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Biomolecular NMR

Structural and interaction studies of RpiB from M. tuberculosis: screening and development of new compounds to a specific target

https://proceedings.science/p/169672?lang=en

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Tuberculosis annually affects about 10 million people worldwide and has been considered an epidemic in several countries. The treatment with old and long-term drugs has generated resistant strains.Thus, searching for new biological targets and inhibitors with high essentiality to the microorganism is paramount to finding specific drugs with low side effects. Ribose-5-phosphate isomerase (Rpi) participates in the non-oxidative branch of the pentose.This protein family has two types, and the RpiB is found in microorganisms with no homologs in humans. Therefore, M. tuberculosis RpiB was chosen as a molecular target for structural and interaction studies using NMR. Recently, our group assigned the dimeric MtRpiB, described the structural and biochemical features of this enzyme, and suggested a mechanism for the uptake of the substrate. This work aims to study the interaction of MtRpiB using Fragment-based Screening using a specific library of 768 fragments by Nuclear Magnetic Resonance (NMR) to understand and search for new compounds. MtRpiB was overexpressed in E.coli BL21(DE3) at 37oC and induced with 0.5mM of IPTG in LB and 15N isotopically labeled minimum medium and purified by affinity and molecular exclusion chromatography. The fragments library was screened using Chemical Shift Perturbation (CSP), Waterlogsy and T2 Relaxation – CPMG experiments using a Bruker AVANCE 500 MHz spectrometer . Each experiment containing pools of 12 compounds was performed free and bounded to MtRpiB using a 10x excess fragment:protein (232mM:20 mM). Positive interaction (Hit) was considered when at least two experiments showed differences between free and bound states. Preliminary results showed at least ten fragments with protein interaction. MtRpiB 15N-HSQC was performed in the presence of two fragments that showed interaction. Molecular docking studies are ongoing. These results may provide new information about the chemical space of the protein and could be essential to the development of new specific inhibitors against tuberculosis.

Amplifying chemical shift sensitivity in 19F NMR studies of proteins using a thiol-reactive trifluoromethyl-cyclohexane reporter

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Fluorinated reporters are exquisite probes of protein conformation due to their high receptivity and chemical shift sensitivity [1]. The inherently high chemical shift sensitivity of CF3 reporters can be further enhanced upon incorporating them into a cyclohexane ring, taking advantage of the anisotropic environments intrinsic to the ring on the axial/equatorial positions [2]. In this work we evaluated fluorinated cyclohexanes as conformation-sensitive and cysteine-specific tags for protein labeling. The tag was designed after evaluating the chemical shift dispersion (δ19F) of cis and trans-3-trifluoromethylcyclohexanol (cCyOH and tCyOH) in solvents of differing polarity, where the trans isomer exhibited significant Δδ19F. A thiol-reactive version of tCyOH was synthesized by the insertion of a bromoacetyl moiety (tCyCO). tCyOH was next conjugated to the alpha subunit of a G protein, Gsα, together with a control, 2-bromo-N-(4-(trifluoromethyl)phenyl)acetamide (BTFMA), (a conventional C19F3) tag. The 19F NMR spectra of the 19F-tagged protein were then recorded under a variety of

conditions to evaluate the chemical shift response and thus, δ19F. The tCyCO exhibited improved resolution of states and improved solubility. Our conclusion is that the CF3 reporter on trans-3-trifluoromethylcyclohexanol undergoes rapid interconversion between an axial and equatorial environment, each of which is distinguished by a unique chemical shift. Different microenvironments in the protein likely influence this equilibrium, giving rise to a large chemical shift dispersion.
Acknowledgments to CAPES, CNPq and Mitacs Globalink for the financial support.
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Application of NMR to study the polymerization mechanism of FtsZ and its inhibition by natural and synthetic modulators

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FtsZ, a cytoplasmic GTPase protein, is a key enzyme in the cytokinesis process. Several proteins act as positive or negative modulators of FtsZ, promoting the assembly of FtsZ to form the Z ring or inhibiting FtsZ polymerization and cell division. The mechanism of action of these regulatory proteins is still poorly understood at the structural level. Therefore, we intend to characterize the conformational and protein dynamic changes associated with FtsZ polymerization and to map the interactions of FtsZ with negative modulators, by Nuclear Magnetic Resonance (NMR). Our data showed structural dynamic of FtsZ-GDP in important regions to interact with another monomer (polymerization). We also observed important chemical shift perturbation (CSP) of FtsZ in presence of different ligants: i) MciZ show CSPs in the C-terminal region of FtsZ, which it is in agreement with our previous crystal structure. Furthermore we identified other CSPs in the helix7, loop T7 and in the N-terminal of FtsZ; ii) PC190723 caused CSPs in the C-terminal region, next to H7, also in agreement with crystal structure, and some CSPs in the nucleotide region, showing its allosteric effect; iii) PC170942 showed CSPs in the C-terminal region, also next to H7 and in the nucleotide region. Previous data showed that PC17 did not inhibit the FtsZ-GTPase activity, we believe that PC17 also bind in the C-terminal region with allosteric effect in the nucleotide region; iv) MinC caused CSPs in the C-terminal region of FtsZ, corroborating with our previous genetics data; v) GTP resulted in small CSPs and new peaks in the spectra, which suggest a tendency of oligomerization. Understanding the polymerization mechanism of FtsZ that is essential to cell division, widely conserved among prokaryotes and evolutionarily distant from tubulin, FtsZ is pointed as a good target for the design of new antibiotics with selectivity to bacterial infections treatment.

Balancing Promiscuity and Selectivity - Determining Factors Governing G-Protein Selectivity and Efficacy for the Adenosine A2A Receptor

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Biological processes are tightly regulated by diverse mediators including hormones and neurotransmitters. To mediate a response, these molecules must interact with the receptor allowing the transfer of information from the outside to the inside of the cell. G protein coupled receptors (GPCRs) are the largest family of receptors that can transduce signals through the membrane. Due to their role in many metabolic pathways and in diverse diseases, GPCRs are targeted by approximatively 30% of the drugs on the market. The adenosine A2A receptor (A2AR) is a prototypical class A G protein coupled

receptor (GPCR) belonging to a superfamily of 7-transmembrane proteins. Like many GPCRs, A2AR is promiscuous and couples to several G-proteins. In addition to its cognate G protein, Gs. Here,19F NMR identified facets of the receptor that dictated selectivity in agonist-stimulated A2AR-Gs and A2AR-Go complexes in phospholipid nanodiscs. TM6 adopted two activation states, whose differing outward displacements proved compatible with the larger and smaller volume H5 helices of Gs and Go, respectively. TM7 adopted an activation intermediate in addition to two activation states (A_1^TM7 and A_2^TM7) in the A2AR-G protein complex. While the nucleotide-free A2AR-Gs ensemble was biased toward the fully active A_1^TM7state, the A2AR-Go ensemble was characterized by a dynamic inactive/intermediate fraction. TM7 activation states were correlated to distinct NPxxY configurations and an allosteric network connecting helix-8 (H8), known to influence G-protein coupling. Spectra of the H5-helix in both A2AR-Gs and A2AR-Go reveal extended and compact states of G. The cognate Gs subunit is more biased toward the active (extended) state of the G-protein, corroborating differences in efficacy. Enhanced sampling MD simulations and allostery provide a mechanistic explanation for the observed promiscuity and enhanced selectivity of A2AR for Gs.This work was supported by the Canadian Insitutes of Health Research (CIHR) and by Japan, Science and Technology Agency

Biophysical studies of the pH effect on the α-helix induction in the intrinsically disordered region of the viral phosphoprotein involved in the interaction with the M2-1 protein of hRSV

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The human Respiratory Syncytial Virus (hRSV) is one of the main causative agents of acute respiratory infections in newborns. During its replication cycle, a crucial interaction occurs between the M2-1 antitermination factor and the P phosphoprotein, specifically between the core domain of M2-1 (cdM2-1) and the intrinsically disordered region (IDR) of residues 90-110 from P (P90-110). The present work aimed to characterize the effect of pH on the induction of α-helix of the IDR P90-110. For this purpose, the formation of α-helix was induced by titrations of cdM2-1 at pH 7 and TFE at different pH conditions (from 3 to 10), and this formation was investigated by circular dichroism (CD) and NMR spectroscopy. The results show that the TFE titration provided an increase in the negativity of the molar ellipticity at 222 nm, indicating the formation of α-helix in the P90-110 peptide structure characterized by a cooperative transition. At acidic pHs, especially at pH 4 (isoelectric point), the conformational transition from random structure to α-helix induced by TFE showed greater cooperativity than at basic pHs. The difference spectra between the CD signal of the P90-110/cdM2-1 complex and free cdM2-1 reported a secondary structure gain in α-helix of P90-110 in the interaction with cdM2-1, presenting a cooperative helix-coil transition similar to the induction of α-helix conformation promoted by TFE at pH 4. 1H-1H COSY, TOCSY, and NOESY spectra of P90-110 collected in presence of TFE-d3 at pH 4 revealed from 1Hα resonance assignments that the residues of IDP P90-110 adopt a conformation in α-helix. Based on the CD and RMN results of peptide titrations with TFE and cdM2-1, it can be suggested that the interaction interface between P90-110 and cdM2-1 is a microenvironment with more acidic characteristics that favor the protonation of negatively charged residues.

COMPARABILITY STUDY OF BOVINE HEPARINs

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Heparin is a drug with several effects, among them, we have the anticoagulant that is commonly used in the clinic. Belonging to the glycosaminoglycan family, it can be obtained exclusively from animal sources, which can be of ovine, bovine or porcine origin. Currently, pork intestine is the main source of heparin extraction, with China responsible for 50% of its world production. This scenario of production from a single type of herd and concentrated in a single country generates insecurity in its supply, leading to the search for new sources. Brazil is the largest producer of unfractionated heparin (UFH) obtained from bovine intestine, and this product has an anticoagulant activity 50-60% of the UFH from swine intestine, so its pharmaceutical formulations exhibit higher mass content to reach the same potential anticoagulant of porcine intestine preparations. Given this scenario, it is necessary to use tools in order to expand the heparin options present in the consumer market. One of the tools that can be used for this purpose is the comparability of biological products, which is based on an ANVISA manual and is based on a study between an innovative product and a reference product already available in the pharmaceutical market. Techniques will be applied to verify the structural, physical-chemical and biological activity likelihood between the analyzed preparations. Nuclear magnetic resonance is one of the spectroscopic techniques that can be used to verify the structure, as it makes it possible to explore the magnetic properties of atomic nuclei and verify the properties of where they are contained. The study will contribute to the knowledge of possible discrepancies, particularities or similarities with the preparations obtained from a national industry and another from an Argentine industry.

Dissecting the structural and dynamical properties of the N-terminal domain of the nucleocapsid protein of SARS-CoV-2 that contributes to the specificity toward the transcription regulatory sequences.

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The nucleocapsid protein (N) of human beta coronaviruses (hCoV) is responsible for nucleocapsid assembly and other essential regulatory functions. Its N-terminal domain (N-NTD) interacts and melts the double-stranded transcriptional regulatory sequences (dsTRS), regulating the discontinuous subgenome transcription process, an essential feature for the virus cycle. There is scarce structural information on the specific binding of dsTRS and single-stranded RNA (TRS+ and TRS-) to the N-NTD [1]. To better describe the dsRNA melting and its role in the template switching, we measured the binding and the melting activity of dsTRSs of different ORF of the SARS-CoV-2 and non-specific RNA sequence. We used 3 different constructs, N-NTD, N-NTD plus the serine and arginine-rich motif (N-NTD-SR), and the N-NTD flanked by the two intrinsically disordered regions, the N-terminal, and the linker (N-NTR). We showed that the melting activity was only observed for the specific TRSs, in which the specificity depends on the sequence. Moreover, we determined the importance of the IDR for the melting activity and binding affinity. Theoretical studies by our group showed the importance of a tweezer-like motion at the binding cleft for melting activity [2]. To approach this experimentally, we measured the 15N CPMG relaxation dispersion of N-NTD of SARS-CoV-2, MERS-CoV, and HKU1-CoV and showed that they all present conformational equilibrium at the binding cleft. We determined that the exposure of hydrophobic surfaces at the open conformation is probably correlated to the binding and melting events. To reinforce this hypothesis, we collected the 19F CPMG in presence of RNA and

19F CEST due the presence of tryptophan at the binding cleft. This approach reveals the conformational selection and dynamics pathway through the RNA chaperon activity. Altogether, our data contribute to the understanding of how promiscuous RNA binding proteins such as N protein can exert specific functions.

Dynamic Properties of the Dengue Virus Capsid Protein Bound to Nucleic Acid through Invisible States

https://proceedings.science/p/169642?lang=en

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Dengue virus (DENV) is a mosquito-borne flavivirus considered a challenge to public health by WHO. The structure of DENV serotype 2 (DENV2) has been solved by cryo-electron microscopy, revealing an icosahedral symmetry on the viral envelope. There is no structural information for the nucleocapsid (NC). One hypothesis raised to explain this behavior was the presence of a dynamic equilibrium in the NC. The capsid protein of DENV2 (DENVC2) presents itself as a stable dimer, composed by four intertwined α-helices and a flexible intrinsically disordered N-terminal region. DENVC2 interacts with viral RNA, having a key role in the packaging of the viral genome. Our group has been studying the NC assembly in vitro, demonstrating the formation of organized but very dynamic nucleocapsid-like particles by the addition of simple oligonucleotides such as 5 'GGG GG 3' (5G)1,2. We aim to understand the dimer/oligomer balance in the capsid assembly. Since the saturation of DENVC2 with nucleic acid leads to precipitation, we used nuclear magnetic resonance (NMR) to study the bound conformation in semi-saturated states. Using NMR in solution and a 5G:DENVC2 molar ratio <0.5, in which only soluble oligomers are formed, we measured the protein signal intensity decay, exposure of the amide groups to the solvent by sPRE and the 15N relaxation dispersion data via CPMG. We showed that the intensity of protein signal decreases with the addition of 5G, leading to the formation of invisible excited species. The characterization of these species by CPMG presented significant changes in the aromatic skeleton at the symmetry axis of DENVC2. These data present a fast dimer/oligomer equilibrium, and this approach revealed transient states of the nucleocapsid structure undetectable by other methodologies.

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Epitope mapping for the rational design of immunotherapeutics using Nuclear Magnetic Resonance (NMR)

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Antibodies are glycoproteins that play a critical role in the humoral immune response and serve as key components of the adaptive immune system against various diseases. The primary structure of antibodies exhibits remarkable diversity, reflecting their ability to recognize and bind antigens derived from pathogens. Their main functions include the neutralization of antigens and the eradication of microorganisms by opsonization. Because of their high specificity and recognition ability, studies investigating antigen-antibody interactions have become indispensable in the pharmaceutical industry.

These studies have facilitated the development of novel the design and development of epitope peptides for diagnostic purposes. The Covid-19 pandemic underlined the urgent need for the scientific community to identify new targets for vaccines, therapeutics, and diagnostics. In this study, we employed nuclear magnetic resonance (NMR) spectroscopy for epitope mapping in order to identify the regions of the immunogenic SARS-CoV-2 nucleocapsid (N) protein that interact with the polyclonal antibodies obtained from patient sera from the Belo Horizonte metropolitan region. The observing changes in the NMR spectra [1H,15N]-HSQC of the antigen upon binding to the antibodies, specific amino acids, or residues involved in the interaction were determined. Our aim was to understand the binding interface between the antigen and the antibodies. The dynamics of the complex provided information flexibility or rigidity of the binding interface. We further conducted paramagnetic relaxation enhancement (PRE) experiments with gadolinium to explore the dynamics and conformational properties of the free protein and protein-antibody complex, providing valuable insights into solvent accessibility. We expressed and purified the N protein to proceed with these analyses and employed affinity chromatography with protein G to purify plasma from multiple Covid-19 convalescent individuals. The comprehensive findings obtained from this study will improve our understanding of the antibody-antigen binding, stability, and functional properties and provide valuable insights for vaccine development.

INHIBITION OF β-LACTAMASE OXA-143 BY THE REACTION PRODUCT WITH MEROPENEM

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Enzymes belonging to the Carbapenem-Hydrolyzing Class D β-lactamases (CHDLs) confer bacterial resistance to β-lactam antibiotics and are often associated with outbreaks involving the pathogenic bacteria Acinetobacter baumannii. In this context, the biophysical and biochemical characterization of the enzyme/substrate and enzyme/inhibitors interaction constitutes a powerful tool for developing drugs that these enzymes cannot hydrolyze and/or new inhibitors. The Nuclear Magnetic Resonance (NMR) technique allows the analysis of direct and indirect (allosteric) structural perturbations and the effects of conformational changes in the enzyme caused by the interaction with the substrate/inhibitor. We evaluated the inhibition of CHDL OXA-143 by the product of the reaction with the carbapenem antibiotic meropenem (hydrolyzed meropenem - hMER) and described its effect on the structure and dynamics of the enzyme. 1H-NMR kinetic experiments were carried out to evaluate how hMER interferes with the catalytic efficiency of the enzyme towards ampicilim and meropenem. Chemical shift perturbation (CSP) were performed through15N-HSQC and 15N-TROSY titration experiments. In addition, Deuterium Hydrogen Exchange Mass Spectrometry (HDX) experiments were also performed to evaluate structural and dynamic modifications. The kinetic profile towards ampicillin indicate that low concentrations of hMER are enough to inhibit the penicillinase activity of OXA-143. In addition, our meropenem results show that this enzyme's carbapenemase efficiency is also affected in the presence of hMER. Furthermore, the NMR titration experiments indicate that the OXA-143 has a low affinity to the hMER, which blocks the active site as it accumulates in the solution. HDX data indicate that hMER decreases the flexibility of motifs essential for catalysis while increasing the dynamics at the active site, which seems counterproductive. These results indicate that hMER acts as a competitive inhibitor by blocking the active site and decreasing the dynamics of essential motifs around the binding site.

Interaction Features Between the Recombinant disintegrin, Jararacin, with Platelets: NMR studies and In vivo Activities.

https://proceedings.science/p/169601?lang=en

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Disintegrins are small cysteine-rich peptides found in snake venom, capable of modulating a broad range of transmembrane receptors known as integrins, heterodimeric receptors that have a key role in mediating physiological and pathological processes, such as platelet aggregation and thrombosis. Integrins, thus, have become a therapeutical target and disintegrins can be used as tools to provide new information about physiopathologies related to this receptor. The objective of this study was to express and analyze the effect of recombinant disintegrin, jararacin (rJarc), upon platelets. For this, we expressed the disintegrin on a Pichia pastoris system. The yeast was transformed with the vector pPIC9 containing the synthetic gene of jararacin. The disintegrin was expressed and secreted in the cultured media and then purified by molecular exclusion chromatography. Also, 15N-rJarc was expressed and purified. We confirmed the molecular mass by mass spectrometry and their correct folding by 1H nuclear magnetic resonance spectra and circular dichroism. NMR studies, in the presence or absence of platelets, revealed changes in rJarc structure and the residues involved in the platelet-desintegrin interaction. We, then, generate dockings based on AlphaFold and compared it with our findings in NMR, to generate in the future experimental models that corroborate the interaction mechanism between rJarc and the Integrins. These models will help us to a better understanding how this disintegrin interacts with platelets integrins, simulating the interaction in a more real way. Following this data we evaluate the ability of rJarc on an arterial thrombosis model, ex vivo platelet aggregation and bleeding assays revealed the ability to prevent thrombus formation and impaired platelet hemostasis at 5mg/kg dose. In conclusion, rJarc was expressed and can be used to get more information on the inhibition mechanism of integrins.

Investigation of the Type IV Secretion System (T4SS) substrate recognition mechanism using NMR spin relaxation experiments

https://proceedings.science/p/169613?lang=en

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The Type IV Secretion System from the bacterial pathogen Xanthomonas citri (T4SSXac) is a nanomachine specialized on the secretion of toxins that kill other gram-negative bacteria. VirD4 is a cytoplasmic coupling protein that recruits T4SS substrates to be translocated. All T4SSXac effectors contain a C-terminal domain called XVIPCD, that binds to the coupling protein VirD4 AAD domain. We have recently solved the NMR structure of the XVIPCD domain, however, the VirD4-AAD interaction mechanism remains poorly understood. NMR spin relaxation methods are extremely useful tools for studying functional protein dynamics and protein interactions. We have been using 15N spin relaxation experiments to study the XVIPCD-AAD interaction. The following NMR experiments were carried out on samples of XVIPCD or AAD in the free state and in the presence of substoichiometric concentrations of the unlabeled partner, at 600 and 900 MHz: 1) 15N R2 dispersion using a TROSY-CPMG pulse sequence; 2) 15N chemical exchange saturation transfer (CEST) experiments. CPMG data showed that a large region of the XVIPCD construct visits a low populated state (p = 4 - 5%) with exchange rates kex = 386 - 580 s-1 at the buffer conditions used (pH = 8.0). The 15N chemical shift differences between major and minor populated XVIPCD states, Δω, showed a reasonable correlation with calculated XVIPCD 15N secondary chemical shifts. In contrast, most of the AAD residues did not show any evidence of dynamic processes at the time scale probed by the CPMG experiment. The equilibrium

between native and unfolded XVIPCD states is consistent with the observation that this domain tends to unfold at pHs < 8. The occurrence of exchange between free and bound XVIPCD (AAD) remains unclear based on the current data.

Ixolaris and Factor-X interaction: structural and dynamic investigation by NMR

https://proceedings.science/p/169657?lang=en

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Coagulation activated by tissue factor (TF) is involved in the maintenance of physiological hemostasis and also in cardiovascular disease and thrombosis. After tissue injury, blood clotting begins with TF exposure, which interacts with Factor VIIa to form the FVIIa/TF complex. This complex will activate Factor X (FX) leading to Factor Xa, culminating in prothrombin's activation into thrombin and subsequent formation of fibrin. Initiation of the blood coagulation cascade by FVIIa/TF is controlled by of tissue factor pathway inhibitor (TFPI), a Kunitz-type inhibitor. Several exogenous coagulation inhibitors from the salivary gland of blood-sucking invertebrates have been characterized, like Ixolaris, a protein with sequence homology to TFPI found in Ixodes scapularis saliva that consists of 2 Kunitz domains (K1 and K2) where K2 is strikingly dynamic and encompasses several residues involved in FX binding (Francischetti et al., 2002). Our research group determined the three-dimensional structure and dynamics of Ixolaris and described the structural basis for recognition of FX, and this was the first report revealing the structure-function relationship of an anticoagulant that targets a zymogen serving as a scaffold for TF inhibition (De Paula et al., 2019). In the current work, we expressed the recombinant 2H, 13C, and 15N isotopic labeled Ixolaris to investigate the formation of the structural details of FX and Ixolaris complex at atomic resolution by Magnetic Nuclear Resonance (NMR). Through NMR TROSY experiments, we determined the relaxation parameters (R1, R2 and HetNOE) and the temperature coefficients for Ixolaris. We then titrated Ixolaris with FX in a 1:1 ratio and mapped the chemical shift perturbation (CSP) caused by the FX/Ixolaris complex formation. Additionally, we measured the dynamics parameters and temperature coefficients of FX/Ixolaris complex at concentrations of 50 and 70 µM. The perdeuteration of Ixolaris improved the characterization of the complex. We will further study the interaction with FVIIa/TF.

NMR STRUCTURES OF A COMPUTATIONALLY DESIGNED PEPTIDES DERIVED FROM Cry10Aa TOXIN

https://proceedings.science/p/169611?lang=en

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According to the World Health Organization (WHO), diseases resulting from Antimicrobial Resistance (AMR) already cause at least 700,000 deaths per year worldwide [1]. In this perspective, antimicrobial peptides (AMPs) have emerged as strong candidates to replace conventional drugs, which are no longer sufficiently effective.

The Cry10Aa toxin has been identified as a protein source for generating a new antimicrobials peptide, as this toxin has shown effective results in combating insect pests and controlling bovine mastitis. In this

regard, the α-helix 3 of the Cry10Aa toxin was used as a template in the Joker algorithm for generating new antimicrobial peptides. Six new variants were generated, and their biological activities were evaluated, along with the original peptide AMPCry10Aa. Among the new variants, the AMPCry10Aa_5 peptide demonstrated the best results in terms of minimum inhibitory concentration against gram-positive and gram-negative bacteria. AMPCry10Aa (IINVLTSIVTPIKNQLDKYQ-NH2) is a peptide composed of 20 amino acid residues cut from the α-helix 3 of the Cry10Aa toxin. AMPCry10Aa_5 (IINVKTSLKTIIKNALDKIQ-NH2) is a synthetic peptide consisting of 20 amino acid residues derived from the sequence of the parental peptide, AMPCry10Aa.

