

CEUM 2022, Warsaw

Poster abstract book

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1. Electric Field Driven Surface Potential Tunability Probed by Scanning Probe Microscopy: An Effective Way to Tune the Surface Potential of the Polymers

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Surface Potential has prime importance in diverse fields of science, from cell adhesion to contact electrification. Conventionally, the techniques like surface chemical functionalization, ion injection, layer-by-layer deposition, and plasma etching are used to tune the surface potential of a material. However, these are complex multistep processes that need sophisticated techniques; moreover, long-time surface potential retentivity is also one of the challenging tasks here. We have proposed an effective single-step method to tune the surface potential of material by just reversing the direction of the electric field in an electrospinning setup, a nano/micro-fiber production unit where in situ electrical poling is happening during the production of the fibers, thus provides the high-stability in long-time retentivity of the surface potential. An atomic force microscopy-based non-contact scanning kelvin probe microscopy has been utilized to investigate the surface potential. Furthermore, we have observed that our proposed method is implementable to all the polymers, which are subject to producing the fibers in the electrospinning unit.

2. Cross-correlated spin relaxation in NMR studies of proteins

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Intrinsically disordered proteins (IDPs) are highly dynamic proteins which play key roles in signaling and regulation of biological processes. Importantly, IDPs are not random coils, but transiently adopt certain structural motifs, related to their functions.[1] Thus their residual structure is worth studying. Nuclear magnetic resonance (NMR) is the only method which provides such information at atomic scale. Nonetheless, high dynamics leads to fast changes of the chemical environments of nuclei, which leads to averaging of chemical shifts, hampering the spectral analysis. The solution to this problem is using methods of high dimensionality, 4D and 5D, which reduces spectral crowding.[2]

One of the structural parameters that can be determined with NMR is dihedral angles distribution of the protein's backbone. For this we can use cross-correlated relaxation (CCR) rates. They are determined separately for individual amino acid residues and are calculated using the peak intensities.[3] Several types of CCR constants are required to determine the preferred conformation of individual residues.

We have developed seven 4D experiments to measure different CCR rates and successfully validated them on a well-known protein, ubiquitin (PDB code 1d3z). Right now, we are conducting the measurements of an IDP, osteopontin.

Acknowledgement:

This work was supported by a grant from the Polish National Science Centre (MAESTRO, 2015/18/A/ST4/00270).

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3. Improvement of cancer contrast in MRI using double action nanoparticles in the mouse animal model

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Magnetic Resonance Imaging (MRI) has been used for early cancer detection and treatment monitoring due to its excellent soft tissue contrast, yet in clinical settings contrast agents are used for cancer diagnosis. While MRI contrast may be provided solely by tissues themselves, due to differences in their relaxation times, contrast agents shortening T₁ and T₂ relaxation times further improve detection of small pathologies such as early stages breast cancers [1,2].

To improve the tumor contrast we have developed core/shell NaDyF₄/NaGdF₄ nanoparticles changing both T₁ and T₂ relaxation times of surrounding water molecules and conjugated them with tumor specific antibodies and proteins. The relaxation times (T₁ and T₂) of the nanoparticles with various core/shell sizes and concentrations were measured at 9.4T and 3T to find the optimum T₁/T₂ ratio for MRI. T₁- and T₂-weighted images using core/shell nanoparticles of the animal models of brain, breast and prostate cancer were collected and combined to provide enhanced contrast and edges. The contrast agents consisting of the core/shell nanoparticles with the optimal core and shell sizes are being developed to provide improved tumor contrast when the T₁ and T₂-weighted MR pulse sequences are applied.

Acknowledgments:

This work was funded by the National Science Center, Poland. Grant numbers: Harmonia no. 2018/30/M/NZ5/00844

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4. NMR spectroscopy – a versatile tool for GPx mimetic studies

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In recent years an increased interest was given to organoselenium compounds containing organic groups with pendant arms capable for N → Se intramolecular interactions, due to their increased stability and improved properties such as enzymatic mimics, e.g. for Glutathione peroxidase (GPx). [1] As a continuation of our previous studies on diorganoselenium(II) compounds containing organic groups with pendant arms and/or pyrazole moieties,[2-4] we employed UV-Vis spectroscopy to investigate their antioxidant behavior via the Tomoda method.[5,6] In order to investigate the reaction mechanism of the compounds in the considered system (reaction between tiophenol and hydrogen peroxide), we employed multinuclear NMR spectroscopy. Given that the chemical shift in the ⁷⁷Se NMR spectra has specific values for certain classes of compounds, ⁷⁷Se NMR spectroscopy is an useful tool for the characterization of organoselenium compounds.[7] The obtained results show that the reactions occur via selenoxide intermediates which are stabilized by intramolecular N → Se interactions and such a behavior was sustained by DFT calculations as well.

