NMRDG Postgraduate Meeting 2025

UCL School of Pharmacy, 29-39 Brunswick Square, London

Monday 16th June 2025

15:30 Registration desk opens (**SoP Reception**)
Poster setup

16:15 Welcome & Plenary 1 (**G10 Chandler House***)
Decoding amyloid signalling in necroptosis by cryoprobe-detected solid-state NMR
Miguel Mompeán (Institute of Physical Chemistry, Madrid)

17:00 Poster session 1 (**SoP Square Lounge**)

18:00 Pizza and drinks (**SoP JCR Bar**)

* Chandler House is a short walk from the School of Pharmacy:

Tuesday 17th June 2025

9:00 Arrival and coffee (**SoP Square Lounge**)

9:30 Plenary 2 (**SoP Maplethorpe Lecture Theatre**)
Order and disorder in protein-RNA interactions: telling the tale of the La-related proteins through NMR and biophysical characterisations
Sasi Conte (King's College London)

10:10 *^{19}F- and ^{18}F-labelled carnitines: a dual approach using NMR and PET imaging to visualize carnitine utilisation and metabolism*
Ella-May Hards (King's College London)

10:30 Flash presentations

10:40 Coffee

11:00 *Chemical shift imaging NMR to investigate DNA i-motif folding*
Jonathan Hewitt (University of East Anglia)

11:20 *Investigating water-accessibility in holocellulose nanofibrils using fast MAS solid-state NMR*
Jairah Lubay (University of Warwick)

11:40 *Characterising the intrinsically-disordered C-terminal domain of calreticulin by solution-state NMR spectroscopy*
Kleopatra Savvidou (University College London)

12:00 Discussion forum

12:20 Lunch

13:00 *Understanding water adsorption in CALF-20 via ^{1}H, ^{2}H and ^{17}O NMR spectroscopy for carbon capture applications*
Louie Lovell (University of Birmingham)

13:20 *Using TROSY-based NMR experiments and deep learning to characterise aromatic residues in large proteins*
Darshil Kapadia (University College London)

13:40 *Towards an experimental toolbox for MAS NMR of paramagnetic lanthanide & actinide organometallics*
Katherine Bonham (University of Manchester)

14:00 *Generative machine learning for automating structure elucidation in synthesis*
Zheqi Jin (University of Bristol)

14:20 Poster session 2 + coffee

15:20 *Solid-state NMR of roxithromycin in crystalline and gel state*
Svetlana Pavlovic (University of Warwick)

15:40 *NMR characterisation of the intrinsically disordered protein JPT2: structural insights and microtubule interactions*
Kirsten Silvey (UCL)

14:00 *Enhanced signal discrimination in SABRE hyperpolarised ^{1}H benchtop NMR*
Gregory Yule (University of York)

16:20 Prizes and closing remarks

16:30 Meeting closes

Plenary Talks

PL1	Miguel Mompeán	Decoding amyloid signalling in necroptosis by cryoprobe-detected solid-state NMR
PL2	Sasi Conte	Order and disorder in protein-RNA interactions: telling the tale of the La-related proteins through NMR and biophysical characterisations

Oral Presentations

O1	Ella-May Hards	^{19}F- and ^{18}F-labelled carnitines: a dual approach using NMR and PET imaging to visualize carnitine utilisation and metabolism
O2	Jonathan Hewitt	Chemical Shift Imaging NMR to Investigate DNA i-Motif Folding
O3	Jairah Lubay	Investigating Water-Accessibility in holocellulose Nanofibrils using Fast MAS Solid-State NMR
O4	Kleopatra Savvidou	Characterising the intrinsically disordered C-terminal domain of calreticulin by solution-state NMR spectroscopy
O5	Louie Lovell	Understanding water adsorption in CALF-20 via ^{1}H, ^{2}H and ^{17}O NMR spectroscopy for carbon capture applications
O6	Darshil Kapadia	Using Deep Learning and TROSY-based NMR to Characterise Aromatic Residues in Large Proteins
O7	K.L. Bonham	Towards an experimental toolbox for MAS NMR of paramagnetic Lanthanide & Actinide organometallics
O8	Zheqi Jin	Generative Machine Learning for Automating Structure Elucidation in Synthesis
O9	Svetlana Pavlović	Solid-State NMR of Roxithromycin in Crystalline and Gel State
O10	Kirsten Silvey	NMR Characterisation of the Intrinsically Disordered Protein JPT2: Structural Insights and Microtubule Interactions
O11	Gregory J. Yule	Enhanced signal discrimination in SABRE hyperpolarised ^{1}H benchtop NMR

Posters

P1*	Anupama Acharya	Nuclear spin-state transport in nonlinear kinetic processes
P2*	Jack Bercovici	Ultrawide and multinuclear NMR: Pushing the boundaries of existing hardware with Seedless optimal control pulses
P3	Jasmine Cornish	A drug-like small-molecule selectively induces multimerisation of an oncogenic disordered protein
P4*	Ronan P. Cosquer	Characterising ^{13}C Spectral Assignments and Substituent Distributions of Hydroxypropylmethylcellulose Acetyl Succinate Using Dynamic Nuclear Polarisation Nuclear Magnetic Resonance Spectroscopy
P5*	Arthur Cottrell-Purser	Slicing Solvent Signals Out of Mixture Spectra
P6	Ellie Davies	Spin-System Selective 2DJ Spectroscopy
P7*	Ireno Demmangngewa	Structural characterisation of co-translational folding H-Ras intermediates by ^{19}F NMR
P8*	Danni Dong	NMR studies of MERS-CoV non-structural protein 10 as a potential drug target
P9*	Daniel Gorman	Improved Light Uniformity using Etched NMR tubes for high throughput NMR experiments
P10	Sophia Hazlett	Quantitative Analysis of Fast Ligand Dissociation Kinetics Using ^{19}F $R_{1\rho}$ Relaxation Dispersion
P11*	Izzy Hehir	*In situ* SABRE hyperpolarisation for mixture analysis in benchtop NMR spectroscopy
P12*	James London	Sequence Structure Relationships of C-terminal linker histone tails and their impact on DNA condensation
P13	Faud Mahamud	Characterisation of Dynamic Hairpin DNA Switches in *E-coli*
P14	Meshezabeel A. Narciso	Development of a ^{19}F NMR fragment screening platform at UCL
P15*	Neelam Sehrawat	Modelling dissipative magnetization exchange dynamics in magnetic resonance
P16	Ananya Singh	Solid-state NMR spectroscopy reveals temperature-dependent glucan remodelling in fungi
P17	James Williamson	Differential Self-Assembly of Sequence-Isomeric Phosphoestamers
P18	Calvin Yiu	Web-IMPRESSION: Fast, Accessible, DFT Accurate NMR Predictions for Chemical Shifts and Couplings At Your Fingertips

* Flash presentation

O1. ^{19}F- and ^{18}F-labelled carnitines: a dual approach using NMR and PET imaging to visualize carnitine utilisation and metabolism

Ella-May Hards[1], Richard Edwards[1], Sofia N. dos Santos[1], Thomas R. Eykyn[1,2,3] and Timothy H. Witney[1]

[1]School of Biomedical Engineering & Imaging Sciences, King's College London, St Thomas' Hospital, London, SE1 7EH, UK. [2]British Heart Foundation Centre of Research Excellence, King's College London, UK. [3]Department of Chemistry, King's College London, UK

Introduction

L-carnitine (LC) is crucial for β-oxidation, translocating fatty acids across the inner and outer mitochondrial membranes[1]. Altered carnitine transport and metabolism are associated with heart disease[2] and cancer[3]. Here, we developed a ^{18}F or ^{19}F labelled carnitine derivative, fluoromethylcarnitine (FMC), to investigate its uptake in non-small cell lung cancer (NSCLC) *in vivo* using PET and its downstream metabolism *in vitro* via ^{19}F-NMR.

Methods

Dynamic PET/CT imaging (Mediso) was performed following i.v. injection of 3 MBq [^{18}F]FMC in mice bearing ~100 mm^3 subcutaneous H460 tumours (*n*=4/group). For metabolite analysis, 50 µM ^{19}F-FMC in RPMI was incubated with H460 cells for 24 hours (h) before harvesting, with ^{1}H decoupled ^{19}F NMR spectroscopy performed using an ultra-shielded Bruker 14.1 T spectrometer equipped with a QCI-F cryoprobe at 298 K.

Results and Discussion

[^{18}F]FMC PET imaging showed healthy tissue distribution that closely matched LC biodistribution, with rapid extraction by the kidneys and redistribution to the liver. There was high [^{18}F]FMC uptake in H460 tumours (4.1 %ID/g at 2h), exceeding levels in blood and muscle. This allowed clear tumour delineation in PET images. The metabolic fate of [^{19}F]FMC, which was unknown, was confirmed using ^{19}F-NMR in H460 cells incubated with FMC. A characteristic triplet displaying a 2-bond scalar coupling $^2J_{FN}$ to the symmetric spin-1 ^{14}N nucleus showed novel fluorinated species that retained the quaternary ammonium moiety, indicating intracellular metabolism into likely acetyl-FMC and acyl-FMC-conjugated fatty acids.

