, **2024**, *9*, 100219.

[2] M. Tišma, A. Bucić-Kojić and M. Planinić, Chem. Biochem. Eng. Q., **2021**, 35, 139–156.

CHARACTERIZATION OF "GRAŠEVINA" AND "PLAVAC MALI" WINE VARIETIES BY NMR SPECTROSCOPY, HPLC AND GC-MS

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"Graševina" and "Plavac mali" are among the most important and widely cultivated grapevine varieties in Croatia and possess significant value. "Graševina" is the most widespread white grape variety in Croatia, accounting for approximately 42 % of total wine production, and is predominantly cultivated in the wine-growing regions of Slavonia and the Croatian Danube area. "Plavac mali" is the most significant Croatian red grape variety, representing around 7 % of the total wine production, and is mainly cultivated in central and southern Dalmatia.

Nuclear magnetic resonance (NMR) spectroscopy is currently considered one of the most advanced and reliable methods for the analysis of complex mixtures such as wine. NMR enables the identification and profiling of various metabolites present in wine, including organic acids, alcohols, amino acids, carbohydrates, and phenolic compounds.[1,2] Due to the simple sample preparation and convenient analysis, chromatography coupled with mass spectrometry (MS) was also frequently used technique for analyzing individual wine compounds and metabolites. Hence, the aim of this research is the characterization of Croatian wine varieties "Graševina" and "Plavac mali" using one- and two-dimensional NMR techniques, together with HPLC and GC-MS.

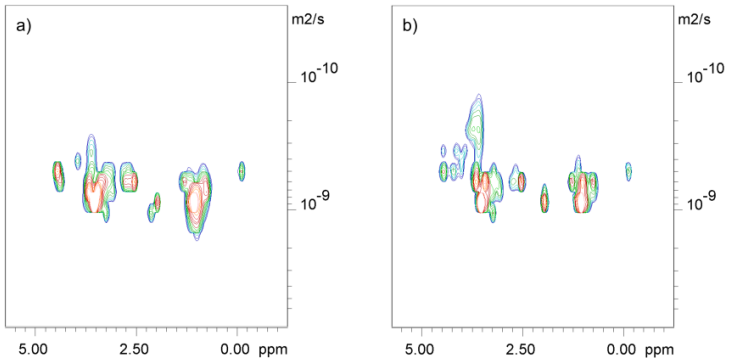

Figure 1. Representative DOSY NMR spectra of a) "Graševina" and b) "Plavac mali" varieties.

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REFERENCES

[1] A.-M. Jagatić Korenika, A. Jeromel, I. Tomaz, T. Jednačak, S. Rončević, I. Nemet, I. Primožič, T. Hrenar, P. Novak, *Food Chem.: X* **2024**, 21, 101162.

[2] J. Parlov Vuković, T. Jednačak, L. Divjak, K. Smetko, M. Tišma, A.-M. Klarić, A.-M. Jagatić Korenika, A. Bucić-Kojić, P. Novak, *Croat. Chem. Acta* **2025**, 98(2), 127–132.

CONFORMATIONAL MOBILITY OF A CALIX[6]ARENE DERIVATIVE AND ITS CATION COMPLEXES STUDIED BY NMR SPECTROSCOPY

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Calix[n]arenes are widely studied hosts in supramolecular chemistry, particularly due to their high binding affinity and selectivity for diverse cations. However, larger calix[*n*]arenes have received considerably less attention than the smaller analogues. Calix[6]arenes are much more flexible than their smaller counterparts, and can adopt numerous conformations, such as *cone*, partial *cone*, 1,2-alternate, 1,3-alternate, 1,2,3-alternate, 1,3,5-alternate etc. NMR spectroscopy is quite elucidative in investigating the conformational flexibility, and can indicate slow or fast conformational interconversion, especially when kinetic and/or variable-temperature measurements are carried out.[1]

In this work, the cation-binding properties of hexadiethylamide calix[6]arene derivate **L** were quantitatively investigated using fluorimetry, spectrophotometry, and microcalorimetry. The NMR spectra revealed the conformational mobility depends on the complexed cation and the solvent used.[2] Conformational locking induced by cation binding was examined for alkali and alkaline earth metal ions in acetonitrile and methanol. The experimental findings were correlated with the stoichiometries and structures of the complexes predicted by molecular dynamics simulations.