Acknowledgements:

Financial support of UEFISCDI project no. PN-III-P4-ID-PCE-2020-1028 is highly acknowledged.

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5. NMR sugar profile of honey from endangered bee species

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Bees are winged insects, known for their role in pollination. Over 20000 bee species are known in the world, only less than 10 are honey bees. Most common honey bee is *Apis mellifera*, which is mostly found in Europe, Africa, and Asia. Numerous bee species found in tropical and subtropical regions, primarily in South America, Africa, and Australia, are the stingless bees. While the honey bees belonging to the genus *Apis* comprise ~ 44 recognized subspecies, more than 550 species of stingless bees are e.g. included in tribe Meliponini. All share the ability to make honey and wax combs. A lot of information about specific behavior and characteristics is well known, however, the sugar composition of honeys produced by diverse genus and tribes is only poorly studied so far.

Honeys from four different *Apis mellifera* subspecies (*A. m. ligustica* – from Italy, *A. m. mellifera* – from Germany, *A. m. ruttneri* – from Malta, *A. m. rodopica* – from Bulgaria) and from two stingless bee species (*Meliponula ferruginea* – from Tanzania and *Scaptotrigona Mexicana* – from Mexico), were analysed using NMR spectroscopy. Their saccharide profile was determined by ¹³C spectra and compared using principle component and cluster analysis. Both methods show high potential to distinguish honeys from various bee species based on distinctive differences in the sugar compositions thus providing additional opportunities for consumer inclination to correct designation of natural food products.

Acknowledgements:

This research was funded by the Project BG05M2OP001-1.002-0012-C01: Centre of Competence „Sustainable utilization of bio-resources and waste from healing and aromatic plants for innovative bioactive products“, financed by Operational program „Science and education for smart growth“, co-financed by the European Union through the European structural and investment funds.

6. Investigating the miscibility of thermodynamically immiscible polymers by solid-state NMR

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The phase mixing efficiency of ABS and PET before and after SPD was investigated by ^1H spin-lattice relaxation time measurements both in the laboratory and in rotating frames (T_1 , $T_{1\rho}$) using solid-state ^{13}C cross-polarization magic-angle spinning NMR experiments. NMR data were obtained using the Bruker AVANCE III WB 400 NMR spectrometer.

It is well known that each phase in immiscible polymer blend with phase separation has a unique characteristic ^1H relaxation time. The relaxation time is almost the same for each polymer phase in miscible polymer blends that exhibit nanoscale phase mixing. According to the two polymer components, the original ABS/PET mixture displays two different ^1H spin-lattice relaxation times in the laboratory frame (T_1). On the other hand, the T_1 values for the ABS and PET phases in the ABS/PET blend after severe plastic deformation processing are rather close. This could mean that the polymer mixing takes place within the interphase at the nanoscale, or in a range of tens of nanometers.

Acknowledgments:

This research was funded in part by National Science Centre (Poland) under the grant 2021/43/B/ST8/01443.

7. Simultaneous Reversible and Irreversible Parahydrogen NMR Signal Enhancement

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Although Magnetic resonance (MR) is widely employed as a powerful non-invasive research tool, it suffers from poor sensitivity, limiting further MR applications.[1,2] Hyperpolarization (HP) techniques can significantly enhance the MR signal.[3] The most prospective HP methods are irreversible hydrogenative Parahydrogen Induced Polarization (hPHIP) and Signal Amplification by Reversible Exchange (SABRE).[4] These two methods are complementary to each other, as SABRE can hyperpolarize some molecules which hPHIP cannot, and vice versa. The methods are based on the same source of hyperpolarization -parahydrogen. In this context, the general question is how to combine SABRE and hPHIP, what will happen when they are combined and how the interplay between them can be manipulated. Therefore, we have investigated the issue of the combination of hPHIP and SABRE.[5]

As a molecule for SABRE-hPHIP 3-ethynylpyridine was chosen because it contains two fragments: pyridine and acetylene units. Pyridine unit can undergo SABRE, while the triple bond can be hydrogenated -thus, hPHIP is possible. As a catalyst, [Ir(COD)(IMes)Cl] (where IMes is the N-heterocyclic carbene ligand = 1,3-bis(2,4,6-trimethyl-phenyl)imidazol-2-ylidene and COD = 1,5-cyclooctadiene), was used as this catalyst is used for SABRE[6] and can also facilitate hydrogenation[7].