Conclusion

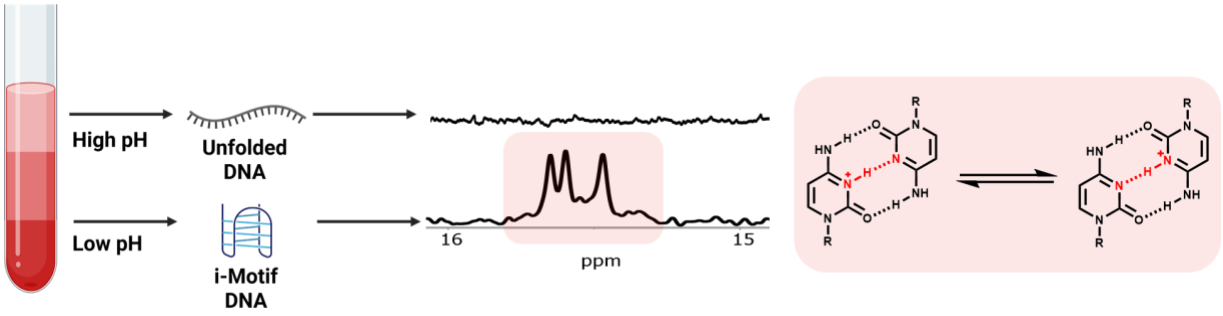
This dual approach, using ^{19}F- and ^{18}F-labeled carnitine derivatives, provides unique insights into perturbed LC metabolism in NSCLC, from its intracellular transport and metabolism to whole-body utilisation.

O2. Chemical Shift Imaging NMR to Investigate DNA i-Motif Folding

Jonathan Hewitt,[1] Zoë A.E. Waller,[2] and Matthew Wallace[1]

[1]School of Chemistry, Pharmacy and Pharmacology, University of East Anglia, England. [2]School of Pharmacy, University College London, England

Nucleic acid sequences rich with cytosine are capable of folding into non-canonical intercalated structures called i-motifs (i-Ms) upon hemi-protonation of the cytosine bases. Since their discovery in 1993,[1] i-Ms have garnered interest in the pharmaceutical and biomedical fields, with their role in disease development and their potentail as therapeutic targets. Their optimal utilisation, however, has been limited by incomplete understanding of their stability and folding behaviour in physiological contexts. As protonation is a pre-requisite for their formation, pH is a commonly used metric indicative of i-M dynamics. The pH at which *ca.* 50% of the oligonucleotide is folded – transitional pH (pH_T) – offers an easy way to systemcatically compare folding behaviours of different strands. By utilising NMR chemical shift imaging (CSI),[2] a pH gradient can be established within a single NMR tube containing an i-M-forming sequence. The pH can be tailored to span a range which allows for a transition from a fully unfolded oligonucleotide sequence (*e.g.*, at low pH), to maximum folding, through to fully unfolded again (at a high pH). This novel method of probing DNA folding is capable of acquiring more data points, and thus providing more time-resolved information than current techniques (*e.g.*, circular dichroism and UV spectroscopy). This technique can be futher optimised to quantitatively probe the effects of potential stabilising and destabilising agents such as metal ions, molecular crowding agents and ligands.



References

(1) Gehring, K.; Leroy, J.-L.; Guéron, M. *Nature* **1993**, *363* (6429), 561–565.

(2) Wallace, M.; Adams, D. J.; Iggo, J. A. *Anal Chem* **2018**, *90* (6), 4160–4166

O3. Investigating Water-Accessibility in holocellulose Nanofibrils using Fast MAS Solid-State NMR

Jairah Lubay,[1] Rosalie Cresswell,[2] Ray Dupree,[2] Parveen Kumar Deralia,[3] Paul Dupree,[3] Steven P. Brown,[2] Józef R. Lewandowski[1]

[1]Department of Chemistry, University of Warwick, Coventry, UK. [2]Department of Physics, University of Warwick, Coventry, UK. [3]Department of Biochemistry, University of Cambridge, Cambridge, UK

Cellulose is the primary structural component of plant cell walls, composed of β-1,4-glucose chains that assemble into microfibrils. Understanding how water interacts with cellulose at the molecular level is critical for applications in biofuels, biomaterials and plant biology. However, probing site-specific water accessibility in cellulose microfibrils remains challenging due to the heterogeneous nature of the plant cell wall and spectral overlap in conventional solid-state NMR experiments.

We use xylanase-treated holocellulose nanofibrils (hCNFs) of poplar wood and 100 kHz magic angle spinning (MAS) solid-state NMR at 100 kHz to perform 2D T_2-filtered water-edited inverse cross-polarisation experiments with variable mixing times.[1,2] Performing water-edited experiments at fast spinning has several benefits compared to performing them in a more traditional moderate spinning frequency: 1. The contribution of spin diffusion is minimised, rendering the Nuclear Overhauser effect (NOE) as one of the most important mechanisms for polarisation transfer. This results in a much better spatial resolution compared to what can be obtained at low spinning; 2. Proton detection provides a significant sensitivity boost, accelerating these typically insensitive experiments (from days to hours for the discussed here samples); 3. Including the ^{1}H dimension helps to disperse signals for different cellulose environments.

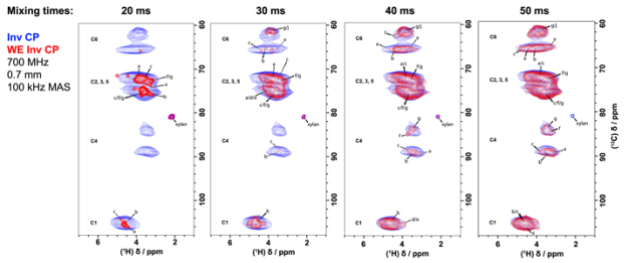


Figure 1: 2D hCH Inverse CP (blue). 2D Water-Edited hCH Inverse CP (red) with different mixing times

Our results aid to establish the spatial hierarchy for the different cellulose environments within the fibrils and highlight the potential of fast MAS NMR for high-resolution, site-specific studies of water and biopolymer interactions. This approach lays a foundation for future investigations into hydration properties and solvent exposure of complex cell wall materials, which can also be extended to other biological molecules such as proteins.

References

(1) White, P.B., Wang, T., Park, Y.B., Cosgrove, D.J. and Hong, M., 2014. Water–polysaccharide interactions in the primary cell wall of Arabidopsis thaliana from polarization transfer solid-state NMR. Journal of the American Chemical Society, 136(29), pp.10399-10409.

(2) Bougault, C., Ayala, I., Vollmer, W., Simorre, J.P. and Schanda, P., 2019. Studying intact bacterial peptidoglycan by proton-detected NMR spectroscopy at 100 kHz MAS frequency. Journal of structural biology, 206(1), pp.66-72.

O4. Characterising the intrinsically disordered C-terminal domain of calreticulin by solution-state NMR spectroscopy

Kleopatra Savvidou,[1] Jiwoo Kim,[1] Pete Simpson,[2] Christopher A. Waudby[1]

[1]School of Pharmacy, University College London, London. [2]MRC Biomedical NMR Center, Francis and Crick Institute, London

Proteins that are destined for the secretory pathway are translocated co-translationally into the ER lumen for further processing prior to acquiring their native structure. The lumen provides an oxidising and calcium-rich environment with an abundance of molecular chaperones. In parallel with translocation, nascent polypeptides undergo N-linked glycosylation, which mediates progression along the glycan quality control (GQC) pathway. Proteins that ultimately fail to fold are directed to the ER-associated degradation (ERAD) pathway for proteasomal degradation[1]. Calreticulin (CRT) is a bifunctional chaperone protein that assists protein folding along the GQC pathway and has been reported to bind calcium[1]. CRT is comprised of three domains: a split N- and C-terminal lectin domain; an extended, proline-rich 'P' domain; and an acidic C- terminal domain (CTD), predicted to be intrinsically disorded[1,2]. Structural studies have been performed on the lectin and P domains of CRT individually, but little experimental information on the CTD is known[1,2,3]. Here, we have generated constructs of the human CRT CTD and successfully established the first protocols for its expression and purification. We will report our progress for complete assignment of the challenging low-complexity sequence using combination of standard triple-resonance and HNN/HNH experiments. This further permits the analysis of the residual structure and backbone dynamics using ^{15}N relaxation measurements. Additionally, we investigate its interactions with divalent cations to provide mapping binding sites and affinities and examining their impact on the residual structure and dynamics. Together, this prepares the way to a complete characterisation of the internal dynamics and interactions of the domain.

References

1. Kozlov, G. & Gehring, K. Calnexin cycle - structural features of the ER chaperone system. *FEBS J.* 287, 4322–4340 (2020).

2. Chouquet, A. *et al.* X-ray structure of the human calreticulin globular domain reveals a peptide-binding area and suggests a multi-molecular mechanism. *PLoS One* 6, e17886 (2011).

3. Villamil Giraldo, A. M. *et al.* The structure of calreticulin C-terminal domain is modulated by physiological variations of calcium concentration. *J. Biol. Chem.* 285, 4544–4553 (2010).

O5. Understanding water adsorption in CALF-20 via ^{1}H, ^{2}H and ^{17}O NMR spectroscopy for carbon capture applications

Louie Lovell, Danielle Johnson, Shrestha Banerjee, Neil Champness, Dominik Kubicki, Melanie Britton

School of Chemistry, University of Birmingham, Birmingham, B15 2TT, United Kingdom

The removal of carbon dioxide (CO_2) from our atmosphere is pivotal in the battle against climate change. CALF-20 is a metal-organic framework that is a highly promising material as a solid sorbent of CO_2.[1] It is durable, scalable and has a relatively high capacity and selectivity for CO_2, even in wet gases. The competitive adsorption of CO_2 and H_2O is an important factor in its use as a carbon capture material, however, the interaction between the water and the CALF-20 framework is poorly understood.[2] In this work, we use static and Magic Angle Spinning (MAS) NMR to experimentally determine, for the first time, the atomic-level structure of confined water in this material, characterise its absorption with isotope specificity and quantify its dynamics, as a function of relative humidity and time.

We prepared CALF-20 samples, exposed to a range of relative humidities (%RH = 0 - 100) and employed ^{1}H, ^{2}H and ^{17}O NMR to monitor local structure. Figure 1a shows a set of representative static and MAS ^{1}H spectra at selected %RH. We find that ^{1}H NMR line shapes are highly sensitive to %RH, suggesting changes in the underlying local structure at different humidity levels. Finally, a combination of static variable-temperature ^{2}H and ^{17}O MAS measurements quantified water dynamics, which indicated water is substantially hindered compared to bulk liquid water.