In this sense, both AMPCry10Aa and AMPCry10Aa_5 peptides had their three-dimensional structures determined by 2D NMR due to their recognition as promising antimicrobial drugs. The AMPCry10Aa and AMPCry10Aa_5 peptides revealed an α-helical folding between residues Ile2-Gln20 and Val4-Ile19, respectively. The structural results showed good convergence of the obtained low-energy structures, evidenced by the low RMSD values of 0.4641 ± 0.1467 and 0.1393 ± 0.0467 for AMPCry10Aa and AMPCry10Aa_5, respectively. Finally, the determination of the peptides' three-dimensional structures through NMR provided a better understanding of their mechanism of action, allowing the association of structural and dynamic characteristics of the molecule with its biological function.

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NMR-based fragment screening to search for antiviral candidates at the viral RNA and phosphoprotein binding site in the globular domain of the M2-1 protein of human Respiratory Syncytial Virus

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The human Respiratory Syncytial Virus (hRSV) is one of the main causes of acute respiratory diseases such as bronchiolitis and pneumonia in newborns, children, and elderly. So far, there is no effective vaccine against hRSV and the only commonly offered licensed treatment, a monoclonal antibody directed to viral fusion protein, is a costly drug that has had adverse reactions. The hRSV M2-1 protein is an important transcriptional anti-termination factor of the viral polymerase complex. The role of M2-1 in the context of this complex is determined by the interaction of its globular domain (dgM2-1) with viral messenger RNA and phosphoprotein. This scenario of intermolecular interactions makes dgM2-1 a potential target for the development of inhibitors of the viral replication cycle. In this sense, this study aimed to identify ligands (molecular fragments) with the potential to inhibit the interaction site of dgM2-1 with RNA and phosphoprotein. For that, the systematic approach of NMR-based fragment screening was used, selecting the most promising fragments from the DSI-PL Enamine library based on measurements of chemical shift perturbation (CSP), transverse relaxation time (T2), and WaterLOGSY. The five fragments identified as a hit had at least two positive parameters used for the screening. From this set of ligands presenting promising interaction with dgM2-1, four of them showed CSP greater than 3 Hz while two of them were greater than 6 Hz. All hits showed positive results for the T2 experiments but only two had positive cases for WaterLOGSY. Further analyses are required to characterize the binding site of these ligands, residues involved in the interaction and dissociation constants, and the development of this research contributes to the design of an alternative strategy to combat infections

caused by hRSV targeting dgM2-1 since its results provide relevant molecular information for potential drugs with antiviral action against this virus.

Probing chromatin function with NMR

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The fascinating thing about chromatin is that fulfills two conflicting roles: on one hand it compacts the DNA, represses unwanted transcription, and protects our genetic information from damage, on the other hand it attracts a myriad of proteins to regulate DNA damage repair, replication, and gene transcription. How these proteins recognize, modify, assemble, remodel or disassemble nucleosomes, the fundamental building block of chromatin, is one of the key questions in chromatin biology.
Our lab focusses on solving the molecular mechanisms underlying chromatin function using an NMR-driven integrative structural biology approach. In this talk I will highlight our recent results in the study of nucleosome assembly during DNA repair. We uncovered that intrinsically disordered histone chaperone APLF can single-handedly assemble the histone octamer and transfer it to the DNA to form nucleosomes, in contrast to the prevailing model of stepwise assembly. I will further present our recent work on the mechanism of nucleosome remodeling by ATP-dependent chromatin remodeler ISWI. This protein machine can translocate the DNA in the nucleosome to alter the spacings between nucleosomes within chromatin. We show that the ISWI ATPase domain (75 kDa) is an intrinsically dynamic machine that causes wide-spread conformational changes and alteration of histone-DNA contacts in the nucleosomes. These data provide crucial support for the twist-diffusion mechanism of remodeling and highlight the nucleosome as a plastic, allosteric unit. Finally, I will present some of our experiences in solution NMR at 1.2 GHz.

Production and characterization of an anti-IgG nanobody

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Nanobodies are single domain antibodies (sdAbs) derived from camelid heavy chain antibody [1,2]. They are characterized by their small size (~12-16 kDa), high stability, solubility, and specificity against antigens [1-4]. Therefore, nanobodies hold great biotechnological potential, with application in diagnosis and therapy. In this work, the recombinant production of a nanobody (126 amino acids – called sdAb-mrh-IgG) that recognizes the Fc region of IgG in rats, mice and humans was evaluated in Escherichia coli transformed with pET25b plasmid coding the sequence of sdAb-mrh-IgG, under different conditions (temperature, time, culture media, strain) after induction with isopropyl-beta-D-thiogalactoside. Considering the amount of soluble recombinant protein and time of the process we selected one protocol and followed with the recombinant [13C,15N]-sdAb-mrh-IgG production for NMR analysis. The cell growth was performed in M9 medium, enriched with 13C and 15N, overnight, at 37 °C. The [13C,15N]-sdAb-mrh-IgG was purified by immobilized metal affinity chromatography followed by a size-exclusion chromatography. The conformational stability of the [13C,15N]-sdAb-mrh-IgG was assessed by monitoring thermal denaturation using circular dichroism and chemical denaturation using intrinsic tryptophan fluorescence. The denaturation of [13C,15N]-sdAb-mrh-IgG is cooperative and has a melting temperature of 70,5 oC, representing a very stable conformation. A set of 2D and 3D NMR spectra was acquired for the assignment of the protein at 298 K using a 600 MHz and 900 MHz spectrometers (Bruker Avance III) equipped with pulse-field Z-axis gradient triple-resonance

probes. The 3D spectra were collected using non-uniform sampling [5]. The NMR spectra were processed with NMRPipe and analyzed with CCPNMR, available on NMRbox. Most of the NMR signals are narrow and well dispersed, typical of stable protein conformation. So far, we found 91% of the backbone nuclei (CHα, CO, and HNα) and 48% of the 13CHn side chain moieties.

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Prospection, Structure and Interaction Studies of NS2B protein and NS3 protease domain of Zika and Yellow Fever Virus: Searching for new compounds

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Flavivirus are arthropod-borne pathogens responsible for significant medical diseases, like, Dengue (DENV), Yellow fever (YFV), and Zika (ZIKV). These viruses could generate different symptoms, such as acute febrile illness, significant neurological disorders, and high mortality risk. The NS2B of Flaviviruses is a transmembrane non-structural protein that interacts with the NS3 protease domain through the soluble portion, acting as a cofactor to the catalytic activity. The NS2B-NS3 complex is responsible for processing the viral polyprotein, essential in viral replication, being an attractive target for developing antiviral drugs. This work aims to determine the structure of NS2B protein and study the interaction of NS2B-NS3 complex, especially ZIKV and YFV, to select through NMR fragment-based screening a new inhibitor candidate. Prospecting studies of NS2B and NS3 proteins from flavivirus were performed using bioinformatics tools. The ZIKV NS2B-NS3 complex was cloned into pET-Duet, while the YFV NS2B and a soluble portion were cloned into pET-28a. Expression tests were performed using different parameters, and the NS2B-NS3 complex purification was achieved using nickel affinity, anion exchanger, and size-exclusion chromatography. All experiments were monitored by 15% SDS-PAGE. The optimal ZIKV NS2B-NS3 expression condition was observed in E. coli Rosetta with 1 mM IPTG at 37ºC for 16h. The best expression condition of YFV NS2B and soluble portion is ongoing. ZIKV NS2B-NS3 complex will be isotopically labeled with 15N e 13C for structural determination by NMR. The interaction studies using fragment-based screening will be performed using a fragment library of 768 compounds and analyzed by STD, Waterlogsy, T2-CPMG, and CSP experiments. The structural and interaction studies with NS2B-NS3 complex and integral NS2B from different flaviviruses could be essential to the development of new specific inhibitors.

STD and WaterLOGSY NMR to explore the interaction of flavonoids with Leishmania donovani nucleoside hydrolase for the development of new preclinical candidates for visceral leishmaniasis

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Leishmaniasis is a neglected disease caused by protozoa of the genus Leishmania, which are transmitted by female sandflies infected with the parasite. Brazil is the country with the highest number of cases of visceral leishmaniasis (VL) in Latin America, which is the severe form of this disease, caused by the Leishmania donovani species, which is the main etiologic agent of VL. High toxicity, adverse effects and drug resistance to available treatments reaffirm the urgency of searching for new drugs. In this sense, a nucleoside hydrolase enzyme not identified in mammals, responsible for obtaining nitrogenous bases for the synthesis of DNA and RNA of the parasite, proves to be a potential therapeutic target[1,2]. In this study, 39 synthetic and natural flavonoids were screened against L. donovani nucleoside hydrolase (LdNH). The enzymatic assay was monitored in a UV-visible spectrophotometer (SpectraMax M5 Molecular Devices®) at 293nm[3]. Among the 39 substances evaluated, 17 inhibit the enzyme above 50% and are being evaluated by STD and WaterLOGSY NMR for structure-activity studies. The spectra were obtained according to the sequences described by Mayer and Meyer and Dalvit et al., with water signal suppression by DPFGSE. STD-NMR spectra were acquired with 2s saturation pulse, irradiating at -2020.5 Hz on resonance and 15970.9 Hz off resonance to produce the difference spectrum. WaterLOGSY spectra were obtained with a selective pulse of water with 25 ms and a power of 2 dB. All spectra were acquired with 3072 scans. The results show that the regions of the A and B rings of flavonoids are responsible for the protein-ligand interaction. Thus, it is possible to propose structural modifications in the C-ring region, to produce more potent inhibitors in the search for new preclinical candidates.

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Structural analysis of the interaction between the GRB2-SH2 domain and peptides derived from H2AX histone

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The homology-directed repair (HDR) pathway is a highly precise mechanism for repairing DNA damage. Histone H2AX plays a crucial role as a DNA damage marker in the HDR pathway. When a double-strand break occurs in DNA, H2AX is phosphorylated in the serine-139 and tyrosine-142 residues, recruiting proteins including the MRE11 protein, essential for the initiation and effectiveness of DNA repair. The interaction between H2AX and MRE11 is mediated by the adapter protein GRB2 through its SH2 domain. In this study, we aimed to structurally evaluate the interaction between the GRB2-SH2 domain and H2AX peptides by nuclear magnetic resonance (NMR) and computational simulations. GRB2-SH2 domain was expressed in E.coli BL21(DE3) and purified by affinity chromatography and size exclusion. The interaction with H2AX phosphopeptides was investigated using 15N-HSQC spectroscopy through chemical shift perturbation (CSP) analysis, along with computer simulations. NMR analysis of the GRB2-SH2 domain in the presence of H2AX peptides revealed that there is no interaction with H2AX_pSpY and H2AX_pS peptides, but interaction occurs with H2AX_pY peptide, which indicates that

the interaction specifically relies on phosphorylated tyrosine. In the GRB2-SH2/H2AX_pY complex, CSP in residues A68, S88, E89, G93, S96, L97, F108, and K109 suggest conformational changes enabling peptide binding. Computational results support the experimental data and provide insights into the mode and stability of the phosphopeptide interaction. This study provides molecular-level insights into the microenvironment of peptide interaction and its structural impact on the protein. Peptides with phosphorylated serine and tyrosine, or only phosphorylated serine, showed no interaction, while the peptide with phosphorylated tyrosine exhibited interaction. The mapping of GRB2-SH2/H2AX_pY interaction through CSP suggests involvement of both site I and site II, with site I recognizing phosphotyrosine and site II contributing to interaction stability.

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Structural and comparative studies of ZIKV NS2B protein in different micelles

https://proceedings.science/p/169673?lang=en

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ZIKA virus belongs to the Flaviviridae family that has structural similarities with other viruses of medical importance, such as Dengue virus (DENV), Yellow Fever (YFV) among others. In 2015, Zika outbreak in Brazil was severe and rapidly widespread worldwide, being characterized as a priority for the World Health Organization (WHO). The virus infection causes mild symptoms but could cause neurological diseases, such as fetal microcephaly and Guillain-Barré Syndrome. ZIKV particle has three structural and five non-structural proteins. The non-structural transmembrane protein, NS2B, works as a cofactor of NS3 protease, which is essential for viral replication working as a crucial target for new antiviral drugs. Recently, our group assigned and determined the structure of NS2B in presence of SDS micelles by NMR, using Rosetta. To better understand the behavior of NS2B in the presence of different micelles, this work aims to determine and compare the structure and dynamics of NS2B in the presence of DHPC micelles.NS2B was expressed in a minimal medium supplemented with Yeast Nitrogen Base (YNB). The optimal expression conditions were achieved using E.coli BL21(DE3) strain and induction at OD600nm 1,0 using 1 mM of IPTG at 37ºC for 4h. After expression, the protein was found insoluble in inclusion bodies, followed by cell lysis, NS2B was extracted using 1% SDS micelles, and purified by nickel affinity and size exclusion chromatography. NS2B protein structural stability was studied by intrinsic tryptophan fluorescence under physical and chemical denaturing conditions in presence of SDS micelles. Studies of NS2B in presence of DHPC micelles will be performed to compare the structural behaviour of NS2B, which remains stable in SDS micelle. These results with comparative studies in DHPC micelle could bring important information about the protein features paving the way to the interaction studies with NS3 protease and to the screening of fragments by NMR.

Structural and dynamics studies of FKBP12 from Different Microorganisms: Biological Target for Inhibitors Against Tuberculosis and Neglected Diseases

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Neglected Tropical Diseases (NTDs) are found in tropical and subtropical world regions and constitute a substantial public health problem. The tuberculosis epidemic annually affects about 10 million people worldwide. Bacterial resistance and the lack of more effective therapy have become a considerable concern. Thus, the investigation of new molecular targets and the development of more potent drugs against these diseases is crucial. FKBP12 proteins, an essential peptidyl-prolil-cis-trans-isomerase to numerous disease-causing microorganisms, are reported as potential biological targets. They differ about 40% in their primary sequence compared to their human ortholog. This work aims to study the structure and dynamics of FKBP12 from different microorganisms and compare it to Homo sapiens ortholog. Prospection studies using the primary sequence of human FKBP12 were done using bioinformatics tools. FKBP12 from Mycobacterium tuberculosis (MtFKBP12) and Leishmania infantum (LiFKBP12) were selected. Previously, the group performed structural, dynamics, and interaction studies with MtFKBP12. LiFKBP12 were cloned into pET28a and transformed in E. coli strains. Expression tests were performed using different parameters and were monitored by 15% SDS-PAGE. Results and discussion: LiFKBP12 best expression condition was achieved using BL21(DE3) strain and induction with 0.5 mM of IPTG (OD600>1.0), at 18oC for 4 hours. Purification methods were applied using two steps: affinity and size-exclusion chromatography. His-tag was cleaved with TEV protein (1:10) using dialysis for 16h at 4oC. LiFKBP12 protein will be isotopically labeled with 15N and 13C to perform all experiments required for NMR assignment and dynamics studies. These studies will bring essential information about specific regions of FKBPs that will be important to the studies of fragment screening for the search for new compounds against Tuberculosis and NTDs.

STRUCTURAL BASIS OF NUCLEIC ACID BINDING TO THE COLD SHOCK DOMAIN OF THE GLYCINE-RICH PROTEIN AtGRP2

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AtGRP2 (Arabidopsis thaliana glycine-rich protein 2) is a glycine-rich, RNA-binding protein that plays key roles in abiotic stress [1] and flowering time regulation [2]. AtGRP2 consists of an N-terminal cold shock domain (CSD) and two C-terminal CCHC-type zinc knuckles interspersed with glycine-rich regions. Despite the wealth of information on AtGRP2 function, the molecular mechanisms underlying its biological roles are largely unknown. Here, we investigated the structure, dynamics, and nucleic acid binding properties of AtGRP2-CSD. The 2D [1H,15N] HSQC spectrum of AtGRP2-CSD1-79 revealed the presence of an unfolded state in equilibrium with the folded state. Addition of eleven residues at the C-terminus stabilized the folded conformation. The three-dimensional structure of AtGRP2-CSD1-90 revealed a β-barrel composed of 5 antiparallel β-strands. The first half of the C-terminal extension is ordered within the structural ensemble and exhibits low flexibility. We observed a network of NOE connectivities between residues V82 and N85 and residues I43, R44, S45, G47, and F48, suggesting that interactions between the C-terminal extension and the β3-β4 loop stabilize the CSD fold. Titration experiments revealed that AtGRP2-CSD1-90 binds nucleic acids through a preformed platform of solvent-exposed residues located in strands β2 and β3, as well as the β3-β4 loop. Binding affinity is higher for DNA than RNA oligonucleotides, with a clear preference for T-rich sequences. 15N-{1H} NOE

values revealed a loss of flexibility for residues R49, S50, and Leu51, in the β3-β4 loop, upon T7 binding. In addition, T7 binding increased the R2/R1 ratio for residues in the binding interface. The largest R2/R1 values were observed for R49 and K22, which may compose an initial protein-DNA encounter complex. These results shed light on the mechanism of nucleic acid recognition employed by AtGRP2, creating a framework for the development of biotechnological strategies to increase plant resistance to abiotic stresses.

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STRUCTURAL CHARACTERIZATION AND DNA BINDING PROPERTIES OF THE RRM DOMAIN OF THE PLANT GLYCINE-RICH PROTEIN AtGRP7

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AtGRP7 (Arabidopsis thaliana glycine-rich protein 7) is a glycine-rich, RNA-binding protein that plays a crucial role in plant growth, development, and abiotic stress response. AtGRP7 consists of an N-terminal RNA recognition motif (RRM) followed by an intrinsically disordered, glycine-rich region. Despite AtGRP7 significance in cold adaptation and flowering time regulation, the biochemical mechanisms underlying its function are largely unknown. Here, we employed biophysical techniques to investigate the structure, dynamics, and DNA binding properties of AtGRP7-RRM. The RMM domain of AtGRP7 (residues 1-90) was cloned into RP1B and expressed in Escherichia coli BL21 DE3 as a His6-fusion protein. Following purification by nickel-affinity and size exclusion chromatography, AtGRP7-RRM was characterized using circular dichroism, NMR and fluorescence spectroscopy. Circular dichroism revealed features consistent of a typical RRM fold, comprising a mixture of α-helices and β-sheets. AtGRP7-RRM displayed a melting temperature of 38 oC, indicating relatively low stability. Using multidimensional, triple resonance NMR, we unambiguously assigned 90% of the backbone resonances. Notably, some NH resonances were missing for residues Met1, Ala2, Ser3, Gly4, Trp17, Arg43, Gln85, Ser86, Arg87, Gly88, Ser89, Gly90, likely due to conformational dynamics or solvent exchange. Upon titration of a 7-nucleotide DNA ligand, previously identified as a specific binding site, we observed shifts in amide resonances. Analysis of the binding interface revealed that surface-exposed residues in the □2-□3 loop and strands □1 and □5 are involved in DNA interaction. The fluorescence emission spectrum of AtGRP7-RRM exhibited a maximum at ~349 nm, indicating solvent exposure of Trp17. Increasing concentrations of DNA resulted in fluorescence quenching, and AtGRP7-RRM interaction with DNA occurred with an apparent KD of 17.9 ± 4.2 μM. 15N relaxation parameters indicated fast backbone motions in the □2-□3 loop, suggesting that loop flexibility is correlated to DNA binding. This is an important first step toward the structure determination of AtGRP7-RRM, which will shed light into its DNA recognition mechanism.

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Structural Dynamics Study of Thermophilic Proteins by NMR - TTHA0849 of Thermus thermophilus

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The PR-10 family shares the tertiary arrangement β-α2-β6-α which forms a hydrophobic cavity. The biological function of these proteins is diverse, and furthermore, they can bind through the hydrophobic cavity to various molecules and participate in enzymatic processes. Structural studies showed that the hydrophobic cavity is partially inaccessible to ligands in solution, and conformational fluctuations are essential for the accessibility of the binding site. These proteins need to modulate between open states that expose the hydrophobic cavity for the ligand but increase aggregation possibilities and more stable closed states. The Bet v 1 protein, the first PR-10 studied in our laboratory, is derived from the pollen of Betula verrucosa and is responsible for the development of allergic processes. Through 15N relaxation experiments, our group showed that Bet v 1 assumes different conformations in the free state that might represent the cavity opening, permitting the ligand to enter and bind to it.
TTHA0849 was found in the Thermus thermophilus genome and presents a PR-10 fold that forms a hydrophobic cavity and high structural similarity with Bet v 1, with considerable differences. The cavity of the TTHA0849 has more residues with hydrophobic and bulky side chains and less volume available for the accommodation of ligands. This work aims to characterize the structural dynamics of TTHA0849 in its free and bound state and compare it with Bet v 1. We used intrinsic fluorescence, CD, and NMR to evaluate the structure, dynamics, and interaction with hydrophobic compounds. TTHA0849 recombinant protein was obtained and purified by chromatography, and our data showed it has high thermal stability and chemical resistance. Furthermore, we have evidence of complex formation with compounds. The backbone assignment is almost complete (93 %), and relaxation parameters (R1, R2, and hetNOE) were collected in different temperatures and are being analyzed.

Structural Studies of Zika Virus NS3 Protease and NS2B Membrane Protein: Expression, Purification and Nanodiscs Assembly

https://proceedings.science/p/169670?lang=en

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Zika virus (ZIKV) is a member of the Flaviviridae family, transmitted to humans by mosquitoes, and has become a significant health concern after the 2015 epidemic in Brazil and quickly spread to other regions. The infection can cause severe neurological diseases, such as fetal microcephaly and Guillain-Barré syndrome in adults. The viral complex NS2B-NS3 is essential for virus replication and represents an attractive drug target. This work aims to assign and study the dynamics of ZIKV NS3 protease (NS3pro) by Nuclear Magnetic Resonance (NMR) and determine the structure and dynamics of integral NS2B assembled into nanodiscs. NS3pro was cloned into pET-28a and transformed into E. coli strains. Expression tests for NS3pro were performed using different parameters to determine the best expression conditions. The protein was purified by nickel affinity chromatography and concentrated by ultrafiltration with a 10 kDa pore membrane. As a test for further NMR experiments, bacteria were grown using the best conditions in M9 minimal medium supplemented with yeast nitrogen base (YNB). All experiments were monitored by 15% SDS-PAGE. The results showed that NS3pro best expression conditions were in E. coli BL21(DE3) with 1 mM IPTG at 37°C for 4 hours or 18°C for 16 hours. The insoluble protein goes to inclusion bodies being extracted with SDS 1%. The purification steps are ongoing using nickel affinity and size-exclusion chromatography. NS3pro was expressed in minimum medium, purified and concentrated for activity and interaction studies. Membrane proteins are

unstable in solution, therefore, a membrane mimetic such as nanodiscs is needed for dynamic and structural investigations. We are producing different constructions of membrane scaffold proteins (MSPs) to produce nanodiscs with low diameters to study the structure of NS2B and its interaction with NS3. In conclusion, this work could pave the way for the search for new compounds with antiviral activity.

STRUCTURE ELUCIDATION OF PURIFIED CRYSTALINE MANNAN ISOLATED FROM AÇAÍ SEEDS (Euterpe oleracea Mart.)

https://proceedings.science/p/169630?lang=en

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Açaí (Euterpe oleracea Mart.) is a typical fruit from the Amazon Forest, whose seed are 85% of the fruit's weight. Almost half of the dry mass of this abundant agroindustrial waste is mannan polysaccharide, and 90% of the glycosil residues composition of the seed is mannose. However, the mannan from the seeds is water-insoluble and recalcitrant to sustainable deconstruction by enzymatic action. Therefore, evaluate the structure of this mannan can help to understand the limitations of the seed industrial processing. The açaí seeds were fractionated to obtain a purified fraction of mannan, analyzed by different complementary methods. The NMR analysis of the purified mannan results in δ from 1H/13C HSQC spectrum which are in agreement with signals of 1,4-linked β-D-Manp. The 13C spectra had six carbon signals assigned to a β-(1→4) D-mannan, as the 1H/13C HMBC confirms the presence of β(1→4) glycosidic linkage between Manp units by the cross peaks at 4.73/78.0 ppm (H-1/C-4) and 3.88/101.8 ppm (H-4/C-1). Given the difficulty to work with a water-insoluble sample, 13C CPMAS solid state NMR evaluated mannan. The identified linear mannan is known to have crystal structures, polymorphs I and II, which were identified in the sample, since the two signals corresponding to carbon 5 of both forms were detected at 70.2 and 70.9 ppm, respectively. The signals intensity shows that polymorph I is in greater concentration. The diffraction profile of the sample was comparable to a crystalline form of linear mannan I, also contained in mannan from ivory nuts. The HPAEC-PAD analysis showed that there is also glucose and galactose content, in low amounts, in the mannan sample. Therefore, this unprecedented identification of the purified linear mannan structure from the açaí seed will allow the establishment of strategies to reduce the crystallinity of the mannan.

