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[1] Structural insights into TAZ2-mediated CBP/p300 recruitment by the first activation domain of the melanogenic transcription factor MITF

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The microphthalmia-associated transcription factor (MITF) is a master regulator of development and differentiation within the melanocyte lineage. However, aberrant MITF activity can lead to multiple malignancies such as skin cancer, where it plays a key role in modulating the proliferation and invasiveness of melanoma. MITF is a basic helix-loop-helix leucine zipper transcription factor that binds to specific gene promoters via a central DNA-binding domain. MITF also recruits transcriptional co-activators, such as the histone acetyltransferase CREB-binding protein and its homologue p300 (CBP/p300) through an N-terminal acidic transactivation domain (TAD), however the details of these interactions are not yet fully understood. In order to gain insight into the mechanisms of gene regulation by MITF, we investigated the structure and functional interaction between MITF-TAD and the transcription adapter putative zinc finger (TAZ2) domain of CBP/p300. In mammalian-one-hybrid assays MITF transcriptional activity was enhanced in the presence of co-transfected CBP/p300 and abolished upon deletion of residues within the MITF-TAD. Peptide microarrays indicated that no one residue of MITF is essential for TAZ2 binding, however, deletion of multiple residues in MITF-TAD ablated its ability to bind TAZ2. NMR-based chemical shift mapping experiments determined that MITF-TAD interacts with the same surface of TAZ2 as the adenoviral protein E1A, which has been shown to inhibit MITF function. We determined that E1A and MITF-TAD directly compete for CBP/p300 through the TAZ2 domain using NMR-based titrations, pulldown, and mammalian-hybrid assays. Understanding mechanistic details regarding the interaction between MITF and its co-activators is fundamental to our understanding of gene regulation by MITF and may outline a potential new strategy to inhibit MITF function.

[2] ^{31}P and ^{17}O Single-Crystal NMR Characterization of Halogen-Bonded Cocrystals

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Halogen bonding is a noncovalent interaction between the elevated electrostatic region of a halogen atom and an electron donor. This interaction is highly directional and tunable, and therefore it has gained an increasing amount of attention in diverse applications, such as organocatalysis, crystal engineering etc.[1] Analysis of single-crystal NMR (SCNMR) spectra, which can reveal the combined effects of quadrupolar coupling, magnetic shielding, and spin-spin coupling, allows for the measurement of the tensor magnitude and orientation for these anisotropic NMR interactions in the molecular frame. Here, ^{17}O enriched triphenylphosphine oxide and three of its halogen-bonded cocrystals[2] featuring 1,4-diiodotetrafluorobenzene and 1,3,5-trifluoro-2,4,6-triiodobenzene as halogen bond donors have been characterized by ^{31}P and ^{17}O single-crystal NMR spectroscopy. ^{31}P chemical shift tensors, ^{17}O chemical shift tensors, ^{17}O quadrupolar coupling tensors, and ^{31}P-^{17}O indirect nuclear spin-spin (***J***) coupling tensors are reported for P=O···I halogen bonds. The angles between the direction of the unique component of the NMR tensors (chemical shift tensors and quadrupolar coupling tensors) and O···I halogen bond correlates with the deiviation in linearity of the P=O···I halogen bond. There is also clear decrease in anisotropy and an increase in asymmetry of the ***J***(^{31}P,^{17}O) coupling tensors attributable to the formation of iodine-oxygen halogen bonds.

This work, encompassing the first ^{17}O single-crystal NMR studies of halogen bonds, helps in our understanding of the correlation between the electronic structure of the halogen bond and NMR propertie and provides a novel probe of halogen bonds.

A single-crystal analysis software, SCFit, was developed to fit the chemical shift and quadrupolar coupling in two ways: (i) through a 'free fit' process where all tensor parameters are freely optimized or (ii) through a 'constrained fit' process where the principal components of the tensors may be fixed to values known previously via the analysis of powder samples. A comparision between the two methods provides an extra check on the quality and consistency of both powder and SCNMR data.

[3] The Drug Discovery Platform of the RI-MUHC: Applications for NMR, LC-MS and MALDI IMS

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The Drug Discovery Platform (DDP), based in Montréal, Québec, provides structure-based drug design, synthesis and bioanalysis using a state-of-the-art facility for solution and High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy, together with Liquid-Chromatography Mass Spectrometry (LC-MS) and Matrix-Assisted Laser Desorption/Ionization (MALDI) Imaging Mass Spectrometry (IMS). Our services are offered to local and international, academic and industrial researchers. With our drug discovery and analytical expertise, the platform identifies and validates promising new biological and chemical entities (including biomarkers) for life-threatening diseases. We also provide access and training to users on a broad range of cutting-edge technologies such as our 400 and 600 MHz NMR spectrometers equipped with HR-MAS and CryoProbes, along with a variety of LC-MS systems (Triple Quad, Q-TOF and Ion Trap) and MALDI-TOF MS & IMS. These technologies along with some recent applications will be presented in this poster.