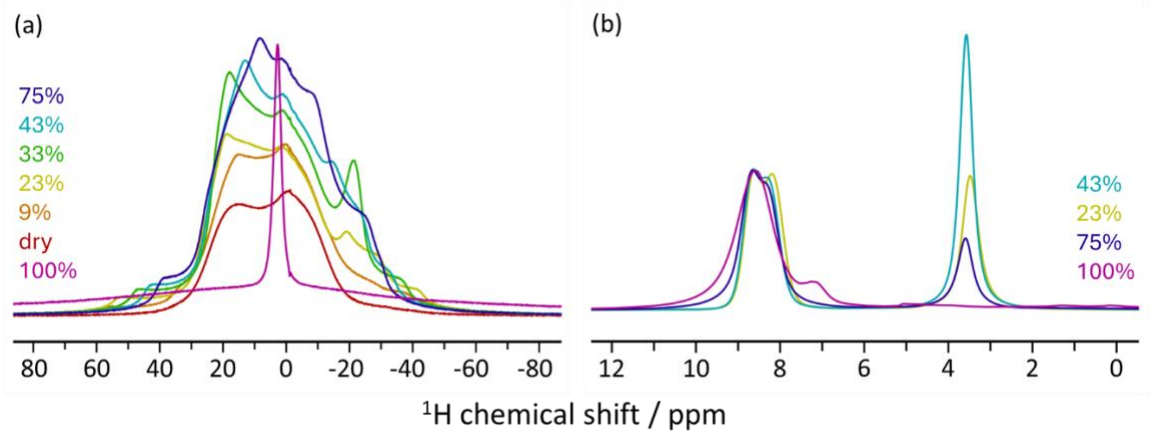


Figure 1. (a) Static and (b) MAS ^{1}H NMR spectra of CALF-20 after exposure to a range of relative humidities.

References

1 J. Bin Lin, et. al., *Science (1979)*, 2021, **374**, 1464–1469.

2 C.-H. Ho et. al., *ACS Appl. Mater. Interfaces*, 2023, **15**, 48295.

O6. Using Deep Learning and TROSY-based NMR to Characterise Aromatic Residues in Large Proteins

Darshil Kapadia[†1,2], Charles Buchanan[†1,2,3], Vaibhav Shukla[1,2], Alon Wenger[4], Rina Rosenzweig[4], D Flemming Hansen*[1,2]

[1]Department of Structural and Molecular Biology, University College London, UK. [2]Francis Crick Institute, London, UK. [3]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany. [4]Department of Chemical and Structural Biology, Weizmann Institute of Science, Israel

Aromatic residues are critical for biological processes such as phase separation, ligand binding, protein folding and enzymatic activity. However, aromatic moieties are challenging to study by NMR for three major reasons: (1) they display pronounced peak splitting due to large scalar couplings; (2) line broadening due to fast relaxation, and (3) highly overlapped spectra. Aromatic Transverse Optimised SpectroscopY (TROSY) has improved the resolution of these spectra when combined with advanced labelling strategies[1,2]. However the study of larger proteins, while preserving the structure and function of aromatic residues, remains inaccessible to modern NMR experiments. Here we present a deep neural network (DNN) capable of processing aromatic TROSY NMR spectra to yield high resolution, decoupled ^{13}C-^{1}H correlation maps on uniformly ^{13}C labelled proteins, allowing functional and mechanistic insights into aromatic residues of substantially larger proteins.

A major bottleneck for deep learning is generally the need for large, high quality, curated datasets. Recently, we and others have demonstrated that the robust mathematical and physical frameworks underpinning NMR enable synthesis of unlimited realistic training spectra for a specific NMR processing task. Thus, we can train a DNN, in this case FID-Net-2 [3], to produce high-resolution datasets or for analysis tasks. Crucially, FID-Net-2 accurately predicts uncertainties, which resolves a common reliability problem of deep learning approaches[3]. We move away from generating human-interpretable spectra to designing sensitive, information-dense NMR experiments, perfect for pattern recognition by deep learning approaches. For example, we provide an additional spectral plane with coupling evolution to aid the model in distinguishing multiplets from other peaks. Additionally, in prioritising sensitivity, we don't filter the aromatic anti-TROSY state and instead task our model to recognise and collapse these signatures.

We generated approximately 60 million 2D training spectra and trained our network on two NVIDIA A100 GPUs. We demonstrate successful results on a variety of targets, including a 50 kDa biological chaperone, and a titration experiment between Hsp70 and one if its client recruiting proteins. Presenting this ability to study aromatic moieties in significantly larger proteins, we hope to enable a new class of aromatic-led interactions to be probed with high sensitivity. This work presents an exciting combination of NMR and deep learning. NMR's robust theoretical frameworks allow unlimited training data generation, the fidelity of pulse programs can be exploited to generate unique 3-dimensional features useful for a specific processing task, and the ability to predict uncertainties does not compromise downstream analysis. We anticipate deep learning approaches to become a staple of NMR processing, to create a surge of new approaches to study dynamics, and to unlock a larger part of the proteome for NMR.

References: (1) Pervushin, K., et al. (1998) *J. Am. Chem. Soc.* **120**(25): 6394-6400. (2) Miyanoiri, Y., et al. (2020). Biochim. Biophys. Acta **1864**: 129439. (3) Shukla, V. K., et al. (2024). *Sci. Adv.* **10**: eadr2155. (4) Karunanithy, G. and D. F. Hansen (2021). *J Biomol NMR* **75**: 179-191. (5) Karunanithy, G., et al. (2024). *Nature Commun.* **15**(1): 5073

O7. Towards an experimental toolbox for MAS NMR of paramagnetic Lanthanide & Actinide organometallics

K.L. Bonham;[1] J. Baldwin,[2] T.R.C. Thompson,[2] G.K. Gransbury,[2] G.F.S. Whitehead,[2] I.J. Vitorica-Yrezabal,[2] N.F. Chilton,[2,3] D.P. Mills;[2] H. Futcher,[2] A.J. Wooles,[2] J.A. Seed,[2] S.T. Liddle;[2] R. MacKenzie,[2] C. Deakin,[2] C. Goodwin;[2] D. Lee[1]

[1]Dept. Chem. Eng., Univ. Manchester, Oxford Rd, Manchester. M13 9PL, UK. [2]Dept. Chem., Univ. Manchester, Oxford Rd, Manchester. M13 9PL, UK. [3]Res. Sch. Chem., Au. Natl. Univ., Sullivans Creek Rd, Canberra, ACT. 2601, AU.

The Lanthanides (Ln) & Actinides (Ac) are highly relevant to technologies to realise a low-carbon future, such as high-performance magnetic materials and sustainable energy generation methods[1,2]. Rational design of such materials requires a thorough understanding of their structure & bonding, which is strongly dependent on the materials' electronic arrangement. This would seem to indicate NMR is an ideal spectroscopic characterisation tool owing to the influence of electron density distribution on chemical shifts, yet the properties that give these materials their molecular engineering potential are the same properties that frustrate their analysis, through NMR and calculation methods alike[3].

This presentation shall summarise the author's experience on the limitations of "standard" solid-state NMR analytical approaches across a variety of Ln & Ac complexes, and the building of a toolbox (e.g. WCPMG-MAS[4], Adiabatic Double-Echo[5]) with which to analyse these materials (including comparison of where the techniques would hinder the analysis rather than help; see Figure 1 for one example).

References

(**1**) Q. Yang, T. Zhong, Z. Tu, L. Zhu, M. Wu, X.C. Zeng, *Adv. Sci.*, 2018, **6**(1), 1801572. DOI: 10.1002/advs.201801572. (**2**) R.M. Grossi, *Climate Change and Nuclear Power 2024: Financing Nuclear Energy in Low Carbon Transitions*, International Atomic Energy Agency, Vienna, 2024 DOI: 10.61092/iaea.sgyh-rjoq. (**3**) A.J. Pell, G. Pintacuda, C.P. Grey, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2019, **111**, 1–271. DOI: 10.1016/j.pnmrs.2018.05.001. (**4**) J. Koppe, M. Bußkamp and M.R. Hansen, J. Phys Chem A, 125(25), 5643-5649. DOI: 10.1021/acs.jpca.1c02958. (**5**) R. Aleksis, J.P. Carvalho, A. Jaworski, A.J. Pell, *Solid State Nucl. Magn. Reson.,* 2019, **101**, 51-62. DOI: 10.1016/j.ssnmr.2019.05.001

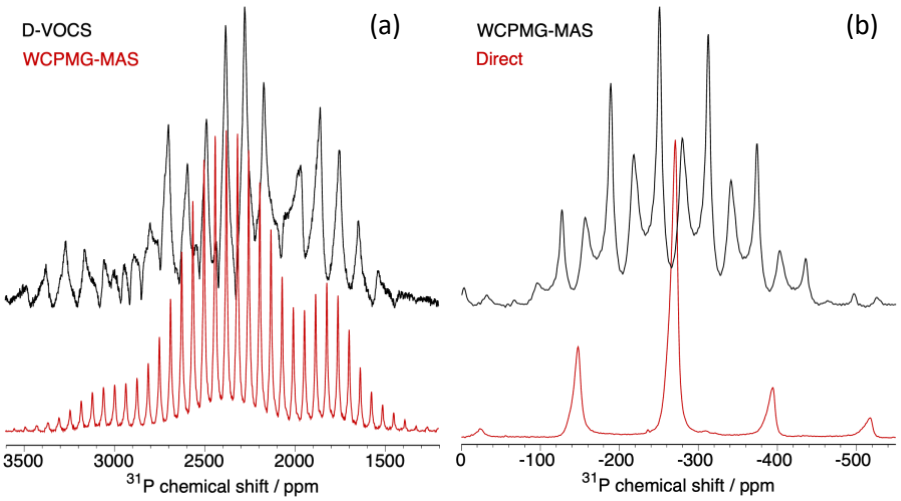


Fig. 1: Comparison of ^{31}P direct-excitation (VOCS) MAS and WCPMG-MAS NMR of $[Ln(P(SiMe_3)_2)_3(THF)_2]$ for Ln = (a) Nd, and (b) Sm, showing rejected spectra in black and accepted in red.