In our experiments, we observed simultaneous SABRE and hPHIP. As time progressed, both effects were step-by-step suppressed. The possible factors which caused the suppression of hPHIP and SABRE were discussed. In particular, the possible scenarios of the deactivation of the catalysts were presented. We have also observed that the reaction mechanism of simultaneous SABRE and hPHIP hyperpolarization could be modulated by the initial activation of the catalyst with a co-ligand. In particular, the hPHIP effect can be suppressed entirely, but stable and efficient SABRE in return was observed.

To conclude, hPHIP-SABRE is possible, and the interplay between hPHIP and SABRE can be modulated. However, further research is required to understand hPHIP-SABRE more comprehensively, which can facilitate the utilization of hPHIP-SABRE in medical diagnostics and catalysis.

Acknowledgments:

This work was financially supported by the National Science Centre in Poland (OPUS 11 2016/21/B/ST4/02162, SONATA-2011/03/D/ST4/02345).

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8. Solution behavior and solid state structure of new di- and triorganolead(IV) complexes with tetraphenyldichalcogenoimidodiphosphinato ligands

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Tetraorganodichalcogenoimidodiphosphinato ligands exhibit a large variety of coordination patterns,[1] but only a few studies on the synthesis and structural characterization of organolead(IV) complexes with such ligands were reported so far.[2-4] Single-crystal X-ray diffraction studies on dimethyl- and diphenyllead(IV) derivatives with imidodiphosphinato ligands revealed an O,O-bidentate coordination to lead,[3,4] thus leading to six-membered inorganic chelate rings. In order to investigate the structure of organolead(IV) complexes and the coordination behavior of this type of ligands, new di- and triorganolead(IV) compounds with tetraphenyldichalcogenoimidodiphosphinato ligands were synthesized. The new compounds were characterized in solution by multinuclear NMR spectroscopy and for several of them the solid state structure was determined by single crystal X-ray diffraction. For selected compounds the dynamic behavior in solution was investigated by variable temperature NMR experiments. The decomposition process of the triorganolead(IV) derivatives was studied by time-related NMR experiments.

Acknowledgements:

This work was supported by the Romanian Ministry of Education and Research (PN-III-P4-ID-PCCF-2016-0088).

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9. Linezolid co-crystals structure elucidation by joint use of high-resolution solid state NMR and crystal structure prediction (CSP) calculations

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Mechanococrystallization of linezolid, a wide-range antibiotic, results micro-crystalline powders, in some of them makes impossible the application of single crystal X-Ray diffraction in structural elucidation.[1,2] We expect a joint use of high-resolution solid-state NMR and crystal structure prediction (CSP) calculations can be successful. However, linezolid has conformational freedom by Having four rotatable bonds, some of which can assume many discreet values poses a challenge in establishing its conformation in a solid phase also the CSP-NMR protocol meets serious obstacles, including ambiguities in NMR signal assignment.[2,3] We demonstrate a possible way of avoiding these obstacles and making as much use of conformations of LIN inside the crystal. CSP calculations yield model crystal structures examined against experimental solid-state NMR data, leading to a reliable identification of the most probable molecular arrangement. Our work led to the crystal structure elucidation of a co-crystal of linezolid (LIN) with 2,3-dihydroxybenzoic acid, however, the challenging case of a co-crystal of LIN with 2,4-dihydroxybenzoic acid led to the identification of the most probable conformations of LIN inside the crystal.[3]

Acknowledgements:

This work was financially supported by Polish National Science Centre under Sonata 14 grant No. UMO-2018/31/D/ST4/01995. The Polish Infrastructure for Supporting Computational Science in the European Research Space (PL-GRID) is gratefully acknowledged for providing computational resources. A purchase of a Panalytical powder X-Ray diffractometer used to obtain results included in this publication was supported by funds from the EU Regional Operational Program of the Lodz Region, RPLD.01.01.00-10-0008/18.