[4] - Peptides from *Fusarium graminearum* associated with NRPS gene clusters

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Fusarium graminearum is a broad host pathogen causing *Fusarium* Head Blight (FHB) in diverse cereal crops. The virulence and host range of *F. graminearum* is associated with its ability to secrete an arsenal of proteins and toxic secondary metabolites, including the regulated mycotoxin deoxynivalenol as a virulence factor. The *F. graminearum* genome harbors an array of unexplored biosynthetic gene clusters that are often co-induced with the *TRI* genes. Two recent publications have introduced the structure of a linear octapeptide, dubbed Fusaoctaxin A, associated with *NRPS5* and *NRPS9* from *F. graminearum*, that has been shown to be a virulence factor. We recently reported the structures of Gramillin A and B, cyclic peptides with an unusual anhydride linkage that are biosynthetic products of the *NRPS8* gene cluster and are host-specific phytotoxins. As part of a metabolomics study on virulence of *F. graminarum* in wheat, we also observe the presence of Fusaoctaxin A, but associated with two related peptides, all regulated to some extent by *Tri6*, the transcription factor that also regulates trichothecene production. These other related peptides have m/z = 787 and 757 as compared to Fusaoctaxin A (m/z =773). In the course of isolating ^{15}N-enriched gramillins for structural determination., these three peptides were also observed to be ^{15}N-labelled. ^{15}N enriched samples were produced by simply substituting nitrogen sources in *F. graminearum* liquid cultures with ^{15}N-ammonium chloride. Initial LC-MS, 1D and 2D NMR, including ^{1}H-^{15}N-^{13}C HNCO, have been used to characterize the isotopically enriched m/z = 787 peptide.

[5] AHNAK C-terminal membrane binding

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AHNAK, also named as desmoyokin, is a very large protein whose majority of its protein interaction sites are located on the C-terminal domain. Among its many functions, AHNAK plays a role in cell membrane repair. The dysferlin membrane repair complex contains a small complex, S100A10–annexin A2, which initiates membrane repair by recruiting binding site comprising 20 amino acids in the C-terminal region of AHNAK protein (pAHNAK) to the membrane, before forming a platform which can initiate membrane repair. However, no molecular data are available for the membrane binding of the various proteins involved in this complex. Therefore, the present study investigated the membrane binding of AHNAK to elucidate its role in the cell membrane repair process.

An artificially synthesized peptide (pAHNAK) was applied to Langmuir monolayer model which mimics the cell membrane. The binding parameters were determined at 20 °C by surface tensiometry to characterize the interaction between pAHNAK and different phospholipids. The insertion depth of pAHNAK into different phospholipid monolayers was then studied by ellipsometry at 20 °C in order to determine the ellipsometric

angle values. The interaction of pAHNAK with lipid bilayers was studied at 20 °C and also at 37 °C to mimic the physiological temperature of the human body, using ^{31}P solid-state nuclear magnetic resonance (NMR). The bilayer model contained a mixture of three monounsaturated phospholipids, with or without pAHNAK as a control. To identify the chemical shift anisotropy (CSA) of individual lipids in the fluid phase, phosphorus recoupling of chemical shift anisotropy (PROCSA) was applied. The interaction between pAHNAK and each phospholipid was represented by the changes of each polar head group CSA, informing on the influence of pAHNAK on their fluidity.

Langmuir monolayer model studies indicated pAHNAK preferentially interacted with unsaturated phospholipids, and more especially, it strongly interacted with phospholipids that comprised negatively charged polar heads and monounsaturation. A preferential interaction order for pAHNAK was suggested as 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS) > 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) > 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). This result was confirmed by ellipsometry studies as the insertion depth of pAHNAK into the phospholipid monolayers appeared to follow the same trend. Studies of solid-state NMR results revealed two different interaction modes: at 20°C, pAHNAK significantly impacted the polar head group of DOPE, whereas at 37°C, it was able to insert into the acyl chain of DOPS.

In conclusion, in a physiological environment at 37°C, AHNAK can probably interact with monounsaturated phospholipids with negatively charged polar head groups, with its insertion into their acyl chains. This finding represents a first step in an improved understanding of AHNAK membrane behavior. Future studies of the membrane interactions of other proteins in the dysferlin membrane repair complex will help to complete the map of membrane repair, potentially allowing the identification of the conditions that result in changes to these membrane bindings and possibly the loss of protein function.

[6] Probing allosteric regulation of pyruvate kinase by ^{17}O quadrupole-central-transition NMR spectroscopy

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Quadrupole central transition (QCT) nuclear magnetic resonance (NMR) spectroscopy is a new approach of detecting ^{17}O ($I = 5/2$) NMR signals from biological macromolecules in aqueous solution. In this study, we used the ^{17}O QCT NMR method to probe allosteric regulation of pyruvate kinase (PK, a tetramer of 237 kDa) by observing ^{17}O NMR signal changes from a PK-oxalate-Mg^{2+} complex. We also tested the role of ATP and amino acids in oxalate binding to PK. We demonstrated that phenylalanine has an inhibiting effect on PK-oxalate binding and alanine has potential reversing effect on this inhibition. The ^{17}O NMR methodology demonstrated in this study may be applicable to other enzymes.

