ABSTRACTS (LECTURES)

NMR POWDER CRYSTALLOGRAPHY

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We will discuss various aspects of progress towards powder crystallography by NMR. Using high-resolution proton spectroscopy of powders, we present the refinement of the three-dimensional structure of organic compounds, at natural isotopic abundance, obtained by an approach that in the first step combines molecular modeling (MM) in the Xplor-NIH program with experimental proton spin-diffusion data (PSD) obtained from high-resolution solid-state NMR of protons. This approach enables us to refine the molecular structure of b-Asp-Ala at natural abundance and in powder form to obtain a group of structures with an average rmsd of 0.1Å, and which deviates from the known structure by only ~0.6Å. Additionally, the conformation of thymol in its crystalline arrangement is investigated following the same MM-PSD optimization scheme. Thymol is a monoterpene phenol found in oil of thyme, with strong antiseptic properties. Due to the ease of obtaining large crystals, it was among the first systems studied by crystallographers, even before the advent of X-Ray methods. We then show how the PSD-MM structures can be used as a starting point for further refinement based on plane wave DFT geometry optimization and chemical shift calculations. This procedure results in structures that are identical to the known X-ray structure to within <0.2Å, and its validity is confirmed by comparing the DFT calculated chemical shifts for ^{1}H and ^{13}C with the experimental shifts. We observe a substantial improvement in the agreement between the calculations and experiments after DFT structure optimization. We will also invoke aspects of small molecule crystallography involving paramagnetic metal centers as reference points for determination of structures in organometallic coordination complexes.

CRYOGENIC INVESTIGATION OF MOLECULAR HYDROGEN IN FULLERENES BY MEANS OF NMR, IR AND INS

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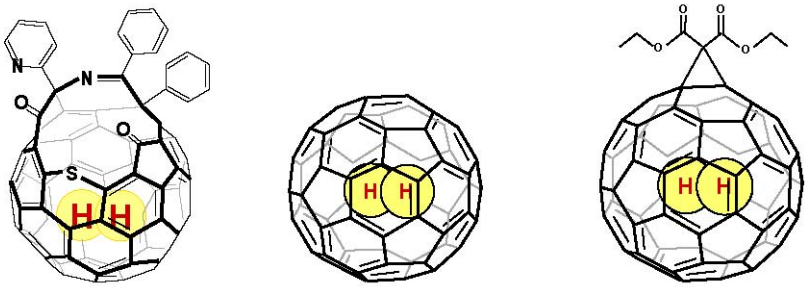
We present our recent work on remarkable molecular complexes, containing endohedral hydrogen molecules in fullerenes:



These systems are molecular scale examples of the particle-in-a-box quantum mechanical problem. The size and the symmetry of the cage are non-trivial parameters and deeply affect the quantum dynamics of the molecular hydrogen molecules inside the modified fullerenes. The properties of these systems are investigated by means of variable temperature solid state NMR, infrared spectroscopy and inelastic neutron scattering. The experimental data are compared with predictions from analytical calculations and quantum-mechanical calculations.

USE OF PERMUTATIONS FOR DYNAMIC-NMR: ROTATION OF A NON-C_2-SYMMETRICAL SUBSTITUENT ON A C_2-SYMMETRICAL CONFORMATION AZACALIX[4]ARENE RING

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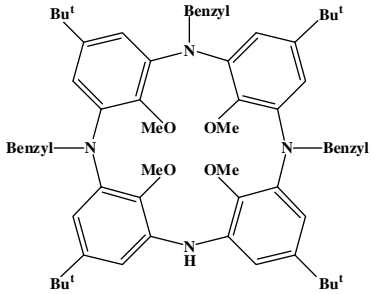
Many molecules that we study by NMR spectroscopy are non-rigid and undergo rapid conformational exchange, or a cycle of structures via single-bond rotation or by concerted molecular motion. It is useful to describe the local site symmetry around nuclei and then produce mathematical sets (permutation sets) illustrating how the nuclear environments cyclically interconvert at the Fast Exchange Limit. In this manner we can categorize a specific nucleus in a time-averaged structure in terms of *dynamic homotopicity, dynamic enantiotopicity, or dynamic diastereotopicity.* This will be illustrated by the dynamic stereochemistry of the rotamer interconversion of *d*- and *meso*-tartaric acid, and by a benzyl-group rotation ligated to a rigid C_2-symmetrical conformation rigid azacalix[4]arene system.