Study of conformational dynamics of the encounter complex formed between the core domain of hRSV M2-1 protein and the intrinsically disordered region of viral phosphoprotein P using NMR spectroscopy

https://proceedings.science/p/169660?lang=en

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In the replication cycle of the human Respiratory Syncytial Virus (hRSV), the interaction between the M2-1 anti-termination factor and phosphoprotein P is important for the success of viral transcription. The M2-1 protein plays a crucial role in preventing the RNA-dependent RNA polymerase replication complex, viral polymerase plus the cofactor phosphoprotein, from disassociating upon reaching the

codon stop, increasing transcriptional efficiency. The M2-1/P interaction occurs between the core domain of M2-1 (cdM2-1) and the intrinsically disordered region (IDR) of residues 90–110 of P (P90-110). The present work aimed to characterize the conformation dynamics of the encounter complex formed between the cdM2-1 and the IDR P90-110 using NMR spectroscopy. For this purpose, it was analyzed the changes in the chemical shift perturbation (CSP) of the 15N cdM2-1 at different concentrations of the peptide P90-110 until reached saturation, as well as analyzed 15N CPMG relaxation dispersion (RD) at undersaturation concentration of the peptide. The CSP and CPMG-RD results revealed concomitantly that the residues Val127, Asn138, Gln144, Lys150, and Val156 in helix α3, α4, and α6 of the cdM2-1 are directly involved in the M2-1/P interaction interface, presenting the highest values of CSP and significant relaxation dispersion profiles, while 15N CPMG-RD data identified exclusively the residues Ser133, Thr145, Leu149, Arg151, and Thr160 involved in the encounter complex formed between the protein and the IDR. These results indicate that the formation of the M2-1/P complex is dynamic with both cdM2-1 and IDR P90-110 undergoing structure, population, and interconversion rate changes in order to interact. The study of such complexes helps in the structural understanding of proteins and intrinsically disordered regions (IDPs and IDRs) enabling new strategies for drug discovery. In further experiments, such dynamics will be investigated by targeting the P90-110 instead of the cdM2-1.

The Pisum sativum defensin 2 (Psd2), a plant defensin, as a model to study the thermostability of surface hydrophobic clusters by NMR

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Plant defensins (PDs) are small peptides, stabilized by 4 disulfide bonds in the well-described cysteine-stabilized αβ-fold (CSαβ-fold). They display antimicrobial activity and are on the front line of innate defense responses developed by plants against pests that threaten their survival. PDs lack a canonical hydrophobic core, displaying almost all hydrophobic residues on the protein surface. The few available studies of the dynamics of PDs show that, despite being highly crosslinked by disulfide bonds, they are highly dynamic in multiple timescales. The exposed hydrophobic residues form surface clusters stabilized by the vicinity of hydrophilic residues and the hydration shell. Here, we studied the Pisum sativum defensin 2 (Psd2) (1, 2) as a model to study the formation and stabilization of these local foldons named surface hydrophobic clusters (SHC). We characterized the temperature dependence of relaxation dispersion profiles (15N CPMG) to describe the complex dynamics of Psd2. These data allow to indirectly study the thermodynamics of SHCs in Psd2. We showed a correlation between residues undergoing conformational exchange and the SHCs. Chemical shift changes between the native ground state and the first thermally accessible excited state enabled us to map the major conformational changes in Psd2 conformational equilibrium. The observation of a cold-driven excited state revealed that SHCs are stabilized by hydrophobic contacts, which are exposed at low temperatures, leading to a favorable decrease in enthalpy compensated by an unfavorable entropy reduction. At higher temperatures, we detected another excited conformer that may play a role in membrane-specific interaction, as previously described for other defensins.

THE SERINE-ARGININE-RICH REGION DRIVES LIQUID-LIQUID PHASE SEPARATION OF THE N-TERMINAL DOMAIN OF hCOV-HKU1 NUCLEOCAPSID PROTEIN

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The coronavirus nucleocapsid protein (N) is a multifunctional RNA-binding protein that plays key roles in nucleocapsid assembly and discontinuous transcription. N consists of two folded domains, namely the N-terminal domain (NTD) and the C-terminal domain (CTD), connected by an intrinsically disordered region containing a serine-arginine-rich motif. Here, we investigated the liquid-liquid phase separation (LLPS) ability of the N-terminal domain of hCoV-HKU1 N (N-NTD-SR) in vitro. Unlike SARS-CoV-2 N-NTD-SR, hCoV-HKU1 N-NTD-SR formed condensates in the presence of nucleic acid alone, indicating higher LLPS propensity. Furthermore, the SR region is critical for HKU1 N-NTD-SR LLPS. Heterotypic phase separation was evaluated using different DNA oligonucleotides, including specific TRS (ssTRS(+); ssTRS(-); dsTRS) and nonspecific (ssNS(+); ssNS(-); dsNS) ligands. All oligonucleotides triggered HKU1-N-NTD-SR condensation. Excess of ssTRS(+) and dsTRS dissolved condensates, whereas the same was not observed for ssTRS(-). dsTRS induced HKU1 N-NTD-SR condensation at a much lower protein:DNA stoichiometry (4:1). The LLPS profile induced by nonspecific DNA was comparable to that of TRS. HKU1 N-NTD-SR:DNA condensates were highly sensitive to NaCl, indicating the significance of electrostatic interactions, while showing resistance to 1,6-hexanediol, suggesting minimal involvement of hydrophobic contacts. Over time, the HKU1 N-NTD-SR:DNA heterotypic condensates displayed a slow fusion process, suggesting their highly viscoelastic nature. Fluorescence recovery after photobleaching (FRAP) confirmed the demi-liquid property of condensates. To determine the oligomerization site that leads to LLPS, 2D [1H,15N] HSQC spectra of HKU1 N-NTD-SR were collected at increasing protein concentrations. In addition, a gadolinium complex was used to induce paramagnetic relaxation enhancement in both the dilute and condensed phases. These results suggest that the SR tail engages in specific contacts relevant to oligomerization and phase separation. Overall, these results provide insights into the molecular mechanisms underlying the regulatory functions of the coronavirus N protein in viral transcription and replication.

Acknowledgments: FAPERJ, CNPq, CAPES

THE USE OF 19F-NMR AS A PROBE TO MEASURE INTER-DOMAIN DYNAMICS AND REGULATION OF GRB2 ACTIVITY

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The growth factor receptor-bound protein 2 (Grb2) is an adapter protein that participates in the activation cascade of some receptor tyrosine kinases. Grb2 forms complexes that activate the mitogen-activated protein kinase (MAPK) signaling pathway, leading cell differentiation. Furthermore, this protein is of paramount importance to prevent these receptors from being activated without extracellular stimuli from growth factors. Grb2 has 217 amino acid residues, 25KDa in its monomeric state, and an SH2 domain flanked by two SH3 domains (cSH3 and nSH3). In this work, we evaluate the hypothesis that the microenvironment modulates the interdomain dynamics and can regulate Grb2 activity. We used effectors that change the protein solvation and known molecular partners to measure protein dynamics using the nuclear magnetic resonance (NMR). The protein expression with isotopic labeling of the atoms 15N and 19F was used E. coli BL21 DE3 strain. We purified Grb2 by nickel affinity followed by a molecular exclusion. The NMR experiments consist of measuring the interaction with

peptides derived from the SOS1 recognition region (VPPPVPPRRR) to the SH3 domain of Grb2 and phosphopeptide from the pEGF recognition region (EpYINSQV) by the SH2 domain. We measured the 19F relaxation parameters (R1, R2) of the with 5-Fluoro-L-Tryptophan (5F-Trp) labeled Grb2 of the free protein in the presence of SOS1, pEGF, and/or PEG400. The 19F measurements provided information on the dynamics of each of the 19F-tryptophans present in the protein. The addition of the pEGF elicited chemical shift changes of W60 and W121, while the addition of the SOS1 peptide changed in W193 and W194. Titration with PEG400 showed a significant line broadening of W60. The W60 is in the dimerization interface and the effect of PEG400 resembles the allosteric effect of the pEGF, which is the trigger for monomerization, suggesting that the solvation shell modulates the allostery.

TOWARD THE STRUCTURE DETERMINATION OF THE C-TERMINAL DOMAIN OF YEAST PDP3

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Histone post-translational modifications (PTMs) modulate chromatin structure by facilitating the interaction between chromatin-modifying complexes and nucleosomes. In budding yeast, the NuA3 histone acetyltransferase complex binds specifically to H3K4me3 and H3K36me3 through Yng1 and Pdp3, respectively. Subsequently, Sas3 catalyzes the acetylation of H3K14, which initiates transcription at a subset of genes. Pdp3 comprises an N-terminal PWWP domain responsible for chromatin association, and a folded C-terminal domain of unknown function connected by an intrinsically disordered region. Here, we aim to elucidate the three-dimensional structure of the C-terminal domain of Pdp3, identify structural homologs, and propose a function for this domain in chromatin remodeling. The C-terminal domain of Saccharomyces cerevisiae Pdp3 (residues 194-303) was cloned into RP1B and expressed in Escherichia coli BL21 DE3 as a His6-fusion protein. Purification of Pdp3C involved nickel-affinity chromatography followed by TEV protease cleavage of the His6 tag. Additionally, Pdp3C was purified through a second nickel-affinity step and subsequent size-exclusion chromatography, resulting in its elution as a monomer. Multidimensional NMR spectra were acquired for a ~800 µM 15N/13C-labeled Pdp3 sample, including 2D [1H, 15N] HSQC, 2D [1H, 13C] HSQC, 3D HNCA, 3D HNCO, 3D HNCACO, 3D HNCACB, 3D CBCA(CO)NH, and 3D HBHA(CO)NH. Analysis of these spectra allowed unambiguous assignment of 90% of the backbone resonances. Chemical-shift derived secondary structure propensities indicated that Pdp3C adopts a fully α-helical conformation. To assign side chain resonances, 3D CC(CO)NH, 3D HC(C)H-TOCSY, and (H)CCH-TOCSY experiments were performed, and the side chain assignment is currently underway. Our data provide a basis for investigating the three-dimensional structure and dynamics of Pdp3C, which will enhance our understanding of its functional role within the NuA3 histone acetyltransferase complex in yeast.

Toward the structure of the FF1 Domain of the P190A RhoGap Protein in the excited state

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P190-A RhoGAP (Rho signaling guanosine triphosphatase activation protein) is the only cytoplasmatic protein that contains FF domains in tandem. FF domain is involved in phosphoserine recognition, and it

is present in proteins that act in the signaling pathway. P190A is regulated by extracellular inputs. Growth factors such as platelet-derived growth factor (PDGF) lead to the phosphorylation of Y308, which results in the dissociation of P190A of the transcription factor TFII-I, ultimately leading to the nuclear internalization of TFII-I and activation of c-fos and inducing cellular proliferation. Y308 is buried in the hydrophobic core of the FF1 domain of P190A and we want to understand how it becomes accessible for the kinase. We aim to determine the structure and dynamics of the excited state of the FF1 domain of the P190A. FF1 was expressed in E. coli strain BL21(DE3) and double-labeled with 13C and 15N. We purified by nickel-affinity followed by gel filtration with a cleavage step between them using the Tobacco Etch Virus (TEV) enzyme. We acquired conformational exchange saturation transfer experiments (CEST) for 15N, C´, Cα, Cβ, and HN, enabling the almost complete assignment of the backbone protein backbone in the excited state. We are now optimizing the methodology for determining the residual dipolar coupling (RDC) at the excited state using Trosy/Anti-Trosy relaxation dispersion. To compare the chemical shift of the excited state with the unfolded state, we assigned the FF1 at 4.5 and 8 M urea. These data allowed us to unambiguously show that the excited state is not the unfolded state. This data enables the understanding of the importance of the conformational equilibrium of the FF1 domain to undergo the signal transduction pathway.

Acknowledgements: CAPES, FAPERJ, CNPq

Towards the mechanism of jarastatin (rJast) inhibition of the integrin αVβ3

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Disintegrins are cysteine-rich proteins found in snake venoms. These proteins selectively bind to integrins, which play a key role in the regulation of many pathophysiological processes. In this study, we report the structure, backbone dynamics, and determine the binding site to integrin αvβ3. We measured the amide backbone relaxation parameters 15N (15N R1, 15N R2, and 1H-15N heteronuclear NOE) and the 15N Carr-Purcell-Meiboom-Gill relaxation dispersion (CPMG-RD). We observed that dynamics play an important role in the interaction. Many residues at the interface of interaction with integrin αvβ3 are undergoing conformational exchange or thermal flexibility. We observed the involvement of the N-terminal domain in the interaction along with the C-terminal domain containing the loop where the RGD (Arg-Gly-Asp) binding motif is located. Finally, we performed NMR-derived docking (Haddock) of the rJAST and αvβ3 complex, using information from the literature and our experimental CSP and dynamics data. To obtain information about the stability of the rJAST/αvβ3 complex, the NMR structures of rJAST (PDB: 8S9E) and αVβ3 ectodomain (PDB: 4MMX) were used as initial structures for molecular dynamics simulations (MD). Three replicas of a 100 ns MD simulation of the rJast/αVβ3 complex were performed, starting from the lowest energy structure of the docking model, monitoring the root mean square deviation (RMSD) in function of time. The RMSD remained below 0.6 nm in all replicas, indicating that the rJast/αVβ3 complex is stable throughout the trajectories, with the N-terminal and C-terminal domains remaining associated with the integrin. The stable interactions between rJast and αVβ3 during the MD simulation were also analyzed.
Acknowledgments: FAPERJ, CNPq, CNRMN

Transient diffusive protein-protein interactions in live cells - functional optimisation on meso- and microscopic levels.

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A striking feature of nucleic acids and lipid membranes is that they all carry net-negative charge, and so is true for the majority of intracellular proteins. It is suggested that the role of this negative charge is to assure a basal inter-molecular repulsion that keeps the cytosolic content suitably 'fluid' for function. Unlike the situation in test tubes, any functional protein-protein interaction in the cytosol is subject to competition from the densely crowded background, i.e. surrounding stickiness. At the nonspecific limit of this stickiness is the 'random' protein-protein association, maintaining profuse populations of transient and constantly interconverting complexes at physiological protein concentrations. The phenomenon is readily quantified in studies of the protein rotational diffusion, showing that the more net negatively charged a protein is the less it is retarded by clustering. In-cell NMR can be used to study how specific protein properties modulate this cross-talk with the cytosolic environment, and conversely: How constant collisions and formation of ransom transient encounter complexes are affecting basal protein properties. Random encounters between proteins in crowded cells are by no means passive, but found to be under selective control, which e.g., enables proteome solubility, optimise the diffusive search for interaction partners, and allow for an adaptation to environmental extremes. Interestingly, the residues that modulate the random encounters act mainly mesoscopically through protein net charge, meaning that their detailed signatures vary across organisms with different intracellular constraints. To further examine such variations, we compare the diffusive behaviour of one bacterial and two human proteins in the E. coli and the human cytosols, using in-cell NMR relaxation. We find that proteins that 'stick' and whose signals become broadened beyond detection in E. coli are generally less restricted in mammalian cells. This dynamic protein-protein interplay is under evolutionary control and
finely tuned across organisms to maintain optimal physicochemical conditions for the cellular processes. The emerging picture is then that specific cellular function relies on close competition between numerous weak and strong interactions, and where all parts of the protein surfaces are involved. The outstanding challenge
is now to decipher the very basics of this many-body system: how the detailed patterns of charged, polar and hydrophobic side chains not only control protein-protein interactions at close- and long-range, but also the collective properties of the cellular interior as a whole.

Unraveling the Contribution of Side-Chain Residues to Lunatin-1 Peptide's Antitumor Activity: A Comprehensive Structural Study via Ala-Scan Strategy

https://proceedings.science/p/169594?lang=en

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The unique composition of peptides combines various physicochemical properties that allow the design of new biopharmaceutical products. Lunatins are a family of bioactive peptides derived from scorpion venom (Hadruroides lunatus), with antimicrobial and antitumor potential. The lunatin-1, a hydrophobic 13-mer peptide with three aromatic residues and a C-terminal amidation, was subjected to Alanine scan (Ala scan) to evaluate the contribution of amino acid residues to the antitumor activity. Thirteen lunatin

analogs were synthesized, and we showed that a glycine-4-alanine substitution improved antitumor activity, whereas a lysine-7-alanine substitution significantly decreased it. The structural effects of the residues substitutions in the peptide have not been previously described. Here, we show by NMR assignment and structural calculations, that the glycine-4-alanine improves the folding of the alpha-helix of the peptide in SDS-d25, which could explain the higher antitumor activity. While the parental peptide, lunatin-1, is partially helical and has an unfolded N-terminal, the analogous peptides have enhanced helical structures, in comparison. Furthermore, the low activity of the analogous lunatin-1T12A appears to be due to the absence of the phenylalanine-12 next to phenylalanine-13, resulting in a change in the structural arrangement of the last residue ring. Our results demonstrate the importance of the composition of residues, apart from their arrangement in the primary structure, in a bioactive peptide and the importance of the rationalization process in the design of a peptide. In addition, the complete folding of lunatin-1G4A appears to be the answer to improved antitumor activity. The family of lunatins appears to be a good model for a better understanding of peptide design in nature and how we can use this information to improve our in silico models for peptide design.

Hyperpolarization/DNP

DNP Surface Enhanced Solid-State NMR Spectroscopy: Recent Developments and Applications

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While the fundamental principles of Dynamic Nuclear Polarization (DNP) and the first proofs of concept were demonstrated 70 years ago, it is only recently that this method became a game-changing technology to overcome the sensitivity barrier of solid-state Nuclear Magnetic Resonance (NMR) spectroscopy under Magic Angle Spinning (MAS). For the last twenty years, ongoing advances, notably in the instrumentation, have democratized this approach, with renewed application breakthroughs. DNP enhanced solid-state NMR has notably developed as a powerful approach for an in-depth structural characterization of functionalized surfaces and materials. In this presentation, we will first describe unique DNP NMR methodologies to disclose, with atomic resolution, individual surface structures in complex multi-site environments, a long-standing challenge in the field of heterogeneous catalysts (2-3).
The efficiency of the continuous-wave DNP critically depends on the structure and properties of the polarizing agents (PA) hosting the free electrons (4). In this presentation we will also review our recent effort at designing PA with improved efficiency, especially at high magnetic field and very fast MAS frequencies.

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Imaging

Deuterium MRI: The next tool for cancer detection?

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Deuterium Metabolic Imaging (DMI) is a promising tool for studying tumor metabolism or metabolism in general. DMI monitors the uptake of [6,6'-2H2]-glucose by tumors, resulting in the formation of HDO and [3,3'-2H2]-lactate as result of the Warburg effect. The most challenging aspect of DMI is the low signal-to-noise ratio (SNR), which is due to the low Larmor frequency of 2H and the low concentrations of the targets. In this talk, I will present the concepts behind DMI and discuss some of our recent efforts to improve the SNR.

KEYWORDS: Deuterium Metabolic Imaging, Magnetic Resonance Spectroscopic Imaging, SSFP

MAGNETIC RESONANCE IMAGING AS A TOOL TO ENLIGHTEN THE UNDERSTANDING OF THE BRAIN CLEARANCE

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Studies on the human brain still fascinate the research community with every new discovery made due to the complex organization of the brain architecture required for precise functioning in performing a variety of tasks. Until the beginning of the 2010s, there was a poor description regarding how the waste clearance was held to keep the brain tissue safe and free to work properly. With the recent advent of the experiments carried out by Iliff et al and by Nedergaard and Goldman it was raised the hypothesis that the brain also has its own clearance mechanism. In that sense, the cerebrospinal fluid (CSF) flow and the water exchange across two important membranes, the bloodbrain and blood-cerebrospinal fluid barriers (BBB and BCSFB) were raised as the topic of several new questions to be answered.
In the process of understanding these freshly described brain clearance mechanisms, neuroimaging tools have played a key role, along with histopathological studies. In especial, MRI emerged as viable alternative in the halfway of spatial resolution and invasiveness to the patients. While initial MRI measures of the brain clearance and the water exchange across the brain barriers were mostly performed with contrast enhanced methods, including the injection of contrast agent via lumbar intrathecal application, novel non-invasive MRI techniques are being optimized to access this desired quantitative information.
This talk will cover the physical mechanisms beyond the MR signal designed to image the CSF flow and the times for the water to cross from the blood vessels reaching the brain tissue or the CSF by crossing the BBB and the BCSFB, respectively

MRI of Porous Media

https://proceedings.science/p/169615?lang=en

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Matrix acidization is a widely used technique in oil reservoirs that involves the application of acid to rocks, creating pathways called wormholes that enhance fluid flow. However, there is still a need for comprehensive information about the characteristics of these wormholes. Magnetic Resonance Imaging (MRI) has emerged as a valuable tool in this field. However, the evaluation of wormholes and fluid dynamics in acidized rocks has been challenging due to the limitations of conventional techniques in providing spatial information. In this study, we propose advanced MRI imaging methods to address this challenge and provide valuable insights into porous media. Our focus is on both static and dynamic analyses of these media. We can extract detailed information about rock morphology by employing state-of-the-art image analysis techniques, specifically identifying and characterizing wormholes. Additionally, we have developed novel methods to investigate fluid flow within the rocks, enabling the generation of velocity maps that offer a better understanding of flow patterns within the wormholes. These findings demonstrate the significant potential of MRI in studying porous media, providing unique insights into fluid dynamics, particularly in wormholes. Our results not only confirm the hypothesis that MRI imaging can significantly contribute to the study of porous media but also highlight its distinct advantages compared to other established techniques. The spatial information provided by MRI complements and, in some cases, surpasses the capabilities of conventional methods, making it an invaluable tool for understanding fluid behavior in porous media, particularly in the context of wormhole formation by matrix acidization.

Patient-specific steady-state CFD in healthy cerebral arteries using MRI: analysis with ASL CBF

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Computational fluid dynamics (CFD) is widely used in research fields such as biomechanics and medicine to study the cardiovascular system. Although magnetic resonance imaging (MRI) can provide patient-specific information for simulations, combining CFD and MRI for cerebral arteries remains a challenge in the field. Arterial spin labeling (ASL) is an MRI technique that enables the estimation of cerebral blood flow (CBF), and is beginning to be explored in CFD for parameter estimation or validation in complex and time demanding simulation models [1, 2]. The purpose of this study is to perform CFD simulations using MRI images in healthy cerebral arteries and to compare them with CBF value estimated with ASL in a simple steady-state model. MRI images were acquired from a single healthy subject on a 3T Siemens Prisma scanner (Siemens Healthineers, Erlangen, Germany). Using SimVascular software [3], we modeled the arterial anatomy with time-of-flight (TOF) MR angiography image. An average flow rate was calculated with the 2D phase-contrast MRI (2D-PC) that served as inlet boundary condition for our steady-state simulation. For the outlet boundaries, we considered a total resistance satisfying P=RQ and Murray's law to manage the resistance splitting. Finally, with ASL, we compared the perfusion split given by a vascular territory atlas with the flow rate split in the circle of Willis (CoW). Simulations were performed using SimVascular supercomputing gateway [4]. The most similar flow splits in the CoW were in the anterior and posterior cerebral arteries regions. However, the cerebelar and middle cerebral arteries regions presented a difference with respectively an overestimation and underestimation in comparison to the perfusion splits. One possible reason for these differences may be the structural modeling restriction around the proximal regions in the CoW. In conclusion, we presented a comparison of CFD with ASL in a simple and fast model.
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Quantitative MRI techniques in clinical research: from in vivo to postmortem MRI

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In this talk I will present how we apply some of the quantitative NMR techniques in clinical research, by in vivo MRI, to address specific clinical questions of the brain. In particular, I will show some examples of spectroscopy and diffusion techniques, applied to psychiatric diseases like bipolar disease and obsessive-compulsive disorder. Furthermore, I will bring up an example on how postmortem MRI can help us to understand the origin of in vivo MRI signal (i.e. in susceptibility mapping), so that we can develop non-invasive imaging biomarkers of neurodegeneration to study the aging population.