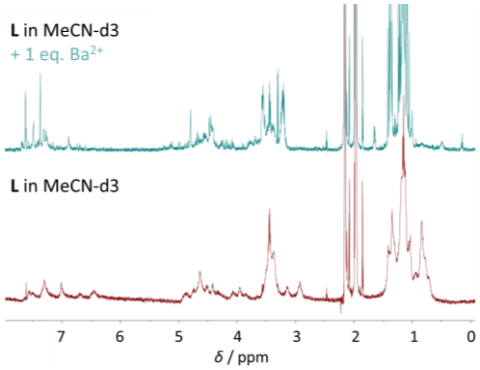

Figure 1. ^{1}H NMR spectra of the free **L** and the Ba**L**$^{2+}$complex in deuterated acetonitrile.

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REFERENCES

[1] A. Casnati, P. Minari, A. Pochini, R. Ungaro, W. F. Nijenhuis, F. de Jong, D. N. Reinhoudt, *Isr. J. Chem.* **1992**, 32, 79–87; V. Iuliano, C. Talotta, P. Della Sala, M. De Rosa, A. Soriente, P. Neri, C. Gaeta, *Molecules* **2023**, 28, 4749.

[2] A. F. Danil de Namor, T. T. Matsufuji-Yasuda, K. Zegarra-Fernandez, O. A. Webb, A. El Gamouz, *Croat. Chem. Acta*, **2013**, 86, 1–19; S. Fanni, F. Arnaud-Neu, M. Anthony McKervey, M-J. Schwing-Weill, K. Ziat, *Tetrahedron Lett.* **1996**, 37, 7975–7978; U. C. Meier, C. Detellier, *Dalton Trans.* **2003**, 4574–4579; A. Casnati, L. Baldini, N. Pelizzi, K. Rissanen, F. Ugozzoli, R. Ungaro, *J. Chem. Soc. Dalton Trans.* **2000**, 19, 3411–3415.

INCLUSION OF PROPOFOL IN β-CYCLODEXTRIN DERIVATIVES: THERMODYNAMIC AND NMR INSIGHTS

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Propofol (2,6-diisopropylphenol) is one of the most widely used intravenous general anesthetics, yet its extremely low aqueous solubility forces administration as a lipid emulsion, a formulation associated with pain on injection and further adverse effects. Cyclodextrins, biocompatible macrocyclic hosts capable of binding diverse hydrophobic and amphiphilic guests within their apolar cavity, offer a route to lipid-free aqueous formulations.[1] So far, binding of propofol has been characterized almost exclusively by the phase-solubility method, which delivers only an apparent complex stability constant and no reliably obtained thermodynamic reaction parameters. Namely, the determination of $\Delta_r H°$ and $\Delta_r S°$ in such studies usually relies on the temperature dependence of the equilibrium constant (linear van't Hoff plot) which usually fails in systems for which $\Delta_r C_p° \neq 0$, and the few reported complexation enthalpies and entropies disagree even qualitatively.[2]