NUCLEAR MAGNETIC SPIN NOISE IN RESONANT CIRCUITS

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Nuclear Magnetic Spin Noise was predicted in 1946 by Bloch [1] as an intrinsic property of finite ensembles of spins. With modern high-resolution NMR spectrometers, in particular with cryogenically cooled probes, spin noise observation is straightforward and has been used to image spin density without rf-excitation and irrespective of longitudinal relaxation [2]. Recent experiments in our labs concern the fundamentals of spin noise spectroscopy. Quantification of spin noise turns out to be a complex issue due to effects of radiation damping and tuning offset.

The dependence of spin noise spectra on the properties of the receiving resonance circuit deviates significantly from what is predicted by Ernst and McCoy [3].

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Acknowledgements

This research is supported by the Austrian Science Funds FWF, project P19635-N17, a grant by the US NSF (CHE-0550054), the ÖAD (WTZ (AT-HR) and the Croatian Ministry of Science, Education and Sports (project 098-0982929-2917)

STRUCTURAL DEPENDENCIES OF PROTEIN BACKBONE $^{2}J_{NC'}$ COUPLINGS

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Protein folding can introduce strain in peptide covalent geometry, including deviations from planarity that are difficult to detect, especially for a protein in solution. We have found dependencies in protein backbone $^{2}J_{NC'}$ couplings on the planarity and the relative orientation of the sequential peptide planes. The dependences are observed in $^{2}J_{NC'}$ couplings of seven proteins and are also supported by DFT calculations in a model tripeptide. Findings indicate that elevated $^{2}J_{NC'}$ couplings may serve as reporters of structural strain in the protein backbone imposed by protein folds. Such information, supplemented with the H-bond strengths derived from $^{h3}J_{NC'}$ couplings, provides useful insight the into the overall energy profile of the protein backbone in solution.

LIGAND-PROTEIN INTERACTIONS AS SEEN BY NMR SPECTROSCOPY

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Understanding molecular recognition processes is one of the key issues in science. On this basis, it is important to access simple procedures that permit the investigation of ligand binding to biological receptors in a robust manner. It has been demonstrated that saturation transfer difference (STD[1,2]) experiments can be successfully applied to determine the "active" chemical groupings of the ligands that are located in and interact with the binding site of the target protein. Moreover, the binding constant (K_d) can also be obtained in competition titration in which the STD signals of the ligand with unknown affinity and that of the reference ligand with known affinity are monitored during the titration. However, overlapping signals of ligands or of ligands and receptors can pose difficulties and may preclude the general use of these experiments.

We herein present novel STD NMR methods[3,4] that overcome the overlapping problem taking advantage of ^{15}N or ^{13}C (or both) labeled protein or ligands. We demonstrate that the proposed STD experiments can be applied when strong spectral overlap of the interacting molecules occurs. In case of competition titration study isotope edited and filtered STD experiments can be used to separate the STD effects of the two ligands even with completely overlapping signals.

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NMR STUDIES OF STRUCTURE AND CATION BINDING TO DNA QUADRUPLEXES

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G-quadruplexes are non-B-DNA structures consisting of four strands that can be formed by G-rich nucleic acid stretches in the presence of cations. Such G-rich segments are found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells. NMR has proven itself as an exceptional method to ascertain the nature of Hoogsteen hydrogen bonds within G-quartets and furthermore contribute experimental data for 3D structure determination of the G-quadruplex structures.[1] Cation binding and localization is of great importance for formation and structural integrity as well as stability of G-quadruplexes.[2-5] The use of heteronuclear NMR has enabled us to localize $^{15}NH_4^+$ ion binding sites between pairs of adjacent G-quartets and in addition study kinetics of their movement inside different G-quadruplex structures. Our results demonstrate that $^{15}NH_4^+$ ion movement from a G-quadruplex into bulk solution is influenced by several factors including steric restraints imposed by loop residues.[3-5]