[7] Mechanism of PKA Activity Modulation by Carney Complex A211D and Acrodysostosis A211T Mutations

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[8] Inhibition of α-Synuclein Aggregation by Extracellular Chaperones

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the deposition of abnormal protein aggregates, known as Lewy bodies, in neurons. The principal component of Lewy bodies is alpha-synuclein (α-syn), a protein that is predominantly expressed in the brain and is responsible for the formation of toxic aggregates [1]. The molecular etiology of PD is currently unclear, but previous studies suggest that misfolding of α-syn and the consequent formation of toxic oligomers are the main reason for the neural damage observed in PD [2]. Although the factors that influence the oligomerization of amyloid proteins, such as α-syn, are not fully understood, previous studies have determined that extracellular proteins with chaperoning activity may inhibit amyloid aggregation [3,4,5]. However, their mechanism of action is not fully understood. Here, we propose to investigate how α-syn oligomer toxicity varies in the presence or absence of different chaperone proteins with known anti-amyloid activity. Using a combination of solution NMR with dynamic light scattering, fluorescence spectroscopy, cell viability assays, and electron microscopy we plan to interrogate the complexes formed by α-syn and the library of chaperone proteins. Results from this study will facilitate the determination of conserved structural elements required for the recognition and consequent elimination of α-syn oligomers. Moreover, understanding the molecular mechanism of these chaperones will serve as a foundation for the design of novel and more potent inhibitors of α-syn oligomers in PD.

[9] Towards ^{19}F NMR of membrane proteins in the native lipid environment

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For the last 50 years, detergent solubilization has been the main method to purify membrane proteins. Although this has yielded lots of success there are significant limitations that imposes constraints on the study of membrane proteins. In particular, detergent solubilization prevents the study of complex interactions between a membrane protein and its native lipid environment. In recent years, it's been shown that styrene maleic acid (SMA) can been used to directly extract membrane proteins from the membrane into styrene maleic acid lipid particles (SMALPs). This method allows for the active protein and its native membrane environment to be extracted without the use any detergents. In this system we are interested in studying membrane protein conformational dynamics within the native lipid environment using solution state ^{19}F NMR. We describe our progress to apply SMALP technology with site-specific incorporation of ^{19}F labels to study conformational dynamics of the GlpG rhomboid protease integral membrane protein.

[10] Non-Additivity of Ligand Binding Affinities and Partial Agonism for Protein Kinase G (PKG)

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Protein kinase G (PKG) is a major protein involved in eukaryotic cyclic GMP (cGMP) dependent intracellular signaling, playing a regulatory role in such processes as cell differentiation, platelet activation, memory formation and vasodilation. Notably, the signaling pathways controlled by PKG are often distinct from those regulated by cyclic AMP (cAMP), and so the selective activation of PKG by cGMP rather than cAMP is critical. However, the mechanism of cGMP-*vs.*-cAMP selectivity in PKG is only limitedly understood. Previously, we showed that cAMP is a partial agonist for PKG, and we elucidated the mechanism of cAMP partial agonism through comparative NMR analysis of the apo, cGMP- and cAMP-bound forms of PKG CNB-B. In the current work, we assessed the contributions of two key cGMP-specific interactions to PKG CNB-B binding and activation by performing comparative NMR analyses of PKG CNB-B bound to cGMP, and the cGMP analogues cIMP, 2-NH_2-cPuMP and cPuMP. Using an approach developed in our lab, we also formulated an explanation for an apparent non-additivity observed among the binding affinities of PKG CNB-B for the cGMP, cIMP, 2-NH_2-cPuMP and cPuMP ligands, which was found to be influenced by variations in the conformational propensities of both the protein and the ligands – a phenomenon we refer to as "mutual protein-ligand conformational selection".

[11] HDM2 binds HDMX via a novel sequence element

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HDMX is a central regulator of p53 that inhibits the protein's transcriptional activity and promote HDM2 mediated proteasomal degradation. Recently, it was shown that a central region (WW domain) of the HDMX could bind to its N-terminal domain. Since HDM2 also contains a N-terminal domain (PBD) that is homologous to the N-terminal domain of HDMX, we hypothesized that the WW domain will also interact with HDM2. ITC result shows that the isolated WW domain binds to N-terminal domain of HDM2 with high affinity and p53. NMR spectroscopy indicates that the WW domain binds to the hydrophobic pocket of the PBD. ITC and NMR result suggest that WW domain competes with p53 N-terminal region for PBD binding. Our results suggest that the WW domain of HDMX is an important regulating element for HDM2 and p53 interaction.