In this work, we examined the inclusion of propofol by β-cyclodextrin and three pharmaceutically relevant derivatives, 2-hydroxypropyl-, randomly methylated- and sulfobutylether-β-cyclodextrin in water, combining isothermal titration calorimetry, UV and NMR spectroscopy. Mutually consistent complex stability constants were obtained by means of all three methods, and the complete set of standard reaction quantities ($\Delta_r G°$, $\Delta_r H°$, $\Delta_r S°$, $\Delta_r C_p°$) was attained from calorimetric experiments. ^{1}H NMR titrations provided additional information on the inclusion depth and guest orientation inside the cavity. All four receptors form 1:1 complexes of moderate and comparable stability, but rim substitution significantly affects the enthalpic and entropic contributions to $\Delta_r G°$. The results obtained from calorimetric titrations over a wide temperature range indicate that the complex stability virtually does not change across a pharmaceutically relevant temperature window, with a considerable variation of $\Delta_r H°$ and $\Delta_r S°$ with temperature (enthalpy-entropy compensation).[3,4] Insights gained from these studies provide useful information on the distribution of bound and free propofol at various temperatures and further implementation of these highly soluble receptors in formulations.

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REFERENCES

[1] T. Loftsson, M. E. Brewster, *J. Pharm. Pharmacol.* **2010**, 62, 1607–1621.

[2] G. Trapani, A. Lopedota, M. Franco, A. Latrofa, G. Liso, *Int. J. Pharm.* **1996**, 139, 215–218; G. Trapani, A. Latrofa, M. Franco, A. Lopedota, E. Sanna, G. Liso, *J. Pharm. Sci.* **1998**, 87, 514–518; M. K. M. Babu, T. N. Godiwala, *Pharm. Dev. Technol.* **2004**, 9, 265–275.

[3] F. Biedermann, W. M. Nau, H.-J. Schneider, *Angew. Chem. Int. Ed.* **2014**, 53, 11158–11171.

[4] K. Leko, M. Hanževački, Z. Brkljača, K. Pičuljan, R. Ribić, J. Požar, *Chem. Eur. J.* **2020**, 26, 5208–5219; A. Usenik, K. Leko, V. Petrović Peroković, Ž. Car, R. Ribić, K. Pičuljan, M. Hanževački, J. Draženović, J. Požar, *J. Mol. Liq.* **2023**, 388, 122774; A. Usenik, M. Alešković, S. Roca, I. Markuš, M. Šekutor, J. Požar, *New J. Chem.* **2023**, 47, 18745–18755.

Rijeka is a city of rich history and dynamic present, new perspectives and the joy of life.

Alongside its newly acquired benefits as a tourist centre that has escaped from its former image of a port and industrial city, it adds old ones as well –excellent connections by road, boat, rail or plane, both as a means of arrival and as a starting point for new journeys. In the historical image of the city, from prehistory and the Gothic, and then the baroque Rijeka and the strong influences of the Austro-Hungarian monarchy, centuries have been deposited and each and every one of them has left its mark in its architecture, creating an interesting city nucleus rich in historical monuments. Trsat, a part of Rijeka, is a very special place for citizens of Rijeka and their guests, as the oldest Croatian Marian shrine and a destination for many religious people, including the Holy Father John Paul II. in 2003. Rijeka has a rich history yet is not its slave. Instead it pulsates with a dynamic rhythm of everyday life, which you can feel in the best way in the very centre of the city, on the always vibrant central promenade, Korzo. You sit and people, images and events are passing by... But even when the lights go out and many cities go silent, Rijeka lives intensely in cafes and restaurants with excellent gastronomic offers and, of course, in clubs where the party goes on until late at night for young people. Rijeka's calendar of events is overflowing throughout the year so regardless of the season, guests will find interesting and attractive music, drama, art ... performances, as well as urban clubs for the younger generations that offer their kind of entertainment. Together with the traditional but always interesting summer events program, manifestations such as Summer at Gradina (Ljeto na Gradini) and others will definitely occupy your attention as well. Visitors of all generations to the aforementioned events will enjoy a rich program of drama, music and art performances prepared by the most famous artists from Croatia and around the world. However, Rijeka lives intensely on its coast with its beautiful beaches. Exploring the city via planned walking routes or bicycle trails is also interesting. Rijeka, to put it simply, has many faces -all of them are interesting and seductive and all of them are meant for you.

https://visitrijeka.croatia.hr/en-gb

ADRIATIC NMR

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