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STRUCTURE, FUNCTION, AND DYNAMICS OF THE MASON-PFIZER MONKEY VIRUS CAPSID PROTEIN

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The high resolution structure of the N-terminal domain (NTD) of the retroviral capsid protein (CA) of Mason-Pfizer monkey virus (M-PMV), a member of Betaretroviruses family, has been determined by NMR. The M-PMV NTD CA structure is similar to the other retroviral capsid structures and is characterized by a six α-helical bundle and an N-terminal β-hairpin, stabilized by an interaction of highly conserved residues, Pro1 and Asp57. Since the role of the β-hairpin has been shown to be critical for formation of infectious viral core, we also investigated the functional role of M-PMV β-hairpin in two mutants i.e. ΔPro NTDCA, and D57A NTDCA, where the salt bridge stabilizing this structure was disrupted. NMR data obtained for these mutants were compared to that for the wild-type. The main structural changes were observed within the β-hairpin structure, within the helices 3 and 5, and in the loop connecting the helices 2-3. This observation is supported by biochemical data showing a different cleavage pattern of these two mutants by M-PMV protease in comparison to the wild-type. Despite these structural changes the mutants with disrupted salt-bridge are still able to assemble into immature, spherical particles. This confirms that the mutual interaction and topology within β-hairpin and the helix 3 might correlate with the changes of interaction between immature and mature lattice.

EPITOPE MAPPING OF MACROLIDE ANTIBIOTICS IN INTERACTIONS WITH RIBOSOMES

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Macrolide antibiotics are therapeutically important molecules that are effective inhibitors of bacterial protein biosynthesis. The increasing resistance to ribosome-targeting antibiotics has become a global problem and much effort is now directed toward new and more potent classes of drugs to overcome resistance mechanisms. An effective approach to overcoming this problem is to understand the principles of how these drugs interact with the ribosome. Recently available crystal structures of some ribosome-macrolide complexes have thrown new light on the binding mechanisms and hence provide a good basis for the design of new ligands and inhibitors. However, when analysing solid state structures of ribosome-macrolide complexes one should keep in mind the discrepancies between structures obtained for halophilic archeon *H. marismortui* and *D. radiodurans*. The proposed models differ significantly even though ribosomal 50S subunits of the two bacteria have drug binding sites whose sequences are highly conserved. Therefore, important steps in the process of drug design should also include elucidation of the solution-state structures of free and bound ligand molecules since the structural features of the complex may not be exactly the same in solution and in the solid state.

One and two-dimensional NMR techniques have proven usefull in conformational analysis and structure-based inhibitor design. Here, an approach which combines NMR parameterts such as spin-spin coupling constants, nOe and relaxation times coupled with molecular modelling is employed to study free conformations of macrolides. Furthermore, an application of transferred nOe and STD experiments provides information on the bound state conformation and binding epitopes. The knowledge gained from these studies can serve as a platform for the design of novel compounds with an improved biological profile.

FLUORINE-19 NMR IN DRUG RESEARCH

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Among the numerous marketed pharmaceuticals more than 150 drugs are fluorinated compounds [1,2]. The occurrences of a fluorine substituent in commercial drugs continuously increase and today it can be estimated that globally 20-25% of drugs contain at least one fluorine atom. This is a huge frequency when comparing with other halogen containing drugs and especially when one considers the fact that there are virtually no natural products that contain fluorine. Incorporation of fluorine atoms in drugs are driven by the special properties of fluorine atom such as strong electronegativity, small size and the low polarisability of the C-F bond that can significantly impact the behavior of drug molecule in biological environment[3].