O8. Generative Machine Learning for Automating Structure Elucidation in Synthesis

Zheqi Jin,[1] Mohammad Golbabaee,[2] Craig Butts[1]

[1]School of Chemistry, University of Bristol, Cantock's Cl, Bristol, BS8 1TS. [2]School of Engineering Mathematics and Technology, University of Bristol, Ada Lovelace Building, Tankard's Cl, Bristol, BS8 1TW

Accurately elucidating molecular structures from Nuclear magnetic resonance (NMR) spectroscopy is of pivotal importance in chemical synthesis and drug discovery. Current methods, such as Computer-Aided Structure Elucidation (CASE)[1], are based on 'library search' and cannot interpret unseen molecules, thus limiting the accuracy of elucidation. Therefore, we want to make use of machine learning methods, so that the relationship between NMR spectroscopy data and molecular structures can be learned, and the process of structure elucidation can be automated.

In this project, we train our Graph Neural Network-based model, IMPRESSION (Intelligent Machine PREdiction of Shift and Scalar Information Of Nuclei)[2], to predict 2D molecular structures from their chemical shifts and coupling constants (Fig. 1a). Using chemical shift and atom type information extracted from NMR spectra, each atom is represented as a floating node in a graph with a feature vector (Fig. 1b). Every two atoms are then connected by an edge, also represented by a feature vector containing coupling constant information, forming a fully connected graph (Fig. 1c). Finally, the IMPRESSION model classifies whether each edge represents a bond, thus generating the 2D connectivity of the molecule (Fig. 1d).

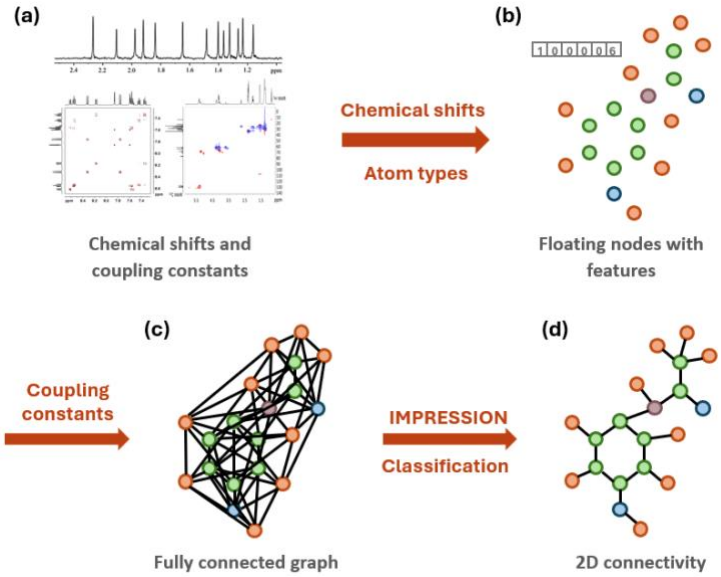


Fig 1. The workflow of IMPRESSION model predicting the 2D structure of a molecule from the chemical shift and coupling constant information

References

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2. Gerrard W, Bratholm LA, Packer MJ, Mulholland AJ, Glowacki DR, Butts CP. IMPRESSION–prediction of NMR parameters for 3-dimensional chemical structures using machine learning with near quantum chemical accuracy. Chemical Science. 2020;11(2):508-15.

O9. Solid-State NMR of Roxithromycin in Crystalline and Gel State

Svetlana Pavlović,[1,3] Maria Adobes Vidal,[2] Steven P. Brown[3]

[1]Department of Chemistry, University of Warwick, CV4 7AL, UK. [2]Novartis, Basel, Switzerland. [3]Department of Physics, University of Warwick, CV4 7AL, UK.

Roxithromycin (Active Pharmaceutic Ingredient – API) is a semi-synthetic drug that belongs to the group of macrolide antibiotics. It is broadly used for fighting infections caused by Gram-positive and Gram-negative bacteria, and atypical microorganisms. The structure of roxithromycin consists of a 14-member lactone ring with an N-oxime side chain, and two sugar molecules L-cladinose and D-desosamine, attached to the ring.[1] It has been reported that a mixture of Roxithromycin and particular excipients forms a gel like film during the dissolution process in acidic conditions.[2], [3] This phenomenon can slow down release of API from the formulation. Even though the gel formation behaviour has been reported, the mechanism behind it is still unclear, to our knowledge.

Recently, a ^{13}C-^{1}H HETCOR MAS NMR spectrum of Roxithromycin has been reported.[4] In this study, we have used MAS NMR to look at Roxithromycin at its crystalline state and in a gel formed with specific excipients.[2] The gel was studied by recording ^{13}C spectra using ^{1}H-^{13}C CP and INEPT and direct polarization experiments. Here, we present Gauge Including Projector Augmented Waves (GIPAW) calculations and ^{1}H detected ^{13}C-^{1}H correlation of Roxithromycin, acquired at 1 GHz with a spinning frequency of 100 kHz.

References

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O10. NMR Characterisation of the Intrinsically Disordered Protein JPT2: Structural Insights and Microtubule Interactions

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Intrinsically disordered proteins (IDPs) constitute 30% of the human proteome and are central to numerous cellular processes and diseases. However, their lack of stable structure poses significant challenges for mechanistic understanding and therapeutic targeting. Jupiter microtubule associated homolog 2 (JPT2) is a poorly characterised protein, predicted by AlphaFold2 to be intrinsically disordered. JPT2 is overexpressed in multiple cancer types, implicated in calcium signalling, and known to associate with microtubules, yet structure, dynamics, and mechanisms remain largely unknown.

In this work, I use a suite of biophysical approaches, with a strong emphasis on NMR spectroscopy, to demonstrate that JPT2 is indeed intrinsically disordered. I will share ^{13}C-detected NMR strategies that enabled high-confidence backbone assignment of this highly proline-rich protein, which exhibits extensive spectral overlap. Additionally, I will share preliminary NMR data on JPT2's interaction with microtubules, offering early mechanistic insights into the functional role of this enigmatic IDP.

O11. Enhanced signal discrimination in SABRE hyperpolarised ^{1}H benchtop NMR

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Benchtop NMR spectrometers are a cost-effective, portable alternative to traditional high-field NMR spectrometers based on superconducting magnets. However, benchtop NMR spectroscopy inherently suffers from reduced sensitivity as a direct result of the lower magnetic field strengths (1–2 T). Furthermore, a reduced Larmor frequency combined with the field-independence of scalar coupling leads to broader and more complex multiplets and peak overlap, particularly for ^{1}H nuclei whose chemical shift range is narrow. In mixtures, even of relatively simple molecules, resolution between multiplets can be impossible to achieve from standard pulse and acquire experiments.

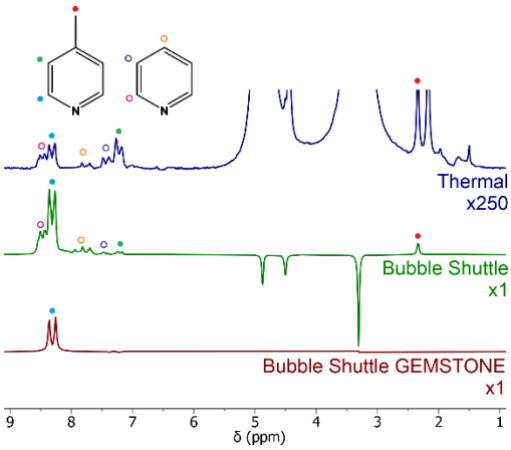

Figure 1: 62 MHz bubble shuttled SABRE GEMSTONE on 50 mM 4-methylpyridine, 50 mM pyridine, 5 mM IMes in methanol. Overlapped ortho resonance of 4-methylpyridine was isolated via GEMSTONE.

Signal amplification by reversible exchange (SABRE) hyperpolarisation can be used to overcome the intrinsic low sensitivity of benchtop NMR spectroscopy.[1] SABRE is a catalytic process that transfers the high spin order of *para*hydrogen (pH$_2$), the singlet nuclear spin isomer of molecular hydrogen, to a target substrate in solution. By generating non-equilibrium spin state populations within the substrate, its detectable NMR signals can be transiently increased by orders of magnitude.[2] In SABRE, spontaneous polarisation transfer occurs in a weak field (typically 6 mT for transfer to ^{1}H nuclei) over a period of *ca.* 10 s prior to detection in the NMR spectrometer. The chemical exchange process is reversible and therefore the same sample can be re-polarised multiple times through supplying fresh pH$_2$.

Peak overlap in ^{1}H benchtop NMR spectroscopy can be overcome through the use of ultra-selective observation methods such as GEMSTONE (gradient enhanced multiplet selective targeted observation NMR experiment),[3] or alternatively through removing the contribution of homonuclear scalar couplings to the spectrum using pure shift NMR.[4] Pure shift and GEMSTONE are complementary; the exact chemical shift of each resonance is identified using pure shift, allowing each multiplet to be individually targeted and resolved using GEMSTONE. These methods both suffer from significant sensitivity penalties and therefore benefit from being combined with hyperpolarisation.[5]

In this work, GEMSTONE and pseudo-2D interferogram Zangger-Sterk pure shift NMR experiments are implemented and optimised on 1 T (43 MHz), 1.4 T (60 MHz), and 1.8 T (80 MHz) benchtop NMR spectrometers for analysis of mixtures. We explore how these sequences perform when combined with SABRE hyperpolarisation, using an automated sample shuttling approach to re-hyperpolarise the sample between each step of the experiment.