Quantum Sensing for advancing in quantitative Magnetic Resonance Imaging techniques

https://proceedings.science/p/169661?lang=en

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Quantum technologies such as quantum sensing are expected to revolutionize medical diagnosis. Quantum sensors will serve applications ranging from thermometry to diagnostic imaging with sub-micrometer resolutions [1-6]. Traditional non-invasive magnetic resonance imaging (MRI) techniques are limited to millimeter-scale spatial resolution, which falls short in capturing relevant details of tissue microstructure and disease processes that operate at molecular and microstructural scales governed by quantum physics. To address this, we leverage the intrinsic nuclear spins of biological molecules, such as water protons, as quantum sensors to quantitatively characterize the underlying microstructure efficiently and with high precision using magnetic resonance techniques [1,3,5]. Drawing upon concepts from quantum information sciences, including quantum control and information theory, we non-invasively quantify deep tissue microstructure parameters, such as cell sizes or axon diameters, which are approximately 100 times smaller than the imaging resolution [5,6]. Here I present first-principles experiments in preclinical and clinical scanners implemented in phantoms, mouse and human brains [6-9]. These results open up a new avenue for MRI to advance in extracting quantitative, and fast microstructural information from images.

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Instrumentation

MAGNETIC FIELD-CYCLING IN NMR: THE RELEVANCE OF THE TIME- SCALE. NMR RELAXOMETRY

https://proceedings.science/p/169936?lang=en

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Cycling the magnetic field in NMR experiments allow to explore two important features: the direct access to large time scales and, the Larmor frequency dispersion of different parameters. NMR relaxation experiments can profit from these possibilities allowing, for example, to measure the spin-lattice relaxation time (or rate) dispersion in a wide Larmor frequency range. This particular relaxometry technique has been successfully used as key analytical tool at both scientific and industrial environments [1]. A continuous improvement of the associated hardware and data interpretation, in addition to a tested consistency with other experimental techniques, favored the adoption and extension of the technique for new applications.

Food analytics [2], biomedical [3], oil & gas industry [4] and polymer science [5] are among those applications where the field-cycling NMR relaxometry technique is showing an increasing impact.

In this talk we will briefly address the main features of the field-cycling relaxometry method and we will show its potentiality through selected exemplary cases.

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ANALYZING PRODUCTION SAMPLES WITH BENCHTOP NMR

https://proceedings.science/p/169938?lang=en

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Benchtop NMR spectrometers have seen great advancements in magnet performance and capabilities to be implemented in a wide scope of applications. The high performance and compact size of benchtop NMR spectrometers shift the practice of NMR analysis to bringing the spectrometers to the samples, instead of the traditional approach of bringing the samples to the dedicated high-field NMR facilities.

This approach enables the placement of benchtop NMR spectrometers next to the reactors, or in the process environment to provide real-time NMR data of samples in non-deuterated solvents. This presentation will discuss recent advances in the solvent suppression techniques on benchtop NMR spectrometers that enable the 1D and 2D analyses of analytes and complex mixtures in protonated solvents. Overall, the compact size and versatility of benchtop NMR spectrometers enables the integration of powerful NMR techniques into processes that weren't accessible by NMR before.

Doing NMR and NQR outside the box

https://proceedings.science/p/169572?lang=en

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NMR and NQR play a key role in several research, development, and innovation areas. In this presentation, non-traditional applications of NMR and NQR developed at LEAR (1) will be shown, highlighting the use of these experimental techniques in the study of magnetic materials and eco-friendly porous media, as well as in quantum computing. It is not an easy task, as commercial spectrometers are not fully prepared for "outside the box" applications, requiring the development of Ad Hoc instrumentation. This encourages us to innovate in the area. To carry out this task, we have a team of physicists, engineers, and technicians, in addition to excellent mechanics and electronics workshops at the Institute of Physics of São Carlos – University of São Paulo.

(1) LEAR: High Resolution NMR Spectroscopy Laboratory (https://ifsc-lear.weebly.com/)

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DREAMTIME NMR USED FOR IDENTIFYING THE PRODUCTS OF THE ACID AND BASIC DEGRADATION OF SOFOSBUVIR IN A MIXTURE – A COMPLETE STUDY

https://proceedings.science/p/169598?lang=en

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The recently developed technique Designed Refocused Excitation and optional Mixing for Targets In vivo and Mixture Elucidation (DREAMTIME)1 aims to study complex mixtures of compounds, whose signals in the 1H-NMR spectrum suffer from low intensity and spectral overlap, improving molecular specificity compared to conventional spectra. Combining doubly selective excitation with multiple amplitude- and phase-modulated waveforms, DREAMTIME allows one or more target compounds to be simultaneously selected in a mixture and, by using spectral "filters", the unwanted components are cancelled. This study aimed to optimize the implementation of the DREAMTIME sequence applying it to the analysis of compounds resulting from the degradation of the drug Sofosbuvir in acidic and basic conditions without the need for physical separation of its components to identify their structures. The degradation products were identified by 1H, HSQC and COSY 1H-1H experiments together with the DREAMTIME technique. DREAMTIME itself allowed to obtain the individual 1H-NMR spectra with selective signals of 8 compounds in the acid degradation mixture and 5 compounds in the basic degradation. These results demonstrated how the technique allows the recovering of the signals of the selected compounds, solving the superposition of these signals in the mixture with the initial drug and other degradation products.

HELIUM MARKET IN BRAZIL HAS SEVERAL CHALLENGES ONCE IT IS CONTINENTAL SIZE COUNTRY

https://proceedings.science/p/169937?lang=en

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In this presentation we will clarify some points of Helium Brazilian Market and clear some of last year public bids, offering some tips and suggestions about what is important do consider when buying helium to have better conditions.

Improving the Resolution of and Scope of Problems Available to Low Field NMR

https://proceedings.science/p/169579?lang=en

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In this presentation “low field” implies the use of non-cryogen consuming, non-superconducting permanent or electromagnets ranging in strength from the 0.5 G Earth’s magnetic field (425 Hz) to 1,000 G (43 MHz). A survey of new mathematical, mechanical and electrical approaches to improving the resolution of both chemical shift - based spectra and relaxation - like graphs at low field will be provided. The mathematical methods consider data treatment alternatives to the Fourier and inverse Laplace transform that can be used to improve the quality of measured data by both filtration and basis set projection. Both the mechanical and electrical strategies improve the spectral resolution in solid samples with magic angle sample rotation and heteronuclear decoupling with a commercially available benchtop spectrometer. Applications of these methods to problems in process control, biomass extraction, environmental monitoring, etc. in real factory environments and in the “field” will be described.

THE FIRST NMR LABORATORY RECOGNIZED ON THE GLP SYSTEM IN BRAZIL

https://proceedings.science/p/169563?lang=en

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Quality management is a relevant tool for research laboratories frequently associated with ISO-IEC17025 (International Electrotechnical Commission - International Standardization Organization) for laboratory analysis or (GLP) Good Laboratory Practices for field essay study. When a laboratory implements a quality standard this implies the development of several Standard Operating Procedures (SOPs) that guarantee the same practice for any laboratory activity including staff training, equipment calibration, and qualification. These well-established protocols mitigate the occurrence of errors and support the evaluation of the quality of routine activities, providing reliability in the analytical results. Together with the methods validation as well as the determination of the uncertainty of the analytical measurements, this process reinforces the quality of work carried out in the laboratory. The purpose of this work was to adapt the Nuclear Magnetic Resonance Laboratory at the Federal University of São Carlos into fulfilling the requirements of the GLP system, which includes the qualification of the NMR equipment and the development of validation processes according to the official guides. One of the biggest challenges was to achieve the qualification NMR spectrometer because the laboratory had to develop this protocol by itself or contract a foreign company to do this work, this last one would be an onerous choice. The qualification protocol for the 9.4 and 14.1 T equipment (AVANCE III model) was

developed by the laboratory supported by Bruker® company protocol, using certificated standards samples - acquired from Bruker®. The inspectors from the National Institute of Metrology Standardization and Industrial Quality (Cgcre - INMETRO) approved this protocol. Finally, in May of 2023, the NMR laboratory at the Federal University of Sao Carlos became the first laboratory in Brazil to be recognized in the GLP system by the Cgcre – INMETRO for NMR analysis. From now, this work could be replicated at other NMR Laboratories.

Materials

Combination of 1H time domain NMR , 13C solid-state NMR and infrared spectroscopies as a tool for elucidating degradation mechanisms in constructing polymers.

https://proceedings.science/p/169640?lang=en

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The degradation of constructing polymer is a central issue in materials science and engineering. While chemical modifications due to degradation can be assessed by many spectroscopy techniques, such as solution and solid-state NMR, infrared spectroscopy, and Raman spectroscopy, changes on the polymer dynamics and microstructure are usually assessed by thermal analysis, X-ray diffraction and many different NMR methods.
In this context, 1H Time Domain Nuclear Magnetic Resonance (1H -TD-NMR) has become a central technique for evaluating changes in the dynamics and microstructure of polymer, providing information about changes in the molecular chain mobility, crystallinity and crosslinking due to chemical, thermal, and physical degradation. In this work, we discuss how a combination of 1H TD-NMR, 13C solid-state NMR, FTIR and Raman spectroscopies can be efficiently used to analyses many features of polymer degradation. 1H TD-NMR techniques, such as the joint analysis of Free Induction Decay (FID) obtained after single pulse excitation and 1H mixed Magic Sandwich Echo (mixed-MSE) were used to evaluate the room temperature mobile and rigid fractions of the polymer. 1H Dipolar Filtered Magic Sandwich Echo (DF-MSE) obtained as a function of temperature was applied to evaluate changes in the mobility transitions and crystalline/amorphous fractions in the samples upon degradation. Complementary, Raman Spectroscopy, Infrared Spectroscopy and 13C NMR CPMAS spectroscopy allowed us to obtain information about the specific chemical sites affected by the degradation. We will discuss how the combination of these methods can give a quite complete picture of the chemical and thermal degradation phenomena in different construction polymers such as polyamides, revealing information about formation of mobility constrained regions, plasticizer extraction and chain crosslinking.

DOE and NMR: a strategy for obtaining an Amazonian technological product with antiglycant potential

https://proceedings.science/p/169617?lang=en

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Pedra-ume-caá (Eugenia punicifolia) is a fruitful shrubby Amazon species that has been reported in the traditional medicine, used for the beneficial effects attributed to the phenolical compounds.1 Its fruits can be submitted to encapsulation processes, aiming to protect their active constituents.2 The present work aimed to evaluate the effect of the drying process (spray-dryer and fluidized bed) with the presence and absence of adjuvant (DSC and CMC) on the yield and chemical composition of the aqueous extract of E. punicifolia fruits through a 2^3 Design Optimization Experiment (DOE). In addition, the eight products were submitted to a digestibility test in order to evaluate the effect of the adjuvant on the in vitro digestion process (pH 7, 2 and 8, respectively) on the chemical composition. The chemical characterization of the products and their derivatives was performed by 1D and 2D NMR (Bruker Avance III, 500 MHz, D2O, zgpr, P1 10.0 µs, D1 1.0 s, PLW9 8.1292 e-5 W, O1 2352.60 Hz, NS 56 and DS 2, 11.74T, 298K). DOE analysis (critical T 2.12) showed that the drying process is the main factor on the yield, whose best yields were obtained by the fluidized bed process (ANOVA, 95%). Multivariate analysis (PCA and HCA) of the NMR chemical profiles of the D2O soluble fractions of the 8 products showed low concentrations of phenolic compounds and different concentrations of sucrose for the different types of products. In addition, NMR analysis of the digested products, after neutralizing the alkaline excess, has shown signs of phenolic compounds with significant intensities. This finding has revealed that the adjuvants corroborate the adsorption of phenolic compounds and sucrose, which are available at an alkaline pH (intestinal). Therefore, the proposed experimental design, assisted by NMR analysis, has made it possible to develop new Amazon products from E. punicifolia fruits.

Incorporation of Gallium Into Bioactive Glasses: New Structure/Function Relations Uncovered by Solid State NMR Techniques

https://proceedings.science/p/169618?lang=en

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When it comes to bioactive glasses and glass-ceramics, the incorporation of additives can enhance performance. Gallium, in particular, stands out for its antibacterial potential, attributed to the similarity between Ga3+ ions and Fe3+ ions. However, there is limited understanding of how gallium's structural incorporation in these glasses relates to their bioactive properties. To address this, Nuclear Magnetic Resonance (NMR) spectroscopy provides a quantitative and flexible approach, offering insights into the structural functions of introduced elements in the glass network. This study focuses on using NMR techniques to investigate gallium-doped bioactive glasses produced through melt-quenching and sol-gel processes, with compositions [(49,16-x)SiO2 – (23,33)Na2O – (25,79)CaO – (1,72)P2O5 – (x)Ga2O3] and [(80-x)SiO2– (15)CaO – (5)P2O5– (x)Ga2O3], respectively. The samples underwent NMR studies for 29Si, 31P, 23Na and 71Ga nuclei, which revealed the formation of Ga-O-Si bonds in both compositions. It was also observed that gallium is mostly 4-coordinated, indicating its role as a network former. In the case of melt-quenched samples, 31P results indicate a proximity effect between P and Ga atoms. As for the sol-gel derived samples, 29Si and 31P spectra indicate that the formation of Ga-O-Si bonds reaches a limit, leading to the formation of Ga-O-P bonds with increasing gallium content. Further research is being conducted to explore the structure upon partial crystallization of the melt-

quenched samples , allowing for a comparison of bioactivity dissolution rates between these glass-ceramics and the two aforementioned compositions.

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Incorporation of Niobium into Photonic Glasses: New Structure/Function Relations Uncovered by Advanced Magnetic Resonance Techniques

https://proceedings.science/p/169619?lang=en

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While niobium-containing oxide glass are used in several technological applications, especially optical glasses, the exact structural role of Nb, which can serve either as a network modifier or as a network former, in these glasses is still ill-understodd. Solid-state nuclear magnetic resonance (NMR) has been proven to be a powerful tool for structural elucidation of glasses, due to its element-selectivity, inherently quantitative character, and its focus on local order [1]. From the NMR point of view, niobium features one of the most NMR-sensitive nuclei (93Nb) which is 100% natural abundant. Nevertheless, it suffers from a large nuclear quadrupolar moment, and the 93Nb NMR spectra are dominated by strong quadrupolar interactions, resulting in excessive line broadening and poor resolution. These challenges can be addressed by techniques involving fast MAS, wideband excitation methods and dipolar recoupling techniques [2]. Here, we report results on glasses in the system Na2O-Nb2O5-P2O5 from two compositional series. Advanced NMR experiments have been used to characterize the local environments of sodium, phosphorus and niobium with the aim of obtaining new structural insight towards the development of new structure-function correlations.
Acknowledgements:
This work is supported by the São Paulo Research Foundation (FAPESP) under grants numbers 2013/07793-6 (CEPID program) and 2022/01937-5
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MAGNETIC RESONANCE OF THE RARE EARTHS: EXPERIMENTAL RESULTS AND APPLICATIONS TO STRUCTURAL STUDIES OF RARE-EARTH DOPED GLASSES

https://proceedings.science/p/169675?lang=en

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Disordered materials, such as defective crystals, glasses, and nanocomposites are of great importance in materials science and technology, as key functional properties are directly linked to the lack of translational symmetry. Designing the physical properties of materials to technological demands requires detailed knowledge of their structural and dynamic properties. For the investigation of the solid state, nuclear magnetic resonance (NMR) is an ideal complement to the various diffraction (x-ray, synchrotron, neutron) techniques, by its specific property of being element-selective, inherently quantitative as well as selective to the local environment. An additional strength of the solid state NMR approach lies in the opportunity of tailoring the effective Hamiltonian by manipulations in physical space (magic angle sample spinning) or spin space (multi-dimensional NMR), offering a toolbox of complementary selective averaging experiments.

Owing to the paramagnetic nature of many rare-earth containing materials, there are severe restrictions to their structural characterization by NMR. A review of the various approaches in the literature and ~60 years of experimental characterization will be given. In addition, we will present a comprehensive NMR/EPR strategy developed in our laboratory for the structural study of rare-earth dopants in glasses. This strategy includes: (i) standard single- and double resonance NMR of diamagnetic mimics, (ii) NMR studies of paramagnetic interactions affecting nuclei in the vicinity of the dopants, and (iii) EPR spectroscopy of the dopants themselves, exploiting electron-nuclear magnetic hyperfine interactions via electron spin echo envelope modulation (ESEEM) and hyperfine sublevel correlation (HYSCORE) spectroscopies. It will be shown how the spectroscopic observables can be correlated with photophysical parameters extracted from luminescence and excitation spectroscopies.

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Multinuclear solid-state NMR characterization of fluid catalytic cracking catalysts containing AlPO4 as active matrix

https://proceedings.science/p/169608?lang=en

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Fluid catalytic cracking (FCC) is a remarkable and profitable conversion process in oil refineries as it turns heavy and low-priced feedstock into more valuable products, mainly gasoline and liquefied petroleum gas. The main FCC catalyst constituents are the Y zeolite in its ultra-stable form (USY), often exchanged with lanthanum (REUSY), and the matrix, typically composed of clay, alumina and silica. The development of new matrices can improve the hydrothermal stability of fluid catalytic cracking catalysts, provide better selectivity to cracking products and reduce coke yields. In this work, amorphous, mesoporous and stoichiometric aluminum phosphate (AlPO4) were investigated as active matrix to achieve these purposes.1
NMR experiments reveal the positive effect of AlPO4 synthesized at stoichiometric Al/P ratio at low pH, when mixed with REUSY, since framework dealumination is attenuated, according to 27Al and 29Si MAS. This effect is related to the migration of polyphosphate species to the zeolite in the calcined (fresh) composite, as evidenced by 1H and 31P MAS. The interaction between AlPO4 and Y zeolite improves zeolite stabilization under steaming, preserving more Brönsted acid sites. Mutually, it avoids AlPO4 crystallization, ensuring its catalytic properties for gasoil pre-cracking. Results suggest that FCC catalysts containing AlPO4 are potential alternative materials for increasing liquid yields and isoparaffins fractions. Besides, they can be attractive materials for reactions involving more easily crackable feedstock, such as renewable sources.1
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Solid-state NMR characterization of graphene oxides used for electrochemical applications

https://proceedings.science/p/169637?lang=en

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Graphene oxide (GO), reduced graphene oxide (rGO), and other graphenic materials exhibit several promising properties for use in electrochemical devices such as batteries, supercapacitors, line filters, fuel cells, and electrochemical sensors. The presence of heteroatoms and functional groups has been reported as highly relevant for the electrochemical properties of GO, rGO, and related materials. Solid-state nuclear magnetic resonance (NMR) spectroscopy has been extensively used in the past years in studies involving graphenic materials, especially regarding the chemical characterization of the functional groups present in GO. NMR methods are also promising for studies of carbon materials used in electrochemical devices, allowing investigations about ion diffusion processes, electrode/electrolyte interactions, and others. This work describes the solid-state NMR characterization of several GO- and rGO-based materials used for electrochemical applications involving supercapacitors and electroanalytical sensors. The analysed materials include: phosphorus/sulfur-enriched rGO papers; lyophilized GO powders and dried GO papers; GO powders obtained from suspensions submitted to different sonication conditions; rGO samples prepared at different temperatures. These materials had their electrochemical properties recorded using methods such as cyclic voltammetry, galvanostatic charge/discharge cycles, and electrochemical impedance spectroscopy. The details of the chemical structure of these materials revealed by the solid-state NMR spectra (using different probe nuclei, such as 13C and 31P) were used to support the interpretation of the results of the electrochemical analyses and to achieve a deeper understanding of the mechanisms responsible for the observed electrochemical performance.

Solid-state NMR with fast MAS and proton detection for the structural characterization of organic-inorganic hybrid materials

https://proceedings.science/p/169635?lang=en

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1H isotope presents a great challenge in solid state NMR, due to high-order dipolar interaction Hamiltonian terms (either multi-spin dipolar interactions or cross-terms involving dipolar coupling and magnetic shielding tensors), which do not vanish completely, but rather become smaller with increasing MAS frequency. As a result, protons are rarely used, in spite of the desperate need for the study of a variety of systems that are not suitable for high resolution investigation using other techniques, such as X-ray crystallography and solution NMR spectroscopy. This scenario is changing with the increasing popularity of fast (> 60 kHz) MAS probes, which are now commercially available [1]. Such high spinning rates allows the suppression of higher-order dipolar terms, improving the proton spectral resolution. Although the internuclear interactions, important for correlation experiments, are suppressed by ultra-fast MAS, they can be reintroduced by appropriate pulse sequences. In the present discussion, we will show the combination of fast MAS with the plethora of experiments available in solid and liquid state NMR for the characterization of H chemical environments in some amorphous organic-inorganic systems. These results are combined with conventional solid-state NMR techniques giving complementary structural insight. The following systems will be discussed: (i) amorphous metal-organic compounds with Eu-based luminescence; (ii) Organosilica materials for organic electronic applications; (iii) Photopolymerizable Aluminum-Phosphate-Silicate Sol–Gel Hybrid Materials for Additive Manufacturing; (iv) Highly luminescent organosilicate xerogel doped with Ir(III) complex.
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Study of Asphaltenes Structure and their Aggregation Properties

https://proceedings.science/p/169587?lang=en

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Petroleum has been used by civilization since a long time ago and its importance has grown with time. Its greatest use is as an energy source, in addition to being used as feedstock in the manufacture of other inputs. Due to its being a complex mixture it is necessary to refine the crude oil. Some organic compounds can precipitate during this process and generate clogging inside the pipelines, raising the production costs, becoming a concern.

Asphaltenes are one of the responsible for the occurrence of precipitation along the entire oil production chain. This class of compounds can present two types, island and archipelago; a better understanding of their structures is required to predict their behavior in the precipitation process. Thus, studies of asphaltenes using spectroscopy analysis are increasing.

In this work, spectroscopic techniques such as FTIR, raman and nuclear magnetic resonance were applied to four asphaltenes, two from stable oils (AP2 and AP4) and two from unstable oils (AP1 and AP3). The values obtained from Raman showed a larger size of the aromatic nucleus for the asphaltene AP3. FTIR analysis indicated a high polarity for the unstable asphaltenes. The data obtained from 1H NMR spectra, showed a lower amount of aromatic hydrogens for AP1, corroborating the results of aromaticity from FTIR. For stable asphaltene AP2, it was observed a smaller substitution in the aromatic core and a larger amount of hydrogens in the aliphatic chain. With this information, it is possible to correlate the aggregation behavior of asphaltenes through the interaction between their structures given by the higher aromaticity, polarity and the aromatic core.

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UNDERSTANDING SOME PROCESSES ON SURFACES OF CATALYTIC MATERIALS USING NMR-DFT COMBINATION

https://proceedings.science/p/169677?lang=en

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Computational calculations, based on Density Functional Theory (DFT), have been used with success to simulate NMR parameters and to understand the structure and the properties of materials, including surface of active phase and support of catalysts. The methodology uses the gauge including projected augmented wave (GIPAW) and can be applied on slab models of surface structures. Several effects are relevant for good models, and consequently, for good NMR simulations. Once the direct comparison among theoretical and experimental data allows to confirm a valid structural model, the combination of MNR parameters with other properties, calculated from electronic density, can be used to discuss interesting applications. In addition, calculated thermodynamic potentials can be used to confirm the stability of active sites, formation of intermediates on surfaces and adsorption enthalpy and free energy. In this conference, examples of simulations of active sites on material surfaces, poisoning of catalysts and adsorption of molecules used as probes will be shown.

Validation of a 29Si NMR semiquantitative method for determination of the average molar mass in silicone prothesis residues

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Polydimethylsiloxane (PDMS), commonly referred to as silicone, is a silicon-containing polymer used in a broad range of applications, including medical devices for prosthetic purposes. Among the analytical methods for characterizing polymers, Gel Permeation Chromatography (GPC) is traditionally used to determine average molecular weights and molecular weight distribution of polymeric materials. However, the underlying principle of separation based on the hydrodynamic polymer volume rather than molecular weight remains a disadvantage in GPC. In addition, there is need for suitable measurable monodisperse standards (as it is a relative method) and great dependency in finding the right solvent systems and column type. One of the main advantages of NMR over GPC, is its absolute characteristics, not requiring the use of standards of known molar masses, since the concentrations of the groups can be obtained directly from the integration of the respective peaks. The NMR technique makes it possible to identify and quantify nuclei of elements that form polymeric chains in different chemical environments, such as polymers based on PDMS, thus making it possible to distinguish between the number of observable nuclei in end groups of PDMS and its intermediate groups, thus allowing to calculate of the numerical average molar mass ($\bar{M}n$) of polymer. This study aimed to present an example of the validation of an alternative method based on high resolution 29Si NMR for determination of $\bar{M}n$ in linear PDMS in prothesis residues using proficiency tests based on the DOQ-CGCRE-008 (INMETRO) and EUROLAB Guide. 29Si NMR data were recorded at 25°C, 90° pulse, 5 sec recycle time, 4096 scans using a 9.4 Tesla Bruker Avance III HD spectrometer. Proper selectivity was achieved, $\bar{M}n$ of residuals in the order of 17,472 g. moL-1 with limit quantification of 25 mg. g-1 and typical uncertainty of 3.0 % leading to satisfactory results for the intended application.