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10. dGAE (297-391) tau filaments seen by solid-state NMR

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Tauopathies are the most troublesome of all age-related chronic conditions, as the development of therapies has an extremely low success rate compared to other chronic diseases. Tau protein is a common molecular denominator of tauopathies, which is found as insoluble deposits in specific brain parts. Pathological tau is a promising molecular target to tackle. However, the field still lacks a deeper understanding of the structural details of tau filaments. Recently, it has been shown that truncated tau construct dGAE (297-391) can form filaments that closely resemble paired helical filaments derived from Alzheimer's disease brain tissue (Al-Hilaly et al., 2017; Al-Hilaly et al., 2019; Lovestam et al., 2022).

Here, we aim to determine the global fold dGAE tau filaments using solid-state NMR. Moreover, we aim to investigate whether chemical shifts of dGAE and seeded by dGAE full-length tau filaments differ.

Double-labeled proteins were expressed in *E. coli* and purified using a combination of CEX, IMAC, and SEC. A set of 2D and 3D solid-state experiments was recorded to assign secondary structures and investigate the global fold of filaments. The macroscopic features of the tau filaments were also examined by AFM and negative stain EM and complimented with various biophysical experiments.

11. The assessment of the distribution of drug-loaded polyelectrolyte nanocarriers by the ^1H or ^{19}F Magnetic Resonance Imaging

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The main disadvantages of chemotherapy (or more generally pharmaceutical therapies) include toxic side effects and the development of resistance to the chemical agents, which is related to the non-specific targeting of therapeutic agents. Although novel approaches are constantly proposed, there are still many obstacles to overcome. Among drug delivery systems, polymeric nanocarriers for passive targeting based on the EPR effect are intensively investigated. Modification of such nanocarriers with imaging agents introduces the possibility of the observation of drugs kinetics and the assessment of the efficiency of the therapeutics transport to the site of action. Among various available imaging modalities, Magnetic Resonance Imaging (MRI) has become a powerful tool for non-invasive imaging due to its high spatial resolution and its inherent safety. The present contribution aimed to investigate the possibility of detection of new theranostic polyelectrolyte nanocarriers by standard, proton magnetic resonance imaging, as well as ^{19}F MRI.

Various types of nanocarriers were formed via the layer-by-layer technique, i.e. by the deposition of ionic and cationic polyelectrolyte shell layers on nanoemulsion drops. MRI contrast agents were either embedded in their shells or encapsulated in cores. In particular, small particles of iron oxide (Fe_3O_4) as were used negative type; Gadolinium ions as positive type, and NafionTM polymer or fluorouracil (FU) as hot-spot type of MRI contrast agents. Both the ^1H and ^{19}F MR spectroscopy/relaxation measurements, as well as ^1H and ^{19}F imaging, were performed at the 9.4 T BioSpec 94/20 preclinical MRI scanner (Bruker BioSpin, Germany), equipped with BGA60-S gradient coils and 35 mm birdcage RF coil, controlled by Paravision 5.1 software. Additionally, a small transmit-receive ribbon solenoid RF coil (ID of 14 mm), which can be tuned either to ^1H or ^{19}F resonant frequency was used for ^{19}F experiments.

The presented results prove the distribution of drug-loaded polyelectrolyte nanocarriers can be reliably assessed by the ^1H or ^{19}F MRI. Due to excellent contrasting properties, proved by high values of molar relaxivities r_1 and r_2 , polyelectrolyte nanocapsules with contrast agents of relaxation type (Fe_3O_4 and Gd) should be suitable for the use in standard clinical MRI setting. However, due to close to zero natural background, the use of ^{19}F MRI as the detection method presents an interesting alternative to classic ^1H -MRI with relaxation-based contrast agents by providing excellent contrast and enabling straightforward quantification of the drug delivery. Regardless of the unfavourable characteristic of NafionTM for the MR imaging, i.e. multiple resonances in MR spectrum and very short T_2 time, it was possible to reliably visualize nanocarriers' distribution in the sample. As the fluorouracil has an NMR spectrum with only one resonance line and relatively long T_2 relaxation, better SNR was achieved within the same acquisition time. This allowed us to shorten the acquisition time to as low as 4 minutes while keeping SNR high enough. From the perspective of the potential preclinical and clinical applications, this suggests the possibility of in-vivo tracking at a 5-FU therapeutic dose.