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P1. Nuclear spin-state transport in nonlinear kinetic processes

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NMR and MRI techniques are frequently used to study chemical kinetics, particularly in metabolic processes within living systems; many such processes are higher order in nature (e.g. the Warburg effect [1]). Modelling the spin dynamics of such nonlinear reactions has remained challenging because fundamental equations of motion in quantum mechanics for the quantum state [2] and density operator [3] are linear. However, the law of mass action in chemical kinetics [4] and Navier-Stokes [5,6] equations in hydrodynamics are not fundamental; they are statistical approximations and can be non-linear. This incompatibility creates insidious difficulties in theoretical descriptions of systems where quantum processes coexist with chemical kinetics and spatial transport, for example in spin chemistry, magnetic resonance imaging of complex metabolic and hydrodynamic processes, and – our predicament here – nuclear magnetic resonance in microfluidic chips. I will report our progress with this problem in the context of NMR, where good approximate solutions exist under the assumption that nuclear spin states do not influence hydrodynamics or chemical kinetics. We found numerically stable solution methods for problems involving simultaneous non-linear kinetics, diffusion, flow, and quantum mechanical evolution in non-trivial spin systems. The results are used to model magnetic resonance spectroscopy and imaging in microfluidic chips.

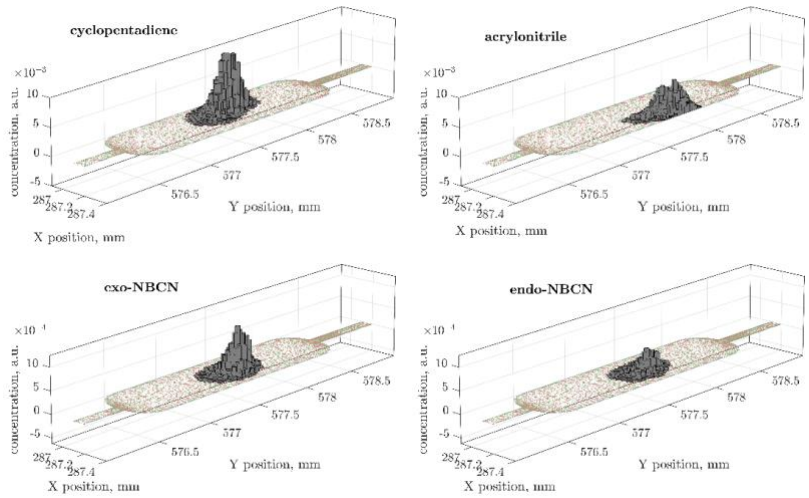

Figure 1. *Stop-frames of spatial dynamics and chemical kinetics simulations of the Diels-Alder cycloaddition of cyclopentadiene and acrylonitrile in a microfluidic chip. The initial condition is drops of cyclopentadiene and acrylonitrile in adjacent regions in the upper part of the chip. As the reagents flow downwards and mix, the reaction produces unequal quantities of exo- and endo-norbornene carbonitrile. The resulting time dependence of all concentrations in all Voronoi cells of the mesh is used in the subsequent stages to generate the non-linear kinetics superoperator at every time point in the spin dynamics simulation.*

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P2. Ultrawide and multinuclear NMR: Pushing the boundaries of existing hardware with Seedless optimal control pulses

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In high resolution NMR the inability to produce uniform excitation across a broad bandwidth hinders data acquisition. This poses a significant problem for 59Co which has a 20000ppm chemical shift range which poses issues for quantitative data collection. In addition, the bandwidth limitations of pulses typically limits high resolution NMR to the nuclei of a single isotope per channel. To bypass these limitations, we used Seedless to design phase modulated optimal control pulses that could either cover the ultrawide bandwidths required, or that would simultaneously excite different regions of frequency space from a single channel and carrier frequency.

Using Seedless simulations, we improved the fidelity of 5000ppm wide pulses, varying finite length, pulse duration, pulse power, and spin density, which we tested on the 59Co reference. We then expanded the excitation to 20000ppm and this pulse was validated by the simultaneous excitation of [Co(CO3)3]3- (14140ppm) and [Co(CN)6]3- (0ppm) in a single coaxial sample, on a 400MHz Bruker spectrometer with a room temperature BBFO probe. For the multinuclear work, we used Seedless to design pulses that can excite multiple narrow bandwidths at a megahertz distance from the carrier frequency which we validated on a 400 MHz Bruker spectrometer with a BBFO probe. Here, we simultaneously excited discontinuous regions of frequency space corresponding to 13C (100.6 MHz), 23Na (105.8 MHz), and 59Co (94.9 MHz) to produce a 1D spectrum with resonances almost 10 MHz apart. This multi-nuclear methodology enabled acquisition of different combinations of nuclei, including 25Mg-43Ca-37Cl (24.5, 26.9, and 32.6 MHz) and 7Li-31P (155.5 and 161.9 MHz).

Our ultrawide work demonstrate excitation of a ~2MHz bandwidth, corresponding to the 59Co bandwidth. These pulses will aid in wide bandwidth excitation in both solution and solid-state NMR, with potential applications including mechanistic insights involving rapid identification of unknown catalytic intermediates and countering broad linewidths and coupling constants. Our multinuclear work has demonstrated selective excitation across a 10MHz range. This has laid the groundwork for simultaneous single-channel multinuclear multidimensional experiments, and the targeting of hard-to-reach nuclei. This will expand the scope of experiments that can be performed on a standard two channel BBO probe, including correlation experiments without proton. Furthermore, this has the potential to expand to other magnetic resonance methods, such as MRI, where Seedless methodologies could enable proton MRI machines to detect 19F labelled probes.

P3. A drug-like small-molecule selectively induces multimerisation of an oncogenic disordered protein

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Intrinsically disordered proteins (IDP) are highly dynamic biomolecules that have historically been considered undruggable due to their lack of stable, well-defined binding pockets. Emerging evidence suggests that IDPs can interact with small, drug-like molecules. However, our understanding of the binding mechanisms is still limited, which hampers the rational development of new IDP-binders. Here we combine multiple biophysical approaches to show that ZZW-115 binds to the intrinsically disordered protein NUPR1 with low micromolar affinity and induces its oligomerisation. In contrast, fluoxetine, a small molecule with similar properties, binds NUPR1 with significantly weaker, millimolar affinity and fails to induce oligomerisation. Microscale thermophoresis shows the binding of ZZW-115 to NUPR1 to be two-phase and whilst NMR struggles to capture the initial small molecule binding event, it reveals local binding-induced dynamic changes in NUPR1 induced by higher molecule concentrations, providing an atomistic picture of how a small molecule can modulate the functional behaviour of a disordered protein. These findings uncover a mechanism by which small molecules can selectively influence the multifunctional roles of "untargetable" IDPs, broadening the scope for therapeutic targeting of this challenging protein class.

P4. Characterising ^{13}C Spectral Assignments and Substituent Distributions of Hydroxypropylmethylcellulose Acetyl Succinate Using Dynamic Nuclear Polarisation Nuclear Magnetic Resonance Spectroscopy

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Hydroxypropylmethylcellulose acetyl succinate (HPMC-AS) is the most widely used polymer in commercially available amorphous solid dispersions (ASDs) due to its ability to aid dissolution of poorly soluble drugs whilst impeding drug recrystallisation.[2] The ^{13}C spectral assignments for HPMC-AS differ in the literature largely due to the significant structural complexity of this polymer but are critical to identify drug-polymer interactions in ASDs containing HPMC-AS.[3,4] A dynamic nuclear polarisation (DNP) enhanced 2D ^{13}C-^{13}C refocused incredible natural abundance double quantum transfer experiment (INADEQUATE) spectrum was obtained to identify the one-bond ^{13}C-^{13}C connectivity in the polymer which confirms the most recent ^{13}C spectral assignments of HPMC-AS (**Figure 1**).[4] Moreover, the spatial distribution of substituents in cellulose-based polymers is known to affect their physical properties and hence the dissolution or absorption of a formulated drug.[5] We used the definitive ^{13}C spectral assignments for HPMC-AS and exploited the relayed-DNP of enhanced 1D cross polarisation (CP) spectra to determine that the HPMC-AS substituents were homogenously distributed for three commercial grades of the polymer.[6] Now NMR experiments performed on ASDs containing HPMC-AS can more accurately correlate observed drug-polymer interactions to specific sites of the polymer.

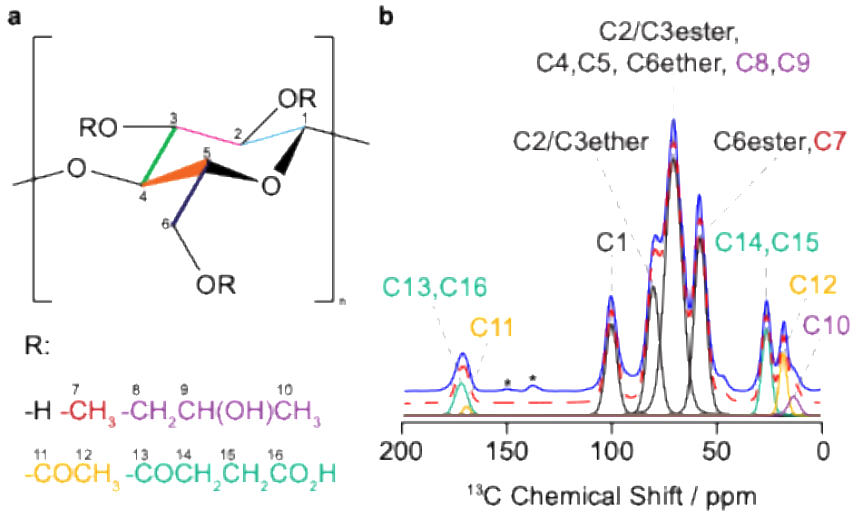

Figure 1: a Structure of HPMC-AS. **b** Full width DNP MAS NMR ^{13}C CP spectrum of HPMC-AS (LF) (blue) with simulated spectrum (red-dashed line). Carbon environments numbered for assignments.