NMR spectroscopy is widely used technique in pharmaceutical industry in all phases of drug development. In the context of fluorine containing drugs ^{19}F NMR is very powerful method for studying such molecules. Fluorine-19 nucleus is very attractive for NMR studies due to the 100% natural abundance, high intrinsic sensitivity and a magnetogyric ratio only slightly smaller than that of proton. The fluorine-19 chemical shifts are also highly sensitive to even subtle changes in the magnetic environment, the property that can be used as a probe for distinguishing closely related molecules. The analysis of complex reaction mixtures, formulated drug products and drug metabolites play an important role in pharmaceutical research. However, the analysis of such samples is often very difficult by severe resonance overlap in proton spectrum. Fluorine-19 NMR is a good alternative in such cases if the molecule contains fluorine atoms. Normally excipients, solvents and biological matrices do not contain fluorine atoms and identification of target molecule is much easier. Solid state fluorine-19 NMR is also very useful for identification and quantification of fluorine containing drug molecules and its polymorphs. The use of NMR based screening in drug discovery is very well known. In recent years it has been demonstrated that ligand and substrate based fluorine-19 NMR screening is a powerful tool for identification of novel active compounds[4].

The overview of fluorine-19 NMR spectroscopy use in pharmaceutical research will be presented.

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ABSTRACTS (POSTERS)

STRUCTURE AND DYNAMICS OF THE ANTIFUNGAL PROTEIN PAF

Gyula BATTA[1], Teréz BARNA[1], Szabolcs SÁNDOR[1], Zoltán GÁSPÁRI[2], Katalin E. KÖVÉR[3], Éva LEITER[4], István PÓCSI[4], Ulrike BINDER[5], Bettina SARG[5], Lydia KAISERER[5], Herbert LINDNER[5] and Florentine MARX[5]

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The small (55 aa, 6.2 kDa) cysteine-rich and basic antifungal protein of *Penicillium chrysogenum* (PAF) induces multifactorial detrimental effects in sensitive fungi. A deeper understanding of the mechanism of action of PAF is likely to lead to the development of novel antifungal drugs. Nearly 90% ^{1}H and full backbone assignment, $^{3}J_{NH,HA}$ couplings from ^{15}N-HSQC and ^{13}CA assignments were obtained at natural abundance. NOE assignments and structure calculation was performed using ATNOS/CANDID in combination with CYANA 2.0. We used combined NOE and S^2 order parameter restrained molecular dynamics simulation (MUMO)[1] for generating realistic ensemble structures. In spite of limited (47%) sequence identity of PAF and AFP[2], the two antifungal proteins exhibit striking similarity: the main PAF fold is composed of five β strands forming two orthogonally packed β sheets sharing a common interface and the six cysteines form three disulfide pairs. However, detailed NMR studies were not capable to unequivocally assign the SS bond patterns either in AFP or in PAF until now. ^{15}N relaxation experiments suggest 3 ns global correlation time and a compact monomeric structure. S^2 parameters from ^{15}N relaxation agreed quite well with chemical shift (RCI, Wishart) based predictions. ^{1}H and ^{15}N CSA/DD cross-correlated relaxation and NH-deuteration rate experiments also corroborate the proposed structure of PAF.

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SOLUTION AND SOLID STATE NMR STUDIES OF CROSS-DIMERIZATION OF NITROSOBENZENES

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Nitrosobenzenes can exist as blue or green monomers or as colorless azodioxy dimers in two forms, (*Z*) and (*E*). In the solid state most of them exist as dimers, while in solution monomer-dimer equilibrium is established. Previous temperature dependent NMR studies have shown that while at ambient temperature the main solution species are monomers decreasing temperature favours the dimeric azodioxy species with both (*Z*)- and (*E*)-forms present in addition to the monomer.[1]

In the present work we studied cross-linking of nitrosobenzenes to asymmetricaly substituted azodioxides (heterodimers) in cases when they are not sterically crowded with large groups in *ortho*-position what was previously stated as the necessary condition for heterodimer formation.[2] The formation of heterodimers was studied in the series of *para*- and *meta*-substituted nitrosobenzenes both in solution (by one- and two-dimensional ^{1}H NMR spectroscopy) and in solid state (by CP-MAS ^{13}C NMR spectroscopy).

Equimolar amounts of two different nitrosobenzenes were dissolved in $CDCl_3$, thus obtained solution was cooled to 218 K and ^{1}H NMR COSY spectra were recorded and analyzed. Heterodimer formation was prooved if obtained NMR spectra were different from the sum of spectra recorded for the solutions of individual components under the same conditions.