Acknowledgements:

This work was partially financed from the Norwegian Financial Mechanism, GRIEG Project 2019/34/H/ST5/00578

12. Harvest time affects antioxidant capacity, total polyphenol and flavonoid content of Polish St John's wort's (*Hypericum perforatum* L.) flowers

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The polyphenol content and antioxidant capacity of hyperforin and hypericin-standardized *H. perforatum* L. extracts may vary due to the harvest time. In this work, ethanol and ethanol–water extracts of air-dried and lyophilized flowers of *H. perforatum* L., collected throughout a vegetation season in central Poland, were studied. Air-dried flowers extracts had higher polyphenol (371 mg GAE/g) and flavonoid (160 mg CAE/g) content, DPPH radical scavenging (1672 mg DPPH/g), ORAC (5214 $\mu\text{mol TE/g}$) and FRAP (2.54 mmol Fe^{2+}/g) than lyophilized flowers extracts (238 mg GAE/g, 107 mg CAE/g, 1287 mg DPPH/g, 3313 $\mu\text{mol TE/g}$ and 0.31 mmol Fe^{2+}/g , respectively). Principal component analysis showed that the collection date influenced the flavonoid and polyphenol contents and FRAP of ethanol extracts, and DPPH and ORAC values of ethanol–water extracts. The ethanol extracts with the highest polyphenol and flavonoid content protected human erythrocytes against bisphenol A-induced damage. Both high field and benchtop NMR spectra of selected extracts revealed differences in composition caused by extraction solvent and raw material collection date. Moreover, we have shown that benchtop NMR can be used to detect the compositional variation of extracts if the assignment of signals is done previously

13. Solid state NMR investigation of CO₂ adsorption on NaX zeolite

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The momentum-gaining drive towards sustainable green society requires a dramatic reduction in the emission of industrial and household gases, such as CO₂. Various methods and materials have been considered and are under development, for CO₂ capture and storage. Faujasite type zeolites (FAU) have been considered as one of the most promising microporous materials for CO₂ capture, due to their relatively large capacity, durability and mainly – high CO₂ binding energies in excess of 40-50 kJ/mol. The heat of adsorption become reduced by factors of, e.g. 2- 3, as the captured CO₂ amount in the zeolite is increased, thus limiting the overall useful capacity of the material under realistic operational conditions. It is therefore of high practical and fundamental interest to elucidate the particular coordination environments responsible for the high binding energy. In the present study we used solid state NMR to investigate the microscopic aspects of ¹³C CO₂ adsorption in pure FAU microcrystalline phase NaX zeolite, prepared from refined natural kaolin by own-synthesis procedure. Samples containing different ¹³C CO₂ amounts were prepared exposing the zeolite to different equilibrium ¹³C CO₂ pressures, spanning wide range of the material adsorption capacity, at room temperatures. Two types of NMR experiments were performed to determine the nature of the adsorbed CO₂ - chemisorbed or physically adsorbed. The 1H- ¹³C CPMAS NMR experiments lead to a selective increase of the signal of chemisorbed ¹³C CO₂ due to possibility for transfer of magnetization from neighboring protons of the zeolite matrix as well as due to its immobilization upon its coordination to Na⁺ ions. These experiments are not suitable for registration of physically adsorbed ¹³C CO₂, since the CP transfer is inefficient due to its higher mobility. To detect the presence of physisorbed ¹³C CO₂, ¹³C spectra with high power proton decoupling were measured. The results show that at low CO₂ pressure (24 mbar) only one type of chemisorbed CO₂ species are present and the quantitative ratio of chemi- to physisorbed CO₂ species is approximately 1:1. At higher pressures the complexity of the chemisorbed CO₂ resonance increases indicating additional binding of the CO₂ molecules to Na⁺ ions located at different positions in the zeolite matrix. The ²³Na spectra demonstrate complex spectral pattern due to the presence of at least 4 different nonequivalent Na sites in the zeolite. Comparison of the line shapes of ²³Na spectra of pure NaX and samples with different CO₂ pressure further suggests the possibility for different binding modes between CO₂ and Na⁺ ions upon increase of the CO₂ loading. The ²⁹Si and ²⁷Al NMR spectra of the studied samples show only minor differences indicating that the zeolite structure is not influenced by the CO₂ loading.