[12] Estimation of Lipid Content in Field Pea Seeds using ^{1}H NMR Spectroscopy

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Field pea (*Pisum sativum*) is being considered as an alternate oilseed crop, and genetic modifications as well as conventional breeding techniques have been employed to improve lipid content in this plant species. However, current chemical extraction methods needed to quantify lipid content are lengthy and require a large sample size. The purpose of this study was to develop a rapid, non-destructive and precise spectroscopic method for quantifying lipid content in pea seeds using high resolution magic angle spinning-nuclear magnetic resonance (HRMAS-NMR). Small sample sizes (7 mg to 8 mg) were used and high-resolution spectra were obtained. To analyse the spectra, a methodology was developed by picking and integrating relevant peaks on the spectrum. Integral values of the peaks were used to quantify the oil content of seeds from select pea varieties and transgenic plants. A calibration curve was obtained by analysing the spectra of pure pea oil ranging from 0.035 mg to 0.35 mg of pea oil (varying 0.5% to 5% of lipid content). The lipid content of each seed sample was calculated by inserting integral values into equations obtained from the calibration curve. Results showed that HRMAS-NMR is an effective tool for assessing oil content in pea seeds. Further research is being conducted to observe the trend of lipid content in wild accessions of pea seeds, which in turn can aid in development of pea as an oilseed crop.

[13] Exploring the structural stability and assembly mechanism of hydrophobin proteins

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Hydrophobins are small, globular proteins with amphiphilic character that are produced and secreted by filamentous fungi. They self-assemble into durable amyloid-containing structures, called rodlets, at hydrophobic-hydrophilic interfaces which create protective, water repellent coatings for fungal spores. Current models of hydrophobin self-assembly predict that hydrophobin monomers undergo a conformational change at a hydrophobic-hydrophilic interface and integrate into a growing rodlet, however the mechanistic details of rodlet assembly are unknown. To investigate the assembly mechanism of hydrophobins, we carried out stability studies with SC16, a hydrophobin isolated from *Schizophyllum commune.* SC16 was recombinantly expressed using *E. coli* and purified by immobilized Ni^{2+} affinity chromatography. NMR spectroscopy was used to determine that the structure of SC16 was minimally perturbed by denaturing (8 M urea) or reducing (2 mM DTT) conditions. Gel filtration chromatography indicated that in solution SC16 exists as a mixture of dimers and tetramers, suggesting that rodlet assembly does not initiate from hydrophobin monomers. Mutant forms of SC16 are being employed to determine the sequences and conformational changes required for rodlet assembly, with gel filtration chromatography used to determine the multimeric state, thioflavin T assays to quantify amyloid formation, and electron microscopy to visualize rodlet formation. By determining which hydrophobin sequences are responsible for their self-assembly, we will know which regions can be modified to add new functionalities to hydrophobins or influence their self-assembly.

[14] Analysis of EPAC1 Inhibition by Non-cyclic Nucleotide Inhibitors: An NMR-Based Approach

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The Exchange Protein Activated by cAMP (EPAC) regulates various signaling pathways within eukaryotic cells [1] by acting as a guanine exchange factor for Rap1 GTPase [2]. Aberrant changes in the activity of EPAC isoform 1 (EPAC1) are associated with the development of ovarian and pancreatic cancer [3], [4]. Several inhibitors have been developed to target EPAC1 including an unconventional uncompetitive inhibitor called CE_3F_4R [5] which targets the enzyme:cAMP complex as well as more novel competitive inhibitors. The advantage of using non-cyclic nucleotide inhibitors is that they escape the termination phase resulting from phosphodiesterase (PDE) hydrolysis. However, the translational potential of these inhibitors is often marred by the weak affinity of these compounds for EPAC1. Here, we propose to build structure-activity relationships for known EPAC inhibitors with the goal to engineer new inhibitors with improved affinities. Using a combination of STD NMR and CHEmical Shift Covariance Analysis (CHESCA) [6], a structure-activity relationship for the various analogues will be developed and the effect of these inhibitors on EPAC1 will be mapped. Results from this study will aid in the development of novel and more potent inhibitors for EPAC1.

[1] B. VanSchouwen, R. Selvaratnam, F. Fogolari, and G. Melacini, "Role of dynamics in the autoinhibition and activation of the exchange protein directly activated by cyclic AMP (EPAC)," *J. Biol. Chem.*, vol. 286, no. 49, pp. 42655–42669, 2011. [2] J. de Rooij *et al.*, "Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP," *Nature*, vol. 396, no. 6710, pp. 474–477, 1998. [3] M. Gao *et al.*, "Epac1 knockdown inhibits the proliferation of ovarian cancer cells by inactivating AKT/Cyclin D1/CDK4 pathway in vitro and in vivo," *Med. Oncol.*, vol. 33, no. 7, p. 73, 2016. [4] X. Wang, C. Luo, X. Cheng, and M. Lu, "Lithium and an EPAC-specific inhibitor ESI-09 synergistically suppress pancreatic cancer cell proliferation and survival," *Acta Biochim. Biophys. Sin. (Shanghai).*, vol. 49, no. 7, pp. 573–580, May 2017. [5] S. Boulton, R. Selvaratnam, J.-P. Blondeau, F. Lezoualc'h, and G. Melacini, "Mechanism of Selective Enzyme Inhibition through Uncompetitive Regulation of an Allosteric Agonist," *J. Am. Chem. Soc.*, vol. 140, no. 30, pp. 9624–9637, Aug. 2018. [6] R. Selvaratnam, S. Chowdhury, B. VanSchouwen, and G. Melacini, "Mapping allostery through the covariance analysis of NMR chemical shifts," *Proc. Natl. Acad. Sci.*, vol. 108, no. 15, pp. 6133 LP – 6138, Apr. 2011.