In the case of cross-dimerization study in solid state, we compared ^{13}C CP-MAS spectra of solids obtained after mixing the equimolar solutions of two different nitrosobenzenes and evaporation of the solvent and sum of spectra of pure homodimers. Differences in these two spectra are indication of heterodimer formation.

We have shown that nitrosobenzenes can form heterodimers in solution as well as in solid state even when they are not sterically crowded with large groups in *ortho*-position.[3] The ability of heterodimer formation is quite different in solid state from that in solution probably because of a considerable influence of packing factors present in the former.

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PEPTIDE – PROTEIN INTERACTION BETWEEN AN UNSTRUCTURED MYOSIN FRAGMENT AND A DIMERIC DYNEIN LIGHT CHAIN

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A dynein light chain (DLC) was identified as a tail domain light chain of myosin-Va (M), which may function as a cargo-binding and/or regulatory subunit of both motor proteins. Various fragments of M tails were analysed in order to determine the binding sites on each fragment. We already showed, that the binding of M occurs on the surface groove on the homodimeric DLC, and two molecules of target M peptide fragments fit in the two binding channels, with no communication between the channels.

In this study we characterized the interaction from the myosin side, using a 27 aminoacid long M fragment with ^{15}N labeling. Assignment of the residues was done from HSQC-TOCSY and HSQC-NOESY measurements. The secondary chemical shift values and the relaxation measurements (T1, T2, heteronuclear NOE) showed that in free form M is an unstructured protein fragment. Upon titration with unlabeled DLC we could detect the binding positions, and we studied the dynamic behavior of the 1:1 complex. These data reveal that the D14-T23 region is responsible for binding, and part of the M fragment becomes structured.

A STUDY OF H-BOND IN GROUP OF NSAID HYDROXAMIC ACID DERIVATIVES

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The conformational behaviour of NSAID series (ibuprofen, fenprofen, ketoprofen, diclophenac and indomethacin) monohydroxamic acids and their intermediates have been studied in DMSO-d_6 solution by one- and two-dimesional homo and heteronuclear ^{1}H and ^{13}C NMR spectroscopy and in solid state using FTIR spectroscopy.

Significant hydrogen bonding effects were observed in both, solid state and solutions. The NMR spectra (^{1}H and ^{13}C NMR, COSY, NOESY, HMBC, HETCOR) revealed that investigated NSAID hydroxamic acids and their corresponding intermediates in DMSO solution are in keto form of cis-(Z) conformer with intramolecular hydrogen bond O-H...O= type. NMR findings observed in DMSO-d_6 solution are in good agreement with FTIR of NSAID hydroxamic acids in solid state.

NMR CHARACTERIZATION OF QUINOLINE-BASED AMINOPHOSPHONATE DERIVATIVES

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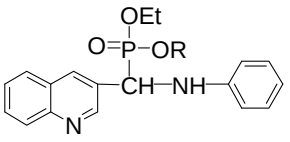
Aminophosphonic acids and their esters have attracted continuous attention because of their wide range biological properties and potential applications in the pharmacological and agrochemical fields [1]. These organophosphorus compounds are also known as good complexing agents for various transition metals, of which platinum group metals form complexes with noticeable antitumor activity [2].

One of the simplest synthetic routes to α-aminophosphonates is the Kabachnik-Fields reaction, a three-component one-pot reaction of amine, carbonyl compound (aldehyde or ketone) and hydrophosphoryl compound [3]. Recently, we focused our attention on the influence of microwave irradiation on the Kabachnik-Fields synthesis of quinoline-based α-aminophosphonates [4]. In studied reactions with 3-quinoline derivatives, along with main products diethyl phosphonates (**1**, **4**), unusual monoester phosphonate derivatives (**2**, **3**, **5**) were isolated. We present here the NMR characterization of compounds **1-5** using various one- and two-dimensional ^{1}H, ^{13}C and ^{31}P NMR spectroscopic methods.