Acknowledgment:

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme E+: Low Carbon Energy for the Transport and Households, grant agreement D01-214/2018 and INFRAMAT Project (Research equipment of Distributed Research Infrastructure), part of Bulgarian National Roadmap for Research Infrastructures.

14. ^{13}C CP MAS NMR spectroscopy in fake drug analysis

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Counterfeit medicines are a growing worldwide problem. According to Global Surveillance and Monitoring System and the World Health Organization (WHO) it is estimated that about 10% of all medical products used in low- and middle-income countries are substandard and falsified (WHO, 2017), but in the last decade this problem has been growing and now affects also highly developed countries. There are several groups of pharmaceutical products that are most commonly falsified. In the case of highly developed countries, preparations for erectile dysfunction are among the most frequently counterfeited medicinal products which, thanks to illegal distribution, could be easily available without the need for medical consultation. From a pharmaceutical point of view, counterfeit drugs pose a health risk due to the possible content of API different from the declared one, differences in the kinetics of drug release, as well as the possible presence of toxic substances. Therefore, development of innovative analytical approaches for falsified drug identification is essential.

The main aim of the study was to assess the possibility of using ^{13}C CP MAS NMR spectroscopy to identify falsified drugs. In our study we showed that ^{13}C CPMAS NMR spectroscopy allows for easy and unambiguous identification of counterfeit products. However, the practical applicability of this technique is limited due to the limited availability of equipment and the cost of measurement. Therefore, in a further stage of the work, the ^{13}C CP MAS NMR spectrum pattern analysis allowed for the identification of structural differences which enabled the development of simple dyeing techniques making the identification of illegal products possible.

15. Linear discriminant analysis reveals hidden patterns in NMR chemical shifts of intrinsically disordered proteins

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Nuclear magnetic resonance spectroscopy (NMR) is the key method to study intrinsically disordered proteins (IDPs). The standard assignment of signals in protein NMR spectra is based on analyzing a set of results from three-dimensional experiments, which provide information on the sequential linkage (correlation) of chemical shifts of individual nuclei, e.g., 3D HNCACB. Yet, even the first step of such analysis, i.e., assignment of observed resonances to particular nuclei, is often very difficult due to low peak dispersion in the spectra of IDPs.

We propose using the advanced statistical method in the assignment process, which can be effectively supported by finding 'hidden' chemical shift patterns, specific for the amino acid residue types. The patterns are found in the training data from Biological Magnetic Resonance Bank (BMRB) using linear discriminant analysis (LDA) and then used to classify spin systems in a fresh alfa-synuclein sample. We show that a procedure can greatly support the analysis of NMR spectra.

16. Solid state NMR characterization of novel polymer/calcium phosphate hybrid materials for biomimetic remineralization of tooth enamel

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Development of new materials for biomimetic remineralization of tooth enamel is a topic of great importance in the dental materials research as a new approach to the prevention, restoration and treatment of defective enamel. Recent studies demonstrated that polymers of appropriate functionality can play a role in controlling mineralization reactions and lead to the desired calcium phosphate phase or mixture of phases.

In the present study novel Polymer/Calcium Phosphate (P/CaP) hybrid materials were synthesized and characterized by solid state NMR spectroscopy. The hybrid P/CaP systems were obtained by in situ precipitation of CaP in a solution of the polymers under different reaction conditions. For comparison pure CaP synthesized under identical conditions as for the preparation of hybrid systems were also investigated. ¹H, single pulse ³¹P and ¹H@³¹P cross-polarization (CP) MAS solid state NMR spectra provided insight to the structural characteristics and chemical composition of calcium phosphate phase formed in studied materials, as a function of sample preparation procedure and synthesis conditions. The NMR data revealed that the pure CaP phase formed under controlled conditions (pH, temperature, maturation time) represent a mixture of crystalline and poorly crystalline (nanocrystalline) hydroxyapatite (HAP). The ³¹P NMR spectrum of CaP phase formed in the absence of polymer under identical conditions as for the synthesis of the hybrid materials, however, represented a complex pattern indicating the presence of mixture of orthophosphates and hydrogen phosphate species. The NMR spectra of the hybrid polymer/CaP sample obtained by in situ precipitation in a solution of polymer demonstrate the formation of amorphous calcium phosphate, clearly evidencing the role of the polymer on the composition and structural properties of the CaP phase.

Acknowledgments:

The financial support of the National Science Fund under project KP-06-H49-6/2020 is gratefully acknowledged. Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation.