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P5. Slicing Solvent Signals Out of Mixture Spectra

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We present a method for improving solvent signal suppression in liquid spectra. Solvent signals can be many orders of magnitude more intense than the resonances of analytes at mM or μM concentrations while water is effectively 110 M in ^{1}H and can give rise to radiation damping and other problematic phenomena. This intensity disparity makes it difficult to detect and quantify the solute. Presaturation by a low-power RF pulse lasting on the order of the solvent T_1 is the most common method of solvent suppression.[1] Such continuous wave irradiation is relatively straightforward to implement, requires no phase cycling, and is much more selective than methods using pulsed excitation such as WATERGATE.[2] Unfortunately, this very selectivity means that for the fringes of the sample where the B_0 field—and thus the Larmor frequency—deviate from the ideal, a residual "hump" of solvent signal will remain, and faraway signal can reside in the baseline.

Presaturation is commonly complemented by using multiple hard pulses such as single-increment NOESY[3] or the specifically designed FLIPSY[4] sequence reduce signal from the sample fringes by exploiting B_1 inhomogeneity in these regions. These methods of volume selection are "plug and play" but require phase cycling and leave the spectroscopist no room to tune the balance between suppression and sensitivity to the solutes.

We instead propose a very short slice-selective spin-echo to achieve suppression in a controllable active volume without the need for prescribed phase cycles. Our method enables reproducible tailored suppression for demanding applications like chemometrics, metabolomics and food analysis.

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P6. Spin-System Selective 2DJ Spectroscopy

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Analysis of ^{1}H NMR spectra is commonly hindered by extensive signal overlap due to the narrow chemical shift range and broad multiplet structures. Selective 1D experiments can be used to target key information and produce spectra that are simpler than the conventional 1D or 2D parent experiment. These are especially useful in cases of mixture analysis, where signals from multiple spin systems routinely overlap, but often lack the resolving power to provide unambiguous separation of signals. Pure shift experiments, including the 1D selective TOCSY-PSYCHE[1] and the recently published TREASURE,[2] have been developed in an attempt to provide the much-needed additional resolution, simplifying wide multiplets to singlet peaks. Although very successful, pure shift approaches remove essential spin-spin coupling information, making confirmation and elucidation of molecular structure and conformation more difficult. An ideal experiment would target one specific spin system, suppressing other signals; provide pure chemical shift information to identify, and determine the number of, chemical environments; and provide coupling information without compromising resolution. A logical approach to this ideal is to incorporate J-resolved spectroscopy[3] into a selective experiment, facilitating signal resolution and aiding mixture analysis.

Here, we introduce a new type of selective 2D NMR method, selective-TOCSY-2DJ spectroscopy, which generates a 2DJ spectrum containing only the signals of a chosen spin system. This approach is compatible both with conventional selective excitation, and with the ultra-selective GEMSTONE[4] element that uses spatial multiplexing to allow the selection of a single frequency even in significantly overlapped spectral regions.

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P7. Structural characterisation of co-translational folding H-Ras intermediates by ^{19}F NMR

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Co-translational folding is crucial for the production of biologically active protein (1), as the in vitro refolding of proteins often results in functionally inactive conformations, as recently demonstrated for the oncogenic H-Ras protein.(2) By lowering the entropic penalty of folding, the ribosome alters the folding pathway of proteins, thermodynamically stabilising folding intermediates that are otherwise only transiently populated or absent off the ribosome.(2–4) Site specific ^{19}F labelling of ribosome-nascent chain complexes (RNCs) has uniquely permitted the observation of different conformational states populated during co-translational folding, including partially folded intermediates of nascent chain HRAS protein (2), and has enabled the determination of atomistic structure if folding intermediates of an immunoglobulin-like domain on the ribosome.(5) In this work, we use ^{19}F NMR to structurally characterise the folding intermediates of HRAS on the ribosome. We rationally designed ^{19}F-label pairs, predicted using AlphaFold3, to engineer chemical shifts sensitive to van der Waal's contact within and between α-helices and β-sheets.(6) Incorporation of these label pairs are enabling insights into the structure adopted by HRAS folding intermediates on the ribosome. Future investigation with molecular dynamic (MD) simulations and hydrogen-deuterium exchange mass spectrometry (HDX-MS) will permit the determination of the structural ensembles of the intermediates, shedding light on their co-translational folding mechanism that is crucial to obtaining their function.

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P8. NMR studies of MERS-CoV non-structural protein 10 as a potential drug target

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Coronavirus nsp10 plays a significant role in RNA-synthesizing machinery by interacting with both nsp14 and nsp16, stimulating their respective 3'-5' exoribonuclease and 2'-O-methyltrasferase activities (Bouvet et al., 2014). While coronavirus non-structural proteins have been popular research topics in recent years since the pandemic, there are still gaps in MERS-CoV nsp10. This study aims to uncover the structure, dynamics and druggability of MERS-CoV nsp10 by solvent NMR. In this research, the structure of MERS nsp10 was studied by the following method: 15N SOFAST and triple resonance spectra (HNCACB, HNCACO, HNCO, HNCOCACB) were recorded for sequential backbone assignments, followed by 13C HSQC and a pair of triple resonance spectra (HCC TOCSY and HHC TOCSY) for sidechain assignments; 15N NOESY and 13C NOESY were also recorded to obtain restraints for structure calculations. To obtain backbone dynamics data in different timescales, 15N Relaxation and Chemical Exchange Saturation Transfer (CEST) studies were carried out. 19F NMR screening was used to identify initial fragment hits, followed by Kd quantification by MST and binding pocket verification by SOFAST. The results show that MERS CoV nsp10 has a conservative and rigid structure, similar to SARS CoV and SARS CoV 2. 15N Relaxation data indicated variation in local flexibility, while CEST shows potential conformational change on the N-terminal helix. This study also identified distinct scaffolds of fragment hits against MERS CoV nsp10, and four binding sites on the protein, revealing MERS CoV nsp10 as an attractive drug target.

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P9. Improved Light Uniformity using Etched NMR tubes for high throughput NMR experiments

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Nuclear Magnetic Resonance (NMR) spectroscopy is a widely utilised analytical technique due to its high reproducibility, non-destructive nature, and ability to provide detailed structural insights, making it particularly suited to studying chemical processes in situ. As photochemistry gains increasing prominence in modern chemistry, integrating controlled light exposure into NMR experiments has become desirable but technically challenging due to the sample's placement deep within the magnet bore. Existing approaches often require permanent probe modifications or sacrifice the high-throughput capabilities essential to routine NMR workflows.

Here, we present an adapted illumination setup that delivers light through the flow cell accessory port at the base of the magnet, enabling illumination without compromising compatibility with standard sample changers.1 In this work we evaluate the light intensity and uniformity of this method using photo-CIDNP (chemically induced dynamic nuclear polarization), a hyperpolarization technique in which light-induced radical pair reactions lead to enhanced NMR signals, providing a sensitive probe of photochemical activity within the sample. Photo-CIDNP enhancements of 155-fold have been observed for 19F NMR using a 700 MHz spectrometer for a 6-fluoroindole, flavin mononucleotide test system. Improved light uniformity was achieved when using etched NMR tubes compared to traditional non-etched tubes, due to improved light refraction into the NMR active volume. The technique was successfully applied to wine samples, where photo-CIDNP enhancements were observed, demonstrating its potential for fingerprinting of metabolomic samples.2

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P10. Quantitative Analysis of Fast Ligand Dissociation Kinetics Using ^{19}F $R_{1\rho}$ Relaxation Dispersion

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Fragment-based drug discovery (FBDD) is a powerful approach for generating small-molecule lead compounds from weak-binding fragments. However, FBDD faces a challenge in detecting and characterising weak interactions (high µM to low mM binding affinities) associated with typical fragment hits, which can push detection methods to their limits, resulting in false positives and false negatives that often go unrecognized.[1] Ligand-observed (LO) NMR is a key tool in this discovery process, and several experiments have been developed, including saturation transfer difference (STD), waterLOGSY, and R_2 relaxation (through CPMG measurements).[2,3] The latter can also be applied to fluorine-containing ligands using ^{19}F spectroscopy. However, translating 'hits' from these experiments into affinity measurements, even only in relative terms, is challenging and inhibits ranking of different compounds. In this work, we propose an alternative approach using ^{19}F ligand-observed NMR to detect binding-induced changes in the rotating-frame relaxation rate, $R_{1\rho}$. We have applied our approach to diverse ligands binding to three protein targets and have successfully extracted dissociation rate constants for both isolated compounds and fragments in mixtures of up to 30 compounds. We have shown that our method can quantify dissociation rates between 5,000 s^{-1} and 60,000 s^{-1}. Importantly, by measuring dissociation kinetics, we provide medicinal chemists with a simple physical quantity free from complex NMR parameters such as tumbling times, cross-relaxation rates, or chemical shift anisotropies. Furthermore, we have developed easy-to-use software with a simple graphical interface to facilitate efficient data analysis. We anticipate this approach may help accelerate early stages of FBDD, facilitating the characterization, screening, and ranking of fragment libraries.

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P11. *In situ* SABRE hyperpolarisation for mixture analysis in benchtop NMR spectroscopy

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Benchtop NMR spectrometers can be advantageous over high-field spectrometers on account of their portability, low maintenance needs and affordability.1 However, these benefits come at the expense of resolution and sensitivity. Hyperpolarisation methods allow the barrier of sensitivity to be overcome as large amounts of polarisation can be generated irrespective of the magnetic field of the spectrometer.