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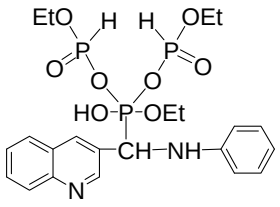

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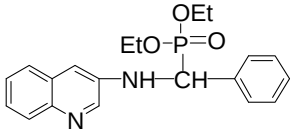

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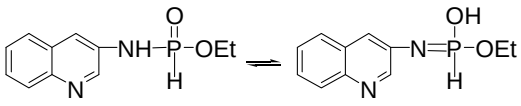

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EFFECT OF WATER ON STABILITY OF GEOPOLYMER SYSTEMS INVESTIGATED BY SOLID STATE NMR SPECTROSCOPY

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Geopolymers that belong to the family of aluminosilicate ceramics synthesized at room temperature have attracted commercial and scientific attention for their superior mechanical properties and relatively low production cost for a long time. Current trends in geopolymer chemistry are to develop new types of geopolymer systems at low energy requirements but still possessing good mechanical properties. One possible way how to achieve formation of such geopolymers is the reaction of metakaolin with a concentrated alkali solution at the room temperature and normal pressure.

Ideally they should have non-crystalline structure, which keeps molecular water inside 3D framework. However, during the time, these materials can undergo gradual transformation to zeolite structures at some circumstances. Stability of system is important for keeping of mechanical properties. This is the reason why this work is focused on the deep investigation of the role of water during these processes.

In this work the stable and unstable systems of geopolymers were tested. For such task we used very powerful method – solid-state NMR. To gain structural information provided by solid-sate NMR we used not only simple one-dimensional experiments on various nuclei like ^{1}H, ^{23}Na, ^{27}Al, ^{29}Si but we also used two-dimensional multiple-quantum experiments that were modified to indirectly detect water molecules that are closed in selected structural units. By this way we were able to resolve additional structural units in geopolymers that are responsible for their instability. We tested large scale of NMR techniques involving also ^{1}H - ^{1}H correlation experiments, REDOR experiments and cross-polarization transfer as well as relaxation experiments to locate and describe properties of clusters of water molecules.

We thank the Grant Agency of Academy of Sciences of the Czech Republic for financial support Grant No. (IAA400500602)

CALCIUM-INDUCED TRIPARTITE BINDING OF INTRINSICALLY DISORDERED CALPASTATIN TO ITS COGNATE ENZYME, CALPAIN

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The activity of the intracellular cysteine protease, calpain, is controlled by both the free intracellular calcium level and its intrinsically disordered endogenous inhibitor protein, an IUP, called as calpastatin. Inhibition of the over 130kDa enzyme by calpastatin is primarily mediated by three short conserved segments of the inhibitor; namely subdomain A, B and C. The exact binding mode of calpastatin to the enzyme responsible for controlling the activity of more than 100 target proteins in the regulation of cell division, differentiation and cell motility, however, has not yet been uncovered. To shed light on the molecular details of binding, we have measured NMR observables, such as secondary chemical shift (SCS) values, HSQC peak intensities, R_2, R_1 and hetero-NOE values of the 141 aminoacid long inhibitor in the absence and presence of calcium and calpain. We have observed both a structural change of the inhibitor caused by calcium binding, and its tripartite binding to the enzyme. The changes in SCS values in the presence of calcium show that two acidic regions at the carboxy-terminal ends of subdomains A and C bind calcium, and probably shift the equilibrium of the subdomains toward the binding-competent alpha-helical conformation. This finding contrasts earlier observations, which suggested a calcium-induced conformational change in calpain structure only. Titration of calcium-bound calpastatin with calpain cause the disappearance of peaks from HSQC spectrum in three regions, corresponding to the three subdomains, which suggests these and only these residues are in direct contact with the enzyme exhibiting slower molecular motion. The two linker regions connecting the above three subdomains, as well as both the N- and the C-terminus of the inhibitor, remain in a disordered state presenting a faster backbone motion. These results suggest a tripartite binding mode, in which the disordered inhibitor wraps around and contacts the enzyme at three distinct points, enabled by flexible linker regions. We suggest that this unprecedented binding mode probably enables a unique combination of specificity, speed and binding strength in protein regulation.