[15] - Mechanism of Allosteric Inhibition of Plasmodium falciparum cGMP-Dependent Protein Kinase

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Malaria is a life-threatening disease responsible for about a million fatalities per year worldwide and about half of the world population is at risk. Most of the malaria deaths are caused by *Plasmodium falciparum*. An essential regulator for the development of *P. falciparum* is the cyclic GMP (cGMP) dependent protein kinase (*Pf*PKG). *Pf*PKG is composed of a regulatory domain and a catalytic domain. In the absence of cGMP, the regulatory domain inhibits the kinase domain. Upon cGMP binding to the regulatory domain, the inhibition is released and *Pf*PKG is activated. Targeting directly the active site of *Pf*PKG poses a major selectivity challenge, since the kinase catalytic domains are highly conserved among eukaryotes. One approach to circumvent this problem is to selectively target less conserved allosteric sites of *Pf*PKG, such as the cGMP-binding domains (CBDs), which can be achieved through cGMP-analogs. Here, we report the mechanism of action for cGMP-antagonists of *Pf*PKG to gain structural and dynamical insight on the otherwise elusive bound-inhibited state of PfPKG. This will provide another avenue to rationally design *Pf*PKG-selective

inhibitors for the treatment of malaria. NMR Chemical Shift Projection Analysis (CHESPA) of *Pf*PKG CBD-D with cGMP-analogs shows that the cGMP-antagonist inhibits *Pf*PKG not through a simple reversal of a two-state active-inactive equilibrium, but through multi-state equilibria sampling distinct holo-inactive intermediate that combine elements of both active and inactive states in different regions of CBD-D. This intermediate state exhibits a disengagement of the lid directly connected to the catalytic domain, making it inhibitory competent. Additionally, through the intact phosphate binding cassette and the pre-lid, the intermediate state provides the inhibitors the avenue for preserving a high affinity for CBD-D. NMR spin relaxation measurements further complement the CHESPA data by probing the dynamical changes within the CBD-D of *Pf*PKG that occur upon inhibition.

[16] Validation of a ^{13}C-satellite free approach to ^{1}H-qNMR

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Quantitative ^{1}H-NMR (^{1}H-qNMR) with an appropriate internal standard is a well-established method for the direct measurements on the analyte of interest. To provide metrologically reliable results, the premise of ^{1}H-qNMR relies on the careful selection of integrals, for both the analyte and the standard, in such a way that the selected integrals are free from exogenous interferences, usually belonging to impurities or ^{13}C-satellite signals of adjacent integrals. The simplest way to identify and avoid these interferences is to decouple the ^{13}C-satellites. Two decoupling schemes were explored to illustrate the benefits of ^{13}C-decoupling for qNMR: GARP and bi-level adiabatic broadband decoupling. GARP decoupling, in particular, has been beleaguered as deleterious to the NMR sample as a source of excessive heat. We present evidence to the contrary by showing that long relaxation delays and optimal acquisition times are key to avoid excessive sample heating under GARP decoupling, while bi-level adiabatic decoupling does not induce any detectable heating effects. We explored and confirmed all these aspects of ^{1}H{^{13}C}-qNMR through the quantitation of dimethyl terephthalate, angiotensin-II, zearalenone and $^{13}C_6$-ochratoxin A via both GARP and bi-level adiabatic decoupling at 400 MHz (9.4 T). In addition, we show that at high field (900 MHz - 21.1 T), only bi-level adiabatic decoupling can be successfully applied for ^{1}H{^{13}C}-qNMR. Our results show the versatility of various ^{13}C-decoupling strategies and their relevance as a complementary tool to ^{1}H-qNMR for purity determination in a metrological setting.

[17] Development of a near infrared calibration model and a nuclear magnetic resonance method for the rapid determination of lipid content in field peas

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The pea plant (*Pisum sativum* L.) is a leguminous cool-weather crop that is well suited for cultivation around the world. Pea crops are used as either forage or dried seed supplement in animal feed, and more recently, as a potential oilseed crop for vegetable oil and biodiesel. This study aimed to develop non-destructive and rapid methods for quantifying and analyzing lipids in improved (higher lipid content) pea crops. Seeds from different pea accessions were processed into small pieces (0.5mm^2) or ground powder before undergoing NMR or NIR analyses. Total lipid content ranged between 0.57%–3.45% and 1.3%–2.6% when analyzed with NMR and NIR, respectively. When compared to lipid content values of the same pea accessions previously determined by traditional extraction with lipid soluble solvents, high correlation values were observed: 0.77 (R^2 = 0.60), 0.56 (R^2 = 0.47), and 0.78 (R^2 = 0.62). Correlation values increased to 0.97 with an R^2 value of

0.99 by the use of the appropriate correction factor. The correction factor was the average ratio of the actual results of each traditional method to NMR. With respect to NIR, an overview of PLS regression analyses confirmed the possible application of this technology for the rapid determination of pea lipid content with trends fitting models often close to an R^2 of 0.95.