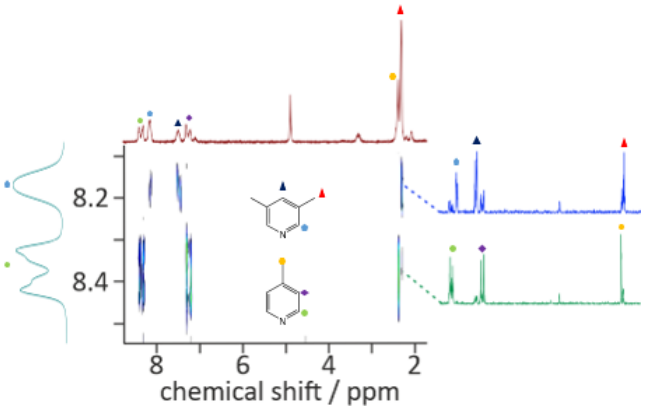

Figure 2: A two-dimensional selective TOCSY spectrum of 4-methylpyridine and 3,5-dimethylpyridine enhanced by RF-SABRE hyperpolarisation. Due to the hyperpolarisation, it was possible to obtain this spectrum with just 2 scans. Right: selective spectra of each compound extracted from the 2D.

This work concerns SABRE (Signal Amplification By Reversible Exchange) hyperpolarisation in which spin order from para-hydrogen (p-H2), the nuclear singlet state of dihydrogen, is transferred onto a substrate via reversible binding to an iridium catalyst.2 SABRE can create large amounts of polarisation in seconds and is relatively cheap when compared to other hyperpolarisation methods. SABRE also allows repeated hyperpolarisation of the sample as the substrate molecules remain chemically unchanged.

SABRE is commonly achieved via the 'shake-and-drop' method, in which the headspace of an NMR tube is filled with p-H2 and shaken in a 6 mT polarisation transfer field, created by a handheld Halbach array, before insertion into the spectrometer.3 This method provides very effective signal enhancement but is arduous to perform and can give irreproducible levels of polarisation. In this work, an entirely in situ method was developed by automatically delivering the gas via a capillary and using radio-frequency (RF) irradiation to satisfy the polarisation transfer conditions. This method, RF-SABRE, has previously been successful at high-field,4 but the lower B0 field and robust hardware of benchtop spectrometers have allowed much greater enhancements (up to 750-fold) to be achieved. Additionally, a new highly effective method to hyperpolarize 13C nuclei using RF-irradiation in situ has been developed.

The automation of the in situ method gives repeatability suitable for two-dimensional experiments such as a hyperpolarized selective-TOCSY as shown in Figure 1. Two-dimensional experiments facilitate the assignment of spectra in spite of the spectral overlap issues associated with benchtop NMR. The methods presented here will contribute to a battery of *in situ* hyperpolarized benchtop experiments capable of differentiating multicomponent mixtures at micromolar concentrations.

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P12. Sequence Structure Relationships of C-terminal linker histone tails and their impact on DNA condensation

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Packaging of eukaryotic DNA utilises several histone proteins to generate condensed macromolecular chromatin structures, thereby controlling gene activity. The basic structural unit of this packaging process, the nucleosome, is composed of an octet of core histones around which 147 bp of DNA are twice wrapped, resulting in 'beads on a string' across broader expanses of DNA [1,2]. Greater compaction is generated by two 'linker' histones, H1 and H5, which associate with the nucleosomal dyad and linker DNA strands [1,3]. The resulting structures, chromatosomes, compact into a virtual continuum of states, that interconvert dynamically between 'open' and 'closed' structures. The more extreme 'closed' structure, the 30nm fibre, is highly ordered and associated with transcriptional inactivity across a range of length scales [4]. A further feature of the interactions between linker histones and the linker DNA is their ability to form fuzzy complexes, which when previously assayed, using a minimal model composed of the H1 C-terminal tail and 20 bp of DNA, resulted in phase separated condensates [5].

The degree to which chromatin is open or closed can be controlled by the particular H1 subtype or variant [1]. The main source of variation is in the long disordered C-terminal region, that is highly amenable to NMR spectroscopy, although its low complexity (35% lysine, 24.0% alanine and 11% proline on average) makes chemical shift assignment particularly challenging. Previous projects in the Stott lab have identified three main factors that control chromatin compaction; positive charge density, lysine:arginine ratio, and the length of proline free regions [5]. We therefore sought to compare the canonical H1.11L C-terminal tail, CH1, with a mutant variant wherein each of the proline residues had been replaced by an alanine, $CH1_{PA}$, designed to mimic natural H1 variants from echinoderm sperm that contain extensive proline free regions and compact chromatin tightly. We have deployed both solid- and solution-state NMR and molecular biophysics methods to examine the effect of proline-free regions on the structure and condensing properties of CH1 in both the free protein and the protein DNA complex in the dense phase of phase separated samples. We achieved a near complete assignment of the free protein using a suite of experiments that is optimal for this ultra-low complexity protein (40.0% lysine, 43.0% alanine), combined with the high resolution that is achievable at 1.2 GHz. We find that despite the high net positive charge of $CH1_{PA}$, it is able to form alpha-helices. Moreover, there is a strong coupling between folding and condensation that we attribute to the associated changes in the charge density of the protein.

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P13. Characterisation of Dynamic Hairpin DNA Switches in *E-coli*

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Antibiotics have revolutionised medicine, saving countless lives since their discovery in the early 20th century.[1] The discovery of penicillin in 1928 started the golden age of antibiotic discovery that peaked in the mid-1950s. Since then, a gradual decline in antibiotic discovery and development and the evolution of drug resistance in many bacteria, especially gram-negative *Escherichia coli (E.coli)*, has led to the current antimicrobial resistance crisis.[2] Identifying and developing new antibiotics, especially those with new modes of action, is imperative to help combat resistance.[1,2] One approach is to identify and target conserved nucleic acid sequences within the regulatory regions of bacteria, capable of forming secondary structures such as G-quadruplexes, i-motifs, or hairpins.[3]

Using bioinformatics, we used known G-quadruplex forming sequences[3] and identified where these were present in gene promoters within *E-coli*. We identified several places where G-quadruplex forming sequences were present in promoter regions where formation could potentially affect gene expression. Our investigation led us to two hairpin sequences in a bi-directional promoter between the genes fepB and entC. The G-quadruplex forming sequences were found to overlap also with two potential hairpins in a region previously identified to form secondary structures.[4] The fepB gene encodes a periplasmic binding protein essential for acquiring ferric enterobactin, a key component in the *E. coli* iron acquisition system, whilst entC is an isochromate synthase that is involved in the biosynthesis of the enterobactin, an effective iron sequestering agent. Biophysical characterisation of these two hairpin sequences from fepB reveals that the sequences can also switch to a competing structure, either a G-quadruplex or an i-motif, depending on the conditions. Our focus on the fepB gene stems from its critical function in iron uptake, a process essential for bacteria growth and survival. Recognising the role of fepB in influencing iron uptake in *E. coli*, we also investigated the potential of iron's two most crucial oxidation states (Fe^{2+} and Fe^{3+}) on the hairpin structures. We show how Fe^{3+}, but not Fe^{2+}, cations switch the conformation of the hairpin sequences, indicating potential of some sort of feedback loop, using Fe^{3+} cations to switch DNA conformation.

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P14. Development of a ^{19}F NMR fragment screening platform at UCL

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Fragment-based drug discovery (FBDD) is a well-established approach to drug development, providing an accessible alternative to high-throughput screening (HTS). While HTS involves screening extensive libraries (>10^5) of drug-like compounds, FBDD utilises smaller libraries (10^2 – 10^3) of small fragment molecules, such as fluorine-containing fragments, to detect ligand binding with protein. Fluorine-containing fragments are ideal due to the sensitivity and simplicity of detection when utilising fluorine ligand-observed nuclear magnetic resonance (^{19}F NMR) spectroscopy [1, 2].

This study involved the validation of the composition and stability of pre-prepared fragment cocktails to ensure reliability and reproducibility of subsequent screening experiments. To support this, a systematic protocol was followed to categorise fragments into three distinct groups through a series of validation tests to ensure clear identification of each fragment. A robust protocol for automated liquid handling was subsequently developed and designed to minimise sample waste and contamination from fragment carryover.

To aid data analysis we have also developed a new data analysis platform, NMRScreen.jl [3], where data on the fragment library, degradation status and results of protein screening was prepared and uploaded onto the programme. This tool provides a user-friendly interface to allow for identification of hits and visualisation of binding events across multiple samples.

We will report on the results for two contrasting protein targets: bovine serum albumin (BSA), for which we obtained a high hit rate of 9%, and the TRXL2 domain of the ER glycoprotein folding sensor UGGT2 which is a novel target with no well-defined binding pockets.

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P15. Modelling dissipative magnetization exchange dynamics in magnetic resonance

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Quantifying the role of the environment in a quantum system of interest has remained an active pursuit for studying the effects of dissipation in a wide-range of problems in chemical physics and spectroscopy. From an operational perspective, the complexities encountered in the description of open systems have ushered in the development of models without explicit consideration of the complete system. To this end, phenomenological descriptions[1,2] that involve the inclusion of exponential damping terms have remained the method of choice. While such methods have gained prominence in providing a qualitative explanation for observations in spectroscopy, they are of limited utility in quantitative studies that involve the estimation of molecular constraints. As an alternative, the present work[3] explores the possibility of understanding the nuances of dissipation found in open systems through analytic methods. Specifically, the magnetization exchange between a pair of spins (say I_1 and I_2) is examined under periodic modulation in the presence of a surrounding bath of other spins. Employing the concept of effective Hamiltonians[4] and utilizing the block-diagonal structure of the derived effective Hamiltonians, analytic expressions (see Fig.1) are derived for describing the effects of dissipation (due to neighboring spins) on the system of interest without increasing the dimension of the problem.