NMR ISOTOPE SHIFT AS A PROBE OF CONFORMATION IN CARBOCATIONS. EXPERIMENTAL ^{13}C NMR SPECTROSCOPY AND GIAO-DFT STUDY OF 1-(2-PROPYL)CYCLOPENTYL CATION

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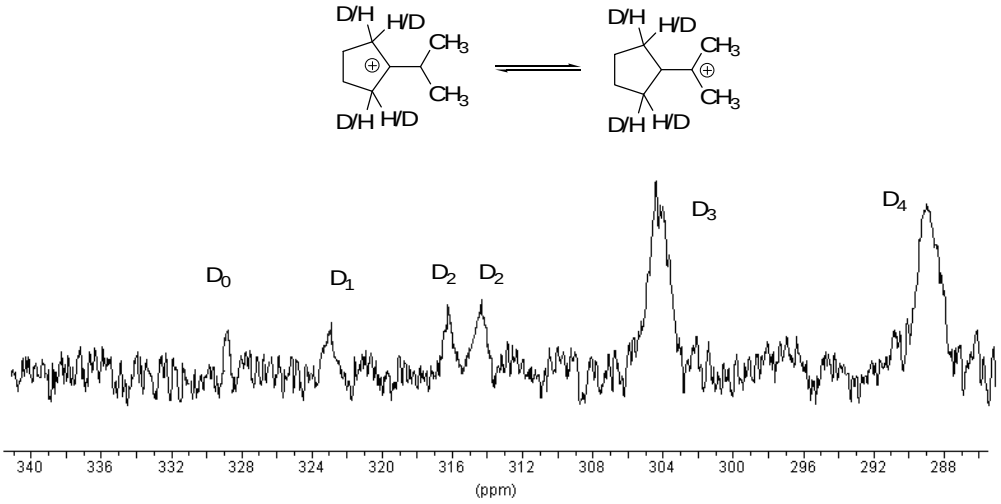
NMR isotope shifts due to β-deuteration (deuterium substitution at a carbon atom adjacent to the carbocation center) have been postulated to be related to the hyperconjugative interaction. C-D bond behaves as a poorer electron donor than a C-H bond in cationic hyperconjugation, resulting in decreased electron density in the π system of the cation and hence NMR deshielding. Typical NMR isotope shifts are additive, but nonadditivity of NMR isotope shifts in carbocations may occur when C-H(D) bonds are involved in hyperconjugation and are also conformationally mobile, because of the imposition of an equilibrium isotope effect on the population of conformers that may have different intrinsic NMR isotope shifts.

In the present work we report the determination of the conformation of the 1-(2-propyl)cyclopentyl cation by use of the additivity characteristics of the $^{2}\Delta C^{+}(D)$ NMR isotope shifts at C^{+} due to β-deuteration. The nonadditivity of isotope shifts indicates that the cyclopentyl cation is nonplanar, and isotopomers of the cation reveal the preferred conformation to be the twist structure. In addition to isotopic perturbation of hyperconjugation, the 1-(2-propyl)cyclopentyl cation undergoes fast reversible nondegenerate hydride shift which results in the averaged ^{13}C NMR chemical shifts of carbocation center.

A theoretical model is developed that provides a possible explanation for the relative magnitude of isotope shifts in this cation**,** based on DFT-GIAO NMR calculations.



^{1}H-decoupled 100 MHz ^{13}C spectrum, at -123 °C in SO_2ClF/SO_2F_2, of 1-(2-propyl)cyclopentyl cation partially deuterated at methylene position (six NMR signals correspond to the carbocation center in each of six different isotopomers).

LOOPS CONTROL CATION MOVEMENTS FROM DNA G-QUADRUPLEXES

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Guanosine-rich nucleic acid sequences are prone to adopt quadruplex structures consisting of stacked planes of four base-paired guanine nucleobases in the presence of cations. These G-rich segments are found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells. NMR is superior tool to ascertain the nature of cation binding between adjacent G-quartets and for 3D structure determination of the G-quadruplex structures.