Metabolomics

Application of NMR in the characterization of compounds and in the determination of the source of starch used in beer production

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The beer market generates over 76.3 billion dollars annually. Beer quality control is necessary for food safety as well as continuous studying to improve beer knowledge. In this regard, NMR is used to characterize and quantify compounds in complex matrices, providing an understanding of how changes in manufacturing methods affect the product. This study aimed to characterize beers produced with different starch sources using NMR. Three beers, each containing 45% Corn, 45% Rice, and 100% Barley Malt, were produced in the laboratory. Samples were analyzed in triplicate on a 9.4 Tesla NMR spectrometer (Bruker®, Avance III). The ^{1}H NMR experiments were acquired using the NOESYGPPR1D pulse sequence and the spectral processing was performed in Topspin® 3.5 software. The characterization and quantification of compounds were performed using Chenomx 8.6 software. Compounds with a factor loading > 0.8 in the first component (PC1) of the Principal Component Analysis were retained for analysis. Comparisons between beers were performed by effect size

measures (ES, standardized mean difference in standard deviation units), with 99% confidence intervals [ES(99%CI)]. 22 compounds were characterized, 13 of which were retained in PC1 (63.1%). Corn beer, when compared to Rice beer and barley Malt beer, presented: higher levels of cytidine [10.4(0.1:20.8) and 15.9(0.4:31.3)], threonine [17.9(0.5:35.2) and 13.9(0.3:27.5)] and tryptophan [13.5(0.1:20.8) and 18.8(0.6: 37.0)]; but smaller than acetate [-20.9(-41.2:-0.7) and -18.0(-35.4:-0.5)]. Additionally, Corn beer had lower levels of adenine [-16.5(-32.5:-0.5)] compared to barley Malt beer, and glucose [-23.0(-45.3:-0.8)] and glutamine [-196.5(-384.7:-8.3)] compared to Rice beer. Only Malt beer showed higher levels of acetate [17.9(0.5:35.3)], adenine [16.5(0.5:32.5)], N-nitrosodimethyllamine [11.5(0,2:22.8)], but lower cytidine [-13.1(-26.0:-0.3)] compared to Rice beer. These results indicate a distinction between the beer's chemical composition, with higher levels of essential amino acids and acetate in Corn and only barley Malt beer, respectively, and lower levels of threonine in Rice beer.

CHARACTERIZATION OF METABOLIC CONSTITUENTS OF BIOFLUIDS BY NMR FOR CLINICAL APPLICATIONS

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Metabolomics or Metabolic Profiling is a methodology which studies the physiology of an organism in a moment of time by measuring concentrations of metabolites or small molecules of complex samples. These samples are biofluids or tissues when the organism is a human or another type of animal, but they can also be cell or plant extracts, food products… A large number of samples is normally required to be able to stablish communalities or differences between groups and they tend to be studied by statistical analysis.
The two main used analytical techniques are mass spectrometry, MS and nuclear magnetic resonance, NMR. MS is often used in hyphenation with different chromatographies, which allows the development of diverse assays to explore a big variety of molecules from polar molecules to lipids. NMR provides a global profile, requires very little sample preparation and it is easily automated. Both analytical platforms are suitable for high throughput analysis and often provide complementary information.
Clinical metabolic profiling has been proven to provide objective information on health status of an individual and have huge potential to be used for diagnosis and prognosis of different diseases. However, to date, these applications have yet to be translated to clinic.
In order to facilitate this translation, a huge effort has been made into standardising the methods of study, increasing the quality control of the data, generating automation pipelines for its analysis, and especially annotating signals. The aim is helping clinical practitioners with the interpretation of the outcomes of the metabolic profiling research. Here I will be providing some examples of how we are contributing to this area within the National Phenome Centre, NPC, in Imperial College London. I will also be presenting some of the more recent clinical applications.

COMPARATIVE ANALYSIS OF EDIBLE OILS USING NMR

https://proceedings.science/p/169603?lang=en

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Vegetable oil is a natural product with a high market value, even more so if it is olive oil, but difficult to control quality. Different analysis methods, including nuclear magnetic resonance, have been used to find ways to improve this situation. Specially for olive oils, high resolution NMR has been used since 1996 [1-3] for characterization and quality control [4-7]. For this work different commercially available

oils and oils were used.
Sample preparation for NMR analysis is relatively simple. In this work 0.4 ml of oil is mixed with 0.2 ml of deuterated solvent, different solvents (DMSO, acetone, chloroform) were used. The samples used in this work are different edible oils that were found in supermarkets.
Standard 1H and 13C [8] NMR spectra were acquired and analyzed using different techniques.
Analysis of spectra in the methyl region revealed significant differences in both 1H-NMR and 13C-NMR spectra. With this, we expect a classification of the samples according to the declared quality of the products and the origin [9].

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Description of the first metabolome of Triatoma infestans (Klug, 1834) using Nuclear Magnetic Resonance

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A doença de Chagas é uma doença tropical negligenciada causada pelo protozoário Trypanosoma cruzi, com cerca de oito milhões de pessoas infectadas e mais de 10.000 mortes todos os anos). A transmissão pode ocorrer por via oral (alimentos in natura contaminados com o parasita), por transfusão de sangue de doadores infectados, por via congênita (transplacentária ou amamentação), por transplantes de órgãos de doadores chagásicos e até por acidentes laboratoriais. Entretanto, o principal modo de transmissão do T. cruzi é através das fezes de triatomíneos (Hemiptera, Triatominae) infectados com o parasita durante o repasto sanguíneo. Na entomologia, as técnicas de metabolômica têm sido utilizadas basicamente para revelar informações que auxiliam no entendimento da fisiologia e do comportamento dos insetos, sendo os estudos de metabolômica em triatomíneos restritos à análise do metaboloma fecal de algumas espécies. Com base no exposto, o presente projeto utilizou a metabolômica para identificar e descrever os metabólitos presentes no trato gastrointestinal de T. infestans. Para o estudo, 20 exemplares adultos foram cedidos pelo insetário do Laboratório de Parasitologia da FCFAR/UNESP, Araraquara-SP. Após 15 dias de jejum, o trato gastrointestinal dos triatomíneos foi coletado e armazenado em freezer a -80ºC e os espectros foram obtidos por meio de aparelho de Ressonância Magnética Nuclear (RMN). Posteriormente, os dados foram processados com auxílio dos softwares Topspin 3.5 (Bruker Biospin, Alemanha) e MestreNova, e a identificação dos metabólitos foi realizada manualmente através da análise dos espectros. A partir dos resultados obtidos referentes à análise metabolômica de insetos não infectados, foi possível identificar 22 metabólitos no trato gastrointestinal de T. infestans, nomeadamente, Treonina, Alanina, Valina, Isoleucina, Lisina, Prolina, Glutamato, Glutamina, Aspartato, Leucina, Acetato, Metionina, Asparagina, Betaína, Colina, Glicose, Manitol,

Tirosina, Serina, Fumarato, Fenilalanina e Triptofano. Portanto, foi caracterizado pela primeira vez o metaboloma do trato gastrointestinal deste vetor. Agradecimento: CAPES e FAPESP 2018/25458-3

Effect of biochar on the metabolome of soybean seedlings (Glycine max L.)

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Biochar application can have varied effects on plant germination, depending on raw material, preparation method, and application rate. However, the molecular mechanisms that lead to this variation have yet to be elucidated. This research aimed to advance the understanding of these mechanisms by characterizing the effect of biochar from sugarcane bagasse on the metabolism of soybean germination. The experiment was conducted using sand containing three types of biochars as substrate, prepared at pyrolysis temperatures of 300 ºC (SCB300), 400 ºC (SCB400), and 600 ºC (SCB600), at rates of 0, 1, 3 and 5% w/w. The number of germinated seeds and the average radicle length were determined 8 days after the incubation. These data were applied to the calculations of Relative Germination (RG), Mean Relative Root Growth Rate (RGR), Germination Index (GI), and dry biomass (DB). To evaluate the metabolome, the DB was subjected to extraction with a mixture of methanol-d4 e D2O (1:1 v/v). The extracts were submitted to metabolomics analysis by Proton NMR Spectroscopy. GR, RGR, and GI increased in all treatments compared to the control. However, these parameters increased linearly and positively with increasing rates only for SCB300 treatment. This may be related to the increased content of soluble salts in the SCB400 and SCB600 samples. On the other hand, the DB increased in all treatments, except for SCB300, at doses of 1 and 3% w/w. Nine metabolites (alanine, asparagine, acetic acid, citric acid, formic acid, fumaric acid, succinic acid, glycerol, and sucrose) were identified and quantified in DB extracts as the most influential for the separation of treatments. The results strongly suggested that biochars accelerated the catabolism of triacylglycerols to sucrose, with a more pronounced effect at higher rates. The authors would like to thank FAPEMIG (APQ-03110-22) for their financial support.

Effects of low and high frequency electromagnetic radiations on the quality of Melipona mondury honey

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Electromagnetic radiations, such as microwaves (MW) and γ-radiation, have been explored as alternative methods for food processing. Their effects on enzymes and microorganisms make them suitable for food preservation. Brazil is home to approximately 300 species of stingless bees, many of which possess significant socioeconomic value due to honey production. Stingless bee honey, characterized by high moisture content, poses a risk of undesirable fermentation. This study aimed to assess the impact of low (MW) and high (γ-radiation) electromagnetic radiations on microorganism inactivation, physicochemical properties, chemical profile, and antioxidant properties of honey produced by the stingless bee Melipona mondury (MMH). MMH was inoculated with Bacillus clausii spores (indicator microorganism), distributed in amber flasks (10 ml), and exposed to MW (20% power, 20 to 80 s) or ɣ-radiation (2.5 to 15 kGy). The growth of total microbiota (aerobic mesophilic

microorganisms and fungi) and B. clausii was determined by the direct count of colonies in Petri dishes. Physicochemical features (Aw, moisture, and total soluble solids) and color intensity were evaluated according to standard methods. Furthermore, the chemical profile of the samples was determined using NMR spectroscopy, while the antioxidant capacity was assessed using FRAP. The results showed that MW eliminated honey microbiota after 60 s of exposure, although it only decreased the viability of B. clausii. In turn, γ-radiation at 5 kGy completely halted microbial growth. The physicochemical parameters remained unchanged regardless of the processing method employed. However, γ-radiation caused significant ($p < 0.05$) alterations in honey color at 10 and 15 kGy. NMR analysis allowed the identification of over 100 compounds, primarily carbohydrates. Notably, samples processed with MW for 80 s exhibited increased levels of hydroxymethylfurfural. These findings demonstrate the effectiveness of electromagnetic radiations, particularly γ-radiation, in eliminating microorganisms with minimal impact on MMH.

EVALUATION OF VARICOCELECTOMY EFFECTS ON SEMINAL PARAMETERS BY NMR-BASED METABONOMICS

https://proceedings.science/p/169578?lang=en

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Introduction: Varicocele may cause damage the proper of testicle and spermatogenesis altering the individual's sperm parameters. However, it is not possible to conclude that varicocele is directly related to male infertility. Varicocelectomy is an alternative to improve seminal parameters and increase the couple's pregnancy probability. However, there are cases in which the treatment is not successful and predicting the outcome of the surgery is challenging. In this study, we used NMR-based metabonomics as evaluate probe to predict the effects of varicocelectomy on seminal parameters of patients diagnosed with male infertility.
Objective: This study aims to build metabonomic models able to predict the improvement of the patient's seminal parameters from the ^{1}H NMR spectra of the blood serum of patients undergoing varicocelectomy.
Methods: Thirty-two volunteers diagnosed with varicocele and infertility were included. All volunteers were undergoing varicocelectomy surgery and had a standard semen analysis and sex hormone measurement. 1H NMR spectra of blood serum were acquired using VNMRS400 spectrometer operating to 400 MHz Spectral data were processed using Statistica 12.0 software to build metabonomic model from LDA formalism.
Results: Clinical data indicated that 17 patients showed improvement of seminal parameters. LDA selected five variables – δ (ppm) 0,85, 1,25, 2,09, 2,57 and 2,61. This metabonomic model presented 84.4% accuracy, 88.2% sensitivity and 80.0% specificity after leave-one-out cross validation (LOOCV).
Conclusion: These partial results indicate that NMR-based metabonomics has potential to be used as prognostic probe for seminal paraments changes after varicocelectomy. We are expanding study population to confirm these findings and to investigate the metabolic pathway involved. This is a minimally invasive procedure which could assist the physician in decision making and advising the patient about the prognosis after surgery.

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HR-MAS NMR analysis of Vero cell metabolism disturbances caused by Chikungunya virus infection and nsP2 inhibitor

https://proceedings.science/p/169582?lang=en

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The arbovirus Chikungunya (CHIKV) is transmitted by Aedes mosquitoes in urban environments, and in human infection, it may trigger debilitating symptoms involving long-term complications, including arthritis and the Guillain-Barré syndrome. The development of antiviral therapies is relevant as no efficacious vaccine or drug has been approved for clinical application. In the search for natural antiviral molecules, Wedelolactone [a coumestan mainly derived from Eclipta prostata (Asteraceae)], has shown potential results against viral proteins and replication processes, which incited us to study it against arboviruses. The metabolome can be considered as biochemical signatures underlying several conditions, including viral infections, therefore, we validated the metabolic signatures of Vero E6 cells through HR-MAS NMR in the following conditions: prior to infection with CHIKV (CC), upon CHIKV infection (CV), and also upon the inclusion of the previously identified nsP2 protease inhibitor, Wedelolactone (WDL), with and without prior CHIKV infection (CWV and CW). The metabolomic analysis by HR-MAS NMR evidenced a metabolic profile of these cells, and also significant changes in the levels of lactate, Myo-inositol, phosphocholine, glucose, betaine, and a few specific amino acids. In CHIKV-infected cells, levels of Myo-inositol and amino acids (proline, phenylalanine and valine) increased, whereas decreases in phosphocholine and lactate levels were observed. Cell treatment with WDL decreased the relative levels of aspartate, while the levels of glucose, betaine and isoleucine increased. Furthermore, the CHIKV-infected cells treated with WDL showed increased methionine levels, while aspartate, betaine, lactate and proline showed lower intensities. Overall, the results demonstrated disturbances in central energy and lipid metabolism by the virus, and a supposedly antioxidant effect of WDL evidenced by altered levels of betaine and methionine, functioning against oxidative stress and viral replication, although further studies are pivotal to confirm such allegations.
Special thanks to FAPESP, process number 2020/03639-6 for supporting this research.

Insights into the Serum Molecular Adaptations in Response to Inspiratory muscle training: A Metabolomics Approach Based on 1H-NMR and UHPLC-HRMS/MS

https://proceedings.science/p/169605?lang=en

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Inspiratory muscle training (IMT) is known to promote physiological benefits and improve the physical performance in endurance sports activities. However, the molecular interactions resulting from adaptations promoted by different IMT prescribing strategies remain unclear. This study investigated the effects of IMT at different intensities on the serum metabolomic profile and its networks with genes and proteins through an integrative bioinformatics analysis of metabolomic data. Thirty healthy male recreational cyclists (30.4±6.5 years) were randomized into 3 groups: low-intensity [(LI-group), 6 cm·H2O of inspiratory pressure, n=7], moderate-intensity [(MI-group), 60% maximal inspiratory pressure (MIP), n=10] and high-intensity [(HI-group), ≈90% MIP (critical inspiratory power), n=11). Blood serum samples were collected before and after 11-week of IMT and analyzed by 1H-NMR (600 MHz, with 5mm-TCI cryoprobe) and UHPLC-HRMS/MS. Data were analyzed using Principal Component Analysis (data dimensionality reduction) and Linear Mixed Models (pairwise comparisons). Metabolite-metabolite, gene-metabolite, and protein-protein interaction networks were explored via web-based tools (Metascape®, MetaBridge®, MetaboAnalyst®). The 1H-NMR and UHPLC-HRMS/MS techniques resulted in 46 and 190 metabolites. After IMT, all groups showed an increase in serum levels of 3-hydroxybutyrate (P<0.001) and 16-hydroxypalmitate (P=0.005), but a decrease in acetoacetate (P=0.007), O-acetylcholine (P=0.009), and citrate (P=0.013) levels. Additionally, serum levels of L-tryptophan also increased in the MI-group (P<0.001), while a reduction was observed in LI-group (P=0.041). This panel of metabolites yielded a list of 21 genes. Metabolites with the highest degree centrality with other genes were serum citrate and 3-hydroxybutyrate. Nonetheless, citrate, O-acetylcholine, and L-tryptophan demonstrated higher degree centrality in metabolite-metabolite interactions. The most enriched pathways derived from protein-protein or metabolite-metabolite interactions were synthesis and degradation of ketone bodies, citrate cycle and amino acid metabolism. These results suggest an increase in the metabolic process of fatty acid derivatives after IMT at different intensities with additional evidence for the involvement of essential amino acids in MI-group.

INSIGHTS ON EFFECTS OF SODIUM NITROPRUSSIDE ON METABOLOME OF SCHIZOPHRENIA ANIMAL MODEL BY NMR

https://proceedings.science/p/169607?lang=en

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The blocked action of glutamate on its receptors mediates the production of neuronal nitric oxide (NO), which leads to the hypothesis that schizophrenia (SCZ) could be related to decreased levels of NO in the brain. The use of compounds containing the NO group, such as sodium nitroprusside (SNP), has been reported in the literature as a possible antipsychotic agent for treating negative SCZ symptoms. SNP reduces psychotic symptoms and improves cognitive functions in patients with schizophrenia. Nevertheless, SNP effects on human or animal metabolomes are not well described. Therefore, we decided to perform a metabolomics study to evaluate the effects of SNP in the SCZ animal model. Spontaneously hypertensive rats (SHR) are animal models of primary hypertension and a great model

for SCZ. We have evaluated the effects of SNP in two different dosages 2.5 mg/kg and 5 0 mg/kg (5 blood serum samples in triplicate for each dosage) in comparison to the control group (4 blood serum samples in triplicate) of SHR to gain insights on metabolome effects of this drug. The experiments on rats were performed at the Federal University of São Paulo in accordance with the Ethics Committee. The NMR data were acquired in a Bruker NMR 600 MHz spectrometer, and chemometrics by MetaboAnalyst. The results from the principal component and the partial least square discriminant analyses pointed to the two NMR spectral regions (6.95-7.50 ppm and 1.30-1.70 ppm) for distinguishing the control group from SHR treated with lower and higher SNP dosages, respectively. It was possible to observe that the higher SNP dosage provoked greater serum metabolome effects in SCZ animals. Our results are in accordance with previous studies. Some altered rat serum metabolites were successfully assigned to isoleucine, lactate, fatty acids, and leucine, among others.
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Insights on Venous Thromboembolism and Antiphospholipid antibody syndrome by NMR

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Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by recurrent arterial or venous thrombosis or obstetrical morbidities accompanied by antiphospholipid antibodies (aPLs). The pathophysiology of APS is complex, containing several pathogenic mechanisms related to coagulation, endothelium, and platelets, which may increase the risk of thromboembolic complications. Our goal was to identify metabolites with the potential to differentiate the groups, helping to understand the biology involved in the disease. Metabonomics allows the understanding of the function of metabolites in the organism, giving important information about biological pathways that are compromised by the disease or lead to alterations linked to it. This study investigated metabolic profiles applying NMR-based metabonomics in serum samples of patients with arterial or venous thromboembolism (VTE) without APS (n = 32), thrombotic primary APS patients (PAPS, n = 32), and healthy controls (HC) (n = 32). Inclusion criteria were objective diagnosis and age above 16 years. Exclusion criteria were secondary APS, infection, rheumatologic, renal, hepatic, or inflammatory disease, as well as the use of corticosteroids. APS diagnosis was defined by persistently positive aPL. Thrombotic APS patients were matched by age and gender to VTE without APS and HC. This comprehensive NMR-based metabolomic study revealed differences in metabolic profiles between VTE and HC, PAPS and HC, as well as between VTE and triple-positive PAPS groups. Obviously, alterations in concentrations of metabolites pointed to changed metabolic pathways of glycolysis, TCA cycle, lipid metabolism, and branched-chain amino acid (BCAA) metabolism in VTE and PAPS patients. These metabolites might be potential biomarkers to differentiate PAPS and VTE patients, including the parameter of aPLs for APS patients. Acknowledgments Sao Paulo Research Foundation, Grant numbers #2016/14172-6, #2014/50867-3, #2018/24069-3, and#2021/07212-0.

Integrated Approach for High-Confidence Compound Identification: Combining DAFdiscovery and NMRfilter

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The identification of structures in complex mixtures presents a significant challenge in natural product and metabolomics research. While the choice between Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) as primary analytical tools is common, the complexity of these systems often demands a multi-technique approach. This presentation introduces the combined use of DAFdiscovery and NMRfilter, offering a tentative solution for high-confidence compound identification in mixtures. DAFdiscovery is a powerful pipeline that integrates MS, NMR, and bioactivity data within a user-friendly Jupyter Notebook environment. By leveraging Statistical Total Correlation Spectroscopy (STOCSY) and Statistical HeteroSpectroscopy (SHY) calculations, DAFdiscovery enables the integration and analysis of diverse data types. NMRfilter, on the other hand, is specifically designed to enhance compound identification in mixtures. The algorithm begins with a list of candidate compounds potentially present in the sample. It simulates the chemical shift data of these candidates and compares them with the user's experimental NMR data. Additionally, it performs a search for heteronuclear and homonuclear scalar coupling networks using HMBC and HSQC-TOCSY data, determining if the signals matched in the HSQC share a unique chemical structure.
The integration of MS and NMR data into a single dataset is promising, providing broader coverage of the chemical space and facilitating confident compound identification. By combining the strengths of DAFdiscovery and NMRfilter, researchers can overcome the limitations of individual techniques and achieve high-confidence compound identification in complex mixtures.

Measuring inflammation and cardiovascular markers at benchtop NMR using diffusion and relaxation edited experiments

https://proceedings.science/p/169939?lang=en

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Molecular phenotyping is an established tool in the systems medicine quiver that captures a snapshot of one’s individual health status and reflects the interaction between genes and external stressors. It uses analytical platforms such as NMR or MS to acquire high fidelity molecular profiles, and complex modelling to extract actionable knowledge. Broad phenotyping, combined with multi-view multivariate analysis, allowed for robust stratification of COVID-19 patients and accurate personalised prediction of disease outcome. It also confirms the critical role played by lipoprotein metabolism in the immune response that is successfully captured by NMR-base lipoprotein parameters. In addition, applying these techniques to a cohort of Australians infected by SARS-CoV-2 revealed a strong alteration in regions of 1D NMR spectra associated with lipoproteins and glycoproteins, and referred as Supramolecular Phospholipid Composite SPC (δ = 3.2 ppm) and Glyc (δ = 2.07 ppm), an established marker of inflammation. The urgent need for very rapid testing, at the early stage of the pandemic, prompted the development of bespoke NMR experiments able to measure this lipoproteins/glycoproteins signature without requiring complex modelling.
Physico-chemical properties of lipoprotein particles, such as diffusion, transverse and longitudinal relaxation rates, are different from other metabolites. Therefore, it is possible to design edited experiments, JEDI (PGPE) to produce a clean lipoproteomic profile devoid of any spectral overlap from low molecular weight metabolites.

Furthermore, we demonstrated that the edited experiment performs equally well at low field using a benchtop NMR, thus effectively translating this COVID19 acute phase signature from high to low field.

Metabolic adaptations in brazilian jet pilots

https://proceedings.science/p/169576?lang=en

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Fighter pilots are subjected large accelerations that overcome the gravitational force (G-Force) cause deleterious damage to the pilots' health 1,2,3. However, it is hoped that with proper training, they are able deal with physiological changes, changes in the circadian cycle, jetleg, g-loc, environmental, chemical and biological stresses1,4,5,8,9, pressurization, vibration, hypoxia, dysbarism, temperature variations among others3,6,7. Our group accomplished a pioneering studied analyzing pilots blood by NMR-based metabolomics in order to elucidate and shed light in the acute or chronic metabolic changes suffered by the pilots during the flight7,8,9. Two independent studies were realized. The first was realized in the Santa Cruz Air Base in Rio de Janeiro. Blood was collected and analyzed by NMR, of a non-pilot control population (N=10), and in two groups of combat pilots (PC): PC 1 ≤1100 flight hours (N=5) and PC 2 > 1100 flight hours (N=5). The second study was carried out at the Natal Air Base (RN). We collected blood, urine, and saliva from pilots (N= 32) immediately before and after the flight and compared acute effects caused by jet flight. Our results revealed an increase in some lipoproteins, massive enhancement of partial thromboplastin time, and in contrast, a decrease in platelet volume, cholesterol, triglycerides, lactate and alanine, and others. Our results showed that jet flight pilots can have metabolic adaptations associated to combat flight time and specific acute effects.