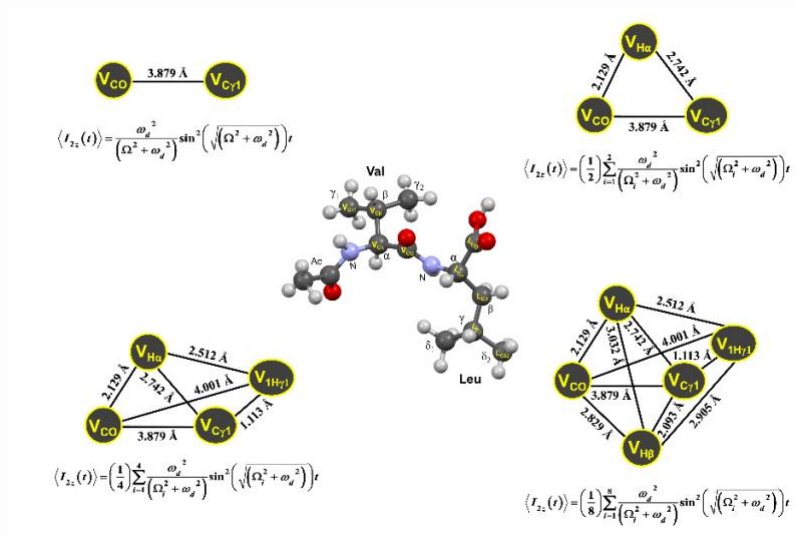

Fig. 1. Signal expressions for various spin systems employing dipeptide N-acetyl-L-valine-L-leucine (VL).

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P16. Solid-state NMR spectroscopy reveals temperature-dependent glucan remodelling in fungi

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Fungal cell walls undergo dynamic remodelling in response to environmental stress, yet the molecular details of these changes remain not completely understood. In this study, we employed ^{13}C-labelled solid-state Nuclear Magnetic Resonance (ssNMR) spectroscopy to investigate the impact of elevated temperature on the glucan architecture of wild-type *Schizosaccharomyces pombe*. Comparative analysis of isolated cell wall and whole cell samples revealed a consistent compensatory shift in glucan composition: β-1,3-glucan levels increased while α-1,3-glucan decreased with temperature in both contexts. However, the total glucan content increased markedly in the isolated cell walls but decreased slightly in whole cells, indicating that thermal stress triggers a redistribution of glucan biosynthesis, favouring the wall. These findings demonstrate that *S. pombe* reinforces its cell wall selectively under stress, a response that is masked in the analysis of whole cells. Our results underscore the importance of targeted sample preparation for capturing localised structural adaptations and highlight ssNMR as a powerful tool for probing in situ polysaccharide remodelling.

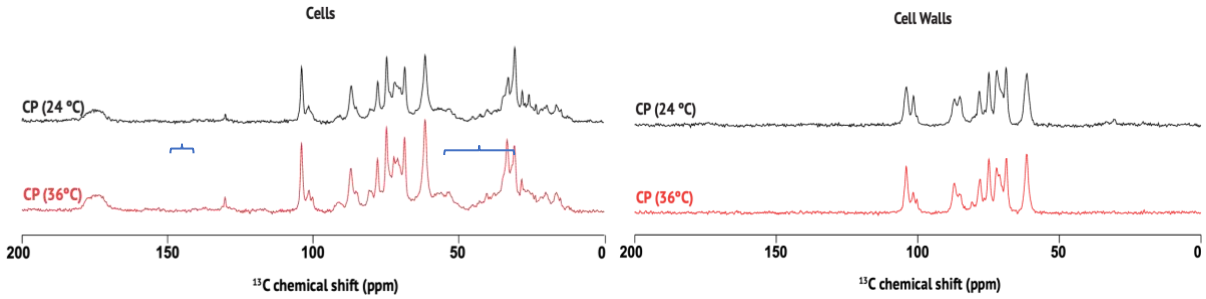

Fig. 1: (left) CP spectra of ^{13}C-labelled *S. pombe* whole cells at 24 °C (black) and 36 °C (red). (right): CP spectra of ^{13}C-labelled *S. pombe* isolated cell walls at the same temperatures (24 °C: black and 36 °C: red).

P17. Differential Self-Assembly of Sequence-Isomeric Phosphoestamers

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Non-natural, sequence-defined polymers could enable fine-tuning of folding and self-assembly programmed through monomer order, analogous to the behaviour of proteins,[1] but we are a long way from being able to reliably predict the conformation and aggregation of any arbitrary precision macromolecule. Sequence-defined oligo/polyphosphoesters (phosphoestamers) can self-assemble into a wide variety of superstructures according to their sequence and provide a useful model for understanding sequence/structure relationships.[2] In this work, we explore this relationship through examining the self-assembly of all possible tetramers comprised of equal ratios of dodecane diol (C12) and hexa(ethylene glycol) (HEG) monomers in a combined experimental/computational workflow using diffusion-ordered NMR spectroscopy (DOSY) and molecular dynamics (MD) simulations (Figure 1). Our results show that self-assembled structures can be reliably programmed and understood using monomer sequence.

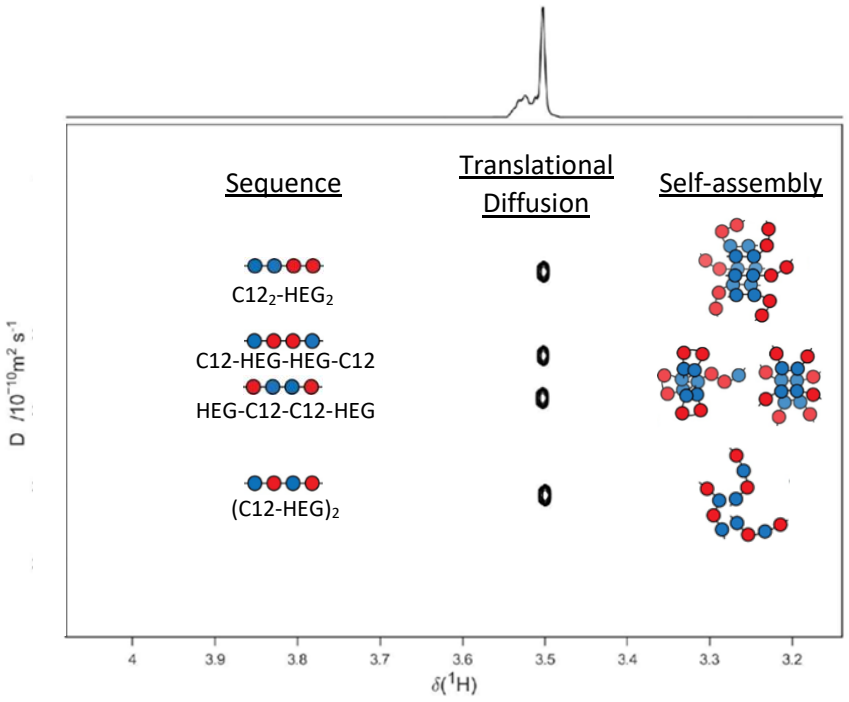

Figure 1. Sequence-isomeric phosphoestamers exhibited differential self-assembly in ^{1}H DOSY NMR and structures were confirmed by subsequent computational analysis.

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P18. Web-IMPRESSION: Fast, Accessible, DFT Accurate NMR Predictions for Chemical Shifts and Couplings At Your Fingertips

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The accurate and rapid prediction of three-dimensional (3D)-aware Nuclear Magnetic Resonance (NMR) properties is essential for elucidating the stereochemistry and conformational dynamics of molecules in solution. Traditional computational approaches such as Density Functional Theory (DFT) provide reliable predictions but are computationally intensive, often requiring days of high-performance computing resources for a single molecule. Empirical and machine learning-based alternatives, while faster, are typically limited in scope - either predicting only a single NMR parameter or being restricted to narrow chemical spaces.

We introduce IMPRESSION-Generation 2 (G2)[1], a transformer-based neural network architecture that significantly accelerates the prediction of multiple NMR parameters simultaneously while maintaining DFT-level accuracy. IMPRESSION-G2 is the first tool of its kind to predict, in a single pass, all NMR chemical shifts and scalar coupling constants for nuclei including $\partial^{1}H$, $\partial^{13}C$, $\partial^{15}N$, and $\partial^{19}F$, up to four bonds apart, directly from a 3D molecular structure. On average, it generates ~5000 NMR parameters per molecule in under 50 milliseconds - representing a speed-up of over six orders of magnitude compared to DFT workflows.

To complement this, the use of GFN2-xTB[2] for geometry optimization allows complete 3D structure generation in mere seconds, enabling full predictive workflows for new molecules that are 1,000 to 10,000 times faster than conventional DFT-based methods. Despite this dramatic acceleration, IMPRESSION-G2 achieves exceptional accuracy across a wide chemical space of small organic molecules (up to ~1000 g mol^{-1}) containing atoms such as C, H, N, O, F, Si, P, S, Cl, and Br. Benchmark results show mean absolute errors of ~0.07 ppm for 1H shifts, ~0.8 ppm for 13C shifts, and <0.15 Hz for $^{3}J_{HH}$ couplings - surpassing existing empirical and machine learning tools. Furthermore, IMPRESSION-G2 demonstrates strong generalisability to entirely independent test sets, reinforcing its robustness for broad real-world applications.

To make this powerful technology widely accessible, we introduce Web-IMPRESSION, a user-friendly online platform that enables researchers to run NMR predictions using IMPRESSION-G2 directly in the browser. Web-IMPRESSION requires only a 3D molecular structure as input and returns accurate chemical shifts and scalar couplings within minutes, even on standard consumer hardware. This accessibility democratises high-throughput NMR prediction workflows, facilitating rapid structural analysis in fields ranging from drug discovery to synthetic chemistry.

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