[18] Mechanochemical preparations of anion coordinated architectures and characterization by solid-state NMR

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The halogen bond has previously been explored as a versatile tool in crystal engineering field and anion coordination chemistry, with mechanochemical synthetic techniques having been shown to be a convenient route towards cocrystals. In an effort to expand our knowledge on the role of halogen bonding [1] in anion coordination, here we explore a series of cocrystals formed between 3-iodoethynylpyridine and 3-iodoethynylbenzoic acid [2] with halide salts. In total, we report 6 new crystal structures prepared by mechanochemical ball milling, with all structures exhibiting a C≡C-I···X^- (X = Cl, Br) halogen bond. Whereas cocrystals featuring a pyridine group favoured the formation of discrete entities, cocrystals featuring a benzoic acid group yielded both halogen and hydrogen bonds. The compounds studied herein were further characterized by ^{13}C and ^{31}P solid-state nuclear magnetic resonance, with the chemical shifts offering a clear and convenient method of identifying the occurrence of halogen bonding [3], using the product obtained from mechanochemical ball milling. Whereas the ^{31}P chemical shifts were quickly able to identify the occurrence of cocrystallization, ^{13}C solid-state NMR was able to identify both the occurrence of halogen bonding and hydrogen bonding. [4]

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[19] – Study of chalcogen-bonded cocrystals of pyridine N-oxide via x-ray diffraction and solid-state NMR

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Chalcogen bonds (ChB) are described as the net attractive interaction between an electrophilic region associated with a chalcogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity, according to the provisional recommendation of IUPAC [1]. The presence of two electron density depleted regions, σ-holes, along the covalent N-Ch bond axes differentiates the ChB from the halogen bond [2]. Herein, electrophilic chalcogen atoms of high polarizability, i.e., selenium atoms in dicyanoselenodiazole and dicyanotelluradiazole derivatives [3], were cocrystallized with a series of electron rich pyridine *N*-oxides. Most of the assemblies are driven by the Ch···O^- synthons as oxygen behaves as a bifurcated ChB acceptor with the two σ-holes present on the chalcogen atoms as characterized by single-crystal X-ray diffraction. Chemical shift tensors extracted from ^{77}Se and ^{125}Te CP/MAS NMR experiments provide a direct correlation between the NMR parameter and ChB geometries.

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[20] - Investigating Water Dynamics Adsorbed in Pillared-Layer Metal-Organic Frameworks via ^{2}H Solid-State NMR Spectroscopy

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Metal-organic frameworks (MOFs) have recently emerged as promising candidates for carbon capture and sequestration. The most striking properties of MOFs are their tunable nature and high surface areas.[1] However, some MOFs have a serious drawback which is the low stability and decreased adsorption capacity upon contact with water. In terms of practical applications, this is a major challenge, since water is ubiquitous in air and in many other industrial applications.[2] Therefore, understanding the effect of water on MOFs is of importance for the design and development of MOFs for practical applications.

Only few studies on water adsorption in MOFs have been reported. Solid-state nuclear magnetic resonance (SSNMR) spectroscopy is a powerful technique that provides valuable insight into the short-range structure and guest molecule dynamics.[3]

Herein, behavior of adsorbed water and its dynamics in a series of pillared-layer MOFs[4] has been studied by ^{2}H SSNMR spectroscopy. The D_2O motional behavior within the MOFs can be determined via simulations of variable-temperature ^{2}H SSNMR spectra. The results revealed that water molecules undergo a π flip-flop rotation around its molecular axis and a four-site jump around the pore cross-section. At temperatures above 20 °C, both motions occur in the fast regime. Upon decreasing the temperature, the exchange rates of both motions decrease until temperature reaches -40 °C, where the four-site jump ceased. We found that the water dynamics is related with the size and the functional groups decorating the pores. While in a small pore the jump rates start decreasing at 0 °C, in larger pores the jump rate started decreasing at -20 °C. This indicates that the water interacts more strongly with the framework when the pore is smaller. When the pores are decorated with amino groups, the jump rate starts decreasing already at 20 °C. The presence of these functional groups decreased the size of the pores and the motion became even more limited.