METABOLIC FINGERPRINT BASED NMR SPECTROSCOPY OF CHARDONNAY GRAPE POMACE

https://proceedings.science/p/169599?lang=en

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Winemaking is a process that generates a large volume of biomass residues, which requires finding ways to add value and use these residues. Grape pomace is the main by-product generated in the winemaking process and just like wine has many bioactive compounds that have benefits to human health, which enables recovery and its application can be enticing to the pharmaceutical, food and cosmetic industries. Several studies have been carried out on the characterization of compounds from grape pomace based on targeted analyses consisting of isolation/identification of specific classes of compounds. In this context, the Nuclear Magnetic Resonance (NMR) technique has been a powerful tool for the non-target analysis of the chemical profile of food matrices, allowing for the simultaneous detection and quantification of various compounds. The purpose of this work was to utilize NMR spectroscopy to get the fingerprint of the grape pomace from the Chardonnay variety, with a focus on mapping the compounds that have the potential to add commercial value to the by-product or to the better application of the residue. From Chardonnay grape pomace ethanolic extract, 18 compounds were identified by 1D and 2D NMR and dates from the literature, without the need for isolation, like alanine, valine, proline, lysine, tryptophan, trigonelline, glutamine, arginine, phenylalanine, isoleucine, histidine, glucose, fructose, sucrose, arabinose, ethanol, propionate and malic acid. The Chardonnay grape pomace spectra showed higher amounts of sugar and lower phenolics compounds amounts, therefore an extensive investigation has been performed to determine phenolics compounds from

Chardonnay grape pomace and 3 phenolic compounds were identified such as gallic acid, epicatechin and resveratrol. Based on the results, this study this study is expected to contribute valuable information to determine the composition of grape pomace, better at aiming destination for their application.

METABOLIC PROFILE STUDY OF DIFFERENT SORGHUM GENOTYPES BY 1H NMR

https://proceedings.science/p/169620?lang=en

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Among the United Nations' seventeen Sustainable Development Goals (SDGs), one ensures access to affordable, reliable, sustainable, and modern energy for all. The use of renewable biomass in products or biofuels as an alternative to fossil fuels meets this goal directly. In Brazil, we can highlight ethanol as one of the main sources of renewable fuel. Lately, the ethanol industry has become the focus of attention with the search for new raw materials to optimize its production, particularly in the periods between harvest seasons Sorghum bicolor (L.) Moench is a wide-world cereal crop known due to its wide adaptability to hard climate conditions, short life cycle, good adaptation to most regions of Brazil, and multiple uses (human food, animal feed, biofuel, and industrial uses). Four sorghum genotypes were obtained to increase the productivity and sanity of grains to be used in bioethanol production. The main differences among them are the tannin contents (SC084, BRS305) and origin: lines (SC084, CMSXS180) or hybrid (BRS501, BRS305). This work aimed to use 1H NMR-based metabolic profiling coupled with chemometric tools to characterize and distinguish four sorghum genotypes: SC084, CMSXS180, BRS501, and BRS305. Sample preparation was carried out according to Kim et al. Protocol (2010), the spectroscopic measurements were done using the Bruker Avance Neo 600 MHz NMR equipment, and the chemometrics analysis was performed by the MetaboAnalyst platform. The genotype SC084 showed a metabolic profile quite different from the others by the enhanced presence of flavan-3-ols/anthocyanins, the lower sucrose content compared to BRS501 and BRS305, and the lipid content and composition. 1H NMR data and PCA analysis also showed that it was possible to differentiate defatted and in natura grains. The authors thank the support from their institutions and the financial support of ANP/FINEP to the PRH program, specially PRH-1.1.

Metabolomic analysis of Staphylococcus aureus: Exploring altered cellular pathways after the application of biogenic silver nanoparticles and antimicrobials using an NMR approach

https://proceedings.science/p/169585?lang=en

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The main objective of this research is to apply metabolomics by nuclear magnetic resonance (NMR) to investigate the effects of biogenic silver nanoparticles and other antimicrobials on pathogenic bacteria to animals and humans, specifically on Staphylococcus aureus (S. aureus). Metabolite identification will be performed in wild-type cells and cells treated with the biological agents, so that the comparative analyses conducted contribute to the discovery of altered cellular metabolic pathways in the cells and reveal the modes of action of antimicrobials. Systemic infections caused by S. aureus, a Gram-positive

bacterium, often involve the disruption of skin integrity and are caused by the proliferation of pathogens or by the action of toxins secreted by these organisms. The emergence and spread of resistance to many classes of antibiotics represent a growing threat to public health [1]. Given the great applicability of biogenic silver nanoparticles, it is indicated that these nanomaterials prove to be a safe alternative for their topical antimicrobial formulation to conventional antimicrobial agents. A recent study concluded that AgNPs do not penetrate human skin, even in clinical cases with damage to the skin surface, demonstrating their wide viability in the pharmaceutical and cosmetic market [2].

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NMR-based metabolomics in biophotonics

https://proceedings.science/p/169575?lang=en

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Low-Level Laser Therapy (LLLT) is widely used clinically for tissue repair, inflammation reduction, pain relief, and treatment of cosmetic dysfunctions. LLLT acts on the absorption of photons by chromophores at the cellular, molecular and tissue levels, resulting in increased cellular activities, cytokine production, growth factors, proliferation, differentiation, and cell migration. However, little is known about the metabolism linked to photobiomodulation. Our work focuses on map the cellular and metabolic profile of skin cells (FGH-fibroblast and MV3-Invasive metastatic melanoma) after irradiation with red laser, 660 nm, 100 mW, with 35 and 75 J/cm2, same protocol for pression ulcer. We did exploratory NMR-based metabolomics to map the intra and extracellular metabolic profile. Also, we evaluate the cells viability by MTT assay, the growth behavior, apoptosis and necrosis by annexin/PI, and cell migration by wound healing. Our results showed the red LLLT increased up to 16% in cell viability in MV3 cells after treatment, as well as a significant change in the energy consumption of the cell, since there are changes in glucose and lactate levels found in cell extracts. Other results will be shown in the poster session.

POTENTIAL BIOMARKERS TO TRACK DRIED-SALTED PIRARUCU QUALITY: A 1H NMR SPECTROSCOPY STUDY

https://proceedings.science/p/169622?lang=en

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Pirarucu (Arapaima gigas) is widely appreciated in the Northern region of Brazil, both in its fresh (FR) and dried-salted (DS) forms – the latter also known as Brazilian cod. DS pirarucu is still produced in an artisanal and empirical fashion, affecting its quality. We aimed to investigate the differences between metabolite profiles FR and SD pirarucu via 1H NMR spectroscopy, targeting metabolites with potential to assess DS product quality. To do that, 5 fresh fish and 5 dried-salted samples were obtained. The meat of fish was pulverized with N2(l) and 50 mg of the material was submitted to direct extraction in D2O (0.05% TMSP-d4). NMR analyses were performed on a Bruker Avance III NMR spectrometer (500.13 MHz) with a BBFO probe, using a ZGPR: 32 (NS), 15s (D1), 65K (TD), 4.08 s (AQ), and 128 (RG). For each sample, 90° pulse (P1), tunning, and matching were calibrated. Chemometrics were performed using PLS Toolbox and MetaboAnalyst. Separation of FR and SD samples was observed in PCA analysis where the two first PCs explained 79.40% of the total variance. Glycine, acetate, lactate, creatine, taurine, inosine, and phosphocholine were responsible for grouping FR samples while leucine, isoleucine, succinate, creatinine, trimethylamine, and hypoxanthine characterized DS samples. Normalized areas of these metabolites were submitted to PLS-DA leading to separation of the groups and pointing out acetate, succinate, lactate, and creatinine as the most important metabolites (VIP scores > 1). Their contents (mmol.g-1) were measured by qNMR resulting in values: 182.18 ± 21.75 and 5.66 ± 1.97 to acetate; 0.76 ± 0.43 and 2.76 ± 1.19 to succinate; 87.22 ± 9.80 and 130.48 ± 37.13 to lactate; not-identified and 9.020 ± 3.74 to creatinine - in FR and SD samples, respectively. Thus, lactate, succinate, and creatinine are potential biomarkers to monitor the quality of dried-salted pirarucu.

Use of python for visualization and processing of NMR spectra

https://proceedings.science/p/169679?lang=en

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Solutions/scripts will be presented using Python and PHP languages that can facilitate and make our research cheaper, avoiding the acquisition of commercial software. These solutions/scripts can be easily shared and adapted for any research group. With the use of these languages it is possible to perform very simple operations such as processing, visualizing and printing a 1D spectrum or two-dimensional spectra and their projections. In addition, more complex methods such as multivariate analysis (PCA), spectral alignment or obtaining kinetic data and electronic scheduling of NMR spectra will also be presented.

Quantitative NMR

Effects of extraction solvents on the 1H NMR profiles of Eugenia punicifolia (Myrtaceae) and their antioxidant potentials

https://proceedings.science/p/169626?lang=en

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In the Amazon region, leaves of E. punicifolia (Myrtaceae) are widely traded as herbal medicines. Previous studies have pointed to organic acids and flavonoids as responsible for the antioxidant potential of this matrix. Increasing the concentration of these compounds through extraction processes can enhance the material bioactivity, which can be decisive for the development of bioproducts. Therefore, this study aimed to quantify the main metabolites in extracts of E. punicifolia leaves using 1H NMR and determine their antioxidant potentials. For this purpose, 3 g of the leaves were separately extracted with ethanol (E), methanol (M), water (A), and ethanol/water (7:3 - E/A). After drying, 10 mg of each extract (n = 3) was dissolved in 550 µL of MeOD-d4 with TMSP-d4 at 2 mM (internal standard). The spectra were acquired at 25 °C on a Bruker Avance III NMR spectrometer operating at 11.75 T, equipped with a 5 mm SmartProbe, using the zgpr pulse sequence: P1 10.62 µs, D1 10.0 s, O1 2350.61 Hz, NS 16, and DS 2. The antioxidant assays performed were DPPH. and ABTS+.. Phenolic compounds, including kaempferol, ellagic acid, quercetin, gallic acid, and myricetin, were identified through NMR spectra analysis. NMR quantitative experiment revealed that the ethanol extract exhibited the highest total phenolic content per gram of dried extract (mM.g-1): E = 59.26, E/A = 45.85, A = 42.07, and M = 21.92. The antioxidant assays suggested the correlation of these contents with the antioxidant potentials of the extracts: E (1952.00 and 1854.59), E/A (1515.10 and 1339.89), A (1506.20 and 1381.41), and M (1364.15 and 1204.34) - DPPH. and ABTS+.. in mM Trolox/mL, respectively. Therefore, the total content of phenolics obtained by the 1H NMR profile can be used as a probe to assist in developing antioxidant bioproducts based on ethanolic extracts of E. punicifolia.

METABOLIC DETERMINATION OF RAW AND FERMENTED CORN GERM BY QUANTITATIVE NMR

https://proceedings.science/p/169590?lang=en

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Corn Germ has been developed to seek maximum utilization of by-products from the food industry, particularly in oil extraction and feed production. There are various limitations for this by-product to be consumed in the human diet, such as high levels of polyunsaturated fatty acids, mycotoxins, and antinutritional factors. Studies have been conducted to break the interaction between relevant nutrients and antinutritional factors to stabilize these by-products through fermentation, generating ingredients suitable for use in the baking industry. (1)
The metabolic profiling of corn germ using the nuclear magnetic resonance (NMR) technique was one of the stages of this project. This approach allows for a deeper understanding of the composition and metabolic transformations occurring during solid-state fermentation, providing essential information for process improvement.
Quantification in NMR is possible due to the direct relationship between the signal intensity and the number of nuclei responsible for the signal. This information is translated through the signal's area, and obtained by integrating it, which reflects the substance's concentration. Following certain experimental conditions for accurate quantification is essential, such as calibrating the excitation pulses, using an appropriate repetition time (> 5 T1), proper signal-to-noise ratio, controlled temperature, and a quantification standard. Probe tuning and field homogeneity are also essential. (2)

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qNMR and GC-MS as an analytical technique for characterization of essential oils of three species of Cymbopogon

https://proceedings.science/p/169665?lang=en

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Cymbopogon species are aromatic plants belonging to the family Poaceae natives to South India and Sri Lanka. Lemongrass is a generic name used for C. citratus and C. flexuosus, which are largely used in folk medicine as anxiolytic, antispasmodic and anti-hypertensive. The main components of C. citratus are geranial, neral and myrcene, while of C. flexuosus are geranial, neral and geranyl acetate. For C. winterianus, known as citronella grass, the major components are geraniol, citronellol and citronellal. This plant is used due to their fungicidal, bactericidal and insect repellent properties. This work reports the quantitative study of the essential oils of C. citratus (CCEO), C. flexuosus (CFEO) and C. winterianus (CWEO) by NMR and GC-MS. The essential oils were obtained from the fresh leaves of each studied species by hydrodistillation in a Clevenger apparatus (triplicate) and stored at 4 oC. Quantitative 1H NMR (qNMR) spectra were measured on a Bruker DRX 500 spectrometer, using the acquisition parameters for each sample: 16 scans, acquisition time of 6s, pulse width of 9.87 □s (90o), relaxation delay 30 s, pre-fixed receiver gain values of 16 and 119,998 data points. The samples (± 20 mg) had their NMR data acquired in triplicate. Antioxidant activity DPPH assay was performed for one sample of each oil. The main components (mg g-1) of CWEO quantified by GC-MS are citronellal (303.5), geraniol (84.7) and citronellol (305.8), while for CFEO are geranial (397.6) and neral (308.8). Geranial and neral were also identified by NMR in CCEO with amount of 460.9 mg g-1 and 320.5 mg g-1, respectively, similar to observed by GC-MS. CWEO showed higher antioxidant activity compared with CCEO and CFEO.

qNMR in Natural Products: Practical approaches

https://proceedings.science/p/169669?lang=en

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Until recently, it was common to observe applications of qNMR (quantitative nuclear magnetic resonance) in samples from the area of Natural Products without the same rigor as analytical works, particularly regarding validation. However, even though new approaches have emerged with the expression of uncertainties involved in the analyses [1-2], some practical details such as sampling plants, extracts preparation and NMR parameters adjustment need to be carefully evaluated. In this sense, this lecture will use the study of qNMR in extracts of Vernonia rubricaulis, guided from previous metabolomic studies, for investigating the compound potentially responsible for the toxicity of this plant in cattle, in order to explore the sample and experimental factors that impact the results.

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CNPq, FUNDECT, CAPES, UFMS

QUANTIFICATION OF SUGARS AND HMF IN HONEY USING NUCLEAR MAGNETIC RESONANCE

https://proceedings.science/p/169593?lang=en

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Honey is widely used in Brazil for medicinal purposes or as a natural sweetener. Honey consists of around 200 substances, including carbohydrates, proteins, phenolic compounds, organic acids etc. Sugars are present in honey in about 85% of the content. The ratio between them is important for verifying the quality of honey and may indicate its ability to crystallize. The amount of HMF (5-hydroxymethylfurfural) is also an indicative of quality. Currently, the quantification of honey analytes is mainly carried out using spectrophotometric or chromatographic method. The use of NMR for quantifying honey analytes is still relatively limited.[1]
The NMR technique is a powerful technique for food analysis and can be used to assess food quality. Validating a methodology requires that the method demonstrates good selectivity, linearity, precision, accuracy, quantification limit, and suitable robustness for the analysis.[2] Therefore, this research aims to conduct a method validation for the simultaneous quantification of sugars and HMF in honey samples in order to compare the results with other techniques. So far, some performance parameters are already evaluated. As an internal standard, maleic acid was used due to its short relaxation time and the fact that its signal does not overlap with others. The concentration range used was 0,07 mg/mL to 2,0 mg/mL. The method showed good selectivity as each 1H NMR spectrum demonstrated that sugars, HMF or maleic acid can be individually quantified without significant overlap. The linearity has already been calculated for glucose and HMF standards, yielding an R^2≥0,995. Statistical analysis and other performance parameters are ongoing.

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Study of types of sugars present in non-centrifugal raw cane sugar by 1H RMN and chemometrics.

https://proceedings.science/p/169653?lang=en

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Non-centrifugal raw cane sugar (NRCS) is a solid product obtained from the evaporation and concentration of sugar cane juice of the genus Saccharum spp. Sucrose is the most abundant component (65 - 85%), followed by reducing sugars (2 - 10%), water (3 - 9%), and other components (< 1%). For NRCS production, two sugarcane varieties were used (RB966928 and RB867515), with ten months of maturation, different optimal cane ripening periods, and different edaphoclimatic conditions. This

choice was based on the possibility of evaluating the influence of these cultivation conditions on the technological quality of the sugarcane raw material used in the production of sugar. High-resolution 1H NMR is considered a fast, easy-acquire, and non-destructive technique for food analysis. 1H NMR spectra were obtained using a 600 MHz Avance III HD Bruker spectrometer, and the parameters: 64 scans, d1=4s, sw=14 ppm, pulse 13.75 µs, and TMSP-2,2,3,3-d4 signal (δ 0.0 ppm) as the internal reference. The sucrose PD45 (98% purity) was used as a reference, their 1H NMR chemical shifts (δ, ppm) were: 5.42, d (J=3.8 Hz, 1H, d); 4.22 (J=8.6 Hz, 1H, d); 4.06 (J=8.6 Hz, 1H, t); 3.91-3.86 (m, 1H); 3.84 (m, 1H); 3.81-3.78 (m, 4H); 3.77 (J=10.1 Hz, 1H, t); 3.69 (s, 2H); 3.57 (J=13.1 Hz; J=3.8 Hz, 1H, dd); 3.48 (J=9.1 Hz, 1H, t). 1H NMR and chemometrics (PCA analysis) data showed that samples 2 and 4 of NRCS from sugar cane juice of the variety RB867515 had a higher amount of reducing sugars (glucose and fructose) when compared to samples 1 and 3 of variety RB966928. 1H NMR results also showed that the influence of the optimal sugarcane ripening period is more important than the different edaphoclimatic conditions.

What do spectroscopy, forensic sciences and data analysis have in common? A metrologist's point of view

https://proceedings.science/p/169596?lang=en

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Reproducible science needs metrology and quality. Without harmonization in procedures and comparability in measurements scientific conclusions can be seriously flawed. Forensic sciences and the industry 4.0 may seem only loosely related at best, however, the need for reliable chemical measurements an unbreakable link between those two fields. Spectroscopic techniques, especially Nuclear Magnetic Resonance (NMR) provide a useful tool aiming at fast and reliable measurements for industry as well as methods of utmost importance both for on-site analysis for police forces and in the development of reference materials for forensic laboratories. With the advance in analytical methods and instruments such as NMR and mass spectrometers (MS), the amount of data generated grows each day and being capable of analyzing and mining all these data is what will ultimately make us go farther. Our recent research at Inmetro has focused on basic metrology for forensic sciences through the production of drug reference materials with innovative synthetic processes, data analysis methods for reliable results in toxicology and fast measurement methods for forensic sciences and food industry. We'll discuss how all of this is united by metrology in chemistry.

Small Molecules

Chemically cross-linked polystyrene gels as aligning media for aromatic solvents

https://proceedings.science/p/169591?lang=en

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Cross-linked polystyrene polymers, obtained by cross-linking with β radiation, can be swollen in many different organic solvents, allowing the measurement of Residual Dipolar Couplings (RDCs) in different

organic solvents by restricted stretching of the gels inside an NMR tube.[1] We present here a chemically cross-linked version of the gel, using 1,4-bis(4-vinilfenoxi)butano as a cross-linker, which can be compressed, in a reversible way,[2] in a 5 mm NMR tube. The gel swells well in aromatic solvents such as benzene-d6 , and pyridine-d5 , as well as in CDCl3. As an example of the performance of this new aligning media, 1DCH RDCs and RCSAs were measured for brucine in a toluene-d6 swollen gel in a range of -13.0 to 8.0 Hz and 0.057 to 0.120 ppm, respectively. Singular-value decomposition fitting of these RDC and RCSA values provided Q factors of 0.052 and 0.076, respectively.

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COMPARATIVE ANALYSIS OF CHOCOLATE POWDER DIFFERENT DATA ANALYSIS METHODS

https://proceedings.science/p/169602?lang=en

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Chocolate is a natural product with high market value, but difficult to control quality. Cocoa has several components such as flavonoids, bioactive compounds that can vary depending on characteristics of fermentation and cultivation.[1]
Different analysis methods, including nuclear magnetic resonance, have been used to find ways to improve this situation. Most studies use cocoa bean extracts [2-4] or ready-made chocolates [5] for high-resolution NMR analyses. This work focuses on the analysis of powdered and chocolate-based chocolates, used in the preparation of beverages and bakery products.
Samples of all products were prepared in DMSO-d6. Due to low solubility of the products and therefore low signal intensities, only 1H and 13C-HSQC spectra were acquired for analysis. In the HSQC spectra, 3 regions can be observed in the spectrum that are common in all samples, but at different intensities. With this, a classification of the components of the samples according to the declared quality of the products is expected. All techniques are well established and have been used regularly in the laboratory for years.

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Evaluation of the relaxation effect by paramagnetic agents in carbonyl compounds

https://proceedings.science/p/169614?lang=en

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Measuring the kinetic isotope effect (KIE) using natural isotopic abundance (13)C-NMR requires a high signal-to-noise ratio for quantitative carbon experiments. This is crucial to minimize any errors that may arise during KIE measurements. Obtaining these spectra can be time-consuming, taking up 30 hours or more, due to the typical range of 5 to 60 seconds of (13)C T1, since the condition of recycled delay (D1) equals to 5 times the relaxation time (T1) of 13C in these experiments must be followed. To reduce the total experimental time for acceptable 2 to 3 hours and keeping the mandatory condition (D1 = 5xT1), relaxation time T1 must be reduced using a paramagnetic relaxation agent. With that in mind this work aims to find the optimal ratio between the quantity of the target molecule and a chosen relaxation agent, to achieve a suitable T1 value that enables faster KIE measurement. To conduct solution-state NMR experiments, we added 2 mmol L-1 of a target carbonyl compound (acetone and acetic acid) in CDCl3 and T1 was measured for (13)C. In the range of 0 to 2% (m/m) of [Cr(acac)3], we observed significant reductions in T1 for acetone: 95.2% for (13)C=O, 91.6% for (13)CH3, and 95.4% for C(1)H3. Similarly, for acetic acid, we observed reductions of 70.7% for (13)C=O, 24.6% for CO2(1)H, and 94.8% for C(1)H3. These results show an important contribution to faster and more accurate KIE measurements. To confirm the dependence of molecular structure and relaxing agent on T1, we intend to perform additional analyzes on different paramagnetic agents and molecules.

HOW IS NMR HELPING THE BRAZILIAN FEDERAL POLICE IN CRIMINAL INVESTIGATION?

https://proceedings.science/p/169680?lang=en

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As crime evolves, so must the forensic techniques used to combat it. We will present the collaboration between the Brazilian Federal Police and the NMR Laboratory of the Federal University of Paraná. Firstly, we delve into the application of Nuclear Magnetic Resonance (NMR) in elucidating the chemical structure of New Psychoactive Substances (NPSs), especially the ones which are thermosensitive and may not be analyzed by Gas Chromatography Mass Spectrometry (GC-MS), currently one of the most widely used techniques in forensic labs. Additionally, the ability of NMR to quantify substances without the need for a chemically identical standard makes qNMR invaluable for producing secondary standards for forensic use. This need prompted the recent acquisition of an NMR spectrometer by the Brazilian Federal Police. Moreover, we examine the impact of this collaboration during various recent police operations. Amid the Covid-19 pandemic, NMR played an important role in the Acquagel Operation (2020), which investigated a company producing hand sanitizers falsely claiming a 70% ethanol concentration while containing as little as 35%. NMR was also crucial in uncovering food fraud during the Xaropel Operation (2021) and the Alcanos Operation (2022), which respectively focused on honey and butter adulteration. In the case of honey adulteration, NMR identified partially inverted sugar and maltodextrin, which was used as a thickener. In butter fraud, margarine and palm oil were added to the milk cream to increase profits. Estimates reveal that honey adulteration resulted in a profit of 3.6 million dollars, while butter adulteration led to 2.4 million dollars in illicit gains. NMR was also used to unveil the adulteration of seized olive oil with other edible oils. Despite NMR's potential, its wider adoption in forensic labs is still limited by cost. However, collaborating with local Universities presents a promising model to effectively incorporate NMR into police investigations.