We have focused on cation interactions with G-quadruplexes. The use of heteronuclear NMR enabled us to localize $^{15}NH_4^+$ ion binding sites between pairs of adjacent G-quartets and in addition study kinetics of their movement inside three different G-quadruplex structures. We were able to demonstrate that $^{15}NH_4^+$ ion movement from G-quadruplex into bulk solution is mainly influenced by steric restraints imposed by loop residues. Thymine residues of the diagonal loops of $d(G_4T_4G_4)_2$ are positioned around the central ion cavity of the outer G-quartets and in such arrangement do not represent a steric barrier for $^{15}NH_4^+$ ions to enter or leave the interior of the G-quadruplex. Such structural features result in a relatively fast movement of $^{15}NH_4^+$ ions into bulk solution. Steric restraints of diagonal loop versus edge-type loop in $d(G_3T_4G_4)_2$ G-quadruplex slow down $^{15}NH_4^+$ ion movement by 12 times. In comparison, steric hindrance of T3 edge-type loops in $d(G_4T_3G_4)_2$ G-quadruplex are responsible for slow $^{15}NH_4^+$ ion movement.

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POYMORPHISM OF ATORVASTATIN

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Atorvastatin is a member of the drug class known as statins, which are used to cure hypercholesterolemia. It is well known, that atorvastatin exhibits extensive polymorphism and majority of the crystal forms is disordered and amorphous. These amorphous or semicrystalline structures are difficult to distinguish by standard spectroscopy techniques. Consequently it is necessary to study the complexity of its polymorphs and amorphous forms by new analytical techniques of NMR crystallography developed for the control in pharmaceutical manufacture. The series of various polymorph of atorvastatin was synthesized and subsequently characterized by ^{13}C CP/MAS NMR and ^{19}F MAS NMR spectroscopy and x-ray diffraction. ^{19}F MAS NMR measurement enable to obtain the spectra of the sample in short time and are supposed to carry the significant structural information especially in tablet forms, because there is no complete with the overlap with signals of placebo. However, the differences in ^{19}F MAS NMR spectra are very slight, difficult to recognize by simple analyze. That is why a factor analysis was applied to the sets of ^{19}F and ^{13}C spectra and x-ray diffractograms of atorvastatin. The aim of the factor-analysis application is to find the similar features in the behavior of different data series. The results of the factor analysis were used to compare the data series and determined their possible correlation. In consequence, the atom groups influenced by each other can be eventually distinguished.

Acknowledgements: The authors wish to thank the Ministry of Education, Youth and Sports for financial support (grant No. 2B08021).

HOW NOT TO LOSE HITS IN LIGAND BINDING TESTS: COMPREHENSIVE OPTIMIZATION INCLUDES TEMPERATURE

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NMR spectroscopy provides various methods to study ligand-protein interactions. Saturation transfer difference (STD) and transferred NOE (trNOE) experiments can be performed with a small amount of non-labeled protein and they are considered to be efficient tools to identify binding molecules. ^{15}N- ^{1}H HSQC measurement, although, requires ^{15}N labeling, provides information about the binding pocket, too (assuming that the resonance assignment is known). Our aim was to optimize the experiments for screening potential peptide ligands for the low molecular weight (14.5 kDa) β-galactoside binding protein galectin-1 and to study the interactions between them.

Galactoside derivatives (lactose and N-acetyl-lactosamine) were used as a positive control for the trNOE, STD and ^{15}N-^{1}H HSQC measurements. Initially, experiments were carried out at 303 K. While ^{15}N-^{1}H HSQC titration worked well, STD and trNOE have not proven the fact of the binding. Experiments were repeated with the same parameter sets at 280 K which resulted in a different outcome: while the sensitivity of the ^{15}N-^{1}H HSQC spectra were not sufficient for the titration, signals of the carbohydrates appeared in the STD and the sign of the crosspeaks in the NOESY spectra has changed both of them proving the binding of the ligands to galectin-1.

Screening at room temperature, using only the STD and trNOE spectra as an indicator of the binding, carbohydrates with an affinity for galectin-1 would not be identified. As a conclusion, it is advisable to perform the binding tests for galectin-1 at a lower temperature and to optimize temperature in case of ligand-protein interaction studies.

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