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[21] - Study of long-range Li+ diffusivity in Li1.5Al0.5Ge1.5(PO4)3 with BPP-PFG NMR

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Sodium (NA) Super Ionic Conductor (NASICON)[1] type $Li_{1+x}Al_xGe_{2-x}(PO_4)_3$ (LAGP) is one promising candidate to replace the flammable liquid organic electrolytes for safer lithium ion batteries (LIBs). This material is a decent conductor with 4×10^{-4} S/cm at room temperature compared to perovskite $Li_{3x}La_{2/3-x}TiO_3$ (highest conductivity reported 2.7×10^{-4} S/cm), Li Super Ionic Conductor (LISICON) (~10^{-6}-10^{-5} S/cm), and Lithium Phosphorus OxyNitride (LiPON) type (~10^{-6} S/cm) solid electrolytes[2]. Although LAGP has lower ionic conductivity compared to argyrodite Li_6PS_5X (X= Cl, Br, I) electrolytes (~10^{-3} S/cm)[2], it is stable under cabinet atmosphere[2], which could be beneficial for applications.

LAGP structure[3], as well as the short-range lithium ions migration pathway within the unit cell[4], have been well studied in the past few decades, however there was no adequate research on long-range Li^+ motion on

this material. Characterizing the long-range Li+ diffusion is more critical to understand the Li^+ transportation in a real battery. Hayamizu and Seki (2017)[5] investigated the long-range Li^+ diffusion in $Li_{1.5}Al_{0.5}Ge_{1.5}(PO_4)_3$ by using pulsed-field-gradient (PFG) NMR, in which the pulse sequence applied was incorporated with stimulated echo (STE)[6] with varying the gradient pulse length δ and the activation energy for the long-range diffusion process is reported to be ~ 0.17 eV[5]. It is believed that the application of STE-PFG in this system might cause eddy current effects due to strong and long gradient pulses. One approach to minimize eddy currents generated during the rapid magnetic field change was to apply bipolar gradient pulse (BPP) coupled with longitudinal eddy current delay (LED)[6]. Instead of applying long and strong gradient pulse, BPP-LED sequence split the long gradient pulse into two short gradient pulses separated by an 180° RF pulse, which is demonstrated to be effective and promising in diminishing STE-induced artifacts[6].

Diffusivity analysis of synthesized $Li_{1.5}Al_{0.5}Ge_{1.5}(PO_4)_3$ sample has been performed using BPP-LED-PFG NMR sequence, and the activation energy of Li^+ diffusion was extrapolated to be 0.29 (±0.01) eV. Parallel analysis was conducted with commercial $Li_{1.5}Al_{0.5}Ge_{1.5}(PO_4)_3$ sample from Toshima Manufacturing Co., Ltd, the same manufacturer that provided samples in Hayamizu's studies[5]. However, the activation energy from the parallel study is 0.283 (±0.003) eV, which is higher but more reasonable compared to the energy barrier reported by Hayamizu et al. This large discrepancy observed in the activation energy of the same material could be caused by the more significant eddy current effects induced using STE in the PFG experiments. PFG NMR method incorporated with BPP-LED component is proved to be a suitable and reliable approach for this class of materials. Comparing activation energies between short-range and long-range diffusivities could also be beneficial in understanding Li^+ transport mechanisms in this material.

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[22] - The WURST of the MOFs

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The BRAINCP-WCPMG technique of Schurko et al.[1] uses broadband WURST pulses to excite wide powder patterns. We use the technique applied to static ^{113}Cd SSNMR to study cadmium-containing metal-organic frameworks.[2] The very different cadmium environments in various metal organic frameworks (MOFs) and in pure cadmium acetate are reflected in their distinct CSA patterns. The technique is also applied to ^{199}Hg, where WURST and other solid-state NMR and computational techniques are used to study a square grid (*sql*) mercury(II) imidazolate framework likely stabilized by C-H···Hg contacts. These contacts provide an explanation of why the *sql* topology is more stable than the previously reported interpenetrated diamondoid (*dia)* framework, now apparently the first example of a disappearing polymorph in MOF chemistry.

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[23] - The effect of the antimicrobial peptide aurein 1.2 on erythrocyte ghosts membranes: deuterium labelling and in situ solid-state NMR studies

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Antimicrobial resistance affects many people's lives as bacteria have become resistant to antibiotics. There is, therefore, an urgent need to propose new drugs with novel action mechanisms. Antimicrobial peptides (AMPs) are considered a promising therapeutic avenue since they act by perturbing the bacterial cell membrane. AMPs must not show any hemolytic effect on human cells such as red blood cells. It is thus of great importance to verify and understand their potential action on eukaryotic cells.
In our study, we have established a protocol to ^{2}H-label equine erythrocyte ghost membranes using deuterated fatty acids, and cell membrane stability was studied by solid-state NMR. The integrity of the phospholipid bilayer was assessed by ^{31}P NMR, and the lipid membrane dynamics were studied by ^{2}H NMR under magic angle spinning (MAS) conditions. About 20% of deuterated fatty acids were incorporated into the ghosts' membranes as determined by GC-MS, and the headgroup profile of the lipid bilayer appeared to be unaltered as determined by solution ^{31}P NMR. The hemolytic activity of the antimicrobial peptide aurein 1.2 on the ghosts' membranes was assessed by the analysis of ^{2}H spectral sidebands as a function of peptide concentration, as well as by static ^{31}P NMR and leakage assays. The hemolytic activity appears at concentrations lower than the minimal inhibitory concentration (MIC) values reported for *Escherichia coli* and *Bacillus subtilis*, which questions the relevance of using such peptide for the treatment of human infections.