LC-SPE-NMR AS A POWERFUL TOOL TO ISOLATE MINORITARY DITERPENOIDS FROM Vellozia pyrantha

https://proceedings.science/p/169592?lang=en

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Natural products isolation has shown great advances, especially with the hyphenation of liquid chromatography with solid-phase extraction and nuclear magnetic resonance (LC-SPE-NMR), allowing to identify minor compounds from natural sources. These compounds are commonly obtained on the µg scale, requiring the use of higher field magnets with cryogenic probes to enhance sensitivity. This study aimed at using LC-SPE-NMR as a tool to isolate minority diterpenoids of Vellozia pyrantha, an endemic plant of Chapada Diamantina National Park known for its flammability and its great flowering capacity after burning events. The resins of Vellozia species are recognized mainly for contain cleistanthane diterpenoids with different degrees of oxidation. The isolated compounds were characterized using 1D NMR (1H/13C/ROESY) and 2D NMR (COSY/HSQC/HMBC) experiments, acquired on a Bruker® AVANCE III 14.1T equipment with a 5 mm TCI cryoprobe. Thirteen new diterpenoids along with six known compounds were obtained by LC-SPE-NMR with amount ranging from 100 to 200 µg. The cleistanthane skeleton was readily identified by a group of signals between δ0.85-1.70 (3 singlets and 1 triplet) attributed to methyl groups attached to sp3 carbons and one singlet between δ2.00-2.30 attributed to a methyl group attached to an aromatic ring. The absence of any of these signals is an indicative of a possible oxidation, commonly as an alcohol or aldehyde but also as an ester bridge. For alcohols, n.O.e experiments defines the relative stereochemistry of the hydroxyls groups. Normally, the oxidation occurs to positions C-1, C-6, C-7, C-11, C-12, and C-17. Positions C-1, C-11 and C-12 usually have hydroxyl groups, C-6 and C-7 hydroxyl and carbonyl groups, whereas C-17 groups hydroxyl, aldehydes, or carboxylic acid. The use of LC-SPE-NMR shows its potential for bioprospecting natural products once it enabled the isolation of 19 minor diterpenes of V. pyrantha and certainly can be used in others sources.

Lyotropic Liquid Crystals based on Bisperylene Imides as Alignment Medium for Obtaining Anisotropic NMR Parameters

https://proceedings.science/p/169577?lang=en

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Measurement of anisotropic parameters, such as Residual Dipolar Couplings (RDCs) and Residual Chemical Shift Anisotropies (RCSAs), have played an important role in structural refinement of organic compounds. A key factor for the obtention of these parameters is the selection of an appropriate alignment medium. Lyotropic liquid crystals, among other systems, have been considered a good alternative for this purpose. We present here three new water-compatible aligning media based on mesogenic bisimides derived from the conjugation of perylenetetracarboxylic dianhydride with the aminoacids L-valine (BPI-LVal), L-leucine (BPI-LLeu) and Glycine (BPI-Gly). We studied the influence of the concentration and the temperature on the formation of the mesophase by monitoring the splitting of the quadrupole coupling of the D2O signal in the 2H NMR spectra. It was determined that the best conditions for using these mesophases as alignment medium corresponds to a solution of 0.5 M at 23ºC for BPI-LVal, 0.3 M at 27ºC for BPI-LLeu, and 0.2 M at 25ºC for BPI- Gly. To evaluate the efficiency of the three nematic phases as alignment media, we used sucrose as a test molecule. The experimental RDCs values were in the range of -54 to 75 Hz, while RCSA values a range of -0.32 to +0.18 ppm were obtained, providing good quality factors that allowed us to study the conformation of sucrose in

aqueous solution.

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Mapping the interaction of the flunitrazepam and its deuterium labelled form with the HSA protein by STD-NMR

https://proceedings.science/p/169655?lang=en

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The literature reports that deuterium labelling of drugs improves pharmacokinetics, pharmacodynamics, toxicology, in addition to reducing dosage1. In this context, a comparative investigation between the interactions of a deuterated and a non-deuterated drug with the Human Serum Albumin (HSA) protein must be investigated and it is the purpose of this work, using deuterated (FLU-D) and non-deuterated flunitrazepam (FLU) with HSA.
The Saturation Transfer Difference STD-NMR2 experiments were performed at 298K on a Bruker Avance III 600 MHz spectrometer, using a molar ratio 1:20 HSA versus ligand.
Several STD experiments were acquired by varying the saturation time (0.5 to 10.0 s), in which the concentration of drugs and protein was kept constant . The results obtained for FLU-D suggests that hydrogens of the trisubstituted ring are closer to the protein. Conversely, we can observe that the methyl group is further from the protein if we compare it with the results obtained from the aromatic hydrogens of the molecule. A similar result was achieved with the FLU molecule.
In order to understand whether there can be variation in the ligand-protein ratio and the saturation time between FLU and FLU-D, the dissociation constant (KD) value was calculated. In this experiment, the ligand concentration was varied between from 0.2 and 1.0 mM, limited by the solubility. The estimated KD for FLU was 0.79 mM and 0.72 mM for FLU-D. There was no significant difference between the KD estimated, which can be explained by the epitope map. Some difference can be observed for full labelling.

Multidisciplinary analysis of structurally complex natural products

https://proceedings.science/p/169606?lang=en

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In recent years an increasing number of natural product structure misassignments have been reported in the literature [1-3], in spite of exceptional advances in spectrometric and spectroscopic instrumentation, in particular of mass spectrometry and nuclear magnetic resonance. In this sense, a major challenge remains to correctly assign the configuration of natural products isolated from various sources [4].
In the course of our biodiscovery program aiming to the isolation of bioactive and structurally unique secondary metabolites from marine invertebrates and from microorganisms, we have faced challenges in structure assignment, specially related to establish the correct configuration of natural products.
In this lecture examples of our recent results will be presented and discussed, illustrating the power of

combining “traditional” NMR structure assignment with the use of advanced tools of NMR calculations, bioinformatics genomic analyses and chiroptical analyses.

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NMR as Part of a Large Effort to Understand Why Hair Cremes Blinded People in Brazil

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The first cases of temporary blindness because of hair cremes were reported in Rio de Janeiro in march of 2022. Numerous new cases were reported over the next months, and by mid February 2023 about 1200 cases had been reported, including 1 case of permanent blindness. Due to this development, ANVISA was forced to issue a complete prohibition of all hair creme sales in Brazil until the reasons for this had been investigated.

A first analysis by NMR revealed that all suspect hair cremes had a very high concentration of ceteareth-20, a common emulsifier in hair products and known for being very effective in straightening curled hair. But, irritation tests revealed that ceteareth-20 does not cause temporary blindness. Hence, the possibility of a combination effect was investigated, were the high concentration of ceteareth-20 would act as trigger. A huge variety of possible factors were verified by the Ministry of Health, and a final result is still being assembled.

NMR ligand-enzyme analysis for identification of nucleoside hydrolase inhibitors

https://proceedings.science/p/169584?lang=en

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Nucleoside hydrolases are a strategic target for drug development to treat leishmaniasis, a neglected disease. This enzyme participates in the purine uptake pathway for the synthesis of nucleic acids, being essential for the survival of the parasite. The most potent inhibitors identified for NH are the immucilins, which are nucleoside analogues. As a strategy for identifying new classes of inhibitors, our group is using fragment-based drug discovery and natural products. From an in-house library of 111 compounds using STD-NMR we identified 5 NH ligands, one of which is a low potency inhibitor. From the pooled analysis between the ligands using NOESY, we identified two ligands that bind closely on the enzyme. From the structural features of these two ligands, we used a library of 23 more complex

compounds to identify a more potent ligand. From the biological screening of 214 Brazilian plant extracts, 23 plants showed NH inhibitory activity in more than 50%. Of these, three were selected for their results and lack of prior phytochemical description: Leandra amplexicaulis, Urvillea rufescens and Ormosia arborea. From the bioguided fractionation of L. amplexicaulis and U. rufescens extracts, three flavonoids were identified as NH inhibitors. The use of NMR combined with chemometrics allowed the identification of two new proanthocyanidins as LdNH inhibitors from the O. arborea extract, before isolation, directing the best strategy to purify them. From this identification, a series of flavonoids was synthesized and their activities determined. STD and Waterlogsy data, obtained for the most potent flavonoids, combined with docking studies, provided information on structure-activity relationships.

Nuclear magnetic resonance to evaluate the influence of granulometry and roasters on the chemical composition of coffees

https://proceedings.science/p/169621?lang=en

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Coffee is the second most consumed beverage in the world, its grains have different physical and chemical characteristics, responsible for characterizing its quality. However, it is during roasting that coffee beans become suitable for consumption, since chemical and physical transformations occur that guarantee their flavor, aroma and texture. However, roasting is a complex process, as it is influenced by factors such as temperature variations, type of roaster and physical characteristics of the beans. Thus, the objective of this study is to evaluate the impact of using different types of roasters on coffee beans with different sizes (granulometry) in order to evaluate the chemical composition of the coffee obtained at the end of the roasting process by means of resonance spectroscopy nuclear magnetic (NMR). For that, coffees with four granulometry (sieve 17 above, 15/16, 13/14 and bottom) and two roasters were used. The results were analyzed by principal component analysis, where it was observed that the NMR analysis is capable of discriminating raw coffees with different granulometries. This separation is seen in the score chart, which showed 99.1% of the total variance explained in the first three main components, with sugars and lipids being responsible for the separation. This result proves that coffee beans from the same origin with different sizes have different chemical composition. The influence of two roasters on the coffees of each sieve was also evaluated, where it was noted that, depending on the type of roaster used, the coffee has a different chemical composition, mainly in the caffeine compounds, sugars and chlorogenic acids. These results show the importance of knowing the factors that influence the chemical composition of coffees and their impact on processes such as roasting, so that processes can be optimized and standardized.

Probing Mefloquine-Resin Complexes by C-13 Solid State NMR

https://proceedings.science/p/169570?lang=en

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Mefloquine chlorhydrate is a medicine prescribed for the full course of malaria treatment. When taking for children, usually vomiting occurs and new doses of medication frequently need to be taken. During the course of our research a new pediatric dispersible mefloquine formulations have been developed,

using mefloquine and ion exchange resins. In this communication samples of mefloquine chlorhydrate, polacrilin potassium resin and mefloquine-resin formulations were evaluated in order to probe the presence of complex type structures by C-13 CPMAS NMR. Spectra were acquired on a Bruker WB Avance III400 spectrometer (9.4T), operating at Larmor frequency of 100.4 MHz. Acquisition conditions: pulse sequence – cross polarization magic angle spinning; spinning rate - between 8 to 10kHz; recycle time - between 3 to 60s; contact time for cross-polarization - between 0.1 to 20ms. Glycine was used as reference (C=O 176.03 ppm). An optimized mefloquine chlorhydrate spectrum could be obtained at recycle time of 20s, spinning rate of 9.8 Hz and contact time of 10 ms. All the carbon signals could be clearly assigned, including those linked to fluorine, in the region between 115 and 125 ppm. Prado et al. (2020) published a mefloquine hydrochloride spectrum with a contact time of 1.5 ms, but they did not visualize the signals of the CF3 carbons. Guo et al. (2021) published data for C-13 CPMAS NMR spectra of mefloquine, at a rotation speed of 20 kHz and contact times between 0.5 and 1.4 ms, that was comparable to our optimized spectrum. Comparison of the T1rhoH values for the carbons of mefloquine and polacrilin and mefloquine-resin allows us to confirm the absence of physical mixture and the presence of the complex in a formulated sample of mefloquine-resin.

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Relaxing Time: a T1 study for heteronuclei

https://proceedings.science/p/169624?lang=en

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Longitudinal relaxation (T1) is a NMR phenomenon that implies the return of the bulk magnetization to the equilibrium state (MZ). This process occurs by the fluctuations on the magnetic field in the Larmor frequency of the nucleus, which allows the energetic changes in the excited states of the spins and the lattice. Knowing these times can bring information about the dynamics of the molecules [1] and is associated with the experimental time of quantitative analyses.
To measure the T1, pulse sequences such as the Saturation-Recovery (SR) [2] and Inversion-Recovery (IR) are used [3]. T1 values can vary for each nuclei due to the difference in Larmor frequency between them [4].
This work has the aim to analyze T1 values for the most important nuclei (1H, 13C and 19F) in organic compounds, as well as how a Paramagnetic Agent (P.A), which enhances the relaxation process [5], affects each nuclei. The decrease in the relaxation time T1 by the P.A will be compared. To measure T1 values the saturation time was optimized in SR sequence. This parameter was also compared for different nuclei.

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Searching for minor compounds in olive oil highlighting the polyphenols – a NMR mixture analysis approach

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Vegetable oils and particularly the olive oil are known as a source of important molecules like polyphenols, glycerides and carbohydrates. Polyphenols are essential molecules in the defense system of plants, including antioxidant, antibiotic, anti-inflammatory and antiallergic properties. The analysis of such minor compounds is traditionally done by chromatographic methods, including LC-UV-MS and GC-MS, in order to separate and detect them. However, these methods are time demanding and require for high amount of solvents. In this context, NMR spectroscopy emerges as an alternative method, being non-destructive, with simple sample preparation and using low amount of solvents. Given some limitations of NMR, such as the small spectral window for 1H and lower sensitivity, new experiments like Maximum Quantum (MaxQ) NMR and the recent DREAMTIME NMR can be used as interesting methods for mixture analysis. The purpose of this work is to extract, analyze and identify minor compounds in olive oil, highlighting polyphenols. A methanol/water extraction were done, and the extract was dried, redissolved in CD3OD. The sample was analyzed in a Bruker Avance III 400 machine. The 1D-DREAMTIME, together with other 2D experiments (COSY, TOCSY, HSQC and MaxQ), allowed the identification of some minor compounds, including some aldehydes, carbohydrates and polyphenols (e.g., tyrosol and hydroxytyrosol).

STD-NMR EVALUATION OF α-GLUCOSIDASE INTERACTION WITH SMALL TRIAZOLIC MOLECULES

https://proceedings.science/p/169656?lang=en

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The present study involves the use of STD-NMR technique. It plays an important role in the elucidation of interactions between ligands and enzymes in equilibrium. Binders are possible drug prototype substances. By analyzing the intensity of the STD amplification factors, it is possible to identify the regions of the substances that have the greatest affinity with the enzyme.
In the case of this study of α-glucosidase (maltase) enzyme inhibition, the region of carbohydrate protecting groups emerged as the most intense, suggesting a significant interaction in this area. These enzymes are important in the case of studies of diseases such as type II diabetes and cancer.
This study consisted in screening 3 triazole-glycoside ligands from an a-glucosidase inhibition study[1] carried out by our research group, in addition to the reference substance, acarbose. This screening was carried out in molar excess, in the proportion 1:200 (enzyme:ligand).
The STD-NMR experiments[2] were performed with Bruker AVHD400 equipment, Topspin software, stddiffesgp.3 pulse sequence, alternating ON and OFF resonance frequencies (0 and -50ppm, respectively). This pulse sequence features a spin-lock that saturates enzyme unwanted signals (10ms) and water suppression with gradient-sculpted excitation. Performed with 256 scans, 40dB attenuation of the shaped pulse power and with 0.300s, 0.626s, 1.250s, 2.500s and 5.000s saturation times.
The results obtained of the ligands' STD amplification factor of the difference signals of the methylic and methoxylic protection groups of the carbohydrate revealed a significant interaction with the enzyme. These findings indicate that these specific regions are crucial for interactions and inhibitory activity of ligands towards the α-glucosidase enzyme.

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STUDIES WITH COUMARIN DERIVATIVES AGAINST ALZHEIMER'S DISEASE

https://proceedings.science/p/169564?lang=en

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Alzheimer's disease (AD) is the most prevalent cause of dementia among the elderly population.[1] Although there isn't a definitive cure, many treatments are available that aim to enhance the quality of life for the affected individuals by managing symptoms and slowing down disease progression. [2-3] One approach to treating AD involves boosting the levels of a neurotransmitter called acetylcholine (ACh) in the small gap between the nerve cells, known as the synaptic cleft. This is done by inhibiting the activity of the cholinesterase enzymes. By inhibiting acetylcholinesterase (AChE), for example, it is possible to prevent the breakdown of ACh, allowing it to accumulate and enhance communication between nerve cells, potentially alleviating some symptoms of AD. [4] Previous studies have revealed that coumarin derivatives show AChE inhibition and neuroprotection, suggesting their potential as the basis for developing new drugs for AD. [5-6] In this study, eighteen coumarin-1,2,3-triazole hybrid compounds were synthesized and characterized by Nuclear Magnetic Resonance (NMR). In silico tests were used to predict physical chemistry properties, oral bioavailability and the toxicity of the compounds. The kinetic enzymatic (AChE) is now being studied using Ellman's test and NMR.

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CNPq, FAPERJ, CAPES

Study of the thermal stability of commercially available cannabidiol (CBD)

https://proceedings.science/p/169589?lang=en

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Cannabis sativa has about 60 identified compounds, and the two main pharmacologically active compounds are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Several studies show that CBD helps with motor activity, depression, reduces anxiety and has anti-inflammatory action.1
Due to its pharmacological actions, CBD is present in several treatments, such as Epidiolex® (GW Pharmaceuticals, Cambridge, United Kingdom) used for refractory epilepsy, Dravet and Lennox-Gastaut syndromes. The drug has a concentration of 100 mg/ml of CBD, being approved by the Food and Drug Administration (FDA).2

However, CBD is degraded with temperature, light and auto-oxidation. Studies indicate that CBD samples stored in the dark after three months at room temperature have been shown to contain impurities such as ∆9-THC and ∆8-THC.3
The present work shows the study of thermal degradation of CBD present in a commercial extract. Thus, 1H NMR were performed at temperatures of 35 and 45°C for 12 hours each. Thus, a variation in the region δ = 4 – 5.5 ppm is observed regarding the doubles of the CBD molecule.
Thanks to LabPetro, UFES, FAPES, CAPES and CNPq.
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USE OF NUCLEAR MAGNETIC RESONANCE IN THE DIFFERENTIATION OF POLYPRENYLATED BENZOPHENONE SKELETONS FROM Clusia burlemarxii.

https://proceedings.science/p/169600?lang=en

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The specimen Clusia burlemarxii is a significant source of polyprenylated benzophenones derived from the shikimate and acetate-malonate pathways. After prenylations, geranylations, and secondary cyclizations these compounds generate several polycyclic structures that may exist in a keto-enol equilibrium. This study aimed to provide NMR spectroscopic support to differentiate the skeletons of polyprenylated benzophenones from C. burlemarxii. Compounds were isolated from the hexane extract of the stem of C. burlemarxii and identified through extensive analysis of 1D-NMR (1H, 13C) and 2D-NMR (COSY, HSQC, HMBC, NOESY) obtained on a 14.1T Bruker® AVANCE III equipment with a 5 mm TCI cryoprobe. Four polyprenylated benzophenones were isolated: the hyperibones A and B, which possess a bicyclo [3.3.1] nonane-2,4,9-trione core, and the burlemarxiones A and B, which possess a tetracyclo [8.3.1.03.11.05.10] tetradecane core. In the first skeleton, one 13C NMR signal is typical found between δ 207-210, attributed to the non-conjugated carbonyl at C-9 of the bicycle portion and two singlets in the 1H NMR spectrum between δ 1.0-1.6, corresponding to aliphatic dimethyl singlets correlating in the HMBC with the C-8 quaternary carbon and one C-6 methylene group at δ 1.90-2.10 for Ha and δ 1.45-1.50 for Hb. The second skeleton has an unconjugated C-4 carbonyl between δ 210-211 and a carbinolic carbon (C-6) between δ 85.0-86.0. Through the NOESY correlations, it was possible to observe the differences between the hyperibones, revealing that the two are epimer compounds at C-18. This characteristic justifies the differences in the chemical shifts of both. In the burlemarxiones case, the distinction is in the methoxy group, found in C-2 for burlemarxione A and C-27 for burlemarxione B. This was confirmed through HMBC correlations. Therefore, the use of the NMR technique, along with efficient elucidation strategies, becomes a valuable tool to distinguish these skeletons, considering the complexity of these structures.

Vegetable oil addition in powdered milk – a preliminary regioespecific approach by 13C NMR

https://proceedings.science/p/169612?lang=en

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Food fraud is a significant public health and food security concern. Among food products most commonly associated with fraud, milk ranked second based on scientific articles published between 1980 and 2010 [1]. However, even in the last decade, frauds involving dairy products continue to be prominent [2,3]. In the case of non-dairy fat addition, the literature reports instances of fraudulent inclusion of vegetable oils [4], and even animal fats such as lard [5]. Despite the existence of official methods for detecting adulterants and contaminants, the emergence of increasingly sophisticated adulteration practices highlights the need for continuous study and development of new detection methods. In this context, the literature has consistently proposed the use of novel techniques for identifying various types of adulteration in dairy products. However, there is still a need for methodologies to determine the purity of dairy fats. Therefore, this work demonstrates the potential of 13C NMR spectroscopy for assessing the regiospecific distribution of carbonyls in triacylglycerides present in dairy fats to detect the addition of vegetable oils in powdered milk samples. Authentic samples with and without the addition of vegetable oil were used to investigate the chemical profile of triacylglyceride carbonyls. Preliminary results obtained through semiquantitative 13C NMR analysis indicate that the PUFA content at the sn-1,3 and sn-2 positions is more informative than the butyric acid content alone. Chemometric and relative proportion analyses are currently underway to subsequently compare suspected and/or commercial powdered milk samples.

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TD-NMR

1H time domain NMR as a toll for probing distribution of metal ions adsorbed in modified sugar-cane bagasse bioadsorbents.

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1H time domain nuclear magnetic resonance (1H TDNMR) was used to investigate the Cu+2 ions adsorbed in the pore structure of a bioadsorbent constituted of polycarboxylated units grafted in the cellulose surface of sugarcane bagasse (PSB). Carr-Purcell-Meiboom-Gill (CPMG) and Gradient-T2 (GT2) correlations experiments were applied to probe T2 relaxation and diffusion of water confined in the inner pores of the samples, providing information on the pore sizes and internal magnetic field gradients (IMFG) distributions within the pore structure. T2 distribution profiles from clean bio-adsorbents, i.e., no ion metal adsorbed, were similar to that of pristine sugarcane bagasse (SB), showing components associated to three pore categories. A direct comparison between the T2 distribution of confined water in clean and Cu(II) saturated bio-adsorbent revealed that the mean T2 value of all components were decreased by to the ion adsorption. CPMG experiments with varying echo times

were conducted and processed as described by Hurlimann [1] to estimate the IMFG distributions. The results showed a distribution with three components, which change consistently with the metal ion adsorption, reducing the average values and changing the relative population of each component. More specifically, the results suggested that adsorption occurs on the surface of all pores in the SB structure but is more effective in pores of few micrometers. Two-dimensional G-T2 experiments confirmed the correspondence between the changes in the IMFG and T2 distributions due to the Cu(II) adsortion.
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A rapid and non-destructive 7Li time-domain NMR method for directly lithium determination

https://proceedings.science/p/169650?lang=en

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1H Time Domain NMR (TD-NMR), based on benchtop spectrometers, has diverse applications in industrial and academic fields, as well as in industrial quality control and quality assurance. However, most of these analyzes are based on the characterization of 1H liquids or liquid components of heterogeneous materials. Most applications in TDNMR are for the 1H nucleus, mainly due to its natural abundance in several samples.
Recently, Babos[1] carried out quantifications in samples of bovine supplements, in TD-NMR, using 19F nuclei. Others nucleus are not commonly studied in TD-NMR due to their low natural abundance or low sensitivity with the NMR technique. Thus, the objective of this study was to answer whether it is possible to identify and quantify the 7Li nucleus using TD-NMR. The use of TD-NMR in the identification of nuclei like 7Li requires modifications in the spectrometer, mainly in the probe system. As a proof of principle, we used a B0 = 0.5 T spectrometer and a 10 mm diameter probe system. The intensity of the NMR signals shows an excellent correlation with the 7Li contraction. In this study, the NMR signals acquired with the Carr Purcell Meiboom Gil (CPMG) sequence were evaluated as a function of the 7Li concentration in aqueous solutions. We will also show specific and general procedures to obtain a quantitation curve using 7Li TD-NMR.