17. Analysis of the composition of biodiesel obtained from the esterification of rapeseed oil and goose fat

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To reduce emissions, various biofuels are used as an additive to diesel fuel supplied to internal combustion engines, both automotive and stationary. The energetic properties of biofuels are strongly dependent on their chemical composition identified using various methods, including NMR spectroscopy, chromatography, and others. The present work utilized the ¹H NMR and the ¹³C NMR spectroscopy using the Bruker Avance apparatus and the gas chromatography using a Hewlett Packard series 5890 chromatograph (Agilent Technologies) coupled with a Hewlett Packard series 5972 mass spectrometer. Two biodiesel samples were tested: the first one in the form of popular methyl ester of rapeseed oil and the other in the form of methyl ester of goose fat obtained via transesterification process for the first time in the world at the Lodz University of Technology. The obtained results showed that the methyl ester of goose fat showed the presence of a higher amount of saturated hydrocarbons than in the case of the methyl ester of rapeseed oil. Although the NMR spectrography turned out to be less accurate than gas chromatography, it also allowed specifying the basic components contained in both tested biodiesel samples.

18. ^1H NMR in verification of hemp-seed oil quality

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Currently, there is a growing interest in the health-promoting effects of vegetable oils, used both in the food and pharmaceutical industries. Consumers pay attention to the high quality of the consumed oil, hence there is a constant search for methods enabling quick and routine quality control of vegetable oils. Hemp-seed oil, the main food product obtained from hemp, is most frequently produced by cold pressing of seeds. Hemp-seed oil exhibits health benefits due to its high content of essential fatty acids. It is composed in approximately 80% of polyunsaturated fatty acids (PUFA), mainly of ω -6 linoleic acid (LA) and ω -3 alpha-linolenic acid (ALA). The LA:ALA ratio is ca. 3:1, as it is recommended in healthy diet.

Six samples of cold pressed hemp-seed oils were studied by nuclear magnetic resonance (NMR) and Fourier-transformed infrared spectroscopy (FT-IR). These methods were applied to evaluate the ratio of ω -6 to ω -3 fatty acids and to control the quality of hemp oils. FT-IR method allowed us to distinguish three oils with the highest level of γ -linolenic acid. Additionally, NMR spectroscopy was applied to determine the ω -6 to ω -3 ratio. The outcomes of the NMR experiment are in agreement with GC outcomes measures. The results indicate that NMR and FT-IR may be used in routine evaluation of hemp-seed oil quality as a fast and reliable method of verification of ω -6 to ω -3 ratio and origin of the oil.

19. Structure and Conformational Mobility of 1,3,5-Triazine Derivatives for OLED Applications

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Series of compounds consisting of 1,3,5-triazine core linked to various aromatic arms by amino group were synthesized and characterized. In solution the studied compounds exist as a mixture of two conformers: symmetric propeller and asymmetric conformer, in which the one aromatic arm is rotated around C-N bond. At temperatures below -60°C the VT NMR spectra in DMF-d₇ are in slow exchange regime and the signals of two conformers were elucidated. At temperatures above 100°C the VT NMR spectra in DMSO-d₆ are in fast exchange regime and the averaged spectra were measured. The populations of propeller and asymmetric conformers in DMF-d₇ vary from 14:86 to 50:50 depending from the substituents. The rotational barriers of propeller and asymmetric conformers in DMF-d₇ were measured for all compounds and are in the interval from 11.7 to 14.7 kcal/mol. The DFT calculations of structures of studied compounds either in ground states (conformers) and in transition states (the rotation around the C-N bonds) predict energy differences, which are in good agreement with the experimental rotational barriers. The DFT calculations reveal that the observed chemical exchange occurs by the rotation around the C(1,3,5-triazine)-N bond. The absorption and emission spectra of studied compounds were measured in acetonitrile, dichloromethane and methanol.

Acknowledgments:

The financial support by the Bulgarian Science Fund (UNA-17/2005, DRNF-02/13, KII-06-H29/6) is gratefully acknowledged. Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation.