[24] - What If We Lock the Spider Wrapping Silk Protein

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Aciniform silk, popularly known as wrapping silk, is a class of spider silk which is distinct from the other classes in its composition, structure, mechanical properties and physical role. Aciniform, applied for prey-wrapping and egg-case architecture, is renowned for its exceptional strength and extensibility, making it the toughest silk type and a form that is highly relevant for bioengineering. Unlike other silks that have many repetitions of short motifs (made up of a few residues), the core domain of aciniform silk from *Argiope trifasciata* comprises a highly conserved 200 amino acid-long unit (the 'W unit') repeated relatively few times (≥14). Previous studies from our lab suggest a compact, a-helix-rich *'beads-on-a-string'* architecture for the soluble W unit. Relative instability of one of these helices (H5) noticed in the soluble state and a significant amount of a-helicity retained in the fibre form even after the structural transition led to the hypothesis that localized unfolding originating from this relatively unstable helix could initiate decompaction of W units, favouring an increase in intermolecular interactions, which in turn could trigger β-sheet formation during fibrillogenesis. To test this hypothesis, we introduced two Ser-to-Cys mutations in the W unit: one in H5 and another in the proximal globular core region underneath this helix. This was engineered to form a disulfide-staple to lock the helix to the core when oxidized. Compellingly, the oxidized ("locked") protein forms very thin, irregularly patterned, highly transparent and tenuous fibril-like structures, while the reduced ("unlocked") mutant is fully functional, resembling the native spidroin, forming highly extensible yet strong regularly-patterned opaque fibres. Backbone NMR assignments and ^{15}N-based relaxation experiments are being used to elucidate the effect of the disulphide lock on secondary structure, folding and dynamics of the modular W unit at the residue-level. This is allowing us to test the role of helix H5 in the fibrillogenesis of aciniform spidroin. Understanding of the initiating trigger and the process of fibrillogenesis, in turn, will facilitate the bioengineering of aciniform silk with modified mechanical behaviour.

[25] - Efficient Approaches for Addressing Spectral Ambiguities in Computer Assisted Structure Elucidation (CASE) Systems

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Since their development over 50 years ago, Computer Assisted Structure Elucidation (CASE) systems (or Expert Systems, ES) have significantly facilitated the de novo structure elucidation of both natural and synthesized organic compounds, especially in cases where using the traditional (manual) methods would has been very challenging or even impossible to perform [1,2]. Current ES are based on 1D and 2D NMR spectra, given that the molecular formula has already been determined by HR-MS. At present, there are several free and commercially available CASE systems offering the following main advantages [3]: i) ES deliver all (without any exception) structures which can be deduced from a given set of NMR data; ii) Application of fast empirical methods for NMR chemical shift prediction allows the program to select the most probable structure; iii) If necessary, DFT based chemical shift calculations are used to confirm the selected structure; iiii) ES are now capable of suggesting a 3D model of the elucidated structure.

Despite all the developments, ES are still susceptible to a series of limitations which impede structure elucidation by a human expert. These limitations are mainly associated with the ambiguity of the experimental data, as well as the overlapping characteristic chemical shift values in NMR spectra. Experimental ambiguity

can result from low resolution 2D spectra, which prohibits the confident assignment of the closely spaced signals. Further, ambiguity can often be due to the hybridization state of carbon nuclei that appear in the same regions of NMR spectra. For example, a ^{13}C NMR signal observed at 90 ppm can belong either to a sp^2 or a sp^3-hybridized carbon connected to one or two oxygen atoms. If a molecule contains atoms that can have variable valences (e.g. N and/or P), all their possible valences should be explored during structure generation. Ambiguity can also be observed in some cases where carbon nuclei show no HMBC or COSY correlations; this is especially evident in molecules with hydrogen deficiency which consequently appear as "floating", i.e. potentially could be connected to any other atom. The presence of "floating" atoms significantly increases both the size of the output and the time of structure generation.

In order to remove uncertainty from spectroscopic data, additional experiments are usually carried out; for example high resolution 2D NMR spectra, such as highly inflated NUS or band selective spectra are collected to reliably assign the correlations. Additional spectroscopic data (IR, Raman, UV-Vis) could help to identify the characteristic groups and resolve the hybridization or valence status. However, such solutions are not useful in all cases.

In such cases, the only remaining solution is the exhaustive investigation of all alternatives ensuing from the presence of any ambiguity. For example, if there are five carbon nuclei with signals in the range of 70-120 ppm, then 2^5=32 combinations of hybridization (sp^3 or sp^2) have to be generated and checked. This could significantly extend the structure generation time.

In this poster by taking advantage of recent programming developments, we will present approaches that enable structure elucidation, under conditions where the initial data contains many ambiguous assumptions. Examples will be presented, and the strengths together with the limitations of each approach will be discussed.

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