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Determination of iron oxide content of soils using low-field NMR

https://proceedings.science/p/169681?lang=en

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Iron oxide content in soils is important for soil classification and soil chemistry, as it impacts soils' ability to strongly adsorb phosphate, reducing fertiliser effectiveness.
The traditional method for determining iron oxides in soils is sample digestion with concentrated sulfuric acid and iron determination in the solution; however, this approach is time-consuming (5h for

10 samples), produces roughly 700ml of residue each sample, and employs over 30 chemical reagents.
Because of the inhomogeneity contribution, solid ferri- and ferromagnetic samples degrade the external magnetic field (B0) homogeneity, causing an increase in the observed transverse relaxation rate (1/T2*) of the liquid 1H, even when the sample is not in contact with the test solution.
T1 and T2 are commonly used to determine the concentration of paramagnetic ions in liquids, solids, and living tissues [1-3], however these approaches are time-consuming and/or limited to low iron concentrations and the linear regression for solid samples were poor (R2=0.6).
We suggest using the standard spin-echo sequence in a manganese chloride test solution (1H T1 of 70ms) after inserting a Falcon tube (50ml) with soil samples with known iron oxide concentration (n=12, from 6 to 565g/kg iron oxide). Two soils with high magnetic susceptibility are also tested (they are attracted by regular ferrite magnets).
The obtained echo decays were adjusted by the convolution of a sine function with a Gaussian decay to obtain the T2* and to compensate for small probe mistuning, saving experimental time. The total experimental time was 1.4s, without any sample preparation, that was kept inside the Falcon tube. The regression between 1/T2* and the iron oxide content up to 243g/kg resulted in a R2=0.998. The correlation was maintained, albeit with a lesser inclination, for the two samples with the highest magnetic susceptibility.
[1] https://doi.org/10.1190/1.2399445
[2] https://doi.org/10.1190/1.3386573
[3] https://doi.org/10.1002/cmmi.1610

Development and Characterization of Nanoporous Materials Using Cryoporometry and Nuclear Magnetic Resonance Techniques

https://proceedings.science/p/169641?lang=en

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Time domain NMR (TDNMR) is one the most effective methods for porous media characterization. While pores with dimensions in the range of hundreds of nm to tens of μm can be characterized by various NMR-TD techniques based on relaxation and molecular diffusion, pores with dimensions in the range of tens to hundreds of nm can be characterized using the so-called cryoporometry techniques. In general, cryoporometry explores variations in the melting temperatures of fluids confined in nanoscale spaces, which can be characterized by monitoring the emergence or (disappearance) of the NMR signal from a liquid phase as a function of temperature. This is usually achieved using standard spin echo pulse sequence. We present an alternative approach to perform NMR-cryoporometry experiments using dipolar echoes pulse sequences. By combining a dipolar echo pulse sequence, such as mixed Magic Sandwich Echoes (mixed MSE), with dipolar filters, such as spin echo or Goldman-Shen, the signals from the full, solid + liquid, and the liquid phase can be obtained almost simultaneously. This makes possible to calculated the fraction of the liquid phase at a given temperature directly from the signal intensities, i.e., without the need of an extra experiment for correcting the temperature dependence of the magnetization with temperature, as in the spin echo-based experiments. The approach was tested in controlled pore glasses (CPG) of different pores sizes, using water and terc butanol as probe fluids. In comparison with the experiments performed with standard spin echo, the dipolar echo approach presented some advantages such as faster execution and processing time, easier determination of the temperature range and conditions for avoiding the appearing of supercooled fluid at low temperatures. Additionally, we will show some applications of the approach t to characterize mesopore distribution in materials such porous biopolymers, natural biomass and soils.
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Efficiency of the main solid echo sequences used in 1H time domain NMR for solid characterization solid

https://proceedings.science/p/169649?lang=en

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1H Time Domain NMR (TD-NMR), based on benchtop spectrometers, has diverse applications in industrial and academic fields, as well as in industrial quality control and quality assurance. However, most of these analyzes are based on the characterization of liquids or liquid components of heterogeneous materials.
A major difficulty in TD-NMR experiments to characterize solid materials is the short-time decay of the 1H FID, which occurs in tenths of microseconds (~ 50 microseconds), i.e., in the same order as the dead time of most test heads commercially available NMR probe. This rapid decay is due to the strong homonuclear dipolar interaction between the 1H-1H spins as found in solid materials. For rigid materials it is necessary to use probes with short dead times or use solid echo sequences to intensify the signals from rigid components.
Thus, solid echoes are often used to recover signals from solid components. The aim of this study was to investigate the efficiency of the main echo sequences: Mixed-Magic Sandwich Echoes (mixed-MSE), Rhim and Kessemeier - Radiofrequency Optimized Solid-Echo (RK-ROSE) and Solid-Echo (SE). As a proof of principle, we acquire the polystyrene sample signals with the solid echo sequences at different spectrometer conditions (dead time and hard pulse settings). We will also show specific and general procedures to reliably reach the respective shape parameters (second momentum - M2).

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Monitoring the kinetic of metal ions removal from aqueous solutions by sugarcane bagasse based bio adsorbents using 1H time domain NMR

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Contamination of water by metal ions is a serious environmental problem because it can lead to deleterious effects on the fauna and flora and incur dangerous levels of toxicity for human beings. Many strategies have been developed to produce low cost and efficient materials for adsorption of specific metal ions. One of these materials is a bio adsorbent constituted of succinate pyromellitate units grafted into the surface of cellulose in sugarcane bagasse (SBSPy) [1] to remove Cu(II) and Zn(II) ions from aqueous solutions, in batch and continuous modes.
The characterization of the adsorption kinetics is also an important issue, being usually achieved by taking aliquots of the adsorbent material during the adsorption and quantifying the amount of the metal ion in the solid substrate, using flame atomic absorption spectrophotometry. Nonetheless, there is a lack of methods for characterizing the removal of the metal ion from aqueous solution during the adsorption process.
Thus, the aim of this study was to answer whether it is possible to characterize and monitor the removal of the metal ion from aqueous solutions during the adsorption processes using 1H time domain NMR (1H -TDNMR). As a proof of principle, we probe the Cu(II) adsorption kinetics for SBSPy samples by monitoring the T2 relaxation rate of the water solution, which was found to be proportional to the Cu2+

concentration. Thus, the Cu2+ ions concentration in the aqueous solution could be monitored during the adsorption process, allowing to probe the adsorption kinetics for different initial concentrations of the Cu2+ and temperature. We will also show specific and general procedures to achieve an artifact free absorption curve, which can be used to test adsorption models.
PUB-USP Scholarship, CNPQ (grant 308760/2022-0) and FAPESP
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New applications of low field Nuclear Magnetic Resonance in Soil Science

https://proceedings.science/p/169682?lang=en

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Despite the wide application of low field NMR in different fields, its use in Soil Science is still inconspicuous. To demonstrate opportunities for low field NMR in this area, we will present a few examples of new applications of this powerful and more affordable technique in Soil Science.

Soil plays a vital role in the ecosystem. It provides food, fuels, fibres, medicines, and some essential construction and manufacturing materials. Some examples of how soil renders environmental services are: providing root support, water, and nutrients to plants; supporting the life of a myriad of microorganisms; maintaining water quality by buffering against organic and inorganic contaminants; regulating the discharge of excess rainwater, preventing flooding; and contributing to the equilibrium of carbon stocks as the primary terrestrial surface carbon deposit.
The selected examples will include ordinary CPMG experiments to study metal ion sorption; certification of organic products; fertiliser shelf life, and caking, as well as innovative use of 2D-ILT for kinetic studies using the proper kernel, with the first dimension being the usual T2 and the second dimension being the kinetic one. With this approach, the T2 is correlated to the time constants of the water uptake kinetics.
Furthermore, examples using modern pulse sequences such as Decay due to Diffusion in Internal Field (DDIF) and solid echoes (mixed Magic Sandwich Echo - MSE) will be presented. DDIF experiments were used to determine the pore size distribution of charcoal and soil samples, as well as to estimate the soil water retention curve, saving months of experimental time compared to the conventional method. MSE combined with T1 and T2 filters was used to study, in an unprecedented way, the fertiliser dissolution kinetics in soil samples. This method can be applied for several kinetic studies, from polymer degradation in the environment to controlled release drugs.

Optimization and validation of a rapid method for the determination of oil and oleic acid content in peanut (Arachis hypogaea L.) pre-breeding lines

https://proceedings.science/p/169562?lang=en

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Peanut is an oilseed native to South America that is especially important all over the world. Brazil produced approximately 0.5 million tons in the same harvest.

Oleic acid is an important fatty acid in the composition of peanuts, since the greater its presence in the grain, the greater its durability (shelf life). Furthermore, there is a nutritional advantage of monounsaturated fatty acids. Thus, the aim of this work is the determination of oleic acid and total oil in peanut grains, and the creation of a statistical model by TD-NMR for these parameters.
Soxhlet is the standard method for determining oil content of peanuts (IUPAC). This method presents satisfactory results, but it is destructive, expensive and take a long time to analyze. An alternative to this method is the use of another extraction technique called accelerated solvent extractor (ASE), a fast and efficient extraction process with comparable to Soxhlet.
TD-NMR is an excellent solution for the determination of peanut oil content in relation to the other techniques mentioned. Peanut oil can also be cold extracted from the grain, using a press, to determine the oleic acid content in the grain. An esterification reaction performed in the oil, then an extraction and injection in a GC with an FID. The whole grain was placed in the TD-NMR and analyzed in a CPMG sequence. It was a partial least square regression model (PLS-R), correlating univariate (GC) and multivariate (NMR) data. The accuracy found was adequate for a rapid analysis such as TD-NMR (the highest relative error value is -9.3%), and the model response was good for at least four months.
It is concluded that TD-NMR technique has potential for the measurement of oil content in peanuts, in addition to the specific measurement of oleic acid content in peanuts.

Probing mobility constrains in polymers using 1H double quantum time domain NMR as a method to investigate thermal, chemical or environmental degradation effects.

https://proceedings.science/p/169652?lang=en

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We will discuss the application of 1H double quantum time domain NMR (1H DQTDNMR) to probe distribution of mobility constraints resulting from degradation of polymers. 1H DQTDNMR allows detecting the build-up of double quantum coherences, which can be associated to the density of points with some movement restriction in the sample. Applying this technic at temperatures where a solid phase is absent, the double quantum coherences can be associated to chain crosslinking or entanglement [1]. We will discuss the most common processing procedures, the limitations and advantages of the method considering the different polymer systems, for example elastomers, polymer melts and polymer composites. Finally, we will show examples on how this type of method can help to characterize the degradation process in constructing polymer such as polyamides.

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Study of the Properties of Rubber-Resin Compounds by Solid-State NMR: Effect of The Type of Resin

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Elastomers, produced by vulcanizing polydiene polymers with sulfur and other additives, are extensively used in the tire industry. Resins are often incorporated to enhance the tensile strength, fatigue resistance, and processing of elastomeric products.[1] However, the petroleum-based resins used in the industry are unsustainable and possibly cancer-causing. To address environmental and health issues, biocompatible, non-toxic alternatives, that maintain or enhance the final product's properties, are necessary.[2] Understanding the molecular compatibility and miscibility between new resins and the polymer matrix is crucial, as these factors affect the overall properties of the products. Here, ^{1}H time-domain (TD-NMR) and high-resolution solid-state NMR (SS-NMR) techniques were applied to investigate the dynamics and structural properties of uncured and cured compounds of styrene-butadiene-rubber (SBR) containing three different tackifying resins, two derived from petroleum and one derivative of colophony pine resin. The structural and phase properties of the compounds were investigated by the analysis of ^{13}C SS-NMR spectra and the measure of ^{1}H T_2 relaxation times. Information on the degree of mixing between the resin and SBR components were obtained by ^{1}H T_1 and $T_1\rho$ measurements. Field-cycling NMR relaxometry was used to measure ^{1}H T_1 relaxation times over a wide range of ^{1}H Larmor frequencies and at different temperatures, allowing to investigate dynamics over a wide range of motion time scales. The obtained NMR data were compared with crosslink density and macroscopic properties, which are routinely analysed in industrial settings to understand the effects of formulation and vulcanization conditions on the structure and dynamics of polymer networks.[3]

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Study of the Properties of Rubber-Resin Compounds by Time-Domain NMR: Effect of The Resin Content

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Resins play crucial roles in the tire industry as tackifiers, reinforcing agents, and curing agents. They modify the rheological behaviour of rubber compounds, improving processability and enhancing mechanical properties.[1] The presence of resins alters polymer chain dynamics, impacting the viscoelastic behaviour and the final mechanical properties. To achieve precise product requirements the miscibility between polymers and resins plays a fundamental role.
In this research, we focused on studying the effect of the content of a natural resin on SBR compounds. The resin content was varied from 15 to 45 phr (parts per hundred parts of rubber). To gain insights into the molecular dynamics and structural properties of the elastomeric blends, we employed a combination of ^{1}H Time Domain Nuclear Magnetic Resonance techniques.[2-3] From spin-spin

relaxation times we obtained valuable information on the molecular dynamics of the polymer chains. Additionally, field-cycling NMR relaxometry allowed us to measure the spin-lattice relaxation times across a broad range of ^{1}H Larmor frequencies,[4] from 10 kHz to 35 MHz. The NMR data obtained from these experiments were complemented with a range of other techniques such as rheometric, calorimetric and chemical analyses. These techniques provided a comprehensive understanding of the mechanical, thermal, and vulcanization properties of the elastomeric blends.

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TD-NMR applied to the quality control of fruits of an oleaginous species from the Amazon: Bactris gasipaes

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Pupunha is a fruit from the Amazonian palm tree Bactris gasipaes, present in Amerindian culture. Its pulp contains provitamin A, starch, and oil, with over 50% unsaturated fatty acids, making it valuable for the food, cosmetics, and biodiesel industries. However, the lack of continuous monitoring of vegetable oil quality hinders its commercialization. In this context, green methodologies emerge as viable alternatives, including the analysis of oil and its matrices by TD-NMR. In this study, we aimed to determine the oil content in pupunha pulp using TD-NMR (FID-ECHO sequence), comparing it with the standard Soxhlet extraction method. Additionally, a design optimization experiment (DOE) 2^3 (3x) was conducted to investigate the correlation between pH and spectral profile by TD-NMR (CPMG sequence) after 40 days, under different temperatures, light conditions, and atmospheres. The acquired pupunha fruits (2.5 kg) were cleaned, depulped, and dried in an oven. The oil was extracted using hexane through the Soxhlet method, resulting in a yield of 12.5+/-1.9%. Analytical curves were obtained from the echoes of oil aliquots, interpolating 20 g of dried pulp, revealing an oil content of 13.4+/-0.5%, whose result is statistically equivalent of standard method (T-test, $p < 0.05$). The oil samples were subjected to different temperature conditions (C: -10+/-5 °C, H: 40+/-5 °C), light conditions (D: absence of light, L: presence of light), and atmosphere conditions (N: N2, O: natural atmosphere). Average pH values, CPMG analysis, and 1H NMR of each sample were performed. The CPMG curves were adjusted (biexponential followed by 2nd derivative), and the values of A21, A22, T21, and T22 were obtained. Factorial analysis demonstrated that temperature affected pH, temperature and light conditions affected T21, and temperature, light conditions, and atmosphere affected A22. Therefore, TD-NMR has proven to be a green analytical tool for quantifying and qualifying pupunha oil over time.

The importance of crossover between academy and industry for the advances of TD-NMR and NMR spectroscopy practical applications

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Since the discovery of NMR, many applications have been developed and applied in different industries like food, agriculture, oil and gas, pharmacy, and complex material like polymers among others using time domain NMR and spectroscopy. On this lecture we will discuss some examples from applications developed to solve problems brought from industry to the academy using 1H, 23Na, 31P TD-NMR, 1D and 2D 1H TD-NMR and high field spectroscopy.

The Influence of Alkanes Non-Negligible Interaction with Silica Mesopores on Self-Diffusivity: Insights combining NMR Experiments and First-Principle Calculations

https://proceedings.science/p/169595?lang=en

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Nuclear magnetic resonance (NMR) is a widely employed technique for investigating the dynamics of fluids confined within porous media, enabling the determination of structural and functional properties of complex systems. This versatile method finds applications in diverse fields, including medicine, gas and oil extraction, soil studies, the development of materials for drug delivery, and catalysis, among others. In this presentation, we provide an overview of several applications conducted at the Universidad Nacional de Córdoba in Argentina. Next, we focus on novel findings that combine first-principle calculations with NMR experiments to evaluate the viability and limitations of alkanes as probes for determining tortuosity in polar mesopores. Our first-principle calculations reveal that the interaction between alkanes and the confining silica mesopores is non-negligible, exerting a significant influence on molecular diffusion. Theoretical results are corroborated by NMR experiments. Furthermore, employing NMR two-dimensional relaxation experiments, we establish an empirical threshold for the lower limit of pore diameter. Beyond this threshold, diffusion NMR experimentation may prove inadequate for accurately determining the geometrical tortuosity of porous media.

Using TD-NMR relaxometry to differentiate polymorphism of Active Pharmaceutical Ingredient – a study on anti-helminthic Mebendazole molecule

https://proceedings.science/p/169581?lang=en

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Time domain nuclear magnetic resonance (TD-NMR) plays an important role on material characterization, ensuring quality control on a different type of heterogeneous materials. The spin-lattice (T1) and spin-spin (T2) relaxation times depends on molecular dynamics for samples, in solid or liquid state. One recurrent problem on crystalized materials is the presence of different molecular arrangement, causing the formation of polymorphic materials. It is well acknowledged that different crystallin arrangements tend to present different physical-chemical properties, including stability and solubility. In Active Pharmaceutical Ingredients (API) different polymorphic conformations are intrinsically related with therapeutic efficiency. Therefore, the presence of API polymorphism is a matter of relevance for regulatory pharmaceutical agencies. So far, API polymorphism have been analyzed on TD-NMR in regards of percentage and crystalline structure (eg. solvates, salts, hydrates). Nevertheless, the use of T1 to characterize different polymorphs of the same molecule is sparse. Hence, the present work investigates the relaxometric profile of different polymorphic structures (inactive A and active C forms) of the anti-helminthic Mebendazole (MBZ). The MBZ samples were analyzed on a 0.49 T benchtop Bruker Minispec using inversion recovery pulse sequence. The T1 values were determined by multi exponential fitting and Inverse Laplace Transformation. The results show two T1

values for A form at 1.9 s and 8.4 s and relative area of 23 and 77%, respectively. The T1 values for C form were 1.0s and 6.2 s and relative areas of 23 and 77%, respectively. Therefore, the T1 values determined in benchtop TD-NMR spectrometer can be a low-cost technique for differentiating the polymorphic form A and C of MBZ. Consequently, T1 measured in TD-NMR spectrometers can be a alternative to the X rays diffractometry, that uses ionizing radiation or expensive high resolution solid state NMR spectroscopy.
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Theory/Computation

13C Solid-State NMR associated with multivariate analysis to study degradation of natural organic matter.

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The identification of the organic components involved in the degradation of natural organic matter is crucial for understanding degradation mechanisms. Solid-state 13C nuclear magnetic resonance spectroscopy (ssNMR) has been widely used for analyzing organic compounds, including those related to environmental sensitive systems. However, interpreting 13C NMR spectra can be challenging due to sample complexity, with strong line superposition being a common problem. Thus, the use of multivariate analysis has become almost mandatory, not only to identify organic components, but also to separate samples according to their composition, origin or other physical chemical properties.

Recently, an approach that combines pulse sequence induced T1 spectral modulation with spectral separation based on Multivariate Curve Resolution (PSIM-MCR) was successfully applied to help elucidating the composition of pretreated sugarcane bagasse and constructing polymers[1,2]. The approach explores the sensitivity of solid state NMR to spin interaction and relaxation times, so, in principle, different type of spectral modulation provide by a specific pulse sequences can also be used .

In this presentation an analysis of the spectral separation capability of MCR will be discussed based on the degree of line superposition, line width and signal to noise ratio. The possible extension of the PSIM-MCR approach to decompose solid-state 13C NMR spectra based on properties such C-H coulpling, chemical shift anysotropy, T1rho relaxation times is also considered. The approaches are tested in model samples, such semicrystalline polymer, micro cristalline cellulose and applied to assist the interpretation of solid-state 13C NMR spectra used for characterizing the degradation of organic matter in colonies of fungus growing ants.

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A simple mechanical NMR analog for teaching basic principles of signal detection and processing.

https://proceedings.science/p/169643?lang=en

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The detection and processing of NMR signals are somewhat different from other spectroscopy methods. Thus, it is not uncommon that students in general spectroscopy courses have some misconceptions about these topics. Thus, having an experimental apparatus that can be used in the classroom to explain these concepts may be very welcome.
We developed a compact mechanical oscillator to be used as an NMR analog. It is comprised by a vertical spring with two small magnets, fixed in line 10 cm apart, hanging from a rope. The magnet closest to the spring is inserted into a coil that is part of an RLC circuit powered by a DC source connected to an Arduino controlled electronic switch. A current pulse applied to the coil triggers the oscillation of the magnets-spring system at its natural frequency, emulating the application of RF pulses. The magnet oscillation induces a current in the coil, emulating the acquisition of an FID signal. Because the RLC circuit has a resonance frequency and a transient response the increased detection efficiency when the magnet oscillation occurs on resonance with the RLC circuit (tuning of the NMR probe) as well as the dead time effects can be shown.
The second magnet is inserted into a second coil connected to an electronically controlled potentiometer forming a closed circuit. The magnet oscillation induces a current in the coil, generating an opposing magnetic field, which dampens the magnets-spring system oscillation, emulating the NMR signal decay. Furthermore, controlling the potentiometer resistance, decay shapes observed in NMR systems, such as exponential, stretched exponential and Gaussian, can be emulated. A program written in Phyton controls the systems and also allows basic data processing.
Illustrative examples will be shown, and the system will be available for use during the poster presentation.
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Beyond the Spectrum: Exploring the Hidden Information in NMR Chemical Shifts

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The NMR chemical shift is one the most reliable spectroscopic tools to study molecular electronic structure. The sensitivity of the chemical shift to the chemical environment surrounding the nucleus makes it a coveted molecular signature for structure elucidation.1 However, the potential for deeper insights lies in the combination of NMR with computational chemistry, elevating the chemical shift to a privileged descriptor of reactivity, aromaticity, and molecular interaction strength.2 This presentation will concentrate on unraveling the intricate origin of the chemical shift and gaining a comprehensive understanding of nuclear shielding mechanisms. By delving into these fundamental aspects, I aim to shed light on some substituent effect trends observed in 13C NMR of substituted benzenes3–5 and in charged haloforms6, as well as the paratropicities of p-expanded antiaromatic molecules.7

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How and why do substituents affect 1JCC couplings?

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The molecular complexity of organic compounds (natural or synthetic) leads to spectroscopic challenges in the assignment of their structure. Quaternary carbons and hydrogen-deficient moieties are particularly challenging due to the scarcity of correlations in classical 2D experiments (e.g., HSQC, COSY, HMBC) used for structure elucidation. Structure elucidation of hydrogen-deficient molecules requires use of more complicated and time-consuming experiments, such as 1,n-ADEQUATE and its variations.[1]
Pulse sequences of these experiments contain delays that must be optimized based on the 1JCC (or nJCC) to obtain spectral data suitable for unambiguous interpretation. Wrong delays could lead either to the absence of some 1JCC correlations or to the presence of additional apparent 1JCC correlations that are in fact nJCC. Both scenarios could easily lead to erroneous structural assignments. Understanding relevant 1JCC values is therefore necessary in order to properly set up and acquire experiments of the ADEQUATE family.
Recently developed J-modulated 19F- and 1H-detected dual-optimized inverted 1JCC 1,n-ADEQUATE experiments [2] revealed a significant difference in 1JCC values between 4-(dimethylamino)phthalonitrile and 4-(dimethylamino)-3,5,6-trifluorophthalonitrile. Experimental data indicate that 1JCC coupling is up to 30% larger in the fluorinated molecule compared to its non-fluorinated analog.[2]
Our present work is aimed at developing understanding and rationalizing the impact of fluorine and other typical substituents (i.e., withdrawing or electron-donating groups) on 1JCC coupling constants. Our theoretical investigation suggests that this difference is due to the s character in the CC-bond, which can be rationalized in terms of Bent's rule.
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