20. DNA construct optimization for the NMR structural studies of the 8-17 DNAzyme

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DNAzymes are synthetic enzymes based on DNA, able to catalyze several chemical reactions – especially when substrates are nucleic acids. Thanks to their resistance to degradation, DNAzymes show promise for future use in biotechnology and medicine, as biosensors or therapeutics, e.g. for gene silencing. Unfortunately, their mechanisms of catalytic action has not yet been fully understood, hindering their practical use. The 8-17 DNAzyme, which is the main focus of our research, is able to catalyze the cleavage of RNA strands at precisely defined points in the sequence. It is a DNA metalloenzyme active in the presence of divalent metal ion cofactors such as Pb^{2+} , Zn^{2+} or Mg^{2+} . Surprisingly, the research into 8-17 structure using low resolution techniques, such as CD spectroscopy and FRET, revealed that it appears to possess two very distinct active conformations depending on the metal cofactor present¹. Namely, while Pb^{2+} appears to bind directly to the apo-enzyme, interaction with other activating ions is accompanied by a large scale structural change, towards a more compact fold. When the crystal structures of the apo- and Pb^{2+} -bound forms of 8-17 DNAzyme were finally obtained, they turned out to indeed be identical, while displaying an unexpectedly intricate network of tertiary interactions². However, the second catalytically active conformation of the DNAzyme – induced by cofactors such as Zn^{2+} or Mg^{2+} – remains elusive to crystallization.

In our research we set out to study the folding of 8-17 DNAzyme in the presence of Zn^{2+} using biomolecular NMR methods in solution. Given the large size of 8-17 DNAzyme constructs usually employed and molecular weight limitations of NMR, a careful optimization of the 8-17 DNAzyme construct to be studied turned out to be a necessary prerequisite for any such investigations. Through a process of gradual truncation and sequence-optimization of the DNAzyme's target-binding arms we were able to arrive at a variant composed only 35 nucleotides (with the classic 8-17 constructs counting >65 nt), without affecting its enzymatic capabilities or metal binding, as assessed by gel electrophoresis and CD spectroscopy. The shortened construct turned out adopt a single NMR spectral form in the presence of Zn^{2+} with sharp lines allowing for resonance assignments and distance restraint derivation without isotope enrichment. Restrained MD simulations are now ongoing of elucidate the elusive Zn^{2+} -bound form of the 8-17 DNAzyme.

Acknowledgements:

This work has been supported by the National Science Center (Poland) under grant agreement: [UMO-2018/31/D/ST4/01467] to W.A.. The calculations were performed at Poznań Supercomputing and Networking Center.

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21. Topological preferences of a systematic set of model DNA G-quadruplexes studied by NMR and low resolution techniques

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The principles underlying the folding of G-quadruplexes remain unexplained thus hindering the analysis of biological processes involving them, as well as, efforts toward their application in nanotechnology. The topological architecture of G4 structures depends on many factors and has not been fully understood [1]. The aim of our research is to understand the relationship between the G4 structures and their nucleotide sequences in order to allow for the design of G-quadruplexes with desired conformations. In order to study the relationship between loop length and conformational preferences we have systematically investigated a set of all 64 two-tetrads G4 containing thymidine-only loops of 1-4 length (GGT1-4GGT1-4GGT1-4GG). We applied a combination of biophysical tools (NMR, CD and UV-VIS spectroscopies) to characterize the G4 folds and native gel electrophoresis to determine the number of structures formed in each case. All 64 sequences form G-quadruplexes both in Na⁺ and K⁺ buffers, although their stabilities vary significantly. For sequences adopting a single form structural studies are ongoing.

22. The usage of chemometric methods and NMR spectroscopy in examination of dietary supplements containing Hawthorn fruit (Crataegus)

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The work aimed to investigate the efficacy of a combination of low-field NMR spectroscopy with chemometric analysis to identify and differentiate hawthorn species occurring in preparations containing hawthorn fruit or its extract, available on the Polish market.

¹H NMR spectra of extracts from 14 preparations available on the Polish market containing hawthorn fruit were recorded using a benchtop NMR spectrometer (resonance frequency: 43 MHz). Despite the dominance in the spectra of signals from the solvent the spectra of different preparations showed visible variation. However, classical quantitative analysis with direct use of the spectra could not be performed in all cases due to the lack of visible signals in the phenolic range. PCA analysis established that it was possible to differentiate samples using the phenolic range (6-8 ppm) even in cases where the intensity of signals from the active components of hawthorn was low, while it was not possible to use other spectral ranges to reliably differentiate samples. However, also differentiation using only the polyphenol range did not allow for the classification of samples according to the species of hawthorn used in a given preparation.